In Silico Prediction of the Mechanobiological Response of Arterial Tissue: Application to Angioplasty and Stenting

Colin J. Boyle, Alexander B. Lennon and Patrick J. Prendergast

J Biomech Eng 133(8), 081001 (Aug 26, 2011) (10 pages)

doi:10.1115/1.4004492

History: Received February 08, 2011; Revised June 03, 2011; Posted June 30, 2011; Published August 26, 2011; Online August 26, 2011

Abstract

One way to restore physiological blood flow to occluded arteries involves the deformation of plaque using an intravascular balloon and preventing elastic recoil using a stent. Angioplasty and stent implantation cause unphysiological loading of the arterial tissue, which may lead to tissue in-growth and reblockage; termed “restenosis.” In this paper, a computational methodology for predicting the time-course of restenosis is presented. Stress-induced damage, computed using a remaining life approach, stimulates inflammation (production of matrix degrading factors and growth stimuli). This, in turn, induces a change in smooth muscle cell phenotype from contractile (as exists in the quiescent tissue) to synthetic (as exists in the growing tissue). In this paper, smooth muscle cell activity (migration, proliferation, and differentiation) is simulated in a lattice using a stochastic approach to model individual cell activity. The inflammation equations are examined under simplified loading cases. The mechanobiological parameters of the model were estimated by calibrating the model response to the results of a balloon angioplasty study in humans. The simulation method was then used to simulate restenosis in a two dimensional model of a stented artery. Cell activity predictions were similar to those observed during neointimal hyperplasia, culminating in the growth of restenosis. Similar to experiment, the amount of neointima produced increased with the degree of expansion of the stent, and this relationship was found to be highly dependant on the prescribed

inflammatory response. It was found that the duration of inflammation affected the amount of restenosis produced, and that this effect was most pronounced with large stent expansions. In conclusion, the paper shows that the arterial tissue response to mechanical stimulation can be predicted using a stochastic cell modeling approach, and that the simulation captures features of restenosis development observed with real stents. The modeling approach is proposed for application in three dimensional models of cardiovascular stenting procedures.

Introduction

The restriction of blood flow characterizes many cardiovascular diseases including stroke and myocardial infarction. Revascularization generally involves inserting a catheter into the vasculature and navigating the end of the catheter to the restricted region, where a balloon on the end of the catheter is inflated to force material outward. This process is called balloon angioplasty, and its most significant limitation has been the re-occlusion of the vessel either by elastic recoil of the tissue or by growth of new tissue (neointimal hyperplasia), a process termed restenosis. Stents have become a most successful device for preventing elastic recoil and are used in increasingly complex lesions. These devices are deployed in the occluded region and are designed to prop open the lumen; however, they do not prevent restenosis. There are a wide range of stent designs available, and stent designs have been shown to induce varying rates of restenosis [1-3]. Despite many generations of coronary stents, up to 10% of stenting procedures require revision due to restenosis, a rate that increases to 50% in some high-risk lesions [(4),5]. It can be argued that successful engineering of stents requires a design tool or model that can predict the tissue hyperplasia induced as a function of stent design.

The key determinant of long-term success after revascularization is whether the lumen area reaches an equilibrium value that provides sufficient patency for blood flow. The first event to occur upon expansion of the stent is that the endothelium becomes denuded within the stent region, exposing the underlying

tissue to blood flow and producing a thrombogenic surface. The stresses within the arterial wall are also increased: tissue is stretched circumferentially between struts and compressed radially beneath them. Damage to the laminae and the media can occur [(6),7]. Smooth muscle cells (SMCs) within the media may become necrotic/apoptotic. These combined alterations trigger an inflammatory response [(8),9]. The first phase of the tissue response is characterized by thrombus formation, platelet deposition, and monocyte infiltration into the injured areas. Matrix degrading enzymes and growth factors are expressed by the areas and come from the invading inflammatory cells, platelets, and necrotic/apoptotic SMCs [10]. Matrix degradation encourages surrounding contractile SMCs to express a synthetic phenotype [11]; reduction of MMPs (matrix metalloproteinases—a family of matrix degrading enzymes) by inhibitors have been shown to reduce subsequent restenosis [12]. The SMCs migrate into injured regions and proliferate in response to growth stimuli, characterizing the proliferation phase. The synthetic SMCs produce extracellular matrix components, such as collagen, elastin, proteoglycans, laminin, and fibronectin, which constitute the neointimal lesion. Provided the source of injurious factors recedes, and the lumen does not occlude, the cells in the lesion can resort back to quiescence and the lumen area equilibrates. If, however, the cell proliferation and matrix synthesis are too intense, the neointima can restrict the artery, requiring further revascularization steps. A schematic of restenosis development is shown in Fig. 1.

Advances in arterial tissue modeling, and computational fluid dynamics allow the mechanical stimuli imparted by a stenting procedure to be calculated. Significant advances have been made over the last 20 years to the precision and accuracy of these models, including improved constitutive modeling of tissues [13], more realistic models of stent deployment [(14),15] and the use of patient-specific geometries [(16),17]. These provide detailed information on the stresses induced in the arterial wall due to stenting and allow comparisons between stent designs, in terms of tissue prolapse [18] or induced stresses [(19),20]. These models may then be used to optimize stent design with respect to mechanical

stress [(21),22], and to investigate the effect of structural differences between coronary and peripheral vessels [23]. These studies provide methods to determine the initial stimuli or triggers for restenosis. The reaction to a stent by the arterial tissue, however, is a complex biological process [24], which is difficult (if not impossible) to predict from the initial stimuli described by the mechanical environment post-stenting. It is unclear; for example, which, if any, mechanical stimulus correlates with subsequent restenosis volume. One way forward is to develop mechanobiological models that relate mechanics to biological response (i.e., injury, inflammation, and SMC behavior) in order to simulate the process over time.

Mechanobiological models offer a method for predicting the long-term effects of perturbing the mechanical environment. These models have been successfully applied to bone remodeling [25] and skeletal tissue differentiation [26] and have been investigated for cardiovascular applications [(24),27,28]. The modeling approach where cell activities are modeled using a regular lattice has been of particular utility in modeling processes that are the result of complex cell activities [29]. In these models, individual cells act at a local level based on the local environment, and tissue-level changes occur as a result of the net effect of local cell activity. This allows a wide range of information and complexity to be included through prescribing algorithmic rules for cell behavior and is particularly suitable to modeling the relationships between cell activity and mechanobiological stimuli [(30),31].

In this paper, we ask the question whether or not a mechanobiological model for restenosis in arteries can be developed using the lattice-modeling approach to describe local cell activities. Specifically, we aim to set the model parameters using the clinical results of balloon angioplasty presented by Schwartz et al. [32] and information on the levels of inflammation in patients after angioplasty described by Cipollone et al. [33]. We then test the model by using the same parameters to simulate restenosis in an artery in response to the injury caused

by cardiovascular stenting. If the model can predict reasonable results in these simple cases, then it opens up the opportunity to apply mechanobiological modeling in three dimensional complex environments for the design of cardiovascular stents.

Methods

The growth of new tissue into the lumen of arteries in response to stenting is assumed to be appositional; i.e. due to the deposition of extracellular matrix by SMCs on the lumen surface. Volume addition is brought about by migration into, and matrix deposition at, a previously unoccupied region of space such that the boundary of the tissue is extended. The number of cells available to produce matrix in a region is not constant over time; it is a function of net migration into the region, and the proliferation, the apoptosis, and the phenotype of the present cells. SMC migration can be modeled as a random-walk process [29]—with the constraint that cells only migrate within or on the surface of the tissue. SMCs proliferate in response to growth factors. The rates of proliferation and migration depend on the individual smooth muscle cell phenotype, which is modulated according to the constituents of the extracellular space around the cell: in particular, the amount of intact extracellular matrix—the greater the volume fraction of extracellular matrix, the more likely SMCs are to express a quiescent phenotype. Matrix degrading factors such as matrix metalloproteinases and growth factors (for example, platelet derived growth factor) are produced by inflammatory cells, apoptotic cells, SMCs, and ECs. Mechanical stimuli induce the inflammation through injury/damage—introducing a mechanobiological link. An outline of the simulation technique is given in Fig. 2.

The spatial domain in which cell activities occur is represented as a regular, orthogonal lattice with dimensions $\{X, Y, Z\}$, and a lattice-point spacing, denoted Δx , which is identical in all three directions. At each lattice point, Boolean and continuous variables are defined representing the model constituents. Cells are

represented by a Boolean variable such that a lattice point is either occupied by one cell, or the lattice point is vacant. The variables at each lattice point are then explicitly updated over time based on algorithms for each cell activity, as described below. A boundary condition describes the migration behavior of cells at the edge of a symmetric boundary, such as that found when simulating a circumferential segment of an artery (see Sec. 2.8 below). At these boundaries, a cell in reality ought to be able to move into surrounding tissue, just as a cell from the surrounding tissue ought to be able to migrate into the simulation domain. To simulate this, a cell moving across a boundary is transported to the equivalent point inside the corresponding surface. If the domain is rectangular, this means a cell migrating off the top of the lattice will move to the bottom, i.e., the simulation domain becomes a torus, and cells are conserved within the lattice. In an artery segment model, the boundaries are at an angle to each other dictated by the angle of the segment. In this case, the equivalent point to each boundary point is found by rotating the point by the angle between the boundary lines about the point of intersection of the lines. For a regular orthogonal lattice, the angle between boundaries may mean that the equivalent position may not correspond to a lattice point, in which case the nearest point is chosen.

Stimulus Computation.

The finite element method was used to compute the arterial wall stresses during loading. Arterial tissue was modeled as an isotropic, hyperelastic material using a second-order Mooney–Rivlin strain-energy function

$$\psi = a_{10}(I_1 - 3) + a_{01}(I_2 - 3) + a_{11}(I_1 - 3)(I_2 - 3) + a_{20}(I_2 - 3)^2 \quad (1)$$

Finite element analysis was performed using abaqus (simulia) v6.8. Von Mises stress was used to compute damage accumulation using a log-linear relationship for fatigue-induced damage

$$\sigma = \alpha \log N_f + \sigma_f \quad (2)$$

where N_f is the number of cycles to failure, σ_f is the failure strength (i.e., when $N_f = 1$), and α is a constant. We define a fatigue strength, σ_0 , below which the damage accumulation rate is assumed to be zero. Rearranging, and taking that the rate of damage formation, denoted $\dot{D}$, is given by $\dot{D} = 1/N_f$ (Miner's rule [34]), we get

$$\dot{D} = 10^{-(\sigma_f - \sigma)/\alpha \sigma_0} \quad 0 < \sigma \leq \sigma_f = 0 \quad \sigma \leq \sigma_0 = 1 \quad \sigma > \sigma_f \quad (3)$$

Modeling Inflammation.

The production of, and reactions between, three biological species—growth stimulus (g), matrix degrading factor (m), and extracellular matrix (e)—in response to damage (D) is simulated using a mathematical model. The variables are updated at each lattice point based on their rate equations. The reaction between e and m is approximated as first-order, with the extracellular matrix degrading at a rate proportional to the amount of matrix degrading factor

$$\frac{de}{dt} = -k_{deg}m \quad (4)$$

and the amount of matrix degrading factor (m) reducing correspondingly, but being replenished in proportion to the injury present

$$\frac{dm}{dt} = -k_{deg}m + k_m D \quad (5)$$

In Eqs. 4,5, k_{deg} is the rate of reaction between the species, and k_m is a constant relating the production of matrix degrading factor (m) to damage, D . Growth stimulus production is assumed to be linearly related to damage (via constant k_g) and is assumed to decay exponentially at a rate d_g

$$\frac{dg}{dt} = k_g D - d_g g \quad (6)$$

Damage removal is assumed to be equal to the removal rate of the extracellular matrix (e), so the damage evolution equation is

$$\frac{dD}{dt} = N \cdot \dot{D} - k_{deg}m \quad (7)$$

where N is the number of cycles at a given stress, and $D\cdot$ is the damage accumulation rate (see Sec. 2.1 above).

Modeling Smooth Muscle Cell Activity.

Each smooth muscle cell occupies a single lattice point. Migration is modeled as the movement of a cell from one lattice point to a neighboring, vacant lattice point. The local neighborhood of a cell is defined as the lattice points within a surrounding sphere of radius Δx centered around the lattice point, i.e., a von Neumann neighborhood [35], which includes the orthogonally adjacent lattice points (in a 2D lattice, there are four such neighbors, see Fig. 3). Proliferation is modeled as the creation of a new cell at a vacant lattice point in the von Neumann neighborhood. If a cell has no vacant neighbors, it cannot proliferate, introducing a contact-inhibition effect. The direction of migration and proliferation is random, implemented by selecting from the vacant neighbors of an active cell randomly. These processes could, in principle, be dependant on a chemical gradient; in this study, however, isotropic migration and proliferation are used.

Phenotype Modulation.

The SMC phenotype (φ) is assumed to vary continuously between fully contractile ($\varphi = 0$) and fully synthetic ($\varphi = 1$) and is modulated by extracellular matrix concentration, e . The ideal or homeostatic phenotype is assumed to be given by $\varphi^* = 1 - e_{\downarrow\downarrow}$, where $e_{\downarrow\downarrow} = (\sum_{i=1}^n e_i)/n$ is the average of e within a sphere of radius $2\sqrt{\Delta x}$ (n number of points); i.e., the “Moore” neighborhood [35], which includes both orthogonally and diagonally adjacent lattice points (Fig. 3). The rate of change of phenotype is described by

$$d\varphi/dt = k_d(\varphi^* - \varphi) \quad (8)$$

where k_d is a constant, such that the phenotype of a cell approaches the homeostatic phenotype of its environment at a rate proportional to the deviation of its phenotype from homeostasis.

Proliferation.

Cells are assumed to have an intrinsic maximum proliferation rate (p_{smc}) such that exponential proliferation would occur if there was no contact-inhibition; however, the lattice-modeling approach explicitly provides for contact-inhibition behavior because, as the lattice fills, the number of vacant lattice sites for cells to occupy are reduced. The mitosis rate of a given cell is assumed to depend linearly on the local amount of growth stimulus (g) and the phenotype of the SMC (ϕ). We therefore write an expression for the probability of cell division when it has been ascertained that there is a vacant neighboring lattice, within a timestep Δt , as

$$P_{proliferation} = p_{smc} \cdot g \cdot \phi \cdot \Delta t \quad (9)$$

Migration.

SMCs are assumed to have an intrinsic maximum migration speed v_{smc} . The actual migration speed is linearly related to phenotype, so the probability of a cell moving to a vacant lattice point, within a timestep Δt , is

$$P_{migration} = v_{smc} \Delta x \phi \cdot \Delta t \quad (10)$$

A lattice site is only acceptable for migration if no cell is present and the extracellular matrix is greater than a critical value e_{min} . This means cells will only migrate into a region if there is sufficient matrix to provide a scaffold for cell attachment.

Extracellular Matrix Synthesis.

Cells are assumed to produce extracellular molecules, which assemble around the cell to form extracellular matrix. Material is deposited within a radius r_{max}

of the cell (here, $r_{\max}$ is assumed to be $2^{-\sqrt{\Delta x}}$), i.e. to each lattice point in the Moore neighborhood.

$$\frac{d\phi}{dt} = k_e \phi(r - \delta r) \quad 0 \leq \delta \leq r \quad (11)$$

where δ is the distance from the center of the cell and k_e is a constant.

Due to these equations and the geometry of the lattice, there are three expression rates around a cell: $\delta = \Delta x$ for von Neumann neighbors, $\delta = 2^{-\sqrt{\Delta x}}$ for diagonally adjacent neighbors and $\delta = 0$ for the cell position itself.

Computational Implementation in a Lattice.

The simulation time is divided into equal timesteps, Δt . During each timestep, each cell expresses extracellular matrix and modulates its phenotype. It also selects its behavior from the set {proliferate, migrate, no change}. To ensure that each cell can perform only one of these behaviors per timestep, the timestep must satisfy

$$(P_{\text{proliferationmax}} + P_{\text{migrationmax}}) \leq 1 \quad (12)$$

and from Eqs. 9,10

$$\Delta t_{\max} = 1 / (p_{\text{smc}} + v_{\text{smc}} / \Delta x) \quad (13)$$

To search for a vacant neighboring lattice point, a neighboring direction is chosen at random using a random number generator. If the selected position is not vacant, another position is randomly chosen, and this is repeated until a vacant position has been found, or all positions have been searched.

These algorithms were implemented using the Visualization Toolkit (VTK) code and custom-build classes and methods in c++ (called the MechanoBiology ToolKit, see Lennon et al. [36]). This allows representation of the finite element mesh (an unstructured grid in VTK) and the lattice (image data set in VTK). The

interpolation algorithms from VTK were used in mapping variables between the spatial representations, and the algorithms described above were implemented.

Inflammation Model Testing.

The inflammation model was isolated for study by assuming no SMC activity occurs, reducing the system to a set of coupled ordinary differential equations for e , D , m , and g . We examined the behavior of the model when an initially uninjured tissue is loaded in uniaxial tension. Four cases were analyzed: (i) an instantaneous stress (σ_0) is applied and removed immediately, (ii) a stress (σ) is held constant, (iii) a stress (σ) decays linearly with time, and (iv) a constant stretch ratio (λ) is applied. These give rise to the prescribed conditions

$$(i) \sigma(t) = \sigma_0 \quad 0 < t < t_1 = 0 \quad t \geq t_1 \quad (14)$$

$$(ii) \sigma(t) = \sigma_0 \quad \forall t \quad (15)$$

$$(iii) \sigma(t) = \sigma_0 - kt \quad \sigma \geq 0 \quad (16)$$

$$(iv) \lambda(t) = \lambda_0 \quad \forall t \quad (17)$$

Parameter Estimation.

Material properties were chosen based on porcine coronary artery uniaxial tension tests [19]. The fatigue parameters (Eq. 2) were determined using the data of McLoughlin [37], as shown in Fig. 4. In these experiments, circumferential porcine coronary artery segments were fatigue tested in tension. The samples were subjected to cyclic tensile loading at three mean stress magnitudes (200 kPa, 500 kPa, and 1 MPa), and the number of cycles to failure was recorded. A log-linear model was fitted to this data (σ versus $\log N_f$). Assuming damage would not accumulate in the healthy artery, the lower limit for the fatigue strength of the material is the maximum stress magnitude induced under physiological loading. To compute this, a finite element model of a healthy artery (with inner radius of 1.5 mm and wall thickness of 0.5 mm) was pressurized to

13.3 kPa (mean physiological blood pressure), and the maximum stress induced was taken as the fatigue limit. The mechanical parameters are summarized in Table 1.

Cell migration was set based on in vitro measurements of human SMC migration [38]. The lattice size was set such that $\Delta x = 0.018$ mm, allowing a maximum cell concentration of 3000 cells/mm². The initial concentration, c_{smc} , was set to a density of 1620 cells/mm², based on the data of Tracy [39] for SMC densities in healthy human arteries. In practice, this was achieved by assigning 50% of all lattice points a smooth muscle cell randomly; all cells were assigned a contractile phenotype initially ($\phi = 0$). Cell model parameters are summarized in Table 2.

The inflammation parameters (k_m and k_{deg}) were calibrated to achieve similar inflammation time-courses as found in the study of angioplasty in humans by Cipollone et al. [33]. In this study, a key marker for inflammation (monocyte chemoattractant protein 1 (MCP-1)) was measured in angioplasty patients. It was found that in patients who did not develop restenosis, MPC-1 reduced much quicker than in the restenosis-prone group. For this reason, three inflammation responses were simulated in our computational models: a prolonged (high) and shortened (low) source of inflammation corresponding to the restenosis and no restenosis groups in the above study, respectively, and an intermediate response, which lay half-way between both responses, as tabulated in Table 3.

Balloon Expansion Calibration.

A simulation of balloon expansion was used to estimate matrix production and cell proliferation parameters by comparing the response to human data [32]. In that paper, the number of cells over time postangioplasty was estimated from the restenosis area measurements, and the known concentration of SMCs in restenotic tissue. In the analogous simulation, a 2D cylindrical, isotropic hyperelastic artery with inner radius 1 mm and outer radius 2 mm was

expanded by applying a displacement to the inner lumen surface to achieve an expansion ratio (maximum dilated diameter/initial lumen diameter) of 1.4. The parameters which control cell proliferation (k_g and d_g) as well as the matrix production and migration parameters (k_e and e_{min}) were calibrated to achieve an accurate model match for cell numbers and neointimal area (Table 4). To calculate the mean response of the model, ten simulations were run at each parameter combination, and the mean of the results was calculated.

Stent Expansion.

To simulate stent expansion, an analytical rigid body representing a stent strut with circular cross-section was displaced radially into the wall of the artery (Fig. 5). The stent strut was assumed to have a circular cross-section and a diameter of 150 μm . To analyze the effect of increasing the extent of stent expansion on the degree of restenosis induced, the stent radial deployment was varied between 0.17 mm and 0.88 mm. This was performed for the three different inflammatory responses described in Sec. 2.6 above.

Results

Inflammation Model.

In case (i), where an initial stress is applied and removed, damage decayed exponentially to zero, while extracellular matrix reduced exponentially to a final value of $e(t = 0) - D(t = 0)$ (Fig. 6). Therefore, D , in this case, is a measure of the quantity of injury in the artery which must be removed. In case (ii), when a constant stress is applied, damage never stabilizes and accumulates eventually to one; the rate of accumulation is higher for higher stress magnitudes (Fig. 6). At low stresses, the extracellular matrix is not significantly altered, whereas at high stresses, all the material is removed rapidly. Case (iii) is in between cases (i) and (ii) depending on the stress relaxation prescribed (Fig. 6); the damage accumulates with prolonged stress, whereas with less-prolonged stress, damage can be removed. In case (iv), the stretch to which the artery is exposed

determines whether the damage accumulates or decays and by how much (Fig. 6). A long-term stress-relaxation-type response is found, where the stress reduces to a homeostatic stress-state at a rate, which depends on the initial magnitude of the stretch.

Balloon Expansion Calibration.

The calibration gave a reasonable match to the experimental data of Schwartz et al. [32] in terms of the number of SMCs found over a 350 day period (Fig. 7). In particular, an initial rapid proliferation rate gradually decreased to zero over the course of the simulation. The smooth muscle cells in the injured region modulated their phenotype after approximately 5 days and began migrating, proliferating, and producing extracellular matrix. The maximum proliferation rate achieved was most affected by the parameter $psmc$, while the final cell number and time to peak proliferation rate (approximately 10 days in the baseline simulations) were most affected by the parameters affecting growth factor kinetics (dg and kg).

The simulations produced a final cell distribution in which there was a gradient of cell concentration through the thickness of the neointima, with the injured media having the most cells, and the surface of the neointima having less (Fig. 8).

Analysis of Stent Expansion.

The model predicted a nonlinear relationship between neointimal area and initial lumen gain. Below a lumen area of 6 mm^2 , restenosis was minimal, but for gains greater than that, neointimal hyperplasia and restenosis increased with initial lumen gain (Fig. 9).

The simulation results were not deterministic, allowing a prediction of the variability in the restenosis developed (Fig. 9). The level of inflammation affected the relationship between restenosis and stent area, with high inflammation inducing more restenosis and a greater dependency on stent area with high inflammation, as can be seen from the final slope of the curves (Fig. 9).

Lattice variables are plotted in Fig. 1 for the high inflammation case, indicating lumen narrowing within the stent.

Discussion

A computational simulation methodology has been described, which captures key characteristics of lesion growth within arteries subjected to nonphysiological mechanical forces. It can predict outcomes with respect to how stent expansion affects the final restenosis within an artery. The model produced rapid neointima formation and cell proliferation in the first few weeks post-implantation, approaching a steady state after six months in an artery after balloon expansion. The model could be calibrated against the neointimal area and cell quantity data measured after balloon angioplasty. With this calibration done, the model was then applied to stenting.

In the analysis of stenting, a nonlinear relationship between lumen gain due to stenting and subsequent restenosis was predicted. In a clinical study of the effect of stent expansion on restenosis, a more linear trend was found [40]. The differences between the simulation setup and the clinical environment may explain this discrepancy. In the clinical study, several stent types were used, the targeted lesions were highly variable in structure, and the individual patients may have had varying inflammation responses, all of which contribute to the high amount of variability in outcome. These sources of variability have not been included in the simulations described here. Our study was an attempt at a mechanistic model of the biological processes involved in restenosis using as few

parameters as possible, while remaining physically relevant. The simulation is made up of modular algorithms for key biological processes, which can, in principal, be independently validated.

Previous models have not accounted for cell activity explicitly; instead, volume change has been related phenomenologically (e.g., directly) to stress (e.g., Ref. [28]). However, the complex interactions between stress, cells, and their environment are important—for example, balloon angioplasty cannot be captured by models that are based on a homeostatic stress-state because the deviation from homeostatic stress in the arterial wall is instantaneous and short lived, making it unlikely to be an adaptation reaction. In other cell-based models [(41),24,5], the behavior of the smooth muscle cell is directly linked to their mechanical environment, which assumes a mechanotransduction mechanism. However, injury and inflammation are regarded as key intermediate processes in the restenosis development, and the importance of extracellular species (modeled here as D , e , g , and m) can be seen from the drugs that target them, such as antiproliferative drugs, MMP inhibitors, extracellular matrix production inhibitors, etc. If we are to capture these processes in silico, the explicit modeling of extracellular species is a crucial first step. For example, an antiproliferative drug could be simulated as a substance produced at the surface of the stent, and which diffuses through the tissue. This drug could inhibit the proliferative activity of cells (adding a new term in Eq. 9) in a dose-dependant way and decay over time.

There are limitations with the present modeling approach. The finite element analysis described here idealizes the artery to a single-layered, atherosclerosis free, and isotropic hyperelastic cylindrical vessel. This could be improved with better material models [42] and patient-specific geometries [17]. Another limitation is that the fatigue data are based on tests performed at three test levels in the circumferential direction. More comprehensive data relating the stress/strain in the tissue to injury would allow more accurate predictions. Other

damage-mechanics models may prove more suitable to the once-off injury source, which is a characteristic of both balloon and stent expansion. For this reason, more work is needed to define the injury and damage behavior of vascular tissues. Another limitation is that the inflammation model is based on an assumption that inflammatory factors are produced at a rate proportional to the injury. In reality, the inflammation is brought about by several cell types, each with their own specific proliferation, metabolic, and migratory behaviors. As more is known about the activities of these cells, this information can easily be incorporated by adding new cell types. The algorithms governing smooth muscle cell behavior are rudimentary, as there are many other stimuli for SMC behavior, such as oxygen, nitric oxide, heparin, and many others, as well as direct mechanical signaling through stretch [43]. The extracellular matrix variable currently encompasses all extracellular species, which, in reality, consists of a diverse array of constituents, and each component may affect cell behavior differently. For example, laminin and fibronectin are found to have opposite effects on cell phenotype, and the ratio of these species is shown to change throughout restenosis development [11]. In the simulations presented here, extracellular matrix was implemented as a variable, which sought to define the conduciveness of the environment to maintaining the contractile phenotype. Similarly, the growth stimulus term could be further subdivided into the diverse chemical agents, as could the matrix degrading factor variable. Biomolecular species have previously been modeled as diffusible [(44),45], which has not been considered in this study. However, when considering that many biomolecules have short half-lives, it may not be valid to model their dispersal using diffusion. Instead, the cells dictate where biomolecules are expressed, and the region in which the molecules are present can only be changed by cell migration, phenotype modulation or cell-to-cell signaling, all of which could be simulated in the lattice-based approach.

In this paper, we have not introduced the environmental and genetic variabilities, which characterize many clinical trials. Lesion and artery structure can be expected to vary in a realistic population, and often differing amounts of

inflation pressure are used by surgeons. Patients may also have differing responses to the same stimuli due to genetic variability in cell processes. These effects have been considered recently for skeletal tissue mechanobiology by Khayyeri et al. [46].

The endothelium is not considered here. In a previous paper from our group, the endothelium was modeled as a cell type which constantly proliferated over the lumen surface [30]. In reality, endothelium is also mechanoregulated [47], and this could be incorporated into the future simulations as a mechanically regulated source of inflammatory factors at the lumen surface. For example, the production of growth stimulus and matrix degrading factors at the surface could be dependant on the presence of endothelium, (e.g., based on a surface migration model) and on the shear stress encountered at that position. This would require calculating fluid flow-induced biophysical stimuli (wall shear stress, oscillatory shear index, etc.). A previous model of restenosis used fluid flow induced stimuli to directly affect the proliferative behavior of smooth muscle cells [24], and the inclusion of this mechanism in the model proposed here may be important for accurately modeling the restenotic response of arteries. However, fluid flow-induced alterations alone cannot predict restenosis, as it leads to homeostatic equilibrium when the growth lesion produces physiological fluid flow-induced shear stresses. The time-course of fluid-flow induced diseases, such as vascular remodeling and atherosclerosis, is also typically longer than restenosis. Finally, arterial wall stress is not updated in the simulations applied to the stent, i.e., injury is assumed to occur immediately poststenting, and assumes chronic injury build-up does not occur.

The lattice-based approach offers advantages over other modeling techniques. The simulation is built up of separate algorithms, which perform specific tasks based on biological processes. Providing the inputs to these algorithms can be controlled experimentally, these algorithms can then be validated independently [48]. The migration algorithm, for example, requires that we know the initial

concentration and phenotypes of cells. These independent algorithms are then combined to provide a cell-centered model. The algorithms, although based on simple behavior, when coupled together have been shown to produce much of the complex behavior found in vivo. Having these biological processes, i.e. proliferation, phenotype modulation, etc. allows us to apply the technique to a range of problems involving cardiovascular injury [49]. It may be useful to simulate stents, grafts, and (with more biological rules and the inclusion of fluid flow) even initial atherosclerotic development.

This paper has demonstrated the potential of the lattice-based modeling approach to the simulation of the mechanobiological response of arterial tissue. It was shown that the model captures the key phenomena of inflammation, cell activity, and tissue production, and that it could be calibrated against the experimental results to simulate the balloon angioplasty results in humans. The inclusion of key biological process opens the way for including inherent interpatient variability in these processes, providing a prediction of the rates of success of a stent, rather than a deterministic answer. By applying the fundamental model described here to stenting procedures in 3D, we can optimize the stenting procedure to maximize long-term lumen gain. This represents a step forward from trying to infer what growth will occur from stress analyses and has the potential to include the bioactive properties of drug-eluting stents. In conclusion, this method may be able to examine the interactions and trade-offs between design options (stent geometric design, expansion method and pharmaceutical action) and long-term lumen gain.

Acknowledgements

The authors would like to thank Ruth McLoughlin, whose Masters thesis results were used in the calibration of a key part of the model. This work is funded by a Science Foundation Ireland (SFI) Principal Investigator grant to Professor Patrick Prendergast.

Nomenclature

$c_{smc} =$

initial cell seeding (cells/mm²)

$D =$

damage

$dg =$

decay constant of g (day⁻¹)

$e =$

normalized concentration of extracellular matrix

$e_{min} =$

minimum e value to support cell adhesion

$g =$

normalized concentration of growth factor

$k_d =$

cell differentiation rate (day⁻¹)

$k_{deg} =$

rate of degradation of e (day⁻¹)

$k_e =$

maximum e synthesis rate (day⁻¹)

$k_g =$

rate of production of g (day⁻¹)

$k_m =$

rate of production of m (day⁻¹)

$m =$

normalized concentration of matrix degrading factor

$N =$

number of cycles of loading (day⁻¹)

$p_{smc} =$

maximum cell proliferation rate (day⁻¹)

$r_{\max} =$

radius of cell local neighborhood (μm)

$v_{\text{smc}} =$

maximum cell migration speed ($\mu\text{m day}^{-1}$)

$\alpha =$

slope of Wöhler curve

$\Delta t =$

timestep (days)

$\Delta x =$

lattice spacing (mm)

$\emptyset =$

smooth muscle cell phenotype

$\psi =$

strain energy density

σ_f =

failure strength (kPa)

σ_0 =

fatigue strength (kPa)

EC =

endothelial cell

ECM =

extra-cellular matrix

MDF =

matrix degrading factor

MMP =

matrix Metalloproteinase

SMC =

smooth muscle cell

References

Hoffmann, R., Jansen, C., Konig, A., Haager, P. K., Kerckhoff, G., Vom Dahl, J., Klauss, V., Hanrath, P., and Mudra, H., 2001, "Stent Design Related Neointimal Tissue Proliferation in Human Coronary Arteries—An Intravascular Ultrasound Study," *Eur. Heart J.*, 22 , pp. 2007–2014.

Hoffmann, R., Mintz, G. S., Haager, P. K., Bozoglu, T., Grube, E., Gross, M., Beythien, C., Mudra, H., vom Dahl, J., and Hanrath, P., 2002, "Relation of Stent Design and Stent Surface Material to Subsequent In-Stent Intimal Hyperplasia in Coronary Arteries Determined by Intravascular Ultrasound," *Am. J. Cardiol.*, 89 , pp. 1360–1364. [

Morton, A. C., Crossman, D., and Gunn, J., 2004, "The Influence of Physical Stent Parameters Upon Restenosis," *Pathol. Biol.*, 52 , pp. 196–205.

Bennett, M. R., and O'Sullivan, M., 2001, "Mechanisms of Angioplasty and Stent Restenosis: Implications for Design of Rational Therapy," *Pharmacol. Ther.*, 91 , pp. 149–166.

Lally, C., Kelly, D. J., and Prendergast, P. J., 2006, "Stents," In "Encyclopedia of Biomedical Engineering", Wiley, pp. 3345–3355.

Gunn, J., Arnold, N., Chan, K. H., Shepherd, L., Cumberland, D. C., and Crossman, D. C., 2002, "Coronary Artery Stretch Versus Deep Injury in The Development of In-Stent Neointima," *Heart*, 88 , pp. 401–405.

Kornowski, R., Hong, M. K., Tio, F. O., Bramwell, O., Wu, H., and Leon, M. B., 1998, "In-stent Restenosis: Contributions of Inflammatory Responses and Arterial Injury to Neointimal Hyperplasia," *J. Am. Coll Cardiol.*, 31 , pp. 224–230.

Scott, N. A., 2006. "Restenosis Following Implantation of Bare Metal Coronary Stents: Pathophysiology and Pathways Involved in the Vascular Response to Injury," *Adv. Drug Delivery Rev.*, 58 , pp. 358–376.

Welt, F. G., and Rogers, C., 2002, "Inflammation and Restenosis in the Stent Era," *Arterioscler., Thromb., Vasc. Biol.*, 22 , pp. 1769–1776.

Thyberg, J., Hedin, U., Sjolund, M., Palmberg, L., and Bottger, B. A., 1990, "Regulation of Differentiated Properties and Proliferation of Arterial Smooth Muscle Cells," *Arteriosclerosis (Dallas)*, 10 , pp. 966–990.

Thyberg, J., Blomgren, K., Roy, J., Tran, P. K., and Hedin, U., 1997, "Phenotypic Modulation of Smooth Muscle Cells After Arterial Injury is Associated With Changes in the Distribution of Laminin and Fibronectin," *J. Histochem. Cytochem.*, 45 , pp. 837–846.

Zempo, N., Koyama, N., Kenagy, R. D., Lea, H. J., and Clowes, A. W., 1996, "Regulation of Vascular Smooth Muscle Cell Migration And Proliferation in Vitro and in Injured Rat Arteries by a Synthetic Matrix Metallopro-Teinase Inhibitor," *Arterioscler., Thromb., Vasc. Biol*, 16 (1), pp. 28–33.

Holzapfel, G. A., Sommer, G., Gasser, C. T., and Regitnig, P., 2005, "Determination of Layer-Specific Mechanical Properties of Human Coronary Arteries With Nonatherosclerotic Intimal Thickening and Related Constitutive Modeling," *Am. J. Physiol. Heart Circ. Physiol.*, 289 , pp. 2048–2058.

De Beule, M., Mortier, P., Carlier, S. G., Verhegghe, B., Van Impe, R., and Verdonck, P., 2008, "Realistic Finite Element-Based Stent Design: The Impact Of Balloon Folding," *J. Biomech.*, 41 (2), pp. 383–389. [

Gervaso, F., Capelli, C., Petrini, L., Lattanzio, S., Di Virgilio, L., and Migliavacca, F., 2008, "On the Effects of Different Strategies in Modelling Balloon-Expandable Stenting by Means of Finite Element Method," *J. Biomech.*, 41 , pp. 1206–1212.

Gijssen, F., Migliavacca, F., Schievano, S., Socci, L., Petrini, L., Thury, A., Wentzel, J., van der Steen, A., Serruys, P., and Dubini, G., 2008, "Simulation of Stent Deployment in a Realistic Human Coronary Artery," *Biomed. Eng. online*, 7 (1), pp. 23–23.

Holzapfel, G. A., Stadler, M., and Schulze-Bauer, C. A. J., 2002, "A Layer-Specific Three-Dimensional Model for the Simulation of Balloon Angio-Plasty Using Magnetic Resonance Imaging And Mechanical Testing," *Ann. Biomed. Eng.*, 30 , pp. 753–767. [

Prendergast, P. J., Lally, C., Daly, S., Reid, A. J., Lee, T. C., Quinn, D., and Dolan, F., 2003, "Analysis of Prolapse in Cardiovascular Stents: A Constitutive Equation for Vascular Tissue and Finite Element Modelling," *Trans. ASME*, 125 , pp. 692–699. [

Lally, C., Dolan, F., and Prendergast, P. J., 2005, "Cardiovascular Stent Design and Vessel Stresses: A Finite Element Analysis," *J. Biomech.*, 38 , pp. 1574–1581.

Migliavacca, F., Petrini, L., Massarotti, P., Schievano, S., Auricchio, F., and Dubini, G., 2004, "Stainless and Shape Memory Alloy Coronary Stents: A Computational Study on the Interaction With the Vascular Wall," *Biomech. Model. Mechanobiol.*, 2 , pp. 205–217.

Bedoya, J., Meyer, C. A., Timmins, L. H., Moreno, M. R., and Moore, J. E., 2006, "Effects of Stent Design Parameters on Normal Artery Wall Mechanics," *J. Biomech. Eng.*, 128 , pp. 757–765.

Timmins, L. H., Moreno, M. R., Meyer, C. A., Criscione, J. C., Rachev, A., and Moore, J. E., 2007, "Stented Artery Biomechanics and Device Design Optimization," *Med. Biol. Eng. Comput.*, 45 , pp. 505–513. [

Early, M., and Kelly, D. J., 2010, "The role of Vessel Geometry and Material Properties on the Mechanics of Stenting in the Coronary and Peripheral Arteries," *Proc. Inst. Mech. Eng., Part H: J. Eng. Med.*, 224 (H3), pp. 465–476.

Evans, D. J., Lawford, P. V., Gunn, J., Walker, D., Hose, D. R., Smallwood, R. H., Chopard, B., Krafczyk, M., Bernsdorf, J., and Hoekstra, A., 2008, "The Application of Multiscale Modelling to the Process of Development and Prevention of Stenosis in a Stented Coronary Artery," *Philos Trans. A*, 366 , pp. 3343–3360.

Garcia, J., Doblare, M., and Cegonino, J., 2002, "Bone Remodelling Simulation: A Tool for Implant Design," *Comput. Mater. Sci.*, 25 (1-2), pp. 100–114.

Prendergast, P. J., 2009, "Papers in Biomechanics and Bioengineering," ScD thesis, University of Dublin, Dublin, Ireland.

Lally, C., and Prendergast, P. J., 2006, "Simulation of In-Stent Restenosis for the Design of Cardiovascular Stents," in "Mechanics of Biological Tissue", G.A.Holzapfel and R.W.Ogden, eds., Springer, Berlin, Heidelberg, pp. 255–267.

Rachev, A., Manoach, E., Berry, J., and Moore, J. E., 2000, "A Model of Stress-Induced Geometrical Remodelling of Vessel Segments Adjacent of Stents and Artery/Graft Anastomoses," J. Theor. Biol., 206 , pp. 429–443.

Peréz, M. A., and Prendergast, P. J., 2007, "Random-Walk Models of Cell Dispersal Included in Mechanobiological Simulations of Tissue Differentiation," J. Biomech., 40 , pp. 2244–2253.

Boyle, C. J., Lennon, A. B., Early, M., Kelly, D. J., Lally, C., and Prendergast, P. J., 2010, "Computational Simulation Methodologies for Mechanobiological Modelling: A Cell-Centred Approach to Neointima Development in Stents," Philos. Trans. R. Soc. London, 368 , pp. 2919–2935.

Merks, R. M. H., and Glazier, J. A., 2005, "A Cell-Centered Approach to Developmental Biology," Physica A, 352 , pp. 113–130.

Schwartz, R. S., Chu, A., Edwards, W. D., Srivatsa, S. S., Simari, R. D., Isner, J. M., and Holmes, D. R., 1996, "A Proliferation Analysis of Arterial Neointimal Hyperplasia: Lessons for Antiproliferative Restenosis Therapies," Int. J. Cardiol., 53 , pp. 71–80.

Cipollone, F., Marini, M., Fazia, M., Pini, B., Iezzi, A., Reale, M., Paloscia, L., Materazzo, G., D'Annunzio, E., Conti, P., Chiarelli, F., Cuccurullo, F., and Mezzetti, A., 2001, "Elevated Circulating Levels of Monocyte Chemoat-Tractant Protein-1 in Patients With Restenosis After Coronary Angioplasty," Arterioscler. Thromb. Vasc. Biol., 21 (3), pp. 327–334.

Lee, Y. L., and Pan, J. H. R. B. M., 2005, "Fatigue Testing and Analysis, Theory and Practice", Elsevier Butterworth-Heinmann, Burlington, MA and Oxford, UK.

Chopard, B., and Droz, M., 1998, "Cellular Automata Modeling of Physical Systems", Cambridge University Press is in Cambridge University, Cambridge, UK.

Lennon, A. B., Khayyeri, H., Xue, F., and Prendergast, P. J., 2011, "Biomechanical Modelling of Cells in Mechanoregulation," In "Cellular and Biomolecular Mechanics and Mechanobiology", A.Gefen, ed., Springer, pp. 295–329.

McLoughlin, R., 2008, "Tensile Fatigue Behaviour of Porcine Coronary Arterial Tissue in the Longitudinal and Circumferential Directions," MSc thesis, University of Dublin, Dublin.

DiMilla, P. A., Stone, J. A., Quinn, J. A., Albelda, S. M., and Lauffenburger, D. A., 1993, "Maximal Migration of Human Smooth Muscle Cells on Fibronectin and Type IV Collagen Occurs at an Intermediate Attachment Strength," *J. Cell Biol.* 122 , pp. 729–737.

Tracy, R. E., 1997, "Declining Density of Intimal Smooth Muscle Cells as a Precondition for Atheronecrosis in the Coronary Artery," *Virchows Archiv.* 430 , pp. 155–162.

Farb, A., Weber, D. K., Kolodgie, F. D., Burke, A. P., and Virmani, R., 2002, "Morphological Predictors of Restenosis After Coronary Stenting in Humans," *Circulation*, 105 , pp. 2974–2980.

Caiazzo, A., Evans, D., Falcone, J.-L., Hegewald, J., Lorenz, E., Stahl, B., Wang, D., Bernsdorf, J., Chopard, B., Gunn, J., Hose, R., Krafczyk, M., Lawford, P., Smallwood, R., Walker, D., and Hoekstra, A. G., (2009), "Towards a Complex Automata Multiscale Model of In-Stent Restenosis," in "Proceedings of the 9th International Conference on Computational Science: Part I", Springer-Verlag, pp. 705–714.

Criscione, J., 2003, "Rivlin's Representation Formula is Ill-Conceived for the Determination of Response Functions via Biaxial Testing," *J. Elast.*, 70 (19), pp. 129–147.

Colombo, A., Cahill, P., and Lally, C., 2007, "The Frequency of Cyclic Stretch Determines the Proliferative and Apoptotic Capacity of Vascular Smooth Muscle Cells In Vitro," *Arterioscler., Thromb. Vasc. Biol.*, 27 (6), pp. E74–E74.

Bailon-Plaza, A., and van der Meulen, M. C., 2001, "A Mathematical Framework to Study The Effects of Growth Factor Influences on Fracture Healing," *J. Theor. Biol.*, 212 , pp. 191–209.

Murray, J. D., 2002, "Mathematical Biology", Springer, New York.

Khayyeri, H., Checa, S., Tgil, M., Aspenberg, P., and Prendergast, P. J., 2011, "Variability Observed In Mechano-Regulated In Vivo Tissue Differentiation can be Explained by Variation in Cell Mechano-Sensitivity," *J. Biomech.*, 44 (6), pp. 1051–1058.

Reneman, R. S., Arts, T., and Hoeks, A. P., 2006, "Wall Shear Stress—An Important Determinant of Endothelial Cell Function and Structure—In the Arterial System In Vivo Discrepancies With Theory," *J. Vasc. Res.*, 43 , pp. 251–269.

Prendergast, P. J., Checa, S., and Lacroix, D., 2010, "Computational Models of Tissue Differentiation," in "Computational Modeling in Biomechanics", S.De, F.Guilak, and M.Mofrad, R.K., eds., Springer, Netherlands, pp. 353–372.

Zahedmanesh, H., 2011, "Finite Element and Mechanobiological Modelling of Vascular Devices," Ph.D thesis, Dublin City University.

Appendix I : Figures

Figure 1

Schematic representation of restenosis development. Initially, quiescent SMCs occupy the vessel wall and an intact endothelium exists (a). Upon stenting, the plaque is plastically deformed, endothelium is denuded, vascular tissue is stretched and inflammation is initiated (b). In response, SMCs become synthetic, migratory, and proliferative, producing neointima (c), which may achieve homeostasis, provided the inflammation stimulus recedes and a layer of endothelial cells is re-established (d). Neointimal area is measured as the difference between the lumen area immediately poststenting and the lumen area upon follow-up.

Figure 2

Flow chart outlining the computational method for the simulation of restenosis. The stress may be updated, depending on whether or not the new geometry significantly affects the structural behavior of the artery, and whether the injury stimulus is assumed to occur once in the initial expansion procedure, or is chronic.

Figure 3

The neighboring points around a cell in an orthogonal lattice. In the left-most diagram, VN indicates the von Neumann neighborhood (shown in 3D in the central diagram), and M indicates the Moore neighborhood (shown in 3D in the right-most diagram).

Figure 4

Stress versus remaining life (number of cycles to failure, N_f) for porcine coronary artery. Crosses indicate the experimental data from McLoughlin [37]. The solid lines show the fitted bilinear model; the horizontal line indicates the fatigue strength, $\sigma_0 = 90$ kPa, the y-intercept is the failure strength, $\sigma_f = 1417$ kPa, and the slope, $\alpha = -187$.

Figure 5

The geometry of the idealised artery and stent (left). A cross-section of a symmetrically stenosed artery is shown with six stent struts. A $1/6$ segment is considered due to symmetry and this model is meshed as shown on the right. A displacement is applied to the strut, and the upper and lower surfaces of the mesh are restrained circumferentially.

Figure 6

Case (i): An initial stress is applied and removed. Damage is initiated abruptly, and decays exponentially to zero, while extracellular matrix decays exponentially to $e = e|_{t=0} - D|_{t=0}$. Case (ii): A constant stress is prescribed, leading to complete ECM removal at a rate dependant on the stress applied. Case (iii): A decaying stress is prescribed. Case (iv): A constant stretch is prescribed, which leads to stress-relaxation.

Figure 7

The best-fit approximation to the data of Schwartz [32], found by systematically testing variable values. The solid line indicates the average cell number over ten simulations, with the gray region indicating the variation between runs (± 1 standard deviation). The experimental data (shown as crosses) indicate cell numbers calculated from human PTCA patients.

Figure 8

Diagrams of lattice state over time after balloon angioplasty (i.e., no stent). The background color indicates concentration of extracellular matrix (e). The spheres indicate the positions of cells, while the color indicates phenotype (green contractile to red synthetic). A band of injury forms at the lumen surface due to balloon expansion, from which cells proliferate and migrate into the lumen over time.

Figure 9

The amount of restenosis (neointimal area/initial lumen area*100%) as a function of initial lumen area (i.e., the area within the expanded stent). Five simulations were conducted at each point with variation occurring due to the stochastic cell activity model. In the high inflammation case, parameters controlling inflammation were calibrated to produce long-term inflammation, while in the low inflammation case, these were selected to produce a removal of damage within 15 days.

Figure 10

Diagrams of lattice state over time after stent expansion, showing SMC activation and proliferation leading to lesion formation and restenosis over time. The background color indicates concentration of extracellular matrix. The spheres indicate the positions of cells, while the color indicates phenotype (green contractile phenotype to red synthetic phenotype).

Appendix 2: Tables

Table 1
Mechanical and fatigue parameters

Parameter	Unit	Value
α	kPa	-187
σ_f	kPa	1417
σ_0	kPa	90
N	day-1	1

Table 2
Cell parameters

Parameter	Unit	Value
$csmc$	%	0.5
$psmc$	day-1	0.24
$vsmc$	μm . day-1	0.48
kd	day-1	0.2
$rmax$	μm	0.0255

Table 3
Inflammation parameters, showing high, intermediate and low responses

Parameter	Unit	Value
km	day-1	(H: 0.0043, I: 0.04, L: 0.2)
$kdeg$	day-1	(H: 0.98, I: 0.4, L: 0.9)

Table 4
Balloon angioplasty-derived parameters

Parameter	Unit	Value
kg	day-1	0.04
dg	day-1	0.011
ke	day-1	0.025
$emin$	—	0.11