

REVIEW

Computer-aided design and finite-element modelling of biomaterial scaffolds for bone tissue engineering

BY DAMIEN LACROIX^{1,*}, JOSEP A. PLANELL^{1,2}
AND PATRICK J. PRENDERGAST³

¹*Institute for Bioengineering of Catalonia, 08028 Barcelona, Spain*

²*Department of Material Sciences, Technical University of Catalonia, 08034 Barcelona, Spain*

³*Trinity Centre for Bioengineering, Trinity College Dublin, Dublin 2, Ireland*

Scaffold biomaterials for tissue engineering can be produced in many different ways depending on the applications and the materials used. Most research into new biomaterials is based on an experimental trial-and-error approach that limits the possibility of making many variations to a single material and studying its interaction with its surroundings. Instead, computer simulation applied to tissue engineering can offer a more exhaustive approach to test and screen out biomaterials. In this paper, a review of the current approach in biomaterials designed through computer-aided design (CAD) and through finite-element modelling is given. First we review the approach used in tissue engineering in the development of scaffolds and the interactions existing between biomaterials, cells and mechanical stimuli. Then, scaffold fabrication through CAD is presented and characterization of existing scaffolds through computed images is reviewed. Several case studies of finite-element studies in tissue engineering show the usefulness of computer simulations in determining the mechanical environment of cells when seeded into a scaffold and the proper design of the geometry and stiffness of the scaffold. This creates a need for more advanced studies that include aspects of mechanobiology in tissue engineering in order to be able to predict over time the growth and differentiation of tissues within scaffolds. Finally, current perspectives indicate that more efforts need to be put into the development of such advanced studies, with the removal of technical limitations such as computer power and the inclusion of more accurate biological and genetic processes into the developed algorithms.

Keywords: biomechanics; tissue engineering; biomaterials; finite-element modelling

* Author for correspondence (dlacroix@ibec.pcb.ub.es).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsta.2009.0024> or via <http://rsta.royalsocietypublishing.org>.

One contribution of 15 to a Theme Issue 'The virtual physiological human: tools and applications I'.

1. Biomaterial scaffolds for tissue engineering

Biomaterials development has evolved from first- and second-generation biomaterials, which sought primarily, respectively, biocompatibility and bioactivity with the surrounding tissues, to third-generation biomaterials, where biomaterials are designed to elicit a favourable and controlled bioactive response from the surrounding tissues (Hench & Polak 2002). The control of the host tissue response has led biomaterials researchers to focus on the development of new materials compositions, and to study the biomaterials' physico-chemical surface properties, topographical properties and drug encapsulation capability to favour one response or another depending on the type of interaction required between the engineered and the biological entities (Mikos *et al.* 2006). Although the inflammation reaction due to the presence of a foreign body is a natural process, with the formation of a fibrous layer encapsulating the foreign body (Castner & Ratner 2002), the aim of biomaterials science is to deceive the receiving body by sending signals eliciting the acceptance of the foreign body.

Within biomaterials, tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physico-chemical factors, to improve or replace biological functions. Traditionally, tissue engineering consists of harvesting cells from a patient, expanding them *in vitro* and culturing them into a biomaterial (also called a scaffold) that serves as a structural framework to allow cell attachment, proliferation and differentiation into a controlled phenotype (Langer & Vacanti 1993). Chemical and topographic substrate patternings are recognized as powerful tools for regulating cell functions (Lim & Donahue 2007). These committed cells can form an extracellular matrix to produce the desired tissue within the biomaterial scaffold. Tissue engineering has led to great expectations for clinical surgery or various diseases that cannot be solved with traditional devices, such as materials for osteochondral ossification (Martin *et al.* 2007). The total market for the regeneration and repair of tissues and organs was estimated to be \$25 billion worldwide in 2001 and is expected to rise steadily (D&MD 2001). However, the harvesting and culture of cells in a controlled manner remain a challenge (Kalyanaraman & Boyce 2007). Moreover, cost versus health gain is still high compared with existing technologies, which makes its industrialization difficult. Thus, research in tissue engineering still remains very active but it has failed to realize viable commercial products. The challenges facing industrialization have led the scientific community to focus on the development of cell-free biomaterials capable of eliciting the appropriate cell response without the need for seeding cells within the biomaterials prior to their implantation. Instead, the concept of tissue engineering is used to study cell–biomaterial interactions *in vitro*, with the goal of developing 'smart' biomaterials.

Whichever of the two approaches is used in tissue engineering, there has been a clear need for a more controlled environment for *in vitro* cell culture (Kirkpatrick *et al.* 2007). Better control of the culture conditions can lead to (i) more reproducible methods and therefore a more feasible industrialization of tissue engineering, (ii) the application of physical and chemical cues mimicking the ones perceived by the cells *in vivo*, and therefore allowing a more realistic *in vitro* study of cell–biomaterial interactions, and (iii) the application of controlled physical and chemical stimuli in order to improve cell response in

reaction to the biomaterial. Thus, there has been a plethora of bioreactors developed mainly in-house to improve cell seeding, cell attachment, cell proliferation and cell differentiation (Freed *et al.* 2006). Cell seeding in three-dimensional scaffolds is particularly difficult but essential for the following cellular steps of proliferation and differentiation. Diffusion through culture medium and tissue typically limits oxygen transport *in vitro*, leading to hypoxic regions and limiting the viable tissue thickness (Malda *et al.* 2007). For this reason, one of the most common bioreactors nowadays is the perfusion bioreactor, where a fluid flow (FF) is forced through the scaffold to give a very high seeding efficiency (Holtorf *et al.* 2007). This initial step is critical not only to enhance proliferation and differentiation but also to avoid cell apoptosis by bringing nutrients to the cells. Perfusion has been shown to enhance proliferation for a highly porous critical-size β -tricalcium phosphate (TCP) scaffold with dimensions of 14 mm diameter and 30 mm length, for which static culture leads to hypoxic conditions (Xie *et al.* 2006). The perfusion bioreactor, in addition to its simplicity, also has the advantage of being able to control the FF profile and magnitude within the tubes and, to a lesser extent, within the scaffold. Other types of bioreactors have also been developed and can be used complementarily with the perfusion bioreactor (Martin *et al.* 2004). These can apply other types of mechanical stimuli such as compression, tension, bending or shearing. Overall, a large range of bioreactors have been developed to apply a wide range of stimuli depending on the biomaterial used and the tissues to engineer. It has been shown, for example, that a 10 per cent uniaxial cyclic tensile strain can increase osteogenic differentiation of human mesenchymal stem cells (MSCs) through bone morphogenetic protein (BMP-2) mRNA expression without the addition of osteogenic supplements (Sumanasinghe *et al.* 2006).

The objectives of this paper are to present a review of the scaffold designs currently used and of their characterization using imaging techniques, and to present the finite-element analyses and mechanobiological concepts used to study the mechanical stimuli in biomaterials scaffolds applied to bone tissue engineering. We address the issues confronting researchers when modelling scaffolds for tissue engineering. In particular, we review current concepts to generate accurate computational models of scaffolds using finite-element analysis, and how this may impact on tissue engineering research in the future.

2. Scaffold designs

The design of a scaffold is essential for its correct interaction with cells as indicated above. Moreover, it is essential that it fulfils its *in vivo* function. Biomaterials science has mainly adopted a 'trial-and-error' approach, with modifications being made to an existing design based on experimental *in vitro* or *in vivo* results. Improvements in cell culture conditions and the development of bioreactors have greatly improved the reliability of *in vitro* experiments. Nonetheless, computer-aided design (CAD) can also contribute to the reduction of experimental tests and to shortening the design process of scaffolds (Sun & Lal 2002; Sun *et al.* 2004*a,b*). CAD consists of the design of engineering components through computer techniques based on mechanical design. This technique can be used to design any kind of component or material and could be very useful for the

design of scaffolds for tissue engineering. Previous work on scaffold design was done by Hollister and colleagues, who showed that scaffolds of a defined material (with a given Young's modulus and Poisson's ratio) and a certain volume could match the stiffness or strength of natural tissues under many different kinds of design architecture (Hollister *et al.* 2002; Hollister 2005; Adachi *et al.* 2006). This technique is powerful, since it allows calculation of the mechanical response of a scaffold, and versatile, since a scaffold with different pore sizes or pore types can be modelled. However, this approach of assuming that growth morphologies would present the best or 'optimal' scaffold geometries might no longer be true. Instead, it might be better to define scaffolds with geometries and mechanical properties similar to those found initially in the embryonic state or to those found in a regeneration state as a transition between the current and the targeted morphologies.

The design of a scaffold through CAD is particularly well suited when used in conjunction with a rapid prototyping technique to produce physical scaffolds. Rapid prototyping is the general term used to define a manufacturing technique that consists of the construction of a structure layer by layer to form a totally controllable structure. Rapid prototyping techniques include selective laser sintering, fused deposition modelling, stereolithography, electron beam melting and three-dimensional printing (see Yeong *et al.* 2004 for details). This method is particularly useful for tissue engineering since it allows a very good reproducibility and the production of almost any kind of structure within the limitations of each technique used. Using this technique, it is possible to design a structure that mimics the natural structure to be replaced (Van Cleynenbreugel *et al.* 2002; Hutchmacher & Cool 2007; Smith *et al.* 2007).

3. Scaffold characterization through computed tomography images

It is not always possible or necessary to construct a scaffold using rapid prototyping, and therefore scaffolds for tissue engineering can have inhomogeneous structure. In such cases, it is particularly important to characterize the scaffold geometry to relate the architecture with cell response and tissue formation. Traditionally, scanning electron microscopy or transmission electron microscopy has been used with great success to give very accurate details of the surface of a scaffold (e.g. Kellomäki *et al.* 2000; Charles-Harris *et al.* 2008). However, the use of three-dimensional scaffolds with an internal pore structure has favoured the use of non-destructive characterization techniques such as microcomputed tomography (microCT) or synchrotron tomography (Ho & Hutmacher 2006; Cancedda *et al.* 2007). These techniques are based on the biomaterial being scanned through X-rays crossing the material as the sample rotates within the X-ray beam. A three-dimensional volume is reconstructed from this set of data using filtered back projection (Feldkamp *et al.* 1989). The resolution that can be obtained using such techniques depends on the X-ray source and detector, in combination with the field of view chosen.

Imaging techniques from microCT data have allowed characterization of scaffolds and have been reviewed by van Lenthe *et al.* (2007). In particular, the overall porosity can be calculated along the distribution of the number of pores as a function of its size. Other parameters such as pore anisotropy and scaffold

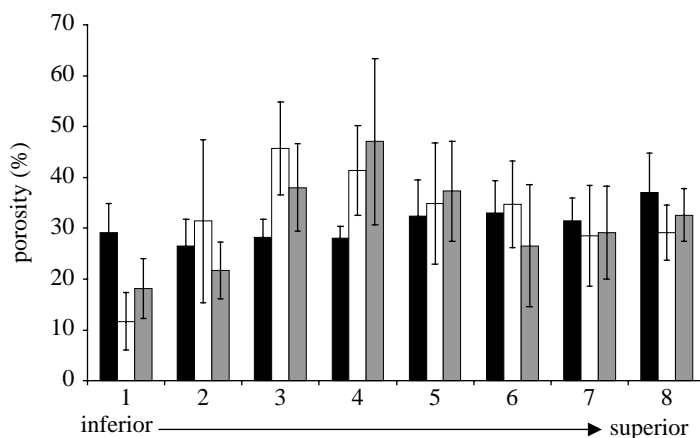


Figure 1. Porosity distribution throughout the height of CaP bone cement samples and CaP-based glass samples. The average is shown with the standard deviation from the inferior part (1) to the superior part (8) of the sample (black bars, porous glass; white bars, TCP non-injected; grey bars, TCP injected). Adapted from Lacroix *et al.* (2006).

permeability can also be calculated. Thus, microCT is a very useful tool to characterize scaffolds in a non-destructive manner. However, owing to the limiting resolution, only pores above a few micrometres are detected and therefore the microporosity in a scaffold cannot be obtained that way. The microCT technique has also been used to monitor three-dimensional mineralization over time in a perfusion bioreactor (Porter *et al.* 2007). High-resolution magnetic resonance imaging (MRI) could also be useful to determine axial fluid velocity within the pores of a scaffold (Swider *et al.* 2007).

MicroCT in tissue engineering is also used for *in vivo* scaffold and tissue characterization. When used on small animals, *in vivo* microCT can be used to evaluate scaffold/tissue integration, tissue formation and scaffold degradation (Komlev *et al.* 2006). *In vivo* microCT scanning has been shown to be both repeatable and reproducible (Voor *et al.* 2008). This can be obtained with a lower resolution than for *in vitro* experiments but has the advantage of characterizing the same scaffold at different time points. Bone tissue formation was characterized within a single scaffold in an *in vitro* experiment by Cartmell *et al.* (2004).

Porous and biodegradable calcium phosphate (CaP)-based bone cement and glass ceramic materials have been characterized by Lacroix *et al.* (2006) using microCT with a resolution of up to 7.8 μm . Cross sections were superimposed using the software MIMICS (Materialise, Leuven, Belgium) to form a three-dimensional reconstruction of the sample. Lower and upper thresholds were used to separate the material from the pores. The samples were then divided into smaller cylinders, 1.15 mm in height and 1.5 mm in diameter. The overall porosity of the samples and the average porosity of each small cylinder were calculated using MIMICS. Porosity calculations indicate that the overall macroporosity of non-injected CaP bone cement, injected CaP bone cement and porous glass are, respectively, 32.2, 30.2 and 30.7 per cent. The porosity of porous glass is more homogeneously distributed than that of CaP bone cement (figure 1). The variability of macropores is also higher in CaP bone cement than in porous glass.

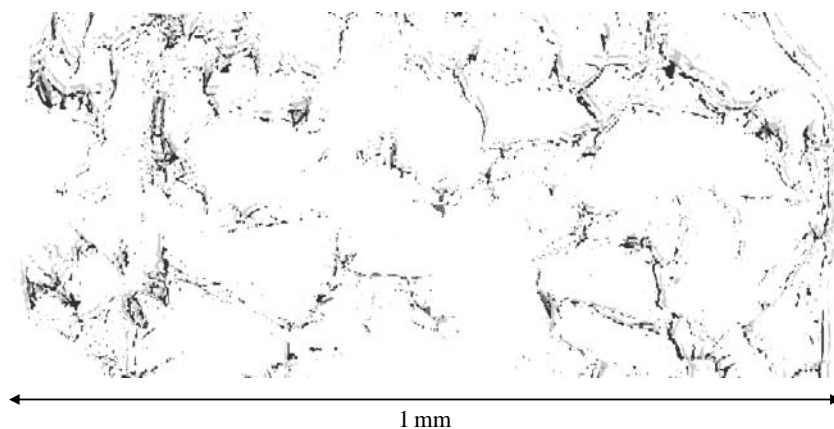


Figure 2. Two-dimensional representation of *in situ* synchrotron axial displacement of 50 μm of a PLA/G5 composite scaffold from the undeformed position (light grey) to the deformed state (dark grey). A colour version of figure 2 is available in the electronic supplementary material.

However, no significant differences could be seen between injected and non-injected CaP bone cements, which confirms quantitatively the similarity of the final product independent of the preparation technique.

A study combining synchrotron X-ray microtomography with simultaneous *in situ* mechanical tests to analyse the microstructure and the deformation of completely degradable composite polylactic acid (PLA)/CaP glass scaffolds was performed by Charles-Harris *et al.* (2007). In this study, internal movement (and therefore strain) within the scaffold could be observed through the imaging technique (figure 2). It can be observed that the ‘trabeculae’ of the scaffold can undergo displacement with or without strain depending on the architecture of the scaffold. Thus, this technique brings a deeper insight into the internal mode of deformation of this scaffold.

4. Finite-element analysis in tissue engineering

Finite-element analysis was first used in tissue engineering for the design of the scaffold to optimize stiffness/geometry as described in §2. More recently, a stress–strain analysis of complete scaffolds has been performed to investigate the state of stress and strain within the scaffolds and its interaction with the surrounding tissues (Jaecques *et al.* 2004). Such an analysis can be used to vary several geometrical or material parameters at the same time and to choose the most suitable ones for the replacement of natural tissues (Van Cleynenbreugel *et al.* 2006).

Finite-element analyses of cylinders of 1.15 mm height and 1.5 mm diameter were carried out on CaP-based scaffolds to calculate the stress–strain distribution throughout larger scaffolds (Lacroix *et al.* 2006). The dimensions chosen were restricted owing to computational limitations but were large enough to fully characterize the scaffold. This resulted in models of approximately 600 000 elements of element length approximately 10–25 μm . This method allowed comparison of the evaluation of microstructure and stiffness with a larger sample (6 mm in diameter and 12 mm in height) from the same material but

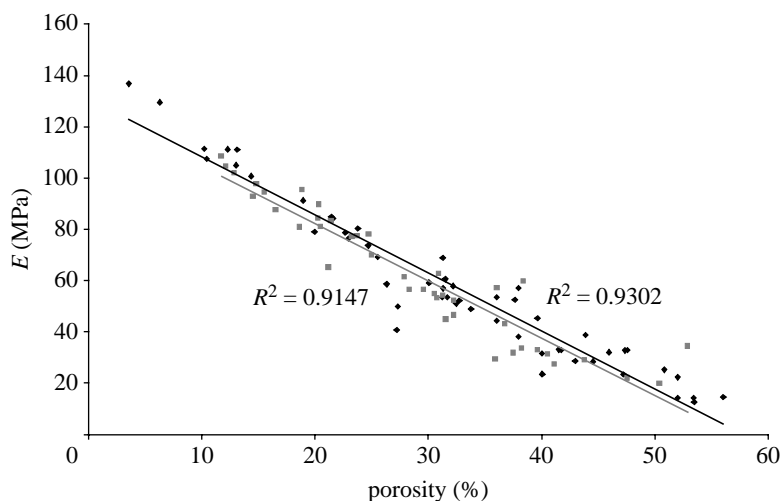


Figure 3. Relationship between effective Young's modulus and scaffold porosity for a CaP bone cement using a manual preparation and an injected preparation. The effective Young's modulus was defined as the axial stress (reaction force divided by area) over the axial strain (0.5%) (black diamonds, non-injected CaP bone cement; grey squares, injected CaP bone cement). Adapted from Lacroix *et al.* (2006).

prepared using two different techniques: moulded manually or injected with a syringe. It was found that there was hardly any difference in the relationship between stiffness and porosity when comparing the method of preparation of the two cements (figure 3). In this study, a linear correlation was used to fit the data points, whereas the stiffness of an open porous material is usually represented as a function of the square of the porosity (Gibson 2005). This discrepancy may be due to the relatively low porosity used for this bone cement (from 5 to 55%), where a linear fit is sufficient to represent correctly the results, given the dispersion of the data. Similar results were obtained by Boger *et al.* (2008) on polymethylmethacrylate bone cement.

Very few studies have performed a finite-element analysis of FF within a scaffold. So far, only simulations of a perfusion bioreactor have been investigated (Cioffi *et al.* 2006; Sandino *et al.* 2008). An FF finite-element analysis has been performed by Sandino *et al.* (2008) on CaP cement cylinders of 1 mm diameter and 2 mm height. For the simulation of the interstitial FF (fluid models), an inlet fluid velocity of $10 \mu\text{m s}^{-1}$ was fixed on the nodes of the entrance side of the meshes, a fluid velocity of zero was fixed on the nodes of the walls of the outer diameter, simulating a confined perfusion system, and the outlet fluid pressure was set as zero on the nodes of the exit side of the meshes. Steady-state Newtonian fluid analyses were performed with a non-slip condition on the walls of the scaffold. Fluid density and viscosity were similar to those of the cell culture medium. The fluid velocity distribution into the pores showed that the FF did not reach all interconnected pores of the samples (figure 4). Moreover, high changes of fluid velocity were observed; there were regions where the fluid velocity was almost zero, and other parts with high-velocity FF. Each pore of the samples had different values of fluid velocity, fluid pressure and fluid shear stress, depending mainly on its position within the scaffold, its size and its

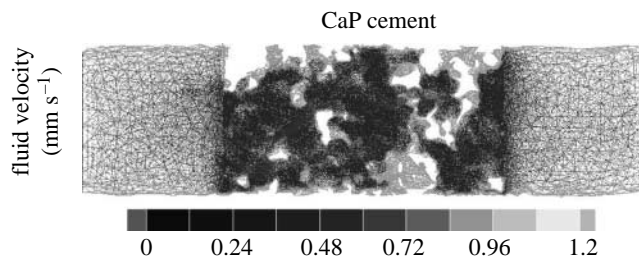


Figure 4. Fluid velocity distribution within the pores of the samples when the FF is simulated. Non-homogeneous fluid velocity is observed. Adapted from Sandino *et al.* (2008). A colour version of figure 4 is available in the electronic supplementary material.

interconnectivity with other pores. When simulating a physiological FF within the scaffold, it was found that maximum FF could increase 1000 times inside the scaffold (Sandino *et al.* 2008). This result therefore shows that the mechanical stimuli through FF sensed by the cells can be much higher than the one applied macroscopically on the scaffold.

5. Mechanobiology in bone tissue engineering

Mechanobiological concepts have been proposed to relate mechanical stimuli to cell differentiation and tissue formation. Pauwels (1960) first proposed a concept based on hydrostatic pressure and octahedral shear strain (SS) to relate fibrous tissue formation and cartilage formation in fracture healing. This concept was developed in a mathematical formulation by Carter *et al.* (1988) and extended to include bone formation as a function of the same mechanical stimuli. Further study by Claes & Heigele (1999) has refined this concept to quantify it with validated finite-element analyses. Another mechanobiological concept was proposed by Prendergast *et al.* (1997) based on shearing stimuli produced by the biphasic nature of tissues: solid phase of collagen and liquid phase of interstitial fluid. Both phases are assumed saturated and the continuity equation for the mixture holds, i.e.

$$\sum_{\alpha=1}^{\nu} \nabla \cdot (\phi^{\alpha} v^{\alpha}) = 0, \quad (5.1)$$

where ν is the number of constituents α ; ∇ is the gradient operator; ϕ^{α} is the volume fraction of the α th constituent; and v^{α} is the local velocity component of the α th constituent. Conservation of linear momentum gives the equation of motion for the α th constituent as

$$\nabla \cdot \sigma^{\alpha} + \rho^{\alpha} \mathbf{q}^{\alpha} + \pi^{\alpha} + \rho_{\Gamma}^{\alpha} c^{\alpha} v^{\alpha} = \rho^{\alpha} \frac{Dv^{\alpha}}{Dt}, \quad (5.2)$$

where σ^{α} is the partial stress; \mathbf{q}^{α} is the body force per unit mass; π^{α} is the rate of momentum supply to the α th constituent; and $\rho_{\Gamma}^{\alpha} c^{\alpha} v^{\alpha}$ is the momentum supply from biochemical reactions, neglected here (Kelly 1964). The constitutive relationships must satisfy thermodynamic constraints (i.e. energy balance and entropy inequality), as described by Mow *et al.* (1980). For a biphasic material in

which the fluid is inviscid and each constituent is isotropic, and where the infinitesimal strain–displacement relationship is assumed, we can write, following Mow *et al.* (1980),

$$\sigma^s = \phi^s pI + \lambda e^s I + 2\mu \epsilon^s, \quad \sigma^f = -\phi^f pI, \quad (5.3)$$

where ϵ denotes the strain and e denotes the dilatational strain; $(-)^s$ and $(-)^f$ denote solid-phase and fluid-phase quantities; p is the apparent pressure; λ and μ are Lamé constants; and I is the unit matrix. A finite-element formulation is applied using the soil analysis capabilities of commercial software programs (MSC MARC (MSC Software, Santa Ana, CA, USA) or ABAQUS (Simulia, Providence, RI, USA)) through an implicit integration based on the full Newton–Raphson algorithm with the relative residual force tolerance convergence set by default to 0.1.

The stimuli investigated are octahedral SS and FF velocity in the sense that both stimuli can produce some distortion of the cell to affect its differentiative activity. Threshold stimuli S were defined by Prendergast *et al.* (1997) by

$$S = \frac{SS}{a} + \frac{FF}{b}, \quad (5.4)$$

where $a=0.0375$ and $b=3 \mu\text{m s}^{-1}$. If $S>3$, then fibrous tissue differentiation occurs; if $3>S>1$, then cartilage differentiation occurs; if $1>S>0.267$, then immature bone differentiation occurs; and if $0.267>S>0.01$, then resorption occurs.

This concept builds on the one proposed by Pauwels since it includes the deviatoric deformation tensor but does not include the hydrostatic tensor and is applied to both phases of tissues. Although the biological processes and mechanical environment can be quite different, this concept was tested with success as a predictive model on various applications such as implant–bone interface (Huiskes *et al.* 1997), fracture healing (Lacroix & Prendergast 2002), osteochondral defect (Kelly & Prendergast 2006), bone chambers (Geris *et al.* 2008a,b) and bone distraction (Isaksson *et al.* 2007). These predictive models establish some initial conditions such as loading and material properties that vary over time in an iterative manner depending on the values of the calculated mechanical stimuli of the mechanobiological concept. Moreover, recent studies on fracture healing have shown that the FF stimuli seemed to be the most determinant in tissue differentiation (Isaksson *et al.* 2006). These concepts are useful to better understand the processes of mechanotransduction and mechanoregulation.

Only recently such mechanobiological concepts have also been applied to tissue engineering. The first application to tissue engineering was done by Kelly & Prendergast (2006) in which a mechanoregulation algorithm for tissue differentiation was used to determine the influence of scaffold material properties on chondrogenesis in a finite-element model of an osteochondral defect. An optimal design was determined by parametrically varying the mechanical properties of the scaffold through its depth. More recently, it has been used for bone tissue engineering in the development of scaffolds based on CaP cement (Byrne *et al.* 2007). Tissue engineering of a three-dimensional finite-element model of a scaffold made of repeated uniform unit cells was simulated with the modelling of the dispersal of the various cell populations in three dimensions. Both cell proliferation and cell migration are based on a stochastic process consisting of a sequence of discrete steps of fixed lengths occurring within a lattice that covers the finite-element domain (Pérez & Prendergast 2007).

A lattice point can be occupied by an MSC, a fibroblast, a chondrocyte, an osteoblast or an endothelial cell. A mechanoregulation model is implemented to simulate MSC differentiation and it was assumed that each lattice point was surrounded by appropriate extracellular matrix. Based on Richardson *et al.* (1992), who observed an exponential increase in stiffness in differentiating tissue, an exponential rate equation is used to better describe the evolution of the Young modulus of the regenerating tissue,

$$E_i = K_i e^{\beta_i t}, \quad (5.5)$$

where E_i represents the Young modulus for tissue phenotype ‘ i ’ (where ‘ i ’ is fibrous tissue, cartilage, immature or mature bone); t is the time; and K_i and β_i are two parameters regulating the shape of the exponential curve (Boccaccio *et al.* 2008). The Young modulus of each element is then averaged over the last 10 iterations in order to account for a gradual change of cell differentiation over time. From one iteration to another, there is therefore a change of mechanical properties of the tissues modelled, reflecting the production or resorption of extracellular matrix by cells. From a computational point of view, the poroelastic analysis is started without initial conditions for each iteration. Rules govern the development of capillaries, and osteogenesis is allowed only within a defined distance (100 μm) of a capillary. It was shown that porosity, Young’s modulus and dissolution rate design variables have a critical effect on the amount of bone regeneration. Scaffolds should be produced that depend on the site of implantation. In a low-load environment (1 MPa), high porosities and higher stiffness but a medium dissolution rate give the greatest amount of bone (figure 5). Alternatively, the initial porosity and rate of dissolution should be lower in a high-load environment (2 MPa) in order to maintain the mechanical and structural integrity of the bone–scaffold system.

Owing to the complexity in implementing these algorithms in structures with non-regular porosity distribution, only static finite-element analyses have been performed so far for tissue engineering in these structures. Nonetheless, the static mechanical stimuli are useful to determine the level of stress or strain within the structure and to relate it to the mechanical stimuli that the cells should feel when seeded within the scaffold. For example, a micro-finite-element analysis of a bone cement scaffold was performed by Lacroix *et al.* (2006), who calculated the mechanical stimuli at the surface of the scaffold. Most nodes have strain stimuli between 1 per cent under compression and 0.5 per cent under tension (figure 6), and fluid shear stress stimuli between 0 and 1×10^{-3} Pa. In the CaP cement scaffold, there are more nodes under tension than in the glass scaffold, and the fluid shear stress distribution at these nodes is also more spread in the CaP than in the glass sample (Sandino *et al.* 2008). The CaP scaffold has regions with a high level of fluid shear stress and/or compressive strain, whereas the glass scaffold has regions with a high level of only one of these stimuli. Owing to the difficulty in creating an automatic mesh for scaffolds with non-regular porosity distribution (Viceconti *et al.* 2004), scaffolds made with rapid prototyping through the repetition of uniform unit cells can be more easily modelled and adapted to multiscale studies. In such studies, as previously discussed, parametric studies can be performed more easily to investigate the effect of pore size, surface-specific area, permeability, etc.

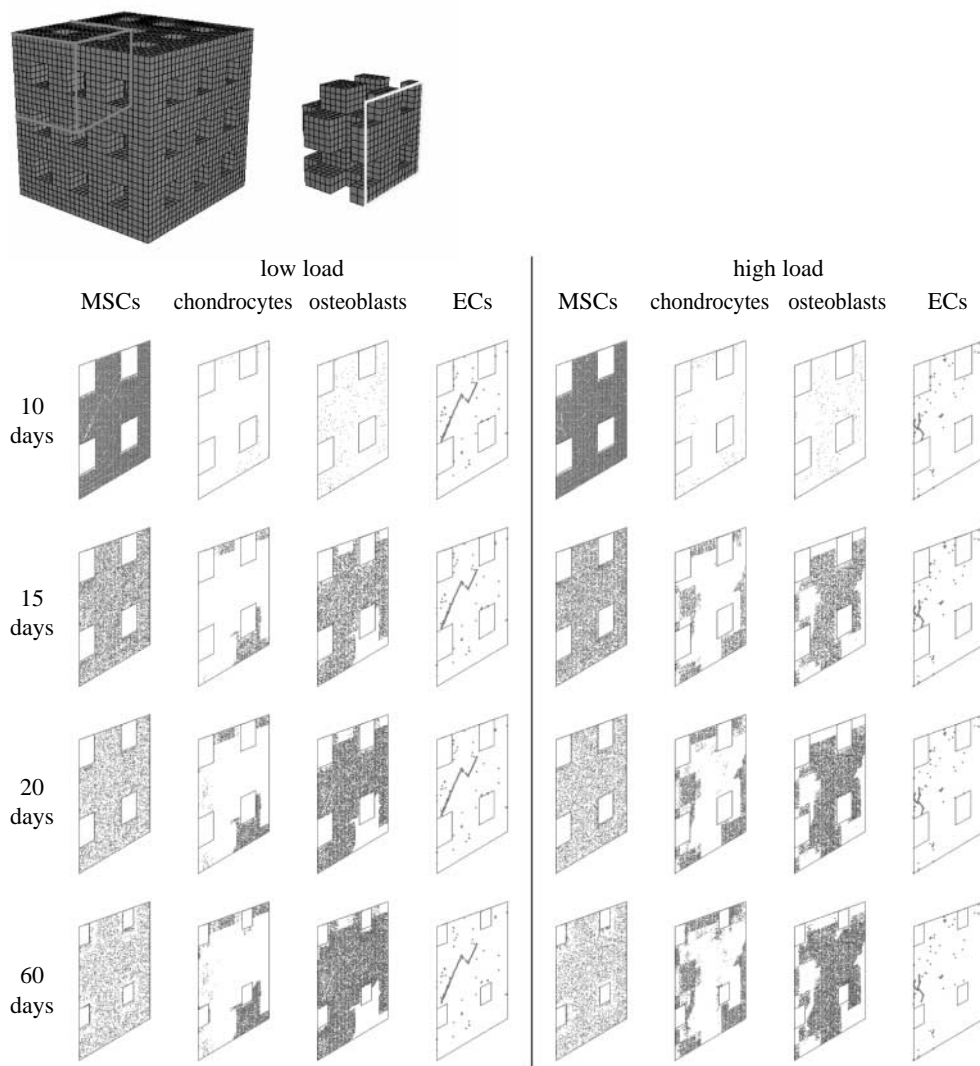


Figure 5. Predicted cell distribution in a printed-type scaffold (dark grey). Only one-eighth of the scaffold needs to be modelled for symmetry reasons. The sections shown are the face of the one-eighth section described by the white outline box. EC, endothelial cell. Note porosity increasing over time due to dissolution of the scaffold biomaterial, initial porosity 50%. Adapted from [Checa & Prendergast \(2008\)](#). A colour version of figure 5 is available in the electronic supplementary material.

6. Future perspectives

In recent years, we have only seen the tip of the iceberg in numerical simulations in tissue engineering. Most studies are still in their infancy, with most of them dealing with static studies of scaffold design or static studies of mechanical analysis. However, more sophisticated analyses are necessary to better understand the effect of mechanical stimuli within the whole body, with its interaction between biological, chemical and other physical cues on the one hand, and

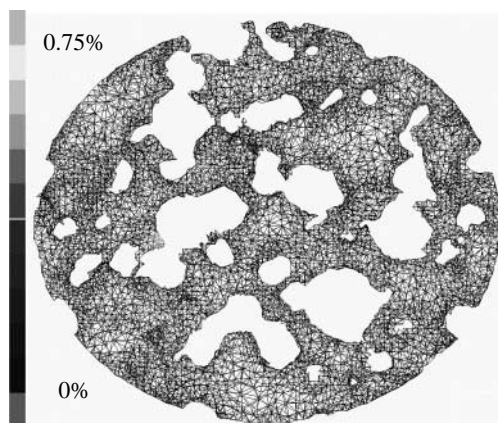


Figure 6. Example of the distribution of octahedral SS in a CaP cement scaffold, showing the strain distribution around the pores and the influence of the homogeneity of the pore distribution. Adapted from Lacroix *et al.* (2006). A colour version of figure 6 is available in the electronic supplementary material.

foreign bodies such as biomaterials on the other. For this, future perspectives of numerical simulations of biomaterial scaffolds for tissue engineering, and, in a more general sense, regenerative medicine, rely on the development of new methods to account for the multiscale dimension of the problems, on the increasing power of computing capabilities, and on the integration of predictive models between biology, materials science and physical stimuli. An approach that accounts for all the different scales at play becomes necessary (Wikswow *et al.* 2006; Sanga *et al.* 2007). Multiscale modelling has been developed in many disciplines (Stoneham & Harding 2003; Coveney & Fowler 2005; Cohen & Harel 2007; Southern *et al.* 2008). Already multiscale analyses have been applied to the heart (Noble 2002), cancer (Bellomo *et al.* 2008), the design of aortic valves (Weinberg & Kaazempur Mofrad 2009), trabecular bone failure (Müller & van Lenthe 2006), teeth (Miura *et al.* 2009) and the human femur (Cristofolini *et al.* 2008). It has recently been applied to tissue engineering for bone regeneration (Sanz-Herrera *et al.* 2009). These approaches will be translated into the clinical side with the development of patient-specific multiscale studies (Kerckhoffs *et al.* 2008).

Future perspectives include the development of computer power. This should inevitably lead to more complex models of higher size being studied. Nonetheless, the use and development of existing tools by the bioengineering community can be pushed forward. The use of the supercomputing grid within the Distributed European Infrastructure for Supercomputing Applications (DEISA) can allow the development of models with high complexity in their resolution or in the management of databases. This can only be done if the models developed are highly parallel, which is not such an easy task with studies of higher complexity. The bioengineering community has a role to play in this development.

More integration is needed between the interrelations of biology, materials science and physical stimuli. Computational models also have a role to play in this direction (Sengers *et al.* 2007). Most of the models so far are rather mechanistic and include very little biology or materials science. For example,

the effect of angiogenesis is crucial for the processes of tissue engineering, as in other biological processes such as wound healing or fracture healing where it has been included (Bailón-Plaza & van der Meulen 2001; Geris *et al.* 2008*a,b*; Peirce 2008). In tissue engineering, this has just been introduced (Checa & Prendergast 2008). Computer models can only be as good as the input data that are fed to them, which need to be correct and sufficient. More experimental tests and biological information are needed to model biological processes more precisely. Most of the interactions between biomaterials and tissues are simulated through the definition of structure parameters, such as scaffold stiffness or pore size, for example. However, physical and chemical surface properties are critical for the development of functional scaffolds for tissue engineering (Castner & Ratner 2002). Therefore, it becomes necessary to include such properties in a computer model to model effectively the integration between biomaterials and surrounding tissues.

As a conclusion, tissue engineering has seen a tremendous expansion through experimental *in vitro* and *in vivo* tests, with the development of new biomaterials that try to elicit a controlled reaction from the surrounding tissues. But the future of computational models applied to tissue engineering is very promising, with the establishment of more powerful and more realistic models that can simulate more accurately the biological processes and the design of biomaterial scaffolds.

The authors acknowledge funding from the European Commission (6FP SmartCaP project, NMP3-CT-2005-013912) and the Spanish Ministry of Science and Education. The following postgraduate students have contributed to parts of this work: Damien Byrne, Stephan Midderhoff and Clara Sandino.

References

- Adachi, T., Osako, Y., Tanaka, M., Hojo, M. & Hollister, S. J. 2006 Framework for optimal design of porous scaffold microstructure by computational simulation of bone regeneration. *Biomaterials* **27**, 3964–3972. (doi:10.1016/j.biomaterials.2006.02.039)
- Bailón-Plaza, A. & van der Meulen, M. C. 2001 A mathematical framework to study the effects of growth factor influences on fracture healing. *J. Theor. Biol.* **21**, 191–209. (doi:10.1006/jtbi.2001.2372)
- Bellomo, N., Li, N. K. & Maini, P. K. 2008 On the foundations of cancer modelling: selected topics, speculations, and perspectives. *Math. Models Methods Appl. Sci.* **18**, 593–646. (doi:10.1142/S0218202508002796)
- Boccaccio, A., Prendergast, P. J., Pappalettere, C. & Kelly, D. J. 2008 Tissue differentiation and bone regeneration in an osteotomized mandible: a computational analysis of the latency period. *Med. Biol. Eng. Comput.* **46**, 283–298. (doi:10.1007/s11517-007-0247-1)
- Boger, A., Bohner, M., Heini, P., Verrier, S. & Schneider, E. 2008 Properties of an injectable low modulus PMMA bone cement for osteoporotic bone. *J. Biomed. Mater. Res. B: Appl. Biomater.* **86B**, 474–482. (doi:10.1002/jbm.b.31044)
- Byrne, D. P., Lacroix, D., Planell, J. A., Kelly, D. J. & Prendergast, P. J. 2007 Simulation of tissue differentiation in a scaffold as a function of porosity, Young's modulus and dissolution rate: application of mechanobiological models in tissue engineering. *Biomaterials* **28**, 5544–5554. (doi:10.1016/j.biomaterials.2007.09.003)
- Cancedda, R., Cedola, A., Giuliani, A., Komlev, V., Lagomarsino, S., Mastrogiacomo, M., Peyrin, F. & Rustichelli, F. 2007 Bulk and interface investigations of scaffolds and tissue-engineered bones by X-ray microtomography and X-ray microdiffraction. *Biomaterials* **28**, 2505–2524. (doi:10.1016/j.biomaterials.2007.01.022)

- Carter, D. R., Blenman, P. R. & Beaupré, G. S. 1988 Correlations between mechanical stress history and tissue differentiation in initial fracture healing. *J. Orthop. Res.* **6**, 736–748. (doi:10.1002/jor.1100060517)
- Cartmell, S., Huynh, K., Lin, A., Nagaraja, S. & Guldberg, R. 2004 Quantitative microcomputed tomography analysis of mineralization within three-dimensional scaffolds *in vitro*. *J. Biomed. Mater. Res. A* **69**, 97–104. (doi:10.1002/jbm.a.20118)
- Castner, D. G. & Ratner, B. D. 2002 Biomedical surface science: foundations to frontiers. *Surface Sci.* **500**, 28–60. (doi:10.1016/S0039-6028(01)01587-4)
- Charles-Harris, M., del Valle, S., Hentges, E., Bleuët, P., Lacroix, D. & Planell, J. A. 2007 Mechanical and structural characterisation of completely degradable polylactic acid/calcium phosphate glass scaffolds. *Biomaterials* **28**, 4429–4438. (doi:10.1016/j.biomaterials.2007.06.029)
- Charles-Harris, M., Koch, M. A., Navarro, M., Lacroix, D., Engel, E. & Planell, J. A. 2008 A PLA/calcium phosphate degradable composite material for bone tissue engineering: an *in vitro* study. *J. Mater. Sci. Mater. Med.* **19**, 1503–1513. (doi:10.1007/s10856-008-3390-9)
- Checa, C. & Prendergast, P. J. 2008 A mechanobiological model for tissue differentiation that includes angiogenesis: a lattice-based modeling approach. *Ann. Biomed. Eng.* **37**, 129–145. (doi:10.1007/s10439-008-9594-9)
- Cioffi, M., Boschetti, F., Raimondi, M. T. & Dubini, G. 2006 Modeling evaluation of the fluid-dynamic microenvironment in tissue-engineered constructs: a micro-CT based model. *Biotechnol. Bioeng.* **93**, 500–510. (doi:10.1002/bit.20740)
- Claes, L. E. & Heigele, C. A. 1999 Magnitudes of local stress and strain along bony surfaces predict the course and type of fracture healing. *J. Biomech.* **32**, 255–266. (doi:10.1016/S0021-9290(98)00153-5)
- Cohen, I. R. & Harel, D. 2007 Explaining a complex living system: dynamics, multi-scaling and emergence. *J. R. Soc. Interface* **4**, 175–182. (doi:10.1098/rsif.2006.0173)
- Coveney, P. V. & Fowler, P. W. 2005 Modelling biological complexity: a physical scientist's perspective. *J. R. Soc. Interface* **2**, 267–280. (doi:10.1098/rsif.2005.0045)
- Cristofolini, L., Taddei, F., Baleani, M., Baruffaldi, F., Stea, S. & Viceconti, M. 2008 Multiscale investigation of the functional properties of the human femur. *Phil. Trans. R. Soc. A* **366**, 3319–3341. (doi:10.1098/rsta.2008.0077)
- D&MD 2001 *Tissue engineering: technologies, markets, and opportunities*, 3rd edn. Drug and Market Development Publishing.
- Feldkamp, L. A., Goldstein, S. A., Parfitt, A. M., Jesion, G. & Kleerekoper, M. 1989 The direct examination of 3-dimensional bone architecture *in vitro* by computed tomography. *J. Bone Miner. Res.* **4**, 3–11.
- Freed, L. E. et al. 2006 Advanced tools for tissue engineering: scaffolds, bioreactors, and signaling. *Tissue Eng.* **12**, 3285–3305. (doi:10.1089/ten.2006.12.3285)
- Geris, L., Gerisch, A., Sloten, J. V., Weiner, R. & Oosterwyck, H. V. 2008a Angiogenesis in bone fracture healing: a bioregulatory model. *J. Theor. Biol.* **7**, 137–158. (doi:10.1016/j.jtbi.2007.11.008)
- Geris, L., Vandamme, K., Naert, I., Vander Sloten, J., Duyck, J. & Van Oosterwyck, H. 2008b Application of mechanoregulatory models to simulate peri-implant tissue formation in an *in vivo* bone chamber. *J. Biomech.* **41**, 145–154. (doi:10.1016/j.jbiomech.2007.07.008)
- Gibson, L. J. 2005 Biomechanics of cellular solids. *J. Biomech.* **38**, 377–399. (doi:10.1016/j.jbiomech.2004.09.027)
- Hench, L. L. & Polak, J. M. 2002 Third-generation biomedical materials. *Science* **295**, 1014–1017. (doi:10.1126/science.1067404)
- Ho, S. T. & Hutmacher, D. W. 2006 A comparison of micro CT with other techniques used in the characterization of scaffolds. *Biomaterials* **27**, 1362–1376. (doi:10.1016/j.biomaterials.2005.08.035)
- Hollister, S. J. 2005 Porous scaffold design for tissue engineering. *Nat. Mater.* **4**, 518–524. (doi:10.1038/nmat1421)

- Hollister, S. J., Maddox, R. D. & Taboas, J. M. 2002 Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. *Biomaterials* **23**, 4095–4103. (doi:10.1016/S0142-9612(02)00148-5)
- Holtorf, H. L., Jansen, J. A. & Mikos, A. G. 2007 Modulation of cell differentiation in bone tissue engineering constructs cultured in a bioreactor. *Adv. Exp. Med. Biol.* **585**, 225–241. (doi:10.1007/978-0-387-34133-0_16)
- Huiskes, R., van Driel, W. D., Prendergast, P. J. & Soballe, K. 1997 A biomechanical regulatory model of peri-prosthetic tissue differentiation. *J. Mater. Sci. Mater. Med.* **8**, 785–788. (doi:10.1023/A:1018520914512)
- Hutchmacher, D. W. & Cool, S. 2007 Concepts of scaffold-based tissue engineering—the rationale to use solid free-form fabrication techniques. *J. Cell Mol. Med.* **11**, 654–669. (doi:10.1111/j.1582-4934.2007.00078.x)
- Isaksson, H., Wilson, W., van Donkelaar, C. C., Huiskes, R. & Ito, K. 2006 Comparison of biophysical stimuli for mechano-regulation of tissue differentiation during fracture healing. *J. Biomech.* **39**, 1507–1516. (doi:10.1016/j.jbiomech.2005.01.037)
- Isaksson, H., Comas, O., van Donkelaar, C. C., Mediavilla, J., Wilson, W., Huiskes, R. & Ito, K. 2007 Bone regeneration during distraction osteogenesis: mechano-regulation by shear strain and fluid velocity. *J. Biomech.* **40**, 2002–2011. (doi:10.1016/j.jbiomech.2006.09.028)
- Jaecques, S. V., Van Oosterwyck, H., Muraru, L., Van Cleynenbreugel, T., De Smet, E., Wevers, M., Naert, I. & Vander Sloten, J. 2004 Individualised, micro CT-based finite element modelling as a tool for biomechanical analysis related to tissue engineering of bone. *Biomaterials* **25**, 1683–1696. (doi:10.1016/S0142-9612(03)00516-7)
- Kalyanaraman, B. & Boyce, S. 2007 Assessment of an automated bioreactor to propagate and harvest keratinocytes for fabrication of engineered skin substitutes. *Tissue Eng.* **13**, 983–993. (doi:10.1089/ten.2006.0338)
- Kellomäki, M., Niiranen, H., Puumanen, K., Ashammakhi, N., Waris, T. & Törmälä, P. 2000 Bioabsorbable scaffolds for guided bone regeneration and generation. *Biomaterials* **21**, 2495–2505. (doi:10.1016/S0142-9612(00)00117-4)
- Kelly, D. J. & Prendergast, P. J. 2006 Prediction of the optimal mechanical properties for a scaffold used in osteochondral defect repair. *Tissue Eng.* **12**, 2509–2519. (doi:10.1089/ten.2006.12.2509)
- Kelly, P. D. 1964 A reacting continuum. *Int. J. Eng. Sci.* **2**, 129–153. (doi:10.1016/0020-7225(64)90001-1)
- Kerckhoffs, R. C., Narayan, S. M., Omens, J. H., Mulligan, L. J. & McCulloch, A. D. 2008 Computational modeling for bedside application. *Heart Fail. Clin.* **4**, 371–378. (doi:10.1016/j.hfc.2008.02.009)
- Kirkpatrick, J. C., Fuchs, S., Hermanns, M. I., Peters, K. & Unger, R. E. 2007 Cell culture models of higher complexity in tissue engineering and regenerative medicine. *Biomaterials* **28**, 5193–5198. (doi:10.1016/j.biomaterials.2007.08.012)
- Komlev, V. S., Peyrin, F., Mastrogiacomo, M., Cedola, A., Papadimitropoulos, A., Rustichelli, F. & Cancedda, R. 2006 Kinetics of *in vivo* bone deposition by bone marrow stromal cells into porous calcium phosphate scaffolds: an X-ray computed microtomography study. *Tissue Eng.* **12**, 3449–3458. (doi:10.1089/ten.2006.12.3449)
- Lacroix, D. & Prendergast, P. J. 2002 A mechano-regulation model for tissue differentiation during fracture healing: analysis of gap size and loading. *J. Biomech.* **35**, 1163–1171. (doi:10.1016/S0021-9290(02)00086-6)
- Lacroix, D., Chateau, A., Ginebra, M. P. & Planell, J. A. 2006 Micro-finite element models of bone tissue-engineering scaffolds. *Biomaterials* **27**, 5326–5334. (doi:10.1016/j.biomaterials.2006.06.009)
- Langer, R. & Vacanti, J. P. 1993 Tissue engineering. *Science* **260**, 920–926. (doi:10.1126/science.8493529)
- Lim, J. Y. & Donahue, H. J. 2007 Cell sensing and response to micro- and nanostructured surfaces produced by chemical and topographic patterning. *Tissue Eng.* **13**, 1879–1891. (doi:10.1089/ten.2006.0154)

- Malda, J., Klein, T. J. & Upton, Z. 2007 The roles of hypoxia in the *in vitro* engineering of tissues. *Tissue Eng.* **13**, 2153–2162. (doi:10.1089/ten.2006.0417)
- Martin, I., Wendt, D. & Heberer, M. 2004 The role of bioreactors in tissue engineering. *Trends Biotechnol.* **22**, 80–86. (doi:10.1016/j.tibtech.2003.12.001)
- Martin, I., Miot, S., Barbero, A., Jakob, M. & Wendt, D. 2007 Osteochondral tissue engineering. *J. Biomech.* **40**, 750–765. (doi:10.1016/j.jbiomech.2006.03.008)
- Mikos, A. G. et al. 2006 Engineering complex tissues. *Tissue Eng.* **12**, 3307–3339. (doi:10.1089/ten.2006.12.3307)
- Miura, J., Maeda, Y., Nakai, H. & Zako, M. 2009 Multiscale analysis of stress distribution in teeth under applied forces. *Dent. Mater.* **25**, 67–730. (doi:10.1016/j.dental.2008.04.015)
- Mow, V. C., Kuei, S. C., Lai, W. M. & Armstrong, C. G. 1980 Biphasic creep and stress relaxation of articular cartilage: theory and experiments. *J. Biomech. Eng.* **102**, 73–84.
- Müller, R. & van Lenthe, G. H. 2006 Trabecular bone failure at the microstructural level. *Curr. Osteoporos. Rep.* **4**, 80–86. (doi:10.1007/s11914-006-0007-4)
- Noble, D. 2002 Modeling the heart—from genes to cells to the whole organ. *Science* **295**, 1678–1682. (doi:10.1126/science.1069881)
- Pauwels, F. 1960 Eine neue theorie über den einflu mechanischer reize auf die differenzierung der stützgewebe. *Z. Anat. Entwickl. Gesch.* **121**, 478–515. (doi:10.1007/BF00523401) [Transl 1980 A new theory concerning the influence of mechanical stimuli on the differentiation of the supporting tissues. In *Biomechanics of the locomotor apparatus* (eds P. Maquet & R. Furlong), pp. 375–407. Berlin, Germany: Springer.]
- Pearce, S. M. 2008 Computational and mathematical modeling of angiogenesis. *Microcirculation* **15**, 739–751. (doi:10.1080/1073968080220331)
- Pérez, M. A. & Prendergast, P. J. 2007 Random-walk models of cell dispersal included in mechanobiological simulations of tissue differentiation. *J. Biomech.* **40**, 2244–2253. (doi:10.1016/j.jbiomech.2006.10.020)
- Porter, B. D., Lin, A. S., Peister, A., Hutmacher, D. & Guldberg, R. E. 2007 Noninvasive image analysis of 3D construct mineralization in a perfusion bioreactor. *Biomaterials* **28**, 2525–2533. (doi:10.1016/j.biomaterials.2007.01.013)
- Prendergast, P. J., Huiskes, R. & Soballe, K. 1997 Biophysical stimuli on cells during tissue differentiation at implant interfaces. *J. Biomech.* **30**, 539–548. (doi:10.1016/S0021-9290(96)00140-6)
- Richardson, J. B., Kenwright, J. & Cunningham, J. L. 1992 Fracture stiffness measurement in the assessment and management of tibial fractures. *Clin. Biomech.* **7**, 75–79. (doi:10.1016/0268-0033(92)90018-Y)
- Sandino, C., Planell, J. A. & Lacroix, D. 2008 A finite element study of mechanical stimuli in scaffolds for bone tissue engineering. *J. Biomech.* **41**, 1005–1014. (doi:10.1016/j.jbiomech.2007.12.011)
- Sanga, S., Frieboes, H. B., Zheng, X., Gatenby, R., Bearer, E. L. & Cristini, V. 2007 Predictive oncology: a review of multidisciplinary, multiscale *in silico* modeling linking phenotype, morphology and growth. *Neuroimage* **37**, S120–S134. (doi:10.1016/j.neuroimage.2007.05.043)
- Sanz-Herrera, J. A., García-Aznar, J. M. & Doblaré, M. 2009 On scaffold designing for bone regeneration: a computational multiscale approach. *Acta Biomater.* **5**, 219–229. (doi:10.1016/j.actbio.2008.06.021)
- Sengers, B. G., Taylor, M., Please, C. P. & Oreffo, R. O. 2007 Computational modelling of cell spreading and tissue regeneration in porous scaffolds. *Biomaterials* **28**, 1926–1940. (doi:10.1016/j.biomaterials.2006.12.008)
- Smith, M. H., Flanagan, C. L., Kempainen, J. M., Sack, J. A., Chung, H., Das, S., Hollister, S. J. & Feinberg, S. E. 2007 Computed tomography-based tissue-engineered scaffolds in craniomaxillofacial surgery. *Int. J. Med. Robot.* **3**, 207–216. (doi:10.1002/rcs.143)
- Southern, J., Pitt-Francis, J., Whiteley, J., Stokeley, D., Kobashi, H., Nobes, R., Kadooka, Y. & Gavaghan, D. 2008 Multi-scale computational modelling in biology and physiology. *Prog. Biophys. Mol. Biol.* **1–3**, 60–89. (doi:10.1016/j.phiomolbio.2007.07.019)

- Stoneham, A. M. & Harding, J. H. 2003 Not too big, not too small: the appropriate scale. *Nat. Mater.* **2**, 77–83. (doi:10.1038/nmat804)
- Sumanasinghe, R. D., Bernacki, S. H. & Loba, E. G. 2006 Osteogenic differentiation of human mesenchymal stem cells in collagen matrices: effect of uniaxial cyclic tensile strain on bone morphogenetic protein (BMP-2) mRNA expression. *Tissue Eng.* **12**, 3459–3465. (doi:10.1089/ten.2006.12.3459)
- Sun, W. & Lal, P. 2002 Recent development on computer aided tissue engineering—a review. *Comput. Methods Prog. Biomed.* **67**, 85–103. (doi:10.1016/S0169-2607(01)00116-X)
- Sun, W., Darling, A., Starly, B. & Nam, J. 2004a Computer aided tissue engineering: overview, scope and challenges. *J. Biotechnol. Appl. Biomech.* **39**, 29–47. (doi:10.1042/BA20030108)
- Sun, W., Starly, B., Darling, A. & Gomez, C. 2004b Computer aided tissue engineering: biomimetic modelling and design of tissue engineering. *J. Biotechnol. Appl. Biomech.* **39**, 49–58. (doi:10.1042/BA20030109)
- Swider, P., Conroy, M., Pédrone, A., Ambard, D., Mantell, S., Søballe, K. & Bechtold, J. E. 2007 Use of high-resolution MRI for investigation of fluid flow and global permeability in a material with interconnected porosity. *J. Biomech.* **40**, 2112–2118. (doi:10.1016/j.jbiomech.2006.10.002)
- Van Cleynenbreugel, T., Van Oosterwyck, H., Vander Sloten, J. & Schrooten, J. 2002 Trabecular bone scaffolding using a biomimetic approach. *J. Mater. Sci. Mater. Med.* **13**, 1245–1249. (doi:10.1023/A:1021183230549)
- Van Cleynenbreugel, T., Schrooten, J., Van Oosterwyck, H. & Vander Sloten, J. 2006 Micro-CT-based screening of biomechanical and structural properties of bone tissue engineering scaffolds. *Med. Biol. Eng. Comput.* **44**, 517–525. (doi:10.1007/s11517-006-0071-z)
- van Lenthe, G. H., Hagenmüller, H., Bohner, M., Hollister, S. J., Meinel, L. & Müller, R. 2007 Nondestructive micro-computed tomography for biological imaging and quantification of scaffold–bone interaction *in vivo*. *Biomaterials* **28**, 2479–2490. (doi:10.1016/j.biomaterials.2007.01.017)
- Viceconti, M., Davinelli, M., Taddei, F. & Cappello, A. 2004 Automatic generation of accurate subject-specific bone finite element models to be used in clinical studies. *J. Biomech.* **37**, 1597–1605. (doi:10.1016/j.jbiomech.2003.12.030)
- Voor, M. J., Yang, S., Burden, R. L. & Waddell, S. W. 2008 *In vivo* micro-CT scanning of a rabbit distal femur: repeatability and reproducibility. *J. Biomech.* **41**, 186–193. (doi:10.1016/j.jbiomech.2007.06.028)
- Weinberg, E. J. & Kaazempur Mofrad, M. R. 2009 A multiscale computational comparison of the bicuspid and tricuspid aortic valves in relation to calcific aortic stenosis. *J. Biomech.* **41**, 3482–3487. (doi:10.1016/j.jbiomech.2008.08.006)
- Wikswow, J. P., Prokop, A., Baudenbacher, F., Cliffel, D., Csukas, B. & Velkovsky, M. 2006 Engineering challenges of BioNEMS: the integration of microfluidics, micro- and nanodevices, models and external control for systems biology. *IEE Proc. Nanobiotechnol.* **153**, 81–101. (doi:10.1049/ip-nbt:20050045)
- Xie, Y., Hardouin, P., Zhu, Z., Tang, T., Dai, K. & Lu, J. 2006 Three-dimensional flow perfusion culture system for stem cell proliferation inside the critical-size beta-tricalcium phosphate scaffold. *Tissue Eng.* **12**, 3535–3543. (doi:10.1089/ten.2006.12.3535)
- Yeong, W. Y., Chua, C. K., Leong, K. F. & Chandrasekaran, M. 2004 Rapid prototyping in tissue engineering: challenges and potential. *Trends Biotechnol.* **22**, 643–652. (doi:10.1016/j.tibtech.2004.10.004)