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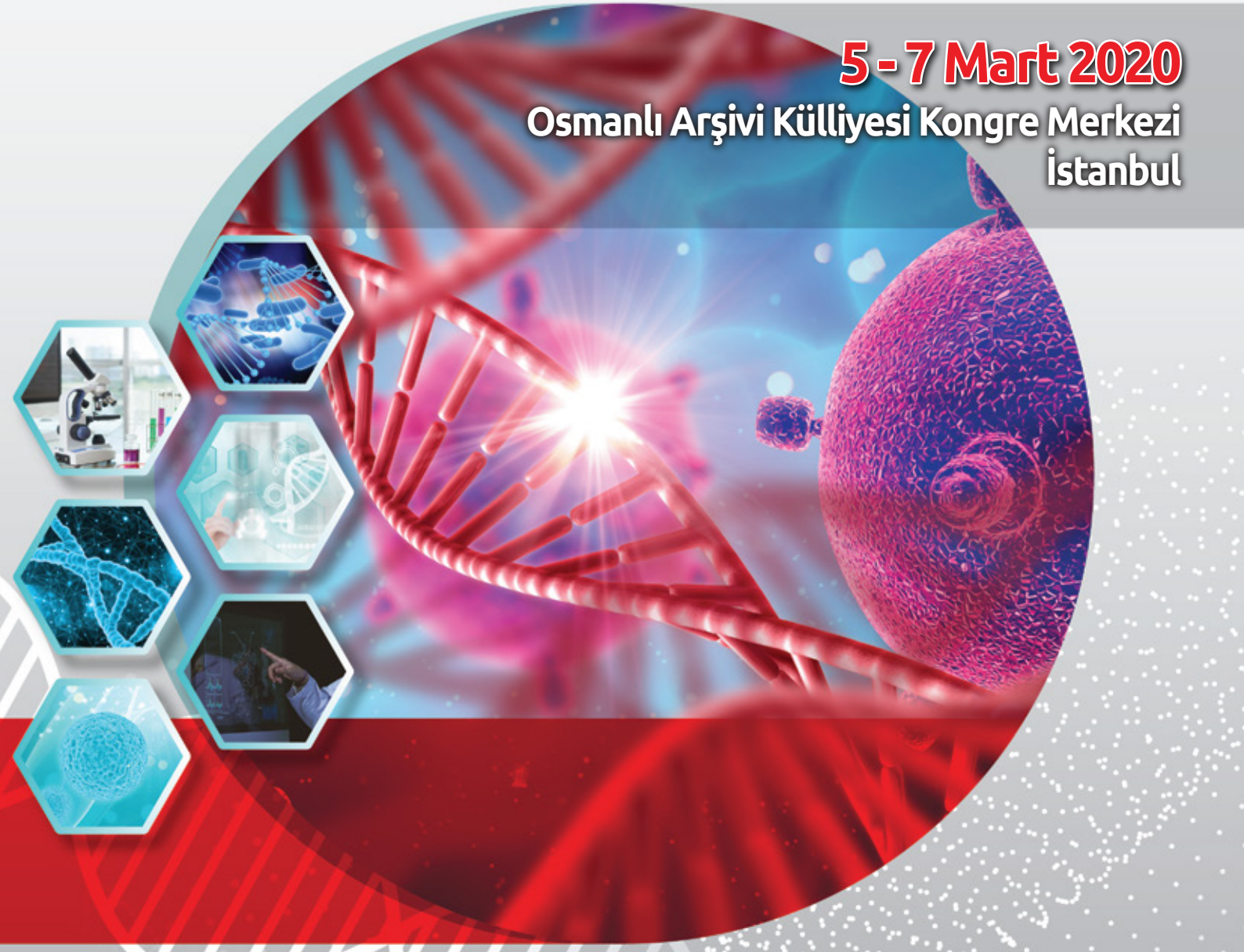

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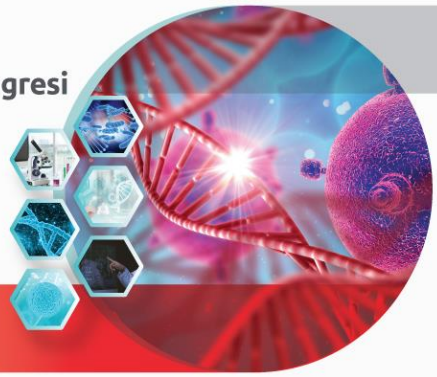

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SS-01

Type of the Paper (Extended Abstract, Meeting Report, Preface, Proceeding, etc.)

Black Phosphorus Based Nanocomposites for Antibacterial Effect against *Staphylococcus aureus* †

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† Presented at the conference title, place, and date.
Published: date

Abstract: BP/Au nanocomposites have been synthesized and used photothermal antibacterial agent against *S. aureus* as a model microorganism. The resulting nanomaterials were characterized by X-Ray Diffraction (XRD) spectroscopy and transmission electron microscopy (TEM) techniques. The antibacterial activity of BP/Au nanocomposites were studied by photothermal effect, bacterial growth curve, colony counting method and live-dead fluorescence staining. The results show that under NIR light irradiation, BP/Au nanocomposites showed antibacterial activity against *S. aureus*. This NIR light driven photothermal antibacterial strategy holds promise for fighting against bacterial infection and paves the way for the investigation of alternative solutions to antibiotic drugs.

Keywords: antibacterial activity; nanocomposites; photothermal effect

1. Introduction

Due to the inadequacy of antibiotics for antibacterial treatment and development of antibiotic resistance, it is crucial to find new strategies that will overcome bacterial resistance issue. Black Phosphorus (BP) is a photosensitizer that has wide absorbtion range in energy spectrum from visible to near-infrared (NIR) light [1]. BP is a non-toxic and biocompatible nanomaterial, which is easily degraded into harmless compounds such as phosphate and phospite [2, 3]. This property shows promise biotechnological and pharmacological applications such as biosensing[4], bioimaging[5] and cancer therapy[6]. However, there are quite few studies on antibacterial activity of BP under NIR light irradiation. Up to now, BP has been used as an antibacterial agent against *E.coli*, *B. subtilis* and *S. aureus* with/without NIR light [7-10]. Recently, BP-Ag/Au nanocomposites have also been used for fighting against MRSA and *E.coli* [11] [12]. In this work, Au nanoparticles (Au NPs) were decorated on BP nanoshets to obtain BP/Au nanocomposites which were characterized by XRD spectra and transmission electron microscopy (TEM). The photothermal antibacterial effect of BP/Au were explored against *S. aureus* by the measurement of temperature increase, bacterial growth curve, colony counting and live/dead fluorescence staining methods. The treatment of either BP or BP/Au under NIR light inhibited the growth of *S. aureus* in the first 5 h. After 5 h BP lost its activity but; BP/Au did not.

This work shows the potency of BP/Au nanocomposites as a photothermal antibacterial agent against pathogen bacteria and makes the way for the exploration of biocompatible nanomaterials as an alternative to antibiotic drugs.

2. Materials and Methods

After BP nanosheets and Au NPs synthesised, Au NPs were assembled onto BP to obtain BP/Au nanocomposites. BP, BP/Au and water (control) were irradiated for 5 minutes by a NIR light source (1 W cm^{-2} ,808 nm). The temperature increase was measured by infrared thermometer (TFA ScanTemp 330). After the BP/Au treatment of the *Staphylococcus aureus* (ATCC 29213) culture in a 96-well plate, samples were irradiated with NIR light for 5 min and incubated at 37° C for 24 h. Antibacterial activity was tested by OD_{600nm} (Optical Density at 600 nm) measurement at a microplate reader (Thermo Scientific Multiskan GO) per hour. After 3h incubation, the samples were diluted to spread on agar petri for colony counting method. For fluorescence microscopy, the samples were inoculated in a 96-well plate and treated with nanomaterials with/without NIR light irradiation. After 3 h incubation, the samples were stained with live-dead bacterial staining kit (LIVE/DEAD BacLight Bacterial Viability Kit)and imaged by a fluorescence microscope Olympus BX51 with DP70 CCD camera.

3. Results and Discussions

3.1. Synthesis and Characterization of nanocomposites

TEM image of BP/Au illustrated in Figure 1 showing that Au NPs were properly decorated on BP nanosheets.

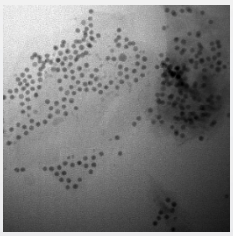

Figure 1. TEM image of BP/Au nanocomposites.

The crystal structure of Au NPs and BP/Au was examined by X-Ray Diffraction analysis. For Au NPs the strongest peak can be seen at 2θ degrees of 38 with several lower intensity peaks (Figure 2a). In Figure 2b, XRD pattern of synthesised BP/Au nanocomposites can be seen. Characteristic peaks of BP are preserved with presence of Au NPs at 2θ degrees of 38.

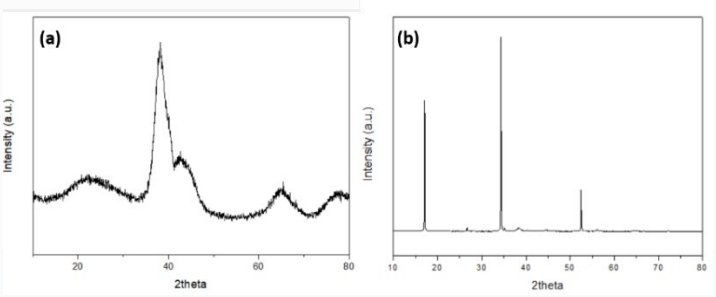

Figure 2. XRD patterns of **(a)** Au NPs and **(b)** BP/Au nanocomposites.

3.2. Photothermal effect

The photothermal effect of BP/Au was examined under NIR light irradiation. As depicted in Figure 3 , after NIR light irradiation, although there was no significant change in the temperature of pure water, the temperature of BP and BP/Au solutions was increased due to their photo-absorbtion ability (BP/Au > BP).

3.3. Antibacterial activity analysis

Figure 4a shows the bacterial growth curve of *S. aureus* after the treatment of BP/Au nanocomposites under NIR light irradiation. As showed in Figure 4b even 5 h after the treatment of nanocomposites, the growth of bacteria was suppressed due to the photothermal effect of BP/Au. The results of colony counting assay were in a harmony with the OD values shown in Figure 4c. Bacterial survival rates after the treatment of NIR, BP/Au and BP/Au under NIR light were 93, 50 and 34 %, respectively (Figure 4d).

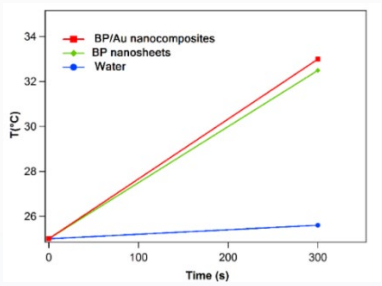

Figure 3. Photothermal effects of BP and BP/Au solutions upon NIR light irradiation. Pure water was used as a control.

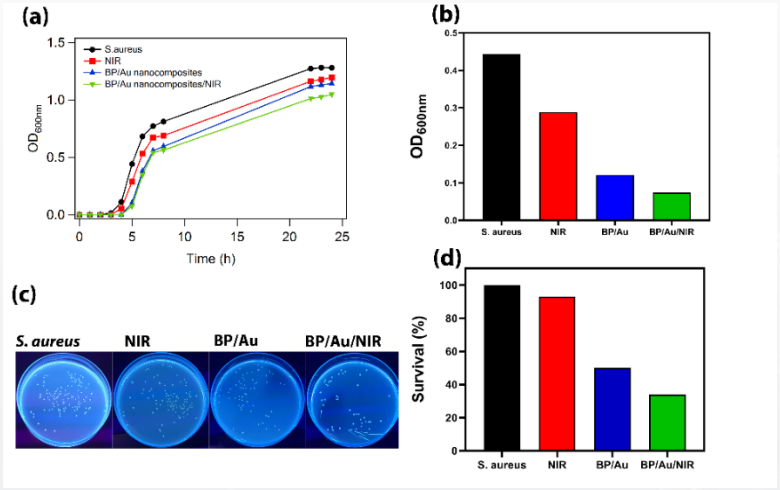

Figure 4. (**a**) Bacterial growth curve of *S. aureus* after different treatments. (**b**) OD_{600nm} values of *S. aureus* after 5 hours incubation. (**c**) Petri images of the bacteria after different treatments and (**d**) the corresponding survival rates (%).

Moreover, the effect of concentration of BP/Au on the bacterial growth during 5 h was examined as shown in Figure 5. As the concentration increased, antibacterial effect has regularly increased.

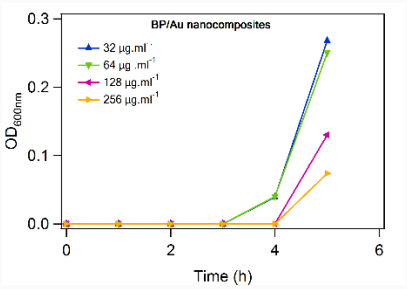

Figure 5. Concentration (µg.ml^{-1}) effect of BP/Au nanocomposites on *S. aureus*.

To further investigation of antibacterial activity of BP/Au, the membrane integrity of the bacteria was imaged by live-dead fluorescence staining kit. The kit containing SYTO 9 and Propidium Iodide (PI) dyes was used to show live and dead bacteria. PI could only permeate through damaged cell and gives red fluorescence. Otherwise, SYTO pass through the live cell membrane and gives green colour. *S. aureus* lost its membrane integrity when treated with BP/Au nanocomposites under NIR light irradiation (Figure 6), probably because of nano-knife effect of nanomaterials [8].

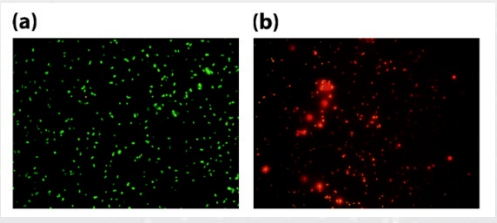

Figure 5. Fluorescence images of **(a)** *S. aureus* and **(b)** after the treatment of BP/Au under NIR light.

4. Conclusions

In summary, BP/Au nanocomposites with the irradiation of NIR light generates heat by photothermal effect which inhibits the growth of *S. aureus*. The antibacterial efficiency was proven with OD_{600nm} values of the bacteria, colony counting results and fluorescence microscopy images. BP/Au nanocomposites was more effective than bare BP nanosheets under NIR light during 5 h, shows the potency of biodegradable nanocomposites to overcome bacterial infection as an alternative to antibiotics.

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Author Contributions: Onder Metin and Huseyin Kucukkececi designed and performed the experiment of nanomaterials synthesis and characterization; Imren Hatay Patir and Ilknur Aksoy performed antibacterial analysis experiments including photothermal effect, OD measurement, colony counting assay and fluorescence staining; Fatih Sevgi, Imren Hatay Patir and Ilknur Aksoy analyzed the data; also Fatih Sevgi contributed reagents and materials tools; Ilknur Aksoy wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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SS-02

Microbiological study of ointments based on silver nanoparticle and phyto extracts

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Summary: One of the important issues of nanobiotechnology is the preparation and delivery of dermatological ointments containing silver nanoparticles and biologically active substances (flavonoids, tannins, antacyanins, polysaccharides). In the study, watery-alcohol-propylene glycol extracts were taken from hibiscus *(Hibiskus sabdariff L.)*, clove wood *(Caryophyllos aromaticus L.)*, and common yarrow *(Achillea millefolium L.)*on the basis of macerry method and silver nanoparticles were prepared oinments in 2 compositions and was studied their antibacterial and antimicrobial features by the method of disk-diffusion to microorganisms ((*Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginoza, Bacillus anthracis, Klebsiella pneumoniae*).Composition I - colloidal solution of Ag NP (nanoparticles) - 0.05-0.1 g; peach oil 12-15g; Twin-80 3-5g; chitosan-4-5g; common yarrow-clove wood-hibiscus extract 2-3ml; citric acid 0,1-0,3g; purified water - as long as necessary; Composition II - colloidal solution of Ag NP (nanoparticles) - 0,5-0,7g; peach oil 12-15g; Twin-80 3-5g; chitosan 4-5g; common yarrow-clove wood-hibiscus extract-5-7ml; citric acid 0,1-0,3g; purified water - as long as necessary. According to the results of microbiological research, the ointment of the composition II had higher activity.

Keywords: *hibiscus - clowe wood - common yarrow extract, silver nanoparticles, chitosan, regenerative ointment, antibacterial and antimicrobial effect*

Introduction: Nanobiotechnology as one aspect of nanotechnology has become a very important area of human activity in recent years. It is well known that nanobiotechnology investigates the possibility of using biological processes at the atomic level in nanotechnology. In addition, nanobiotechnology plays a special role in improving the physical and chemical properties of biomaterials and nanostructures, and enhancing their ability in medicine and in preserving the environment. Recently, there has been very promising results in medical practice using the convergence of nanomaterials with biomaterials. Examples of these results are the injection of medicine into the body, their delivery to the tissues and cells they need, and the elimination of sources of disease. There is great potential for precision and early diagnostics of cancer, as well as other diseases, derived from a mixture of biomaterials and nanomaterials.One of the main directions in this field, is the preparation of nanomedicine and their delivery to target cells [5].

One of the topical issues of nanobiotechnology is the creation of nanomedicine in dermatological diseases. Although there is a wide range of regenerative agents to overcome this problem, there is a need for the preparation of ointments of various metal (Ag, Cu, Ti, Fe,Cd, Au) nanoparticles.Given the urgency of the above, along with silver nanoparticles, it is intended to develop regenerative ointments containing biologically active substances (flavonoids, tannins, anthocyanins, polysaccharides) with broad pharmacotherapeutic properties.

Materials and methods. Colloidal solution containing of silver nanoparticles being antibacterial feature [6],herbs-having regenerative, immunostimulatory properties: common yarrow,clove wood, hibiscus [2,3,4] were used as the research object. The study of antibacterial effects was performed by disc-diffusion method in test cultures of gram-negative and gram-positive bacteria [1].

Discussion and Conclusions:

Three compositions have been proposed for the preparation of ointments in laboratory conditions.Composition I colloidal solution of Ag NP (nanoparticle) - 0.05-0.1 g; peach oil 12-15g; Twin-80 3-5g; chitosan 4-5g; common yarrow- clove wood -hibiscus extract-2-3ml; citric acid 0,1-0,3g; purified water - as long as necessary; Composition II - colloidal solution of Ag NP (nanoparticle)

-0,5-0,7g; peach oil 12-15g; Twin-80 3-5g; chitosan-4-5g; common yarrow- clove wood- hibiscus extract-5-7ml; citric acid 0,1-0,3g; purified water - as long as necessary.

Preparation of ointments was realised according to the following technological scheme.

TP1. Preparation of chitosan gel

TP 1.1.Weighing of chitosan and measuring of purified water.

TP 1.2. Exaggeration of chitosan

TP 2. Dispersion of peach oil with colloidal solution of Ag NP

TP 3. Inclusion of peach oily suspension with Ag NP into chitosan gel

TP3.1. Measuring of Twin-80 emulsifier

TP 3.2. Mixing Twin-80 emulsifier with chitosan gel

TP 3.3. Addition of peach oily suspension with Ag NP to chitosan gel

TP 3.4. Measuring phyto extracts and adding them to the gel mass

TP 3.5. Addition of citric acid

TP 3.6. Homogenization of ointment

To determine the pH of the prepared emulsion ointment, aqueous extract was prepared at a ratio of 1:10 and checked by a potentiometer (pH-6,5-6,8).

As a result of microbiological studies in comparison with plant extracts and ointments, they have been shown to have devastating effects on microorganisms.The preliminary results are given in Table 1.

Table 1.

The effect of Common yarrow- Clove wood- Hibiscus Extract and I-II Ointment on microorganisms

№	*Microorganisms*	*Area of inhibited zone, mm*				
		CCH Extract	Ointment-I	Ointment-II	Control (ethanol-95%)	Control (4% chitosan solution)
1.	Staphylococcus aureus	16	11	14	3-5	9
2.	Esherichia coli	8	8	16	3-5	18
3.	Pseudomonas aeruginoza	21	9	11	3-5	6-7
4.	Candida albicans	21	13	14	3-5	12
5.	Bacillus anthracis	24	3	18	3-5	11
6.	Klebsiella pneumoniae	22	4	17	3-5	14

As a result of the research it was found that the effect of the ointment of the composition II on microorganisms is more active.Given that this composition has an effective antibacterial and antimicrobial properties, studies in this area are ongoing.

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SS-03

Fibroblast Growth Factor-2 Encoding Plasmid Loaded Lipid Nanoparticles: A Potential Gene Delivery System to Accelerate Wound Healing

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Abstract:

The increased prevalence of chronic wounds requires innovative treatment options. In this study, an effective system to accelerate wound healing was aimed to developed and evaluated. For this purpose, FGF-2 encoding plasmid DNA (pFGF-2) selected as a model. In order to achieve the delivery of pFGF-2 and to obtain lower cytotoxicity together with higher transfection, solid lipid nanoparticles (SLNs) were developed as a delivery vector by the melt-emulsification process. The formulated nanoparticles were electrostatically bound to pFGF-2 to form SLN: pFGF-2 vectors. The SLN:pFGF-2 vectors have particle sizes of 88.53 nm, and zeta potential values of 27.8 mV. Their particle characteristics were also visualized using a scanning electron microscopy (SEM). According to cytotoxicity test results, no significant cytotoxicity was observed on L929 cells in the concentration up to 225 µg/mL. Furthermore, in vitro scratch assay demonstrated that SLN:pFGF-2 vector accelerates L-929 cell migration and proliferation in 48h. Considering the role of FGF-2 in granulation formation, re-epithelialization, matrix formation and remodeling; the developed SLN:pFGF-2 vector system may have promises in wound healing therapy.

Keywords

Fibroblast Growth Factor- 2; solid lipid nanoparticle; pDNA; wound healing; gene delivery

Introduction

During the wound healing process, several types of growth factors are secreted, and these growth factors are very effective in wound healing. Among these growth factors, fibroblast growth factor-2 (FGF-2) that signal through FGF receptors regulates a broad spectrum of biological functions, including cellular proliferation, survival, migration, and differentiation(1). Multiple studies have reported the benefits of delivery of FGF-2 for wound healing. However, administration of the FGF-2 onto the wound area needs multiple application doses and causes high cost. Besides, the delivery of growth factor expressing genes is an attractive and long lasting method for wound therapy. In this approach, cells produce the protein through the delivery of pDNA that encodes the needed protein in the wound area and thus pDNA can be continuous source of the growth factor.

The use of therapeutic naked pDNA is not efficient for several reasons including their hydrophilic structure, high molecular weights, and anionic charges, preventing them from crossing cell membranes to reach the action sites. Various strategies have been explored to develop topical plasmid delivery systems such as chitosan/PVP hydrogels, peptide-based carriers and others (2,3). However, pFGF-2 incorporated solid lipid nanoparticles firstly investigated in this study.

In this work, a novel gene therapy based approach proposed to accelerate wound healing. For this purpose, SLN:pDNA vector system for delivery of FGF-2 was developed. The developed formulation characterized in terms of size, zeta potential, and morphology. Cytotoxicity and proliferative effect of the formulation were also evaluated on murine fibroblasts cell line (L929) cell line.

Materials and Methods

pUNO1-mFGF2 (3645 bp) (Invivogen, USA) that encoded mouse FGF-2 was used in this study. The plasmid DNA was amplified in DH5α strain of *E. coli* and extracted by Gene Jet Endo-free plasmid maxiprep kit (Thermo Sci., USA) according to manufacturer's protocol. The purity and concentration of the isolated plasmid DNA were spectrophotometrically assayed at 260 nm by Nanovette (Beckman Coulter, USA). Purity and verification of the isolated plasmid was also checked by diagnostic *Sac*I enzyme digestion study with agarose gel electrophoresis with ethidium bromide staining (1% agarose in tris-boric acid-EDTA buffer, pH 8.0).

SLNs as gene delivery vectors were prepared by the melt-emulsification process. Firstly, oil in water (o/w) microemulsion system was formed with Witepsol W35, DDAB , Kolliphor HS15, Peceol, ethanol, and water. Following microemulsion formation, obtained o/w microemulsion was dispersed in cold ultra-pure distilled water and SLNs were formed when hot microemulsion droplets were met with cold water (4). Gel retardation assay was performed to evaluate the complex formation ability of SLNs. Particle size and zeta potential of SLN formulations and SLN:pFGF2 vectors were measured on Zetasizer Nano ZS instrument (Malvern, UK). Scanning electron microscopy (SEM) was also performed to visualize obtained nanoparticles. The viability of cells was determined by Alamar Blue cell proliferation assay prior to scratch assay. Finally, scratch assay was applied to estimate the *in vitro* wound-healing ability of developed SLN:pFGF2 vector system.

Results and Discussion

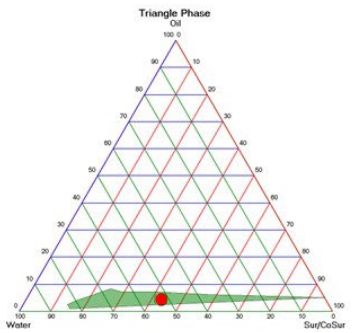
The pseudo-ternary phase diagram constructed by titration with UPH_2O into the oil (Witepsol W35 and DDAB), surfactants (Kolliphor HS15 and Peceol) and co-surfactant

(Ethanol) mixture is presented in Figure 1a. After the construction of the phase diagram, one point in the w/o micro- emulsion region was selected for the final formulation due to its clear appearance and having good stability.

Agarose gel electrophoresis studies were conducted to evaluate complex formation ability of SLNs with pFGF-2. As seen in figure 1b, at 1:1 (v/v) SLN:pFGF-2 ratio (lane 7), migration of plasmid DNA was slightly retarded probably due to an increase in the molecular weight or decrease in the net negative charge of the plasmid DNA as a result of SLN binding.

Once the optimal SLN and SLN:pFGF-2 complex formation ratio was determined, the physiochemical characterization studies were performed. According to DLS measurements, particle size was measured as 44.61 nm for SLNs and 88.53 nm for SLN:pFGF-2 (1:1, v/v) vectors (Figure 1c). SEM images also support the particle size measurement data obtained through DLS method (Figure 1d). Furthermore, SLN formulations have a positive electrical charge in order to be used for pDNA complexation and by addiŞtion of pFGF-2, the surface charge values changed into negative direction due to the negative charge of plasmid, as expected (5).

a.



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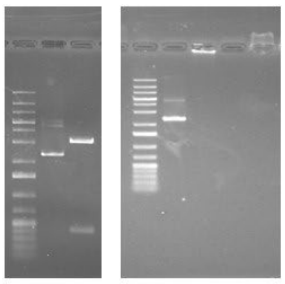

c.

Formulation	Size (nm)	PDI	Zeta Potential (mV)
SLN	44,61	0,63	35,8
SLN:pFGF-2	88,53	0,18	27,8

d.

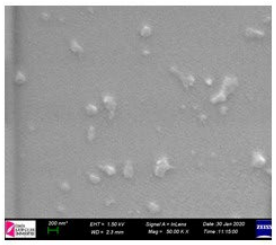

Figure 1. Construction and characterization of SLN:pFGF-2 for wound therapy. **(a)**Pseudo-ternary phase diagram for the system. Gray area represents transparent w/o microemulsion formation region. **(b)** Agarose gel electrophoresis of SLN:pFGF-2 complexes. Lane 1 and 4: 1 kb DNA ladder, Lane 2 and 5: Naked pFGF-2, Lane3: Sac I digested pFGF-2, Lane 6-8: SLN:pFGF-2 complexes for the ratio 0,5:1, 1:1, 2:1(v/v), respectively. **(c)** Particle size and zeta

potential measurement of SLN and SLN:pFGF-2 complex (n=3). **(d)** Scanning electron micrograph of SLN:pFGF-2 complex; scale bar, 200 nm.

All excipients used in this study were selected due to their low cytotoxic properties. The results obtained from Alamar Blue assay support this fact. No significant cytotoxicity was observed on L929 cells up to up to 225 µg/mL concentration (data is not shown).

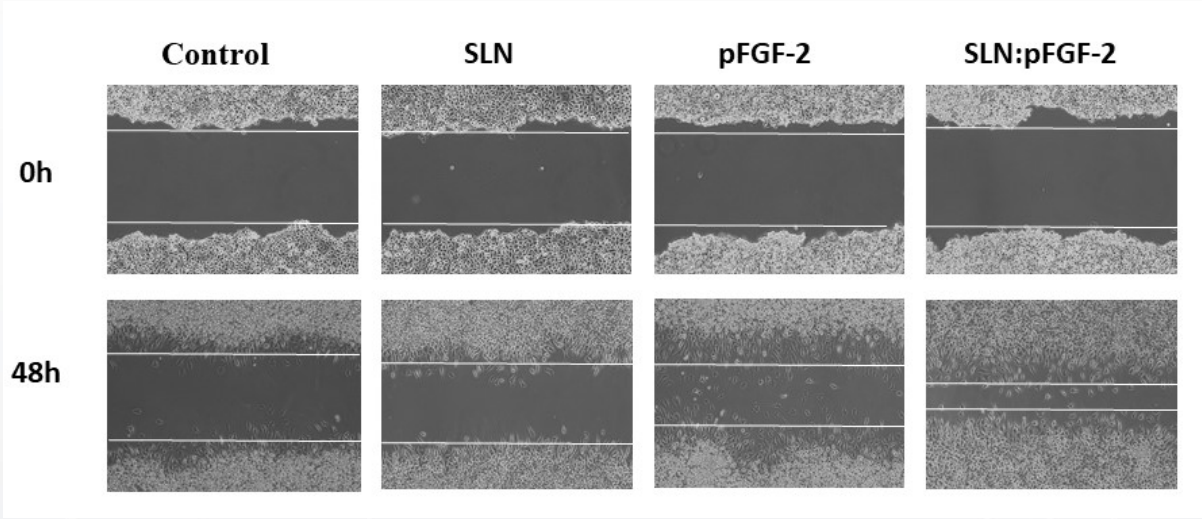

Figure 2. Micrographs of in vitro scratch closure assay of control, SLN, pFGF-2, SLN:pFGF-2 at 0 and 48 hours.

One of the easiest and most convenient methods of monitoring cell migration and proliferation is the scratch assay. To determine the proliferative ability of developed SLN:pFGF-2 system, the scratch closure rate of L929 fibroblast cells was investigated in the culture media with or without SLN:pFGF-2 application. The microscopic images showed that SLN:pFGF-2 accelerate cells migration and proliferation and increased the scratch closure rate in 48 h (Figure 2).

Conclusion

According to the results, the obtained formulation is promising and encouraging in the field of wound therapy due to its potential to transform into a product in the market and the ability to be used in different superficial wounds.

Acknowledgments:
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SS-04

A Novel Concept for Immune Checkpoint Gene Knockout Via Lipid Based Non-Viral Delivery Systems

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Abstract

Immuno-oncology has been involved among the most important cancer therapies after surgery, radiation therapy, and chemotherapy in the last decade. In order for cancer immunotherapy to be successful, the recognition of cancer cells by the immune system needs to be increased. To increase the recognition of cancer cells by genetic modifications could be supplied by gene knock-out of immune checkpoint protein ligands on cancer cells. For this purpose, it is aimed to use the CRISPR/Cas9 gene edition system for the knock-out of the programmed cell death receptor (PD-1) ligand-1 (PD-L1) which is the one of the effective immune check point proteins.

One of the most popular CRISPR/Cas9 gene edition systems is the use of plasmid vectors that expresses Cas9 protein together with target specific single guide RNA. It is common to use viral vectors for the delivery of these relatively large plasmid DNAs into the cell. However, transduction of CRISPR plasmids *via* viral vectors is limited due to their immunogenicity, insertional mutagenesis and safety problems.

For an effective *in vivo* CRISPR/Cas9 based treatment, it is necessary to develop targeted non-viral delivery systems with high transfection efficiency. Solid lipid nanoparticles (SLNs) are one of the important candidates for plasmid DNA transfection due to its low toxicity, efficacy and targetability.

In this study, SLN mediated transfection of a popular CRISPR/Cas9 backbone plasmid (PX458) was used. Physicochemical characterization of the system, complex formation, plasmid DNA protection against nucleases and transfection ability of SLN were investigated. Furthermore, PX459 plasmid was cloned for the knockout of PD-L1 gene and the concept of CRISPR/Cas9 plasmid delivery system for PD-L1 knockout with targetable SLNs has been revealed for the future studies.

Keywords

Immune checkpoint inhibitor; programmed cell death ligand-1; solid lipid nanoparticle, gene knock-out; gene targeting

Introduction

Immunotherapeutic strategies currently under investigation include immune checkpoint therapies, engineered T-cells, monoclonal antibodies, and cancer vaccines. The goal of checkpoint inhibition is to enhance anticancer immune activation by downregulating inhibitory pathways such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or programmed cell death ligand-1 (PD-L1). PD-L1 is a transmembrane protein that inhibits T-cell mediated immune attack through binding to its receptor PD-1 on tumor-specific T-cells. PD-L1 has been reported to be overexpressed in several cancer types, and recent studies showed that blocking PD-1/PD-L1 pathway or decreasing their expression in tumor cells plays an important role in the adaptive immune response [1].

In recent years, clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) has become a popular precision genome editing tool. Originally, CRISPR/Cas9 systems are adaptable immune mechanisms of many bacteria and archaea to protect themselves from invading nucleic acids. For the use of CRISPR-based genome editing for clinical use, several practical issues and technical challenges must be addressed. One of the most important challenges is setting and reaching the target site with efficiency and accuracy to improve specificity and reduce off-target probability [2].

The delivery vectors of CRISPR/Cas9 system are vital for in vivo application. Viral vectors and some physical methods have been investigated for their potential to facilitate in vivo delivery of CRISPR/Cas9 system. Nevertheless, with the rapid development of various non-viral vectors are very likely to provide unique advantages over viral vectors in these fields [3].

One of the widely studied non-viral vectors is solid lipid nanoparticles (SLNs). SLNs are nanosized particulate systems prepared by lipids which are solid at room temperature. Their success to incorporate genes into cells as a non-viral gene delivery system has been proven in many studies [1,4].

Here, we offer SLNs as a non-viral delivery systems for CRISPR/Cas9 mediated gene knockout of immune checkpoint protein PD-L1.

Materials and Methods

Materials

Green fluorescent protein (GFP) encoding CRISPR-Cas9 backbone plasmid pSpCas9(BB)-2A-GFP (PX458) and pSpCas9(BB)-2A-Puro (PX459) V2.0 were gifts from Feng Zhang (Addgene plasmid # 48138, # 62988). Glyceryl monostearate and Macrogol 15 Hydroxystearate were kindly donated by BASF, Switzerland. Mouse fibroblast cell line (L929) was purchased from ATCC, USA. Ethanol, Sodium dodecyl sulfate (SDS) were provided from Merck-Co., Germany. Dimethyldioctadecylammonium bromide (DDAB) was purchased from Sigma-Aldrich Co., USA. Alamar Blue cell viability assay purchased from Thermo Fisher Scientific, USA. All other chemicals were of analytical grade and used as received. Ultrapure water was used in all stages needed.

Vector preparation

SLNs as gene delivery vectors were prepared by the melt-emulsification process. Firstly, oil in water (o/w) microemulsion system was formed. For this stage, microemulsion was prepared by using Glyceryl monostearate as internal oil phase; Macrogol 15 Hydroxystearate as surfactant (S); ethanol as co-surfactant (CoS) and ultra-pure water as the continuous water phase. Pseudoternary phase diagram was drawn by water titration with components of microemulsion over lipid melting temperature (~80 °C). The o/w microemulsion formation area on the phase diagram was determined due to transparency of the mixture. To impart cationic character to the SLNs, DDAB was incorporated into the oil phase of the microemulsion. Following microemulsion formation, obtained o/w microemulsion was dispersed in cold ultra-pure distilled water (0-4 °C) at a ratio of 1:10 (w/v) under magnetic stirring at 1000 rpm. SLNs were formed when hot microemulsion droplets were met with cold water [5].

Gel retardation assay

Gel retardation assay was performed to evaluate the complex formation ability and protection capability of SLNs against serum nucleases. SLN: PX458 vectors were obtained by mixing the constant amount of PX458 (0.1 µg) with the increasing amount of obtained SLNs (10–100 µg) under agitation for 30 min at room temperature, which allows the formation of electrostatic interactions between the positive charges of SLNs and the negative charges of PX458. The resultant complexes were characterized by gel retardation assay.

Particle size and zeta potential measurements

For particle size measurements, SLN formulation and SLN:PX458 vectors were diluted 5-fold with ultrapure water. Measurements were performed on Zetasizer Nano ZS instrument (Malvern Instruments

Ltd., England) using non-invasive back scattering mode with the detector positioned at 173° from the incident beam. Samples were filled in disposable polystyrene microcuvettes. At least three measurements were performed for each sample. Cumulative analysis was employed by the software in order to analyze the autocorrelation function, and Z-average particle size and polydispersity index (PDI) were reported. Electrophoretic mobility of the dispersions was measured in standard zeta cuvettes (Malvern Instruments Ltd., England), and the zeta potential was calculated by the software using Smoluchowsky equation. The data are presented as mean ± S.D.

Morphology of SLN and SLN:PX458 complex

The morphology of SLN formulation and SLN:PX458 vectors were visualized by using Scanning Electron Microscope (SEM, Carl Zeiss 300VP, Jena, Germany). Sample preparation was done by fixing a drop of SLN formulation and SLN:PX458 vector on metal plates by two-sided adhesive tape and then coated with 100 A° thick gold in the brand coating device (Quorum Q150 Res, Lewes, United Kingdom).

Cytotoxicity Analysis

The *in vitro* cytotoxicity of developed nanoparticles was investigated on L929 cell line. Cells were plated in 96-well plates at a density of 5×10^3 cells per well in 100 µL and treated with SLN formulation and SLN:PX458 at increasing concentrations (3, 6, 9, 12, 15 µL/well), with respect to solid lipids) for 24 h. The proportion of viable cells was evaluated by Alamar Blue cell viability assay according to the manufacturer's instructions. Cell viability was calculated by normalizing the fluorescence of media from treated cells to untreated cells.

Cloning PX459 plasmid for PD-L1 gene knockout

Zhang lab general target sequence cloning protocol was followed for the cloning. sgRNAs were designed using online bioinformatics tools [6].

Results and Discussion

The pseudoternary phase diagram that was drawn by water phase titration with components of the microemulsion is presented in Figure 1a. After construction of the phase diagram, a central point in the o/w microemulsion region was selected for the final formulation due to its clear appearance. This was followed by SLNs production. SLN:PX458 vectors were obtained by mixing the constant volume of PX458 (0.1 µg/ mL) with the increasing volume of SLNs and gel retardation assay was performed to determine the optimal complex formation ratio. Gel retardation assay showed that SLN:PX458 ratio 1:1 (v/v) on lane 2 or higher are necessary to bind DNA completely to SLN and block the DNA migration on the agarose gel (Figure 1b).

Once the optimal SLN:PX458 vector ratio was determined, we proceed to the physicochemical characterization of SLNs and SLN:PX458 vectors. DLS measurements were performed in order to investigate the particle size, PDI and zeta potential. As shown in the table, the particle sizes of the SLN formulation is 32.7 nm ± 7.7 and when the PX458 was complexed, the particle size increased to 121.3 nm ± 1.8. PDI can be used to describe the uniformity of nanoparticles. There is not a certain acceptable limit for PDI, however when the PDI value is lower than 0.4 for DLS measurements, the particles are evaluated as monodisperse and both SLNs and SLN:PX458 (1:1, v/v) vectors can be evaluated as monodisperse particles. Zeta potential is the sign of electrostatic interactions between SLN and PX458. Zeta potential of SLNs are positive and measured as 27.8 (±1.73) mV while SLN:PX458 (1:1, v/v) was measured 14.7 (±0.12) mV. The reason for the reduction is due to the effect of negatively charged phosphate chains of nucleic acids to the electrostatic sum up (Figure 1c).

The SEM images confirm the results obtained with the DLS measurements. SLNs showed a spherical morphology and homogenous surfaces with particle sizes consistent with those determined previously (Figure 1d).

Increasing amount of GMS-SLN and GMS-SLN:PX458 vectors were evaluated for cytotoxicity test. No significant cytotoxicity was observed in the concentration range of 3–9 µL/well ($p > 0.05$). Both GMS-SLN and GMS-SLN:PX458 vectors showed a concentration dependent cytotoxicity for the cell viability (Figure 1e).

While optimizing the transfection ability of the using bare GMS-SLN and PX458, the production process of targetable concept nanoparticles continues (Figure 1f). PX459, another widely used CRISPR/Cas9 backbone plasmid, was cloned for PD-L1 knockout and verification of cloning was shown by the Sanger sequencing (Figure 1g).

Conclusion

In this study, it was recommended that SLNs can be an effective delivery system for CRISPR/Cas9 plasmids. With the growing interest in targeted non-viral nanoparticle mediated gene delivery systems and effective results obtained in immune-oncology in recent years may be adopted to *in vivo* gene therapy methods as well.

Acknowledgments

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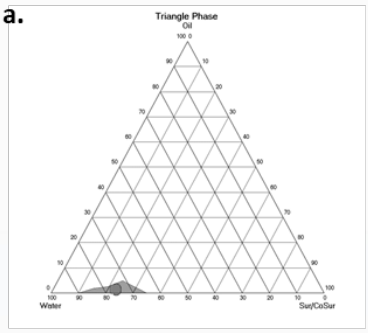

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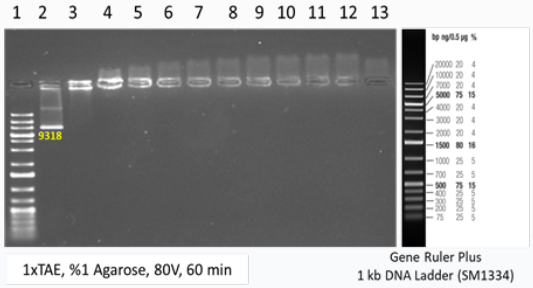

c.

Formulation	*Particle Size (nm) (±SD)*	*Polydispersity Index*	*Zeta Potential (mV) (±SD)*
GMS-SLN	32.7 (7.707)	0.155 (0.011)	27.8 (1.73)
GMS-SLN:PX458	121.3 (1.823)	0.204 (0.006)	14.7 (0.115)

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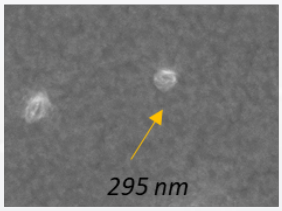

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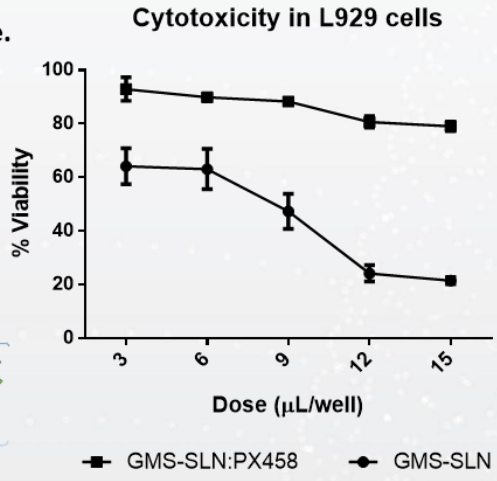

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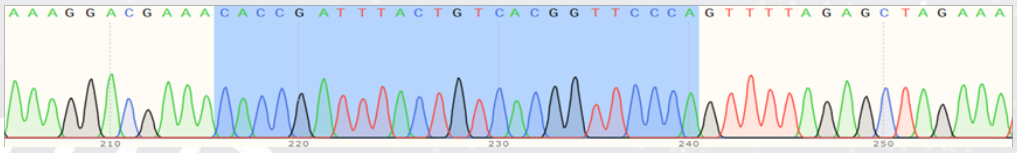

Figure 1. a. Triangular phase diagram of o/w microemulsion. Grey area represents the transparent regions belongs to o/w microemulsion, b. Agarose gel electrophoresis image of gel retardation assay, c. Physicochemical properties of the GMS-SLN and GMS-SLN:PX458 (1:1, v/v), d. SEM images, e. Viability percentages of L929 cells treated with increasing doses of GMS-SLN and GMS-SLN:PX458 (1:1, v/v) vectors. f. Schematic illustration of conceptual targeting delivery system, g. Sanger sequence of cloned PX459 plasmid with PD-L1 gene knockout sgRNA region.

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SS-05

Type of the Paper (Extended Abstract, Meeting Report, Preface, Proceeding, etc.)

Antibacterial Performance of Au-C_3N_4 Nanocomposites

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† Presented at the conference title, place, and date.

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Abstract: The antibacterial activity of Au-C_3N_4 nanocomposites against MRSA, which is one of the nosocomial pathogen bacteria, was investigated. The antibacterial effect of Au-C_3N_4 nanocomposites were analyzed through bacterial growth curve, photothermal effect, oxidative stress assay and live-dead fluorescence staining. The results show that the nanocomposites exhibited antibacterial activity against MRSA by especially ROS production. This effect promises a way to kill bacteria without causing antibiotic resistance.

Keywords: antibacterial activity, MRSA, nanocomposites, photothermal effect

1. Introduction

According to the data of Turkey Health Ministry, the amount of death due to the bacterial infection increases with the effect of increasing antibiotic resistance. Especially in hospitals and intensive care units, the risk of fatal infection increases because of drug-resistant microorganisms. [1] Methicillin-resistant *Staphylococcus aureus* (MRSA) is one the most important nosocomial pathogens. MRSA causes various infections such as soft tissue, deep tissue and skin infections; respiratory and urinary tract infections [2] The main problem is the strong antibiotic resistance of MRSA against to antibiotics. [3] The overuse and misuse of antibiotics led to the development of antibiotic resistance in pathogenic bacteria, especially in MRSA. Thus, alternative drugs have been investigated instead of antibiotics. Nanomaterials are recommended as the current solution for this problem. Nanomaterials do not cause antibiotic resistance as they kill bacteria through Reactive Oxygen Species (ROS) production and photothermal pathway generally under NIR light irradiation. Recently, mesoporous carbon nitride (mpg-C_3N_4) has been shown to act as natural peroxidases. The semiconductor mpg-C_3N_4 possess biocompatibility because it consists of abundant Carbon (C) and Nitrogen (N) atoms in living organisms. In addition to its photocatalytic effect, it is also very good at detecting metal ions. These properties allow mpg-C_3N_4 to form an efficient nanocomposite with noble metals. [4] A drug that overcome antibiotic resistance can be produced with a nanocomposite that can exhibit ROS production and photothermal effect. Many studies have shown that noble metals

such as gold, silver, platinum have enhanced photo-efficiency and a synergistic effect. [5] So far, different combinations have been tried to learn the synergistic effect of nanocomposites containing noble metals. In this study, we investigated the antibacterial effect of Au-C_3N_4 nanocomposites on MRSA and examined the photothermal effect and ROS generation of the nanocomposites and tried to understand underlying mechanism.

2. Materials and Methods

Microbiology Laboratory of Selcuk University Vocational School of Health Services provided *Staphylococus aureus* ATCC 43300. L-Glutathione reduced (GSH), Phosphate buffered saline (PBS) and 5.5′ – Dithiobis (Ellman's reagent) were bought from Sigma Aldrich. LIVE/DEAD BacLight Bacterial Viability Kit was purchased from Thermo Fisher. Tryptic Soy Broth medium (TSB), Mueller-Hinton agar (MHA) were purchased from Merck. All chemicals used without purification.

Synthesis of Au-C_3N_4 nanocomposites were performed at Koc University. Photothermal effect of Au-C_3N_4 nanocomposites investigated with a continuous semiconductor diode laser. Antibacterial activity of Au-C_3N_4 nanocomposites were measured by Optical Density measurement (OD_{600nm}) at a microplate reader. The assay was separated into 4 groups; (1) blank control; (2) NIR; (3) Au-C_3N_4; (4) Au-C_3N_4 + NIR. Oxidative stress assay was achieved with the same groups. Finally, living and dead cells were examined with LIVE/DEAD fluorescence staining.

3. Results and Discussions

In this study, we made photothermal effect assay, antibacterial activity assay of Au-C_3N_4 nanocomposite, oxidative stress assay and live/dead fluorescence staining. As a result of these experiments, we proved that Au-C_3N_4 nanocomposites exhibit lethal effect to bacteria. NIR light has decelerated bacterial growth during 24 hours but it could not show direct lethal effect on MRSA.

3.1 Antibacterial activity analysis

Figure 1 showed the 8-hour growth curve of bacteria for OD_{600}. There were differences between the pre- and post-treatment for 8 hours, but did not show the NIR effect. Furthermore, at the end of 24 hours in Figure 2, it was observed that NIR light slowed down the growth of bacteria. Figure 1a, 1b and 1c showed that Au-C_3N_4 was the most effective treatment. In Figure 1d, the most effective concentration was 128 µg ml^{-1}. Figure 3a also showed the bacteria before treatment, under fluorescence microscopy. In figure 3b, bacteria were killed after Au-C_3N_4 treatment. As illustrated in Figure 3b and 3c NIR light did not affect bacterial death extra apart from Au-C_3N_4.

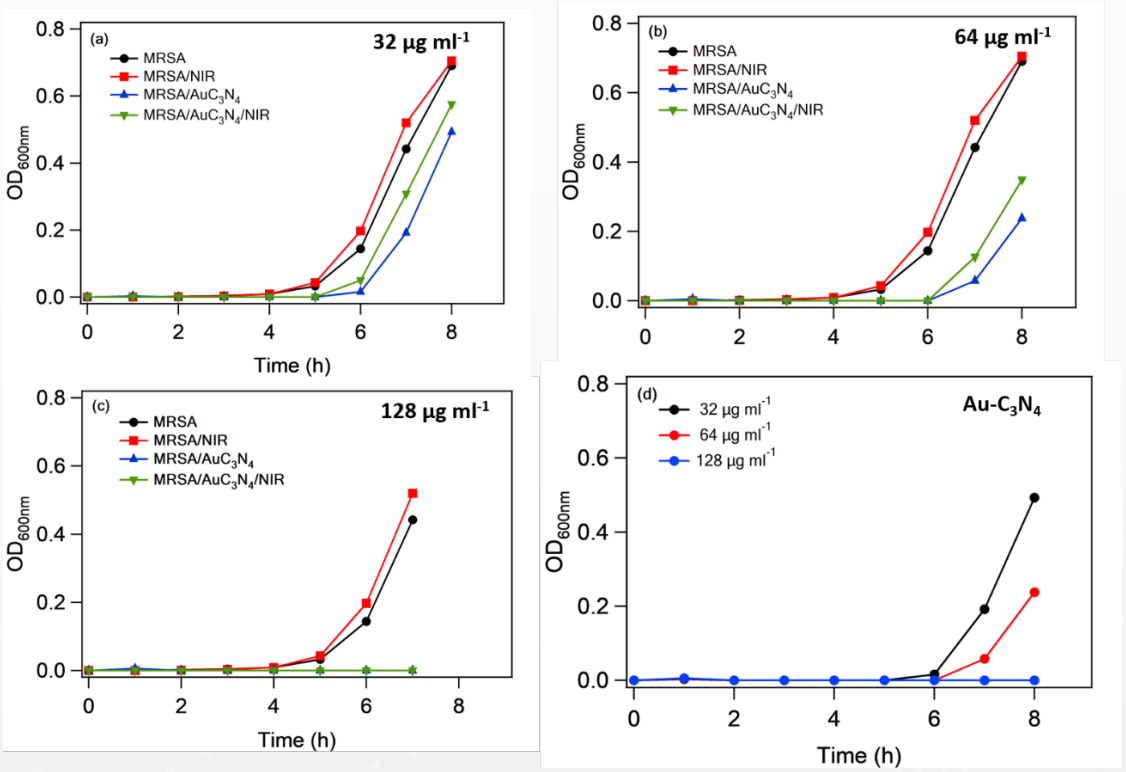

Figure 1. Concentration-dependent OD_{600nm} values of the bacteria. (a) 32 µg ml^{-1}. (b) 64 µg ml^{-1}. (c) 128 µg ml^{-1}. (d) All concentrations of Au-C_3N_4 in the same graphic.

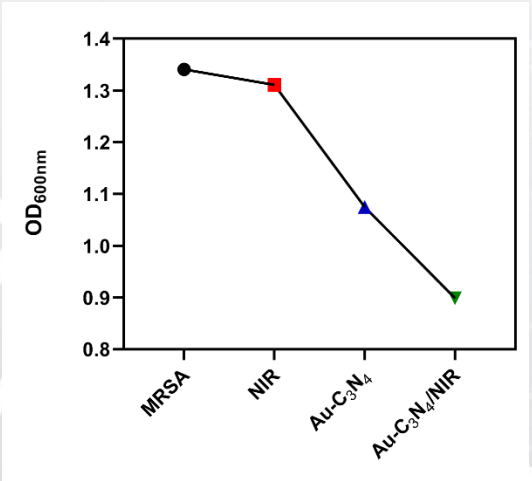

Figure 2. MRSA growth curve after different treatments during 24 hours incubation.

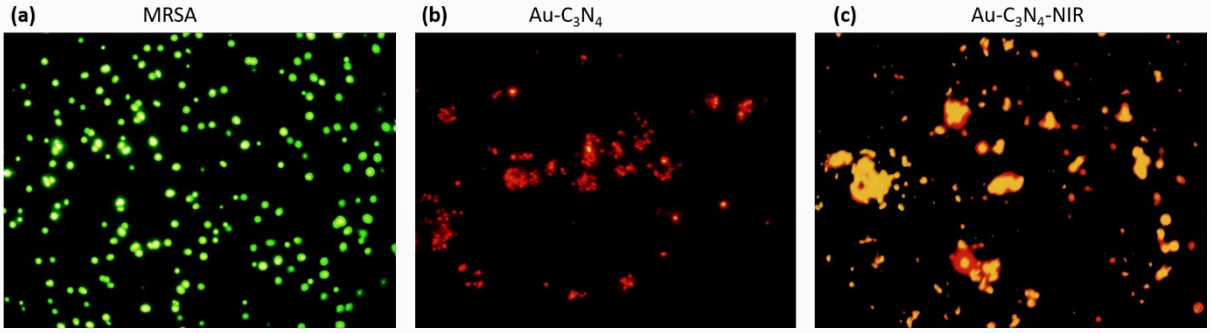

Figure 3. MRSA fluorescence images after treatment of Au-C_3N_4 with and without NIR light.

3.2 *Photothermal effect*

The photothermal effect of Au-C_3N_4 nanocomposites was investigated under exposure of NIR light. The results were shown in Figure 4. The temperature of solutions was increased by the reason of photo-absorbtion ability of Au-C_3N_4. dH_2O was used as a control group. After 5 minute NIR light irradiation the temperature of Au-C_3N_4 solution increased about 4 degrees more than the pure water.

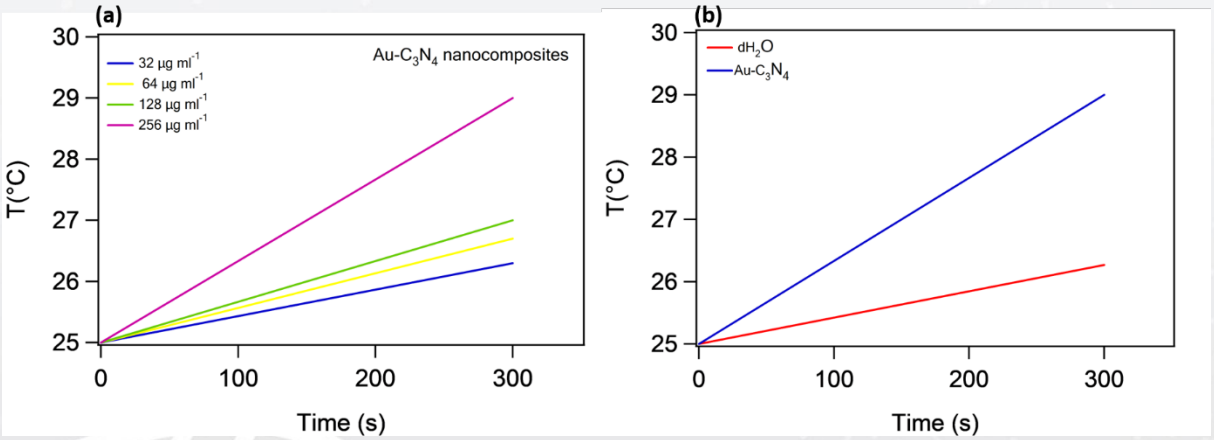

Figure 4. Photothermal effects of Au-C_3N_4 solutions under NIR light. dH_2O was used as a control group.

3.3 *Reactive Oxygen Species (ROS) effect*

GSH concentration was calculated before and after treatment according to the GSH standard curve previously prepared. The percentage of depleted GSH was calculated as shown in Figure 5. GSH concentration decreased by 36 % with the treatment of Au-C_3N_4 under NIR light, while 34 % in dark.

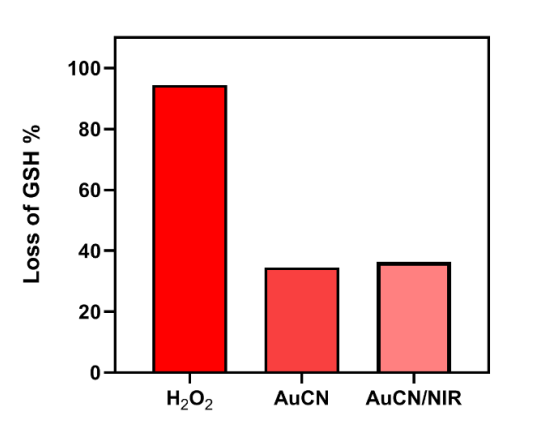

Figure 5. Reactive Oxygen Species (ROS) effect of Au-C_3N_4 and Au-C_3N_4-NIR.

4. Conclusions

In summary, Au-C_3N_4 nanocomposites showed antibacterial effect on MRSA. NIR did not show its effect in the first 8 hours but decelerated bacterial growth in the 24 hours. We believe that the bacterial killing mechanism of Au-C_3N_4 nanocomposite is realized through ROS production. NIR may have a photothermal effect to decelerate the bacterial growth.

Acknowledgments: The authors would like to thank Research Foundation of Selcuk University for financial support of this work. This study is the part of MSc thesis of Esra Ceran.

Author Contributions: Onder Metin and Huseyin Kucukkececi made the nanocomposites synthesis and characterization; Imren Hatay Patir, Taha Kuru, Ilknur Aksoy and Esra Ceran conceived and performed antibacterial analysis experiments including photothermal effect, OD measurement, oxidative stress assay and fluorescence staining; Imren Hatay Patir, Ilknur Aksoy and Esra Ceran analyzed the datas; Esra Ceran wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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SS-06

Title:
Development of a Cationic Nanoemulsion as Non-Viral Vector for pDNA Delivery

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Abstract:

Gene therapy is a promising approach to prevent and treat genetic diseases, based on inhibiting pathogenic gene expression, among others. For that purpose, the genetic material must be delivered to the nucleus, by the use of viral or non-viral carriers, e.g. nanoemulsions (NEs). Cationic NEs (CNEs) are a promising approach for gene or vaccine delivery. NEs are able to form a complex with the negatively charged genetic material like plasmid DNA (pDNA) or small interfering DNA (siRNA) via electrostatic interactions. Here a CNE was prepared with microfluidization by investigation the homogenization duration of 1-10 minutes and characterized in terms of droplet size, polydispersity index (PDI), zeta potential (ZP) and complexation capacity with pDNA.
The droplet size of CNE decreased from 159 nm to 114 nm, just like the ZP decreased from 43.2 mV to 36.6 mV, with increasing homogenization duration from 1-10 minutes. For the PDI there is not a clear tendency obtained, with values between 0.06 and 0.13, indicating a narrow size distribution.
The developed CNE was able to form a complex with pEGFP-C1, which is a prerequisite for a successfull therapy with genetic material like siRNA.
Keywords:
cationic nanoemulsion, microfluidization, non-viral vector, pDNA, complexation

Introduction

Gene therapy is a promising approach to prevent and treat genetic diseases (Verissimo et al, 2010) and is based on the transfer of an exogenous genetic material into the cell by replacing defective genes (Bulst et al., 2012) or inhibiting pathogenic gene expression (Jagani et al., 2013). For a successful gene therapy, the genetic material must be delivered to the nucleus (Nam et al., 2009). For that purpose, two different carriers are suggested: viral and non-viral. Non-viral carriers like nanoemulsions (NEs) were extensively investigated in the latest decades.

Especially, cationic NEs (CNEs) as non-viral carriers are a promising approach for gene or vaccine delivery (Bogers et al., 2015; Brito et al., 2014). NEs consist of two immiscible liquids, oil and water phase. In addition NEs are stabilized by surfactants (Verissimo et al, 2010; Nam et al., 2009) and contain compounds responsible for the cationic charge. NEs are able to complex the genetic material like plasmid DNA or siRNA, which are negatively charged, via electrostatic interactions (Cun et al., 2010; Caracciolo and Amenitsch 2012), useful for gene targeting (Jafari et al., 2012). NEs can be produced with high energy methods like high-pressure homogenization or microfluidization. In addition, non-ionic surfactants will not compete with the cationic compounds of the formulation.

Due to these facts, the aim of this study was to develop and characterize phytosphingosine (PS) loaded CNEs as non-viral delivery system in terms of the complex forming ability of this CNE for pDNA.

Materials and Methods

Materials

Phytosphingosine (PS; 2S-amino-1, 3S, 4R-octadecanetriol) was purchased from Evonik (Essen, Germany). Polysorbate 80 (Tween 80, Merck, Germany) and lecithin (Applichem, Darmstadt, Germany) were chosen as emulsifiers, Octyldodecanol (Eutanol® G, Caesar and Lorenz GmbH, Hilden, Germany) was used as the oil component. Benzalkonium chloride (Signa, St. Louis, USA) was used as preservative.

Methods

Preparation

The CNEs were prepared by microfluidization as described by Başpınar et al. 2015 The oil phase, containing octyldodecanol, phytosphingosine and lecithin, and the

aqueous phase, consisting of distilled water, Tween 80 and benzalkonium chloride, were prepared separately before adding the aqueous phase to the oil phase. Afterwards, the pre-emulsion was obtained by using the high speed stirrer Silverson L5M with 10000 rpm for 5 min and subjected afterwards to the Microfluidizer ML100L. The NEs were homogenized at 25 °C at 600 bar, with different homogenization times (1, 2, 3, 4, 5, 6, 8 and 10 min) to obtain the NEs.

Characterization

Mean droplet size and polydispersity index (PDI) of the formulations were determined by A Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) per photon correlation spectroscopy (PCS) with dynamic light scattering (DLS). The zeta potential (ZP), representing the surface charge of the NEs was determined by measuring the electrophoretic mobility. 40 µl of the sample was added to 40 ml bidistilled water and measured. The Helmholtz Smoluchowski equation was applied for the calculation of the ZP [Muller 1996].

The CNE with appropriate properties like a small droplet size, a narrow size distribution and especially a high ZP of >30 mV was investigated according to its ability to form a complex with pDNA (pEGFP-C1) in ratios between pEGFP-C1:KNE of 1:0-1:9 (1:3).

Results

With increasing homogenization duration the droplet size decreased from 159 nm (1 min) to 114 nm (10 min), just like the ZP decreased from from 43.2 mV (1 min) to 36.6 mV (10 min). On the contrary to these parameter, the tendency of the PDI is not clear with values of between 0.06 (5 min) and 0.13 (2 min). In spite of these fluctuation, all PDI values indicate a narrow size distribution.

The developed CNE was able to form a complex with pEGFP-C1 in a ratio of 1:2.

Discussion

As expected, the droplet size decreased with increasing homogenization time, due to the fact that the energy input into the NEs increased. A small droplet size is essential for a successful delivery of the genetic material to the target. Although

there is not a clear tendency according to the PDI, all values are absolute appropriate, indicating a very narrow size distribution. According to the ZP of the CNEs, a decrease was observed, indicating that higher homogenization duration is not beneficial for the ZP and less homogenization of 1 minute is sufficient to obtain a CNE with a small droplet size of 159 nm , a PDI of 0.09 and a ZP of 43.2 mV.
This can be explained with the fact that with with increasing duration the interfacial film between the oily and aqueous phase is more stable, resulting in the present ZP.
For forming a complex with pDNA is a pre-step before building a complex with siRNA, which is a prerequisite for a successfull therapy with genetic material like siRNA.

Conclusions

The here developed CNEs revealed suitable properties like a small droplet size of less than 200 nm, a narrow size distribution, expressed as PDI <0.2, and a high zeta potentail of >+30 mV, all required properties for a successful gene delivery.
Thus, phytosphingosine is able to form a complex with pDNA in form of CNE, representing an alternative to the well-known cationic agents kile DOTAP etc.

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SS-08

Development of SIRT1 siRNA-carrying liposomes for treatment of prostate cancer

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Abstract:
Sirtuin 1 (SIRT1) is a histone deacetylase (HDAC) from Sirtuin family and high expression of it induces activation of DNA repairing factors eventually leading either tumor suppression or tumor development in cellular context-dependent manner. Since most of the chemotherapeutics induce apoptosis by creating DNA damage in tumor cells and further SIRT1-activated repair of the chemotherapeutic-induced DNA damage results in decreased apoptosis, inhibition of SIRT1 is a promising strategy both in anticancer therapy and preventing chemotherapy resistance. High expression level of SIRT1 in prostate cancer level has been also reported in many cases and it has been confirmed in our context by showing the higher protein levels of SIRT1 in prostate cancer cell lines than normal prostate epithelium cells. As, gene silencing of oncogenes via RNA interference technology is a recent approach in cancer therapy, in this study, siRNA carrying liposomal formulations composed of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) as cationic lipid have been formulated by freeze drying method for SIRT1 inhibition. In addition to characterization of the liposomes, their efficiency on SIRT1 silencing has been shown both on mRNA and protein levels on prostate cancer cell lines LNCaP, Du145, and PC-3. It has also been determined that each liposomal formulation has different levels of efficiency on each cell line so it has been achieved to develop an appropriate nucleic acid delivery formulation for SIRT1 silencing for different metastatic prostate cancer types.

Keywords:
Liposome; SIRT1; nucleic acid delivery; prostate cancer

Introduction

SIRT1 as an HDAC has many non-histone cellular targets such as p53, NFkB, and FOXO leading it to effect significant cellular events such as proliferation, cell cycle, and apoptosis. Further, it also acts as a metabolic sensor in different tissues, effecting a wide range of cellular events including cell metabolism and stress as well as DNA damage response. In addition, SIRT1 has found to be highly expressed in many types of cancer including prostate cancer. Anticancer effects of both SIRT1 activators (resveratrol) and inhibitors (sirtinol) have been determined as a conflicting issue, however, further studies have shown that the effect of SIRT1 on cellular stress and viability varies between normal and tumor cells depending on the presence of other cancer-related factors in the cell. Although function of SIRT1 enables cells to better tolerate oxidative stress levels in normal cells leading its anti-tumoral effect, the same function maintains cell viability in tumor cells despite their high oxidative stress and DNA damage levels promoting tumorigenesis. Therefore high levels of SIRT1 expression in mutated cells increase cell viability as well as cancer risk. Thus, silencing of SIRT1 via RNA interference is a useful strategy in cancer cells to suppress their viability requiring an siRNA delivery system. Non-viral nucleic acid carrier systems are considered to be safe in terms of mutagenicity and immunogenicity. So liposomes are widely studied, as the phospholipids used in their structures are safe and biocompatible. In this project, we aimed to develop efficient liposomal carriers for SIRT1 siRNA and to investigate their efficiency on suppressing SIRT1 protein levels and further cellular effects in prostate cancer cells.

Materials and Method

SIRT1 and non-targeting control siRNA was purchased from Dharmacon. RWPE1, LNCaP, DU145 and PC3 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and propagated as recommended.

Upon determination of the optimum complexation ratio for siSIRT1 and protamine sulphate by gel retardation assay, preparation of a series of siRNA-loaded liposomes was performed by the freeze-drying (lyophilization) technique followed by a sonication process after rehydration of the lyophilizate. The particle size and zeta potential of formulations were measured using Zetasizer NanoZS instrument (Malvern Panalytical, UK). Morphology of the liposomes observed by scanning electron microscopy and scanning transmission electron microscopy. Efficiency of formulations on SIRT1 protein level was detected by western blotting and on mRNA level by quantitative real-time PCR.

Results

SIRT1 expression level has been detected in normal and cancer cell lines of the prostate to check whether this silencing approach is appropriate for prostate cancer cases. It has been shown that SIRT1 protein level in cancer cell lines LNCaP, DU145 and PC3 is higher in comparison to RWPE1 normal prostate epithelial cells suggesting that SIRT1 silencing might be useful to cure prostate cancer cases with high SIRT1 expression. After a series of liposomal carriers have been formulated and characterized, screening their efficiency on SIRT1 silencing was carried out by detection of SIRT1 protein level in 3 prostate cancer cell lines since the ultimate cellular effect of gene silencing is correlated to the decrease in the protein level and detected to be suppressed between 2.5 and 4 folds. However, in order to confirm that the alleviation at protein level was the result of RNA interference, relative SIRT1 mRNA level was analyzed and found to decrease around 4 folds in all 3 cancer cell lines.

Discussion

The main strategy in this study was developing efficient liposomal nucleic acid carriers to suppress SIRT1 expression in order to support a therapy strategy for combinational use of DNA damage inducing chemotherapeutics and siSIRT1-carrying formulation in tumor cells with high SIRT1 level. Upon results showing the suppression in SIRT1, it was concluded that the efficiency of each formulation on distinct prostate cancer cell lines was different and decrease rates between mRNA and protein levels were comparable. So, formulation of efficient siSIRT1 liposomal carriers has been achieved for further cellular analysis such as cell viability, DNA damage responses and apoptosis in case of combinatorial use of the liposomes with DNA-damage inducing chemotherapeutics.

Conclusion

Efficient silencing of SIRT1 by the liposomal carriers in 3 prostate cancer cell lines showing high SIRT1 expression but different p53-expression patterns was expected to improve the personalized treatment strategy of prostate cancer.

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Identification of *Fusobacterium nucleatum* 1 -deoxy-D-xylulose 5-phosphate Reductoisomerase Inhibitor Candidates by Molecular Docking Analyses

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Abstract:
Fusobacterium nucleatum is an opportunistic gram-negative bacteria that is responsible for periodontal diseases and associated with developing colorectal cancer in human. In addition, it has developed drug resistance and promotes chemoresistance. Therefore, drugs that have different mode of actions, should be investigated against this bacterium. *F. nucleatum* 1-deoxy-D-xylulose 5-phosphate reductoisomerase (*Fn*DXR) could be a possible target because it is a key enzyme in methylerythritol phosphate pathway for isoprenoid synthesis which is not present in human. In this study, homology modelling of *Fn*DXR was performed for inhibitor screening analyses by computational methods. 466 enzyme inhibitors and fosmidomycin, which is the inhibitor of DXR, were selected as a ligand library for screening analyses and screened by molecular docking on the active site of *Fn*DXR. According to docking scores, 10 inhibitors were identified as potential *Fn*DXR inhibitors and especially three compounds (PubChem ID: 72968, 104762 and 16886) showed the best docking scores as -7.840, -7.146 and -7.096 kcal/mol. Also, the docking score of fosmidomycin was calculated as -5.446 kcal/mol. As the results showed that these three compounds could be promising lead compounds for *Fn*DXR inhibition and should be evaluated as drug candidates against the bacterium in future drug design studies.
Keywords: Computer-aided drug design, *Fusobacterium nucleatum,* 1-deoxy-D-xylulose 5-phosphate reductoisomerase

1. Introduction

F. nucleatum is an anaerobic, gram-negative rod bacterium, is found naturally in human oral microbiota [1,2]. However, it is an opportunistic pathogen that causes periodontal diseases and is also associated with some diseases including gastrointestinal disorders, artherosclerosis, endocarditis, respiratory and urinary tract infections, organ abscesses etc [2]. Particularly, *F. nucleatum* is abundant in colorectal cancer tissues and is related to this cancer type intensely [2,3]. The bacterium has developed antibiotic resistance against ampicillin and penicillin [4,5]. Also, recent studies showed that *F. nucleatum* contributes chemoresistance in colorectal cancer [6]. Therefore, developing alternative drugs against this bacterium becomes necessary nowadays.

Methylerythritol phosphate pathway (MEP) for isoprenoid synthesis is an essential pathway that is found in most bacteria, especially gram-negative bacteria, some parasites and plants. MEP route is the alternative of the mevalonate pathway that is present in some organism such as animals, fungi etc. [7]. 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) plays an important role in MEP pathway by catalyzing the conversion of 1-deoxy-D-xylulose 5-phosphate (DXP) to 2-C-methyl-D-erythritol 4-phosphate (MEP) by a NADPH-dependent rearrangement [7,8]. Therefore, this enzyme might be a potential druggable target that is important for *F. nucleatum* metabolism [7] and is not found in human host. Fosmidomycin is a natural inhibitor of DXR [8], but some bacteria are resistant against the inhibitor [9,10].

Computational methods in drug design supports the understanding of receptor-ligand interactions and assists to design new therapeutic agents [11]. The first step in structure based drug design is homology modelling of the target protein three dimensional structure that is not identified by experimentally. Then lead drug compounds are predicted by screening analyses on protein models [12]. In this study, *Fn*DXR is selected as a drug target and the enzyme structure was modeled by a computational program. Then, an alternative ligand library was generated and virtual screening on this model was carried out by molecular docking methods. In conclusion, potential *Fn*DXR inhibitor candidates differ from fosmidomycin were predicted by *in silico* analyses and these compounds may be used in further *in vitro* inhibition analyses of *Fn*DXR for identifying lead drugs against this bacterium mediated diseases.

2. Materials and Methods

The homology modeling process started by finding a template protein for *Fn*DXR. NCBI/BLAST server was used to identify this structure by entering *Fn*DXR amino acid sequence as data (Accession no: AAL95520). RSCB Protein Data Bank was used to retrieve the template PDB file format. The target protein model was built by using template protein file and *Fn*DXR amino acid sequence in MODELLER ver9.15 software [13]. The best three dimensional model of *Fn*DXR was chosen among 100 models and minimized by using AMBER ff4SB force field and 2000 steps of Steepest descent algorithm in UCSF Chimera ver1.10.1 software [14]. The generated model was validated by using ERRAT, RAMPAGE, ProSA, ProQ, Verify 3D servers [15-19]. *Fn*DXR model and the template structure were superimposed and the RMSD (root-mean-square deviation) value was determined by UCSF Chimera ver1.10.1 software [14].

Molecular docking analyses were performed by Maestro Molecular Modeling Interface 11.4 (Schrödinger Release 2017-4: Maestro, Schrödinger, LLC, New York, NY, 2017). *Fn*DXR model was prepared by ProteinPrepWizard [20]. 466 enzyme inhibitors and the DXR inhibitor fosmidomycin were retrieved from MESH PubChem data source and this inhibitor library was filtered by selecting the active in bioassays and the Rule of 5 sections (https://pubchem.ncbi.nlm.nih.gov). These selected inhibitors were prepared by LigPrep (Schrödinger Release 2017-1: LigPrep, Schrödinger, LLC, New York, NY, 2017). Receptor Grid Generation was used to identify the grid file of *Fn*DXR model active site. The docking analyses of all inhibitors were carried out into the active site by using Schrödinger Glide standard-precision (SP) mode [21].

3. Results

Mycobacterium tuberculosis DXR (*Mt*DXR) (PDB ID: 2C82_A) [22] was chosen as template protein for *Fn*DXR based on 90% Query cover and 42.37 % identity. MODELLER ver9.15 software [13] was used to build the model by using the PDB file of the template structure and the target amino acid sequence. The three dimensional model of *Fn*DXR was selected based on Discrete Optimized Protein Energy (DOPE) score which was calculated as -46123.81641. After the minimization steps, the model quality was evaluated by web-based servers [15-19]. All validation scores, as shown in Table 1, confirmed that the model was appropriate for the further docking analyses.

Table 1: Validation scores of *Fn*DXR three dimensional model

Protein model	Web-Server							
	ERRAT (Overall quality factor)	ProSA (Z-score)	ProQ[1] (LGscore)	RAMPAGE (Ramachandran plot)			VERIFY3D[2] (%)	RMSD[3]
				Favored region (%)	Allowed region (%)	Outlier region (%)		
*Fn*DXR	89.362	-10.31	6.255	92.0	7.5	0.5	96.15	0.422

[1]Quality is defined with different ranges: LGscore>1.5 fairly good model; LGscore>2.5 very good model; LGscore>4 extremly good model.
[2] The amino acids must be scored at least 80% >= 0.2 in the 3D/1D profile.
[3]RMSD value was calculated by comparing with the 2C82 structure

Active site of *Fn*DXR model was predicted according to catalytic residues (such as Asp151, Glu153, catalytic loop residues 198-205, Ser177, Ser213, Asn218, Lys219 for *Mt*DXR) of some bacteria DXR enzymes that identified by experimentally through X-ray crystallography [22-24]. The 466 selected inhibitors and fosmidomycin (https://pubchem.ncbi.nlm.nih.gov) were docked into the active site by using SP docking mode and interaction poses were sorted according to docking scores. The hit 10 inhibitors were identified based on these scores (Table 2) (Figure 1).

Table 2: Docking and ΔG binding scores of the hit 10 inhibitors (https://pubchem.ncbi.nlm.nih.gov) docked on *Fn*DXR model

PubChem ID	Docking score (kcal/mol)
72968	-7.840
104762	-7.146
16886	-7.096
72828	-6.862
73947328	-6.777
60750	-6.758
135401907	-6.605
5328552	-6.540
546	-6.495
10168	-6.455
572	-5.446

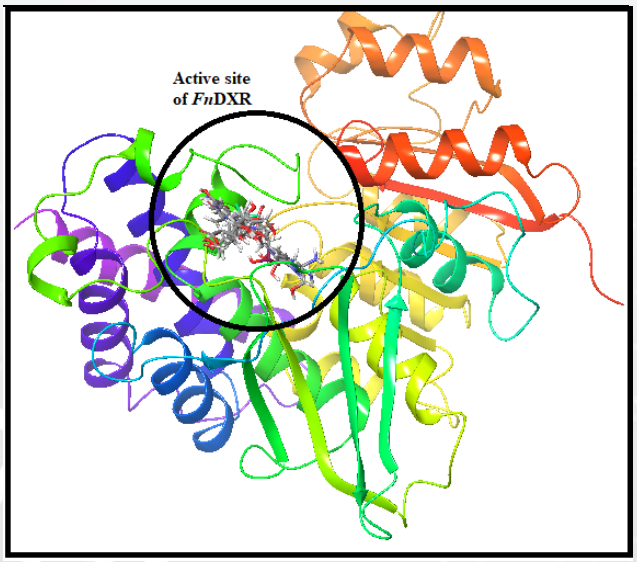

Figure 1: Cartoon representation of the hit 10 inhibitors and fosmidomycin that docked into *Fn*DXR active site.

4. Discussion

DXR may have three conformations as open, closed and super-open forms [22, 23]. The selected template *M. tuberculosis* DXR (PDB ID: 2C82_A) [22] showed quite similarity to the closed conformation of *E. coli* DXR

complexes with 1-Deoxy-D-xylulose 5-phosphate (DXP) and NADPH (PDB ID: 1Q0Q) [23]. Therefore, the interactions between the ligands and *Fn*DXR were predicted more accurately because the flexible loop (residues 198-205, [22]) bent closer to the active site.

In this study, inhibitor screening analyses on *F. nucleatum* DXR were performed by *in silico* docking analyses for the first time. The hit 10 inhibitors were predicted by docking of the selected enzyme inhibitors and fosmidomycin on *Fn*DXR. The hit compounds were observed as more potential inhibitors than fosmidomycin (Table 2). Especially, 72968, 104762 and 16886 compounds among these 10 inhibitors showed the best docking scores as -7.840, -7.146 and -7.096 kcal/mol, respectively. The docking score of DXR inhibitor, fosmidomycin, was found as -5.446 kcal/mol. According to these results, the best three compounds (72968, 104762 and 16886) were predicted as potent *Fn*DXR inhibitors than fosmidomycin for further analyses. As the next step of computer-aided drug design, these compounds will be studied by molecular dynamic simulation and ADME-TOX analyzes. In this way, inhibitor candidates of *Fn*DXR would be able to use for the further *in vitro* inhibition studies.

5. Conclusion

Discovering an alternative drugs against *F. nucleatum* will be necessary because of its pathogenic features [2,3] and *Fn*DXR is chosen as a potent drug target for these studies. This enzyme is responsible for an important ,metabolism of the bacterium and a homolog enzyme of DXR is not located in the host [7,8]. In this study, different compounds apart from fosmidomycin were identified as candidate inhibitors of *Fn*DXR by *in silico* analyses. For the further analyses, derivatives of these compounds can be synthesized and *in vitro* inhibition screening studies can be carried out on this enzyme. In this way, lead compounds may be identified and be used in pre-clinic and clinical studies for the treatment of diseases that are related to *F. nucleatum*.

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SS-10

Chitosan-Based Vector/DNA Complexes for Gene Delivery to HEK 293-T Cells

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INTRODUCTION

Gene therapy is the trasportation of genes into an individual's cells and tissues to treat a disease. Successful gene therapy relies on effective vector system. Viral or non-viral gene delivery systems are vectors that are used in gene therapy. Although, viral vectors demonstrated high transfection efficiency, they have high immunogenicity. The non-viral delivery systems have advantage low immunogenicity. As for that, non-viral delivery systems have advantage low immunogenicity [1, 2].
The ideal gene delivery system must be capable of protecting the DNA until target cell. Chitosan which is a cationic polymer was used as gene delivery system. Chitosan which a natural biopolymer, has been considered to be a good gene carrier which combines biocompatibility. Chitosan has several advantages in comparison with other non-viral vectors in that it is relatively non-toxic and has high transfection efficiency. Chitosan which has cationic charge, it form complexes with negatively charged DNA [3, 4].
In our study, we evaluated the preparation of low molecular weight chitosan (LMW), medium molecular weight chitosan (MMW) and high molecular weight chitosan (HMW) polymers as non-viral gene delivery systems, transfection efficiency and cystotoxicity.

MATERIALS AND METHODS

Materials

Plasmid DNA(26001:LV-RFP) was from Addgene. Low, medium and high molecular weight chitosans were purchased from Fluka. Plasmid DNA purification kit was from Qiagen. TPP sodium was from Sigma.

Plasmid DNA Isolation

Plasmid DNA(Plasmid 26001:LV-RFP) was amplified using *E. coli* DH5α strain. *E. coli* DH5α strains were made competent by $CaCl_2$ method. Red Fluorescent Protein (RFP) coding plasmid DNA were transformed into host cells. The purity of plasmid was checked by 1% agarose gel electrophoresis and concentration of DNA was determined by measuring UV absorbance at 260 and 280 nm.

Preparation of Chitosan-based vector

Chitosan-based vectors (CSV) were prepared by ionic gelation technique with tripolyphospate (TPP) sodium as crosslinking agent. 2 mg/ml chitosan solutions were prepared in 1% (v/v) acetic acid either by middle viscous or highly viscous chitosan polymer. TPP sodium was dissolved in distilled water (0.84 mg/ml) and added into the chitosan solution under magnetic stirring.

Preparation of Chitosan-based vector/pDNA complexes

Chitosan-based vector/pDNA complexes were freshly prepared at different w/w ratios by mixing equal volumes of chitosan-TPP and pDNA stock solution diluted with ultrapure water. pDNA is added on chitosan-based vector solution and after mixing, incubated at 37°C for 30 minutes to form complexes. pDNA binding ability of chitosan-based vector were checked by agarose gel electrophoresis.

Characterization of Chitosan-based vector

The chitosan-based vectors were characterized in terms of size, surface charge, polydispersity index and ability for DNA condensation. The hydrodynamic diameter of chitosan-based vectors were determined by Dynamic Light Scattering (DLS) using a Zetasizer (Malvern Zetasizer ZEN3600). Samples were measured at room temperature in triplicate for vesicle size zeta potential analysis and polydispersity index (PDI) .

In Vitro Transfection Studies

293-T human embryonic kidney cells were cultured in DMEM growth medium at 37°C with 5% CO_2 under humidified condition. 293T cells were seeded into the 24 well-plate at a density of $3x10^4$ cells/well. Chitosan-based vector/ pDNA complexes were freshly prepared at different weight ratio of 1:1, 1:0,5 and 1:0,25 respectively. Chitosan-based vector complexes were added to the 24 well-plate and incubated for 4 h at 37°C. Flourescence signal was observed by the flourescence microscope after 48 hours.

In Vitro Cytotoxicity Assay

Cytotoxicity of different formulations were determined by MTT [(3–(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide blue-indicator dye]-based assay. Briefly, 293T cells were seeded in 24-well plates at a density of $3x10^4$ cells per well. After 24 h incubation at 37 °C in 5% CO_2 atmosphere, the culture medium was replaced with fresh medium alone (negative control) or containing various amounts of each formulation and incubated for 48 h. Following each incubation time, 20 µl of MTT solution (5 mg/ ml in PBS) was added to each well and the cells were further incubated at 37 °C for another 4 h. After that, the culture medium was replaced with 200 µl of isopropanol to dissolve formazan crystals. The optical density of each well was read at 570 and 630 nm using a microplate reader.

RESULTS AND DISCUSSION

Morphology of Chitosan-based vector

The morphological structure of chitosan-based vectors low, medium and high molecular weight chitosans based vectors are observed by optical microscopy in Figures 1A, 1B and 1C, respectively.

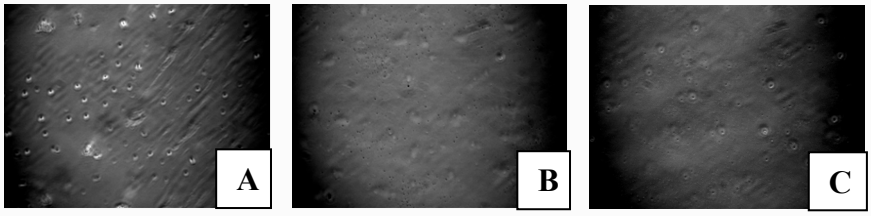

Figure 1. Optical microscopy image of chitosan-based vector (40X magnification).

Preparation of Chitosan-based vector/pDNA complexes

Chitosan-based vector pDNA complexes were incubated at room temperature for 30 min. The size of the vectors prepared of medium molecular weight chitosan were smaller, than that of vectors prepared of high and low molecular weight chitosans. pDNA binding ability of chitosan-based vectors were checked by 1% (w/w) agarose gel electrophoresis. As shown in Figure 2,3 and 4 between 1:1(w/w) and 1:0,25 chitosan-based vector/ LV-RFP can be efficiently complexed by chitosan-based vector/pDNA complexes.

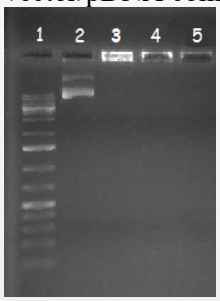

Figure 2. The formation of complexes were analyzed by 1% agarose gel electrophoresis (Low molecular weight chitosan-based vector/ pDNA).

1. DNA Ladder
2. pDNA (Addgene Plasmid 26001:LV-RFP): 7539 bp
3. LMW Chitosan-based vector/ pDNA: 1:1 (w/w)
4. LMW Chitosan-based vector/ pDNA complex: 1:0,5 (w/w)
5. LMW Chitosan-based vector/ pDNA complex: 1:0,25 (w/w)

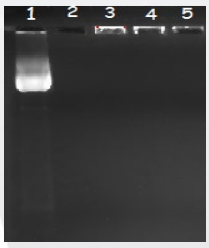

Figure 3. Electrophoretic mobility analysis of complexes. Samples were run on a 1 % agarose gel and stained with ethidium bromide (Medium molecular weight chitosan-based vector/ pDNA).

1. pDNA (Addgene Plasmid 26001:LV-RFP): 7539 bp
2. Medium molecular weight chitosan-based vector
3. MMW Chitosan-based vector/pDNA complex: 1:1 (w/w)
4. MMW Chitosan-based vector/pDNA complex: 1:0,5 (w/w)
5. MMW Chitosan-based vector/pDNA complex: 1:0,25 (w/w)

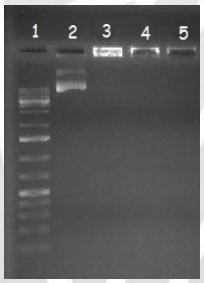

Figure 4. Electrophoretic mobility analysis of complexes. Samples were run on a 1 % agarose gel and stained with ethidium bromide (High molecular weight chitosan-based vector/ pDNA).

1. DNA Ladder
2. pDNA (Addgene Plasmid 26001:LV-RFP): 7539 bp
3. HMW Chitosan-based vector / pDNA complex 1:1 (w/w)
4. HMW Chitosan-based vector/ pDNA complex 1:0,5 (w/w)
5. HMW Chitosan-based vector / pDNA complex 1:0,25 (w/w)

Characterization of Chitosan-based vector

The zeta potentials, size and PDI value are given in Table 1. It is the smallest middle viscous chitosan-based vector. According to Table 1 the zeta potentials of chitosan-based vectors are cationic.

Table1. Particle size and zeta potential values of the vectors.

Formulations	Zeta Potential(mV)	Size (nm)	pDI
LMW – CSV	+26,87	128,7± 0,87	0,647
MMW-CSV	+25,44	100,4	1
HMW-CSV	+23,41	111,3± 1,02	0,469

In Vitro Transfection Studies

As seen in Figure 5 red fluorescent protein expression can be achieved in HEK 293-T cells after 48 hours by chitosan-based vector prepared by either middle viscous or highly viscous chitosan polymer. Either 1: 0,25 (w/w) CSV/pDNA or 1: 0,5 (w/w) CSV/pDNA can be delivered by chitosan-based vector. Transfection efficiency was decreased by using highly viscous chitosan polymer. Transfection did not occur in low viscous chitosan based-vector/ pDNA complexes.

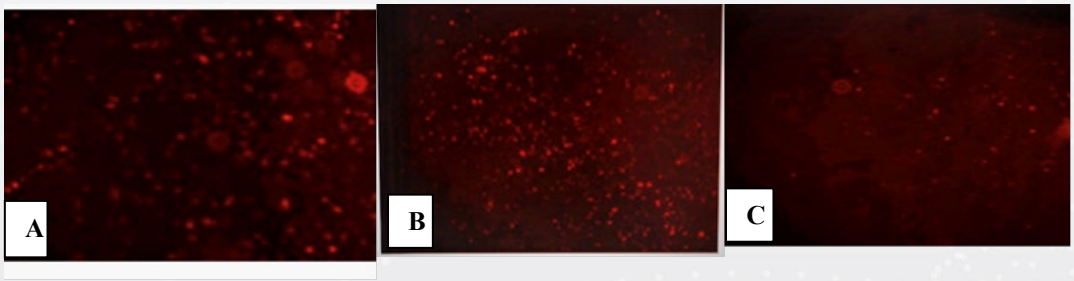

Figure 5. Red Fluorescent Protein Expression in HEK 293-T cells
A: 1: 0,25 (w/w) MMW- CSV/ pDNA complex, 48 hours after transfection
B: 1: 0,5 (w/w) MMW- CSV/ pDNA complex, 48 hours after transfection
C: 1: 0,5 (w/w) HMW- CSV/ pDNA complex, 48 hours after transfection

In vitro cytotoxicity assay

According to Figure 6, middle chitosan vector is less cytotoxic than low and high chitosan vectors in Hek 293-T cells. As seen in Figure 7, all chitosan formulations are non-cytotoxic in HEK 293-T cells.

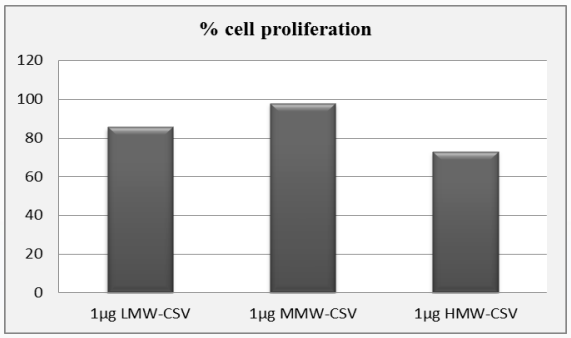

Figure 6. HEK 293-T cells cell proliferation (%)

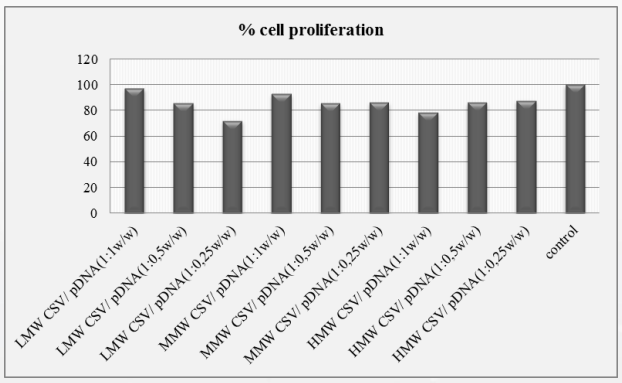

Figure 7. HEK 293-T cells cell proliferation (%)

CONCLUSION

Chitosan-based vectors were prepared to deliver plasmid DNA effectively. The vectors should be non-cytotoxic and can be used as an efficient non-viral gene delivery system. Transfection efficiency depends on the type of chitosan and cells.

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SS-11

Clinical Trials on Biosimilar Products-Current Status from The Point of View of Turkish Medicines and Medical Devices Agency

The adventure of biotechnological drugs which are obtained through genetic modification from living organisms started with the use of human insulin produced by recombinant DNA technology in the early 1980's. In recent years R&D and productive activities of biotechnological drugs have accelerated all over the world. In the development procedure of new drugs against diseases, biotechnological methods have been started to be used more widely than conventional methods. At the same time the number of biosimilar products have increased rapidly. Considering both of the policy documents and the enhancement of the industrial investments, the importance given to the R&D of the biotechnological products in our country increases as in the global market. Consequently, the number and budget of clinical trials performed by biotechnological products are also increased in Turkey. Related studies are mostly performed with monoclonal antibodies and in the field of oncology. These trials are generally phase III but the other phases are also available.

In Turkey national clinical trial legislation is in compliance with international regulations. During the evaluation of clinical trial applications, the research-specific guidelines published by FDA and/or EMA could be used in addition to our local legislation. In clinical trials performed with biotechnological drugs, the period of the insurance should cover five more years from the end of the study.

The acceleration of the R&D of biotechnological drugs together with the whole world will increase the know-how and infrastructure in this attractive and important area. The treatment approaches developed with new biotechnological methods are expected to meet the unmet medical needs.

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3D Microfluidic Platforms for Screening of Nanomaterial Toxicity and Biocompatibility

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Knowledge on the toxicity and potential health risks associated with nanomaterial use is extremely limited, despite the widespread use of nanomaterials in cell and tissue engineering, medical device development, and the encapsulation and delivery of drugs, diagnostics and genes. However, in parallel to their increasing use in clinical applications and the vast variety of their biomedical applications, safety concerns and the number of studies on health risks associated with nanomaterial exposure continue to grow.

Toxicity studies are indispensable in biochemical and medical research and are critical to the successful translation of any formulation including nanomaterials into the clinic. In preclinical toxicity studies, use of animal models is a costly and sometimes inefficient method of generating toxicity data, in addition, there are differences in xenobiotic metabolism between humans and animals (e.g. the different responses by humans and rodents to xenobiotics are explained in part by species differences in Cytochrome P450). Thus, *in vitro* toxicity tests alternative to animal testing are urgently required.

Contrary to conventional 2D culture in which cells rapidly lose differentiated functions, 3D microfluidic platforms have been shown to allow the creation of an artificially engineered, physiologically relevant cell culture microenvironment mimicking the *in vivo* environment, which is not possible using standard plate cultures. Thus, the 3D microfluidic platforms provide a significant new opportunity to replace animal testing as well as to investigate the toxicity and biocompatibility of nanomaterials at the cellular and molecular levels. Herein, the developed 3D microfluidic platforms for the screening of nanomaterial toxicity and biocompatibility will be discussed.

Keywords: Biocompatibility; Nanotoxicity; Organ-on-a-chip; Primary cells; 3D cell culture

Nanomaterials have a vast variety of applications in a vast variety of sectors including biomedical, cosmetics, food, electronics, textile [1, 2]. Safety concerns and the number of studies on health risks associated with nanomaterial exposure continue to grow, in parallel to their increasing use in clinical applications and their vast variety of biomedical applications including cell and tissue engineering, medical device development, and the encapsulation and delivery of drugs, diagnostics and genes [3, 4].

Toxicity studies are indispensable in biochemical and medical research and are critical to the successful translation of any formulation including nanomaterials into the clinic [3, 4]. Nanotoxicology is a new branch of toxicology that addresses the gap in knowledge of toxicity induced by nanomaterials; this new realm of toxicology introduces protocols to access the toxicity induced by nanomaterials; this branch of toxicology includes the basic understanding of the physicochemical effects of nanomaterials and their routes of exposure/uptake mechanisms for toxicity assessment in humans [2, 5]. In preclinical toxicity studies, use of animal models is a costly and sometimes inefficient method of generating toxicity data, in addition, there are differences in xenobiotic metabolism between humans and animals (e.g. the different responses by humans and rodents to xenobiotics are explained in part by species differences in Cytochrome P450). Thus, *in vitro* toxicity tests alternative to animal testing are urgently required; numerous *in vitro* models have been proposed for this purpose, such as organ slices, primary cells, and cell lines [4]. Compared with organ slices (they exhibit a limited lifespan in culture) and cell lines (they do not possess important phenotypic characteristics of the organ tissue with no or very low levels of important drug-metabolizing enzymes and transporters; show much lower expression of organ-specific functions on average; and represent a phenotype from a single donor, which reduces their predictive value for the human population), primary cells are currently the best option for *in vitro* toxicity testing [3, 4, 6, 7].

On the other hand, the dimension of the cell culture is another issue for *in vitro* toxicity testing; to date, traditional two-dimensional (2D) monolayer cells cultured on flat and rigid substrates are used in most cell-based assays. While time-dependent 2D cell cultures have proven to be a valuable method for cell-based studies, they have serious limitations. Since almost all cells are surrounded by other cells as well

as by extracellular matrix (ECM) in a three-dimensional (3D) form in *in vivo* environment, 2D cell cultures do not adequately represent the physiological 3D environment of cells. As a result, 2D cell culture tests sometimes provide misleading and nonpredictive data for *in vivo* responses; many drugs fail in clinical trials, especially during phase III, the most expensive stage of clinical development, due to the lack of clinical effectiveness and/or unacceptable toxicity. These failures are partially attributed to data collected from 2D monolayer culture tests in which the cellular response to drugs is altered due to unnatural microenvironment; thus, *in vitro* toxicity tests mimicking the *in vivo* cell behaviors and providing more predictable results to *in vivo* tests are urgently required [4, 8, 9]. On the other hand, 3D microfluidic platforms have been shown as valuable *in vitro* models that allow the creation of an artificially engineered, physiologically relevant cell culture microenvironment mimicking the *in vivo* environment, which is not possible using standard 2D plate cultures. Thus, the 3D microfluidic platforms provide a significant new opportunity to replace animal testing as well as to investigate the toxicity and biocompatibility of nanomaterials at the cellular and molecular levels [4].

As an example for successful implementation of the 3D microfluidic approach for screening of nanomaterial toxicity and biocompatibility, a practical microfluidic 3D hepatocyte chip shown below was developed for hepatotoxicity testing of nanoparticles using proof of concept studies providing first results of the potential hepatotoxicity of superparamagnetic iron oxide nanoparticles (SPION) under microfluidic conditions [4].

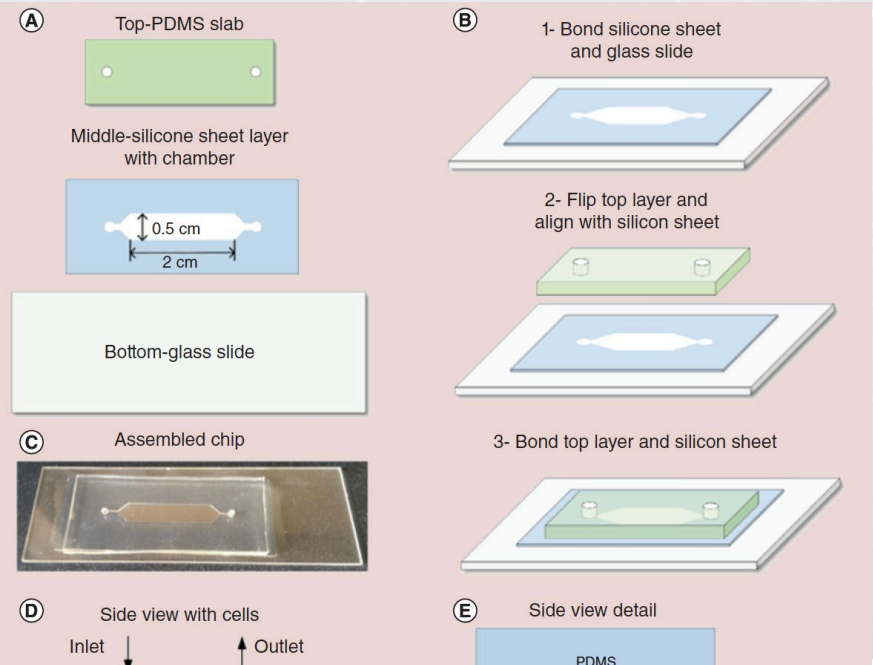

Figure 1. Schematic of the various steps during fabrication of the microfluidic device and the 3D hepatocyte culture assembly within the device. (A) Schematic of the three layers that were used to assemble the device. (B) The bonding process of the three layers by using O_2 plasma treatment. (C) Photo of the assembled microfluidic chip. (D) Side view of the chip with seeding of hepatocytes. (E) Detailed side view of the chip with hepatocytes [4].

The described 3D hepatocyte chip containing primary hepatocyte cells was tested using different concentrations (50, 100 and 200 µg/ml) of SPION in 3-day (short-term) and 1-week (long-term) cultures; compared with standard 2D well plates, the hepatocyte chip with flow provided comparable viability and significantly higher liver-specific functions, up to 1-week; in addition, the chip recapitulates the key physiological responses in the hepatotoxicity of SPION [4].

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SS-13

Synthesis, Bioactivities and Docking Studies of Novel Trimethoxy Substituted Chalcone Compounds

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Abstract: In this study, two new compounds, 6-(3-trimethoxysubstitutedphenyl-2-propenoyl)-2(*3H*)-benzoxazolones (**1, 2**) were synthesized and evaluated their AChE inhibitory and CA (hCA I and II) inhibitory effects. We performed *in silico* molecular modeling studies of the compounds with AutoDock program to evaluate and confirm interactions of the compounds with AChE and hCA II enzymes.

Keywords: Acetylcholinesterase, benzoxazolone, carbonic anhydrase, trimethoxy, docking study.

Introduction: Alzheimer's disease is a progressive brain disease in which behavior disorders can be seen and routine various mental functions practices weaken over time [1]. Although there has been great progress in understanding the pathogenesis of Alzheimer's, there is no cure for Alzheimer disease. The treatments applied are to slow down the progression of the disease and reduce the symptoms. In Alzheimer's disease, acetylcholine levels have been decreased in parallel with the loss of neurons [2]. So, increasing the level of acetylcholine due to acetylcholine esterase (AChE) inhibition in the treatment of Alzheimer's disease is one of the most commonly used treatment approaches. Carbonic anhydrase (CA) is an enzyme family that catalyzes interconversion between carbon dioxide and bicarbonate. CAs have been distributed in many organs/tissues, so they play an important role in many physiological and pathological processes [3]. Thus, inhibition of several CAs is an important strategy for the treatment of several diseases such as epilepsy, glaucoma, obesity and several neurological diseases[3].

Chalcones, 1,3-diaryl-2-propenones, have a wide range of biological activities such as cytotoxic [4], acetylcholinesterase inhibitory [5, 6], and carbonic anhydrase inhibitory [4, 6] activities. On the other hand, benzoxazolones have several bioactivities like anticancer [7], cytotoxic [4], acetylcholinesterase inhibitory [4, 8] and carbonic anhydrase inhibitory [4] activities.

Methoxy group is an important group for the drug design in medicinal chemistry due to being an acceptor of proton in hydrogen bonds [9].

In this study, we synthesized two new compounds, 6-(3-trimethoxysubstitutedphenyl-2-propenoyl)-2(*3H*)-benzoxazolones (**1, 2**) and evaluated their AChE inhibitory and CA (hCA I and II) inhibitory effects. We performed *in silico* molecular modeling studies of the compounds with AutoDock program to evaluate and confirm interactions of the compounds with AChE and hCA II enzymes.

Materials and Methods: First, 2(*3H*)-benzoxazolone was acylated by Friedel-Crafts acylation reaction and so, we synthesized the ketone compound. We synthesized compounds **1** and **2** by Claisen-Schmidt Condensation reaction in basic condition as reported in our previous study [4]. To mixture of the ketone compound and suitable aromatic benzaldehyde [2,4,6-trimethoxybenzaldehyde (**1**) and 2,3,4-trimethoxybenzaldehyde (**2**)] in 1:1 mol ratios in ethanol (5ml), aqueous solution of KOH (10%, 5ml) was added. Reaction content was stirred at room temperature for 24 hours. After the reaction finished, the content of the reaction was poured on cold water and neutralized by HCl (%10). The solid precipitated was filtered and washed with water, then crystallized from acetonitrile. The chemical structures of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS spectra.
Carbonic anhydrase (hCA I and II) inhibition and acetylcholinesterase inhibition assays of the compounds were done as described in our previous studies[10-12]. Compounds were docked at the binding sites of AChE (PDB ID: 1EVE) and hCA II isozyme (PDB ID: 3HS4) by AutoDock program (ver. 4.2.6).

Results: The two compounds designed were successfully synthesized and their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS spectra as shown below. Carbonic anhydrase inhibition and AChE inhibition results of the compounds was shown in Table 1. Docking results of the compounds was shown in Figure 1 and Figure 2.

6-[3-(2,4,6-Trimeyhoxyphenyl)-2-propenoyl]-2(*3H*)-benzooxazolone (1)

^{1}H-NMR (DMSO-d_6) δ (ppm) 12.01 (bs, 1H, NH), 8.08 (d, 1H, Ar-CH=, *J*=15.2 Hz), 7.86 (d, 1H, arom. H, *J*=8.1 Hz), 7.83 (s, 1H, arom. H), 7.82 (d, 1H, =CHCO, *J*=15.2Hz), 7.20 (d, 1H, arom. H, *J*=8.1Hz), 6.30 (s, 2H, arom. H), 3.90 (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3). ^{13}C-NMR (DMSO-d_6) δ (ppm) 188.9, 164.0, 162.1, 155.1, 144.2, 135.7, 135.1, 133.5, 125.8, 120.6, 110.2, 109.5, 105.8, 91.7, 56.8, 56.3. HRMS (ESI-MS) m/z calculated $[M+H]^+$ 356.1129; measured 356.1119.

6-[3-(2,3,4-Trimethoxyphenyl)-2-propenoyl]-2(*3H*)-benzoxazolone (2)

^{1}H-NMR (DMSO-d_6) δ (ppm) 8.05 (s, 1H, arom. H), 8.03 (d, 1H, Ar-CH=, *J*=15.4 Hz), 8.02 (d, 1H, arom. H, *J*=8.2 Hz), 8.01 (d, 1H, arom. H, *J*=8.2Hz), 7.92 (d, 1H, =CHCO, *J*=15.4 Hz), 7.84 (d, 1H, arom. H, *J*=8.0 Hz), 7.81 (d, 1H, arom. H, *J*=8.9 Hz), 7.22 (d, 1H, arom. H, *J*=8.0 Hz), 6.92 (d, 1H, arom. H, *J*=8.9Hz), 3.87 (s, 3H, -OCH_3), 3.86 (s, 3H, -OCH_3), 3.77 (s, 3H, -OCH_3). ^{13}C-NMR (DMSO-d_6) δ (ppm) 187.7, 156.2, 154.9, 153.5, 143.9, 142.2, 138.5, 135.2, 132.6, 126.0, 123.8, 121.5, 120.5, 110.0, 109.8, 108.9, 62.0, 60.9, 56.5. HRMS (ESI-MS) m/z calculated $[M+H]^+$ 356.1129; measured 356.1135.

Table 1. Carbonic anhydrase and AChE inhibition results of the compounds **1** and **2**.

Compounds	KI(μM)		
	AChE	hCA II	hCA I
1	1.4±0.3	11.7±2.0	38.7±13.5
2	3.9±0.9	55.5±2.7	57.9±2.2
AZA*	-	4.4±0.6	30.2±7.8
TAC**	7.9±2.0	-	-

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I and II isoenzymes.
** Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes.

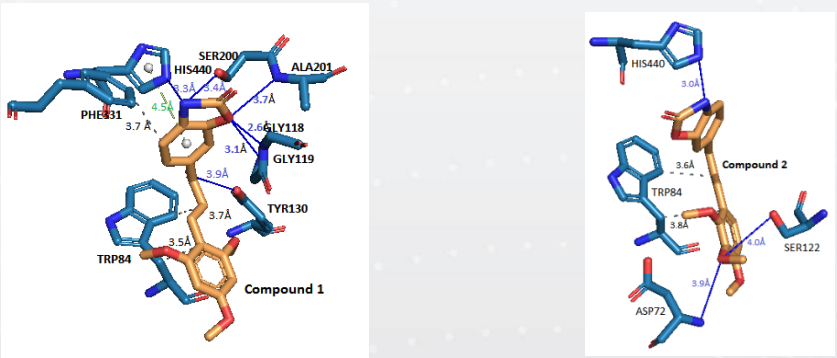

Figure 1. Schematic representation of interactions of compounds **1** and **2** with AChE (1EVE). Green color represents hydrophobic interactions, light blue represents hydrogen bondings, grey represents π-π stacking interactions.

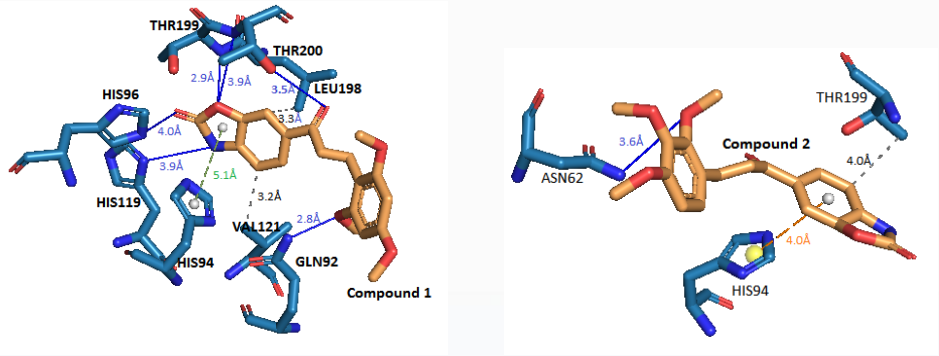

Figure 2. Schematic representation of interactions of compounds **1** and **2** with hCA II (3HS4). Green color represents hydrophobic interactions, light blue represents hydrogen bondings, grey represents π-π stacking interactions, and brown represents π-cation interactions.

Discussion: ^{1}H NMR spectra of the compounds showed that all compounds synthesized were configured *E* configuration, as understood from coupling constant *J*: 15.2 and 15.4 Hz for vinyl protons, observed at 7.82-8.08 ppm. The methoxy protons were observed at the area of 3.0- 4.0 ppm, as expected. ^{13}C NMR and HRMS results confirmed the chemical structures of the compounds, as shown above.

According to AChE inhibition results of the compounds (Table 1), K_i values of the compounds **1** and **2** had higher K_i values compared to reference drug, TAC. K_i value of compound **1** was lower than K_i value of compound **2** (about three times). This result was explained and confirmed by molecular docking studies (Figure 1). According to docking studies, compound **1** had lower binding energy (-6.86 kcal/mol) than compound **2** (-5.67 kcal/mol). Compound **1** interact six hydrogen bonds, three hydrophobic interactions and one π-π stacking interaction with AChE (1EVE) while compound **2** interact by three hydrogen bonds and three hydrophpbic interactions with 1EVE.
Carbonic anhydrase (hCA I and II) inhibition results of the compounds are presented in Table 1. According to the results, compounds **1** and **2** had higher K_i values than reference drug AZA. Compound **1** had lower K_i value than compound **2** towards hCA II**.** This result was explained and confirmed by molecular docking studies with hCA II (3HS4) and results are shown in Figure 2. According to docking studies, compound **1** had lower binding energy (-5.33 kcal/mol) than compound **2** (-4.87 kcal/mol). Compound **1** interact with hCA II (3HS4) by six hydrogen bonds, two hydrophobic interactions while compound **2** interact with 3HS4 by one hydrogen bond, one hydrophobic interaction and one π-cation interaction.
Conclusions: Compound **1** had a lower Ki value than reference drug TAC towards AChE. On the other hand, compound **1** was found to have more potent inhibitory activity both on AChE and CA enzymes than compound **2**. Compounds were docked at the binding site of AChE (1EVE) and hCA II (3HS4) to further validate the inhibitory results. According to the results, compound **1** showed good interaction with the AChE. So, compound **1** can be considered as a lead molecule of this study for further studies.

Acknowledgment: I would like to thanks Prof. Hiroshi Sakagami (Meikai University, Japan) and Prof. Ilhami Gulcin (Ataturk University, Turkey) for their contributions to the bioactivity assays.

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SS-14

Abstract Template

Title: Computational Drug Design Approach and Drug-likeness Evaluation for Bone Cancer Treatment

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Department of Biomedical Engineering, Faculty of Technology, Selcuk University

Abstract:
Bone cancer is a type of cancer mostly seen in teens. In Turkey, approximate 200 people are diagnosed with bone cancer every year. We, in this study, investigated the existing FDA approved small molecule drugs used for bone cancer treatment, their physico-chemical & pharmaceutical properties and their bioactivity scores using in silico modeling approach, Molinspiration and SwissADME cheminformatics and bioinformatics resources. Due to its highest bioactivity score, Methotrexate scaffold was used as a lead molecule, and we investigated fifteen bioisosteric analogs of Methotrexate in terms of biological activity and five drug likeness criteria reported in literature. We, interestingly, observed that silicon incorporated compounds showed a much broader spectrum of bioactivity across the most important drug targets including G-protein coupled receptor (GPCR), ion channel modulator, kinase inhibitor, protease, and enzyme inhibitors than even Methotrexate. Moreover, drug-likeness analysis of our new design compounds and the marketed molecule Methotrexate resulted in a similar behavior. As multi-targeted therapeutic agents are gaining a growing interest, we consider that our new design multi-targeted compound could be a good drug candidate for bone cancer treatment to be tested for further pharmacological investigations.

Keywords:
Drug-likeness; ADME; in silico; bioisostere; bone cancer; silicon

Introduction:

Exponential growth of computer technology and computational tools has significantly decreased both of the duration and the cost of drug development process. We, in this study, discussed an approach to design new drug molecules for treatment of bone cancer and to evaluate them in terms of drug likeness criteria reported in literature. To do that, we used molecular modeling softwares, calculated physico-chemical parameters, and predicted in silico bioactivity scores of the model compounds based on a virtual screening method.

For computational drug-likeness evaluation of a new designed compound, the molecular physico-chemical properties such as lipophilicity, polar surface area, number of hydrogen bond donor and receptor, molecular weight and volume are used as descriptors of the compound. Using those properties, different drug-likeness criteria were reported in the literature by Lipinski (Lipinski, 2001), Ghose (Ghose, Viswanadhan, & Wendoloski, 1999), Veber (Veber et al., 2002), Egan (Egan, Merz, & Baldwin, 2000), and Muegge (Muegge, Heald, & Brittelli, 2001). Among them, Lipinski's rule of five is commonly applied.

Drug molecules were evaluated in terms of their biological activity for the most important drug targets such as G-protein coupled receptors (GPCR), kinase inhibitors, nuclear receptors, ion channel modulators, protease, and enzyme inhibitors by using Molinspiration software. For the detailed drug likeness properties, free online cheminformatics tool SwissADME developed by Swiss Institute of Bioinformatics was used to calculate physico-chemical descriptors and to predict ADME parameters of both approved drug molecules and the design compounds.

Materials and Methods:

The U.S Food and Drug Administration (FDA) approved drugs are obtained from the official website of the National Cancer Institute, the National Institute of Health of USA (NIH), https://www.cancer.gov/. SMILES codes of the FDA approved drugs were obtained from the unique bioinformatics and cheminformatics resource, DrugBank 5.1.1 https://www.drugbank.ca/ (Wishart et al., 2018). Using the SMILES codes of the approved and new design compounds, their bioactivity scores were calculated using Molinspiration and SwissADME softwares. Their drug-likeness scores were evaluated in terms of drug-likeness criteria listed above.

Results and Conclusion:

According to our in silico computational bioactivity analysis, Methotrexate was found to be the most promising molecular compound. Using the molecular scaffold of Methotrexate, four bioisosteric analogues of Methotrexate were shown to have more promising pharmacological properties than the marketed drug based on our in silico bioactivity and drug-likeess analysis.

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SS-15

Analysis Methods Used In Biotechnological Products

Semra KOYUNOĞLU, Handan ÖZTUNCA, Harun KIZILAY, Hakkı GÜRSÖZ

Department of Herbal and Support Products, Vice Presidency of Medicines and Pharmacy, Turkish Medicines and Medical Devices Agency (TMMDA)

Biotechnological product means a product obtained from living organisms. To use biological systems or living organisms and to get benefit from them are the basis of the biotechnology. In medical area, recombinant DNA (rDNA), monoclonal antibody technology, hibridoma technology and gene therapy have been developed by means of understanding of DNA structure in detail. New drugs and vaccines have been started to produce. Biotechnology has been developing by the occurrence of cancer, AIDS, viral infections. All formulation development attempts and related investigations are target to specific disease. Biotechnological drugs make up a large part of the new drugs developed for the treatment of 200 diseases including cancer, alzheimer's, heart diseases, diabetes and rheumatoid arthritis. Drugs used in various treatment areas within the scope of biotechnological drugs include hormones (erythropoietin, somatropin growth factors), insulin, immunomodulators, monoclonal antibodies (mAbs), blood coagulation factors and vaccines. These products are divided into reference biotechnological drugs and biosimilar drugs. Although the active substance of biosimilar and reference biotechnological drugs is basically the same biological substance, they may show some minor differences due to their complex nature and production methods. During the approval process, it should be proved that the biosimilar and reference biotechnological drug does not affect the efficacy and safety of such variability and other differences. Biotechnological products are highly specific in activity and represent less side effects. Breakthrough biotechnological products in human health

are expensive products due to high production and development costs. The production process is complex and requires advanced technology, since the final product, the biological active substance, must be purified by removing it from thousands of other molecules present in the living cell or organism.Turkey is one of the most important centers for the global pharmaceutical industry. However, only certain stages of biosimilar products can be produced in our country, and most of the biotechnological drugs are imported.The growing importance of biotechnology products makes it important to analyze these products. The biotechnological products can be analyzed with numerous methods such as electrophoresis, spectrophotometric, chromatographic, immunological, colorimetry. These techniques are becoming increasingly important in clinical trials.

Keywords: Biotechnological product, analysıs methods used in biotechnological products

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SS-16

Cartilage Regeneration with 3D Woven Scaffolds

Ümran Aydemir Sezer

Suleyman Demirel University, Faculty of Medicine, Department of Pharmacology, Medicine, Medical Device and Dermocosmetic Research and Application Laboratory-IDAL, 32260, Isparta, Turkey

Abstract: Repairing cartilage defects is a challenging area due to the avascular structure of the tissue. Especially regeneration of hyalin cartilage on the joint surface is the focus area of many researchers. The populations of the cells in cartilage which are called chondrocytes decreases with the age, accidents or trauma. As a result, regeneration of cartilage is very difficult. In this study, novel 3D woven scaffolds were produced and then combined with hydrogel for cartilage tissue engineering applications. Starting from the design, woven scaffold structures were produced via polyglycolic acid (PGA) yarn and then combined with hydrogel. Thus, the tissue scaffold which will be ready to be used as functional cartilage tissue engineering product has been designed and obtained in all stages. In conclusion, 3D woven technology could be promising for tissue engineering scaffolds especially for load-bearing areas.

Keywords: cartilage tissue engineering, polyglycolic acid, 3D woven

Cartilage tissue can be damaged by many ways like sudden traumas or repetitive micro-traumas. Avascular characteristics of cartilage tissue make them difficult to regenerate themselves. Varying products to support of regeneration of cartilage tissue have been introduced in literature and clinical applications [1-3]. However, they do not offer desired efficiencies in defect treatments due to their scaffold structures failing to exhibit equivalent mechanical properties to target tissues [3−5]. It has been tried to develop innovative scaffold structures with known characteristics of cartilage tissue through composite materials for achieving high mechanical strength [4]; however, there is still intense research to find out a mechanically strong and porous scaffold structure that can be replaced the real tissue.

Textile modeling begins with geometric modeling of the woven fabric structure which might be expected to have various properties. It would be advantageous to determine whether the material to be produced has the needed properties, or not, by mathematical modeling and computational analysis methods, due to its cost and time efficiencies. The design, then, is examined with specially developed algorithms and analysis method or with existing theories. Computer aided modeling and analysis methods seem to reveal satisfactory results in woven structures [6,7]. Practically, this process is composed of creating a model by available computer aided design/drawing programs and then subjecting the model to test conditions by finite element analysis programs.

In this study, geometric characteristics and porosities of 3D woven preforms produced with PGA yarn in two different designs are calculated by computational methods and their use as composite scaffold structure is discussed along with their mechanical characterization by modeling. In addition to the 3D orthogonal (3DW-O-ZO) model; the 3D-plain-Z-orthogonal (3DW-P-ZO) model is subjected to computational studies and their potentials in cartilage tissue engineering are discussed.

Considering the fact that the PGA fiber is quite thin and of low strength for 3D forming process and due to thickness limitation; the PGA fiber was put into a form that allows to be woven when folded for 100 times. In this study, 3D orthogonal (non-interlaced, 3DW-O-ZO) and 3D plain-Z-orthogonal (semi-interlaced, 3DW-P-ZO) woven fabrics consisting of six warp layers were prepared with the warp and weft PGA tow containing multifilament yarns. In 3D orthogonal preform, layered three yarn sets were used to form the preform as warp (0°) and filling (90°) were laid to each other in the structure plane but Z-yarn bound the warp and filling to the out-of-plane direction. In 3D plain-Z-orthogonal preform, layered warp and filling yarn sets were interlaced at plain pattern but they were locked by Z-yarn with orthogonal pattern.

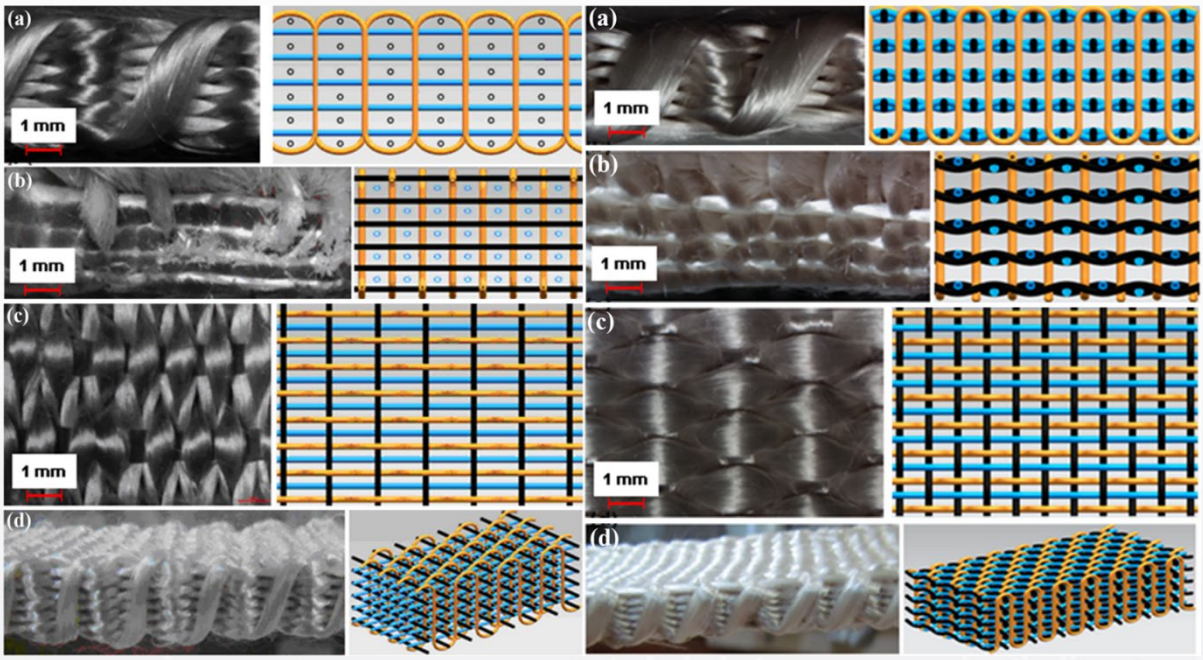

Figure 1. 3D structures of the woven scaffolds.

Correct determination of the mechanical coefficients of the materials is of great importance for the mechanical properties of the orthotropic structure where it is possible to try many different options in terms of both yarn and preforms. The number-one problem that would be encountered in dozens of experiments would be the need for too many layers in the 3D structure at the desired dimensions because the biodegradable yarn to be used is of very small diameter; which causes a growth in the model that ends up with insufficient processing power. Using a high number of finite elements would allow analysis results to be closer to the reality. However, since the software used in this study is still under development, complicated structures could not have been modeled most of the time. The precision of the best result among the computer-calculated results is determined through a comparison of the real test items. However, the prices of yarns, especially the ones made up of biodegradable biopolymers for biomedical use; make this iterative experimental

process quite costly. In this respect, modeling and computational calculations are quite advantageous for developing ideal scaffold structure and making preliminary screening for further biological applications. The modeling results indicate that the 3D plain-Z-orthogonal structure has higher tensile and compressional modulus of elasticity in terms of both warp and weft. However, the transverse tensile modulus of the 3DW-P-ZO and 3DW-O-ZO were higher than those of the longitudinal modulus due to directional fiber volume fraction. The 3D plain-Z-orthogonal structure has higher shear modulus values in x-y, y-z and x-z planes; however, in case of x-y plane, the structure with the highest shear modulus value is the semi-interlaced structure due to the in-plane interlacements.

Comparative cell culture studies under in vitro conditions were conducted with immortalized C-28/I2 human cell line and the results are indicated the scaffold structure supported cell proliferation. The study has revealed applicable, remarkable and promising results for biodegradable artificial organ applications with regards to the 3D preform architectures, their geometric characterization and the fiber volume fraction as well as predictive modeling, which is crucial for the development of innovative and damage tolerance scaffold structures.

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SS-17

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Isolated Glioblastoma mediate exosomes induced strong neuroinflammation in cortex neuron: in vitro study

Abstract

Exosomes are common membrane-bound nanovesicles that contain diverse biomolecules, such as lipids, proteins, and nucleic acids. Glioblastoma, also known as glioblastoma multiforme (GBM), is the most aggressive cancer that begins within the brain. Initially, signs and symptoms of glioblastoma are nonspecific. The emerging evidence of pathogenic mutations associated to neuroinflammation susceptibility, leading to impairment of exosome production and secretion, opens a new perspective on the mechanisms involved in neurodegeneration. The aim of this study is to prove that exosomes of T98 glioma cancer cells cause neurotoxic effect. The Primer neuron cells were grown in neurobasal cell culture medium. As a result of various ultracentrifugation processes, exosomes of the T98 cell line were obtained. Different dose of exosome (1,5,10,50 and 100µg/ml) was applied on neuron cells for 72 hours. The viability, antioxidant and oxidant level were determined by using MTT, TAS and TOS test. As a result of the MTT test, it was determined that 100 µg/ml exosome decrease cell proliferation compared to control ($p<0.05$). Our results show that exosomes induced neuroinflammation in cortex neuron

Keywords: Exosome, T98, MTT,TAS,TOS

Introduction

Exosomes are common membrane-bound nanovesicles that contain diverse biomolecules, such as lipids, proteins, and nucleic acids. They are secreted by nearly all cell typse and cancer cells. Early studies showed exosomes to be a simple tool for the inter- and extracellular transport of different molecule[1].

Neuroinflammation (ND) is a pathologic future of many neurological disorders including Parkinson's disease, Alzheimer's disease, Amyotrophic lateral sclerosis, Huntington disease and also in all central nervous system cancers [2]. Glioblastoma, also known as glioblastoma

multiforme (GBM), is the most aggressive cancer that begins within the brain. Initially, signs and symptoms of glioblastoma are nonspecific. They may include headaches, personality changes, nausea, and symptoms similar to those of a stroke. The recent study suggest exosomes play important role in cancer mediate neuroinflammation becouse of exosomes play a fundamental role on their releasing in extracellular space after endosomal pathway activation, allowing to remove protein and metabolit aggregates besides promoting cellular cross-talk [3]. The emerging evidence of pathogenic mutations associated to ND susceptibility, leading to impairment of exosome production and secretion, opens a new perspective on the mechanisms involved in neurodegeneration. The aim of this study is to prove that exosomes of T98 glioma cancer cells cause neurotoxic effect.

Materials and Methods

Cell Cultures

T98 were obtained from department of medical pharmacology department of Ataturk University (Erzurum, Turkey). Briefly, the cells after centrifuged in 1200 rpm for 5 min were disseminated in 75 cm^2 flask by fresh medium (DMEM, with FBS 10% and antibiotic (Penicillin- Streptomycin, Amphotericin B) 1%) and store at incubator (5% CO2, 95% moisture 37°C (23,24).

Materials and Methods

Cell Cultures

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Primer Neuron Culture

In the study, Sprague Dawley newborn rats were used to obtain neuron cells. Briefly, after the rats were quickly decapitated, the extracted tissues were transferred to 5 mL Hanks'Balanced Salt solution (HBSS) and macro lysis was performed with a scalpel. Then, micro-lysis was performed with Trypsin-Ethylenediaminetetraacetic acid (EDTA) (0.25% trypsin- 0.02% EDTA). Cells were centrifuged at 1200 rpm for 5 minutes and seeded to 96 well plates. Cells will be incubated at 5% CO2 and 37oC for 10 days by changing the medium every 3 days.

Exosome Isolation

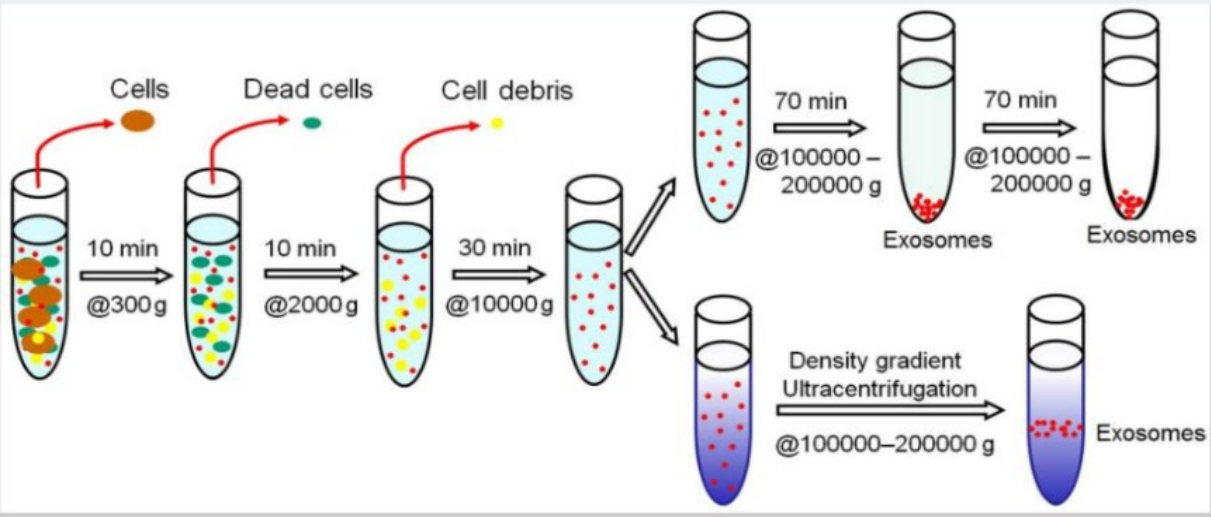

Figure 1. Exosome isolation methods

Exosome Administration

After gaining 85% confluency in 96-well plates the exosomes were added. Exosome (1,5,10,50 and 100 µg/ml) were chosen to be added to the well plate and incubate for 72 hours (incubated in 5% CO2, 95% moisture and 37 °C). For this aim, the experiments groups were divided into following 6 groups: Control group, Exosome (1,5,10,50 and 100 µg/ml).

MTT Assay

The proliferation rate in the neuron was determined by the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The MTT assay was evaluated according to the manufacturer's instructions (Sigma, Munich, Germany). At established time points, the medium was replaced with a fresh one containing 10 µL MTT, and the cells were incubated for 4 h at 37 °C. Then 100 µL DMSO was added to the samples.The MTT reduction/attenuation values for each well were measured spectrophotometrically at 570 nm.

TAS Assay

TAS was investigated by using commercially available kits (Rel assay, Turkey) that were used according to the manufacturer's suggested procedure. The evaluation is made by calculating spectrophotometrically. Briefly, for evaluating TAS, 500 µl Reactive compound 1 was added to each well followed by the addition of 30 µl of specimen and initial absorbance was read at 660 nm (time 0). After the initial reading, 75 µl of Reactive 2 was added to the wells and allowed to incubate at room temperature for 10 minutes. After 10 minutes, a secondary absorbance value was obtained at 660 nm. While distilled water was used for Standard 1 (blank), Standard 2 in the kit was used as the second point for calibrating the relationship of absorbance intensity to pro-oxidants present. The absorbance values acquired were established according to the following formula and TAS standards were detected in Trolox Equiv/mmol L^{-1}.

TOS Assay

TOS was investigated by using commercially available kits (Rel assay, Turkey). The test was done according to the manufacturer's procedure. The evaluation is made by calculating spectrophotometrically. The intensity of the color linked to the quantity of oxidants status. The ingredients in the TOS kits were Reactive 1, Reactive 2, Standard 1, and Standard 2. In order to detect the TOS standard; 500 µl Reactive 1 was incorporated into the wells in which 75 µl plasma specimen was present and later reading the original absorbance value at 530 nm, 25 µl Reactive 2 was incorporated in the equal well and secondary absorbance was read at 530 nm at the end of the waiting duration of 10 minutes at room temperature. Standard 2 in the kit was used for measure Standard 2 value. TOS standards were detected in H2O2 Equiv/mmol L^{-1}.

Results

In this study, the cell viability and proliferation effect of T98 exosome was evaluated and MTT, TAS and TOS were performed after 72 hours of exposure.

MTT

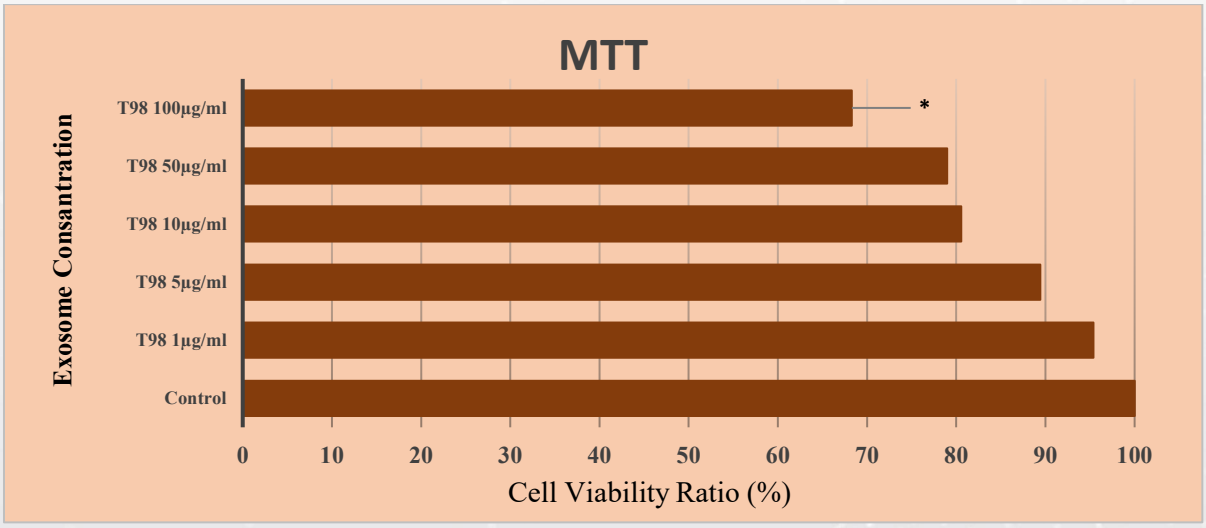

Figure 2: MTT results of neuron cells

Results from the MTT cell viability assay are shown in (Fig. 2) The vitality of the control group was defined as 100% and the other groups were rated accordingly. Exosome groups showed significantly lower cell viability compared with control group. 100 µg/ml exosome showed a statistical difference in comparison to the control group *($p<0.05$)*.

TAS

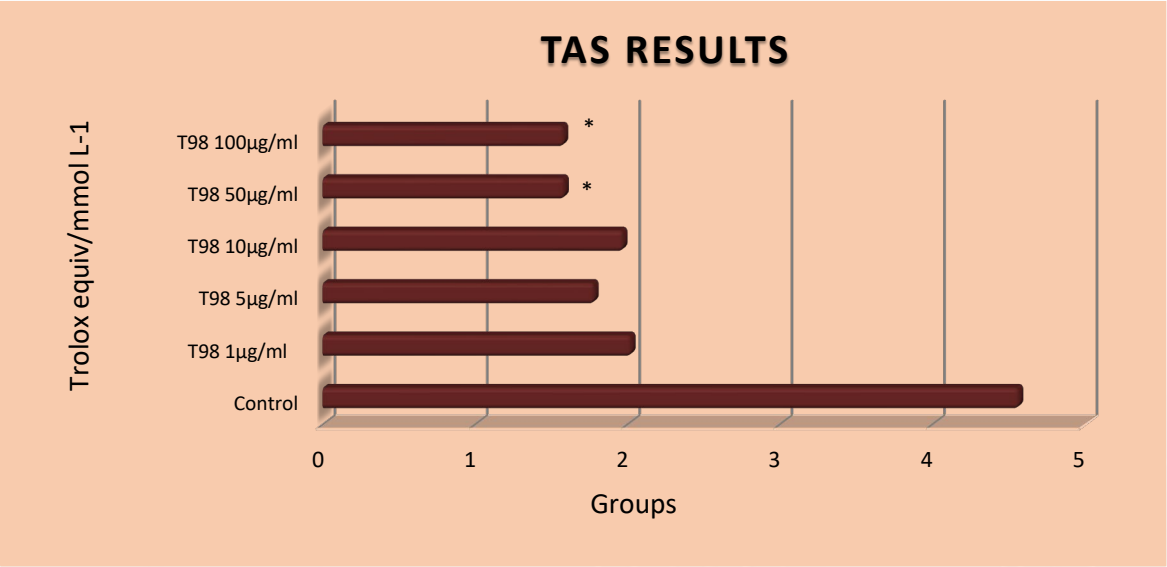

Figure 3. TAS results of neuron cells

We evaluated the TAS test based on Trolox equiv/mmol L-1 (Fig. 3). According to this test, the TAS level of the control group has the highest anti-oxidant level compared to the the other groups. It was found statistically significant in other groups compared to the control group *($p < 0.05$)*.

TOS

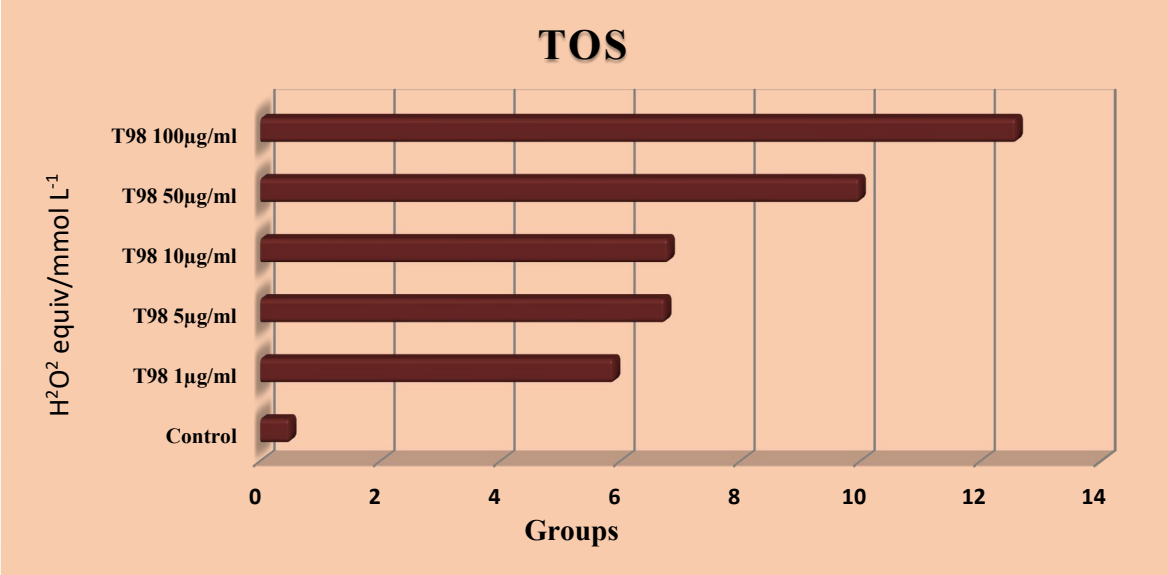

Figure 4. TOS results of neron cells

We evaluated the TOS test based on H^2O^2 equiv/mmol L^{-1} (Fig. 4). According to this test, the TOS level of the higher concentration has the highest oxidant level compared to the control. Our groups were statistically significant compared to the control group *($p < 0.05$).*

Discussion

Exosomes play a crucial role in the brain and nervous system development by transmitting donor cell information throughout the body. On the other hand, due to their capacity to carry pathogenic molecules, exosomes have also been labeled the "Trojan horse of neurodegeneration" given their potential role in disease pathogenesis [4]. A study showed that exosomes transported between neurons in AD patients contain high levels of toxic amyloid-β oligomers. The study also said that blocking the development, release and/or uptake of such exosomes decreased the spread of these oligomers and their toxic effect[5].

Coclusions

As a result, exosomes isolated from glioma cancer line cause neuroinflammation by induction a toxic effect on neurons.

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SS-18

Fabrication of Propolis Loaded Sodium Alginate Scaffolds Using Three Dimensionally (3D) Printing Technology and Antibacterial Investigation for Wound Treatment

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Abstract

Additive manufacturing or three-dimensional (3D) printing is a popular technology in recent years due to providing advantages. This technology allows the manufacturing of customizable, biocompatible, patient-specific, quite comprehensive, mechanically stable and rapidly fabricated products in medical fields. Especially, 3D printed tissue scaffolds are popular in regenerative medicine and tissue engineering applications. In this study, it was aimed to produce 3D printed, biocompatible and propolis incorporated alginate scaffolds to use in wound treatments. For this, 10, 20, 40% (v/v) propolis concentrations were added to 4.5% (w/v) of alginate solution. All scaffolds were cross-linked with $CaCl_2$ solution. The optimized propolis loaded alginate solutions were tried to print with different properties and different parameters. Also, the antibacterial assay of 3D printed, propolis incorporated alginate

scaffolds was investigated. *Escherichia coli* and *Staphylococcus aureus* strains were used for this test. The results displayed that scaffolds have excellent antibacterial effect due to propolis extract. 3D printed scaffolds are promising for further tissue applications.

Keywords: alginate, 3D printing, propolis, tissue scaffold, wound healing

1. Introduction

Additive manufacturing (AM) or three-dimensional (3D) printing is an advanced technology that used for obtaining as a matter the output of a three-dimensional model. [1, 2]. Dental applications, biomedical devices, anatomical modeling, regenerative medicine, tissue engineering scaffolds, and drug formulations are the most popular medical fields for 3D-printing technology. Especially, regenerative medicine and tissue engineering applications use this technology for wound treatment [3]

Alginate is the most preferred natural anionic polymer that is used for biomedical applications due to its biocompatibility, low toxicity and low cost [4]. Propolis (bee glue) is a resinous substance collected by honey bees from leaf, exudates, bud etc. of different plants to build, isolate and protect bee hives [5]. The propolis has antibacterial [6-8], antiviral [9], antifungal [10], antioxidant [11] properties therefore it is a good candidate for using as a therapeutic substance in biomedical fields.

This study aims to construct 3D printed, biocompatible and propolis incorporated alginate scaffolds to use in wound treatments. Also, antibacterial assay was investigated for using these scaffolds in wound treatment.

2. Materials and Methods

Sodium alginate (SA) with an average molecular weight of 216.121 g/mol was purchased from Sigma-Aldrich (Istanbul, Turkey). Calcium chloride dihydrate ($CaCl_2.2H_2O$) was purchased from Merck (Darmstadt, Germany) for using as a crosslinker. The propolis extract was kindly provided from SBS (Scientific Bio Solutions) Incorporated Company, Istanbul, Turkey).

Methods

The SA and SA-Propolis solutions in different concentrations were prepared. 10, 20, 40% (v/v) propolis concentrations were added to 4.5% (w/v) of alginate solution.The prepared propolis based SA solution was taken to 10 mL syringe for 3D printing. After printing, the $CaCl_2$ solution was used for crosslinking.

The antibacterial assay was determined by disk diffusion method by using *E.coli* and *S.aureus* strains.

3. Results and Discussion

The scaffolds were printed with optimized parameters. The tests were continued with successfully printed and optimized scaffold that have best properties as shown in **Figure 1**. After printing, antibacterial assay was investigated against both Gram-positive and Gram-negative bacteria strains. The results showed that 3D printed scaffolds have excellent antibacterial activity against *E.coli* and *S.aureus* strains due to propolis content. All zones' diameters were closed to control antibiotic drug (ampicillin) zones by the help of propolis.

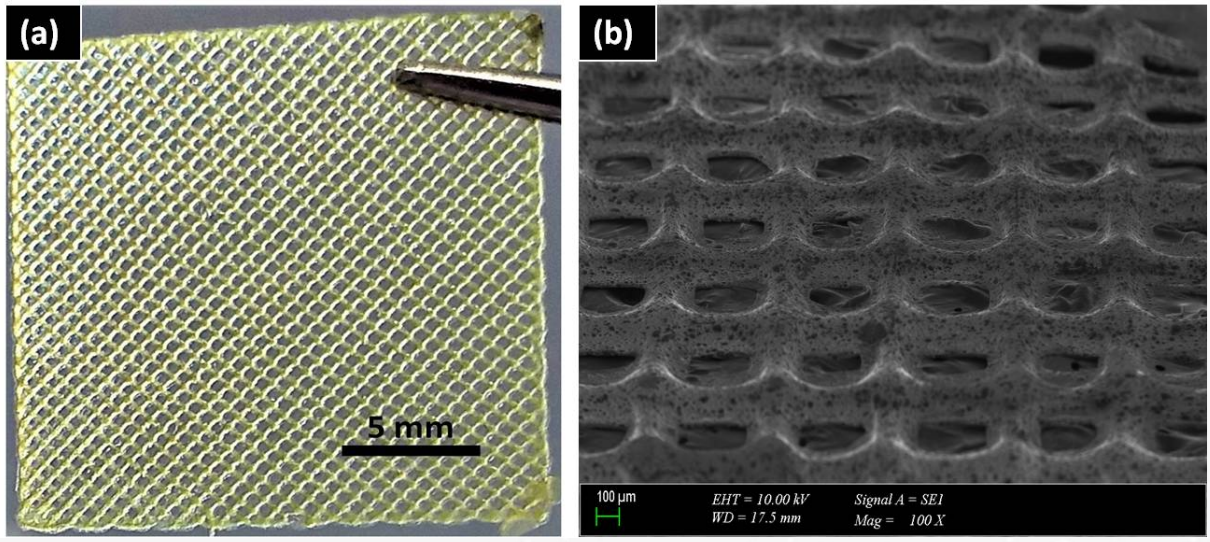

Figure 1. (a) 3D printed, propolis loaded sodium alginate based scaffold **(b)** SEM image of 3D printed, propolis loaded porous, and sodium alginate scaffold

4. Conclusions

In this study, the propolis loaded SA scaffolds were three dimensionally printed and produced scaffolds' antibacterial assay was also investigated. According to results, 3D printed scaffolds have excellent antibacterial effect due to propolis effective substance. The propolis loaded 3D printed scaffolds can be used in wound treatments by developing for further studies.

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SS-19

Can minocycline be used in the development of an alternative biomaterial to biodentine?

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Abstract

Dental Pulp Stem Cells (DPSCs) are neural crest-derived cells with many differentiating capacaties for cell therapy. Biodentin It is used for endodontic repair and pulp closure. Minocycline (MINO) is a member of the bacteriostatic and broad-spectrum antimicrobials group of tetracyclines. The main purpose of this study, MINO is to compare the viability of DPSCs with Biodentine and investigate whether there is a proliferative effect on DPSCs. The DPSC was grown in normal cell culture medium. Different dose of Biodentine (2-4-8-16 and 32 µg/ml) and MINO (2.5-5-10-20 and 40 µg/ml) was applied on DPSC for 24 hours.The viability, antioxidant and oxidant level were determined by using MTT,TAS and TOS test.As aresult of the MTT test, it was determined that 20 µg/ml MINO increased cell proliferation compared to control *($p<0.05$)*.This study, MINO promises to be an alternative biomaterial to biodentine.

Keywords: Biodentine, dental pulp, minocycline.

Introduction

Dental Pulp Stem Cells (DPSCs) are neural crest-derived cells with an outstanding capacity to differentiate along multiple lineages of interest for cell therapy (1). These cells are referred to as DPSCs when extracted from permanent teeth, and as stem cells from human exfoliated deciduous teeth when extracted from first-generation teeth during childhood (2).

Biodentin is a calcium silicate-based bioactive material (3). It is used for endodontic repair and pulp closure and has good biocompatibility with DPSCs (4). However, clinicians face the difficulty of placing this material in a low pH environment due to inflammation (5). Variations in the pH at the time of placement could affect the physical and chemical properties of Biodentine (6,7,8). Limited information is available on the possible cytotoxicity of this bioceramic material (9,10).

Minocycline (MINO) is a member of the bacteriostatic and broad-spectrum antimicrobials group of tetracyclines. It is effective against both gram-positive and gram-negative microorganisms, including most spirochaet and many anaerobic and facultative bacteria. MINO occupies bacterial cells, respectively, from external passive diffusion, internally active transport, reaching the surfaces of ribosomes, and inhibiting protein synthesis. MINO has many topical forms and has been used in periodontal therapy (11).

The primary aim of this study was to evaluate the viability of DPSCs when in contact with MINO in comparison with Biodentine. The secondary aim is to investigate whether MINO has a proliferation effect on DPSCs. For this purpose, we designed the current study in 12 different groups and we used MTT, TOS and TAS method for 24 hours.

Materials and Methods

Cell Cultures

DPSC were obtained from department of medical pharmacology department of Ataturk University (Erzurum, Turkey). Briefly, the cells after centrifuged in 1200 rpm for 5 min were disseminated in 96 well plate by fresh medium (DMEM, with FBS 10% and antibiotic (Penicillin- Streptomycin, Amphotericin B) 1%) and store at incubator (5% CO2, 95% moisture 37°C.

Drug Administration

After gaining 85% confluency in 96-well plates the drugs were added. Biodentine (2-4-8-16 and 32 µg/ml) and MINO (2.5-5-10-20 and 40 µg/ml) were chosen to be added to the well plate and incubate for 24 hours (incubated in 5% CO2, 95%

moisture and 37 °C). For this aim, the experiments groups were divided into following 12 groups: Control group, positive control group, Biodentine (2-4-8-16 and 32 µg/ml) and MINO (2.5-5-10-20 and 40 µg/ml).

MTT Assay

The proliferation rate in the DPSC was determined by the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay using DPSCs seeded into 96-well plates at a density of 2 × 103 cells per well. The MTT assay was evaluated according to the manufacturer's instructions (Sigma, Munich, Germany). At established time points, the medium was replaced with a fresh one containing 10 µL MTT, and the cells were incubated for 4 h at 37 °C. Then 100 µL DMSO was added to the samples.The MTT reduction/attenuation values for each well were measured spectrophotometrically at 570 nm.

TAS Assay

TAS was investigated by using commercially available kits (Rel assay, Turkey) that were used according to the manufacturer's suggested procedure. The evaluation is made by calculating spectrophotometrically. Briefly, for evaluating TAS, 500 µl Reactive compound 1 was added to each well followed by the addition of 30 µl of specimen and initial absorbance was read at 660 nm (time 0). After the initial reading, 75 µl of Reactive 2 was added to the wells and allowed to incubate at room temperature for 10 minutes. After 10 minutes, a secondary absorbance value was obtained at 660 nm. While distilled water was used for Standard 1 (blank), Standard 2 in the kit was used as the second point for calibrating the relationship of absorbance intensity to pro-oxidants present. The absorbance values acquired were established according to the following formula and TAS standards were detected in Trolox Equiv/mmol L-1.

TOS Assay

TOS was investigated by using commercially available kits (Rel assay, Turkey). The test was done according to the manufacturer's procedure. The evaluation is made by calculating spectrophotometrically. The intensity of the color linked to the quantity of oxidants status. The ingredients in the TOS kits were Reactive 1, Reactive 2,

Standard 1, and Standard 2. In order to detect the TOS standard; 500 µl Reactive 1 was incorporated into the wells in which 75 µl plasma specimen was present and later reading the original absorbance value at 530 nm, 25 µl Reactive 2 was incorporated in the equal well and secondary absorbance was read at 530 nm at the end of the waiting duration of 10 minutes at room temperature. Standard 2 in the kit was used for measure Standard 2 value. TOS standards were detected in H2O2 Equiv/mmol L-1.

Statistically Analysis

The statistical analysis was done by one-way analysis of variance (ANOVA) and Tukey's LSD using the SPSS 22.0 software. $P<0.05$ was considered as statistically important distinction for whole tests.

Results

In this study, the cell viability and proliferation effect of MINO was evaluated compared to biodientine and MTT, TAS and TOS were performed after 24 hours of exposure.

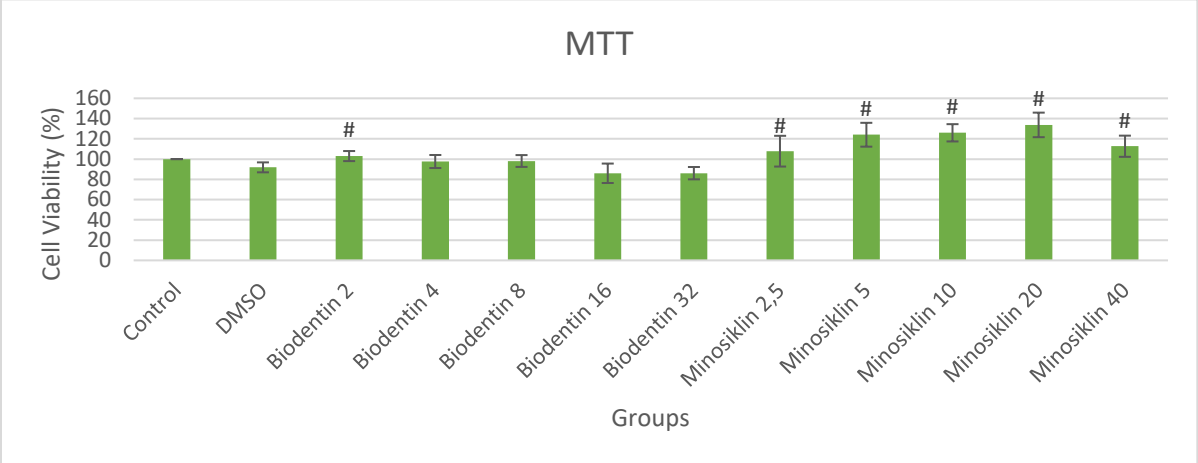

Figure 1.

Results from the MTT cell viability assay are shown in Fig.1. The vitality of the control group was defined as 100% and the other groups were rated accordingly. Biodentine groups showed significantly lower cell viability compared with MINO groups. Our results show MINO (150 µg/ml) group has the highest rate of viability in DPSCs in comparison to other treatments (%). In addition, all MINO groups showed a statistical difference in comparison to the control group *($p<0.05$)*.

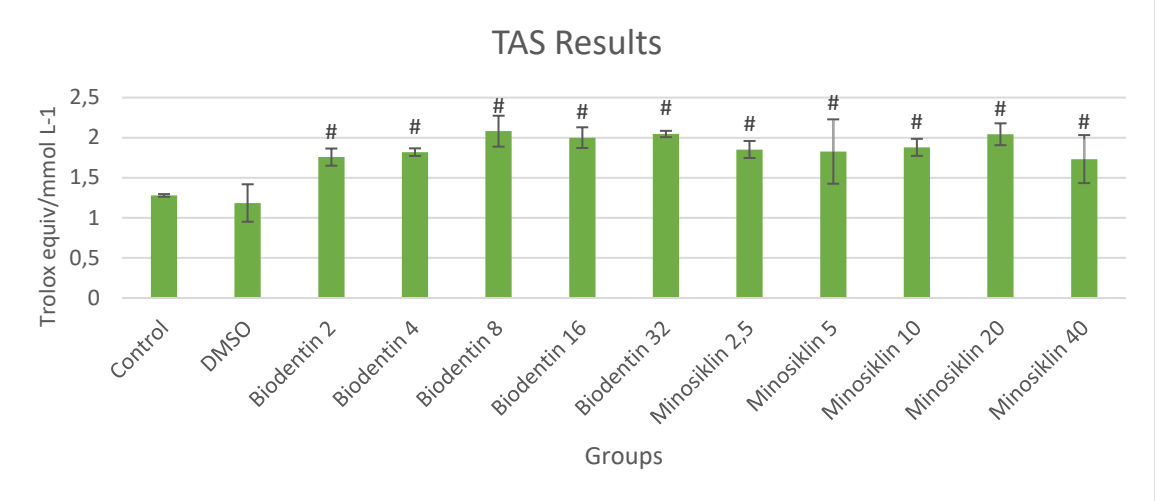

Figure 2.

We evaluated the TAS test based on Trolox equiv/mmol L-1 (Fig. 2). According to this test, the TAC level of the control group were 1.3 Trolox equiv/mmol L-1. Among groups, MINO (20 µg / ml) and Biodentin (8 µg / ml) show that they have the highest antioxidant capacity compared to control. In addition, it was found statistically significant in other groups compared to the control group *(p <0.05)*.

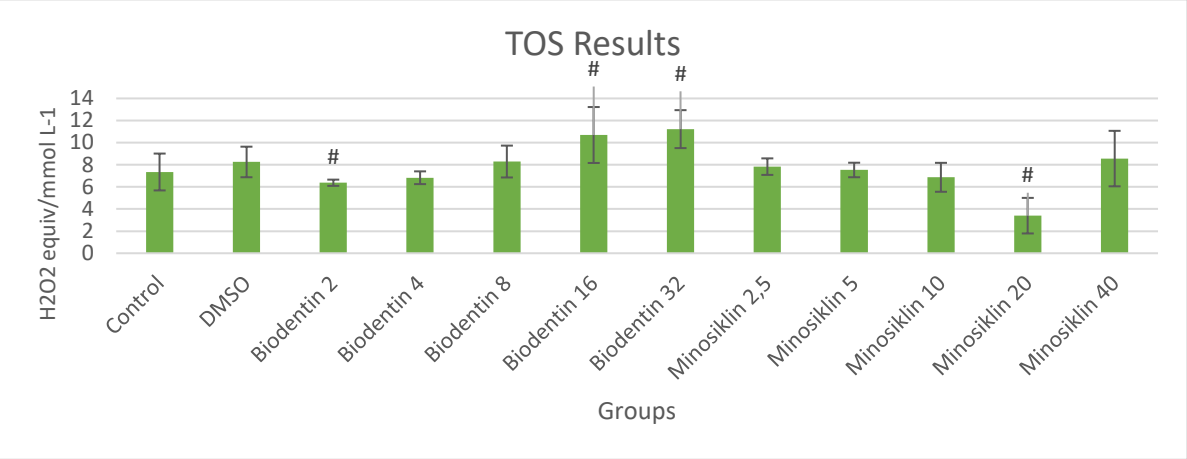

Figure 3.

We evaluated the TOS test based on H2O2 equiv/mmol L-1 (Fig. 3). According to this test, the TAC level of the control group were 7 H2O2 equiv/mmol L-1. Among the groups, the Biodentine (16 µg / ml) group shows that it has the highest oxidant level compared to the control. In addition, Biodentin (2-16 and 32 µg / ml) and MINO (20 µg / ml) groups were statistically significant compared to the control group *(p <0.05)*.

Discussion

DPSCs represent a specific population of stem cells for orthopedic and dentistry applications because of their easy extraction from the harvested dental pulp after routine surgical practice, the achievement of osteogenic differentiation using simple in-vitro protocols and an appreciable interaction with biomaterials (1). The current study was designed to investigate the effects of Biodentine and MINO on cell viability on DPSCs.

Youssef et al. Investigated the cytotoxic effect of pulp sealing materials on DPSCs. This study revealed that when the closure material Biodentine was not added to the cell growth medium, it showed variable cytotoxicity against DPSCs compared to control (12). Similarly, Bortoluzzi et al. who have found that cell viability was significantly affected in the presence of Biodentine (13). In another study, Luo et al. observed that the proliferation of DPSCs is increased with Biodentine at 2 mg / ml and 0.2 mg / ml and is reduced at 20 mg / ml, which suggests that the concentration of the capping material may affect cell viability (14).

Reynolds et al. suggested that the discoloring effect of MINO can be minimized by coating the dentinal tubules in the pulp chamber with a bonding agent (15). In addition, the review carried out by Kahler & Rossi-Fedele analyzed the tooth discoloration in 80 studies of regenerative endodontic procedures, including 379 treated teeth. The results demonstrated a strong association of discoloration with the use of triple antibiotic paste containing minocycline (16). Nevertheless, the clinical studies of Chan et al., without minocycline, revealed more than a half and 44% of the treated teeth were discolored, respectively (17).

In the present study, we evaluated the proliferative effects of Biodentine and MINO on DPSC via MTT assay. We have shown that MINO significantly increases the viability of cells compared to the biodient *($p < 0.05$)*. Also, this study aimed to evaluate the oxidative/antioxidative potential of Biodentine and MINO with TAS and TOS assays. Biodentine (8 µg / ml) and MINO (20 µg / ml) group caused significant increases in TAS level compared to the control value. On the other hand, the TOS level of Biodentine (32 µg/ml) treatment group caused a significant increase compared to the control value. According to our data, MINO significantly increased cell proliferation compared to biodentine.

Conclusions

As a result, we report that MINO increases the proliferation and viability of DPSCs. MINO was less cytotoxic compared to Biodentine at low concentrations. MINO promises to be an alternative biomaterial to biodentine.

This study is supported by Tubitak with Project number 218S666.

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SS-20

'In Silico' Mechanism Discovery for Sporadic Amyotrophic Lateral Sclerosis and Computer Aided Drug Design

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a progressive disease characterized by degeneration in the upper and lower motor neurons of the brain and spinal cord. About 10-15% patients with ALS have a familial background and 85-90% is sporadic. There are two major genetic variations discovered for inherited ALS, which are hexanucleotide repeat expansion in C9orf72 (40%) and SOD1 mutations (20%). The mutant SOD1 gains toxic functions for neurons. Mutant SOD1 proteins enter the membrane of mitochondria and bind the apoptotic B-cell lymphoma 2 (BCL2) which is an oncoprotein that plays a role in apoptosis.

Purpose: Neuroinflammatory processes play an important role in the diseases development and progression in sporadic cases. However, anti-inflammatory treatments are effective on protecting neurons. We are, then involved in the investigation of process of unknown mechanism of neuroinflammation in ALS.

Methodology: We discovered a new protein network with "In silico" analysis using computer aided methods. With the protein network we have discovered, it can transform the natural processes of microglia functions from neuroprotective to neurotoxic, resulting in the downstream process of p38 and JNK MAP kinases and apoptosis. Therefore, the key proteins in our proposed network are promising for new ALS therapies. Using computer aided methods we designed new blockers for binding site of signals proteins in our proposed mechanism that might lead through novel molecules, in which the development of progressive further clinical studies (phase I). Proteinlerin RCSB PDB

veritabanındaki 3D yapıları indirildi. Ligand bağlanma yerleri Chimera'da tespit edildi ve potansiyel blokerler Bioluminate Schrodinger'da tasarlandı

Result: Using a data set including 243 ALS patients and 718 controls we learned a model with Bayesian Structure Learning (BSL). We test the model with a test data and observed that we can classify ALS patients with an accuracy of 0.743. Three different proteins are detected higly expressed in ALS patients and also regulates the TLR2 signaling pathway.

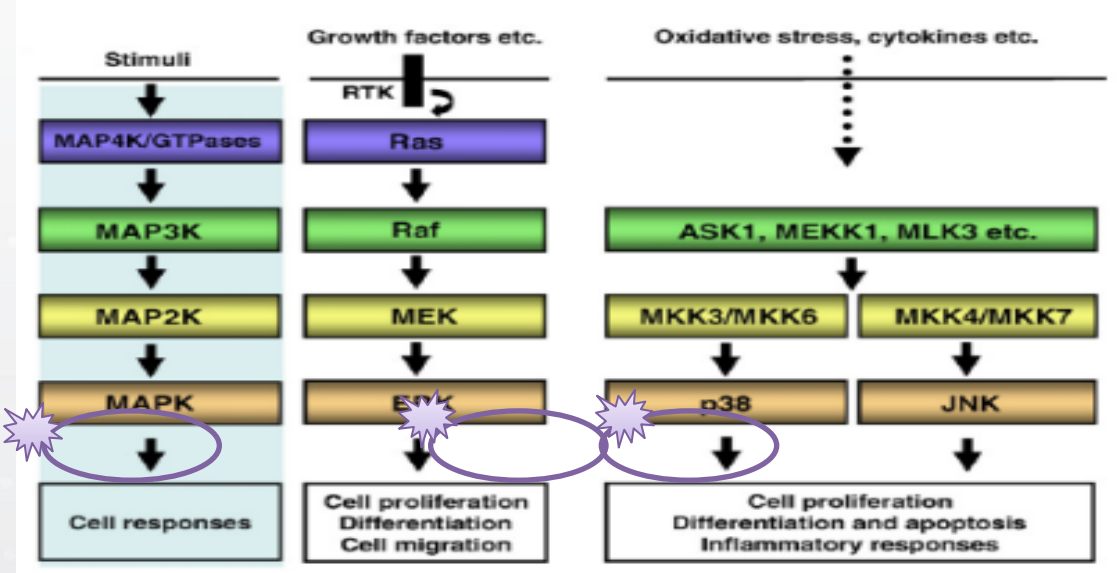

Figure 1: MAPK signal paths (Kim EK, Choi EJ. Biochimica et Biophysica Acta 2010)

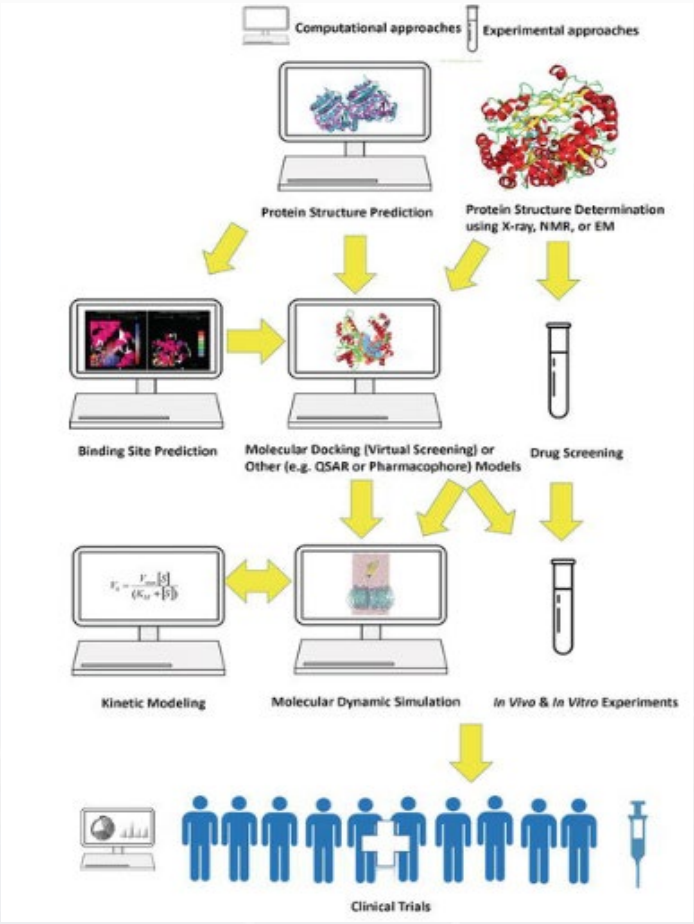

Figure 2: Computer aided methods for new blockers or drugs (By Mark Andrew Phillips, DOI: 10.5772/intechopen.72898, 2017)

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SS-21

Title:
Intra Cellular and Extra Cellular Effects of Nano Drug Delivery Systems

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Abstract:
Effects of Nano Drug Delivery Systems (NDDS) are directly related to the location that their uploaded drug molecule is released. According to the application route, NDDS are divided to systemic and local delivery systems. Systemic application of NDDS need to be ended up in the targeted organ, tissue or cell to successfully show their activity, however, locally administered NDDS are generally consisting of muco-adhesive particles that simply release the drugs in the application side. In both cases size, shape, charge and the chemical constituents of the NDDS define its fate in terms of cell internalization. The internalized nano particles will release their payload in the internal environment f cells, while the payload of the others will be released to the outer environment and will be introduced in the blood circulation or remain in the outer matrix. The latter case is rarely desired and generally causes toxic effects.

Keywords:
Nano Drug Delivery System, Targeted Cancer Therapy, Local Nano Medicine, Systemic Nano Medicine, Cell internalization, Size, Shape, Charge, Biocompatibel, Biodegradable

1. Introduction:

Nano Drug Delivery Systems (NDDS) are particularly new but very fast growing area of drug delivery systems. They are supra-molecular structures which are able to deliver therapeutic or imaging agents to the targeted tissue and in some cases they are just used to enhance the aqueous solubility of the active molecules. In the latter case the active ingredient is released to the systemic blood circulation. In both cases the release of payload in the cell's outer matrix or inner environment changes the

effects of the active ingredient. Accordingly, when the NDDS is degraded in the systemic circulation, or, targeting the desired tissue via blood veins is not easily possible, the NDDS is administered locally. Intraurethral administrations are good examples for this case. These kinds of NDDS are generally muco-adhesieve paerticles which provide release and accumulation of the drug molecules at the side of administration. However, NDDS which provide intravenous administration are generally used to target a special pathologic side in the living body such as cancer tumors. It is crucial for these particles to successfully internalize the cells of the targeted region and release the payload inside of the desired cells. Otherwise, the payload, generally being toxic, will be released to blood circulation, causing numerous side effects.

Cell internalization property of NDDS is affected by their size, shape, charge and the chemical constituents. In this presentation different nano particles are compared regarding their intra and extra cellular effects and these effects are discussed as consequences of their ability in the cell internalization.

2. Materials and Methods:

Natural molecules with strong fluorescence emission property were used as the template drug molecule in preparation of drug loaded NDDS. Three different NDDS were synthesized. Sterically Stabilized Micelles consisting 1,2- distearoyl-sn -glycero -3- phosphoethanolamine-N- [methoxy (polyethylene glycol) -2000] (SSM), micelles made of polylactide-co-glycolide (PLGA) and nano carriers consisting natural layered clay structures, namely Nano-Clay (NC) were used as NDDS in this study.

SSM was prepared by film preparation and rehydration method, while the active ingredient was included in the film strucrure. PLGA micelles and NC particles were prepared using precipitation method in both of which the active ingredient was in traduced to the system in the organic solvent.

Cell internalization was studied using fluorescence microscope or by conjugating the fluorescence molecules to the drug delivery systems and measuring the fluorescence emission of the internalized particles.

The extra cellular effects of the NDDS was followed as consequences of the biological effects of the payload at the extracellular environment such as interaction with the cell surface receptors.

The synthesized NDDS were studied both *in-vitro* and *in vivo* and the *in vivo* application route was oral and intravenous.

3. Results

SSM and PLGA nano micelles possess sizes around 15 and 150 nm respectively, while it is possible to tune the size of NC from 4 to 600 nm. Although the shape and the surface charge of particles are important factors, we demonstrated that, it is the size of NDDS which mainly defines the success of these vehicles in delivering drug molecules to their targets. Particles with sizes larger than 300 nm are neither able to be absorbed from the intestinal membrane nor can target cancer tumors via Enhanced Permeability and Retention (EPR) Effect. This is where NDDS with sizes less than 100 nm can successfully internalize to the cells, those with diameters below 40 nm can enter the cell nucleus, and those that are smaller than 35 nm can pass through the BBB. Surface modification of NDDS with macromolecules (peptides or poly-saccharides) enhances their cell internalization, only if their size is not changed.

4. Discussion

Although Nanotechnology has been defined as the science of engineering particles with sizes less than 100 nm, the progress made in this field, especially in the development of nano-drug delivery systems (NDDS) has widened this definition to cover the particles with larger sizes up to 500 nm. The biological system of a human body with natural defenses against pathogens acts as a barrier on the way of NDDS in a variety of application routes from local to systemic treatments. Intestinal absorption, efflux pumps, mononuclear phagocytic system (MPS), blood-brain barrier (BBB), the resistance of the cell membrane against nanoparticle internalization and many other barriers prevent the body from benefiting from the payload of NDDS. Our studies showed that the size less than 300 nm is curtail for successful delivery of active ingredients in oral iv administrations. Some studies have also shown that MPS is unable to remove nano-particles with sizes larger than 100 nm via phagocytosis, while, other studies mention that successful treatment of cancer by co-application of nano-chemotherapeutics with anti-angiogenetic drugs requires employing particles with sizes smaller than 50 nm. Thus, it could be concluded that the disease defines the size and the size affects the disease.

5. Conclusion

The sizes less than 300 nm is important in successful delivery of the active molecules using NDDS, while neutral or positive surface charge and bio-degradability and bio-compatibility of NDDS enhance the success of these vehicles in carrying the payload.

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SS-22

Title: Progesterone at High Doses Reduces the Growth of U87 and A172 Glioblastoma Cells: Proteomic Changes Regarding Metabolism and Immunity

Authors:

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Abstract:

Our group was the first to show that a progesterone analog, medroxyprogesterone acetate could block growth, enhance procarbazine chemosensitivity and induce cell kill and invasion blockage in C6 rat, U87 and U251 human GBM cells, respectively. In this study, we aimed to investigate the effects of the native hormone progesterone on the growth of human glioblastoma cells and to characterize the proteomic changes

associated with the progesterone-induced growth inhibition in glioblastoma. Progesterone significantly inhibited growth of human GBM cells in accompaniment with prominent changes in cellular metabolism, telomerase and innate immunity pathways.

Keywords:
Glioblastoma; progesterone; antineoplasticity; proteomics; metabolism; immunity

Main Text:

Introduction: While pregnancy may accelerate growth of glioblastoma multiforme (GBM), parity and progesterone (P4)-including treatments (i.e. hormone replacement therapy) reduce life-time risk of GBM. In parallel, low and high doses of P4 exert stimulating and inhibitory actions on GBM growth, respectively. Antineoplastic effects of high dose P4 on GBM is not illuminated. In this investigation, we evaluated the effects of the high dose P4 (100 and 300 µM) on the proliferation and proteomic changes in human U87 and A172 GBM cells.

Materials and Methods: The xCELLigence system was used to assess cell proliferation. Cellular proteins were separated by 2D gel electrophoresis and peptides were determined using Rapiflex MALDI-TOF/TOF mass spectrometry and searched against the UniProt human reference proteome database.

Results: P4 inhibited the growth of both GBM cells in a dose-dependent manner and decreased mitochondrial 60 kDa heat shock protein and T complex protein 1 subunit gamma, which involve in cell proliferation and telomere maintenance, respectively. P4 decreased mitochondrial ornithine aminotransferase, an enzyme with

protumorigenic functions. P4 significantly increased proteins involved in MHC Class-I peptide loading and IL-12 signaling.

Conclusions: P4 reduces the growth rate of GBM cells and as it is nontoxic in comparison to classical chemotherapy, it can be harnessed as a novel treatment in GBM. A logical strategy would be employing “P4-sensitizing” drugs which would further augment antineoplasticity of this hormone.

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SS-23

Evaluation of the effect of solid and cationic lipids on siRNA delivery systems

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Abstract

This study aims to develop nanoparticles with different types of solid and cationic lipids and evaluate them as siRNA delivery systems. For this purpose, we prepared a series of lipid nanoparticles by combining cationic lipids (DOTMA, stearylamine, DDAB, and Esterquat-1) with solid lipids [Precirol ATO 5 (P), Compritol 888 ATO (C), Cetyl palmitate 15 (CP15)] by a modified hot microemulsion method. The developed cationic solid lipid nanoparticles (cSLNs) were characterized in terms of particle size, size distribution, and zeta potential using a Malvern NanoZS Instrument. After determining the complexation ability of cSLNs with siRNA, the Resazurin assay was conducted to evaluate and compare their cytotoxicity. The cSLNs which successfully bind siRNA and show low cytotoxicity were further investigated by in vitro cellular uptake (fluorescence microscopy) and gene silencing (Western Blot) studies. According to characterization measurements, all formulations had a small particle size (< 200 nm), narrow size distribution (polydispersity index, PDI< 0.5), and high positive zeta potential (> +25 mV). The cSLN with Esterquat-1 was eliminated from further studies due to the poor complexation capacity. The use of cSLN with stearylamine was found inappropriate because of its high toxicity on both DU145 and PC-3 prostate cancer cells. None of these formulations showed effective gene silencing on DU145 cells. Only cSLN including P:C mixture with DDAB or DOTMA were uptaken by PC-3 cells and showed silencing efficiency. This study shows the importance of the lipid type selection for siRNA delivery systems and how this changes the transfection efficiency of these systems.

Keywords: Cationic solid lipid nanoparticles, cytotoxicity, gene delivery systems, RNA interference, siRNA, transfection efficiency.

Introduction

The development of drug delivery systems has special importance in order to deliver them effectively and safely to the patient. For gene delivery purposes; cost-efficient, easily producible and scalable non-viral delivery systems are considered as an alternative to viral vectors, which may have high immunogenicity and severe side effects. One of these non-viral systems is lipid-based carriers, which are generally preferred for the delivery of oligonucleotides such as siRNA because of their biocompatibility. Thus, many of them are being tested in clinical trials, and a lipid-based siRNA carrier called patisiran (ONPATTRO™, Alnylam Pharmaceuticals) has been approved by the FDA recently.

Among the lipid-based carrier systems, cationic solid lipid nanoparticles (cSLNs) have been used for gene silencing in recent years due to their advantages such as low toxicity, long stability, ease of permeating into cancer cells via their small sizes. Therefore, it is worthwhile to develop new cSLN carrier systems and evaluate their potential use for gene delivery purposes.

Materials and Methods

We prepared cSLN formulations with water, solid lipid(s), a cationic lipid, surfactants, and a co-surfactant by modifying the hot microemulsion method. The amount of each ingredient is kept the same for all formulations. However, the type of solid lipid (Precirol ATO 5, Compritol 888 ATO or Cetyl palmitate 15) or cationic lipid (DOTMA, stearylamine, DDAB, and Esterquat-1) is different between each other. After preparing the formulations, the characterization of cSLNs was performed using the NanoZS instrument (Malvern Panalytical) to measure their particle size, homogeneity, and net surface charge. The complexation capacity of cSLN formulations with siRNA was evaluated by agarose gel electrophoresis. The cytotoxicity of the selected cSLNs and their siRNA complexes was determined by Resazurin (Alamar Blue) Assay in both DU145 and PC-3 prostate cancer cells. Subsequently, the cellular uptake (fluorescence microscopy) and gene silencing efficiency at the protein level by immunoblotting (Western Blot) were determined in these prostate cancer cells.

Results

Six different cSLNs were successfully prepared by a modified hot microemulsion method. According to characterization measurements, all formulations had a small particle size (< 200 nm), narrow size distribution (polydispersity index, PDI< 0.5), and high positive zeta potential (> +25 mV) values.

The cSLN with Esterquat-1 was eliminated from further studies due to the poor complexation capacity with siRNA. The use of cSLN with stearylamine was found inappropriate because of its high toxicity on both DU145 and PC-3 prostate cancer cells. None of the developed cSLN formulations showed an effective gene silencing on DU145 cells. The cSLN including Compritol 888 ATO with DDAB did not transfect PC-3 cells and formed aggregate like structures according to its fluorescent microscopy images. Slight uptake of nanoparticles by PC-3 cells was observed by changing the solid lipid to Cetyl Palmitate 15. Only, cSLN including the mixture of Precirol ATO 5:Compritol 888 ATO solid lipids with DDAB or DOTMA cationic lipids were uptaken by PC-3 cells. As consistent with the results from the cellular uptake assay, they showed gene silencing activity at the protein level.

Discussion

In this study, using different cationic lipids or solid lipids did not remarkably change the physicochemical characterization of the developed cSLN formulations, i.e., the measurements of size, PDI, and zeta potential are all within the desired values. However, complexation capacity, cytotoxicity, the transfection efficiency of these cSLNs are significantly different.

Conclusions

This study shows the importance of the solid lipid and cationic lipid selection for siRNA delivery systems, and how it impacts the complexation capacity, cytotoxicity, transfection efficiency of these systems.

Acknowledgements

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SS-24

Production of Alginate Hydrogel Films Containing *Hypericum Perforatum* as Wound Dressing Material

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Abstract

The use of therapeutic herbals for the treatment of various wounds has been an important research topic for a long time. The incorporation of herbal extracts in wound dressing materials is an important concept that has been researched recently. In this study, sodium alginate films containing *Hypericum perforatum* extract were prepared by the solvent-casting methods and examined wound healing potential with cell viability and scratch assay, film swelling properties were studied also. Results show that *H. perforatum* incorporated films have better supporting cell viability and wound healing compared to films produced using alginate only.

Keywords: alginate, wound healing, *Hypericum perforatum*

1. Introduction

Wound dressings have been used for many years to protect the wound from various chemical and microbial contaminations. In addition to the protection function, a suitable wound dressing for wound healing is should be gas permeable, supply a moist environment in the wound, prevent infection and remove excess exudate and promote tissue regeneration [1]. Particularly biocompatible, biodegradable natural polymers are widely used for this purpose [2]. Alginate is a natural polymer derived from seaweed and is frequently used as wound dressing material since its biodegradability, biocompatibility and ability to form hydrogels. Alginate is commonly used biopolymer as wound dressings materials in the form of film, sponge, or hydrogel [3]. The design of wound dressings that can release bioactive components such as herbal agents, growth factors or drugs is one of the most remarkable new approaches recently [4, 5]. *Hypericum perforatum* also known as St John's Wort, a member of the Hypericaceae family, is a valuable plant with a wide variety of therapeutic effects such as anti-inflammatory, anti-microbial, anti-oxidant, promoting wound healing and pain relief [6]. No study has been found on the

incorporation of *H.perforatum* extract, known for its wound-healing effects, into the alginate film as a wound dressing and to examine its effectiveness on fibroblast cells. The aim of this study is to produce *H. perforatum* extract including alginate films as a wound dressing material and investigate wound healing potential in fibroblast cell culture by using scratch assay and viability assay.

2. Materials and Methods

2.1. Alginate Film Production

Alginate (Al) and *H. perforatum* extract (Immunat Herbal Medicine Inc.) incorporated alginate (Al/HPE) films were produced using solvent casting method [7]. Briefly, in the first step, alginate solutions (1% w/v) were casted into 6 well-plate and dried at room temperature for 24 h. After then, 3 ml of 5% calcium chloride solution, as a crosslinking agent, was added to the dried films for 2 hrs. After gel formation, the films were washed twice with distilled water and dried at room temperature for 24 hours. For Al/HPE films same protocol were followed, while alginate solutions (1% w/v), with 3 different concentration (0.25, 0.5, 1% v/v) of *H. perforatum* extract, were used in the first step of experiment.

2.2. Swelling test

For determination of swelling degree (SD%), films with 4 cm in diameter, were placed into 20 mL of phosphate-buffered saline (PBS) (pH 7.4) and incubated at 37 °C. At certain periods of time (10, 20, and 60 min), the swollen films were taken from PBS and excess water is removed from the surface of the films with filter paper. The swelling degree (SD%) of the films was calculated using the following formula:

$$SD\% = \left(\frac{Ww - Wd}{Wd}\right) x100$$

Wd and Ww are the dry and wet weight of the films, respectively. The results were given as mean SE ± standard error.

2.3. *In Vitro* Cell Viability Assay

In vitro effect of Al and Al/HPE films on L929 mouse fibroblasts cell viability was evaluated by the XTT assay according to the indirect extraction method of ISO 10993-5-2009 with some modifications. UV sterilized film samples were kept in DMEM containing 10% fetal bovine serum (FBS) and 100 mg/ml penicillin/streptomycin for 24 hours at 37º C for indirect extraction. Briefly, cells in DMEM (10% FBS and 100 mg/ml strep.) were seeded in 96 well-plate at the density of 1×10^4 cell/well and incubated at 37º C in an atmosphere of 5% CO_2 for 24 h. The cell culture medium were discarded and medium containing film extract at different dilution ratios (25:75, 50:50, 75:25 and 100:0) were added. At the end of the 24 hrs incubation, the medium was replaced with 0.1% (v/v) PMS and 0.04% (w /v) XTT

containing medium and incubated at 37º C for 4 hrs more. The optical density were assayed with multiplate reader at 450 nm. and the cell viability was calculated using the equation:

$$\%Viability = \left(\frac{Optical\ density\ (OD)\ of\ treated\ cells}{OD\ of\ control\ cells}\right)x100$$

Experiments were performed in triplicate and data was represented as mean ± standard error (SE).

2.4. *In Vitro* Wound Healing Test-Scratch Assay

L929 cells were seeded into 48-well plates and incubated at 37º C in an atmosphere of 5% CO_2 until 80-90% confluency. The well surface was slightly scratched in a line by a p100 µl pipette tip. The cells were washed with medium to remove unattached cells and then incubated with medium containing film extracts for 24 hrs. Scratches were followed with an inverted microscope with ×100 magnification at different time intervals (0., 4. and 24. hrs).

3. Results and Discussion

3.1. Film swelling properties

The swelling degrees of the films are shown in the Figure 1. The film samples presented a high swelling degree in PBS for a short period of time. As seen in Figure 1 film %SD values are 475.8, 536.6, 412.5 and 410 for 1% Al,1% Al/0.25%HPE, 1% Al/0.5% HP and 1% Al/1%HPE respectively in 10 min. When the swelling degrees of the films are examined it is thought that the films reach the swelling balance in about 60 minutes because the SD values in the 20. and 60. minutes are quite close to each other and the changes in the weight of the films are observed after the 90. minutes during the experiment. Another result, addition of 0.25% HPE to alginate films is not affect to SD% of films while 0.5 and 1 % HPE addition cause decrease SD% of films.

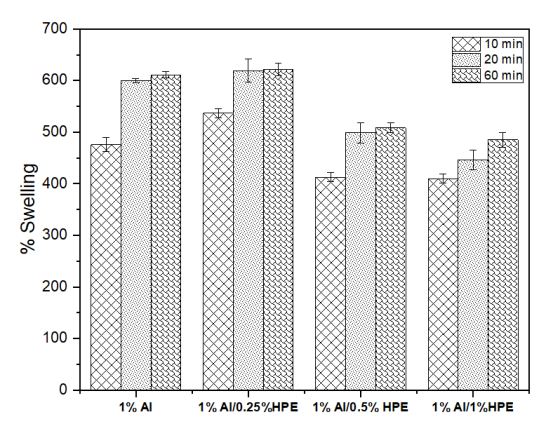

Figure 1. Swelling degree of Al and Al/HPE films.

3.2. Cell Viability

The effect of film samples on cell viability is shown by the XTT results in Figure 2. Compared to the control, it was observed to 1% Al / 1% HPE have a positive effect on cell viability all dilutions of the film. According to the ISO 10993-5-2009 standard, it was concluded that the films do not have a toxic effect on L929 cells since cell viability does not decrease below 80% in all dilutions (except 100/0 1% Al) of the films.

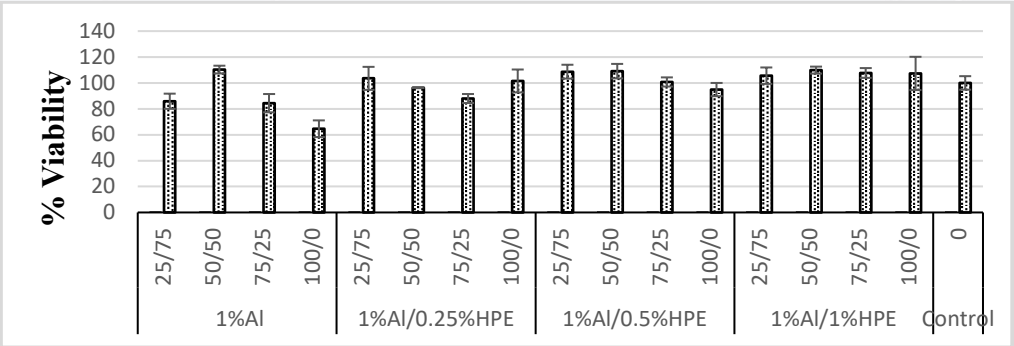

Figure 2. XTT cell viability assay.

3.3. Wound Healing Test-Scratch Assay

Scratch assay was used to evaluate the potential of the produced films as wound dressings material. Figure 3 shows that microscope images of scratches that treated with extracts of 1%Al, 1%Al/0.5% HPE and 1%Al/1% HPE film and control at 0., 4. and 24. hours. Compared with the control, although the scratch area of treated with 1%Al film has a number of decreased at the end of the 4. hrs, the closing of the scratch has not observed at the 24 hrs. In both cases, 1% Al/0.5%HPE and 1%Al/1%HPE were observed that scratch closure rate to be better than 1% Al film extract.

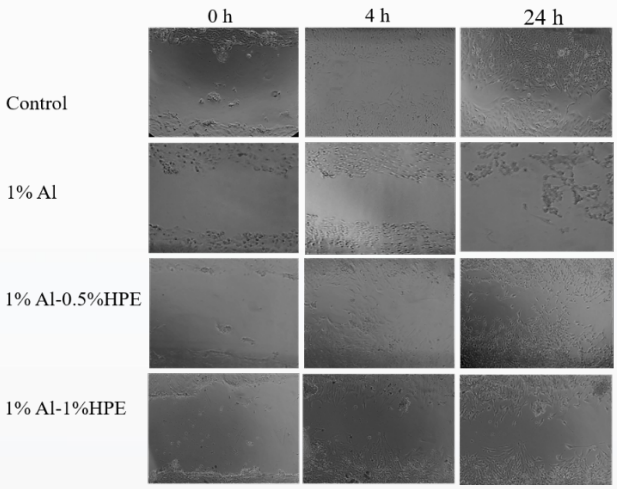

Figure 3. Wound Healing Test-Scratch Assay results.

4. Conclusions

Hydrogel films consisting of alginate and *H.perforatum* extract were generated for wound dressing applications. Produced alginate and alginate/*H.perforatum* extract hydrogel films had examined wound healing potential in fibroblast cell culture by using scratch assay and cell viability assay, swelling properties were studied also. Increasing *H.perforatum* extract content in alginate hydrogel films led to a decrease in swelling degree, on the other hand, it had caused positive effects on cell viability and wound healing thanks to the therapeutic properties of *H.perforatum* extract. These results suggest that alginate/*H.perforatum* extract hydrogel films have important potential as dressing material that supports wound healing.

Acknowledgement

The authors thanks to Immunat Herbal Medicine Inc.for providing *H.perforatum* herbal extract.

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SS-25

Investigation of the Effects of Allopregnanolon on DPSCs in New Dental Biomaterial Production

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Abstract: Dental pulp stem cells, which are popular for the repair of pulp regeneration, have an important place in recent studies in the development of new biomaterials. One of the most important situations when developing these materials is that they must not be cytotoxic. Allopregnanolone is a neuroprotective drug that occurs naturally in the human body. Based on this idea, we observed the proliferation in cells by exposing DPSC cells to allopregnanolone.DPSC cultures were obtained and drugs were added to the culture plates after obtaining a 85-90% confluency ratio. Control, positive control and Allopregnanolon concentration (5, 10, 20, 40 and 80 µM) were added to well plates and incubated for 24 hours. After 24 hours 3- (4,5-dimethylthiazole-2-yl) -2,5-diphenyltetrazolium bromide (MTT), Total Antioxidant Capacity (TAC) and Total Oxidant Status (TOS) analyzes were performed for DPSC cultures. Viability was defined as 100% in the control groups and other groups were proportioned accordingly. The positive control group showed 91% viability. The highest viability rate of 147% was observed in the Allopregnanolone groups. Our MTT, TAC and TOS Results Are İn Harmony With Each Other.

Keywords: DPSC, dental biomaterial, allopregnanolon,

Introduction: Dental pulp stem cells (DPSCs) can differentiate into dentin-producing cells that produce restorative dentine in pulp regeneration, and in addition, exhibit numerous potential in osteogenesis, neurogenesis, and angiogenesis regeneration. The use of DPSCs facilitates the development of biotechnology for dental tissue regeneration. Cells in carious or defective teeth should be used as a source of DPSC. It is not important that these teeth to be used are restored

with resin-based composites, glass ionomer cements or filling materials containing zinc oxide eugenol. The cytotoxicity of these materials to dental pulp cells has been well documented in both in vitro and in vivo studies. For this reason, scientists are directed to the search for new biomaterials. In addition to being non-cytotoxic, the material intended to be designed is also important for its proliferation ability. The allopregnanolone; is a naturally produced steroid in the human body and is a drug that affects the brain with a wide range of effects, including anxiolytic, stress-relieving, cognitive, memory impairment, neuroprotective and neurogenic effects. In this study, it was aimed to characterize the in vitro proliferation ability of DPSCs by using different doses of Allopregnanolone to obtain new dental biomaterial in this study.

Materials and Methods: DPSC cultures, was obtained from the Pharmacology section Ataturk University (Erzurum, Turkey). Briefly, cells replicated in a 25 cm^2 flask were treated with trypsin 0.25 EDTA, and then the cells were planted with fresh medium (DPSC medium, FBS 15% and antibiotic 1%) into a 96-well plate and stored in the incubator (5% CO2, 37 ° C). Their medium was renewed every 3 days until the desired cell density was reached. Subsequently, allopregnanolone dissolved in a small amount of DMSO and was used at 5, 10, 20, 40 and 80 µM doses. Seven experimental groups, morphological changes, MTT, TAC and TOS results were examined after 24 hours of treatment of DPSC cells. The results were analyzed by one way ANOVA method in SPSS, IBM 21.00 program.

Results: The dental pulp stem cell viability rate was measured by MTT test. According to our results, viability was defined as 100% in the control groups and other groups were proportioned accordingly (Figure 1). When the positive control group and the control group are compared, there is a difference between them. (** P <0.001) A significant difference was observed in the doses of Allopregnanolone compared to the positive control group in all groups. (* P <0.05) Also, Allopregnanolone did not show cytotoxic effects in DPSC cells and all doses significantly increased cell proliferation.

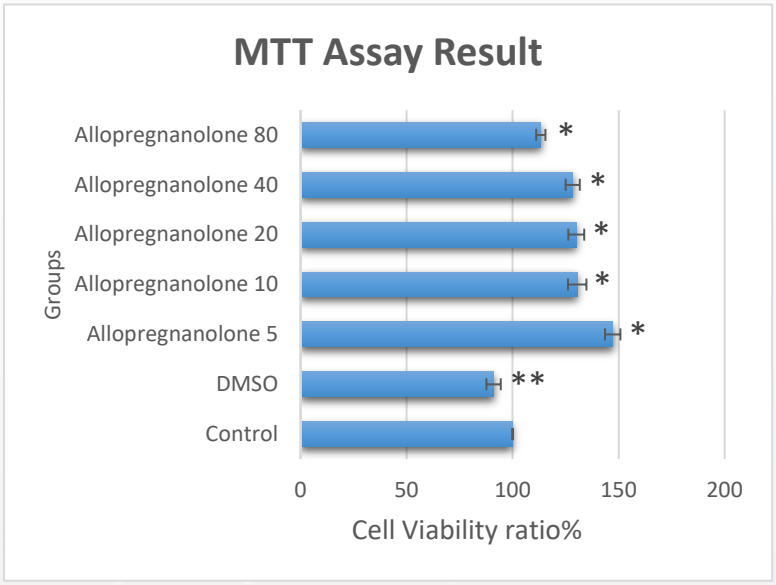

Figure 1. % Viability rates for DPSC - MTT test chart

Dental pulp stem cell TAC test results are shown in Figure 2 based on the Trolox equiv / mmol L^{-1}. According to the results of the test, antioxidant levels were found as 1.27 in the control group and 1.01 in the positive control group, and there was a significant difference between them. (** P <0.001) On the other hand, when the positive control group and Allopregnanolone groups were compared, a significant difference was found between all drug doses. (* P <0.05) The highest TAC value among the Allopregnanolone groups belongs to the 5µM concentration of 1.44 to. In addition, the MTT and TAC results of the Allopregnanolone and control groups are compatible with each other.

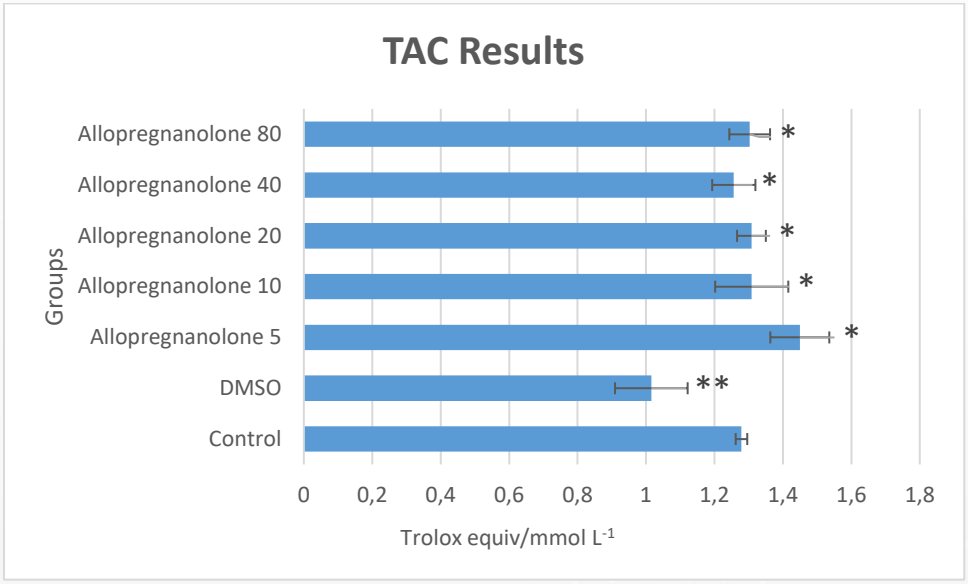

Figure 2. Total antioxidant capacity test values read spectrophotometrically at 660 nm in DPSC culture fluid.

The total oxidant test results obtained in the dental pulp stem cell line are shown in Figure 3 in the H_2O_2 equiv / mmol L^{-1} level. According to the test results, the control group and the positive control group have 7.28 and 8.74 oxidant levels, respectively. These two values are statistically significant (** P<0.001). Compared to the positive control group, all doses of Allopregnanolone were lower and this value was statistically significant (* P <0.05).

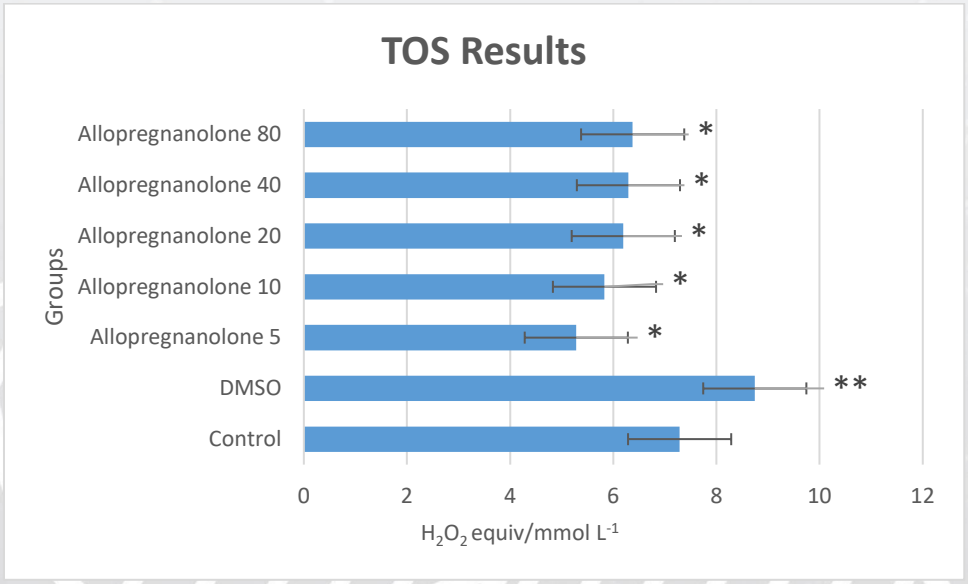

Figure 3. Total oxidant status test values read spectrophotometrically at 530 nm in DPSC culture fluid.

Discussion and Conclusions: Today, we see that biomaterials replace conventional dental materials. Although this change itself is exciting, we have not reached the desired level yet. With this study, we aimed to make a pioneering study to develop a new dental biomaterial and to use allopregnanolone in the structure of this biomaterial. With this first study examining the effects of allopregnanolone on DPSCs, we have realized the first phase of a new dental biomaterial production. Although our results show that allopregnanolone is promising for the production of dental biomaterials, animal studies should be done in the continuation of this study, if a combination of new substances and this drug is tried and found successful. This pioneering study has the potential to create its own clinical use with the planned studies.

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SS-26

Diagnostic Values of microRNAs in Sarcoidosis Using Principal Component Analysis Based Unsupervised Feature Extraction

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Abstract: Sarcoidosis is a complex disorder characterized by parenchymal lung involvement, intrathoracic lymphadenopathy, and extrapulmonary manifestations. Sarcoidosis can cause high mortality and morbidity. MicroRNAs act as post-transcriptional regulators of gene expression and have been studied in various diseases, mainly for their potential use in diagnosis. In this study, we aimed to find the diagnostic value of the principal component analysis (PCA)-based unsupervised feature extraction (FE) method in sarcoidosis. This method reduces the number of variables that accurately separate sarcoidosis and controls without losing biological feasibility. This technique was applied to GSE34608 microRNA expression profile, which was downloaded from the Gene Expression Omnibus (GEO) database that consists of peripheral blood samples of eight sarcoidosis patients and eight healthy controls. We detected a set of microRNA that could successfully discriminate sarcoidosis patients from healthy controls with 81.2% accuracy.

Keywords:
PCA-based unsupervised FE; ROC; sarcoidosis; microRNA

1. Introduction

Sarcoidosis is an inflammatory disorder that affects mostly lymph glands and lungs in the body. These patients have abnormal nodules or masses consisting of inflamed tissues form in specific organs of the body [1, 2]. In people with sarcoidosis have no symptoms; thus, the disease may be found only while a chest X-ray imaging is done for other reasons. This disease affects the lungs in nearly 90% of patients, but it can affect nearly all organs in the body. In spite of increasing advances in studies, the diagnosis of sarcoidosis remains difficult with limited treatment options. Thus, more innovative methods are required to overcome the diagnostic difficulties in sarcoidosis. In our study, we analyzed the blood-based microRNA expression profiles of sarcoidosis patients with the principal component analysis (PCA)-based

unsupervised feature extraction (FE) technique. We aimed to find the diagnostic value of this technique in sarcoidosis.

2. Materials and Methods

We searched the Gene Expression Omnibus (GEO) database by using the following keywords: "sarcoidosis", "Expression profiling by array" and "Homo sapiens". The inclusion criteria were: (1) peripheral blood samples of patients with sarcoidosis and healthy controls were compared, (2) adequate information to perform the analyses. The GSE34608-GPL7731_series_matrix.txt.gz microRNA expression profile was downloaded from the GEO database, which consists of eight sarcoidosis patients and eight healthy controls.

PCA is a mathematical data reduction method and the process of extracting relevant information from a large dataset. We employed the PCA-based unsupervised FE method to determine a set of microRNA that could discriminate sarcoidosis from control groups. In contrast to standard PCA, which integrates the samples, PCA-based unsupervised FE integrates the microRNAs that principal component (PC) loadings and PC scores were attributed to samples and genes, respectively [3]. It has been previously applied to gene expression data obtained from microarray experiments [4, 5].

Firstly, PCA was applied to the centered and scaled data matrix [16x13737] by "prcomp" R code to obtain PC loadings, and "lm" R code was used to calculate p-values for each PC loadings that $p<0.01$ was selected as significant. Then, based on the significant PC scores, "pchisq" R code was applied to calculate the p-values for microRNAs. p-values were adjusted by "p.adjust" R code with the Benjamini–Hochberg (BH) option [6], and significant microRNAs were selected ($p<0.0001$). Following "prcomp" R code was practiced on the expression profile matrix of the significant microRNAs to get the PC loadings and "lm" R code was used to calculate p-values. Finally, based on the PC loadings, the "lda" (Linear Discriminant Analysis) R code was applied to classify the samples into two categories. The cross-validation (CV) was achieved via “leave-one-out” (LOO) technique to prevent overfitting. The performance of PCA-based unsupervised FE method and LDA was validated with an unbiased manner LOO-CV methodology. The redeveloped PCA-LDA algorithm was then used to classify the entire dataset. This process was repeated iteratively until all data were classified [7]. Sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve (AUC) for the optimal cut-point were determined based on discriminant function scores [8]. All statistical analyses were performed by using the R-Studio Software package program [9]. The PCA-based unsupervised FE method workflow with the followed steps is shown in fig. 1.

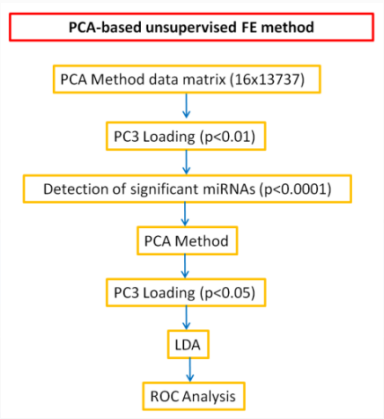

Figure 1. PCA-based unsupervised FE method workflow

3. Results

In our study, the GSE34608 microRNA expression profile was downloaded from the GEO database. Firstly, we performed PCA by applying the "prcomp" to reduce the number of predictor variables, without much loss of information which was achieved by first finding the direction having the largest variance (PC1: 96.8%), and after that finding following directions. Then by applying "lm" R code PC3 was selected with an adjusted p-value 0.0017. After that, based on these PC scores, we calculated the p-values of microRNAs by using the "pchisq" R code, and then we achieved 100 microRNAs with an adjusted p-value<0.0001 by “p.adjust” R code. Secondly, we performed PCA by applying the "prcomp" which was achieved by first finding the direction having the largest variance (PC1: 96.3%), and after that, finding the following directions. Then by applying "lm" R code to 100 microRNAs expression profile matrix, only PC3 was found significant among patients with sarcoidosis and healthy controls with an adjusted p-value 0.003 (Fig. 2).

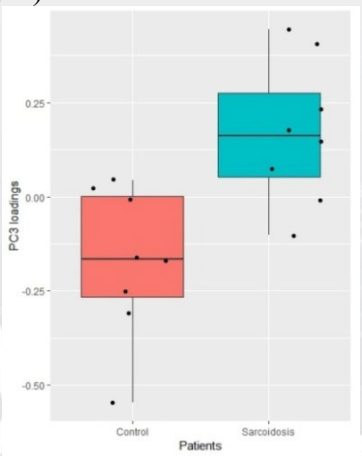

Figure 2. PC3 loading

Then 16 samples (patients and healthy controls) were discriminated into two classes using the "lda" R code. LDA can successfully discriminate healthy controls from patients with sarcoidosis. In a group of 8 healthy controls, one case was misclassified as sarcoidosis, and in a group of 8 sarcoidosis, two cases were misclassified as healthy control (Table 1).

Table 1. Distribution of the healthy controls and sarcoidosis cases

	Control	**Sarcoidosis**
Control	7	2
Sarcoidosis	1	6
Total	**8**	**8**

ROC analysis with a discriminant score produced AUC of 0.836 for discriminating sarcoidosis from healthy control samples with sensitivity, and specificity values 75% and 87.5%, respectively (Fig. 3), and the overall accuracy was found 0.812.

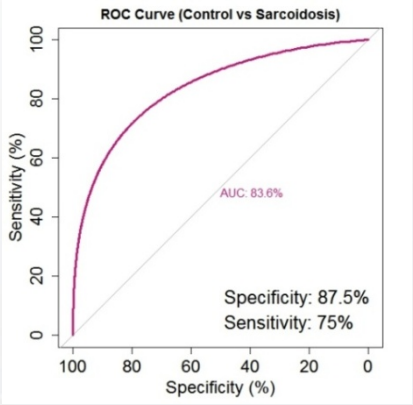

Figure 3. ROC curve comparing patients with sarcoidosis and healthy controls.

4. Discussion

In most sarcoidosis cases, making accurate identification requires invasive procedures [10]. It was suggested that the identification of sarcoidosis biomarkers could solve the problems in the diagnosis and prognosis of this disease [11]. Previous researches focused on angiotensin-converting enzyme to determine a circulating sarcoidosis biomarker; however, the current literature highlights its insufficient specificity and poor sensitivity [12]. Recently, microRNA has emerged as a promising methodology for prognosis and diagnosis in multiple respiratory diseases [13, 14]. Ascoli et al. showed a set of eight-microRNA signature that distinguishes sarcoidosis from controls with 74.8% accuracy [15]. In this study, we investigated whether circulating microRNAs can serve as diagnostic biomarkers for sarcoidosis via the PCA-based unsupervised FE method.

5. Conclusions

In conclusion, we aimed to find the diagnostic value of PCA-based unsupervised FE method in sarcoidosis. We found this technique could discriminate patients with sarcoidosis from healthy controls with 81.2% accuracy.

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SS-27

Emergent Bistability of SREBP Pathway Activation by Inhibition of Negative Feedback Regulation

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Medical Biology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

ABSTRACT

Metabolic control circuitry is complex and robust, resisting perturbations. Negative feedback regulation makes the response time rapid and robust (insensitive to) to variations in component concentrations from cell to cell in the same tissue. SREBP pathway regulates lipid metabolism in mammals and Drosophila. SREBP-1 and -2 are transcription factors regulating cholesterol and triglyceride metabolism with overlapping functions in mammals. Drosophila has one SREBP and it is involved in triglyceride and phospholipid metabolism. SREBP-GAL4 reporter was generated by replacing nuclear part with GAL4 so that upon activation, with UAS-GFP responder, it can be observed where it is activated normally and upon different interrogations in Drosophila. By knocking down specific genes with transgenic RNAi, SREBP activation status can be determined by the reporter. A few genes involved in lipid synthesis when disrupted by RNAi resulted in bistable SREBP pathway activity. In the same tissue some cells display overactivity of SREBP pathway, other cells display diminished pathway activity. Especially observed in positive autoregulatory genetic networks, two states are observed. By disrupting negative feedback regulation, bistability emerged in a multicellular organism. This result may have implications for the HMG-CoA reductase inhibition and its side effects in people. Related genes, results and implications will be discussed.

KEY WORDS: Systems Biology, SREBP Pathway, RNAi, Drosophila, Bistability, Robustness

Introduction

SREBP-1 and -2 proteins in the SREBP signal transduction pathway are transcription factors that regulate cholesterol and triglyceride metabolism in mammals, and there is a single SREBP homologue in Drosophila and regulates triglyceride metabolism (Dobrosotskaya, Seegmiller et al. 2002). SREBP proteins form complex on the endoplasmic reticulum membrane with SCAP protein and are kept inactive by proteins called Insig. Upon the activation signal (fatty acid, phospholipid, cholesterol deficiency), the SREBP-SCAP complex is separated from insigs and transferred to Golgi, where it is cut

from two places by two proteases (S1P and S2P). The N-terminal, which is released and is a transcription factor, goes to the nucleus and regulates the expression of genes that affect lipid metabolism (Brown and Goldstein 1997; Rawson 2003).

Material and Methods

Drosophila melanogaster is an excellent model organization for genetic screening for a long time (Rubin 1988). The transgenic SREBP-GAL4 driver developed in Drosophila by replacing the nuclear part with GAL4 shows the activation status of the SREBP pathway with the UAS-GFP responder (Kunte, Matthews et al. 2006). By crossing the flies with these two transgenes with different transgenic RNAi flies, the effect of other genes on the activation of this pathway can be discovered.

Results

Metabolic control circuitry is complex and robust, resisting perturbations. Negative feedback regulation makes the response time rapid and robust (insensitive to) to variations in component concentrations from cell to cell in the same tissue. A few genes involved in lipid synthesis and so end-product inhibition when disrupted by RNAi resulted in bistable SREBP pathway activity. In the same tissue some cells display overactivity of SREBP pathway, other cells display diminished pathway activity. Especially observed in positive autoregulatory genetic networks, two stable states are observed.

Conclusions and Discussion

By disrupting negative feedback regulation, bistability emerged in SREBP pathway in a multicellular organism. This result may have implications for the HMG-CoA reductase inhibition and its side effects in people.

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SS-28

Title: Novel immune and molecular strategies in treatment of brain tumors with special emphasis to glioblastoma

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Abstract:

Glioblastoma multiforme (GBM) has an extremely grave prognosis. Despite optimal surgery, radiotherapy and temozolomide chemotherapy, the average survival is around 15 months. The additive survival benefit of temozolomide is only around 3 months. When the patients are resistant to temozolomide, additional options include irinotecan chemotherapy and the anti-angiogenic agent bevacizumab. Unfortunately, the survival benefits of these agents are again limited to few months. Therefore, it is of essential importance to develop novel strategies in the fight against GBM. We performed a librarial analysis in electronic databases including NCBI and clinicaltrials.gov and collected data together to reveal new options in the treatment of this highly fatal neoplasia. We mainly focused on four

approaches including immunotherapy, molecular targeted therapies (smart drugs), combinatory repurposal strategies and locoregional treatment strategies including nano-technological and/or direct intratumoral applications.

Keywords:
Glioblastoma multiforme; immunotherapy; molecular therapy

Main Text:

Introduction: At first, we evaluated efficacy of the already applied chemotherapeutics on GBM. Then, we aimed to reveal novel treatment strategies in management of GBM including immunotherapy, combinatory repurposal strategies and locoregional antineoplastic agent delivery.

Materials and Methods: Electronic databases such as NCBI/PubMed and clinicaltrials.gov were screened with relevant keywords (i.e. immunotherapy, checkpoint inhibitor, repurposal, retargeting, combinatorial treatment, locoregional delivery) to gather data regarding novel treatments.

Results: Trial RTOG 04-20 determined the efficacy and toxicity of simultaneous temozolomide (TMZ) and radiation therapy (RT) followed by adjuvant TMZ combined with irinotecan given for 12 cycles compared with historical controls of adjuvant TMZ alone given for 6 cycles in GBM. Median overall survival for all eligible patients was 16.9 months in comparison to 13.7 months of the historical control (P = .03). Thus, even in a combinatorial protocol, the contribution of

irinotecan to survival is not higher than 3 months. Adding bevacizumab to temozolomide may add a further survival benefit, but this is also limited to a duration of 4.5 months (PMID: 32020475). A phase III clinical trial, Checkmate 143, revealed that nivolumab, which targets PD-1, did not provide survival benefits in comparison to bevacizumab in recurrent GBM patients. On the other hand, the Ivy Foundation Early Phase Clinical Trials Consortium's randomized, multi-institution clinical trial demonstrated that another PD-1 inhibitor pembrolizumab may extend overall survival. There exists limited immune cell infiltration in GBM and GBMs harbour only 30-50 non-synonymous mutations, which render them "immunologically cold". Exploitation of the full repertoire of cancer antigens-that is, both non-mutant antigens and neoepitopes-may provide more efficient immune therapies.

Hence, in the phase I trial GAPVAC-101 of the Glioma Actively Personalized Vaccine Consortium (GAPVAC), highly personalized vaccinations with both types of cancer antigens were integrated into standard care. For that purpose, ERC1671, an allogeneic/autologous therapeutic glioblastoma (GBM) vaccine is developed, which includes whole, inactivated tumor cells mixed with tumor cell lysates obtained from the patient and three GBM donors. The addition of ERC1671/GM-CSF/cyclophosphamide to bevacizumab resulted in a clinically meaningful survival benefit with minimal additional toxicity. Nimotuzumab, a monoclonal antibody against EGFR acted effective in EGFR-expressing gliomas especially when the tumors had higher levels of phosphorylated ribosomal protein S6. Some Phase-II studies showed efficacy of dendritic cell vaccines; in particular,

$IDH1^{WT}TERT^{MT}$ glioblastomas with low B7-H4 expression were more responsive to GSC dendritic cell vaccine-based specific active-immunotherapy. CAR-T cells targeting antigens such as EGFR vIII and IL-13Rα2 are tested in current clinical trials, similar to a clinical trial with HER2-targeting CAR-NK cell therapy.

Intratumoral delivery of the recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO) provided promising survival benefits in GBM. Vocimagene amiretrorepvec (Toca 511) is a gamma-retroviral replicating vector encoding cytosine deaminase that, when additionally with extended-release 5-fluorocytosine (Toca FC), results preclinically in local production of the anticancer agent 5-fluorouracil, which is directly tumoricidal and which can deplete immune-suppressive myeloid cells. Multiyear durable responses were encountered in some GBM patients treated with Toca 511 + Toca FC in a phase I trial, and the treatment will be further evaluated in a randomized phase III trial. Purpose DNX-2401 (Delta-24-RGD; tasadenoturev) is a tumor-selective, replication-competent oncolytic adenovirus which resulted in dramatic responses with long-term survival in recurrent GBMs that are likely due to direct oncolytic effects of the virus followed by triggering of an immune antiglioma response.

Entotherapy is an image-guided drug-eluting biopolymer microcylinder platform releasing temozolomide and it is tested in the treatment of canine brain tumours. Besides, all these novel strategies, repurposal of agents - which are not being used as anticancer agents - provided noteworthy results in pilot studies,

which include disulfiram and metformine and combinatorial strategies with these agents may be plausible.

Conclusions: Despite all the disappointing results, it is certain that a new era is rising regarding more efficient treatment of GBMs. Multimodal treatment strategies will likely provide the most efficient results. To obtain maximal survival benefits in intractable cancers such as GBMs, clinicians and translational basic scientists shall work together.

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SS-30

Fiber Optical Sensor Based Lab on a Chip Device Platform for Blood Coagulation Measurements

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Abstract:

Blood coagulation time measurement devices are routinely used in clinics. Patients have genetic diseases, heart implantations, by-pass operations are treated with vitamin-K antagonists as anticoagulation medicine and have to monitor blood Prothrombin Time (PT) and the international normalised ratio (INR) values frequently to dose the medicine and inhibit strokes or internal bleeding. Existing point-of-care devices use indirect electrochemical measurement methods and might be affected by other chemicals and medicines. Tarabios device performs direct optical measurement via two optical fibers pointing towards each other operating in a microfluidic channel. First preclinical trials were completed in Koc University Hospital with 50 patient blood. Then, plot study with a group of 160 patients were performed at Kosuyolu Hospital, Istanbul. Comparison of our PT/INR measurements with commercial devices, manual measurement and hospital laboratory results has shown that this direct working principle of our device provides higher quality results compared to current commercial devices and within limits of ISO17593:2001 standarts.

Keywords:
Blood Coagulation, Clinical Blood Researches, Lab-on-a-chip, Optical Biosensor, Point of Care Diagnostics, Prothrombin Time / International Normalized Ratio

1. Introduction

Monitoring blood clotting time has an important role in the follow-up and treatment of several cardiovascular diseases. Coagulation tests are routinely performed in clinics for patients undergoing surgical operations such as by-pass [1], taking anticoagulant treatments with Vitamin-K antagonists[2], having cardiac problems in order to dose their therapies and avoiding risks such as stroke [3].

Oral anticoagulant medications are widely used to prevent atrial fibrillation, pulmonary embolism, phlebothrombosis and other diseases in order to keep it between normal values in the blood clotting time. Abnormal blood clotting time may lead to thrombus formation or bleeding risk[4]. Anticoagulant medicines should be used to minimize these possibilities, and the dosage of these drugs should be adjusted according to the blood clotting test results.

Blood clotting time (PT/INR) measurements performed with hospital laboratory equipment, table top devices, point of care devices, self-care devices and manual slide methods. Measurements of hospital laboratory equipments are based on direct mechanical detection using plasma from centrifuged blood and require time for this process. Portable point-of-care (PoC) coagulation monitoring devices measures using shorter time and less volume of blood (10 to 20µl finger prick sample) and unlike laboratory devices, measurement is made with whole blood without plasma extraction. PoC coagulation measurement devices commonly use optical or electrochemical measurement principles. The working principle of most commercial devices is based on electrochemical measurement. As the blood clots, the electrical resistance of the blood changes and those electrochemical methods indicate the clotting time by this change. This is an indirect method and have limitations regarding to drug interactions or some blood parameters [5].

In this study, a portable device named Tarabios using direct optical method is developed to assess coagulation time with the aim of fast and accurate measurement.

2. Materials and Methods

2.1 Cartridge Unit

The optical reader system consists of electronics and cartridge components. Optical microfibers lay inside the microfluidic channel in the disposable cartridge shown in Fig1.a. Tips of two separate fibers pointing each other with a gap between them. Microfluidic channel conducts the dropped blood the where first fiber is immersed. While this nickel piece coated first fiber oscillates in the magnetic field, the second fiber picks the transmitted light. The gap between those fibers stays clear by the fluid stop region design of the cartridge shown in Fig 1.b. Thus, transmitted light has a clear path and lower noise [5].

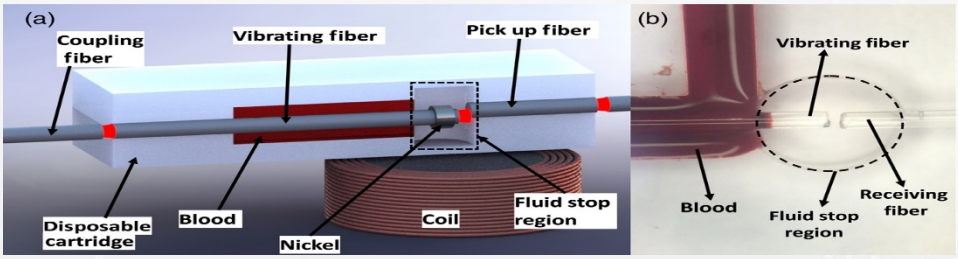

Fig. 1. (a) A cross sectional 3-D sketch of the cartridge with electrocoil. (b) Top view microscope picture of the fluid stop region with blood in the microfluidic channel.

2.2 Optical Measurement Principle

As clot formation occurs during blood coagulation viscoelasticity changes and causes differences in resonant frequency and vibration amplitude.[6] Resonant frequency is inversely proportional to the fluid viscosity. While blood coagulates, viscosity increases and resonant frequency decreases.[7] On the other hand, fibrin formation in the clot causes increase the elasticity. This change of the elasticity decreases the vibration and increase the resonance frequency.[8] In light of that information, the optical reading principle is based on the amplitude of light transmitted from the oscillating fiber to the pick-up fiber. Change of the amplitude in a constant frequency range belonging to the blood is detected.

2.3. Clinical Trials

Preclinical trials are completed in Koc University Hospital with a group consisting of 50 patients. Afterwards, pilot study conducted in Koşuyolu Hospital with a group of 160 patients who are different in age and having different therapies, medicines and health conditions. Samples are collected with a syringe and applied to reagent coated cartridges of Tarabios device, strips of the commercial devices and on manual measurement glass slides; and simultaneously transferred to hospital laboratory in a citrated tube. Obtained measurement results compared with hospital laboratory, manual measurement, and two other commercial point-of-care device results according to ISO17593:2007 standarts.

ISO 17593:2007

INR Range	Allowable Difference within 90% of all results
< 2	± 0.5 INR
2.0 – 4.5	± 30%
≥ 4.5	No criteria set

3. Results and Discussion

Clinical data are collected from 160 patients with different PT/INR levels ranging from 0.8 to 12. High INR measurement were of particular importance as it is more difficult to achieve accurate results in such cases. When the INR level higher than 4.5, electrochemical measurement based point of care devices measurement error increases and fails to measure over INR value of 8.

For each patient, we carried out measurements using 5 different methods: Tarabios method, laboratory equipment, manual measurement, two portable PoC commercial instruments. All measurements were bench marked against the laboratory equipment results. Commercial instruments employed an electrochemical measurement principle and TARABIOS cartridges employed a viscosity based direct measurement principle. TARABIOS measurements were within the margin of error for all 160 cases. 141 measurements were within ISO limits for commercial instruments 1 and 2. Commercial instruments failed in 19 cases (10 using antibiotics, 6 with low

hematocrit levels, 3 with both conditions) due to use of indirect electrochemical measurement method.

4. Conclusion

The results of the clinical studies proved that commercial devices using the indirect electrochemical method were adversely affected by conditions such as blood values or some medications, while our device using the direct optical method was able to measure under the same conditions.

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Trace Element Analysis in Formalin-Fixed Paraffin-Embedded Cancer and Healthy Thyroid Tissues by ICP-MS Method

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Abstract
Papillary Thyroid Cancer (PTC) is a type of thyroid cancer whose incidences have increased all over the world. Changes in biological element concentration have been observed in a number of pathological conditions. In this study, the aim is to determine trace element concentrations of the cancerous and healthy sample tissues of patients diagnosed with PTC using the ICP-MS method. Samples of Formalin-Fixed Paraffin-Embedded (FFPE) tissue were subjected to deparaffinization with xylene and rehydration with ethanol. Al, Cr, Mn, Fe, Hg, Co, Cu, Zn, Se, Ag, Sb and Pb levels in the thyroid tissues were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results were compared with the control group and it was found that Ag level in tissues with thyroid cancer was significantly lover ($p < 0.05$) compared to healthy tissues. These results suggest that trace elements play an important role in the etiology of thyroid cancer.
Key words: Thyroid cancer, trace elements, inductively coupled plasma - mass spectrometer, FFPE tissue

INTRODUCTION

Thyroid cancer is the most common endocrine system cancer with an annual number of 122,000 cases and constitutes 2% of all cancers and 95% of all endocrine system cancers. While the incidence of thyroid cancer is increasing day by day, advances in early diagnosis and treatment decrease the mortality rate (Bondeson, 2004; Cossu et al., 2013; Udul et al., 2018). Approximately 85% of thyroid carcinomas constitute papillary thyroid cancer (PTC) (Erol et al., 2010). It is three times more common in women than men in all age groups except for childhood are. (Malloy and Cunnane, 2008).

The World Health Organization has identified 15 different variants of papillary thyroid cancer. In recent years, it has been shown that only 50% of PTCs are classical variants and the remaining 50% are other histological variants (Topaloğlu et al., 2018). The most common type of other histological variants is the follicular variant and is similar to follicular cancer. The main distinction is made according to the differentiation in the core. (Adaş et al., 2012; Jameson and De Groot, 2010). Although Hurthle Cell or oxyphilic type reflects the findings of Hurthle cell cancer, its core structure is similar to papillary thyroid carcinoma. For this reason, Hurthle Cell Cancer is classified as a variant of both follicular and papillary type (Adaş et al., 2012; Tuttle et al., 2011).

Trace elements act as enzymes in biological systems or as catalysts in chemical reactions that take place inside cells. Therefore, inadequate or excessive intake of a large number of elements known to cause a large number of diseases, including various types of cancer (Aras and Ataman, 2007). Beryllium (Be), chromium (Cr), cobalt (Co), nickel (Ni), arsenic (As), cadmium (Cd), antimony (Sb), lead (Pb), silver (Ag) and platinum (Pt) to cancer are the main elements known to cause. The effects of manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se) and strontium (Sr) elements on cancer development have not been proven yet (Carvalho et al., 2007; Çelen et al., 2011). Most trace elements affect carcinogenicity by inducing ROS production and oxidative stress. (Gupte and Mumper, 2009) One of the most important mechanisms causing tissue damage is oxidative stress, which is defined as the overproduction of reactive oxygen species (ROS). ROS, which contains all metabolites of molecular oxygen, has been shown in many studies in vivo and in vitro, which can directly damage biomolecules in either reversible or irreversible damage (Başkol et al., 2007; McCord, 2000).

The correct diagnosis is very important for the treatment of cancer. In diagnoses made in some cancer cases, the difference of up to 40% between expert opinions reveals the difficulty in making cancer diagnoses correctly. This situation reveals the importance of studies that can help diagnose cancer. It has been reported in many studies that trace element level in biological materials may have an important place in cancer diagnosis and treatment (Schwartz, 1975).

In this study, we aimed to perform trace element analysis in healthy and cancerous FFPE tissue samples of 23 patients diagnosed with PTC using the ICP-MS method. Another aim was to statistically reveal the relationship between thyroid cancer and trace elements together with the three most common variants (classical, follicular and Hurthle cell).

MATERIAL AND METHOD

Chemicals

All chemicals used were preferred as suprapur. Ethanol (purity >99%) and Xylene (purity >99%) were obtained from Sigma-Aldrich (St. Louis, MO). Multiple standards containing 26 elements (100 ml in 5% HNO3), 100 mL Internal Standard (100 mL in 10% HNO3), Tune Solution (500 ml in 2% HNO3), Nitric acid (purity >65%) and hydrogen peroxide (purity >31%) were purchased from Merck (Darmstadt, Germany) and were used without further purification.

Sample Preparation Procedures for Analysis of Trace Elements in FFPE Tissues

In our study, tissue samples with tumor and control region FFPE belonging to patients with thyroid cancer were obtained from the archive of the Department of Medical Pathology, Faculty of Medicine, Atatürk University. Analysis of archive tissues embedded in paraffin belonging to patients diagnosed with thyroid cancer started with the removal of paraffin. FFPE archive blocks were cut in micROSon device and 4 µm thick slides were obtained. Samples, each containing 16 slides, were divided into 2 parts for more smooth deparaffinization and taken into different eppendorf tubes (2 mL). Samples were heated to 60 ° C and 1 mL of xylene was added and 1 min. It was mixed with vortex at 300 rpm. 5 minutes to precipitate the tissues and remove the xylene phase. It was centrifuged at 14000 rpm. After the xylene phase was removed with a pipette, the samples were dried in a centrifugal vacuum

concentrator (45 ° C-30min-water mode) to completely remove the solvent. The same procedure was repeated 3 times to completely remove the wax. For the rehydration of the samples, 1 mL of 100% ethanol was added, vortexed and centrifuged at 15000 rpm. The ethanol phase was discarded from the sample with a pipette and dried in a centrifugal vacuum concentrator (45 ° C / 10 min.-alcohol mode) to completely remove the ethanol. Then, the same process was repeated with 95% and 70% ethanol solutions, respectively. To complete the rehydration process, the same process was repeated with water and dried in a centrifugal vacuum concentrator (45 ° C / 30 min.-water mode).
For trace element analysis, the tissues whose deparaffinization processes were completed and weighed and placed in tubes with screw caps (15 ml). 400µL nitric acid and 100µL hydrogen peroxide (supra pure) solutions were added to them. It was burned in a microwave device at 120 degrees for 120 minutes to ensure that the tissues were completely in solution. After burning, 9.5 ml of deionized water was added to the samples and vortexed in order to complete the sample volumes to 10 ml in order to make the calculations more easily.

ICP-MS Analysis of Trace Elements

In order to test the performance of the device (Agilent 7700 a series ICP / MS) for analysis, first of all, using tune solution Y (Yttrium), Li (Lithium), Co (Cobalt), Tl (Thallium), Er (Erbium) tune setting is done. Standards were prepared with 2% nitric acid solution in increasing concentrations containing elements to be analyzed before measurement (Al, Cr, Mn, Fe, Hg, Co, Cu, Zn, Se, Ag, Sb, Pb) (0, 0.625, 1.25, 2.5, 5, 10, 20 ppb) and then these were introduced to the instrument ready for analysis. Calibration curves are plotted. After evaluating the standard curves, samples and control groups were introduced to the device and the device was commanded to be read. During the analysis, in order to correct the deviations in the calibration line, Indium, Scandium, Germanium, Bismuth elements representing the periodic table are given to the internal standard device. The samples were then read by the method (for the desired elements) and the results were obtained in ppb. Later, the obtained results were converted to ppm and statistical analyzes were done.

Operating conditions for the ICP-MS device

The system, at a radio frequency of 1550 W, at a flow rate of 4.3 mL/min, nickel sampler skimmer cone, 15 L/min argon (Ar) plasma gas flow rate and 1 L/min and 0.99 L/min, respectively. Auxiliary and carrier gas operated at flow rate. For the measurements, 20-fold diluted samples with an 8% sampling depth and 0.40mL / min sampling rate were treated with 1% HNO3 in 15 mL conical tubes.

Statistical analysis

For data analysis, the Software Package for Social Sciences (IBM SPSS Statistics), version 22.0 was used. Independent sample t-test and paired sample were used to compare trace element concentrations in thyroid tissues of cancer and non-cancerous patients. T-test was used to compare trace elements in the cancerous and non-cancerous tissues of each individual. PCA (Principal components analysis) was performed to understand whether there are multiple connections between trace elements. Statistically, $p < 0.05$ values were considered significant, and $p < 0.001$ values were considered very significant.

RESULTS

Standards were prepared with 2% nitric acid solution in increasing concentrations containing elements to be analyzed before measurement (Al, Cr, Mn, Fe, Hg, Co, Cu, Zn, Se, Ag, Sb, Pb) (0, 0.625, 1.25, 2.5, 5, 10, 20 ppb) and calibration curves were plotted. When the calibration curves were evaluated, it was observed that all R values were above 0,99. After evaluating the standard curves, samples and control groups were introduced to the device and the device was commanded to be read. During the analysis, in order to correct the deviations in the calibration line, Indium, Scandium, Germanium, Bismuth elements representing the periodic table are given to the internal standard device. The graphic of the international standards is given in Figure 1.

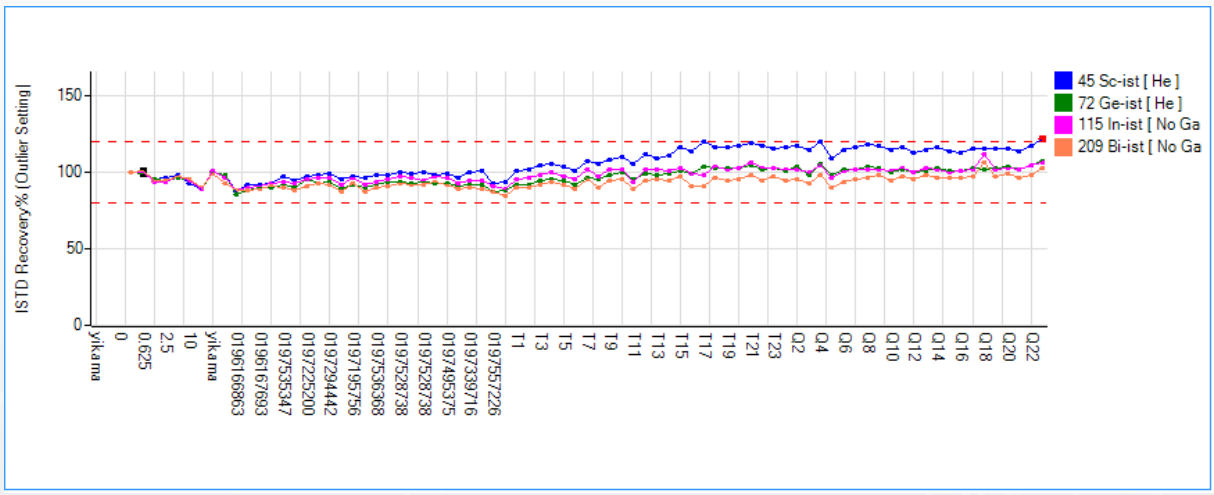

Figure 1. Graphic of internal standards

ICP-MS device is given by calculating the results of the analyzed tissues in ppb. However, the amount of tissue remaining after deparaffinization and rehydration of paraffin-embedded tissues is not the same. For this reason, the tissues that are removed from wax are weighed and recorded, and the values (ppb) obtained after analysis are calculated according to these weighing values and calculated as µg / gr tissue (ppm). The spectrum of a sample taken from ICP-MS device because of trace element analysis of a sample is given in Figure 2.

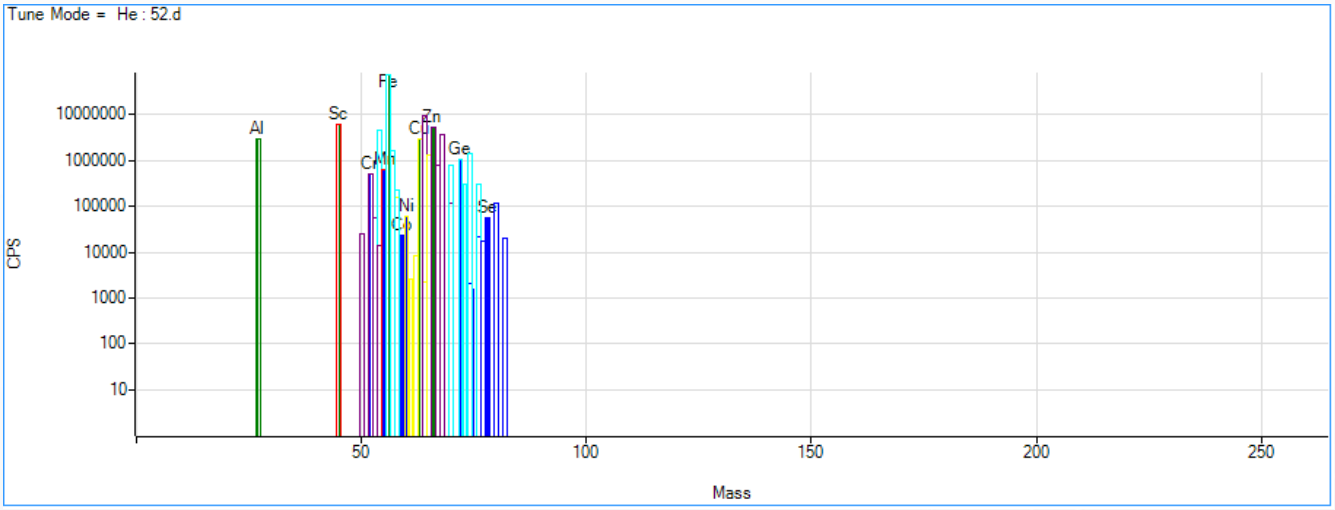

Figure 2. ICP-MS spectrum of a Sample

The results obtained as a result of trace element analysis made in cancerous and healthy tissues are given in Table 1. As a result of this statistical analysis, while the average values of Al, Cr, Co, Cu, Ag, Hg and Pb elements decreased in cancer tissues compared to healthy tissues, the values of Mn, Fe, Zn, Se and Sb elements increased compared to healthy tissues. However, it was determined that only the value of the Ag element expresses a statistically significant difference ($p < 0.05$).

Table 1. t Test Results of Trace Elements

Trace Elements	Cancerous Thyroid Tissues Mean± SD	Healthy Thyroid Tissues Mean ± SD	P values
Al	15.28±14.59	16.75±15.48	0.741
Cr	1.38 ±0.25	1.50 ±0.69	0.441
Mn	1.64±0.69	1.40±1.17	0.401
Fe	458.45±634.40	448.52±759.47	0.962
Co	0.10±0.07	0.10±0.11	0.946
Cu	99.99±152.10	109.87±130.61	0.814
Zn	121.69±79.77	97.82±41.97	0.211
Se	3.22±0.80	3.16±1.18	0.848
Ag	**1.29±0.81**	**2.15±1.68**	**0.033**
Sb	317.45±212.78	272.66±183.98	0.449
Hg	0.78±0.85	0.87±1.03	0.744
Pb	0.38±0.21	0.43±0.35	0.512

After examining the change of trace elements in cancerous and healthy tissues, the results obtained by performing statistical analysis in order to determine the relationship between the three most common variants of Papillary thyroid cancer in terms of trace elements in cancerous and healthy tissues are given in Table 2.

Table 2. Changes of Trace Elements in Cancer and Healthy Tissues According to Variants.

Trace Element	Variant	N	Cancerous Tissue Average±SD		Healthy Tissue Average±SD		P Value
Al	Follicular	20	16.06	±11.38	20.01	±19.59	0.588
	Classical	18	10.94	±5.23	11.80	±11.28	0.839
	H.Cell	8	23.08	±30.98	19.77	±12.12	0.849
Cr	Follicular	20	1.30	±0.20	1.65	±0.98	0.293
	Classical	18	1.43	±0.25	1.27	±0.25	0.215
	H.Cell	8	1.46	±0.34	1.63	±0.43	0.552
Mn	Follicular	20	1.75	±0.57	1.72	±1.58	0.952
	Classical	18	1.43	±0.56	0.95	±0.49	0.074
	H.Cell	8	1.81	±1.21	1.58	±0.99	0.777
Fe	Follicular	20	300.1	±160.9	308.6	±186.6	0.914
	Classical	18	361.5	±215.9	642.4	±1213	0.504
	H.Cell	8	1072.53	±1461.1	362.2	±91.61	0.369
Co	Follicular	20	0.10	±0.06	0.08	±0.03	0.352
	Classical	18	0.07	±0.02	0.07	±0.03	0.869
	H.Cell	8	0.18	±0.11	0.23	±0.25	0.710
Cu	Follicular	20	136.66	±219.64	125.9	±155.8	0.900
	Classical	18	48.60	±41.74	49.88	±37.9	0.946
	H.Cell	8	124	±84.31	205.0	±157.3	0.399
Zn	Follicular	20	160.29	±107.46	93.55	±38.85	0.081
	Classical	18	97.32	±30.95	86.27	±43.25	0.542
	H.Cell	**8**	**80.04**	**±20.13**	**134.5**	**±33.92**	**0.033**
Se	Follicular	20	3.13	±1.03	3.22	±1.49	0.884
	Classical	18	3.31	±0.58	2.73	±0.77	0.091
	H.Cell	8	3.23	±0.74	4.00	±0.69	0.182
Ag	Follicular	20	1.53	±0.89	2.06	±1.71	0.395
	Classical	**18**	**0.91**	**±0.40**	**1.55**	**±0.82**	**0.049**
	H.Cell	8	1.58	±1.14	3.75	±2.39	0.153
Sb	Follicular	20	293.45	±172.89	239.0	±176.1	0.494
	Classical	18	361.03	±269.91	243.4	±134.5	0.259
	H.Cell	8	279.40	±196.28	422.6	±265.8	0.419
Hg	Follicular	20	0.88	±0.74	1.00	±1.19	0.799
	Classical	18	0.66	±1.06	0.68	±0.98	0.971
	H.Cell	8	0.76	±0.76	0.95	±0.90	0.747
Pb	Follicular	20	0.30	±0.13	0.48	±0.41	0.203
	Classical	18	0.39	±0.23	0.30	±0.19	0.379
	H.Cell	8	0.52	±0.28	0.60	±0.46	0.774

When trace element concentrations of healthy and cancerous tissues of cancer patients with classical variants are analyzed, Cr, Mn, Zn, Se, Sb and Pb values increase in cancerous tissues and Al, Fe, Co, Cu, Ag and Hg values are higher in healthy tissues. However, it was determined that only the value of the Ag element expresses a statistically significant difference ($p < 0.05$). While the average value of the Ag element in healthy tissues was 1.55, it decreased to 0.91 in cancerous tissues and p value was calculated as 0.049. While the concentrations of Al, Cr, Fe, Se, Ag, Hg, and Pb in the cancerous tissues of patients with follicular variants decrease compared to their values in healthy tissues, Mn, Co, Cu, Zn and Sb values increase in cancerous tissues. However, these changes do not represent a statistically significant difference ($p < 0.05$). When trace element concentration changes in healthy and cancerous tissues of patients with Hurtle Cell variant were examined, Cr, Co, Cu, Zn, Se, Ag, Sb, Hg and Pb amounts decreased in cancerous tissues compared to healthy tissues. Al, Mn and Fe values are higher in cancerous tissues than healthy tissues. When the P values are examined, the only value that expresses a significant difference from these changes belongs to the element Zn with 0.033. While Zn Hurtle Cell variant has an average of 134.5 in healthy tissues of cancer patients, it decreased significantly to 80.04 in cancer tissues of these patients.

In the data analysis of the trace elements, the main components were analyzed to determine what percentage of the total variation can be explained and which components can explain most of the total variation, and the values found are given in Table 3.

Table 3. PCA (Principal Components Analysis) Results of Trace Element Analysis.

Component	Total	Variance %	Addition Variance %	Total	Variance %	Addition Variance %
1	4.968	41.398	41.398	4.968	41.398	41.398
2	1.397	11.645	53.043	1.397	11.645	53.043
3	1.313	10.938	63.981	1.313	10.938	63.981
4	0.982	8.186	72.167			
5	0.882	7.353	79.520			
6	0.809	6.739	86.259			
7	0.522	4.351	90.610			
8	0.369	3.078	93.687			
9	0.283	2.358	96.046			
10	0.259	2.158	98.203			
11	0.148	1.235	99.439			
12	0.067	0.561	100.000			

When Table 3. is analyzed, it was seen that the total variation in the data set was explained by the first three factors of 63.981%. 41.398% of the total variation in the data set is explained by the first factor, 11.645% of the second factor and 10.938% of the third factor. It is understood from the weight values that these three components are Al, Pb and Ag. The total variance explanation rate of the other 9 components was found to be 36.019%, which were not considered important.

DISCUSSION

Papillary thyroid cancer is the most common type of thyroid cancer with a rate of 80%. Papillary microcancer covers cancers 1 cm below the tumor diameter. It is seen in women of all age groups three times more than men, except in childhood. The incidence of papillary thyroid cancer is greatest in women of reproductive age (Adaş et al., 2012; Carr et al., 1998; Guerrero and Clark, 2011; Haselkorn et al., 2000; Liu et al., 2001; Malloy and Cunnane, 2008; Schlumberger, 1998).

Cancer continues to be regarded as one of the most important public health problems of today despite innovations in diagnosis and treatment. Various reasons such as the incidence of cancer increasing day by day and high mortality rate increase the importance of cancer today. One area that is widely used in cancer research is trace elements. Trace elements function in many important biological functions in the living organism. Concentrations of trace elements in the body are important. The concentrations of each of them in the body fluids and tissues and the amount that should be taken into the body daily are different. As a result of their insufficient or excessive intake, cellular functions are impaired and various diseases occur. Cancer is one of these diseases. Although trace elements have been investigated in many types of cancer, it has not been fully clarified whether they have cancer-causing or cancer-pROSective effects. Trace element levels differ in many types of cancer. Although beryllium, chromium, cobalt, nickel, arsenic, cadmium, antimony, lead, silver and platinum are known to cause cancer, the effects of manganese, iron, copper, zinc, selenium and strontium elements on cancer development have not been proven yet. (Araz, 2014). Most trace elements affect carcinogenicity by inducing ROS production and oxidative stress (Gupte and Mumper, 2009).

More than twenty chemical elements affect the normal physiology of the thyroid gland. The concentration of these elements in the thyroid gland is higher than in other tissues. (Zaichick et al., 1995). In a study on samples taken from different thyroid tissues; Co, Zn, Cu and Mo concentrations were higher in thyroid carcinoma tissues compared to other tissues, and Fe and Ni elements were high in non-neoplasm tissue (Salimi, 2004). Valademir Ye et al. examined the Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn levels of maling and bening thyroid tumors with instrumental neutron activation analysis; found that Ag, Co, Hg, I and Rb contents were higher in paranodular tissue than the standard for malignant and benign nodules and there was a slight deficiency in the nodules compared to the standard (Zaichick et al., 1995). Se measurements were performed with Total Reflection X-Ray Fluorescence method in tissues with various thyroid diseases and selenium levels in thyroid cancer tissue were lower than other thyroid disorders (Kucharzewski et al., 2002). In a study by H. Al-Sayer et al; It was observed that there was no significant change in zinc, copper, iron, magnesium, manganese and selenium between tumor tissue and juxta tumor thyroid tissue, but serum levels of both Zn and Cu increased significantly (compared to preoperative levels) after removal of cancerous thyroid tissue (Al-Sayer et al., 2004). In our study, it was observed that Ag concentration decreased significantly in cancerous tissues, while other elements did not show a significant change in terms of healthy and cancerous tissues.

As can be seen, the literature is full of contradictions in terms of results in trace element analysis with cancerous and healthy biological materials. Differences in the literature on increases or decreases in trace metal concentrations of cancerous tissues compared to non-cancerous tissues; It has been stated that there may be several reasons such as tissue based dry or wet weight, the basis of analysis methods affecting different sensitivities and accuracy, and difficulties in taking the sample representing the cancerous or non-cancerous area (Yaman, 2006). We used tissues with FFPE in order to reduce these negativities and to analyze many retrospective patients. The use of FFPE tissues was effective in

overcoming the difficulties in taking the sample representing tissue-based dry or wet weight and cancerous or non-cancerous area.

It has been suggested that another factor, which is shown as the reason for the differences in the literature, may result from analysis methods (Yaman, 2006). ICP-MS method was used to overcome this negativity. Because ICP-MS is a frequently used method for the analysis of trace elements. AAS, which is used for trace element analysis, has gradually outstripped other methods in terms of its advantages over ICP-OES systems. Detection limits provided by ICP-MS can not be provided by other methods and isotope analysis cannot be made. With the ICP-MS device, it can be worked on by providing linearity in a very wide range (ppt-ppm range). It provides a significant superiority over both methods in terms of analysis time (Caner, 2014).

CONCLUSION

As a result of our study, it was determined that the amount of Ag element in cancerous tissues decreased significantly compared to that of healthy tissues. In addition, Zn and Ag concentrations were significantly changed in healthy and cancerous tissues in terms of variants of PTC. Cancer tissues with Zn Hurtle Cell variant decreased significantly compared to healthy tissues, and Ag concentration decreased in cancer tissues with Classical variant compared to healthy tissues. PCA analysis was applied to the trace element analysis results, which lists the components whose components decrease gradually starting from the ones explaining the change of data the most and thus the most effective variables on the data can be visualized. In the last of this, we determined that 63.981% of the total variation in the data set was explained Al, Pb and Ag. These results will lead to studies on the diagnosis and treatment of cancer patients. In our study, we think that the relationship between trace elements and cancer will emerge more clearly by using FFPE tissues and preferring the ICP-MS method and will reduce the contradictions in the studies on the relationship of PTC with trace elements. We also believe that this study will shed light on research on cancer etiology.

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Biological Activities of PCL/Quercetin Biocomposites For Food Packaging Applications

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Abstract

The aim of this study was to prepare quercetin(Que) loaded poly(epsilon-caprolactone) (PCL) based biocomposites for food packaging applications. The biocomposites containing 1, 2.5 and 5% Que were synthesized by melting method. Firstly, the biocomposites (PCL/Que) were characterized with some physicochemical analyses such as FTIR-ATR, UV-Visible light transmittance and water contact angle measurements. Secondly, biological activities of the biocomposites were tested due to antibacterial and antioxidant activities by adherence and radical scavenging methods, respectively. The PCL/Que biocomposites were exhibited a good hydrophobicity, and set off barrier against UV light transmittance. Antibacterial activity of the biocomposites were determined to show a strong activity against *Staphylacoccus aureus* and *Escherichia coli*. The biocomposites also demonstrated high radical scavenging activity against DPPH and ABTS radicals.

Keywords: Poly(caprolactone); quercetin; biocomposite; food packaging.

1. Introduction

Food package is a product that is shelf life extender of foods, to ensure that they are stored under suitable conditions, and to transport by isolating them completely or partially from the external environment until they reach to the consumer. The package in contact with the food should have some features,which arelisted as follows; it should keep the product clean and prevent contaminants, decrease nutrient losses, protect the food during the transport. As long as it is kept on the shelf, the packaging material should protect the food product from physical and chemical hazards and should not be capable of reacting with food. It shouldsupport the product in the best way and be easy to apply[1], [2].PCL is a synthetic polymer which is biodegradable, biocompatible, stretchable and can be shaped into a film, especially preferred as a biomaterial, especially utilized in the food and pharmaceutical industry[3]. Quercetin is a plant flavonol from the flavonoid polyphenol group that is a strong natural antioxidant biomolecule. Flavonoids can be used directly to scavenge O_2^- and -OH by single electron transfer[4].Recently, interest in the use of biomolecules in the packaging industry has increased due to the unique properties such as antibacterial, antioxidant and barrier to UV ligtht.

This paper was aimed to design a smart cold chain food packaging material. By loading quercetin on PCL films, it was aimed both extending the shelf life of foods and increasing their resistance against the external environment conditions. Accordingly, FTIR-ATR, optical contact angle, UV-visible transmittance, antibacterial and antioxidant analyses were applied to determine their characteristic properties as a novel food packaging material.

2. Materials and Methods

All chemicals including PCL (MW: 80000) and quercetin were purchased from Sigma-Aldrich.

2.1 Biocomposites Preparation

PCL and PCL/Que biocomposites were synthesized in a film form using a twin-screw micro-compounder explore (DSM Xplore, Micro 5cc Twin Screw Compounder). The micro-compounder condition, the temperature of extruder was adjusted in the range of 100–110°C. After PCL/Que mixtures at certain ratios were loaded into micro-compounder, the mixtures were homogenized with stirring at 100 rpm for about 5 min. Then, PCL-based thin films were obtained.

2.2 Characterization

FTIR-ATR measurements for the biocomposites were performed with a Perkin Elmer Spectrum 100 in the transmittance mode and the wavelength range of 4000 to 650 cm^{-1}.

Optical contact angle measurements of the biocomposites were performed using the Attension Theta Lite instrument. The contact angle measurements were made at specific time intervals for a single drop of 10 recordings per second at a sensitivity of ± 1°. Measurements were made using pure water with a microsyringe with a stationary drop volume of approximately 10 µL.

The UV-vis spectra of PCL and its biocomposites in the wavelength ranges of 200–700 nm were measured using a UV–vis spectrophotometer (PerkinElmer Lamda 35).

2.3 Biological Activities

Antibacterial activity assays were performed using *E. coli*, a Gram-negative strain, and *S. aureus*, a Gram-positive strain. Stock cultures of *E. coli* (ATTC-8739) and *S. aureus* (ATCC-6538) were breaded on Muller Hinton Agar (MHA) medium to provide breeds. Bacteria prepared for inoculation were prepared as 10^5 CFU ml^{-1} in Muller Hinton Broth (MHB). The biocomposites were then incubated at 37°C for 3 h in MHB medium. After the incubation, The biocomposites were cleaned with distilled water, and were added in buffer solution. Finally, 10 µL of each PBS (pH7.4, phosphat buffer solution) were inoculated in MHA and

incubated at 37ºC for 18-24 h. The colonies were counted. Bacterial suspensions were used as positive controls[5].

The scavenging activity of DPPH (2,2-diphenyl-1-picryl hydrylase) of the biocomposites were measured by UV-visible spectrophotometer. For DPPH solution prepare, 0.024 g of DPPH was weighed and dissolved in 200 mL of methanol. 10 mg of the sample was added in 5mL DPPH. It was then stored in a dark place for 1 h. The antiradical (antioxidant) activity was then determined at 517 nm by UV-Visible spectrophotometer.

ABTS radical scavenging activity of the biocomposites were performed by the method of Re et al.[6]. $ABTS^{+}$ radical solution was prepared using equal volumes of 7 mM ABTS salt and 2.4 mM ammonium persulfate and standed in dark for 24h. The solution was then diluted with MeOH until an absorbance of 1.70±0.01 at 734 nm was obtained. This absorbance was recorded as control. For the sample analysis, 2990µL of the $ABTS^{+}$ solution and 10 mg of sample were added in a 3 mL cuvette. DPPH and ABTS radical scavenging activity (%) of the biocomposites were calculated with the following formula;
Antioxidant Activity (%) = [1- (absorbance of sample / absorbance of control)] x 100.

3. Result and Discussion

The FTIR spectra of quercetin, PCL and PCL/Que were shown in Figure 1. As the amount of quercetin increases, new peaks are formed at 3371 - 3257 cm^{-1}. This peak is due to the O-H stretching of the hydroxyl group in the quercetin. The 2900 - 2850 cm^{-1} peaks are the C-H stretching peaks in both PCL and quercetin. The strongest peak in the spectrum is C=O stretching at 1720 cm^{-1}. The peaks in the range of 1657 - 1600 cm^{-1} are the C = C stretching group that increases in proportional to the weight of quercetin and is due to the presence of quercetin. The C-O stretching found in all compounds is observed clearly as 1161 cm^{-1} peak.

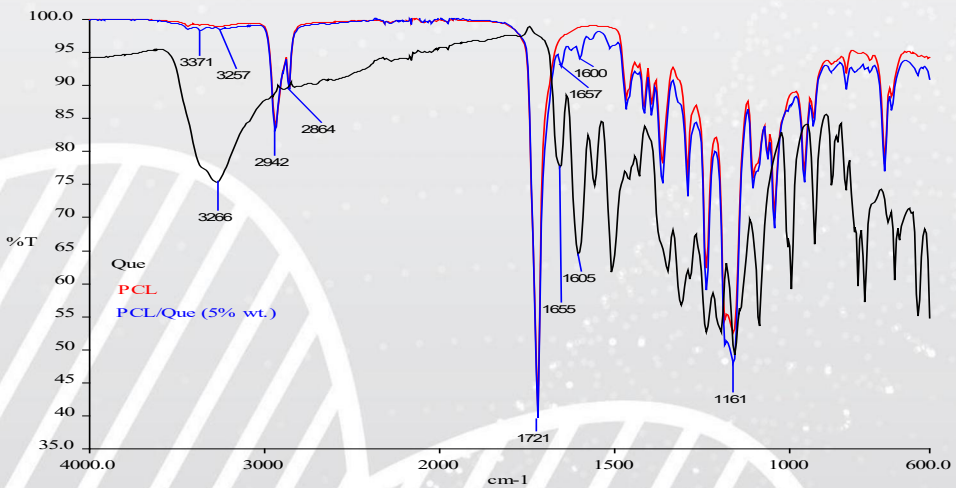

Figure1. FTIR spectra of quercetin, PCL and PCL/Que(5%wt.).

The optical properties of PCL and PCL/Que biocomposite films were evaluated using the light transmittance in the range of 200–700 nm by spectrophotometer. The transmittance of the films decreased with the increase of amount of Que. The Visible light transmittance of the films prepared with at 1% Que was determined to decrease as 10% ratio, however the films could block the UV light in the ratio of 50%. According to the results, the othersblockedUV light completely, but they have decreased only 10-15% of visible light transmittance. The spectra of films preprared at higher Que loading exhibited lower optical clarity, indicating that there was a strong scattering of Que resulting in lower transparency of the UV light. Consequently, it can be said that the biocomposites had lower optical transparency than that of PCL film.

Water contact angle results were given in Table 1. PCL based films were indicated to have a hydrophobic character. Also it was determined that the hydrophobicity of the PCL films was increased with the incorporation of quercetin. In the packaging industry, packages with high hyrophobicity is preferred. This feature increases as the amount of quercetin increases.

Table1. contact angle measures and antioxidant activity results of PCL and the biocomposites.

Samples	contact angle (º)	DPPH	ABTS
PCL	77.6±3,2	10,2±0,9	14,3±2,2
PCL/Quercetin (1% wt.)	77.8±1,8	31,2±2,1	35,4±3,5
PCL/Quercetin (2.5% wt.)	82.1±0,6	63,2±1,2	76,4±2,5
PCL/Quercetin (5% wt.)	86.9±0,8	92,0±0,1	97,6±1,6

Even if the biomaterials do not have any bacterocyte activity, they should prevent to hold on to the surface them. The purpose of the adherence test was to examine whether the bacteria hold on to the surfaces of PCL and biocomposites. According to the results given in Figure 3, PCL and biocomposites prevented both of the bacteria from adhere to their surfaces. Two bacteria species were used in the study due to their difference of compatibility with the environment. Gram positive *S. aureus* hold on less to the surfaces of PCL and biocomposites, while gram negative *E. coli* adhered slightlymore. In general terms, it has been found that both species of bacteria were quite difficult to adhere on the surfaces of PCL and biocomposites.

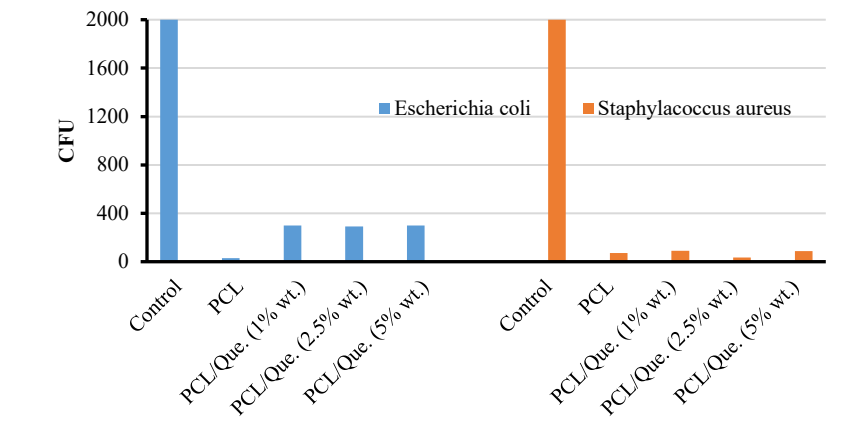

Figure2.Result of adherent colony number of *E. coli* and *S. aureus* on the PCL and PCL based biocomposites

Antioxidant activities of PCL/Que biocomposites, synthesized as antioxidant packaging materials were analysed by the reduction or neutralisation of stable free radicals of DPPH and ABTS due to the antioxidant efficacy of Que[4]. In this study, biodegradable materials based on PCL incorporated with 5 wt% of Que showed antioxidant activity against both DPPH and ABTS 90% over. The results showed that antioxidant activity increased depending on the amount of increased Que (Table 1). These results were comparable with the previous works [7]. It has seen that quercetin was not degraded after the synthesis of the biocomposites and remained its stability since the biological activity of quercetin in the biocomposites was high.

4. Conclusion

In this paper, PCL/Que (5% wt.) showed the best results as a food packaging material due to the highest hydrophobicity and strongest barrier to UV ligth. Moreover, it shows that both 97% antioxidant activity and resistance against bacterial species are important biological activities in terms of applicability.

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Quantification of Chlorogenic Acid in *Galium verum* subsp. *verum* (Yanıkotu) Extract Using HPLC and Investigate to It's Proliferative Effect on Human Dermal Fibroblast Cell Line

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ABSTRACT

Galium verum subsp. *verum* (GV) (Rubiaceae; Yanıkotu) is an herb that generally used in traditional medicine, and rich in flavonoids. In this study, it is aimed to determine chlorogenic acid (CA) in aerial part of GV and investigate it's the effect on proliferative effect on cell viability by using human dermal fibroblast (HDF) cells. For this purpose, quantitative determination of CA in methanol extract of GV collected from Erzurum Köşk province was performed by a The High Performance Liquid Chromatography (HPLC) method. The chromatographic separation was achieved by using acetonitrile and water containing 0.4 % orthophosphoric acid (95:5 h/h) on C18 column at 330 nm. The relative quantity of CA in methanol extract of GV was determined as 8.405±2.145 µg/mg extract. It is known that CA benefit in wound healing because it has anti-bacterial, antiviral, antifungal, antioxidant, anti-inflammatory properties. So, the proliferative effects of GV extract containing CA was evaluated on HDF cells by using MTT assay. MTT assay showed that a dose range between 1µg/mL-1 ng/mL of methanol extract of aerial part of GV have a very significant positive effects on HDF cell proliferation. However, more detailed studies to investigate this effect of GV should be done in *in vitro* and *in vivo* animal models.

Keywords:
Galium verum subsp. *verum*; Chlorogenic Acid, Proliferative Effect

INTRODUCTION

Galium verum subsp. *verum* (Rubiaceae family, Lady's Bedstraw, Yellow Bedstraw, Yoğurt otu, Yanıkotu) [1, 2] is widely used in traditional medicine for pathological conditions, diuretic, choleretic, against diarrhea, some stomach complaints, gout, epilepsy [3, 4] and to treat burn wound healing [1, 5]. In clinical studies GV is used for treating thromboembolic disease with anticoagulants because of flavones (Diosmetin) [6, 7]. Diosmetin has also anticancer activity [8]. And also GV compared to vitamin C has been reported to show very high antihaemolytic activity [9]. In an *in vitro* study has been shown GV has very strong scavenger activity reducing the DPPH ($IC_{(50)}$=3.10), OH radical formation ($IC_{(50)}$=0.05), neutralising H_2O_2 ($IC_{(50)}$=4.98 A µg/ml), inhibition of LP ($IC_{(50)}$=11.69 A µg /ml), phenolics (2.44-4.65 mg, flavonoids quercetin equivalents/g dry extract) [10] [3] [11]. The main active components of GV are flavonoids [6], iridoids, flavonol glycosides, tannins, enzymes [2], vitamins, chlorogenic acid, salicylic acid [7], antraquinones [4]. Chlorogenic acid [1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3- (3,4-dihydroxycinnamate); $C_{16}H_{18}O_9$] has anti-bacterial,

antiviral, antifungal, antioxidant, anti-inflammatory, antidiabetic and anti-carcinogenic properties [12-14]. In an *in vivo* study evaluating the effectiveness of CA on wound healing, it has been reported that CA has a high wound healing capacity due to its strong antioxidant capacity and accelerates the wound healing process by increasing collagen synthesis in different phases of wound healing [15]. In recent years, herbal products have become the focus of researchers for both cosmetic and therapeutic purposes. There is no study in the literature examining the effect of GV on wound healing. Therefore, in this study, it was aimed to determine of CA amount and investigate the proliferative effect of GV collected from Erzurum province on cell viability using HDF cells.

MATERIALS AND METHODS

Reagents and Solutions: CA were purchased from Sigma (USA). HPLC grade methanol and acetonitrile were supplied by Merck (Germany) while analytical grade orthophosphoric acid was purchased from Sigma-Aldrich (USA).

Plant Materials: The *G. verum* subsp. *verum* (Figure 1) were collected in July 2018 from Erzurum Söğütyanı Village and diagnosed by Leyla GÜVEN. The location of the plant is 40º 6’ 13” North 41º 24’ 32” East-1890 m.

Figure 1. The appearance of the GV plant in nature (Illustrated by Dr. Lecturer Leyla Güven).

Plant Extraction Procedure: The air-dried and powdered (50 g) the aerial part of GV plant was extracted with methanol (500 mL × 3) at room temperature. The methanolic extract was concentrated and dried under reduced pressure to give a residue. For HPLC analysis, 1 mg from obtained extract was weighed, dissolved with 10 mL methanol and sonicated for 30 min. The solution (0.1 mg/mL) was filtered through a 0.25 mm membrane filter and injected into

HPLC Analysis of CA: An Agilent 1200 Series HPLC instrument was equipped with a G1311A/Quat Pump and G1314B/VWD detector was used. The chromatographic separation was achived on an BRISA LC2 C18 column (250 × 4.6 mm, 5-µm particle size). Before starting the HPLC analysis, working solutions (WC; 0.5-100 µg/mL) and quality control (QC; 1, 20 and 80 µg/mL) solutions for CA were prepared in acetonitrile and stored at 4°C until analysis. The isocratic elution with a mobile phase consisting of acetonitrile and water containing 0.4 % orthophosphoric acid (95:5 h/h) was used for HPLC analysis of CA. The detection wavelength was set at 330 nm. Chromatographic separation has been done at 5 min run time by using 1 mL/min flow rate and 10 µL injection volume and 25 °C column temperature.

Cell Culture Conditions: Human dermal fibroblast cell line (Adult (HDF); ATCC® PCS-201-012™) were grown in DMEM supplemented with 20 IU/mL of penicillin, 20 µg/mL of streptomycin, 2 mM of L-glutamine, and 10% (v/v) FBS and the cells were incubated at 37ºC, 5% CO_2.

MTT Analysis: HDF cells were seeded at a density of $1x10^4$ cells/well in 96-well plates and incubated for 24 hours at 37 °C and 5% CO_2. After the incubation, the cells were treated with the cell culture medium: Control (C; untreated cell), 0.5% DMSO (DMSO control; DC), The methanol extract of GV (in concentration range of 0.1 ng/mL-1 mg/mL) in the cell culture medium. The methanol extract of GV was dissolved in 0.5% DMSO. Then, the plates were incubated at 37 °C and 5% CO_2 for 24 h. After 24 h, 10 mg/mL MTT solution was added to each well and incubated for 4 h in incubator. After incubation, 100 µL of DMSO solution was added to each well and the plates were shaken gently. Later, the absorbance (A) was determined at 570 nm and the cell viability (%) was calculated as follows:

$$\text{Cell viability } \% = [\text{ A of sample (at 570 nm)/A of control (at 570 nm)}]x100$$

RESULTS

Quantification of CA in GV Plant by HPLC: HPLC analysis, which is powerful quantification method, was used in this study to determine the CA level in methanol extract of aerial part of GV. CA showed the maximum peak at 330 nm and the retention time was 2,7 min according to the obtained chromatograms. The Calibration curve was obtained by plotting between peak areas and CA concentrations. The equations for calibration curves for CA were obtained as PA = 32.176x + 10.654 (R^2 = 0.9993; n:6) in 0.5-100 µg/mL concentration range for determination of CA. The method precision and accuracy were determined by analyzing the QC samples (1, 20 and 80 µg /mL) the intra-day (6 times per day) and inter-day (6 times once daily for 6 days). The precision of method was given the RSD % ((standard deviation/mean) x 100) and found as less than 1.15 %. The accuracy of method was given the RE [(found concentration-known concentration)/known concentrationx100] and found as less than ±2.13. With acceptable these values, the method is accurate and precise for determination of CA. The limit of quantification (LOQ) was calculated as 10xσ(standard deviation of *y*-intercepts)/ S (slope of the calibration curve) and found as 0.09 µg/mL, respectively. The developed and validated HPLC method was applied to quantify CA in methanol extract of aerial part of GV plant. The obtained chromatograms from samples were given the **Figure 2**. The relative quantity of CA in methanol extract of GV plant was determined as 8.405±2.145 µg/mg extract.

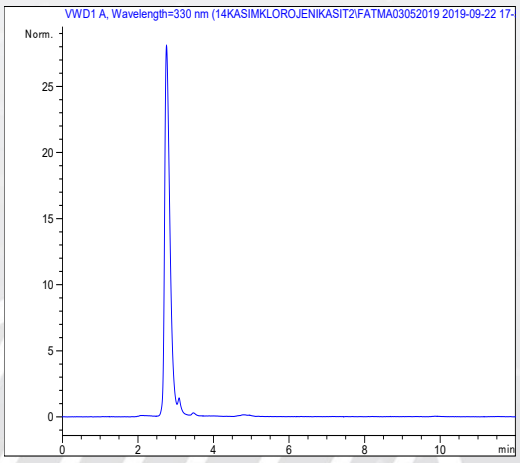

Figure 2: HPLC chromatograms of the obtained from methanol extract of aerial part of GV plant

MTT Assay Results: The proliferative effect of methanol extract of aerial part of GV was determined in HDF cells by using MTT assay. Various concentrations were used to study the cytotoxicity effect of extract (0.1 ng/mL-1 mg/mL) on HDF cells, as shown in Figures 3. In our study, a dose range between

1µg/mL-1 ng/mL of methanol extract of aerial part of GV at 24 hours had a significant stimulatory effect on the migration of the HDF cells (Figure 3). In this study, methanol extract of aerial part of GV has been shown to have a dose-dependent proliferative effect on HDF cell line for the first time.

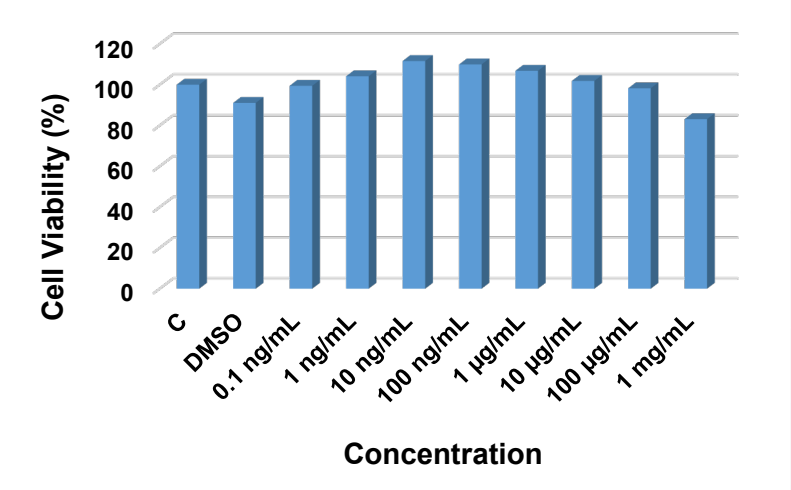

Figure 3: Effect of methanol extract of aerial part of GV on the viability of on HDF cells. The cells were treated with methanol extract of aerial part of GV at different concentrations and their proliferation was assessed by MTT assay (mean±standart deviation; C:control (untreated cells).

DISCUSSION

Our country is very rich in terms of plant species. The number of plant species growing in Turkey is about 10,000. Many species are also used among the people for medical purposes. One of these species is *Galium* species. *Galium* species are used in the treatment of burns in traditional treatment. In a study to show this, *G. odaratum* was compared to sulfadiazine in rats with a second degree burn. Topical treatment continued for 14 days. Histologically evaluated wound healing was found to be better in the water fraction [5]. The above mentioned biological effects of *Galium* species are due to the active compounds contained in these plants. The main active components of *G. verum* are flavonoids [6], iridoids, flavonol glycosides, tannins, enzymes [2], vitamins, chlorogenic acid, salicylic acid [7], antraquinones [4]. Caffeic and chlorogenic acids were identified in all *Galium* species. The richest source of chlorogenic acid being *G. odoratum* (10.387 +/- 45.67 µg /mg) [4]. Chlorogenic acid, p-coumaric acid and ferulic acid were identified in extracts of all *Galium* species in different amounts [16]. In this study, the quantification of CA has been successfully determined in the methanol extract of GV plant collected from Erzurum Köşk province. The CA content was calculated as 8.405±2.145 µg/mg extract. In an *in vivo* study evaluating the effectiveness of CA on wound healing, it has been reported that CA has a high wound healing capacity due to its strong antioxidant capacity and accelerates the wound healing process by increasing collagen synthesis in different phases of wound healing [15]. In our study, a dose range between 1µg/mL-1 ng/mL of methanol extract of aerial part of GV at 24 hours had a significant stimulatory effect on the migration of the HDF cells. It should be thought that this effect was due to the content of chloragenic acid in it. In this study, methanol extract of aerial part of GV has been shown to have a dose-dependent proliferative effect on HDF cell line for the first time.

CONCLUSIONS

Our results show that, methanol extract of aerial part of GV increased cell migration and can be accelerated wound healing and has been shown to be dose-dependent proliferative effect on the HDF cell line for the first time. In addition, CA was determined by HPLC in GV plant grown in the Erzurum province and the amount of CA in GV is similar to the amount of CA in other *Galium* species. The methanol extract of aerial part of GV and CA may be a candidate agent in the treatment of wound healing as a single or in combination with other drugs, components or molecules. For this reason, more detailed

studies should be designed *in vitro* and *in vivo* animal models to investigate the most safest and effective dose of methanol extract of aerial part of GV.

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SS-34

DETERMINATION OF SERUM PREPTIN LEVELS IN PATIENTS WITH KNEE OSTEOARTHRITIS AND COMPARISON WITH HEALTHY INDIVIDUALS

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ABSTRACT

Osteoarthritis (OA) is a multifactorial disease characterized by joint degeneration in adults. Inflammation and bone metabolism have an important role in the mechanism of OA. Preptin is newly isolated peptide hormone that has anti-inflammatory properties and osteoblastic activity as a regulatory compound in bone metabolism. This study was designed to determine serum preptin levels in patients with knee OA (KOA) and to investigate the relationship between preptin and KOA by comparing with healthy individuals. Eighty patients with KOA and 30 healthy controls (HC) admitted to the Orthopedics and Traumatology Clinic of Erzurum Regional Training and Research Hospital were included in the study. Blood samples of these individuals were taken and serum samples were separated. Knee radiographs were taken and KL grading was carried out using the radiographic findings. Body mass index (BMI), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and hemogram parameters were recorded. Preptin levels in all serum samples were determined by enzyme-linked immunosorbent assay (ELISA). There were no significant differences observed in the demographic characteristics of KOA patients and HC. Serum preptin levels were lower in KOA patients compared with HC ($p>0.05$). However, there was a significant decrease in preptin level in the KL grade 4 ($p<0.01$). In addition, serum preptin levels showed a significant negative correlation with CRP. The negative relationship between KOA and serum preptin level was demonstrated in this study. The determination of preptin levels may provide a new perspective for severe KOA.

Keywords: Knee Osteoarthritis, preptin

INTRODUCTION

OA is the most common chronic, a degenerative joint disease characterized by degeneration of cartilage in joints covered with synovia, joint pain, motion stiffness, local tenderness, and various degrees of inflammation, [1]. OA is the most common form of arthritis in the world, its incidence increases with age and usually involves the knee joint. Pain and stiffness are the most obvious symptoms in the joints [2]. In the world, with the increase of aging and obesity, OA will become an important health problem that will affect a large part of the society in the near future. Age is a strongly related risk factor that 80% of people over the age of 65 experience pain associated with OA and dysfunction in the joints [3, 4]. It is estimated

that the incidence of OA will double by 2020 due to the increasing aging population in the world and is becoming an important medical, financial burden for the world budget [5]. Therefore, the early diagnosis of this disease is very important. Some factors were identified as the reason of OA like the inflammation and altered biochemical conditions but the pathogenesis of OA is still poorly known. Nowadays, knee radiographs are used as diagnostic criteria [4]. 'Kellgren and Lawrence (KL) classification' is commonly used radiographic classification for determining the severity of knee OA. [7]. Even though the pre-radiographic period should be determined for early diagnosis of the disease, there is no specific laboratory finding specific to OA. Studies on determining which molecule or molecules can be used in diagnosis that will provide early diagnosis of the disease are current study topics. Adipose tissue-derived adipokines play an important role in cartilage and bone homeostasis. For this reason, adipokines have become the focus of attention in studies conducted for the pathophysiology of OA, because adipokines have been considered to be important mediators that provide an inflammatory connection in OA [6, 7]. Preptin, a newly isolated 34-amino-acid peptide hormone that is secreted with insulin and amylin from pancreatic beta-cells, has emerged as a regulatory compound in bone metabolism. In one study, it was reported that the administration of preptin was increased bone area and mineralizing surface in adult mice and it contributes to bone anabolism. According to some research, serum preptin levels could be related to alterations in inflammation and bone health [8]. Although the literature has pointed to a relation between adipokines and OA, preptin has not been investigated. In this study, we aim to determine serum preptin levels in patients with KOA and to investigate the relationship between preptin and KOA by comparing with healthy individuals.

MATERIALS AND METHODS

Ethics statement: Approval for this study was obtained from the ethics committee of the medical faculty of Ataturk University Erzurum, Turkey (date: 07.06.2018; number: 197). All procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000. Informed consent forms were signed by all the participants before conducting the study.

Subjects: The study group consisted of 80 KOA patients (except traumatic arthrosis with secondary arthrosis and patients with rheumatic diseases like rheumatoid arthritis and metabolic disease) and 30 HC, matched in terms of age, sex, and BMI. All the participants were evaluated in the Orthopedics and Traumatology Polyclinic of Erzurum Regional Training and Research Hospital, Erzurum, Turkey. The subjects were aged between 45 and 75 years. Blood samples were taken from all the KOA patients and HC at the time of presentation to the hospital. Anteroposterior knee X-ray radiographs were obtained from all the participants, and they were evaluated by Dr. Gundogdu.

Radiographic assessment of knee OA: All patients and controls underwent anteroposterior knee X-ray radiographs. Radiographs were then analyzed by Dr. Gundogdu **(Figure 1)**. The X-rays were then rated using the KL grading criteria as follows:

Grade 0: There was no evidence for OA.

Grade 1: Suspicious contraction of the joint space and possible osteophyte formation.

Grade 2: Definite osteophyte but an unimpaired joint gap.

Grade 3: Multiple osteophytes, tightness of the joint space, sclerosis and bone deformation may be present.

Grade 4: Wide osteophytes, severe constriction of the joint space, severe sclerosis and obvious deformities in the bone margins.

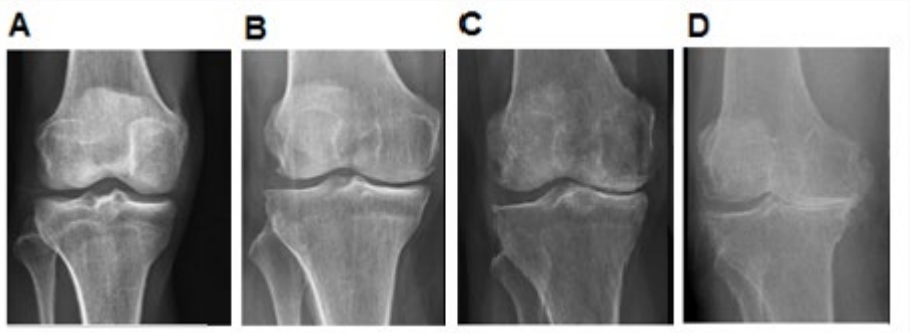

Figure 1. Radiographic imaging according to KL grade scale. A: Grade 1, B: Grade 2, C: Grade 3, D: Grade 4

Biochemical Parameters: The routine blood parameters (C-reactive protein (CRP, mg/dL), erythrocyte sedimentation rate (ESR, mm/h), white blood cell (WBC), neutrophil (K/µL), lymphocyte (K/µL), thrombocyte (K/µL), platelet (K/µL), and monocyte counts (K/uL)) were evaluated in both groups. The blood neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were calculated. The blood samples were centrifuged for 20 min at 6.000 rpm. The serum samples were stored at -80°C until preptin analysis.

The ELISA Method for Determine of Serum Preptin levels: Multiscan GO 51119300 model UV-Visible spectrophotometer (Thermo Scientific, Massachusetts, USA) and a 96-well ELISA kit (Elabscience, Wuhan, China) were used to determine the preptin levels in serum samples in accordance with the manufacturer's instructions. The linearity of the method was in the range of 0-4000 pg/mL concentrations. The limit of detection values of the method was 62.5 pg/mL. The relative standard deviation of intra-day and inter-day precision for plasma preptin was < 4.63 % and < 6.99%, respectively.

Statistical analysis: The demographic and biochemical parameters of KOA patients and HC were evaluated by using descriptive statistical analysis. Independent samples *t-test* was used to compare the KL scores of both KOA patients and HC. The correlations between preptin levels and laboratory parameters of KOA patients and HC were evaluated by using Pearson's correlation analysis. p-value < 0.05 was regarded as statistically significant.

RESULTS

Demographic Data of KOA patients and HC: 80 KOA patients and 30 HC were evaluated in this study. There were no significant variations between KOA patients and HC in the mean age, body height, weight, and BMI in all groups ($p > 0.05$). Thus, the ages, body height, weight, and BMI were all matched. The mean sedimentation of KOA patients was significantly higher compared with HC.

Serum Preptin Levels of KOA patients and HC: The serum preptin levels in KOA patients and HC were determined with the ELISA method by using the ELISA kit. Preptin was determined by means of UV (A) absorbance values measured at 450 nm. The calibration curves obtained by plotting A_{450} values versus concentration show linear relationships. The curves showed good linearity and the regression equation and correlation coefficient was found as $A = 0.0006C + 0.0661$ and 0.9936 at the 0-4000 pg/mL concentration ranges of preptin, respectively. (C: the preptin concentration (pg/mL) and A: the absorbance of preptin). The preptin levels in the KOA patients were lower compared with HC but the difference was not significant (403.18±78.24 pg/mL and 422.95±101.08 pg/mL respectively ($p > 0.05$)) **(Figure 2).** The KOA patients were stratified according to their KL grades: mild-to-moderate KOA (KL grade1-3) and

severe KOA (KL grade 4). The preptin values in severe KOA and mild-to-moderate KOA were 344.15±38.80 pg/mL and 416.48±75.35 pg/mL, respectively ($p < 0.01$) **(Figure 3).** Serum preptin levels in patients with severe KOA were significantly lower as compared to mild-to-moderate KOA. In addition, serum preptin levels showed a significant negative correlation with CRP.

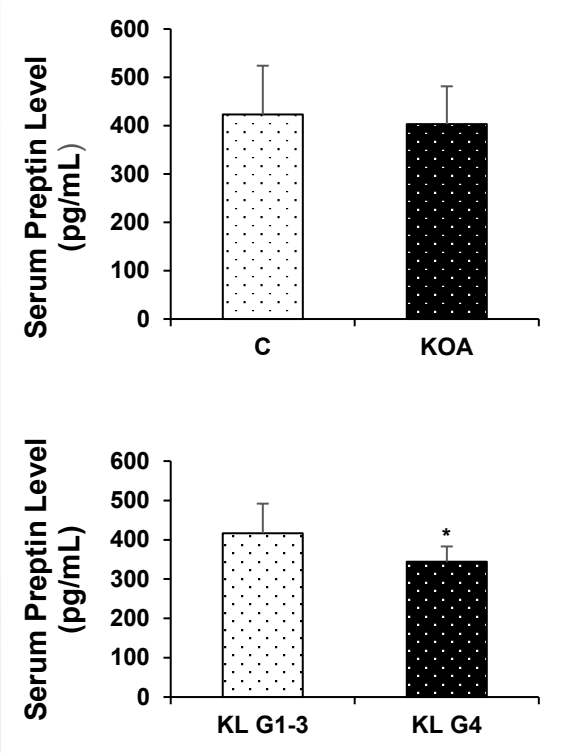

Figure. 2. Serum levels of preptin in healty control and KOA patients (Results are given in mean ± SD, p>0.05, C: Healty control, KOA: knee osteoarthrithis)

Figure 3. Serum levels of preptin in healty control and KOA patients according to KL grade (*Statistically significant in comparison with K-L grade 4 and other grade, Results are given in mean ± SD, p<0.01)

DISCUSSION

OA is a chronic and degenerative disease characterized by degeneration of articular cartilage and disruption of the normal balance between periarticular bone formation and destruction events [1]. OA is the most common form of arthritis in the world, its incidence increases with age and usually involves the knee joint [9]. However, the pathophysiology of OA is still not fully explained. It also causes alterations in subchondral bone [10]. Nowadays, knee radiographs are used as diagnostic criteria but it can only detect damage that occurs after cartilage destruction. Therefore, a pre-radiographic period should be determined for early diagnosis of the disease [6, 7, 11]. Adipokines are expressed in cartilage and bone and also play an important role in inflammation. Therefore, they have become target molecules with the idea that they can be used as biomarkers in the diagnosis and treatment of many diseases. [7, 12]. Because adipokines play an important role in bone and cartilage homeostasis, systemic (plasma or serum) and local (synovial fluid) adipokine levels are thought to be associated with cartilage degeneration and synovial inflammation [13-15]. Preptin, an adipose tissue-derived adipokine, has been shown to stimulate osteoblast proliferation and inhibit osteoblast apoptosis. In a study with rats, preptin administration has

been shown to increase bone area and mineralized surface. Li et al. showed that blood preptin levels decreased in elderly male patients with osteoporosis [16]. In another study, serum preptin level was found to be lower in postmenopausal women compared to premenopausal women [17]. In the literature, most studies have used an ELISA to evaluate serum levels of preptin. The assay is easy to use and sensitive, thereby allowing rapid analysis of samples without any pretreatment. Thus, this method was preferred in the present study. An ELISA was used for the determination of serum preptin levels in the control and KOA patients.

In this study, serum preptin levels in the KOA patients were lower compared to HC but this decrease was not statistically significant. It was thought that this decrease may be due to consisting of OA patients mostly of postmenopausal women in accordance with the literature. In addition, when we classified the patients according to the KL classification, it was found that the preptin level was decreased statistically significant in patients with severe KOA compared to the patients with mild-moderate KOA.

CONCLUSIONS

Serum preptin levels were decreased in KOA patients as compared with HC. Serum preptin levels in patients with severe KOA were significantly lower as compared to mild-to-moderate KOA. Accordingly, serum preptin levels showed a negative correlation with both the BMI. In conclusion, it could provide a new perspective on the relation between serum preptin levels and severe KOA. (Ataturk University Scientific Research Project No: TSA-2018-6796).

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PS-001

Acquisition of regenerative phytoextracts and their study of microbiological properties

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One of the topical issues facing medical science is the development of phytoextracts that have regenerative effects in the treatment of various etiological skin diseases, allergic rash, and inflammatory wounds, which are widespread and growing day by day.Taking into account the mentioned urgency, the aim is to obtain new ingredients and study their microbiological properties based on regenerative effects of medicinal plants grown and spread in the environmental conditions of Azerbaijan.
Object and methods of research.In practice Sabdarif hibiscus (Hibiskus sabdariff L.), clove wood (Caryophyllos aromaticus L.), and common yarrow (Achillea millefolium L.) were used.The optimum conditions for the production of phyto-extracts were selected: extraction method - maserry, extraction time - 24 hours,extractant-propylene glycol-alcohol(PGA)(5:95), raw material-0.5mm. Within these conditions, mono- and multi-component phyto- extracts have been developed. Monocomponent Phytoextracts: Composition-1 (PGA Extract of Clove buds Composition -2 (PGA Extract of Yarrow flower); Composition -3 (PGA Extract of Hibiscus flowers); Multicomponent Phytoextracts: Composition -4 (Clove + Yarrow + Hibiscus PGA Extract; Composition -5 (Clove + Yarrow PGA Extract); Composition-6 (Yarrow + Hibiscus PGA Extract); Composition -7 (Clove + Hibiscus PGA Extract). The development of microorganisms at a dilution rate of 1: 400 and 1: 800 was recorded.Composition 6 - Clove + Hibiscus (Alcohol Extract) - Has a weak effect. It inhibits the development of microorganisms only in concentrate. In other dilution rates, the development of microorganisms was noted.Composition7- Clove + Hibiscus (alcohol extract) - medium activity. Effect of concentrating and dilution at 1: 100 and 1: 200 ratio.The development of microorganisms at a dilution rate of 1: 400 and 1: 800 was recorded.CONCLUSION: During the microbiological studies, it was found that phytoextracts had a sufficient antimicrobial and antifungal effect. In view of the above, it is considered advisable to develop appropriate medicinal forms based on these extracts and study the regenerative effects in the treatment of dermatological diseases.

Anahtar Kelimeler: regenerative, phytoextracts, nails, hibiscus, dandruff, antimicrobial and antifungal effects

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PS-002

Phytochemical characterization and antioxidant activity of the methanolic extract of Urtica dioïca L. leaves

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Urtica dioïca L. is an endemic species in Algeria, important for its pharmacological properties since it is recognized as being anti-cancer according to the declarations of the world health organization. We wanted to highlight this property and research in vitro, its antioxidant activity as well as the specific chemical components whose leaves are rich in polyphenols including anthraquinons. The present study aims to make a qualitative characterization of these phenolic compounds responsible for the antioxidant activity of the plant. On the methanolic extract of the leaves. To research the antioxidant activity in the mathanolic extract of the dried leaves of Urtica dioïca. We used the trapping method at 2, 2 DiPhenyle-1-Picryl-Hydrazyle (DPPH). The results obtained show significant antioxidant activity. With this antioxidant property, this plant should be taken as a natural source and an alternative treatment for certain cancers that could be treated in a gentle and effective way.

Anahtar Kelimeler: Urtica dioïca L., methanolic extract, polyphenols, anthtraquinons, antioxidant activity.

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PS-003

Characterization of bioactive components of Crataegus monogyna Jacq. leaves and its antibacterial activity against pathogenic germs

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Crataegus mongyna Jacq., is a species endemic to eastern Algeria, belonging to the Rosaceae family. It is one of the plants used in traditional medicine in Algeria thanks to its many therapeutic virtues. The present study aims to characterize the bioactive components in the methanolic extract of hawthorn leaves by phytochemical screening of the various components of which it is rich. We also looked for its Antibacterial activity research is carried out in vitro on Gram positive and Gram negative microbial strains represented by Micrococcus flavus, Bacillus subtilis and Lysteria monocytogenes. The recommended method is that of diffusion in a solid medium or antibiogram. The phytochemical screening results show the richness of the hawthorn leaves in polyphenols. The results of the antibiogram show significant antibacterial activity. This property, well used, could be used as an alternative treatment for certain pathologies because it is of value for public health.

Anahtar Kelimeler: Crataegus mongyna Jacq., leaves, methanolic extract, bioactive components, antibacterial activity

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PS-004

Antibacterial activity of two essential oils obtained from species of the genus Thymus against multidrug-resistant germs

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Thymus hirtus and Thymus ciliatus are aromatic plants from southern Algeria. They are used in traditional medicine for the many therapeutic properties. In order to demonstrate their value as a source of bioactive natural substances, we evaluated their antibacterial activity. The method we have advocated is that of the aromatogram. We first extracted the essential oil from stems, flowers and dried leaves. This operation is carried out using a standard apparatus used for the extraction of essential oils. Thin layer chromatography showed us the chemical screening of the plant. Indeed, it appears that the main chemical components are carvacrol and thymol. To mount the aromatogram, we used multi-resistant pathogens: Klebsiella pneumoniae, Pseudomonas aeroginosa, Serratia marcessenc, Acinetobacter baumanii, Staphyloccus aureus, Escherichia coli and Proteus vulgaris. The results of the aromatogram indicate that the crude essential oils of Thymus hirtus and Thymus ciliatus, as well as their different degrees of dilution show a significant antibacterial activity on the selected strains. From these results, we can conclude that the multidrug-resistant pathogens we selected are sensitive to the crude and diluted essential oils of the two species studied. Thymus hirtus and Thymus ciliatus are aromatic plants that constitute an important source of bioactive natural substances that can be a solution for certain infectious pathogens, especially those caused by multidrug-resistant germs.

Anahtar Kelimeler: essential oils, activity, antibacterial, multidrug-resistant germs, aromatogram

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PS-005

A genomic data of novel antibiotic producer AGBY19 to control of pathogenic bacteria

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Increasing multidrug-resistance to pathogens, particularly to *Staphylococci*, *Enterococci* and *Streptococci*, is a major problem, resulting in significant morbidity, mortality and healthcare costs. In recent years, only a small number of effective novel antibiotics against Gram-positive bacteria has been approved. Searching of novel antibiotics has gained importance to overcome this issue threating human being.

In this study we have identified a non-pathogenic bacterium belonging *Serratia fonticola* strain using whole shot gun genome sequence analysis and has been labelled as "AGBY19". Genome analyses of AGBY19 revealed that the bacteria harbours different gene clusters comprising flanked regions associated with the production of secondary metabolites providing antibiotic properties against bacteria. 30 genes associated with production of bacteriocins and proteases in studied genome with their detection e-values and products was determined. These genes might be used for recombinant DNA technology to produce new antibiotics and enhance the efficacious of known existing antibiotics in pharmacology.

Therefore, our strain has remarkable importance as alternative and effective antibiotic producer and further studies are required to understand of its inhibitory effect on different pathogens.

Keywords: whole genome sequencing, multidrug-resistance, novel antibiotics, bacteria, pathogen

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PS-006

A study on prophage regions and antibiotic resistance genes in whole genomic mapping of *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286

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Bacteriophages and genetic elements, such as prophage-like elements, pathogenicity islands, and phage morons, make up a considerable amount of bacterial genomes. Their transfer and subsequent activity within the host's genetic circuitry have a significant impact on bacterial evolution. The spontaneous induction of prophages can lead to competitive advantages and influence the lifestyle of bacterial populations or the virulence of pathogenic strains. One of this advantages is antibiotic resistance among pathogens, which is an emerging problem in every country. Development of new novel antibiotics has gained importance to solve this issue.

In this study, we have screened whole genome sequence of a human pathogenic bacteria; *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286 for prophage, and antibiotic resistance regions by bioinformatics tools. The results indicated that there are 8 different prophage clusters in major genome of bacteria. Four regions are in intact position, 1 region is incomplete and 3 regions are sorted as questionable. Also plasmid genes are screened and interestingly, one plasmid (pKPHS1) is found to be a bacteriophage (PHAGE_Salmon_SSU5). In other 5 plasmids no prophage regions were found. The screening for antibiotic resistance genes in major genome and plasmids varied different genes against fosfomycin, beta-lactam, aminoglycoside, sulphonamide, tetracycline, trimethoprim antibiotics. The plasmid (pKPHS1), which is a bacteriophage, has beta-lactam resistance gene (blaCTX-M-14) that is thought to help the bacteria for acquiring resistance.

Further studies are needed to understand the relationship of bacteria and bacteriophages/ prophages. We assume that the grounded prophages integration regions in bacterial genome related to evolution for horizontal gene transfers show accordance with several ecologically significant genes involved in stress tolerance, antimicrobial resistance, and novel metabolic pathways. Moreover, the high frequency of genetic changes within prophages also allows proportionate probability *de novo* genesis of genetic information. These regions used tracking of strains and to evaluate differentiation of bacterial populations seems very effective tool. This

relationship might be used for development of designed target-oriented bacteriophages against human pathogenic bacteria as a new concept of drug design.

Keywords: antibiotic resistance, bacteria, bacteriophage, pathogen, prophage, whole genome sequencing.

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PS-007

Nano-PCR Detection of S. pyogenes with Amino Acid Conjugated Magnetic Beads

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S. pyogenes also known as Group A Streptoccoccus (GAS) is a Gram-positive pathogen that causes a wide variety of infections in humans. It is an extremely dangerous bacteria, so there is an urgent need for rapid and precise detection methods for S. pyogenes. Polymerase chain reaction (PCR) is commonly used to identify specific DNA sequences of pathogens. However, the specificity and sensitivity of PCR need to be improved. Due to the special physical and chemical properties of nano-sized materials, nanoparticle-assisted PCR (nano-PCR) has been developed and shown to improve specificity, sensitivity and efficiency of PCR. In this study, we aimed to design a novel nano-PCR assay by using magnetic beads conjugated with glycine, lysine, and bovine serum albumin (BSA). We used Dynabeads M-280 Tosyl-activated beads (ThermoFisher) and performed the conjugation reactions as suggested by the supplier. We amplified the speB gene of S. pyogenes in the presence of nanoparticles. We performed nano-PCR with different concentrations of template DNA and nanoparticles. Then, we monitored the PCR products on 1.0 % agarose gel. As a result, we showed that nano-PCR with a detection limit of 1 ng DNA was superior to the conventional PCR.

Keywords: polymerase chain reactions (PCR), nano-PCR, pathogen detection

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PS-008

Investigation of Wound Healing and Antimicrobial Effects of ZnO Nanoparticles Synthesized Using Mentha longifolia L. Essential Oil

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Nanoparticles (NPs) are used in many fields such as medicine, industry and textile with the developing technology. Some researchers think that biological systems and the environment will be negatively affected by chemically synthesized NPs. Obtaining NPs by green synthesis is advantageous in this regard and offers more reliable, inexpensive, environmentally friendly and effective products. ZnO is an n-type semiconductor metal oxide classified by FDA among safe metal oxides. In this study, Mentha longifolia L. essential oil (MLO) used in the synthesis of ZnO NPs has bioactive properties. By taking advantage of these bioactive properties, we aimed to synthesize ZnO NPs, which will accelerate wound healing and are expected to execute antimicrobial effects against important clinical pathogens.
Aerial parts of Mentha longifolia L. were collected from Alanya / Antalya, diagnosed in the biology department and stored after being dried. The plant parts were ground, essential oil was obtained using the clevenger device. Zn (SO)4.7H2O was used as the precursor for synthesizing ZnO NPs. 2,876 gr Zn(SO)4.7H2O were dissolved in 49 ml of distilled water. 1 ml of MLO was added to the solution and left in the magnetic stirrer for 4 hours for the reaction. After 4 hours, 50 ml of 2 M NaOH was added to the solution and held overnight in a magnetic stirrer (60°C) for reaction. The precipitate formed at the end of the period was centrifuged (20000 rpm, 20 min) and supernatate was poured.
Disc diffusion method was used to determine the antimicrobial properties of ZnO NPs. NPs executed bactericidal effect against MRSA ATCC 67106, Acinetobacter baumannii ATCC BA1609, Escherichia coli ATCC BAA-2523, Streptococcus salivarius ATCC13419, Pseudomonas aeruginosa ATCC 9070, Streptococcus mutans ATCC35668.
Human fibroblast cell line was used for cell culture studies. At the end of the dose studies repeated twice in the form of serial dilutions, in vitro wound model was created for 11 different concentrations (1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625, 0.001953125, 0.0009765625 µg/ml). A microscope image was taken every 3 hours for 48 hours. 3 doses (0,03125, 0.015625, 0.0078125 µg/ml) supplied the wound line to close in less than control.

Keywords: Nanoparticles, Essential Oil, Wound, Antimicrobial

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PS-009

Automated Detection of HER2 Gene Amplification in Whole Slide Fluorescent In Situ Hybridization (FISH) Images in Invasive Breast Cancer

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The presence of human epidermal growth factor receptor 2 (HER2) mutations in breast cancer, which is the most common type of cancer in women today, provides prognostic information to the pathologist and enables targeted treatment to be applied to the patient. According to clinical trials, Trastuzumab, a monoclonal antibody against the HER2 receptor, significantly increases patients' survival when used in the treatment of breast cancer patients with HER2 overexpression. The aim of this study is to evaluate automatically the Fluorescent in situ hybridization (FISH) result which is used to detect the HER2 gene mutation precisely, thereby to prevent the inter-observer variation during the manual FISH evaluations of pathologist and to obtain more objective, accurate and fast results.Thus, the correct evaluation of HER2 status will prevent getting wrong chemotherapy or false treatment with Trastuzumab. Whole slide HER2 FISH images were obtained for this study from the pathology laboratory of Acibadem Maslak hospital. The algorithm is designed based on the ASCO-CAP guideline, which is universally used by all the pathologists to evaluate the HER2 status. HER2 FISH algorithm is developed by using OpenCV framework which is based on C++ programming language. FISH image analysis consists of two main stages which are cell nucleus detection and signal detection. In order to avoid the problems caused by staining, which complicates the analysis, some pre-process techniques have been applied before nuclei and signals are detected. In order to reduce signals noise pre-process is applied which includes contrast and brightness optimization and various specific filters (White-tophat, Gaussian, Laplacian). Then, dapi detection / segmentation and signal detection were performed by using specific image processing filters, adaptive thresholding method and watershed method. In the result of the analysis, HER2 copy number, chromosome 17 (CEP17) copy number, HER2 / CEP17 ratio and FISH status are given. When the analysis is run on the whole slide image (approx. 10 mm2), 25000-30000 nucleus and the signals in the cores are successfully found within 5-10 minutes. With this study, we purpose to provide accurate results that support patient survival by standardizing all pathological FISH assessments and minimizing errors caused by the observer.

Keywords: Breast cancer, Fluorescent in situ hybridization, HER2/CEP17 status, image processing

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PS-010

Antisense Oligonucleotide Therapies For Spinal Muscular Atrophy

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Antisense oligonucleotides are new therapeutic agents for the discovery of potential drugs in the treatment of neurodegenerative diseases. (Bennet et al.,2019). Spinal muscular atrophy (SMA) is a destructive degenerative neuromuscular disorder characterized by loss of spinal cord motor neurons, muscle atrophy and infant death or severe disability (Wood et al.,2017). They range in severity from neonatal onset with quickly progressive weakness and early mortality (SMA-1), to onset in infancy (SMA-2), to adolescent/adult onset with painless clinical course (SMA-3/-4) (Neil and Bisaccia,2019). A deficiency in SMN protein results in loss of motor neurons in the spinal cord, defective neuromuscular junctions, and atrophy of skeletal muscles. Researchers; the splicing of SMN-2, a gene containing exon 7, discovered that enough SMN protein can be produced to improve symptoms of the disease (Passini et al.,2017). Antisense oligonucleotide studies for spinal muscular atrophy began with cell culture experiments designed to test hypotheses that antisense oligonucleotides may affect the insertion of exon 7 and the SMN-2 gene can be induced to make stable SMN protein (Corey,2017). These experiments identified ASOs that can increase full-length SMN-2 production in cell-free splicing assays and cultured cells (Lim and Hertel,2001; Hua et al,2007). In subsequent in vivo experiments, these findings were tested in transgenic mice and found that antisense oligonucleotide could increase SMN protein levels, rescue the mutant phenotype and reduce disease symptoms (Passini et al,2011). Recent data from phase 2 and phase 3 clinical trials have demonstrated that nusinersen (Spinraza), an antisense oligonucleotide drug, is effective for treating spinal muscular atrophy (Finkel et al.,2016). Recently, nusinersen, an antisense oligonucleotide that corrects SMN-2 splicing and thereby increases full-length SMN protein, has been approved by the FDA and EMA for spinal muscular atrophy therapy (Torrent et al.,2019). In the last study, it was found that HDAC inhibitors (LBH589) can strengthen the activity of nusinersen with synergism in the treatment of spinal muscular atrophy (Pagliarini et al., 2019).

Keywords: antisens oligonucleotide, spinal muscular atrophy, SMN protein, nusinersen

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PS-011

Cellular activities of erlotinib contining nanotailored ZnO quantum dots

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Fibrosarcoma which occurs predominantly in the area around bones or in the soft tissues is one harsh type of malignant tumor. Chemotherapy remains insufficient in many cases because of the absence of targeted mechanism, side effects and the necessity of high doses in current medicine. Erlotinib hydrochloride(ERL) is one of the orally applied tyrosine kinase inhibitors. Tyrosine kinases are of significance in sarcoma [1,2]. We developed ERL loaded and targeted chitosan nanoparticles. They were also combined with dexketoprofen trometamol(DT) to decrease the dose of ERL and decorated with ZnO quantum dots (ZnO-QDs) to enhance their antitumoral effect on tumor cells. The hybrid nanoparticles were obtained with a size of 161.6±96.9nm and a negative surface charge at the surface was observed (-0.98±0.88mV). The encapsulation efficiency was 48% and 98% for ERL and DT, respectively. The drug release from hybrid nanoparticles was observed to be slowly reached up to more than 30% in 24hours. Antitumoral activity was also measured on WEHI164(ATCC®CRL-1751™) cells which harvest from mice after inducing the tumor with methylcolantrene to reflect the effect of nanoparticles on tumor. The hybrid nanoparticles showed 54.1±5.3% of antitumoral activity while bare nanoparticles remained in 20.7±11.3% of that. ZnO-QDs was also showed antitumoral activity in accordance with previous literature [3]. These hybrid nanoparticles dispersions were orally applied to methylcolantrene-induced tumor bearing mice. Their tumor tissues were evaluated for tyrosine kinase and cyclooxygenase inhibitor activity and folate receptor activity. Tyrosine kinase inhibitory effect limited to 36.1±0.4% due to the low solubility of ERL, while cyclooxygenase inhibitor effect reached to 90.39±6.1% due to the high solubility of DT. Folate receptor activity was 95.5±0.1% for hybrid nanoparticles. Cell culture studies on WEHI164 cell lines underline the presence of antitumoral efficacy in ZnO-QDs and the effectiveness of hybrid nanoparticles. The results based on the study of the tyrosine kinase inhibitor effect supported that. Folate activity emphasized that active targeting successfully achieved. However, these quite promising results fell short of the goals because of the low solubility of ERL. Further studies should be focused on the solubility enhancement of ERL.
This study is financially supported by TUBITAK, No.213M675.

Keywords: ZnO quantum dots, chitosan, nanoparticles, erlotinib hydrochloride, dexketoprofen trometamole, fibrosarcoma

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PS-012

3D printing in biomedical field

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3D printing and printers have gain importance in the biomedical applications in accordance with advancing technology. 3D printing is a manufacturing method in which objects are made by fusing or depositing materials such as plastic, metal, ceramics, polymer, powders, liquids, living cells in layers to produce a 3D object by using a 3D printer. 3D printing can be based on different technics such as; stereolithography, fused deposition modelling, selective laser sintering, selective laser melting, 3D ink jet printing/thermal inkjet, laminated object manufacturing (1,2). 3D application in biomedical field can be classified as surgical planning, prostheses, medical education and training, medical research and tissue printing (3). Among the main usage examples of 3D printing are given as the production of biomodels (artificial models of human body parts, e.g. bone, dental prosthesis, etc.). The development of artificial models for the different individual anatomy of humans provides many benefits in the field of treatment. These can be indicated as more accurate diagnosis, better planning and testing, more effective guidance during operation, increased quality and accuracy, testing new medical methods and technologies and conducting activities such as research, education, and exercise more effectively. There is considerable potential in bio-printing for the development of tailor-made treatments, treatment-related tests, medical research and tissue and organ transplantation (4-5). In this study, potential technologies and usage of 3D printers in biomedical field are presented.

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Keywords: Tailor-made treatments, Treatment-related tests, Bio-printing, 3D printer, Tissue engineering, Bioengineering

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PS-013

Manufacturing and quality control of biosimilar medicines

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Biosimilar medicines are biotherapeutics that are similar in quality, safety and efficacy to previously licensed reference biotherapeutics (1). Since the exact production method of the reference biological product is not known, different processes are mostly used in the production of biosimilar drugs. The slightest change in any stage of production can cause differences in the product. Among the factors, affecting production can be listed as; host cell selection, fermenter type, ambient conditions, broth, substances used for cell culture, fermentation method and purification method (2). Good manufacturing practices (GMP) requirements for biological and biosimilar products are higher than for small molecules. The similarity should be demonstrated by comparative quality, non-clinical and clinical tests (1,3). While non-clinical tests can be grouped as physicochemical characterization, biological characterization, pre-clinical and pharmacodynamic/pharmacokinetic tests, clinical tests can be grouped as pharmacokinetic-pharmacodynamic tests, all reliability and effectiveness studies and clinical studies. Biosimilar products have high degree of similarity to the reference products but they are not accepted as bioequivalent products (4). In this study, manufacturing methods, critical parameters in manufacturing and quality control of biosimilar medicines are evaluated in accordance with the related regulations and standards.

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Keywords: Biosimilars, biotherapeutics, manufacturing methods, good manufacturing practices/GMP, quality control

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PS-014

Investigation of Antimicrobial Activity of a New Generation Nanocomposites on *Escherichia coli*

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Nanomaterials have rendered potential effect on controlling bacterial infections [1]. Nowadays, especially two-dimensional (2D) semiconductors, such as graphene, titanium dioxide (TiO2) and molybdenium disulfide (MoS2) have gained tremendous interest in the field of biotechnology [2]. Different from graphene and other semiconductors, Black Phosphorus (BP) nanosheets has thickness-dependent bandgap 0.3 eV for bulk to 2.0 eV for single-layer showing wide absorption range including UV, entire visible and near infrared light region [3-5]. Benefiting from its strong photothermal conversion and enhanced production of reactive oxygen species (ROS) under NIR light irradiation, BP shows promise for antibacterial applications [6]. In this study, we conjugated BP with Au NPs to obtain BP/Au nanocomposites and investigated antibacterial efficiency on *Escherichia coli*, as a model microorganism. Antibacterial activity studies of BP nanosheets and BP/Au nanocomposites were performed through Optical Density at 600 nm (OD600nm) measurement, oxidative stress (GSH depletion) and photothermal conversion assay. Fluorescence microscopy technique was used to monitorize live and dead bacterial cells after the treatment of nanomaterials under NIR light irradiation.

Keywords: antibacterial efficiency, black phosphorus, photothermal conversion

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PS-015

Microbiological quality control analysis of biological and biotechnological medicines

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Microbiological quality control analyzes of biological and biotechnological medicines are carried out according to the methods specified in internationally accepted pharmacopoeias such as the European Pharmacopoeia (EP), the British Pharmacopoeia (BP) and the Unites States Pharmacopoeia (USP). These medicines are grouped as sterile and non-sterile products. Non-sterile products should be within microbiological limits prescribed by pharmacopoeias. The quality of the products are analyzed by microbiological counting and specific microorganism tests. The microbiological quality of non-sterile biological and biotechnological medicines is determined according to EP Chapter 5.1.4, 2.6.12, 2.6.13; USP Chapter 61, 62, 1111; BP XVI-D. The products that should be sterile are analyzed by sterility test and the presence of aerobic, anaerobic microorganisms and yeast and molds are analyzed. Sterility test is applied according to the EP Chapter 2.6.1, USP Chapter 71; BP XVI-A. Sterility testing is based on the 14-day incubation of the product in the medium and as a result of incubation, there should be no growth in the media. In these analyzes, neutralization processes are applied against antibiotic and antimicrobial preservatives according to the method compliance parameters specified in the pharmacopoeias. In this study, microbiological quality control parameters which are necessary for biopharmaceutics are evaluated in terms of internationally accepted pharmacopoeias and scientific literatures for microbiological analysis methods that are generally compatible with standard tests.

Keywords: Biological medicines, microbiological quality, bioburden, sterility, pharmacopoeial tests, antimicrobial efficacy

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PS-016

Analysis of biological/biotechnological medicines in pharmacopoeias

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Advances in the scientific and technological field have significant contribution to the development and manufacture of biological and biotechnological medicines in recent years. Research and development studies in the biopharmaceutical field bring diversity of quality control methods along with the formulation and manufacturing method of biological/biotechnological medicines. Although there are some standardized and validated quality control methods given in the internationally recognized pharmacopoeias, there are many in house methods of biopharmaceutical product owners that can only be used as internal quality control methods by them. The main international sources for quality control methods of biopharmaceutics can be given as pharmacopoeias (1-4), International Organization for Standardization-ISO standards (5) and Organization for Economic Co-operation and Development-OECD methods (6). Between the pharmacopoeias European Pharmacopoeia, United States Pharmacopoeia, British Pharmacopoeia and Japanese Pharmacopoeia are mostly accepted ones. In the scope of above mentioned pharmacopoeias the analyses of biological and biotechnological medicines can be grouped as physical, chemical, pharmacological and microbiological controls. Those include identity, quantity and potency tests, thermostability, viral control tests, total and bound protein and purity (impurity) controls, sterility test, bacterial endotoxin and pyrogenicity test to prove their efficacy and safety. In this study, analysis methods for quality control of biological and biotechnological medicines in pharmacopoeias are evaluated in terms of biopharmaceutical product groups.
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Keywords: Biological, biotechnological medicines, efficacy, safety, quality, pharmacopoeial methods

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PS-017

Investigation of Neuroprotective Effect of Genticic Acid Against Glutamate Excitotoxicity in Primary Cortical Neuron Culture

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Glutamate is an excitatory amino acid and is one of the main neurotransmitters of the mammalian central nervous system. It is present in a fairly high concentration in the brain, and 50% of neurons use glutamate as a neurotransmitter. Excessive lowering of glutamate can lead to disruption of normal excitation, while its excessive rise may disrupt calcium homeostasis, leading to excitotoxicity and ultimately cell death. Glutamate has been shown to cause neuronal degeneration in the neuron body, dendrites, and glia. Genticic acid is an anti-oxidant with a simple molecular structure; aspirin catabolite, tyrosine catabolism byproduct, endogenous is synthesized as an anti-microbial siderophore; it is also abundant in many fruits. In this study, it is aimed to reveal the neuroprotective effects of genticic acid in primary cortical neuron cultures exposed to glutamate excitotoxicity. Primary cortical neurons were obtained from newborn Sprague Dawley rats. Glutamate excitotoxicity was created by applying glutamate at a concentration of 10^{-5} M to the culture medium. Subsequently, the cells were incubated for 24 hours by applying certain doses of genticic acid. Cell viability level was determined by MTT analysis method. According to the results of MTT analysis, it has been revealed that genticic acid administered at low doses has a protective effect on neurons against glutamate excitotoxicity. According to the results of the study, genticic acid is thought to have a protective effect against glutamate excitotoxicity in primary cortical neuron cells and can be used as a therapeutic agent against glutamate excitotoxicity.
Keywords: Genticic acid, glutamate, neuroprotective, primary neuron culture.

Keywords: Genticic acid, glutamate, neuroprotective, primary neuron culture.

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PS-018

Laboratory requirements for microbiological controls of biological and biotechnological medicines

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Laboratory requirements, which are defined by standards should be provided for microbiological control of biological and biotechnological medicines. These are necessary for reliable analyses results and risk of contamination. Especially the minimum requirements for sterility testing are the acceptance criteria for the test, and they are defined in the European Pharmacopoeia-EP Chapters of 2.6.1 and 5.1.9, and the Unites States Pharmacopoeia-USP Chapters of 71, 1208 and 1116. In microbiological control laboratories (MCL) the sterility testing environment mainly requires a Class A laminar flow cabinet located within a Class B clean room or an isolator. Classification of Class B environment and Class A cabin are explained in the standards of International Organisation of Standardization-ISO (ISO 14644). According to ISO 14644 the active and passive microbiological control of the environment, and the microbiological control of the surfaces and equipment should be checked routinely. Settle plate, contact plate, swab control, finger prints method, active and passive microorganism count methods can be used for these controls. In other parts of MCL, the prevention of microbial contamination is the main necessity and to provide this there should be separated areas and a biosafety cabinet, which are specified in ISO 17025 and related standard TS 13134. In terms of related standards briefly in a microbiology control laboratory; i. time-space separation should be made for the risk of cross-contamination in the studies performed, ii. equipment, wall, floor building materials in laboratories should be suitable for use in a microbiology laboratory, iii. preparation, test, incubation, identification and decontamination areas must be defined and separated, iv. the microbiological control of the procedures performed at each stage should be checked for suitability, v. sterilization and decontamination control, positive and negative microorganism control, microorganism culture control, microbiological control of the environment, growth promotion control, sterility control should be performed and recorded. There should be daily and monthly hygiene plans applied in laboratories. Disinfection and decontaminations procedures should be performed regularly and the environment should be checked microbiologically. In this study MCL design considering microbiological control tests for biotherapeutics, requirements and monitoring are evaluated in the scope of related standards and pharmacopoeias.

Keywords: Microbiology test laboratory, laboratory design, microbiological environment controls, classification areas, biotherapeutics, sterility laboratory

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PS-019

Design of Gold Nanorod Based Localized Surface Plasmon Resonance (LSPR) Biosensor for Detection of Bioanalytes

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Localized surface plasmon resonance (LSPR) based detection has been widely used in recent years for biosensor applications since it's label free, cost effective and relatively simple. LSPR is highly sensitive to the refractive index change in the local dielectric environment of nobel metal nanoparticles and the red shift of the maximum absorption wavelength is an indicator of the detection of the molecules. Gold nanorods (GNRs) are excellent tools for LSPR biosensors due to their unique optical properties. In this work, GNR-based LSPR biosensor surfaces have been developed for detection of model bioanalytes as a proof-of-concept study. GNR patterned glass surfaces were first prepared. Self-assembled monolayers of functional alkanethiols were then formed on GNR patterns. Antibodies were conjugated onto funtionalized GNR patterns for specific detection of antigens. The sensor chips which were tested with varying antigen concentrations between 0.1 nM-10 μM showed linear response. A λmax shift of 2 nm was observed with the detection limit of 1 nM antigen concentration. Sensitivity of biochips was determined as 298 nm/RIU. GNR patterns modified with non-specific antibodies showed no antigen binding. In conclusion, GNR-patterned biosensor surfaces were proven to be selective, sensitive and practical.

Keywords: Gold nanorods, biosensor, localized surface plasmon resonance, label-free detection

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PS-020

Standards and quality control testing of surgical sutures

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Dating back to ancient times, sutures are used by health professionals to close cuts, wounds, insicions on the skin, internal tissues, organs and blood vessels in human or animal body for contributing to their healing process after injury or surgical operation. They keep tissues together allowing body to deliver necessary healing agents to the problematic regions to be repaired. Surgical sutures can be classified according to their raw materials, manufacturing methods, etc. They can be absorbable or nonabsorbable, natural or synthetic based on their structures and content. They can also be classified as monofilament, multifilament/braided and pseudo filament according to their manufacturing process (1). The healing properties of surgical sutures can be improved by loading therapeutic agents having anti-inflammatory, analgesic, antibacterial, antibiotic, etc. effects (2,3). Either sutures or drug-eluting sutures along with their needles should meet the quality requirements, which are indicated in the related international standards such as the standards of International Organization for Standardization-ISO (4), European Standardization Organizations (5) and the American Society for Testing and Materials-ASTM (6). They should also meet the quality requirements of sutures stated in the pharmacopoeias such as European Pharmacopoeia-EP (7) and the United States Pharmacopoeia-USP (8). In this study, quality requirements for surgical sutures and quality control testing methods are evaluated in terms of related international standards and pharmacopoeias.

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7. https://www.edqm.eu/en/news/10th-edition-100-102-subscriptions-now-open
8. https://www.uspnf.com/

Keywords: Surgical sutures/threads, smart sutures, standards, pharmacopoeias, quality control, test methods

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PS-021

Drug eluting medical devices

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Drug eluting medical devices have gained great importance in recent years, mainly due to advances in materials and technology allowing design and development of multifunctional medical devices including wearable, inserting and implantable smart medical devices. Drug eluting medical devices can increase the potential success of wound dressings, scaffolds, sutures, stents, urinary catheters, bone prosthesis, central venous catheters, intrauterine devices, etc. owing to the improvement of their biocompatibility and, the physiological and immunological response of the body and the healing of related tissues. Medical devices can be loaded with anti-inflammatory, analgesic, antibacterial, antibiotic, regenerative agent, contraceptive agent, etc. either inside/inner parts or on their surface. In addition to the physical and functional properties of loaded medical devices, elucidating of the drug releasing profile of them is also important for achieving their intended use. In this study drug eluting medical devices are presented in terms of their manufacturing process and application areas in the medical field.

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Keywords: Drug-eluting, smart medical devices, wearable, inserting, implantable

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PS-022

Animal tests for quality control of biopharmaceutics and medical devices

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Quality control tests are necessary to prove efficacy and safety of biopharmaceutical products and medical devices. Among these tests animal tests are mainly used to evaluate the potency and toxicity the products. Potency tests for different products such as Multiple Dilution Mouse Challenge Method and Neutralization Test (Effective Dose -ED50, Protective Dose 50-PD50) are used for the efficacy of products. Acute toxicity test, pyrogen test, in vivo sensitization, in vivo irritation, genotoxicity, in vivo biocompatibility, chronic toxicity test, hemocompatibility is used for safety. These tests are mainly taking part in the European Pharmacopoeia, the United States Pharmacopoiea, Japanese Pharmacopoeia, International Organization for Standardization-ISO Standards and Organization for Economic Co-operation and Development-OECD methods. In this study, in vivo animal tests for the quality control of biopharmaceutical products and medical devices are compared and evaluated in the scope of related regulations.

Keywords: Biopharmaceutics, medical devices, quality control, animal tests, potency, safety

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PS-023

Vaccines produced by recombinant DNA techniques

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Biological substances designed to provide specific protection against a particular disease are defined as vaccines. Traditional (conventional) vaccines, which are considered the basis of vaccines used for active immunity; live attenuated, inactive (dead) and inactive toxin (toxoid) vaccines and all of the organism is used in these vaccines. With the development of biotechnological methods, recombinant protein vaccines and DNA vaccines began to be produced. Thus, vaccines can be developed against viruses that cannot be cultured. Genes encoding an immunogenic protein in recombinant vaccines are isolated and then transferred to a recipient cell. After the recombinant antigen is expressed in this vector, it is purified from the produced cells by biochemical techniques. Hepatitis B and Influenza (influenza) vaccines are examples of these vaccines. DNA vaccines consist of synthetic DNA containing the gene encoding the disease-agent protein. This DNA is usually injected into intramuscular or intradermal. The antigen (DNA) is directly expressed in a similar way to viral infection and initiates an immune response in the host (1-3,6). In this study, vaccines that are produced by recombinant method, method of manufacturing, the advantages and disadvantages of recombinant vaccines compared to conventional vaccines, and their evaluation in terms of quality assurance are presented (1,4,5).

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Keywords: Vaccine, recombinant DNA, manufacturing, quality assurance and control

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PS-024

Therapeutic biological actives from marine sources

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The high biological diversity of oceans and advances in technology has gained the opportunity to obtain new bioactive compounds, which can be used as new therapeutic agents. Many approaches used to acquire them such as metamogenomics, genome mining and heterologous biosynthesis approaches (1). There have already existed marine based natural products approved by some authorities (2). In addition, there are many ongoing studies on many isolated marine bioactive compounds in different stages of preclinical and clinical studies. Among the remarkable therapeutic activities of these compounds are anticancer/antitumor, anti-inflammatory, immunomodulatory, analgesic, antiviral and antibiotic activities (1,3). Moreover anti-oxidative, anti-thrombin and anti-diabetic activities of some marine bioactives are investigated (4). In this study, recent developments in therapeutic biological actives from marine sources are presented in details with their isolation, therapeutic activities and pharmaceutical development for being a medicine.

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Keywords: Therapeutic bioactive compounds, marine sources, anticancer, isolation, pharmaceutical development

PS-025

Recent developments in advanced therapy medicinal products

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Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells, which use for the treatment of disease and injury. ATMPs can be classified into four groups as gene therapy medicines, somatic-cell therapy medicines, tissue-engineered medicines and combined ATMPs that contain one or more medical devices as an integral part of the medicine (1). Since the potential of ATMPs to provide marked and durable responses, often with just a single treatment, for a diverse array of serious diseases and disorders, in recent years, related innovators and authorities have focused on their development and evaluation (2). In this line main issues such as clinical (trial, efficacy, safety), financial (funding, reimbursement), human sources (qualified), regulatory (product dossier and evaluation), scientific (research, study design, knowledge gap) and technical (manufacturing, quality standards, starting materials) issues have studied and monitored (1-5). In this study, recent developments in ATMPs and their evaluation are presented.

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Keywords: Advanced therapy medicinal products, development, manufacturing, clinical trial, quality standards

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PS-026

The Effect of Anti-PD-L1 Treatment on Metastasis in PD-L1 High and Low Tumor Cells

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BACKGROUND: PD-L1 expression by tumor cells has been linked to poor prognosis and a better clinical response to anti–PD-1/PD-L1 therapy in a wide variety of cancers. Anti-PD-1 and anti-PD-L1 monoclonal antibodies (MoAb) have been used in the routine treatment of many solid tumors, but only 25% of patients respond successfully to this therapy. Despite being studied extensively in tumor immune evasion, tumor intrinsic roles of PD-L1 and its regulatory roles in EMT, tumor initiation, treatment resistance and metastasis are not precise.

OBJECTIVE: The aim of this study was to investigate the association between PD-L1 expression levels and EMT-related genes in tumors.

METHODS: Eleven different human tumor cell lines were screened for PD-L1 expression by flow cytometry. We have tested the three different doses (40, 20, and 2 µg/ml) of anti-PDL1 (avelumab) on tumor cell lines with PD-L1 expression over 50% (LNCAP; prostatic carcinoma and MDA-MB-231; breast cancer) and the cell lines with low or negative PD-L1 expression (less than 2%) (DU145; prostatic carcinoma and MDA-MB-435; melanoma). The expression levels of EMT-related genes and proteins of tumor cells were examined by RT-PCR, ELISA, and Western Blotting.

RESULTS: Anti-PD-L1 MoAb administration significantly reduced the TGFB1 and bFGF expressions in both PD-L1 positive and negative cells. On the contrary, E-cadherin and EGFR expressions were increased in PD-L1 high cells and decreased significantly in PD-L1 low cells ($p < 0.05$).

CONCLUSION: This study demonstrates that EGFR and E-cadherin expressions were enhanced by anti-PD-L1 antibody in PD-L1 high tumors, that suggests, a relationship between PD-L1 expression levels in tumors and tumor progression, metastasis development, and treatment efficacy. This relation may provide a rationale for the possible combination of anti-PD-L1 and EGFR targeted agents in patients with PD-L1 high tumors.
Keywords: Anti-PD-L1, EGFR, Metastasis, PD-L1

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PS-027

Tissue engineering bioreactors

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Over the years; cell culture has been used in medical sciences. There are many disadvantages in the 2D cell culture such as not mimicking the in-vivo environment, mass transfer, gas exchange, waste management, inability to real time monitoring of culture medium, harvesting the cells by enzymatic methods and eliminating the cell products from the medium during replacement (1,2). Bioreactors are 3D culture systems developed to eliminate the disadvantages of the static culture (3). Bioreactors can be utilized to tailor made treatment, organ support systems, increasing the number of cells before autologous cell implantation, in-vitro tissue/disease modelling in pharmaceutical research and producing recombinant human proteins, vaccines, drugs and tissue grafts (4). There are several types of commercially available bioreactors such as spinner flasks, rotating bed/wall bioreactors, perfusion bioreactors, mechanical stimuli bioreactors and hollow fiber membrane bioreactors (5). In this study, potential technologies and usage of bioreactors in tissue engineering are presented.

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Keywords: Tissue engineering, bioreactor, bioengineering, biomedical engineering

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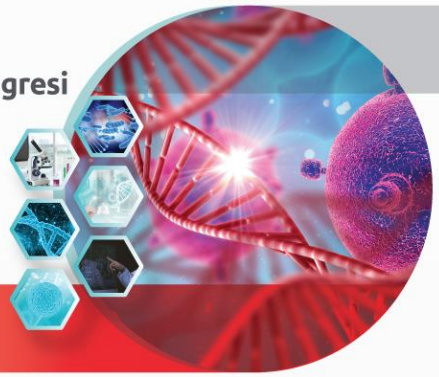

PS-028

Biotechnological Vaccines And New Approaches

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Vaccination is one of the most effective prevention measures in the fight against infectious diseases. Although has been used successfully for many years, the effects of conventionel vaccines like toxoid or inactive vaccines are limited or short-term, booster vaccination is required at certain time intervals. Furthermore attenue vaccines have a risk that will spread to the environment and regain the pathogen feature of the apatogen vaccine strain. Therefore developing new types of vaccine models that do not carry these risks, do not need repeated vaccinations and are free from side effects have gained importance in recent years. Biotechnology makes it possible to significantly improve the quality of vaccines and expand clinical practice.
Recombinant vaccine technology includes recombinant expression of proteins and viral vectors. Safety risks can be eliminated as production is achieved in a shorter time in more controlled bioprocesses than conventional methods. With the development of transient expression systems based on plant viruses provides another alternative platform for vaccine production. In 1986, the approval of recombinant hepatitis B vaccine, which is subunit vaccine, was a landmark in the development of the vaccine innovation system. Then new type of vaccines started to develop that are subunit vaccines, nucleic acid-based vaccines such as DNA, mRNA, viral vector vaccines and dendritic cell vaccines, edible vaccines which are produced edible format such as a plant, fruit or subproducts derived from this plant.
Conventional vaccines are only used to prevent infectious diseases. However, the development of biotechnological vaccines is trying to prevent many noninfectious human diseases such as cancer, type I diabetes mellitus, Alzheimer disease, allergies and drug addiction. In particular, tumor vaccines have an important place rather than other treatment methods in the fight with cancer. Therefore, to improve vaccine quality and clinical practices, vaccination studies should continue to be developed with combined vaccine techniques using both traditional and biotechnological methods.

Keywords: Biotechnology, recombinant vaccines, vaccination studies

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PS-029

Ulipristal – Hydroxyurea – Temozolomide Synergism in Blocking Growth of U373 Human Glioblastoma Cells

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Glioblastoma multiforme (GBM) is among the malignancies with worst survival rates and there exist not many chemotherapy options except temozolomide and irinotecan. Hence, development of innovative new treatment options is of essential need. In this study, we aimed to determine the effects of ulipristal (a progesterone antagonist), temozolomide and hydroxyurea on the growth and redox status of human U373 glioblastoma cells in vitro in order to develop an endocrino-chemotherapy protocol for GBM.

Keywords: Glioblastoma, hydroxyurea, MTT, temozolomide, total antioxidant/oxidant status, ulipristal

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PS-030

Outlook forecasting of pharmaceutical spending – insights from new drug launches, biosimilars, and growth in the use of specialty medicines

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The unprecedented expansion of access to healthcare globally over the past ten years – ranging from hundreds of millions of people in low- and middle-income countries getting access via government programs and/or rising incomes to the reduction in the uninsured populations in most countries.The global outlook for medicine use and spending affects the prospects of life sciences companies, insurers and the health of populations around the world. This study includes the latest predictions for the global pharmaceutical market along with geographic, therapy area and channel perspectives. It assesses the impact of new drug launches, biosimilars, and growth in the use of specialty medicines. The study also highlights ten areas to watch over the next several years that impact the use of and cost of medicines including estimated patent/exclusivity expiry dates for some of the best-selling biologicals.

Keywords: Biologics, biosimilar, health economics, pricing, reimbursement, research and development.

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PS-031

Gene Expression Profile Analysis in C57bl / 6 (B6) and Balb / C Models of Cystic Fibrosis

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INTRODUCTION: Cystic fibrosis (CF) is a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, affecting many organs such as the intestine, liver, pancreas and causing various symptoms. CF, also known as mucovisidosis, is an inherited autosomal recessive disorder (Figure 1). CF lung disease; Inflammation and infection due to neutrophil height consist of damage cycles that destroy the respiratory tract and prevent gas exchange. Data of mice with null mutation in the Cftr gene (BALB / c and C57BL/6) were evaluated.
PURPOSE: The aim of this study was to evaluate gene expression profiles for nonfunctional Cftr gene in C57BL / 6 (B6) and BALB / c models and to reveal the direct or indirect effects of the relationships between genes.
METHODOLOGY: In our study, we compared the 100 genes known to be associated with the disease for two models using the GenBank Overview-NCBI-NIH database in BALB/c and C57BL/6 mice. 12 BALB/c (5 controls, 7 patients); 11 C57BL/6 (5 controls, 6 patients) were included in the study. The relationship between genes was evaluated using the R database. Log-fold change (LogFC) values were taken into account when determining expression levels. LogFC -2.4, $p<0.05$; logFC 2,382, $p<0.05$ values were taken as criteria for determination of high expression levels.
RESULT: Models showed up to 36 gene up regulation; 64 genes showed down regulation. The Pyridoxal-dependent decarboxylase domain containing 1 (Pdxdc1) gene shows both up-regulation and down-regulation for two models. Arsk, Rab6b, Vnn1 genes show down-regulation for 5 BALB/c mice; 7 BALB/c showed up-regulation for the mouse (Figure 2).
DISCUSSION: The Pdxdc1 gene, which is frequently studied in BALB / c and C57BL / 6 mice, was found to be non-discriminatory for CF disease, contrary to the literature. More detailed investigations are needed for the location of the Arsk,Rab6b,Vnn1 genes in CF.
Resources:
-J.Buchanan, 2018, Microbial infection in cystic fibrosis, PATHOGENS & DISEASE, Paul Queen's University Belfast, UK.
-Haston CK, Cory S, Lafontaine L, Dorion G, Hallett MT, 2006, Strain-dependent pulmonary gene expression profiles of a cystic fibrosis mouse model, Physiol Genomics. 25(2):336-45.
-GenBank Overview-NCBI-NIH

Keywords: Cystic Fibrosis, Gene Expression, C57bl/6 (B6) and Balb/C Models

Figure 1:Function of CFTR gene in healthy and cystic cell(Paul J. Buchanan, Queen's University Belfast, UK)

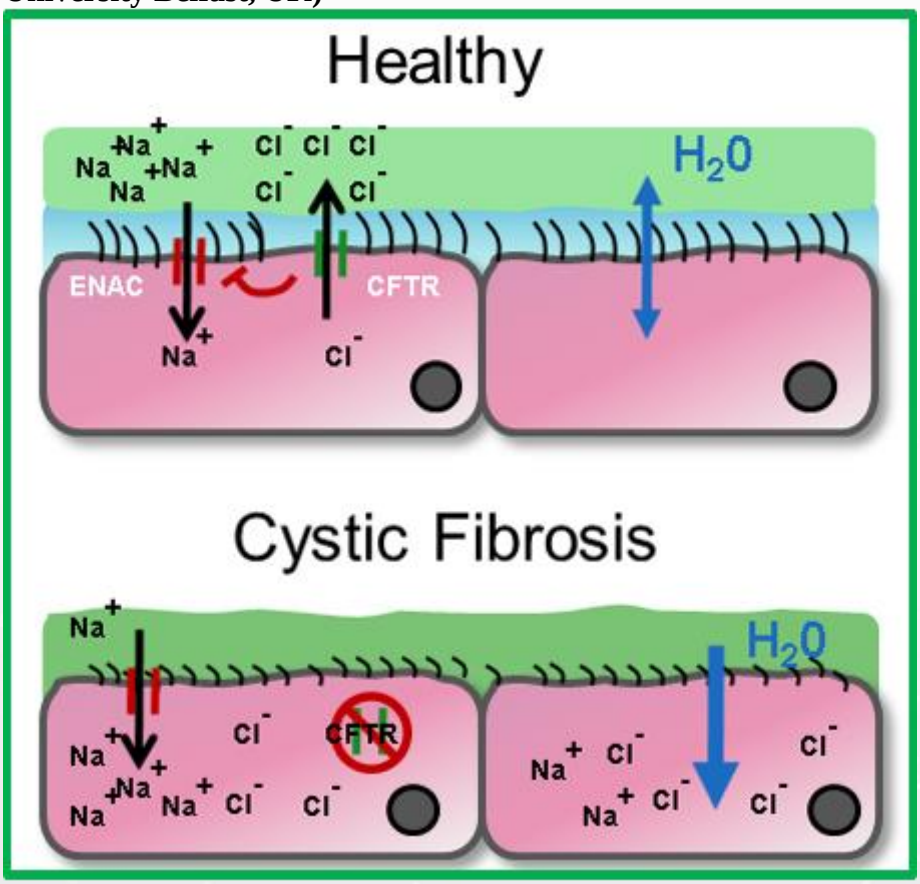
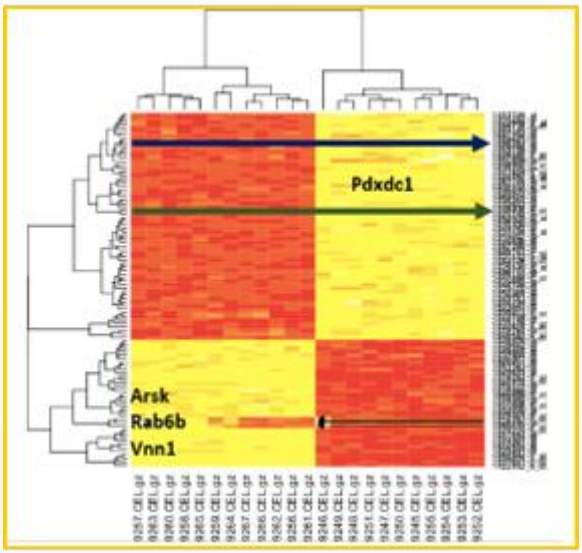


Figure 2: Evaluation of inter-gene relationships


Pdxdc1
Arsk
Rab6b
Vnn1

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PS-032

The Role of Anti-Angiogenic Treatment on Controlling the Metastasis

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Vascular endothelial growth factor (VEGF) and its receptors (VEGFR1 and VEGFR2) are known for their critical role in tumor angiogenesis. There is also evidence about their effect on tumor growth and metastasis as well, which could be done through the formation of new blood vessels and inducing metastatic related cytokines. In this study, we aimed to investigate the effect of axitinib, an anti-angiogenic drug inhibiting tyrosine kinases of the VEGF/VEGFR pathway, on the expression of metastasis-related cytokines secreted from tumor cells. For this study, MCF-7 (human breast cancer cell) and HT-29 (human colon cancer cell) cell lines were used. Cells were treated with three different doses of axitinib (103, 10, and 10-2nM). The expression of metastasis-related cytokines such as IL-17, IL-6, TGF-B, and NOS3 at the gene level was investigated by qPCR and at the protein level by ELISA. VEGFR1 and VEGFR2 gene expression levels were investigated by qPCR and protein levels by western blot and flow cytometry. We found a positive correlation between VEGFR1 and VEGFR2 with IL-17, IL-6, and NOS3, that suggests a promoting role for immunosuppressive tumor microenvironment. However, TGF-beta was negatively correlated with VEGF receptors. Axitinib decreased VEGFR1 and VEGFR2 expression levels in both cell lines. Likewise, it decreased the expression of IL-17, IL-6, and NOS3. On the other hand, the expression level of TGF-b was increased with axitinib. In conclusion, it may seem like anti-angiogenic treatment decrease the metastasis via suppressing IL-17, IL-6, and NO production in tumor cells; however, increasing the EMT as a result of increasing TGF-b level will result in metastasis. This may provide a rationale for the combination of anti-angiogenic treatments, immunotherapies, and TGF-b pathway inhibitors.

Keywords: Metastasis, İmmunosuppression, Anti-Angiogenic, VEGF, VEGFR1, VEGFR2

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PS-033

Investigation of the anticancer activity of coenzyme Q10 on the neuroblastoma cell line

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Coenzyme q10, also called Ubiquinone, is expressed as a hereditary antioxidant substance known to play a role in different biological activities. There was a relationship between the deficiency of coenzyme q10 and the emergence of several types of cancer. However, it is obvious that this substance is the subject of many scientific studies in the prevention and treatment of cancer. There is no study about Coenzyme q10 on neuroblastoma cell line, which has proven efficacy in breast cancer. This study was carried out to determine the anticancer activity of the coenzyme q10 on the neuroblastoma cell line. Neuroblastoma, SH-SY5Y cell line, which is one of the most common tumor types in childhood, was grown in laboratory conditions under suitable aseptic conditions. Coenzyme q10 was applied on the relevant cell line in a positive and negative controlled manner, with a sufficient number of repetitions in 5 different doses (15, 30, 60, 120, 200 µg / ml). 24 hours after the applications, MTT cytotoxicity test was performed. Changes in the cellular level were recorded with the xCelligence imaging system, which simultaneously reveals changes in cells treated with coenzyme q10 second by second. According to the results obtained in this direction, it was determined that the coenzyme q10 substance increased in anticancer activity at increasing doses. As a result of MTT cytotoxicity test, the most effective dose in terms of anticancer efficacy was determined to be 200 µg / ml. It has been determined that changes in the cellular level obtained with the xCelligence imaging system also provide data in parallel with the MTT cytotoxicity test.

Keywords: Neuroblastoma, coenzyme q10, xCelligence

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PS-034

Construction of Integrated Matematical Model for Gut Microbiota and Simulations for Autoimmune Diseases

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Istanbul Medeniyet University

The gut microbiota has an important role in the protection of human health in various diseases, and studies on the gut microbiota have gained importance for its diagnostic and treatment potential. Thousands of microbial species of the gut microbiota involved in complex cross-feeding with host cells and with each other. Metagenomic studies identify taxonomic groups of dominating microbial species in parallel to various physiological and pathological conditions; from epilepsi to obesity. Other works concentrate on genome-scale metabolic modeling (GSMM) of multi-species environment of gut flora in order to simulate certain physiological conditions, metabolite exchange, diet-microbiome and host-microorganism interactions between intestinal microorganisms. Here, we addressed a novel approach to merge these two common approaches to construct integrated GSMM for gut microbiota in parallel to autoimmune diseases. By doing so, presence of particular biomarkers for autoimmune disease will be investigated with the integrated GSMM at the metabolic resolution.

Keywords: Human gut microbiota, Genome Scale Metabolic Modeling (GSMM), Autoimmune diseases

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PS-035

An effective agent for neuroblastoma cancer: Syringic acid

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Syringic acid (SA) is a phenolic compound found in many fruits and vegetables. This compound is known to have antioxidant, antimicrobial, anti-inflammatory, neuroprotective and hepatoprotective effects. In many studies performed in SA, it was found that this substance exhibited antimitotic, apoptosis, inhibiting cell migration and decreasing cell proliferation on many cancer lines. However, in studies conducted to date, the effect of neuroblastoma cancer line, which is an extremely aggressive cancer type, has not been revealed. For this reason, this study was carried out to determine the anticancer activity of SA on the neuroblastoma cell line. For this purpose, the SH-SY5Y neuroblastoma cell line was grown in aseptic conditions in a suitable laboratory environment. When sufficient confluency was achieved, the SA compound was applied on the neuroblastoma cell line in a positive and negative controlled manner with sufficient repetition. 24 hours after applying SA in four different doses (12.5, 25, 50, 100 µg / ml), applications were analyzed by the MTT cytotoxicity test. Simultaneously, the same applications were monitored with the xCelligence imaging system by observing the changes at the cellular level. The data obtained revealed that SA exhibited more effective anticancer activity at lower doses. According to the results of MTT cytotoxicity test, it was determined that the highest anticancer efficacy was at the dose of 25 µg / ml. These data correlate with the data obtained from the xCelligence imaging system.

Keywords: syringic acid, neuroblastoma, xcelligence

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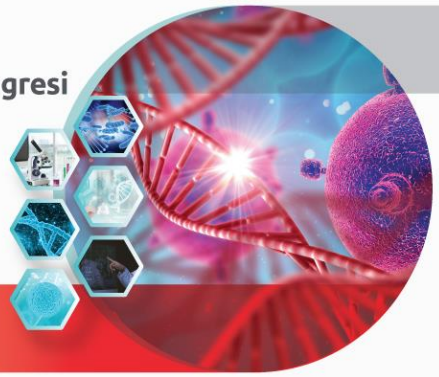

PS-036

Investigation of anti-tumor activity of Bacopa monnieri, known to be a neuroprotective agent, on neuroblastoma

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Neuroblastoma (SH-SY5Y), a malignancy of the developing peripheral nervous system that affects infants and young children, is a complex disease. Bacopa monnieri (Bacopa), has long been used in Indian traditional medicine (Ayurveda). Although both animal and clinical studies supported its role as a memory enhancer, sedative, analgesic, anti-epileptic and anti-inflammatory agent, recent studies have shown to be cytotoxic in several cancer cell lines including colon, lung and breast cancer. However, the anti-cancer effect of bacopa on neroblastoma has not been investigated yet. The aim of this study is to investigate the antitumor activity of Bacopa on SH-SY5Y. The SH-SY5Y cell line was grown in the normal cell culture medium. Different doses of Bacopa (10, 20, 40 and 80 µg/mL) were applied to SH-SY5Y cancer cell lines for 24 hours. The cytotoxicity was determined using both by MTT assay and real-time monitoring of cell viability by the xCELLigence system. According to our results, 40 µg / mL Bacopa was found to decrease cell viability compared to the negative control group (DMEM added group only) and positive control group (DMSO + DMEM). Also, the results of real-time monitoring of cell viability by the xCELLigence system correlate with mtt result ($P < 0.05$). CONCLUSION: Our studies have found that Bacopa has anti-tumor activity. Based on these findings, we think that the use of Bacopa will become widespread in the future in order to reduce the use of chemotherapeutics used in cancer treatment and to eliminate their side effects.

Keywords: Neuroblastoma, Bacopa, xCELLigence

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PS-037

Instant examination of the effect of citicoline on the neuroblastoma cell line

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Citicoline is an essential endogenous substance. It plays a role as a precursor in the synthesis of phosphatidylcholine, one of the cell membrane phospholipids, by the Kennedy pathway. Two essential components are revealed in oral or parenteral use. These released components, cytidine and choline, cross the blood-brain barrier and act on the central nervous system. In recent years, studies on citicoline have proved its neuroprotective properties with caspase-3 inhibitory activity.
Neuroblastoma is a type of cancer with a high prevalence in patients under 15 years of age. In this study, the anticancer activity of the citicoline on the SH-SY5Y neuroblastoma cell line was investigated. Citicoline was tested on 5 different doses (25, 50, 75, 100, 150 µg/mL) on the neuroblastoma cell line developed in cell culture. MTT solution, which provides viability measurement in the spectrophotometer device by transforming into a formazan crystal in live-cell mitochondria, was applied on the cell line 24 hours after dosing. Instantaneous counts of cells growing on 96 well plate surfaces with gold-plated bases were measured by Xcelligence to measure cellular viability. In this procedure cells in contact with the electronic sensor act as insulators that produce resistance to current flow. This resistance formed on the electrodes is expressed in Cell Index (CI). CI is a dimensionless value of the cell population that is in contact with the electrode. This measurement was performed simultaneously with the MTT test. These two measurements were shown similar results for the anticancer efficacy of citicoline at low doses.

Keywords: Xcelligence, Neuroblastoma, Citicoline

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PS-038

Advanced therapy medicinal products and health technology assessment principles and practices for value-based and sustainable healthcare across the European healthcare markets

Seray Nacar, Muhammet Ali Sahip, Asena Ayşe Genç, Tuğba Dolu, Banu Bayar, Harun Kızılay, Hakkı Gürsöz
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Advanced therapy medicinal products are medicines for human use that are based on genes, tissues or cells allowed by the European Medicines Agency. They offer groundbreaking new opportunities for the treatment of disease and injury. Several advanced therapy medicinal products have recently received marketing authorization in Europe, a trend that is likely to continue and will accelerate advanced therapy medicinal products are beginning to reach European markets. But, questions are raised about their value for patients and how healthcare systems should pay for them. Navigating the emerging challenges of gene therapies can be a daunting task across the entire healthcare space, and not just for biopharmaceutical companies. This study examines the current health technology assessment landscape for advanced therapy medicinal products and the emerging health technology assessment appraisal frameworks.

Keywords: Advanced therapy medicinal products, cross country comparison (France. Germany. Italy. Spain and the United Kingdom), healthcare funding, health technology assessment, pricing and reimbursement.

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PS-039

Addressing the health technology assessment of biosimilar pharmaceuticals

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Regulatory and reimbursement authorities worldwide increasingly employ Health Technology Assessment to assess the social consequences of using a particular medical intervention. Definitions of Health Technology Assessment vary between authorities. Health Technology Assessment helped develop more transparent and explicit methods of allocating limited healthcare resources. Across European countries, differences exist in biosimilar policies, leading to variations in uptake of biosimilars and divergences in savings all over Europe. The aim of this article is to provide an overview of the different initiatives and policies that may influence the uptake of biosimilars, undertaken by health authorities in different European countries and also examines the current Health Technology Assessment landscape for biosimilar and biosimilar policies.

Keywords: Biologics, biosimilar, health economics, health technology assessment

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PS-040

The importance of the biotechnologic and biosimilar products, and the market situation in Turkey and the World

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Department of Economic Assessment and Medicines Supply Management, Turkish Medicines and Medical Devices Agency, Ankara,Turkey

Biotechnological and biosimilars are defined in different ways by many regulatory agencies. In Turkey, these products are defined as “a product produced or extracted from a biological source that requires a combination of physicochemical biological tests with the manufacturing process and control to determine the quality and quality of the active substance”. Besides the treatment of chronic diseases, it is also used in the treatment of acute diseases. Examples of such innovative products include products such as recombinant blood products, hormones, monoclonal antibodies, vaccines, therapeutic enzymes, cytokines. Since biotechnological and biosimilar products are in protein structure, the most important criterion to be considered in their use is immunogenicity. The resulting immune response may vary depending on the factors associated with the disease, the patient, the drug. Therefore, the immunogenicity of these products should be evaluated in detail before they are licensed. Since the quality of the biotechnological and biosimilar product is highly affected by the production processes, the formulation, production and sterilization designs of these products are very important. Therefore, new technologies and new processes developed for these products cause increasing costs. Biotechnological and biosimilar products fall under the status of specially conditioned products in our country and are priced under the terms of the “The Legislation on the Pricing of Medicinal Products For Human Use”. According to the Legislation, both biotechnological and biosimilar products are price 100% of the external reference price. In conventional products, with the release of the first generic product to the market, price of the reference product (original product) is reduced to 60% of its external reference price. On the contrary, the price of biotechnological products is not reduced to 60% when the biosimilar is released. Thus, these products, which have high research, development and production costs and increasingly take place in the world market, are tried to be offered to our patients as treatment options in our country's market. With the expiration of the patent period of the original biotechnological drugs, the number of biosimilar products is gradually increasing. Our Ministry supports the production and exportation of these products.

Keywords: Biotechnology, biosimilars, marketing, pricing.

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PS-041

Plant stem cells in cosmetic products

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Epidermal stem cells are the main stem cells responsible for the permanent renewal of the epidermis. In recent years, studies have interest to find suitable methods for stimulation of the epidermal stem cells especially for an anti-aging effect. The most interesting among these studies is performed on plant stem cell culture technology. Plant-derived substances have many positive effects on the skin and have been used as main base in many cosmetic products for centuries. However, biologically active plant-derived substances produced in very small amounts by plants. Therefore another important use of plant stem cell culture is providing the high yield production of plant-derived substances. Plant stem cell culture are “new generation” of high quality natural products, produced by the modern plan biotechnology methods. Plant stem cell cultures are the source of a number of factors that support skin protection and regeneration processes, thereby preventing skin aging. Also in vitro and in vivo studies have shown that biotechnologic plant stem cell extracts usually showed stronger activities than the plant extracts obtained by the classical methods. Plant stem cell extracts derived from different plants have varying biological properties. Plant stem cells extracts from apple (Malus domestica) argan (Argania spinosa), alpenrose (Rhododendron ferrugineum), grape vine (Vitis vinifera), samphire (Crithmum maritimum) and tomato (Lycopersicon esculentum) have been studied clinically until today. Today, there are many and various cosmetic products containing plant stem cells, available on the market. Considering current data for cosmetic products containing plant stem cells in Turkey, 250 cosmetic product notification of which 21 are domestic and 229 are import are observed in The Product Tracking System of Turkish Medicines and Medical Devices Agency.
Consequently, plant stem cells appear to be a promising material for use in cosmetology because of their protective and regenerative properties. Studies, conducted to date, confirmed the potential of these cells in anti aging effect and the revealed their beneficial effects on epidermal stem cells, which is crucial to the process of skin renewal.
The aim of this poster presentation was to provide a review of the recent progress in plant cell technology for cosmetic application.

Keywords: anti-aging, cosmetic, cosmetic products, stem cells, plant-derived substances, plant stem cells

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PS-042

Introducing Boron Nitride Nanotubes To Drug Delivery Platform For The Treatment Of Cancer

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Among the cancer treatment options, chemotherapy plays a significant role in eradication and/or prevention of the cancer. The lack of selectivity of the drugs for tumor tissues and the development of systemic toxicity can be considered as limitations for the therapy. Minimizing the dose of drug, improving pharmacological profiles and the targeting to specific cells may fulfill the requirements of current approaches in cancer treatments [1,2]. Boron nitride nanotubes (BNNTs) which possess an excellent biocompatibility and a lower cytotoxicity can be regarded as promising materials in biotechnology [3]. In this study, we aimed to develop PEGylated, folic acid-bounded and imatinib mesylate, dexketoprofen trometamol loaded BNNTs. Simply BNNTs were dispersed in DMF and added dropwise into the chitosan solution which contained all formulation components. After all, BNNTs were observed to be coated with chitosan and all other components. The entrapment efficiency of imatinib mesylate was 89.4% and that of dexketoprofen trometamol was found to be 80.9%. It was hypothesized that the molecular recognition of cell specific receptors, targeting to tumor tissues and enhanced antitumoral activity can be achieved with these BNNTs. MTT cell viability test on WEHI164 cell lines which are originated from fibrosarcomas were also performed. The cell viability of BNNTs were 53.7%. These functionalized BNNTs immobilized the tumor growth comparing with the control group. BNNTs also inhibited the tyrosine kinase enzyme (63.8%). Their cyclooxygenase inhibitory effect was 89.7%. The interaction of BNNTs with the folic acid receptor was 92.2% that supports molecular recognition and active targeting. We also investigated the tissue distributions of BNNTs because the introduction of new inorganic materials (BNNTs) in biomedical research needs to knows its biosafety and their interactions with living matter. BNNTs showed tendency to accumulate in the tumor and liver. In conclusion, we demonstrated that prepared BNNTs have a great potential as a drug carrier

system, this high drug loading capacity, biocompatibility and exceptional high surface area of BNNTs can lead some extraordinary surface interactions with tumor cells.
This study is financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK,No.213M675).

Keywords: nanoparticles, boron nitride nanotubes, cellular activity, cancer treatment

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PS-043

Effects of Curcumin On Cell Proliferation In Neuroblastoma: An In Vitro Study

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Neuroblastoma (NBL) is the most common solid extracranial cancer found in children, appearing in the tissues of the sympathetic nervous system. NBL accounts for 15% of childhood malignancies and more deaths than any other cancer. Therefore, an effective treatment for NBL is still needed. Recently, natural compounds with low toxicity have gained increasing attention in the treatment and development of cancers. Thus, concomitant use of natural compounds with chemotherapeutic drugs represents an attractive strategy to increase the effectiveness of cancer treatment compared to single-drug treatment. Curcumin is a natural compound with anti-inflammatory and anti-cancer properties with promising potential for the development of therapeutic drugs for the treatment of cancer as well as neurodegenerative diseases. Here, we investigated the study of the antiproliferative effect of curcumin using the SH-SY5Y cell line. The SH-SY5Y cell line was grown in normal cell culture medium. Different doses of curcumin (50, 75, 100 and 150 µg/mL) were applied to SH-SY5Y cancer cell lines for 24 hours. The cytotoxicity was determined using both by MTT assay and real-time monitoring of cell viability by the xCELLigence system. As a result, the 150 µg/mL curcumin dose applied was found to reduce cancer cell viability by compared to the control ($P<0.05$). According to our findings, the cytotoxicity of curcumin, MTT test and real-time monitoring were in line with xCELLigence. This study showed that curcumin could be evaluated as a promising anti-cancer agent for NBL.

Keywords: Curcumin, SY-SH5Y, xCELLİgence.

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PS-044

Development of Nanoemulsion Containing Walnut Oil Essential Oil and Cytotoxic Agents for Cancer Treatment: İn Vitro Study

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INTRODUCTION: Recently, nanoemulsion formulations have attracted a lot of attention due to their potential in a wide variety of applications, including pharmaceutical, cosmetic and food industry applications. Nanoemulsions with a droplet size of 10 to 600nm are produced by mixing two immiscible liquids with or without an emulsifier containing ultrafine droplets. Hydroxyurea is an antineoplastic drug used in the treatment of other disorders such as essential thrombocythemia and desmoid tumors. On the other hand, 5-Fluorouracil (5-FU) is one of the most widely used drugs in anticancer treatment. Walnut tree is a perennial herb consisting of many components, especially its leaves are used as a source of medicine in traditional medicine. We aimed to determine the effects of walnut leaf essential oil, 5-FU and HU medicines on neuroblastoma cells by creating a single formulation with nanoemusions. Materials- METHODS: Neuroblastoma cells, Ataturk University (Erzurum, Turkey) was obtained from the department of Pharmacology. After 85-90% confluence rate was obtained in neuroblastoma cultures, drugs were added to the culture plates. The wells were exposed to two types of nanoemusulation formulations made with walnut leaf essential oil doses of 10-3, 5-FU (2.5, 5, 7.5 ve 10µg/mL) and Hydroxyurea (25,50,75 ve 100µg/mL) for 24 hours. One of the groups contains walnut leaf essential oil, while other nanoemusion groups are placed in walnut leaf essential oil as a control group. The 14 groups formed were applied to the neuroblastoma cell culture, after 24 hours it was read at a wavelength of 570nm. Methylthiazole diphenyl tetrazolium (MTT) results were analyzed by one way ANOVA method in SPSS, IBM 21.00 program.
RESULTS: In our control group, the vitality was defined as 100% and the other groups were rated accordingly. When compared with the control group, the nanoemusure group with WLEO 10-4 concentration 5-FU 2.5µM and HU 25µM showed the lowest viability rate with 28%. Discussion-CONCLUSIONS: In the studies of Salehi et all, they found that essential oils represent a feasible and efficient approach to nano emulsification, modulating drug release, reducing volatility, increasing bioactivity, and reducing toxicity. In our study, it was observed that the survival rate of cancer cells decreased significantly.
Keywords: Nanoemulsion, Walnut Leaves Essential Oil, Hydroxyurea, 5-FU

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PS-045

In vitro Examination of the Antiproliferative Effects of Ulipristal Acetate and Aloe Barbadensis Miller on the Neuroblastoma Cell Line

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OBJECTIVE: Anti-proliferative effects of ulipristal acetate (UPA), well known for inhibition or delay of ovulation in women, have been observed. The relevance of plants worldwide continues to increase in terms of health. Aloe barbadensis (AB), a plant that has attracted attention recently, has been revealed by studies such as wound healing and angiogenesis. In our study, we aimed to examine the effects of these substances by observing how they change cell proliferation.
MATERIAL-METHOD: Neuroblastoma cell line (SH-SY5Y) were obtained from the medical pharmacology department of Ataturk University. Aloe barbadensis plant extract was obtained from Nurbal, Istanbul. After 85-90% confluence rate was obtained in neuroblastoma cultures, drugs were added to the culture plates. Groups of 25, 50, 100, 200 µM UPA and 100, 150, 200, 250µg/mL aloe barbadensis were prepared both separately and in combination. The 14 groups formed were applied to the neuroblastoma cell culture, after 24 hours it was read at a wavelength of 570 nm. Methylthiazole diphenyl tetrazolium (MTT) results were analyzed by one way ANOVA method in SPSS, IBM 21.00 program.
RESULTS: In our control group, the vitality was defined as 100% and the other groups were rated accordingly. UPA ve AB decreased viability of neuroblastoma due to dose increase. The decrease in viability with similar results was also observed in combination groups. The group with the lowest viability rate was obtained in UPA 25 µM (88%) and AB 100 µgr / mL (86%) groups. The lowest viability rate observed in the proliferation of neuroblastoma was observed in the UPA200 µM + AB 250 µgr / mL group with 53% ($P < 0.001$).
DISCUSSION and CONCLUSION: So-Jin Shin et al examined the proliferative effects of UPA on leiomyoma cells. In their study, they observed that UPA used at doses of 1 and 10 µM significantly reduced proliferation. In our study, we obtained similar results and observed effective results.

Keywords: Aloe Barbadensis Miller, Antiproliferative, Neuroblastoma, Ulipristal Acetate

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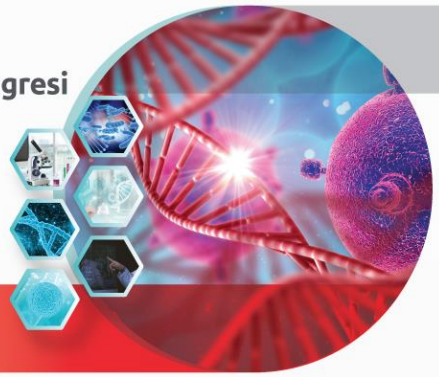

PS-046

Preparation and Characterization of A Nanoemulsion Containing Soy Extract Obtained By Plant Cell Culture Technology Against Hyperpigmentation

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Pigmenter disorders are the third most common dermatological disorder and cause significant psychosocial impairment in patients due to their visibility. They are common in middle-aged women and are related to endogenous and exogenous factors, such as use of cosmetics and perfumes, and exposure to sun radiation. These disorders are difficult to treat, hence, the need for skin lightening agents including cosmeceuticals. Due to the potential side effects of current treatments, there is a rising trend towards the development of effective and safe formulations containing naturally derived extracts for hyperpigmentation. Soy (glycine soja) has evidenced to be both powerful and safe. A number of skin care products containing soy are available to improve hyperpigmentation. Skin lightening effect was demonstrated to be seen after 12 weeks of twice daily application.
Unfortunately, new plant derived ingredients are limited because several plants of cosmetic interest are not to be used due to following facts: the plants contain toxic metabolites, the plants grow too slow and a seasonal harvesting is not possible, the concentration of plant constituents differ from harvest to harvest or the plant is endangered and not allowed to harvest. With the plant cell culture technology we bring complete new aspects in the development of novel cosmetic plant derived actives.
For this purpose, we aimed to develop a nanoemulsion containing cell-cultured soy extract to benefit from the anti-hyperpigmentation potantial of soy sufficiently. Based on the results of the preliminary stability tests (termal test and centrifugation test), 8 formulations were selected which were suitable for further studies. When the mean droplet size and PDI values of the selected formulations were examined, it was observed that the formulations had acceptable mean droplet sizes (minimum 128.5±1.6 nm; maximum 265±0.9 nm) and PDI values (minimum 0.057±0.006; maximum 0.186±0.003). The formulation composition containing 5% w/w soy extract with 10% (w/w) surfactant (Pluronic® F-68) and 10% (w/w) cosurfactant (Transcutol® HP and Lauroglycol™ 90) was selected as the optimized formulation with a 30 minute sonication time. Consequently, the use of cell cultured soy extract laden nanoemulsion via topical skin route may provide an effective strategy in the treatment of hyperpigmentation.

Keywords: hyperpigmentation, nanoemulsion, plant cell culture, soy extract, topical

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PS-047

An Application in Medicine with Deep Learning Approach and Estimation of Related Factors

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AIM: In this study, it is aimed to classify breast cancer and identify related factors by applying deep learning method on open access breast cancer dataset. MATERIALS-METHODS: In this study, 11 variables related to open access breast cancer dataset of 569 patients shared by the University of Wisconsin were used. The deep learning model for classifying breast cancer was established by 10-fold cross-validation method. The performance of the model was evaluated with accuracy, sensitivity, specificity, positive/negative predictive values, F-score and area under the curve. Factors associated with breast cancer were estimated from the deep learning model. RESULTS: Accuracy, specificity, AUC, sensitivity, positive predictive value, negative predictive value, and F-score values obtained from the model were %94.91, %91.47, 0.988, %96.90, %95.42, %95.14, and %96.03, respectively. In this study, when the effects of the variables in the dataset on breast cancer were evaluated, the three most important variables were obtained as area mean, concave points mean and symmetry mean, respectively. CONCLUSION: The findings of this study showed that the deep learning model provided successful predictions for the classification of breast cancer. In addition, unlike similar studies examining the same dataset, the importance values of cancer-related factors was estimated with the help of the model. In the following studies, breast cancer classification performances can give more successful predictions thanks to different deep learning architectures and ensemble learning approaches.

Keywords: Breast cancer, artificial intelligence, deep learning, classification

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PS-048

Detection of Valine-Glutamic Acid Alteration *via* FAM-Labelled Probe and Streptavidin-Conjugated Magnetic Particule In Pregnant Sickle Cell Anemia Patients cffDNA

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INTRODUCTION: Sickle cell anemia (SCA) is an autosomal resessive hematopoetic genetic disease associated with valine (V) to glutamic acid (E) alteration at 6. amino acid within the Hemoglobin beta subunit (HBB) gene. Early detection of genetic diseases is essential via non-invasive prenatal testing (NIPT). Recently, NIPT technology is focused on cell free fetal DNA (cffDNA) isolation and detection of fetal mutations before early second-trimester period of pregnancy. Our aim in this study was to detect fetal HBB gene mutation using cffDNA from maternal serum, through both 5' FAM-labelled mutant HBB gene probe and streptavidin-coated magnetic nanoparticles by using chemiluminescence detection system.
MATERIALS-METHODS: cffDNA isolation was performed from 10 ml whole blood samples of pregnant control and sickle cell anemia patients by MagMAX Cell-Free DNA (cfDNA) Isolation kit (ThermoScientific, Catalog number: A29319). HBB gene was amplified from isolated cffDNA samples and biotinylated by Biotin 3'-End DNA labeling kit (ThermoScientific, Catalog number: 89818) through manufacturer's instructions. Dot-blot method was used to detect 5'-FAM labelled probe and streptavidin-coated magnetic nanoparticles to capture 3'-biotinylated HBB PCR products.
RESULTS: Pregnant HBB wild type (wt) and mutant (mut) women were included in the study. Total 30-42 ng/µl cffDNA was isolated from 10 ml blood samples. HBB wt gene product (3-0,3 ng) was amplified by PCR and %78-30 biotinylation was achieved respectively. HBB mut gene products (42-0,042 ng) were hybridized by 5'-FAM labelled mutant HBB probe and binding affinity was determined by dot-blot and visualized by Chemi-Doc MP. We also determined HBB mut probe binding efficiency by streptavidin-coated magnetic nanoparticles and 3'-biotiynlated cffDNA by chemiluminescence.
CONCLUSIONS: By this study, we determined that chemiluminescence detection system might be used for diagnosis of HBB gene mutation (V/E) after cffDNA collection from serum and streptavidin-coated magnetic nanoparicules are benefical for single nucleotide mutation in SCA.

Keywords: cffDNA, mutation, Sickle Cell Anemia, streptavidin-conjugated magnetic nanoparticule

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PS-049

Fast Curable and Bio-inspired Surgical Adhesives for Sternal Closure: Eliminates Disadvantages of Steel Wire

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OBJECTIVE: Sternal closure is the reunification of the thorax that opens after open-heart surgeries. Currently, mechanical methods (steel wire) are used for sternal closure, and high mortality rates are observed due to the sternum not being closed properly [1,2]. The reason for this high number of deaths are factors such as disadvantages of mechanical methods and infections. In the study, a bioadhesive formulation was developed to eliminate these problems. In this context, a bioadhesive with fully biocompatible, biomimetic design, UV-curable and injectable form, with fast and strong adhesive properties was developed. MATERIAL-METHODS: The formulation includes a two-component, UV-curable polyurethane-acrylate system. The first component in the formulation is an injectable pre-polymer containing double-bonded ends. The second component is composed of dopamine methacrylamide and UV-curing agent. Formulation was cured in 5-15 minutes by exposure to UV light with dopamine methacrylamide and photoinitiator. RESULTS: The final adhesives were characterized structurally, thermally and morphologically. In this context, the adhesive strength of the adhesive formulation was found as 783 kPa in calf bone as ex vivo, and its strength was determined at least two times higher than commercial adhesives. Furthermore, in advanced biochemical analyzes of the formulation, the biocompatibility against L-929 cells was 83%, and in vivo biocompatibility in Wistar albino rats was found to the same as commercial polypropylene. Finally, in the sternal closure model with rats, it showed significantly lower inflammation compared to steel wire and commercial adhesives and showed very close results to control. CONCLUSION: As a result, fast curing, high adhesive strength, antibacterial and biocompatible adhesive formulations were developed and used in sternal closure.
-This project was supported by TUBITAK-KBAG-116Z501.
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Keywords: Sternal closure, tissue adhesive, polyurethane-acrylate, fast-curing, biocompatibility

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PS-050

Make Hospitals Green: A proposal for regulation on reprocessing of single use medical devices

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Reprocessing of single use devices (SUDs) for reuse has been a common approach since the late 1970s. With the aim of cost and waste reduction almost in all countries reprocessing has been happening whether with or without regulations and quality standards. The purpose of this study is to provide high quality reprocessing with an efficient regulation.

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2-Soylu, I., Tek Kullanımlık Tıbbi Cihazların Yeniden İşlenmesinin Risk ve Faydalarının Araştırılması, Diğer Ülkelerde Uygulanan Modellerin İncelenmesi ve Etkin Uygulama Modelinin Belirlenmesi, Uzmanlık tezi, Sağlık Bakanlığı, 2018.

Keywords: disinfection, medical device, reprocessing, single use device, sterilization

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PS-051

Development of Ciprofloxacine Loaded-Antibacterial Hydrogels for The Treatment of Vesicoureteral Reflux (VUR)

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Vesicoureteral Reflux (VUR), which is defined as urine escaping from the bladder to the ureter, is a disease that can produce dangerous results especially among children and women. Surgical interventions are mostly used for its treatment which causes some problems. Therefore, in order to overcome these problems, searches for new treatment methods continue. Recently, treatment has been brought to the agenda by injections that prevent urine leakage by compressing the urinary canal. In these treatments, polydimethyl siloxane microspheres or dextranomer-hyaluronic acid formulations are mostly used. However, in these applications, it is important that the injected structure remains adherent and does not move in the area where it is applied. Otherwise, if the injected formulation spreads, it can not perform its compression. Another target to be exceeded in such applications is the continuity of the treatment once the application is performed. In order to this, the continuity of the treatment should be naturally achieved by either providing a tissue formation during the application of a synthetic and biocharging polymeric structure or narrowing the enlarged duct entrance. At the same time, removing the area to be applied from infection risk is another important issue. Therefore, in this study, hydrogel systems with antibacterial mechanical barrier properties were prepared. For this, injected barriers in hydrogel form containing 80-250 µm dextranomer particulate structures were prepared. The hydrogels were formed by changing the mesh structure, especially the pore amount, pore size and cross-linking ratio, and swelling structures at different rates. The structures obtained were doped with different rates of ciprofloxacine and provided a structure capable of releasing antibacterial and medication. Characterization of the structures obtained was carried out by Fouriyer Transform Infrared spectrophotometer (FTIR) and scanning electron microscope (SEM). Thermal properties were determined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) techniques. Swelling levels and water holding capacity of the synthesized structures were determined. In addition, drug release studies were carried out and antibacterial properties were controlled by disk diffusion technique. According to our results, ciprofloxacine loaded-antibacterial hydrogels may have a good alternative for VUR treatment.
This project (TKP-2019-1955) was supported by Inonu University.
The authors would like to thank EKSPO FARMA Pharmaceutical Medical Industry and Trade Company for providing hydrogels.

Keywords: Vesicoureteral Reflux, Ciprofloxacine, Polymeric microspheres, Cross-linked polymers, Hydrogel

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PS-052

Investigation of the relationship between rosacea and gut microbioma

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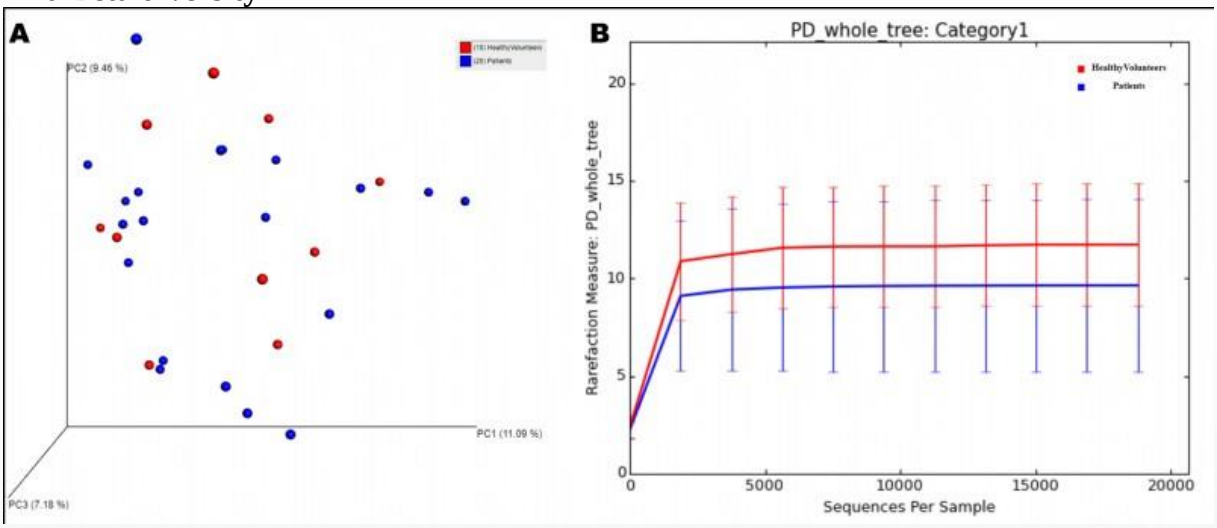
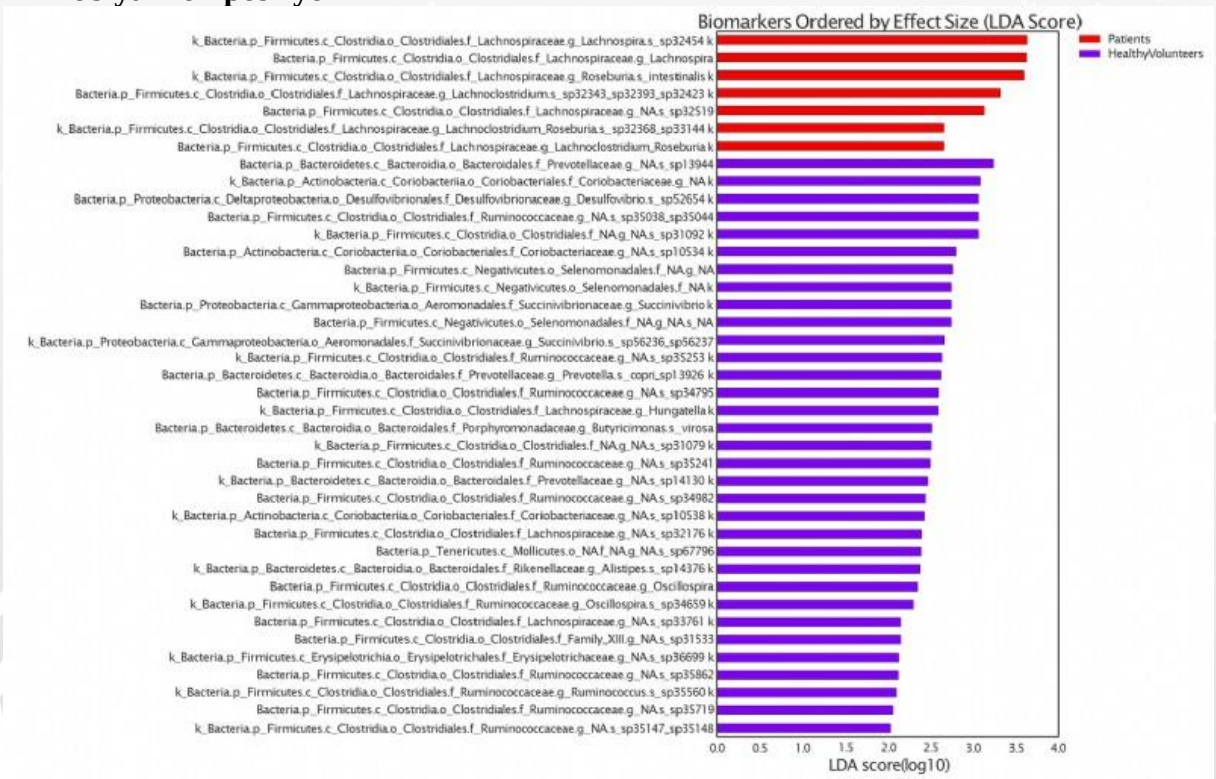
Rosacea is a common chronic inflammatory dermatosis that affects about 10% of the population. Although various environmental stimulants and endogenous factors have been shown to stimulate the innate immune response and abnormal neurovascular signaling in the etiology, the variety of clinical forms leads to a poor understanding of the pastophysiology of rosacea. In this study, we aimed to determine the relationship between rosacea disease and intestinal microbiome. For this purpose, 20 patients with clinical diagnosis of rosacea in the Education and Research Hospital of Sakarya University and 10 healthy volunteers with age and sex matched to the control group were included. 16s rRNA sequence and metagenomic analyzes were performed from faecal samples. We determined the relationships between various changes in the rosacea clinic and intestinal microbiome. According to the results of metagenomic DNA analysis in rosacea patients according to healthy volunteers; Lachnospira, Lachnoclostridium, Roseburia and Roseburia intestinalis were found to be higher and Coriobacteriaceae, Ruminoococcaeae, Butyricimonas virosa and Clostridiales bacteria were found to be higher. These differences were thought to be related to indirect dysbiotic pathways with rosacea clinic. Since only one study examining the relationship between rosacea and intestinal microbiome can be reached in the literature, it is needed to have more and more sampling studies in order to make sense of the microorganisms that stand out.

Keywords: Next generation sequencing, Microbiome, Rosacea

Alfa Beta diversity



mikrobiyal komposizyon



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PS-053

Determination of Antibiotic Susceptibility of Gram Positive and Gram Negative Bacteria by Flow Cytometry

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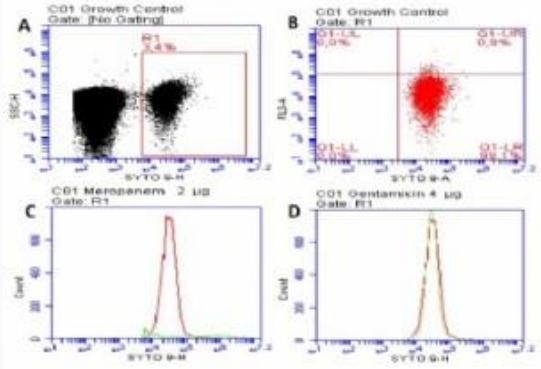
BACKGROUND: Today, antibiotic resistance has become a global problem. It is very important to determine the sensitivity of antibiotics in order to prevent antibiotic resistance and to guide the treatment earlier. In this study, it was aimed to shorten the time to determine the in vitro antibiotic susceptibility of gram positive and negative bacteria by using flow cytometry method.
Materials/METHODS: In this study, six standard strains and 11 clinical isolates were examined. Antibiotic susceptibility of all strains; VITEK 2 automated system was tested with liquid microdilution and flow cytometric method. Antibiotic dilutions were determined according to the current EUCAST guidelines. Prior to flow cytometric analysis, suspensions of microplate bacteria were incubated at 120 rpm and 35-37 ° C for 2 hours and then taken into 100 µl reading tubes. Then, 10 Syl Syto 9 and 10 Propl Propidium iodide dye were added to each tube and incubated for 15 minutes in dark environment. In flow cytometric analysis, reproduction control wells and antibiotic wells were compared by overlapping on the FL1-H graph and a reduction in bacterial count of at least 55% in antibiotic wells was accepted as the sensitivity criterion.
RESULTS: The antibiotic susceptibility determined by flow cytometric method and the agreement between BMD and VITEK 2 system were as follows; two E. coli strains (92.3%, 84.6%), four K. pneumoniae strains (88.7%, 81.8%), two P. aeruginosa strains (73%, 73%), two A. baumannii complex strains (81.8%, 81.8%) for two S. aureus strains (86.1%, 81.2%), three Enterococcus spp. strain (81.8%, 88.8%)(Figure).
CONCLUSIONS: Although antibiotic susceptibility tests performed with flow cytometric method showed a correlation between BMD and VITEK 2 system, it was found that the gram negative bacteria had higher compliance than gram positive bacteria. Flow cytometry shortens the antibiotic susceptibility test result time by an additional 12-14 hours. However, it has not yet been standardized to be used in all laboratories. More detailed and comprehensive studies are needed to determine antibiotic susceptibility by flow cytometry.

Keywords: Gram positive, Gram Negative, Flow cytometry

Dot blot graphical images of the gating process of reproduction control wells by flow cytometric method of K. pneumoniae 700603 ATCC strain



Amikacin and ceftazidime susceptibility overlay graphics (Red Peak: Reproduction Control Well, Green Peak: Antibiotic Well) A: Gating procedure on cells stained with fluorescent dyes

in the SSC-H / FL1-H (Syto 9-H) channel of Klebsiella pneumoniae ATCC strain 700603. B: Dot blot plot of cells in the FL3-A (PI-A) / FL1-A (Syto9-A) channel of gated cells. Result: Sensitive. C: Ceftazidime 4µg / ml sensitivity to show the overlay graph. Result: Resistant. D: Cefoxitin screening (ESBL screening) susceptibility status overlay graph to show. Result: ESBL Positive

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PS-054

Estimation of Risk Factors Related To Heart Diseases with Multilayer Perceptron Model

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İnönü Üniversitesi

INTRODUCTION: Cardiovascular disease is a class of diseases that involve the heart or blood vessels. Heart disease refers to many diseases such as coronary heart disease, heart failure, and heart attack. Every year, approximately 647.000 people die in the United States from heart disease (1). Genetic and environmental risk factors have been identified as a result of numerous studies for the determination of heart disease risk factors (2). In this study, the Multilayer Perceptron (MLP) model was constructed to predict the risk factors related to heart disease in both genders.
MATERIALS-METHODS: The dataset used in this study were was taken from the www.kaggle.com/ronitf/heart-disease-uci. The relevant dataset consisted of 270 individuals, 13 predictors (demographic and clinical) and 1 response/target variable (Presence presence and absence of heart disease). MLP model was constructed to predict heart disease. Model performance was evaluated using overall accuracy, the area under the ROC (Receiver Operating Characteristics) curve (AUC), sensitivity and specificity metrics. IBM SPSS Statistics 252 software was used in the analysis.
RESULTS: The performance metric values for accuracy, AUC, sensitivity and specificity were obtained with 95% CI, 0.876 (0.79-0.937), 0.935 (0.877-0.992), 0.921 (0.786-0.983) and 0.843 (0.714-0.93), respectively.
Discussions: The MLP model, trained using 13 predictive variables, showed a remarkable performance to predict/classify heart disease. Also according to the relevant model findings; blood pressure, number of major vessels colored by fluoroscopy and cholesterol variables were found to be the three most important variables in the diagnosis of heart disease. Due to the predictive performance in this study, the MLP model can be recommended to clinicians as a clinical decision support system.
Key words: Heart disease, Multilayer Perceptron, Risk Factors, Prediction, Clinical Decision Support System.

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Keywords: Heart disease, Multilayer Perceptron, Risk Factors, Prediction, Clinical Decision Support System.

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PS-055

ASSESSMENT OF ASSOCIATION RULE MINING USING INTEREST MEASURES ON THE GENE DATA

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[2]İnönü Universitesi

Data mining is discovery process of beneficial information, not revealed from large-scale data beforehand. Association rules are one of data mining techniques. Support and confidence are basic measures in order to assess unearthed rules via that technique. Rules, used for support and confidence measures, are correct but not so strong. Therefore, interest measures are used, besides two basic measures, with the aim of obtaining stronger rules. The main goal of this study is to constitute a strong association rules by using the studied data of 225 patients suffering from acute myocardial infarction (AMI) and getting treatment in coroner intensive care unit of Firat University Department of Medical Faculty. The patients have also carrying the genotyping of the FNDC5 (rs3480, rs726344, rs16835198) polymorphisms. Apriori algorithm which is one of the association rules methods was used to obtain the rules. After analyzing data in the scope of confidence and support measures, 159 rules are revealed. For reaching stronger rules, interest measures such as lift, conviction, certainty factor, cosine, coefficient of correlation (phi) and mutual information measures are applied, and as a result of that, 21 of the rules are qualified as strong. In this context, stronger rules can be discovered by using interest measures beside basic measures.

Keywords: Data Mining, Association Rules, Apriori Algorithm, Interest Measures, Gene Expression Data.

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PS-056

Drug Screening and Modeling for Tumoral Cells by Using 3D Printing

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Nisantasi University

Development of high-throughput technologies was brought a new perspective for the production of biotechnological drug in pharmaceutical sectors. These technologies were started to develop with using compatible cell culture techniques based on liquid handling to analyze compenents of drugs. Recent advances in 3D technology and modelling was opened a new section for researches in in vivo and in vitro 3D-cell based assays as well as production of scaffold biomaterials by using 3D-bioprinting. Studies have been showed that 3D cellular model systems had various opportutinities for investigating cellular matrix competents, interactions between cells as well as diffusion kinetics. However, the imaging systems for visualing of 3D-based high-throughput systems were not still developed. Here, our aim to describe the need for advanced modeling and visualition system which is compatible with 3D models to supply efficancy of drug screening and improve understanding of cancer modeling.

Keywords: cancer engineering, 3D modeling, cancer cells, drug screening