"Living Cell Factories"- Electrosprayed Microcapsules and Microcarriers for Minimally Invasive Delivery

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Abstract

Minimally invasive delivery of *"living cell factories"* consisting of cells and therapeutic agents has gained wide attention for next generation biomaterial device systems for multiple applications including musculoskeletal tissue regeneration, diabetes and cancer. Cellular-based microcapsules and microcarrier systems offer several attractive features for this particular purpose. One such technology capable of generating these types of systems is electrohydrodynamic (EHD) spraying. Depending on various parameters including applied voltage, biomaterial properties (viscosity, conductivity) and needle geometry, complex structures and arrangements can be fabricated for therapeutic strategies. In this paper, we outline the advances in the use of EHD technology specifically in the manipulation of bioactive and dynamic material systems to control size, composition and configuration in the development of minimally invasive micro-scaled biopolymeric systems. The exciting therapeutic applications of this technology, future perspectives and associated challenges are also presented.

Keywords: Hydrogels; Microencapsulation; Microcarriers; Electrohydrodynamic; Core-Shell

Introduction

The arena of biomedical delivery systems has propagated in recent years following advances in polymeric materials science but also manufacturing technology, which has significantly enhanced biomedical therapeutics. Areas such as hormone and *'short half-life'* peptide delivery in diabetes [1], regenerative ventures in cartilage, bone and neurodegenerative disorders [2] have all benefitted. The early advances in *'living cell factories'* have improved the pharmacokinetics and administration of drugs and consequently patient compliance and comfort (for further details, see review article [2b]). In the past few years, advances in the fields of biomaterials and cell therapy, design of novel tissue engineering approaches and improvements in the fabrication of application tailored and advanced micro- and nano-carriers for protein and drug delivery have been some of the highlights. The progress in these areas has synergistically fuelled the advances in the 60-year-old encapsulation technology opening new avenues of work. The ability to create *'living cell factories'* by combining hydrogels and cells can now facilitate cell transplantation in a permeable system while isolating the cells from the host's immune system has resurrected the concept of allogeneic-based therapies. Such approaches eliminate the need for immunosuppression in allogenic cell delivery or toxicity in host cell exposure to drugs and more targeted delivery mechanisms [3]. Since the work of Biscglie in the early 30s who transplanted tumour cells enclosed in a polymer membrane into porcine abdominal cavity demonstrating cells were not destroyed by the immune system [4], to successful practice of immobilizing xenograft islet cells to control diabetes in small animal models in the 70s and 80s [5], advances have been rapid.

At present there is an overwhelming resurrection in microparticle fabrication technology with the design of biomimetic and biodegradable microcapsule and microcarrier systems which can be easily combined with cells and growth factors advancing the traditional tissue engineering model (**Figure 1a and 1b**). Direct needle injection of cells without

biomaterial protection has the propensity to expose cells to excessive shear forces, risks cell leakage and elicit an undesired host-immune response. Encapsulation systems enhance the protection and transport of cells and drugs to targeted tissue sites, promote cell integration and consequently tissue repair and regeneration.

Microcapsule and microcarrier systems provide a larger surface area for cellular attachment, provide cell protection against excessive mechanical stresses and simulates an *in vivo* environment [6]. It also offers the advantage whereby these culture systems can be directly injectable biodegradable cell systems, allowing cell repopulation or augmentation of cell population through growth factor or drug release agents aimed at regeneration. With advanced fabrication techniques, various conformations of matrix core-shell, liquid core-shell, hollow core, coatings (**Figure 1c**) [2b] have all fuelled new ventures in tissue engineering with co-culture concepts involving multiples cell types or guided cell implantation with growth factor supplementation as *'bio-organs'* [7]. These injectable systems have gained attention due to the advantages of minimally invasive delivery and operational convenience in regenerative surgery (for further details, see review article [8]). Furthermore, smart matrices with the incorporation of thermal [9], pH [10] sensitive materials or ferrous magnetic materials [11], hold the potential for *in vivo* localised cell release in conjunction with therapeutic agent targeting through remote activation in a multitude of biomedical areas **(Figure 1d)**.

One such technology capable of generating these types of structures is electrohydrodynamic (EHD) spraying. Depending on various parameters including applied voltage, biomaterial properties (*e.g.* concentration/viscosity, conductivity) and needle geometry complex structures and arrangements can be fabricated for therapeutic strategies. Herein, we address this cutting edge technology, recent advances and highlights in the development of micro-scaled biomaterial systems injectable systems.

2.1 EHD spraying technology

Electrohydrodynamic (EHD) spraying is a one-step system that requires no additional solvents other than those usually present in suspension and is carried out under ambient conditions [12]. During EHD processing a flowing liquid is subjected to a high electrical potential difference, which is applied between the source of the flow and an earthed substrate (**Figure 1ai**). Depending on the physicochemical properties of the solution being electrosprayed, beyond a certain critical charge the imposed electrical forces overcome the effects of surface tension acting on the liquid drop by accumulating charges of the opposite sign on its surface. As the potential is increased the deformed liquid adopts a conical shape, known as a Taylor cone from which a fine jet develops breaking up into charged droplets that are attracted towards the substrate [13]. It is capable of fabricating particles from micrometres to millimetres in size with a narrow distribution [13c]. As such, it is a scalable technology that has potential for application in the field of tissue engineering and regenerative medicine**.** The Taylor cone formation phenomenon can be divided into three stages: (1) dripping zone (applied voltages have not overcome the effects of surface tension) (2) transition zone (partial or intermittent jet formation) (3) jet forming zone (**Figure 1aii**). When an aqueous polymer solution containing cells in suspension is passed through the EHD system the cells/therapeutic agents can be entrapped in the solution droplets that are ejected from the needle tip. If the target is a liquid that reacts with the polymer solution, gel transition is induced (*e.g.* ionotropic crosslinking) with cells/therapeutic agents encapsulated within [13b].

A number of studies have examined the potential of EHD as a suitable technology for regeneration therapies. For example, bio-electrospraying (BES) is a microencapsulation approach similar to EHD spraying driven by high intensity electric fields applied between a submerged, charged needle and a grounded electrode [12, 14]. In one study, a viable cell

population of >70% was achieved post-encapsulation over a time period of three weeks [14e]. With regards to stem cells, BES did not have any detrimental effect on the viability also or functional capacity of cells to home to the bone marrow compartment after injection into a peripheral vein where they successfully engrafted and differentiated to produce various cell types that make up blood [12]. In another study, whole multicellular organism encapsulation was performed with no adverse effects on the development, viability, or fertility of Drosophila embryos [14d]. In other works, a recent study by Liaudanskaya et al. demonstrated that cells encapsulated by the EHD method are subject to mild stress conditions, but are able to recover in a short time-frame and maintain their ability to self-assemble in 3D constructs mimicking an in vivo morphogenesis [15]. Sahoo et al demonstrated bone marrow stem cells (BMSCs) electrosprayed at 7.5kV retain their multipotency and differentiate into adipogenic, chondrogenic, and osteogenic lineages although cell viability decreased with increasing applied voltages [16]. Furthermore, other studies focusing on applied voltage, needle diameter, polymer concentration and cell density effects confirm that the EHD processing technique does not impose detrimental effects on cell viability [13b, 16], and that applied voltages between 0kV and 20kV as well as flow rate had no obvious effect on human stem cell survival [17]. To this end, fabricated functional living constructs may be explored for repair and replacement of damaged or degenerating tissues or organs, and/or for delivery of personalised medicines in a controlled and localised manner.

With regards to in vivo work, BES luciferase-expressing N2A cells have been successfully implanted in an in vivo mouse tumour model for non-invasive real time bioluminescent imaging and no significant differences were detected between BES cells and untreated cells at any time point, establishing that BES has no deleterious effects on cells [14a]. Furthermore, tumour growth was visually detected on day 13 in both groups of mice showing that BES did not affect the ability of N2A cells to form tumours in vivo.

2.2. System Processing Parameters

In parallel with the physicochemical properties of the polymer solution, the system processing parameters determine the EHD mode and the subsequent size of the microparticles formed. System parameters include applied voltage, flow rate, needle gauge and the distance over which the potential difference is applied (working distance). At low applied voltages partial or intermittent jet formation occurs while at higher applied voltages a sustained and continuous jet can be formed resulting in a narrow particle size distribution of microcapsules. Needle diameter also has an effect on microcapsule diameter; large inner diameter (lower gauge) generates microcapsules of larger diameter compared to a small inner diameter (higher gauge) at the same operating voltage **(Figure 1aiii)**. With regards to working distance, there is a threshold above and below which microcapsules cannot form. Importantly, cell survival is significantly reduced when longer working distances are used. Paletta *et al* showed a reduction in cell viability of approximately 10% when the distance between the needle and the collecting dish was increased from 6cm to 11cm [18]. One explanation the authors provide is that a greater distance leads to higher evaporation rates of cell-containing droplets, resulting in an increased salt concentration and therefore decreased cell survival. It is worth noting that system parameters are required to be optimised for each polymer type being investigated due to differences in solution concentration/viscosity and conductivity, which will impact on the microcapsule size and distribution that can be achieved.

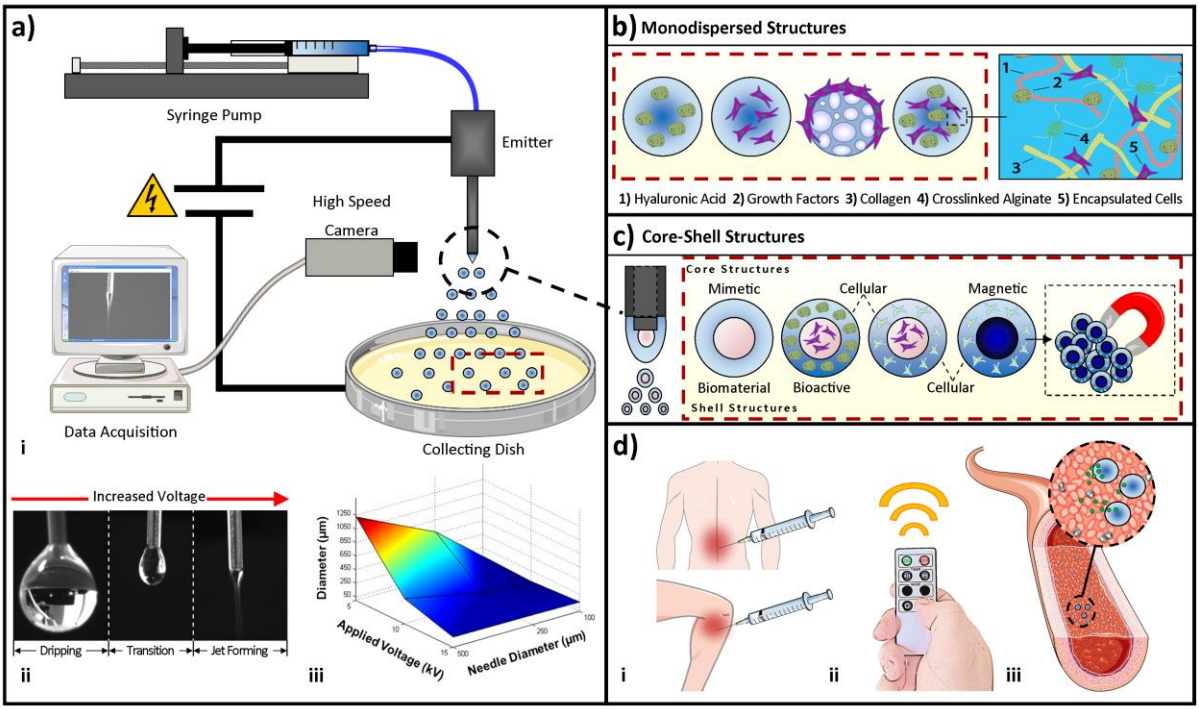

Figure 1. a) (i) Schematic illustrating single needle arrangement for production of microcapsules and microcarriers of uniform size using electrohydrodynamic (EHD) technology. **(ii)** Taylor cone formation with increasing applied voltage illustrating dripping zone (applied voltages have not overcome the effects of surface tension) (2) transition zone (partial or intermittent jet formation) (3) jet forming zone. **(iii)** influence of applied voltage and needle diameter on microcapsule size. **b)** Schematic representations of microcapsules and microcarrier morphologies formed with various constituent configurations in monodispersed spraying mode. **c)** Coaxial spraying and different combinations and conformations of core-shell microcapsules. **d)** Potential application areas of microcapsules and microcarriers **(i)** Injectable regenerative therapies **(ii)** Remote activation devices **(iii)** Controlled spatio-temporal drug or cell release.

3. EHD Compatible Biomaterials

The selection of an appropriate biomaterial is essential in the design and development of tissue engineering approaches and injectable drug delivery systems using EHD spraying technology. An ideal biomaterial should be biocompatible, biodegradable and should not interfere with the homeostasis within the host tissue. With regards to cellular therapy, it is essential that cell delivery vehicles maintain cell viability throughout the injection procedure. Hydrogels have been predominantly utilised and can provide a shielding effect on cells during needle injection [19], providing support for cells. Furthermore, therapeutic agents and factors (*e.g.* growth factors, trophic cells, drugs, genes and proteins) may be incorporated for sustained delivery making them ideal delivery biomaterials.

Many natural polymers including alginate [20], agarose [21], atelocollagen [22], fibrin [23], collagen [24], chitosan [25], and pectin [26] have been investigated in the development of minimally invasive injectables (**Figure 2a**). Alginate is purified from brown seaweed and is made up of a family of linear copolymers containing blocks of α-L-guluronic acid (G) and β-D-manuronic acid (M). The most common method to prepare an alginate hydrogel is by combining the solution with ionic cross-linking agents, such as divalent cations (Ca^{2+}). The divalent cations bind to G blocks of the alginate chains, the G blocks of one chain form junctions with adjacent G blocks in what is termed the *'egg-box'* model of crosslinking, resulting in a gel structure [20, 27]. Alginates are among the most frequently employed biomaterials for encapsulation due to their abundance, convenient gelling properties and high biocompatibility [28].

Pectin is a natural polysaccharide secreted from plants and bacteria and has been proposed to stimulate bone tissue regeneration when acting as a cell carrier vehicle. It is a widely available bioactive polymer and FDA approved which may emphasise its

translatability. Its use as a single material or in combination with other materials in carrier and capsule systems may allow for an improved injectable formulation [26].

Chitosan is another biomaterial that is being explored due to its biocompatibility and favourable degradation kinetics [29]. Chitosan is a linear polysaccharide composed of glucosamine and N-acetylglucosamine formed from the deacetylation of chitin, with the degree of deacetylation being directly proportional to cell attachment [25, 30]. In addition to its cell compatibility, it has also been shown to exert an antibiotic effect and has demonstrated that it can be combined with antibiotics for sustained release maintaining clinical efficacy for three months [31]. Alginate, pectin and chitosan can all be successfully crosslinked through ionotropic gelation making them ideal biomaterials for EHD processing.

Negotiating permeability with stability and degradation kinetics in an application specific manner is a key step in choosing the right biomaterial. By adjusting permeability, the capsule should still possess adequate mechanical stability, immune evasion and permit adequate exchange of nutrients and metabolic waste products [3]. Mass transfer across a membrane is governed by thermodynamic parameters that need to be tailored to the type and size of solutes, membrane thickness, etc. [32]. This is determined by the molecular weight cut-off (MWCO) of the capsule membrane which is critical to diffusivity and influx rate of nutrient supply concomitant with efflux of molecules [33]. The micro-scale operating range used to process natural biological materials results in biocompatible degradation by-products which can be excreted without the risk of immunogenicity or the risk of an autoimmune response. Biocompatibility should be maintained by ensuring adequate purification of materials [34], neutralising the cation content which can otherwise invoke inflammatory responses [2b] and ensuring degradation products do not elicit a host tissue response. The diameter and material properties of the microcapsule/microcarrier can also influence the immune response and controlled geometry and topography is critical [35]. As microparticles

degrade, the degradation kinetics of the biomaterial also determines the controlled release profile of the entrapped agents. Furthermore, the degradation kinetics must match the rate of cellular tissue reconstitution [2b]. Biopolymer remodelling allows space for synthesis of matrix internally in carrier systems and increases surrounding membrane permeability in capsule systems. This also enables interactions between the cellular and matrix constituents in adjacent microcapsules/microcarriers whereby biochemical signalling and direct ligand-based interactions both result in microparticle aggregation and tissue formation (for further details, see review article [36]).

Physicochemical properties of the polymer are essential considerations when using EHD such as surface tension, electrical conductivity and viscosity of the solution, as these properties determine the optimal system processing parameters such as voltage, flow rate, needle size, and tip to collector distance, hence reflecting the subsequent size of microcapsules formed. Permittivity and viscosity play key roles in transmitting the electrical force through the material and surface tension is important in controlling size and distribution of droplets formed [37]. For example, increasing alginate concentration results in higher viscosities, requiring the voltage and flow rate to be altered in order to achieve a stable spray or microparticles of a specific size [38]. Furthermore, biomaterials may have various gelling mechanisms such as thermally sensitive gels, which solidify due to temperature change, photo resistive gels solidify with ultra violet (UV) light exposure, and ion based gels which solidify in the presence of cations. The precise gelation mechanism needs to be considered and adapted accordingly when electrospraying with different materials. For example, pectin responds to pH depending on its origin [39], methylcellulose responds to temperature [40], and alginate is UV responsive as demonstrated by Guo et al who showed an increasing porosity in 3-D alginate scaffolds when exposed to ultrasound leading to improved growth of

chondrocytes [41]. Thus, microcapsule and microcarrier preparation can vary depending on material and desired physical parameters for specific applications.

4. EHD compatible delivery cargo

EHD technology has been explored for spraying with different cell types such as adipose-derived stem cells (ADSCs) [42], primary human monocytes [43] and hepatocytes [44]. The same technology may be used to spray different cell types simultaneously in multicomponent or multicompartment capsular structures to facilitate complex tissue regeneration. Furthermore, the delivery of cell types in combination with growth factors, vitamins, drugs and non-living cargo can be coordinated to serve as therapeutic agents in regenerative ventures. The half-life, degradation, charge, density and other biochemical properties of this cargo is important to consider [8]. System processing parameters must be optimized accordingly for specific cargo being delivered with electrosprayed microparticles. For example, altering the concentrations of the cargo will influence the viscosity of the material suspensions which affect optimal operational parametric windows of voltage for a stable spray to be attained and the flow rates required to achieve this. When varying neuronal cell concentration from $1x10^4$ to $1x10^7$ cells, Jayasinghe (2011) found that the voltages and flow rates needed to be optimised. Specifically, the voltage ranged between 1-30kV and flow rates of $1x10^{-7}$ to $1x10^{-12}$ m^3 s^{-1} were explored to achieve a stable spray for immortalized or primary cultures of neurons [45].

5. Microparticle systems

5.1. Microcapsules

Cellular microencapsulation (**Figure 2b**) involves the immobilization of cells in a biomaterial, which holds the potential to overcome recurring limitations such as immunogenicity, by way of physically blocking molecules larger than a critical size and

allowing the diffusion of small sized molecules [5, 46]. Also, in the case of stem cells the biomaterial niche may offer the benefit of directing cell differentiation towards the desired lineage [46c, 47], enabling the cells to produce bioactive factors with synergistic intercellular interactions, without being damaged due to surrounding shear forces [47b].

The advantage of immune-isolation conferred by microcapsule systems allows for the use of banked allogeneic cell lines, which presents a more commercially viable solution in tissue regeneration strategies. This further opens up avenues for applications using genetically modified cells, which can augment tissue formation through in vivo paracrine growth factor signalling. In this way, 'living cell factory' systems can be delivered through microencapsulation for therapies such as diabetes, back pain, bone healing and osteoarthritis in a cost-effective manner. One study demonstrated that microcapsules made up of ultra-high−viscosity alginate cross-linked with Ba^{2+} and stabilized with human serum albumin (HSA) protect adult rat and human islets against xenorejection after transplantation [48]. Another study demonstrated survival of islet allografts in streptozotocin-induced diabetic rats by encapsulating individual islets in protective, biocompatible alginate-polylysine-alginate membranes [49]. Although, much of the work has focused on diabetes (for further details, see review article [50]), this technology could easily be expanded or adopted in other fields of regenerative medicine. For example, the implantation of microencapsulated genetically modified non-autologous cells delivering a recombinant therapeutic product has been successfully employed in preclinical animal studies involving delivery of hormone or enzymes to treat dwarfism [51], lysosomal storage disease [52] or hemophilia B [53].

Long-term storage through cell cryopreservation may address the commercial challenges associated with the scaling-up of the EHD process while maintaining a controlled environment with rigorous quality controls. Cryopreservation has become an attractive method for microcapsule banking and has shown promising results to date [54] offering the

potential for *'off-the-shelf'* availability and pre-designed deliverability mechanisms for on-demand clinical applications and therapeutic strategies. This step will be essential in advancing cell microencapsulation technologies to enter human clinical trials and eventually become a real clinical therapeutic strategy.

5.2. Microcarriers

The microcarrier culture system offers multiple solutions for enhancing the proliferative and phenotypic expression of anchorage dependent cells. Porous microcarriers (**Figure 2c)**, the scaffold equivalent to the hydrogel microcapsules possess large surface area for cell growth with a surface area of 1g of microcarriers comparable to fifteen 75 cm^2 flasks, a culture system that is both space saving and cost effective [55]. Transplanted cells on microcarriers can proliferate *in situ* to achieve cell numbers within a short period without significant de-differentiation making them ideal candidates as cell delivery systems.

Microcarriers, in our laboratory, are fabricated using EHD in a similar fashion to microcapsule manufacture with an additional lyophilisation step to induce surface porosity, increase surface area and facilitate long-term storage. This allows for an *'off-the shelf'* product for future seeding with cells or soak-loading with growth factors. In this way they can potentially be used for 3D in vitro regeneration or in vivo culture or for sustained growth factor release with improved temporal control [2b]. Commercially available microcarriers (fabricated using alternative technologies such as oil-water emulsion, solvent extraction-evaporation), such as clinically used dextran (cytodex), collagen coated (CultiSpher) [56], poly (lactic-co-glycolic acid (PLGA) [57], poly-(L)-lactic acid (PLLA) [58] and hydroxyapatite [59] systems are being investigated for multiple cell delivery applications for tissue regeneration in blood vessels, muscle, liver, neurons, skin, bone and cartilage. Details of investigative applications can be found in a review by Li et al (2015) [60]. Importantly, EHD

spraying technology could potentially be scaled-up to fabricate these types of microcarrier systems also. This *'off the shelf'* concept is an additional advantage offered by microcarrier systems and allows this technology to be more easily translatable in multiple clinical applications. Examples include bioactive carriers to support cell based regeneration in musculoskeletal, cardiac and pancreatic tissues and controlled drug release especially in the context of '*short half-life*' growth factors such as targeted delivery of biologics to augment healing [7]. Microcarriers can also be used for delivery of therapeutic agents. For example, bFGF and TGFβ1 have been covalently immobilized onto microcarriers to induce chondrogenic differentiation of MSCs [61]. In this way microcarrier systems can be scaled for manufacturing in accordance with good manufacturing practice (GMP) guidelines and provide exciting opportunities in regenerative medicine in the future [2b].

Material and physical parameters used in microcarrier fabrication can affect their function in different applications. Cellular attachment and controlled release of loaded residues is determined by surface topography, porosity, charge density and chemical composition [6]. The porosity of microcarriers can be tailored to match cellular attachment and support, providing large surface areas for 3D culture. Also, it may need to be tailored to the specific cargo being delivered in terms of degradation and subsequent immune response and tissue reconstitution [36, 62]. The chemical composition determines biodegradability, which must be tailored to desired pharmacokinetics in biological delivery matrices. An important consideration is that post-manufacturing sterilization processes must not compromise the structural or chemical integrity of the microcarrier system which may impair subsequent function or bioactivity.

Microparticle systems, microcapsules and microcarriers, (**Figure 2d**) are injectable allowing for easy handling and delivery which makes them an attractive option for minimally invasive cell transplantation and growth factor delivery. By injecting these micron sized

particles into a defect, it overcomes the traditional surgical challenges with placement or suturing scaffolds and allows easier filling of the defect with the potential to improve surgical outcomes [63]. Furthermore, microparticle suspensions allow effective diffusion of nutrients and oxygen to cells and efficient removal of metabolic waste products. To ensure optimal diffusive mass transport, diameter of microparticles should be maintained below 400µm [64]. In a comparison of commercially available microcarriers, optimal size distribution of microcarriers was found to be in the range of 150-300µm which facilitated high cell attachment efficiencies [65]. Also, relative pore to cell size ratio is critical for cell 3D environmental perception and subsequent attachment. This allows for improved control over the physical, biological and chemical milieu in '*in vitro*' culture systems (**Figure 2d**) and better exposure to nutrients in the nutrient compromised 'in vivo' scenario [2b].

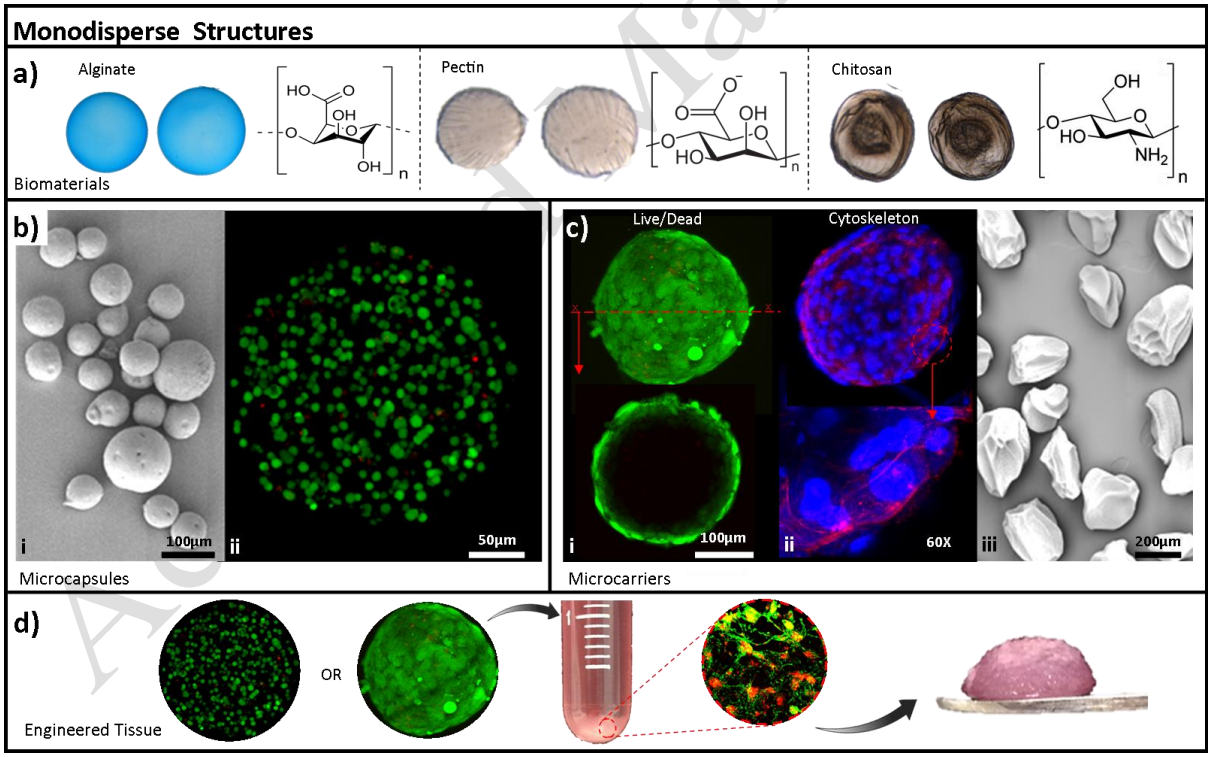

Figure 2. Exemplars of electrohydrodynamic (EHD) spraying using monodispersed- spraying **a)** EHD and ionotropic gelation of different natural hydrogels such as Alginate, Pectin or Chitosan. **b-i)** Scanning electron micrograph (SEM) of microcapsules with **(ii)** encapsulated cells depicting rounded morphology. **c)** Microcarriers with cells attached to surface **(i-ii)** with

spread morphology. **(iii)** SEM image of microcarriers. **d)** Engineered tissue using microcapsules or microcarriers formed by capsule/carrier aggregation with extracellular interactions.

6. Advanced strategies

6.1 Advanced Materials

The advancement and optimization of bioactive biomaterials for cell encapsulation has allowed the modification of materials to control proliferation and differentiation of encapsulated cells or to control drug release kinetics. Although this has largely involved combining or tethering biomaterials with peptide sequences [2b, 66], incorporation of extracellular matrix (ECM) mimetics with preserved endogenous ligands is emerging as a pertinent area. It allows for physiological interactions by recapitulating the *in vivo* environment allowing for improved cell attachment, proliferation, matrix production and phenotypic expression [2b]. Also, they act as powerful delivery vectors for growth factors, which can also recruit endogenous cells with more physiological interactions and support cellular implantation (**Figure 3a**). These materials can be fabricated using a *"bottom up"* approach by combining specific components of ECM such as collagen/proteoglycans [67] or from the *"top down"* by the use of decellularized ECM derived materials where the desired ECM components are maintained and the cellular components are removed. Furthermore, combining different materials can exploit unique properties to improve cell growth kinetics and bioactivity. Similarly functions involving targeted, tailored spatio-temporal growth factor release, interactions with site-specific ligands and material specific resorption rates are important considerations [7].

6.2 Advanced EHD Spraying

Coaxial EHD spraying is an evolution of typical EHD spraying technology that utilises a double-lumen needle to allow for the formation of multi-layered, multi-material systems, with a clear delineation between the outer shell and inner core phases (**Figure 1c**) [37]. Over the past decade, it has been shown to have several advantages over other methods of fabricating core-shell structures as it is a scalable process that produces uniformly sized microparticles with precisely controlled core-shell geometries. The ratio of shell to core volume can be tailored using varying flowrates of the individual solutions with a high encapsulation rate without the use of harsh preparation methods, which could denature fragile cargo [68]. This offers an attractive approach for delivery of drugs and proteins as it facilitates lower initial burst release kinetics resulting in a longer and more controlled sustained release profile [69].

Traditional EHD has been shown to be an effective method for cellular encapsulation [16] however due to the homogenous mixing of the cells throughout the formed spheres some are inevitably contained within the periphery and "visible" to the immune system which may provoke a foreign body response especially when attempting to make smaller spheres with high cell numbers [70]. To avoid this, coaxial EHD has been shown to be capable of encapsulating the cells within an immunoprivileged shell in a single step procedure [71]. Encapsulating multiple components into core/shell structures while yet isolating them from one another is an exciting step forward in the field of tissue engineering and regenerative medicine.

Through combining different materials, distinct material advantages can be exploited. Material modification through multiphase material spraying with core-shell morphology or biofactor responsive matrices through external stimulation with temperature, pH, light, electricity and magnetism hold significant potential for novel applications. For example, the core region could comprise of a specific material that provides a more appropriate substrate

environment to enhance cell bioactivity, while the shell provides protection or a reservoir of growth factors. Hydrogel shells with a liquid core have been developed in a single step manufacturing technique for the culture of embryonic stem cells within the core (**Figure 3a**) [72]. Air, as an alternative to liquids or polymers, may be incorporated allowing for the generation of hollow microcapsules. Their low density and floating mechanism will effectively result in slower release kinetics. In addition, the lower density of hollow microspheres can be exploited to simulate microgravity [73] allowing for optimal biomechanical signalling with improved co-localisation of cells and initiation of specialised cell adhesion molecules (CAMs) and ECM proteins with enhanced 3D tissue reconstitution [74]. This has been successfully employed to engineer functional bone using hollow ceramic biospheres [73]. Core-shell EHD spraying can permit the fabrication of co-culture systems allowing physiological cellular paracrine signalling and reconstitution of complex tissues in a minimally invasive manner. Multiple cell types may be combined in a single capsule to generate 'instructive' co-culture systems or specific biologics can be incorporated into the shell layer and activity can be tailored in a sustained manner **(Figure 3b)** (for further details, see review article [75]). These 'super-specialized' microcapsules with multiple microcompartments can also allow dynamic cell interaction while isolating cell populations in core-shell configurations. For example this is employed to induce prevascularisation at the site of cell transplantation by co-encapsulating angiogenic factors or cells modified to secrete such and controlling release kinetics in response to physiological cues [76]. Structural modification with EHD technology in coaxial and triaxial microcapsules can manipulate material properties with sequential and controlled release profiles allowing targeted delivery of therapeutics. Similarly therapeutic agents can be chemically tethered or soak loaded onto biopolymeric suspensions optimised for control release. Furthermore, incorporating and

manipulating mechanically responsive materials in microcapsules can be used to orchestrate therapeutic release in vivo [76].

Coaxial EHD spraying also allows for the incorporation of a ferromagnetic or super-paramagnetic phase within the sphere, isolated from the cellular component to alleviate toxicity concerns (**Figure 3c**). These have been shown to be capable of undergoing spontaneous aggregation or shape specific aggregation and levitation in the presence of a magnetic field and a similar, but more involved, process has been described previously to culture glioblastoma cells [77]. The development of easy to fabricate magnetic hydrogels opens many potential avenues for tissue engineering as magnetic hydrogels have been shown to increase the metabolic rate of aortic endothelial cells and capable of coaxing them into assuming a capillary-bed like structure [78]. In addition magnetic hydrogel systems can be used to remotely impart mechanical stimuli to the cells which can influence cellular processes and phenotypic processes such as chondrogenesis [11a, 79].

These magnetic gels are also capable of producing large amounts of heat from hysteresis of the magnetic particles when exposed to an external varying magnetic field, and have been shown to be able to reach sufficient levels for therapeutic hyperthermia (43-47°C) [80]. PEGDA-based gels have been developed that allow for the release of chemotherapeutics in conjunction with therapeutic hyperthermia, sowing the possibility of creating an injectable core-shell magnetic system for targeted drug delivery in tandem with thermoablation for treating malignancies.

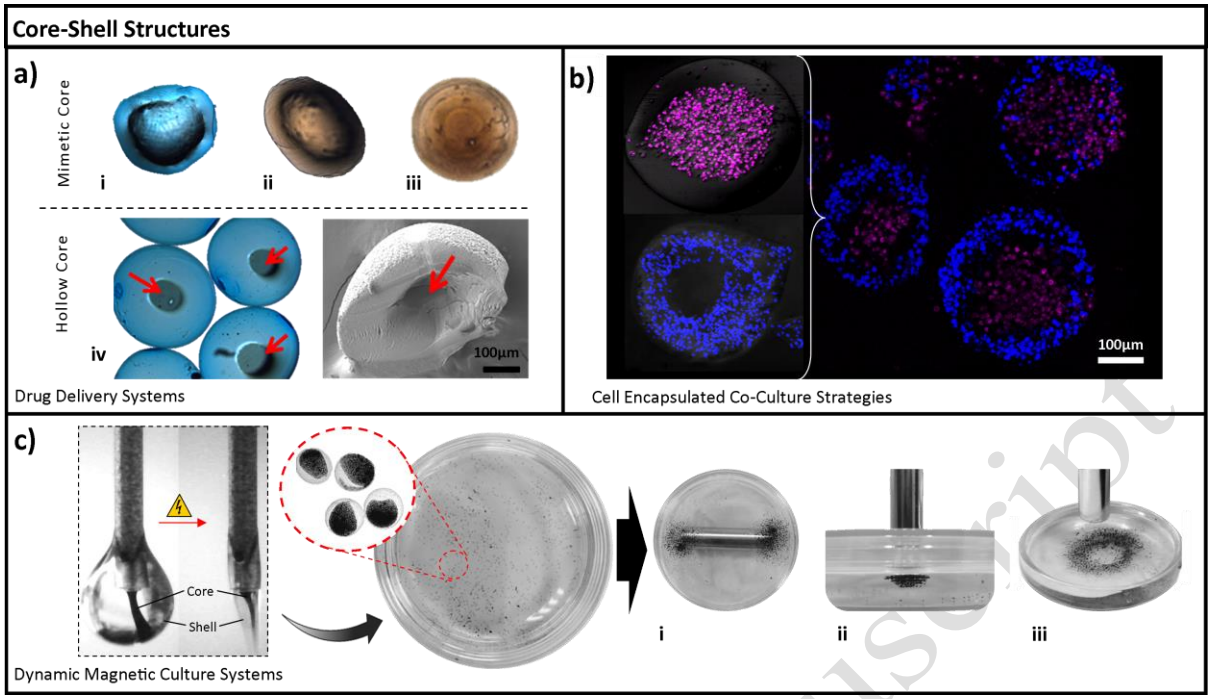

Figure 3. Exemplars of electrohydrodynamic (EHD) spraying using co-axial- spraying to form core-shell structures. **a)** Mimetic materials and combinatory drug delivery systems with **(i)** alginate –collagen, **(ii)** pectin-collagen, **(iii)** chitosan-collagen or **(iv)** fabrication of hollow-core structures. **b)** Encapsulated cell co-culture strategies with core and shell based cell populations (blue= chondrocytes, pink = bone marrow stem cells). **c)** Ferro-magnetic core-shell spheres and microcarriers. Dynamic microsystem cultures facilitating **(i)** co-culture with close proximity paracrine signalling, **(ii)** levitation and mechanical deformation systems or **(iii)** spatial manipulation of constructs.

7. Conclusions and Outlook

Cell microencapsulation technology has only seen its infancy and we expect to see exciting improvements in the next few decades especially in the setting of an ageing population and the increasing burden of chronic and degenerative diseases [2b]. The EHD spraying process can achieve uniform dispersion of growth factors within the polymeric matrix with high loading capacity, minimal drug loss and maintained cell viability and support for therapeutic

applications. FDA recognition of cell-based products as a new category of therapeutics can be exploited with bioactive material support for translational in vivo culture and specific constituent support in multi-compartment therapeutic delivery [32b]. Structural manipulation to exploit differential permeability and degradation properties of various biomaterials in monosphere, coaxial and triaxial structures may be tailored for temporal release kinetics. The mild handling properties of this technology makes it attractive for manipulating biological or bioactive materials into microparticulate form while maintaining integral properties of cells or residues. This positions it well ahead of other modalities of direct cell jet technologies such as ink-jet printing, air-jet printing or laser guided cell writing [81].

As microparticle technology translates into application, further advanced strategies will be investigated in the setting. As highlighted this covers novel manipulation strategies with co-axial core-shell EHD spraying, ferro-magnetic, differential drug release kinetics and cellular co-cultures to generate complex structures and tissues. Encapsulating trophic cells and controlling release of bioactive agents such as growth factors, vitamins, anti-inflammatory agents, antioxidants or using ECM mimetic materials in this layered conformation as instructive materials allows modulation of the tissue microenvironment and improve nutrient and trophic support [32b]. This allows appropriate signalling for cell attachment, proliferation and differentiation to meet the dynamic reciprocity in both the generation of biofunctional tissues and drug delivery. This approach is attractive as it is no more complex biologically or chemically but involves a bit of clever engineering and material manipulation. EHD provides a low cost, high throughput, controlled processing approach as micron sized particles with a narrow size distribution of different materials can be produced with minimal amounts of material for mass manufacturing [82].

Future directions should veer on the development of technically advanced systems to satisfy the demands of GMP guidelines for large-scale transplantation. This involves

controlled systems, operational rigor and improved quality control. Monitoring and retrievability whether through genetic [83], biochemical [84] or magnetic tagging [85] are key areas to investigate in order to alleviate biosafety concerns. Cryopreservation, banking and subsequent culture of cells encapsulated in microcapsule delivery systems represents an alternative to the economically attractive *'off the shelf'* microcarrier concept. With long-term delivery of therapeutic agents, the cost of encapsulated cells is offset by the cost of short half-life therapeutic peptides and the costs involved in organ transplantation. This favourable pharmacoeconomics of EHD based technologies may impact insurance companies who may favour such an approach over traditional therapies creating a paradigm shift in regenerative medicine.

More interdisciplinary and integrated input is required to realise this ambitious goal incorporating expertise from the fields of physicochemistry, tissue engineering, cell biology, pharmaceutical technology, materials science, genetics and medicine [86]. Ultimately the goal is the development of a highly biocompatible polymeric membrane reconciling the application specific benefits of both microcapsule and microcarrier systems to address clinical demands. Given the rapid developments in cell encapsulation and the prospects of tailored, manipulable *'living cell factories'* and regenerative delivery systems, microcapsule and microcarriers systems will continue to spark interest.

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