Computational Mechanobiology in Cartilage and Bone Tissue Engineering: From Cell Phenotype to Tissue Structure

Thomas Nagel and Daniel J. Kelly

Abstract This chapter gives a short overview of computational models in cartilage and bone tissue engineering with a focus on how mechanical cues can regulate tissue regeneration on multiple levels, from cell phenotype to tissue architecture. The chapter begins with a brief review of single cell models with a focus on cell-substrate interactions and cytoskeletal remodelling. After summarising a number of current theories for mechanoregulated tissue differentiation, we explain how such hypotheses can either be corroborated or rejected by attempting to simulate in vivo regenerative events. We then outline a recently introduced model for MSC differentiation based on substrate stiffness and oxygen tension as well as how tissue phenotype and organisation can be explored simultaneously within a computational model. The application of computational models to aid in the design of scaffolds for bone and cartilage repair is demonstrated. We also outline how such models can be used in the design and analysis of bioreactors, demonstrating how changes in tissue structure in response to mechanical loading during bioreactor culture can potentially impact the mechanical properties of the final engineered constructs. The chapter closes with a short overview of multiscale models with relevance to tissue engineering.

T. Nagel (✉) · D. J. Kelly
Centre for Bioengineering, Trinity Biomedical Sciences Institute,
Trinity College Dublin, 152–160 Pearse Street, Dublin 2, Ireland
e-mail: nagelt@tcd.ie

D. J. Kelly
e-mail: kellyd9@tcd.ie

T. Nagel · D. J. Kelly
Department of Mechanical and Manufacturing Engineering,
School of Engineering, Trinity College Dublin, Dublin 2, Ireland

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1 Introduction

> "Classical science is a conversation between theory and experiment. A scientist can start at either end – with theory or experiment – but progress usually demands the union of both a theory to make sense of the experiments and data to verify the theory. Technological novelties such as computer models are neither here nor there. A really good dynamic computer model – of the global atmosphere, for example – is like a theory that throws off data, or data with a built-in theory. It's easy to see why such technological worlds are regarded with such wariness by science – they seem corrupted coming and going. But in fact, these models yield a third kind of truth, an experiential synthesis – a parallel existence, so to speak" [57].

The quantitative evaluation of any experiment is benefited by a model that defines a consistent set of parameters allowing comparisons to be made among results from different experiments. In practice, the experiments or systems in question quickly attain a high degree of complexity that is reflected in the models used to describe them. Intuitive understanding or even analytic solutions of such models are limited to a few and practically usually irrelevant cases. Computer technology in combination with numerical mathematics provides the means of solving complex models under practically relevant boundary conditions. Since the necessary tools have become widely available and affordable, engineers and decision makers in the industrial and public sectors have come to rely on quantitative computer analyses in order to develop products or assess risks more cost effectively and quickly by partially eliminating expensive experiments [86]. Cost-effectiveness and possible liabilities require thoroughly validated and verified models with a sufficient quantitative accuracy and their application domain usually has a high degree of overlap with their validation domain, the only exception being high-consequence systems where full-scale physical testing is never an option [86]. Models in contemporary bioengineering are at a different developmental stage with different modelling challenges taking precedence. The particular difficulties in the interpretation of experimental data related to cells and biological tissues are rooted in the large sample variabilities and the nonlinear, multiphasic, heterogeneous, anisotropic, viscoelastic and often active nature of these tissues as well as the usually large deformations they undergo. However, computational models are being used to analyse the mechanical behaviour of biological tissues leading to the development of a wide range of constitutive models and furthering our understanding of the structure-function relationships of the main tissues prevalent in the musculoskeletal and cardiovascular system.

Besides this tight synergistic coupling of experiment and model *analyses*, computer *simulations* are becoming more wide-spread. In contrast to pure quantitative analyses of a known, i.e. experimentally well defined, system, simulations aim to go a step further. Simulations create a virtual reality in which the modeller has the opportunity to alter certain aspects of the system and its environment to test different hypotheses by observing the effect they have on the system. Therefore, simulations often focus on the time course, i.e. the transient development, of a system rather than analysing one specific state and involve theories from a

spectrum of scientific areas—the focus shifts from parameter identification to explanation. In essence, the modeller tries to ask the system of interest a specific question and seeks to approach a possible answer by conducting in silico experiments. In mechanobiology, where the central question is how cells and tissues respond and adapt to mechanical forces, these simulations can provide a valuable extension of classical experimental methods. Even under very defined and simplified experimental conditions the inherent heterogeneity and adaptivity of biological tissues presents a major hurdle in evaluating and quantifying the stimuli and mechanisms responsible for an observed response. The role of computer simulations in this quest has been defined by van der Meulen and Huiskes [111] as follows:

> "Computational mechanobiologists hypothesize a potential rule and determine if the outcome of this hypothesis produces realistic tissue structures and morphologies, hence *trial-and-error*. If the results correspond well, they might be an explanation for the mechanism being modeled. This method of research is common practice and productive in physics, less common in biology (Huiskes, 1995); although *theoretical biology* is based on this type of approach."

In other words, due to the complexity of mechanobiological systems simple answers are often unlikely from experiments alone and alterations to the experimental system tend to introduce other unknowns. In such cases, simulation environments can help unravel part of the mysteries by approaching a problem in a more systematic and quantitative way. For that purpose, computational engineering methods have been introduced into the field and aim to tackle the multiphysics problem by linking mechanics and various branches of biology.

As the opening quote stated and our short introduction outlined, the approaches, intentions and philosophies behind theoretical models differ between the various fields of science and engineering. In this review, we focus on hypothesis driven simulations that try to explain certain experimental phenomena, the assumptions made in them and the conceptual ideas relevant for the understanding of both their potentials and limitations. For this reason we have excluded purely quantitative analyses of biomechanical behaviour. For computer aided tissue engineering with a focus on CAD, image processing and various computer aided scaffold manufacturing techniques we further refer the reader to the review articles by Sun et al. [109, 110]. Since the field of computational mechanobiology is growing rapidly and the wealth of publications is significant, we narrowed our focus on studies that have relevance to cartilage and bone tissue engineering applications. In particular, our interest lies with the mechanoregulation of mesenchymal stem cell differentiation and the incorporation of tissue architecture into simulations of tissue engineering and regeneration. This chapter is organised into several sections that are intended to highlight different conceptual modelling aspects relevant to tissue engineering applications and as such are often very selective in the literature cited:

- Section 2 introduces some examples of mechanobiological single cell models and exemplary questions currently under investigation, namely the interaction of cells with their substrate. This is done for the purpose of showing how

theoretical models can help overcome particular experimental hurdles and in order to illustrate some aspects of structural reorganisation in cell biology.

- Section 3 introduces the concept of testing theories of mechanoregulated tissue differentiation by performing simulations, where the hypothesis under investigation can be corroborated or rejected by comparing in silico predictions with in vivo results. The main focus will be on simulations of fracture healing and osteochondral defect healing. The section is kept brief with a focus on providing essential historical and conceptual background information. It closes with some recent work from our lab on differentiation guided by substrate stiffness and oxygen tension as well as the incorporation of tissue structure into simulations of tissue regeneration.
- Section 4 is concerned with the application of computational models to tissue engineering and regeneration approaches involving bioreactors and scaffolds. The focus here is on understanding how scaffold heterogeneity and architecture affect mechanical stimuli imposed on cells and the role of modelling in complex design challenges. We further provide an example of tissue adaptation during bioreactor culture demonstrating how modelling approaches somewhat akin to those introduced in Sects. 2 and 3 can be used to determine the role of collagen remodelling on the mechanical properties of engineered cartilage subjected to dynamic compression.
- Section 5 finally bridges the gap between the scales by giving a short overview over multiscale simulations in tissue engineering and regeneration.

2 Single Cell Models and Cell-Substrate Interactions

2.1 Mechanosensing, Cell Rheology and Substrate Effects

When descending to the cellular level the main mechanobiological question relates to the fundamental aspect of mechanosensing—how does the cell sense, integrate and translate mechanical signals of different kinds to evoke a biological response. But cells are not merely passive sensors. Cells actively interact with their environment, probe it, sense it and adapt to it. Cytoskeletal components such as actin filaments and microtubuli undergo continuous polymerisation and depolymerisation depending on cell activity, attachment and external cues. Hence, an inherent problem of elucidating cellular properties and behaviours is the living nature of the cell itself—it interacts with the commonly applied measurement tools such that the measurement itself alters the cellular properties that are to be measured. For that reason, intrinsic properties are difficult to obtain by experimental methods alone and constitutive models that feature the salient aspects of cellular behaviour are required for a thorough evaluation [27]. Since the understanding of mechanotransduction pathways requires knowledge on the mechanics of the cell and its

stress or strain generating mechanisms, the lack of such constitutive models is particularly problematic for the elucidation of relevant signalling cascades [53]. Cellular rheology is therefore a very active field of research where phenomena unknown from traditional engineering materials play a role, e.g. the ability of the "material" to perform work via ATP hydrolysis. While classical equilibrium materials exhibit thermal fluctuations, the cell presents a nonequilibrium material with additional actively driven fluctuations [69]. For a recent review on mechanosensing, candidate signalling mechanisms and their relation to cell rheology see the article by Janmey and McCulloch [53].

Most tissue cells are anchorage dependent, probe their environment by actively pulling on it and respond with cytoskeletal (re)organisation and rearrangement of focal adhesion complexes [29]. Apart from this anchorage dependent behaviour of differentiated cells, mesenchymal stem cells (MSCs) have recently been shown to differentiate into neurons, myoblasts and osteoblasts depending on the stiffness of the substrate onto which they adhere [31]. This differentiation mechanism was shown to be dependent on nonmuscle myosin II and is hence linked to the active contractility of the cells [31]. In contrast to the response to external forces relying on outside-in signalling pathways, sensitivity to passive properties of the extracellular space requires a two-step inside-out outside-in mechanism [29]. Cell contraction and stress-fibre formation are thus fundamental aspects of both the behaviour of differentiated cells and stem cell differentiation itself and are of direct relevance for tissue engineering applications. In this review we chose to highlight a class of models that incorporates some of the active mechanisms described above and appears useful in the investigation of cell-ECM or cell-substrate interactions. Understanding these interactions is important not only for explaining fundamental cell functions, cell remodelling and cell differentiation, but also for the design of biomaterials that support cells and are used in pharmacology, cell culture and tissue engineering.

2.2 Modelling the Environmentally Regulated Dynamics of the Cytoskeleton

Early models of cell contraction relied on static discrete sets of stress-fibres [77] or classical continuum modelling strategies such as thermoelasticity [83], neglecting the dynamic and anisotropic nature of the cell. Deshpande et al. [27] developed a continuum model that incorporates "three key biochemical processes: (i) an activation signal that triggers actin polymerization and myosin phosphorylation, (ii) the tension-dependent assembly of the actin and myosin into stress fibres, and (iii) the cross-bridge cycling between the actin and the myosin filaments that generates the tension." The finite strain constitutive model for the cell was developed based on the homogenisation of the activation and deformation behaviour of a single cellular stress fibre. It has been shown to capture the scaling

of the cellular forces with the substrate stiffness, the development of structural anisotropy dependent on cell shape and boundary conditions and the co-localisation of high concentrations of stress-fibres with focal adhesion sites or externally induced local stress concentrations. From a modelling perspective it is noteworthy that this type of model allows a representation of the cytoskeleton in some ways similar to that in tensegrity models [48] and not usually captured in continuum models. Additionally, the continuum approach offers the advantage that the cytoskeletal arrangement does not have to be pre-defined and is dynamic in nature, i.e. the cytoskeleton can remodel, which is a non-trivial task for tensegrity models.

Cells are attached to the ECM through discrete multi-protein complexes called focal adhesions. These focal adhesions assemble and disassemble depending on the forces exerted upon them and are linked to the formation and dissociation of stress-fibres in the cytoskeleton. The adhesion sites undergo maturation from dynamic to fully reinforced static adhesion sites, passing the stages of initial adhesions, adhesion complexes and focal adhesions. Based on continuum mechanics arguments it has been suggested that with maturation into the static state the adhesion sites loose their sensitivity to substrate stiffness; for more details on adhesion sites in relation to mechanosensitivity see the articles by Fereol et al. [32], Nicolas et al. [85], and Nicolas and Safran [84]. The cell contractility model [27] described previously has been extended and applied to the investigation of focal adhesion dynamics using a thermodynamical approach and uniaxial example problems [28]. The authors of this study stated that: "The fact that many proteins perform both structural and signaling functions hinders the ability of traditional genetic approaches to parse the mechanisms that regulate [focal adhesion] dynamics, since knocking out a protein by genetic manipulation may also eliminate an essential structural component. Consequently, to understand how specific molecular features give rise to the observed behavior it is essential to combine experimental studies of adherent cells—including the use of microscopy to observe the structure and dynamics of the cytokskeleton and [focal adhesions]—with computational models that include the salient mechanics" [28]. Among the successful predictions made by the model was the focal adhesion concentration around the cell periphery as well as the dependence of focal adhesion intensity on cell size and contractility.

The coupled mechanosensitive focal adhesion/stress-fibre model was extended to two dimensions and applied to simulate the effect that a micro-patterned shape of ligands would have on cytoskeletal remodelling and focal adhesion distributions [88]. Both convex ligand patterns where the entire cell periphery adheres to the substrate and various concave patterns with free non-adhered edges were investigated. The model's predictions in terms of stress-fibre alignment and concentration were regarded as satisfactory while some discrepancies in the vinculin[1] distribution were observed.

[1] A protein in the membrane that correlates with the concentration of high-affinity integrins and is involved in their binding to cytoskeletal components.

Uniaxial and biaxial cyclic stretching of cells by 3 and 10% at 0.1, 1.0 and 10.0 Hz were simulated using the Deshpande model in Wei et al. [114]. Corresponding to experimental observations the model predicted stress-fibre alignment perpendicular to the stretch direction and its dependence on the stretch magnitude, the amount of lateral contraction and the frequency. Isotropic stress-fibre architectures were predicted in the biaxially stretched cells. An earlier model [113] had investigated cytoskeletal reorganisation in response to cyclic loading by assuming acting filament remodelling in response to normal strain and filament disassembly when their strain energies reach certain levels below or above their basal attachment values. While that model was capable of predicting experimentally observed alignment patterns, it is incapable of reproducing frequency dependent effects. This is rooted in the purely elastic (i.e. scleronomous) approach taken that neglected transient biochemical signals. While the model by Deshpande and coworkers [27, 28] represents a three dimensional finite strain constitutive framework suitable for finite element implementation, this early model was based on two in-plane normal strains and assumed linear elastic actin filaments [113].

Arrays of flexible micro posts have been used to measure forces exerted by the cells onto a substrate and their distribution within the cell. Due to the dynamic nature of the cells the interpretation of the results and the extraction of meaningful parameters has proven difficult. McGarry et al. [75] simulated smooth muscle cells, mesenchymal stem cells and fibroblasts on different beds of micro posts using the model by Deshpande and coworkers [27, 28]. Certain model parameters such as the maximum tensile stress of a fibre bundle were cell type dependent which has been attributed to the expression of different isoforms of actin and myosin among those cell types and potentially presents meaningful parameters for the interpretation of cell type dependent experimental results. For a single cell type, namely smooth muscle cells, the model could predict a number of experimental observations with the same set of parameters: "(i) the scaling of the force exerted by the cells with the number of posts; (ii) actin distributions within the cells, including the rings of actin around the micro-posts; (iii) the curvature of the cell boundaries between the posts; and (iv) the higher post forces towards the cell periphery" [75]. The experimental and computed actin stress-fibre architecture of a cell on a micropost array is depicted in Fig. 1.

Motivated by observations of fibroblast alignment with directions of tensile strain, cell locomotion along rigidity gradients towards areas of higher stiffness or tensile strain and enhanced cell spreading and cytoskeletal organisation with increasing substrate stiffness, Bischofs and Schwarz [6] developed a model based on the idea that cells have a preference for large effective stiffnesses in their local environment which drives their positioning and orientation. Linear elasticity continuum theory was adopted to model the ECM in order to keep the presented calculations feasible. Cells pull on the ECM with their contractile machinery to extract mechanical information that encompasses the effects of both rigidity and prestrain in the substrate. In order to capture this behaviour, mechanical work was chosen as the fundamental stimulus of the mechanoregulation algorithm. By pulling on the ECM cells build up a force at a focal adhesion site that in

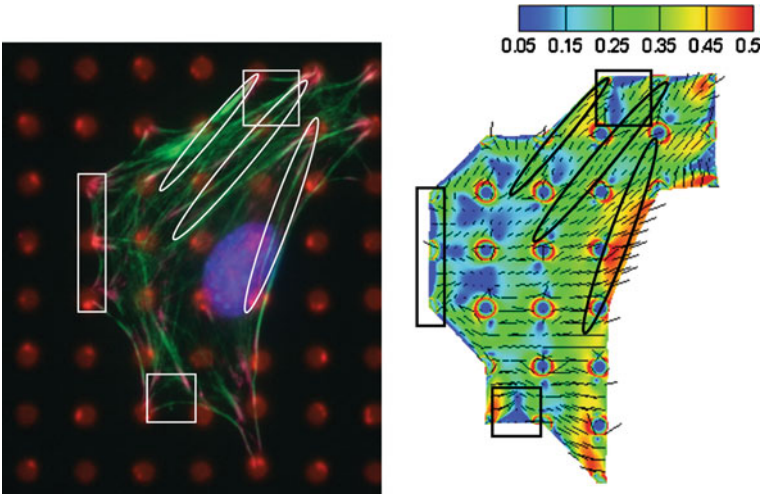

Fig. 1 Steady-state actin stress-fibre distribution in a fibroblast on a micropost array (*left*) and corresponding simulation results based on McGarry et al. [75] (*right*). Contours illustrate the degree of stress fibre formation, vectors correspond to the dominant stress fibre direction. Images courtesy of Dr. Patrick McGarry, Mechanical and Biomedical Engineering, NUI Galway, Ireland

conjunction with a displacement produces that mechanical work. This work can now be minimised by the cell in different ways: If the substrate stiffness increases, the cell has to strain the ECM less to create a given level of force which corresponds to less work being invested. A prestrain of the substrate is shown to lower the work as well and hence corresponds to an effective stiffening. In terms of the wider concepts of modelling the authors make a statement that holds for phenomenological models in general and thus fits the general context of this chapter: "It is important to note that conceptually the principle suggested here does not imply that the cell actually minimizes the work W invested into its soft environment. Instead we suggest here that calculating the quantity W for different situations of interest is an appropriate measure for the kind of information a cell can extract from its elastic environment through active mechanosensing. The real justification of our model will be its success in explaining a large body of experimental data" [6]. Nevertheless, the considerations imply a mechanism by which higher matrix stiffness leads to an increased efficiency in the up-regulation of cell-matrix contact points. Based on the work optimisation principle, the model predicted cell alignment parallel and perpendicular to free and clamped edges, respectively, cell alignment with applied tensile strain and coalignment of cells into strings. Cells were further predicted to have a tendency of moving away from free surfaces and the model can potentially explain certain aspects of cell behaviour that cannot be established based on contact guidance models [3]. Cell alignment in response to ECM alignment, i.e. contact guidance, is relevant for tissue engineering applications and soft scaffold contraction. To allow a theoretical

investigation of contact guidence effects an extensive modelling framework has been introduced by Barocas and Tranquillo [3]. It relies on mixture theory to capture the biphasic nature of the soft tissue equivalents under consideration and accounts for collagen alignment in response to anisotropic deformation, subsequent cell alignment, i.e. contact guidance, and the resulting anisotropic traction exerted by the cells onto the ECM as well as their anisotropic migration.

The described models are taking a major step towards the coupling of a cell's response to its external as well as internal mechanical environment. They have the potential of facilitating a better understanding of the cell's interaction with its environment, including intrusive measurement tools. This is crucial for the adequate interpretation of experimental results and for examining cell rheology. Several aspects might be put onto the research agenda: The incorporation of actual signalling pathways into the models will not only increase their predictive capability, but also allow the investigation of pathological or experimental (e.g. drug related) inhibition of those very pathways. Further, while the models described in this section link mechanics to cellular remodelling and are capable of rationalising a number of experimental observations, the link between biology and mechanics needs to be extended.

3 Stem Cell Differentiation During In Vivo Regeneration

3.1 Testing Mechanoregulation Hypotheses In Silico

The idea that mechanics affects tissue differentiation has been discussed in the scientific community for a long time [89, 95]. By incorporating hypotheses regarding mechanoregulated tissue differentiation into computer simulations of regenerative events (such as fracture healing) that exhibit well defined and repeatable temporal and spatial patterns of tissue differentiation with alternative healing paths depending on mechanical stimulation, these hypotheses can be corroborated or rejected by comparing the in silico predictions to the experimental observations. These approaches therefore constitute a valuable tool in the assessment of potential biophysical regulators of tissue differentiation and may have specific relevance for the incorporation of biophysical stimulation into bioreactor designs for tissue engineering applications. Similarly, such corroborated hypotheses might serve to form the basis for the evaluation of the mechanical environment in scaffolds for tissue engineering (see Sect. 4) The ultimate goal of tissue engineering is to replace or repair damaged tissues in vivo. Quantitatively evaluating the in vivo environment and its effects on the tissues during regeneration therefore has direct relevance to tissue engineering itself.

In accordance with the scope of this chapter we restrict this overview on relatively recent work where theories regarding mechanoregulated tissue differentiation have been tested using modern simulation techniques. Specifically we

focus on some recent developments, namely (i) a tissue differentiation algorithm based on substrate stiffness and oxygen tension; (ii) the simultaneous prediction of tissue structure and phenotype during regenerative events; (iii) performing non-deterministic simulations to capture variability.

Among the mechanical stimuli proposed as potential mechanoregulators are

- Octahaedral shear stress/strain or maximum principal strain and hydrostatic stress (Carter and co-workers [15–17]). Quantitative boundaries were only proposed later by others [50].
- Principal strain and hydrostatic stress (Claes and Heigele [22, 23])
- Octahaedral shear strain and fluid velocity (Prendergast and Huiskes [46, 91])
- Others include certain strain invariants [34, 94] and strain energy density [1].

The model by Carter and colleagues [15–17] was used among others in a study where pseudoarthrosis formation was studied in oblique fractures [71]. Furthermore, this model has been applied frequently in tendon mechanobiology, such as fibrocartilaginous metaplasia formation in tendons wrapping around bony prominences [39, 116].

The theory by Claes and Heigele [22, 23], which is based on Pauwel's ideas [89], was mainly applied to problems of fracture healing. A recent model for tissue differentiation and revascularisation in fracture healing by Simon and co-workers [107] implemented a set of rules into a fuzzy-logic controller to simulate regenerative events in the fracture callus. The limiting factors for healing were found to be revascularisation in stable fractures and an inadequate mechanical environment in unstable fractures. A similar fuzzy logic model was able to predict all stages of trabecular fracture healing including the final remodelling stages to re-establish a trabecular structure depending on the loading direction [106].

The model by Prendergast et al. [91] accounts for the biphasic nature of most biological tissues and as such incorporates fluid flow as a stimulus. Its first implementation into an automated feedback algorithm to simulate the time course of fracture healing was performed by Lacroix et al. [68]. The origin of MSCs, whose migration into the fracture callus was modelled as a diffusive process, was predicted to have a significant impact on the rate at which healing progressed. The model was further able to predict the spatio-temporal sequence of phenotypes occurring during fracture healing. The results provided support for the hypothesis that fluid flow and shear strain are regulators of MSC differentiation during fracture healing. Following this initial corroboration, the model was applied to analyse the influence of fracture gap size and loading on the emerging phenotypes and the healing outcome [67]. Further corroboration was achieved by applying the tissue differentiation model in simulations of other regenerative events. Besides fracture healing [49, 67, 68], it has been successfully used to predict key events during distraction osteogenesis [8, 9, 51] osteochondral defect healing [55, 56], implant integration [36, 46] and pseudoarthrosis formation [43, 81]. Many studies on scaffold aided tissue repair or scaffold design rely on this algorithm since fluid perfusion is thought to play a major role in porous scaffolds (see Sect. 4)

Several comparison studies between the various algorithms named above have been performed with no full corroboration achieved for any of them [35, 49, 50] although the influence of torsional loading on fracture healing could only be captured by considering fluid flow and shear strain as regulators of MSC differentiation [49]. Further reviews of in silico approaches to regenerative medicine, namely for bone and wound healing, can be found elsewhere in the literature [10, 38].

3.2 MSC Differentiation Regulated by Substrate Stiffness

As outlined in Sect. 2, the profound effect of substrate stiffness on cells including the stiffness-dependent differentiation of mesenchymal stem cells into various lineages [31] has attracted considerable attention and is an important factor in the choice of an appropriate biomaterial carrier for tissue engineering applications. Cells furthermore depend on the supply of oxygen and nutrients and different phenotypes thrive under different ambient oxygen tensions. Perhaps most importantly, oxygen tension itself has been shown to be a regulator of stem cell fate [105]. In regenerative events or during progenitor cell based tissue engineering, osteogenesis, for example, relies heavily on sufficient vascular supply, while chondrogenesis has been shown to be favoured under low oxygen conditions [76]. Various tissue differentiation models therefore include an angiogenic component [19, 37, 107].

A recent mechanoregulation model for tissue differentiation [12] developed in our lab is based on two fundamental components: The influence of substrate stiffness and oxygen tension. The model relies on a four-step analysis approach:

1. MSC infiltration into the regenerating tissue is modelled as a diffusive process.
2. A biphasic analysis is performed to evaluate the mechanical environment throughout the regenerating domain.
3. Angiogenesis is modelled as a mechanoregulated diffusive process where the formation of new capillaries is inhibited in regions of high shear strain as determined in analysis step 2.
4. Based on the vascular supply another diffusion analysis additionally accounts for cellular consumption and determines the levels of oxygen tension in the regenerating tissue.

Based on the current distribution of tissues, information on both the local oxygen tension and substrate stiffness is now available in each point. This information is used to predict the cell phenotypes for the next iteration based on the theory described in Fig. 2. The mechanical properties, state of vascular and cellular infiltration as well as oxygen perfusion can now be updated for the next time increment of the analysis. In contrast to most mechanoregulation models, mechanical stimuli such as strain, stress or fluid flow do not exert their influence on MSC differentiation directly in this model. Instead, neovascularisation is inhibited in regions of high strain and the resulting low

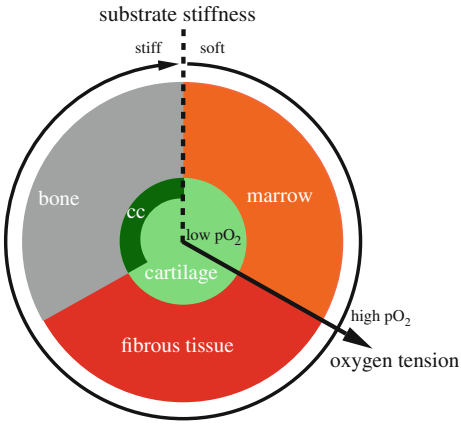

Fig. 2 Regulation of tissue differentiation by substrate stiffness and oxygen tension. Cartilage forms in a low oxygen environment and turns into calcified cartilage (CC) in close proximity to bone. MSCs will differentiate to form marrow (stroma and adipogenesis), fibrous tissue or bone when attached to soft, medium or stiff extracellular materials, respectively. Colour version available online

oxygen concentration results in cartilage formation. Thus, the effect of deformation on stem cell fate is more indirect. Rather than responding to external strain, the cells are assumed to actively probe the ECM in order to determine its stiffness and differentiate accordingly.

Like several other theories, this regulatory model has successfully captured many of the key stages of fracture healing [12]. This illustrates that the corroboration of one hypothesis in a simulation does not allow one to reject others. Extensive assessment of the efficacy of a proposed theory via the simulation of a broad range of experiments is needed to further corroborate the proposed hypothesis.

3.3 Accounting for Tissue Architecture During Skeletal Regeneration

A tissue's structure is crucial for its mechanical fitness. Understanding how the structure evolves during development and regeneration can have important implications for how tissue engineering protocols, scaffolds and bioreactors are designed in order to mimic a desired tissue architecture. The extension of the predictive power of tissue differentiation models to include anisotropy will benefit the investigation of specifically those healing processes and tissue engineering strategies where recapitulating normal tissue architecture is important. For example chondral and osteochondral defect repair critically depends on achieving a native-like zonal structure within the cartilage tissue so that the tissue can endure in-vivo loads.

Cullinane et al. [25, 26] have demonstrated that the mechanical environment during bone defect healing can influence both tissue differentiation and the molecular organisation (collagen fibre architecture) of the repair tissue. They showed that cyclic bending applied daily to an experimental mid-femoral defect

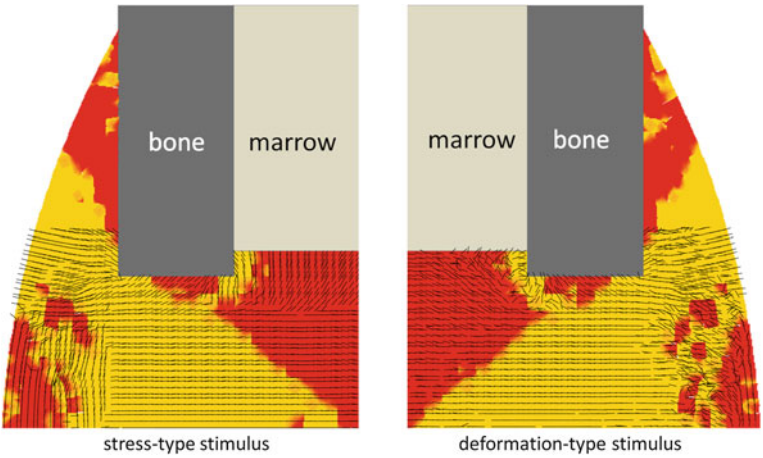

Fig. 3 Distribution of phenotypes (*yellow* fibrous tissue; *red* cartilage) in an idealised model of a fracture callus subjected to cyclic bending. Vector plots show predicted collagen fibre orientation in the region of interest when collagen alignment was regulated by stress (*left*) or deformation (*right*). The predicted orientations of collagen fibres [81] were comparable to those observed experimentally [25]. Colour version available online

results in the formation of cartilage as opposed to bone tissue. These neoarthroses exhibited preferred fibre angles consistent with those seen in articular cartilage. Hayward and Morgan [43] further demonstrated that the patterns of tissue differentiation observed experimentally could be predicted using the mechanoregulation theory of Prendergast et al. [91], providing further evidence to suggest that both strain and fluid flow are key regulators of tissue differentiation. Nagel and Kelly [81] extended the mechanoregulation model of Prendergast et al. [91], as implemented by Lacroix et al. [68], to include a biphasic fibre reinforced constitutive model for soft tissues, where the organisation of the fibre network is regulated by the mechanical environment. Collagen fibres where assumed to align between the principal directions of various stress and deformation measures and depending on the local maximum principal values occurring during a bending cycle. By simulating the effect of bending on bone defect repair, the predicted patterns of differentiation and collagen fibre orientations in the repair tissue could be compared to those observed experimentally [25]. It was demonstrated that mechanoregulation models can be used to successfully predict both tissue differentiation and organisation during skeletal tissue regeneration (Fig. 3).

3.4 Variability in Tissue Differentiation

A high degree of variability is observed in the outcome of tissue engineering as well as in vivo experiments. In the context of tissue differentiation models, this

variability can pose a problem for the corroboration of these theories. Similarly, model predictions can benefit from covering a range of possible outcomes based on statistical considerations of the underlying populations and variabilities. Predicting a range of performance measures for an implanted tissue engineered construct and its probability of success can aid decisions regarding its design or even just whether or not to implant a certain construct. The need for nondeterministic simulations and appropriate validation metrics has long been recognised in traditional engineering, especially in the design of safety relevant systems [86]. These validation metrics should be based on a statistical evaluation of both the experiments and the computations. In bioengineering, the variations that occur are usually larger than those observed in controlled technical systems. Focussing on scenarios for which mechanoregulatory aspects are of importance, for example for load bearing implants, variability arises from environmental factors, such as implant positioning and activity levels, i.e. external loading, as well as genetic factors such as the level of mechanosensitivity.

The variability of bone geometry and mechanosensitivity observed in a population was modelled to simulate the clinical trial of low and high-stiffness intramedullary prostheses in 100 patients [92]. Variable mechanosensitivity was included by modulating the parameter values that determine the onset of bone resorption/deposition in the bone remodelling algorithm used. When only one averaged ideal patient was simulated, i.e. no variability included, the deterministic simulation predicted that the low stiffness implant would migrate less. The simulation of the clinical trial with 100 different patients, however, predicted no statistically significant difference between the two implants. The study concluded, that stochastic simulations might be more validatable against an actual clinical trial and may eventually overcome the lack of falsifiability of this type of model [92].

Modelling certain cellular processes in a stochastic fashion [90] introduces only a minor degree of nondeterminism in the simulation outcomes. Khayyeri et al. [59] simulated tissue differentiation inside an in vivo bone chamber in which the tissue could be loaded in compression and investigated the influence of the load magnitude, implant positioning, blood pressure and bone marrow MSC density on the distribution of phenotypes. The study concluded that these environmental factors were not sufficient to fully explain the high degree of variability observed in the experiments, namely the emergence of two distinct experimental groups. In a later study [61], the authors thus hypothesised that a variable mechanosensitivity between individuals could be responsible for the variability observed in the tissue differentiation experiments. Simulations of the bone chamber experiment were performed and the effect of stochastically up- or down-regulating the cellular process rates for proliferation, differentiation and apoptosis in an individual-specific manner was investigated. Simulation results were highly sensitive to MSC activity. The simulations not only predicted the general patterns of tissue differentiation but also produced a variability akin to that observed in the experiment. Specifically, the simulations predicted the dichotomy, i.e. the emergence of two distinct differentiation patterns. Results such as this can have important implications for the corroboration of mechanoregulation theories.

Instead of focussing on variability, other studies take a subject specific approach. A bone remodelling algorithm based on open system thermodynamics was applied to simulate the pattern of bone mineral density in the proximal tibia in response to gait loading [87]. A subject specific geometry and load, derived from gait analysis, were used. The predicted bone density distribution showed excellent qualitative and quantitative agreement with the subject specific pattern measured by X-ray absorptiometry. In another study, Wolff's law was "inverted" to estimate the loading history of a murine vertebral body via an optimisation approach based on a set of unit loads applied to a subject specific model of a murine vertebral body, the internal structure of which was modelled based on μCT scans [21]. Based on the assumption that bone tissue remodels such that the strain energy density approaches a target value and that the tissue is loaded uniformly, the dominant load case could be extracted both in direction and magnitude. The strain energy density in the trabecular architecture remained highly non-uniform nevertheless. The review article by Geris et al. [38] includes a discussion of patient specificity and variability in in silico approaches.

4 Scaffolds in Bone and Cartilage Repair

4.1 Computer Aided Design of Scaffolds and Bioreactors

Scaffold design is constrained by a large number of design criteria that often compete. This competition implies that there is some optimum design that represents the best compromise between the various requirements. Relevant aspects to be considered in scaffold design include:

- The *material* has to be non-toxic, biocompatible, sterilisable, manufacturable into arbitrary shapes accommodating patient specific defect geometries, and possess biologically favourable surface properties.
- Considerations regarding the intrinsic *mechanical properties* of the material include compliance-matching with the host tissue, sufficient strength during implantation, cellular response to substrate stiffness (see Sect. 2), and expected deformations that potentially act as external stimuli.
- The scaffold *architecture* can serve as a template to guide tissue architecture, including anisotropy [30]. The *porosity* is linked to the apparent properties of the scaffold and is therefore mechanically constrained such that the scaffold maintains its structural integrity. However, higher porosities are favourable for cell seeding and proliferation, tissue ingrowth and mass transport. Porosity also affects the permeability and hence fluid velocities during seeding as well as mechanical loading. Depending on the target tissue, the pore architecture and its interconnectivity has to be suitable for *vascularisation* of scaffold and tissue.

- The *dissolution rate* of biodegradable scaffolds should be optimised to match the speed of differentiation and matrix synthesis by cells within the scaffold. The dissolution rate can be modulated via the relative amounts of the components that make up the biomaterial, which will in turn affect intrinsic properties such as stiffness.

Alterations to the properties above likely affect the mechanical environment in the scaffold and hence the mechanoregulation of biological processes. Due to the multitude of design criteria and their strong coupling, mechanobiological modelling can contribute significantly to finding optimal scaffold properties or limiting the number of physical prototypes to be tested. Provided the knowledge, efficiency and resources in the future, this computational optimisation of scaffolds could be envisaged routinely on a patient-specific basis to maximise clinical success and stream-line the automated production process [56, 72].

Bioreactors are a fundamental part of tissue engineering for two main reasons [72]. First, one approach to tissue engineering involves cell biopsies, ex vivo expansion and culture in a bioreactor to achieve desired tissue properties and subsequent reimplantation. More indirectly, bioreactors bear significant relevance for mechanobiological research. They present model systems in which environmental aspects such as cytokine concentrations, medium composition, pH, oxygen environment and mechanical loading can be much more tightly controlled than in vivo.

Computational models can not only help in the design of scaffolds, but also in the design of bioreactors and the choice of their operating parameters. Several bioreactor designs exist that induce fluid flow in or around the scaffolds, such as direct perfusion, spinner flask or rotating wall bioreactors. Their prime purpose is to enhance culture medium transport to provide the optimal biochemical environment for the cells. However, fluid flow induced shear is a potent mechanical stimulus that can influence the metabolic activity of the cells or even their phenotype. Hence it has been suggested [72] that computational fluid dynamics methods rather than trial and error should be used to establish the flow conditions for the various bioreactor systems, scaffold architectures and cell types. The same concept can of course be applied to related aspects of bioreactor performance such as direct mechanical stimulation by deformation. Other applications include the derivation of culture and rehabilitation protocols derived from the analysis of in vivo conditions [13], the derivation of the cellular environment from the macroscopic bioreactor input profiles [72], the online integration of computational models into tightly monitored commercial tissue culture systems to improve automated control and surgical planning [72] and hence enable a more cost-effective and scalable approach to tissue engineering. In the context of cartilage tissue engineering and bioreactor design we wish to draw attention to the contributions by Raimondi et al. [93] and Bjork et al. [7] in this book for further information.

4.2 Tissue Differentiation in Scaffolds

4.2.1 Optimisation of Scaffold Properties

Significant efforts have been directed at engineering bone and cartilage or enhancing the natural reparative processes by a variety of interventional methods. Scaffolds implanted into osteochondral defects have been used to enhance repair tissue quality but a major unknown are the ideal scaffold properties. The earliest attempt to determine optimal mechanical properties of a scaffold using a computational mechanobiological model was for the repair of an osteochondral defect [56]. The main assumptions of the model were that MSC differentiation as well as cell proliferation and apoptosis were determined by a combination of fluid flow and shear strain [91] and that cell migration could be modelled as a diffusive process. By systematically varying the Young's modulus and the permeability of homogeneous scaffolds it could be shown that optimal values for these parameters exist, with both higher and lower values leading to decreased amounts of cartilage tissue. This result was due to the differential influence of fluid flow and shear strain on cell differentiation. To further enhance repair, i.e. reduce the amount of fibrous tissue and stop the subchondral bone from progressing into the cartilage zone, a bilayered scaffold with inhomogeneous properties was then optimised. The osseous part of the scaffold was modelled as homogeneous while the properties of the chondral part were depth dependent. The study found that the optimal scaffold should have a stiffness that decreases with depth from the superficial to the deep zone and a permeability that increases with depth, i.e. has its minimum at the articular surface. Given the inhomogeneous properties of articular cartilage this scaffold has to a certain extend biomimetic qualities. The study closes with the remark that scaffolds optimised for the in vitro setting may not be well suited for the in vivo environment and vice versa.

A similar approach has been followed to investigate design parameters in bone scaffolds by Byrne et al. [14]. Tissue differentiation was again regulated by fluid flow and shear strain [91], while cellular activities were modelled using the lattice approach [90]. Tissue differentiation inside a bioresorbable scaffold with regular geometry typical for 3D printing techniques was simulated under low and high loading conditions varying three key design variables: Young's modulus, porosity and dissolution rate. The authors were able to determine unique combinations of these parameters that maximised bone formation depending on the loading condition. Especially under high loading conditions the initial porosity and dissolution rate had to be chosen conservatively as to not compromise the structural integrity of the scaffold. High porosities, a medium dissolution rate and a high stiffness gave the optimal results under low loading conditions. This suggests that scaffold manufacturing should be tailored towards the loading conditions at the implantation site.

Scaffold based tissue repair of critical sized defects is often hindered by inadequate vascular supply resulting in a degraded scaffold core and peripheral

tissue formation. Due to its importance in tissue regeneration, the lattice model approach has been extended to include angiogenesis [19] in an attempt to better model the process of bone regeneration. Checa and Prendergast [20] closely followed the approach of Byrne et al. [14] to investigate the effect of cell seeding and mechanical loading on vascularisation and tissue formation inside a scaffold. High and low loading conditions and different seeding densities were simulated and homogeneous seeding compared to only peripheral seeding. It was predicted that reducing the MSC seeding density from 1 to 0.5% greatly improved vascular infiltration and bone tissue formation, whereas under high seeding density conditions only peripheral vascularisation and ossification occurred. Likewise, peripheral seeding was predicted to allow scaffold core vascularisation and subsequent bone formation. While low loading conditions were generally beneficial for bone formation, increasing the applied stress above a certain threshold value was predicted to result in decreased vascularisation and a mainly cartilaginous tissue.

4.2.2 Incorporating Realistic Scaffold Architectures

While the previous two studies modelled a regularly shaped scaffold, an irregularly shaped scaffold inside a loaded in vivo bone chamber was implemented on the lattice level by Khayyeri et al. [60]. Its geometrical representation was based on μCT scans of a highly porous collagen-glycosaminoglycan (collagen-GAG) scaffold. On the level of the regularly structured finite element mesh material properties were homogenised by a rule of mixtures approach using the volume fractions of tissues and scaffold material present in the element's lattice. This approach allows for an easy implementation of the scaffold geometry into the lattice. It is computationally efficient and hence suitable for larger scaffold structures at the expense of micromechanical accuracy of the solution due to the application of the rule of mixtures. The effect of a varying scaffold stiffness on tissue differentiation patterns was investigated. Soft scaffolds were predicted to result in mainly fibrous tissue formation while increasing stiffness values of up to 1 GPa increased the amount of cartilage and bone present in the bone chamber significantly.

A micromechanically more accurate assessment of biophysical stimuli in a μCT based model of an irregular scaffold is necessarily computationally very expensive since a large number of elements is required to mesh the scaffold structure (and the pore space). This approach was followed in Sandino et al. [99] for calcium phosphate (CaP) and glass scaffolds by performing a solid analysis of applied compression and a steady-state Newtonian fluid flow analysis to assess stress, strain, fluid pressure, fluid velocity and flow induced surface shear stress in the scaffold during early bioreactor culture. The heterogeneity was not only reflected in the strain pattern, but also in the fluid velocities: Some pores were never perfused despite sufficient interconnectivity while in others the fluid velocity was

1000 times higher than the inlet velocity. Due to the considerable computational cost only a part of the full scaffold was modelled.

Sandino et al. [100] included angiogenesis and tissue differentiation into a μCT based model of a CaP scaffold section by filling the irregular pore space with a lattice to simulate cell activities. In vitro seeding was simulated by attaching the initial MSC population to the struts throughout the scaffold and compared to In vivo colonisation, where the scaffold was initially cell free and the original MSC population was modelled to reside at the outside faces of the pore network where endothelial cells had their origin in both cases as well. In vivo colonisation led to faster migration and proliferation but affected final tissue distribution and vascularisation of the scaffold only marginally. Despite 70% of the pore volume having a stimulus favourable for ossification, only 40% of that space filled with bone due to vascularisation being restricted to the periphery of the scaffold. Deeper vascular penetration was inhibited by the scaffold walls in the model. When the applied compression was doubled from 0.5 to 1% strain, cartilage was also predicted in external pores. Apoptosis, induced by a mechanical stimulus twice as high as that for fibrous tissue formation, increased from 17 to 22% as the applied strain increased from 0.5 to 1%.

Stops et al. [108] simulated MSC differentiation in a collagen-GAG scaffold based on μCT scans using a combination of FE and CFD analysis. Strain dependent cell proliferation was included and octahaedral shear strain in combination with fluid velocity used to determine MSC fate. Applied strains of 1% and above led to predictions of lower cell densities. By comparing different scaffold strains and fluid inlet velocities the authors were able to determine specific combinations of these two parameters that favoured certain phenotypes. While certain conditions favourable for osteoblasts (final cell fraction 84.9%) and fibroblasts (final cell fraction 73.9%) could be established, none of the combinations proved particularly suitable for robust chondrocyte differentiation (maximum cell fraction achieved 56.7%).

Besides its influence on the mechanical conditions inside the scaffold, the pore size influences cell attachment morphologies. In a combined fluid-elastostatic analysis, Jungreuthmayer et al. [54] simulated the effect of cell attachment modes on the experienced stimuli. Three random sub-volumes of the μCT reconstruction of a collagen-GAG scaffold were numerically seeded with cells that either attached flatly to one strut or bridged two struts by means of cellular processes. A steady-state incompressible Newtonian-fluid flow analysis revealed that pressures and wall shear stresses experienced by the cells were largely independent of attachment mode. A subsequent linear elastic analysis of cells subjected to the pressure and shear loads derived from the CFD simulation revealed that bridging cells underwent approximately 500 times higher displacements than flatly attached cells. Van Mises stresses in bridging cells were about 26 times higher than in flatly attached cells. These results can potentially explain why cells seeded onto 3D scaffolds with different morphologies, which promote various modes of cellular attachment, elicit a dramatically different biological response when subjected to

similar levels of inlet flow. They could also explain the higher flow rates necessary to stimulate cells in 2D compared to a 3D environment [4, 52].

This brief selection of numerical studies on tissue differentiation in scaffolds seeded with mesenchymal stem cells provides a small insight into how quantitative engineering analyses can help understand the heterogeneity of microscopically induced deformations with peak values significantly exceeding macroscopically applied loads. They present a valuable tool to balance the various competing design criteria for scaffolds in a more systematic way compared to experimental trial and error to achieve optimal tissue growth and regeneration. The application will dictate whether a micromechanically accurate representation of the scaffold is necessary or whether the use of a smeared continuum treatment is sufficient.

4.3 Modelling the Bioreactor Environment: From Structure to Function in Engineered Cartilage

4.3.1 Importance of Structure and Composition for Mechanical Fitness

While these studies demonstrate a role of models in predicting phenotypical changes, the mechanical environment also influences the organisation of the tissue. A biomimetic recapitulation of tissue structure has relevance for those tissues in which the architecture is optimised for their loadbearing duties, such as articular cartilage. Cartilage tissue engineering is based either on chondrocytes or progenitor cells induced to undergo chondrogenesis and differentiate towards a chondrocyte-like cell. The chondrocytes then synthesise cartilage specific extracellular matrix components to establish a functional and viable tissue. Common assessment of this functionality is usually based on measuring glycosaminoglycan and collagen II content as well as performing compression tests to determine tissue mechanical properties. Both in native and in engineered cartilage various tissue stiffness measures have been found to correlate with the amount of these two main components constituting the cartilage extracellular matrix. Yet, this correlation of composition and functionality is an oversimplification and neglects the structure-function relationships present. This becomes apparent when considering experiments [5, 44, 45, 70, 73] that report a stiffer cartilage matrix when the constructs were mechanically loaded during bioreactor culture than when it was kept unloaded, but found no differences in the collagen and sulphated GAG content. Yan et al. [117] investigated structure-function relations in tissue engineered cartilage, including minor ECM components and cross-linking proteins into their analysis. They found strong correlations between these usually omitted constituents and the mechanical properties of the constructs as well as an increase in their concentration due to loading. They concluded that low levels of collagen IX and mature collagen cross-linking are a major contributing factor to poor mechanical properties of in vitro engineered cartilage. Thus it could be shown experimentally

that basing composition-function relationships on major ECM components alone does not provide a complete picture. The importance of cross-linking collagen to achieve mechanically mature network has also been shown in cardiovascular tissue engineering [2, 96].

There is another key factor that contributes to tissue mechanical properties. Several experimental studies have suggested that enhanced structural organisation of the collagen network could contribute to the elevated stiffness values observed in bioreactor studies [44, 58, 70, 74]. The effect of structural alterations is however difficult to investigate experimentally, since tissue structure is not easily altered in a tightly controlled manner, biochemical and biomechanical alterations are commonly not easily uncoupled and certain structural features aside from fibre orientation are difficult to quantify. However, from extensive studies on a wide range of load bearing soft tissues, it has become apparent that the collagen architecture in these tissues is not only optimised for the particular load bearing duties but is also able to adapt to changes in the loading environment [33, 63, 97]. Understanding the mechanisms of tissue remodelling will potentially allow us to design bioreactors to control the structure and organisation of engineered tissues.

4.3.2 Modelling the Influence of the Collagen Architecture in Tissue Engineered Cartilage

Several studies exist that model cartilage structure. Collagen remodelling algorithms developed in the cardiovascular biomechanics field have been successfully applied to predict the collagen organisation in tibial plateau cartilage [115]. Khoshgoftar et al. [62] evaluated deformation fields in cartilaginous constructs undergoing various loading protocols: unconfined compression, sliding indentation and combined compression-sliding indentation. Based on the predicted strain fields the latter was hypothesised to provide the most suitable stimulation for the development of a Benninghoff-like zonal structure in the engineered constructs. This native cartilage architecture, in which collagen fibres arcade from an alignment perpendicular to the articular surface in the deep zone to an orientation parallel to the surface in the superficial zone, is crucial for cartilage structure-function relationships and has proven difficult to recapitulate in engineered tissues [64]. The influence of postnatal collagen reorientation on the confined compression behaviour of articular cartilage was investigated with a composition based constitutive model in van Turnhout et al. [112]. Klisch et al. [65] developed a cartilage growth mixture model in which proteoglycans and collagen can grow independently of each other via volumetric mass deposition but are constrained to move together during deformation. More advanced constitutive relations were incorporated later on that included the balance between stresses generated by the proteoglycans and those in the collagen network [66]. The model was successfully validated against biochemical content, tissue volume and tensile moduli from in vitro growth experiments [66]. The effect of collagen orientation on the equilibrium properties of charged and neutral biphasic tissues was investigated in Nagel

and Kelly [78]. While in uncharged tissues, fibres perpendicular to the loading direction cause the highest stiffness in unconfined compression, fibres aligned with the loading direction produce the highest initial stiffness values in charged tissues. This is routed in the pre-stress of the collagen network due to the swelling pressures induced by the fixed negative charges of the GAG molecules. None of these studies, however, investigated potential collagen remodelling mechanisms during bioreactor culture and their effect on the mechanical properties of the engineered cartilage.

In a recent study from our lab we applied a newly developed collagen remodelling algorithm [80] to test the hypothesis that structural changes to the collagen network in response to loading could contribute to enhanced biomechanical properties of dynamically compressed cartilaginous constructs even in the absence of biochemical differences between the free-swelling (FS) and dynamically loaded (DL) groups [79]. Fifty-six days of bioreactor culture were simulated during which proteoglycans and collagen were synthesised leading to a steady increase in material parameters that was equal in both the FS and DL groups. The collagen remodelling algorithm used [79, 80] allowed for changes in the local collagen orientation and its stress-free configuration (i.e. the transition from a buckled fibre with insignificant stress contribution to a tensed cable like configuration with a high stress contribution where the transition point is called recruitment stretch, Fig. 4a) via a multiplicative decomposition of the deformation gradient (Fig. 4b). Collagen reorientation alone was predicted to lead to decreased construct stiffnesses. Remodelling the stress-free configuration of the collagen network increased swelling pressures and altered its state of pre-stress leading to increased mechanical properties. Only when combining both mechanisms could the increased Young's moduli, decreased Poisson's ratios and altered construct geometries be predicted in accordance with experimental observations (Fig. 5). These results provide support for the hypothesis that in addition to various mechanisms such as cross-linking, a structural reorganisation of the collagen network potentially contributes to enhanced mechanical properties, and provides mechanistic insight into the effects of different structural phenomena [79]. The study further demonstrates how constitutive models can add insight to experimental results via a theoretical decoupling of physical mechanisms.

5 Bridging the Gap: Multiscale Models

5.1 The Multiscale Approach

Extensive knowledge on biological processes and their dependence on mechanics is being acquired at all relevant length scales from the biomolecular level up to the tissue and organ level. One challenge is to data mine, integrate, present and distil this huge amount of information and computational information technology is an

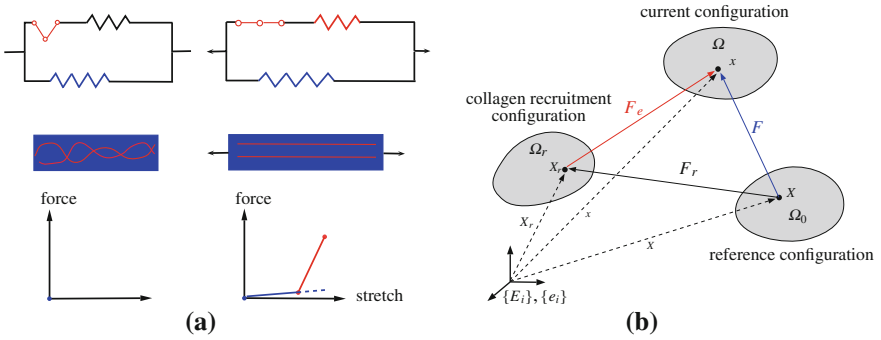

Fig. 4 Illustration of the collagen network's state of tension-compression transition in 1D (**a**) and 3D (**b**). **a** The stress-free configuration of a collagen fibre can be visualised by a one dimensional rheological analogy (*top row*) and the concept of crimp (*middle row*). Once the fibres are uncrimped and the angular joint in the assembly is straightened out, the top spring representing the collagen fibres begins to bear load. After this (recruitment) point the force curve of the idealised material has a higher slope (*bottom row*). **b** A multiplicative decomposition of the deformation gradient F into a recruitment F_r and elastic part F_e allows the definition of appropriate free energy potentials for various constituents and a remodelling of the collagen network's stress-free configuration in 3D. Colour version available online

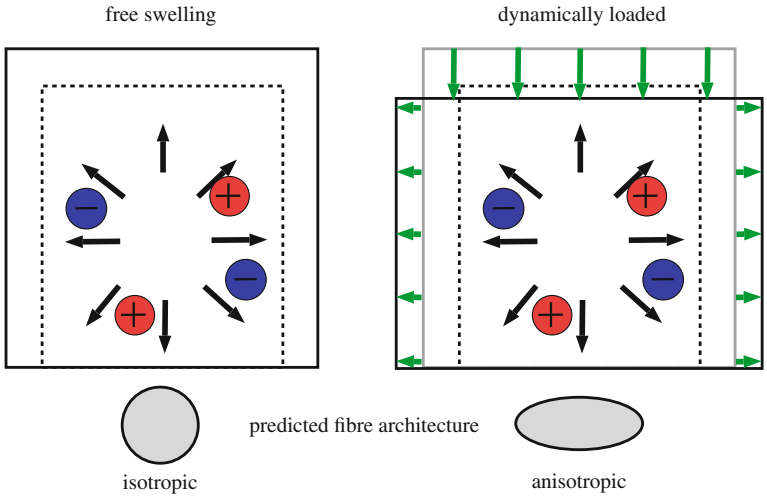

Fig. 5 The fixed negative charges of the glycosaminoglycans lead to an isotropic swelling of the constructs. The FS group therefore has an isotropic tissue architecture. Dynamic compression induces axial compressive strains and lateral expansion. Remodelling leads to horizontal fibre alignment and a similarly anisotropic distribution of the recruitment stretch. In combination, this leads to higher Young's moduli, lower Poisson's ratios as well as lower, wider and more compact samples. Colour version available online

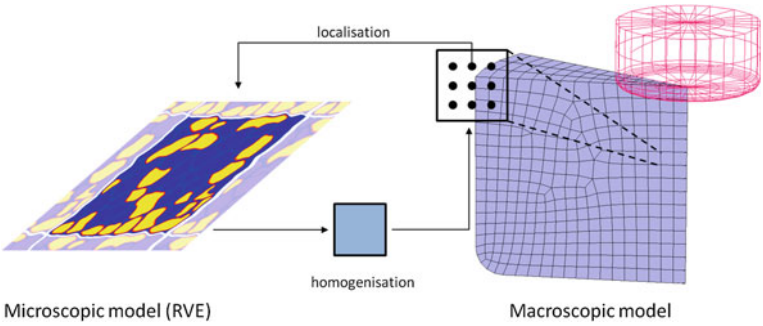

Fig. 6 Schematic illustration of the multiscale approach. Instead of a constitutive model at the integration point level of the macroscopic domain, a localisation rule is used to determine the boundary conditions for a representative volume element (RVE, here example with periodic boundary conditions [82]). The solution of the microscale problem is then homogenised (smeared) and passed up to the macroscopic level again. Colour version available online

indispensable tool in this process [104]. Another challenge is to bridge the scales and integrate the information into multiscale models that are predictive across a range of scales. This presents challenges on all levels: experimental, theoretical and computational. The computational methods for scale bridging and coarse graining have not come to a close and neither have mechanoregulation theories for tissue differentiation. Thus, while a significant body of literature exists at both ends, little has been done at the interface, i.e. multiscale mechanobiological tissue differentiation models.

Bone properties are determined by the intrinsic material properties and morphology of the underlying trabecular structure. Since a single computational model of a full bone that is detailed enough to capture the underlying trabecular architecture does not seem feasible, the problem lends itself to hierarchical multiscale methods. The classic approach (Fig. 6) is computationally very expensive: Starting from a deformation estimate on the macro-model, local boundary conditions are derived for the micro-models (following a localisation rule). Finite element simulations of often complex representative volume elements have to be then solved for each integration point location of the macroscopic domain and the stress response is then homogenised and passed up to the macroscopic model replacing pre-defined constitutive models at this level. This loop has to be iterated until equilibrium is achieved and only then can the next increment be processed. For models of a practically relevant size this approach is only feasible on large parallel computing facilities where the RVE simulations are distributed and solved simultaneously. Hambli et al. [42] introduced neural network computation into multiscale simulations of bone remodelling. Five μCT based models of the trabecular structure served as RVEs and a remodelling algorithm [41] was implemented. These RVEs were loaded with boundary conditions derived from the macroscopic model under known inputs (the amplitude, orientation and frequency of the applied stress) to derive five outputs (the averaged bone density, damage,

elastic modulus and stimulus). These inputs and outputs from 100 factorial designs were used to train a neural network, i.e. determine the weights of its connections. Once trained, the network, which does not contain any a priori rules, is then able to very quickly predict outputs when given a set of input values. This neural network thus eliminates the need for the RVE FE simulations and can be incorporated as a material formulation at the integration point level which speeds up simulations tremendously. The model was applied to the remodelling of a femoral head loaded with 7000 cycles per day for 100 days. Simulations were about 1000 times faster than with the classical approach.

Theoretical models of cells that account for their interaction with the pericellular (PCM) and extracellular matrix (ECM) and are coupled to tissue level models "can provide information on biophysical parameters that cannot be measured experimentally in situ at the cellular level, e.g., the stress–strain, fluid flow, physicochemical, and electrical states in the immediate vicinity of the cell" [40]. By investigating cell-matrix interactions using a biphasic multiscale model, the transient heterogeneity of the microscopic mechanical environment due to the differences in mechanical properties between cells and their ECM could be shown. Adding a PCM to the model had a significant effect on the stress and strain fields within the chondrocytes suggesting a biomechanically functional role for the PCM [40].

A method to couple macroscopic FE and microscopic Voxel-FE simulations to investigate bone regeneration was presented in Sanz-Herrera et al. [101]. Bone remodelling and cell migration were solved at the macroscopic level. The information was passed to the microscopic level and used to simulate bone growth and scaffold resorption in the representative volume elements. Using homogenisation techniques, the mechanical properties derived from the representative volume elements were then passed up again to the macroscopic model. The model was applied to the formation of immature and mature bone in a non-resorbing ceramic scaffold in a rabbit femoral defect. The principal model behaviour and the effect of different macroscopic locations on the evolution of local RVEs was investigated. Later, Sanz-Herrera et al. [102] studied scaffold-aided bone tissue regeneration in a rabbit femur in more detail. Variations of scaffold stiffness, porosity, pore size, degradation mode and seeding were simulated. The femur was modelled macroscopically only and its mechanical properties were dependent on the apparent density of the bone that was allowed to remodel depending on the local strain energy density. Macroscopic properties in the repair zone were derived from microscale analyses of both the solid and the fluid domains of the scaffold. In turn, stimuli and cell densities derived at the macroscopic level were used to determine the rate of microscopic bone formation in the scaffold. The mechanical stimulus used was based on local strain energy density and biomaterial stiffness. Degradation via scaffold hydrolysis was also included. The highest rate of bone formation was predicted in the stiffest scaffold and no bone formation in the softest. Compared to a non-seeded scaffold, pre-seeding led to higher rates of earlier bone formation. Higher rates were also observed for increased mean pore sizes while fast resorption kinetics were predicted to lead to scaffold collapse.

A multiscale approach to scaffold design spanning a number of length scales and hierarchies has been presented in Chan et al. [18] for hydroxyapatite-collagen composite materials. The elastic properties of the HA nanoparticles were determined from first (i.e. quantum mechanical) principles. The constitutive behaviour of the HA-collagen composite was then determined for various volume ratios using microstructural 2D unit cell FE modelling of a HA particle embedded in a collagen matrix. The collected data was used to investigate the behaviour of a 3D RVE of the scaffold architecture in order to optimise pore size, pore density and HA volume fraction for cortical and cancellous bone. While the properties of cancellous bone could be matched, those of cortical bone could not be reproduced. Layer-by-layer scaffold fabrication based on printing techniques was envisioned to manufacture the computationally optimised scaffold.

A middle-out multiscale approach to assess fracture risk in a proximal femur and an outline for appropriate verification and validation methods was introduced by Cristofolini et al. [24] spanning several scales from the body to the cell level. The central part of the approach is the organ model of the bone where the fracture occurs. This model and its boundary conditions are informed by the body level model establishing the musculoskeletal loads during daily activity. Downstream, the organ level model also relies on the tissue level model where constitutive relations are used to derive stresses and strains as well as the risk for material failure (fracture). The tissue level model is coupled to the cell level model, that provides the material properties for the tissue level model and relies on the input of biophysical stimuli from the tissue level model to simulate bone cell activity and remodelling. This type of hierarchical approach potentially allows the investigation of a multitude of factors as diverse as pharmacological treatment and subject specific gait patterns and activity levels on the fracture risk of a bone. Currently however, many questions regarding the individual component models remain unresolved and under investigation. For any clinical use the various levels of the model also need to be coupled in a fully automated and robust fashion to improve usability.

5.2 Tissue Structure in Multiscale Simulations of Tissue Regeneration

Bone and cartilage are anisotropic tissues and as such scaffolds can provide environmental cues that guide subsequent anisotropic tissue formation [30, 47]. Microscopic tissue structure has been incorporated into models investigating the biomechanics of tissue behaviour. Sander et al. [98] report a recent example in the context of tissue equivalent mechanics. Based on orientation and alignment measured using polarised light imaging unique 3D fibre networks were created in each point of the cell-compacted collagen gels. Based on constitutive parameters defined at the fibre level and fit to macroscopic off-axis hold tests, the model was

then able to predict both the macroscopic response to equibiaxial stretch tests as well as the non-affine and heterogeneous fibre network rearrangement as measured during deformation using polarimetric fibre alignment imaging. The model thus allowed for the simultaneous prediction of experimental data acquired at multiple length scales and provides insight into how macroscopically applied loads translate into highly heterogeneous fibre deformations, both tensile and compressive, which have relevance to cellular mechanotransduction.

Relatively few studies have combined architectural considerations with models of tissue differentiation and regeneration of osseous and chondral tissues. The multiscale bone growth model by Sanz-Herrera et al. [101, 102] was extended to study the effect of pore structure on directional bone growth [103]. Scaffolds with idealised isotropic (spherical) and anisotropic (ellipsoid) pore structures as well as realistic pore structures were simulated and anisotropic elasticity and permeability tensors derived from the homogenisation process and passed to the macroscopic scaffold domain. While the net rate of bone formation was found to be similar for the various architectures once they were sufficiently interconnected, the initial anisotropy determined the directions of bone growth. This was caused by the influence of the pore architecture both on the microscopic deformation and on cell migration/fluid perfusion.

Trabecular healing in a vertebral body was investigated using a multiscale approach in Boccaccio et al. [11]. The spinal segment L3-L4-L5 with a mild wedge fracture in the L4 vertebra was used to derive the poroelastic boundary conditions for representative volume elements in the repair zone. An idealised trabecular structure with a 0.5 mm diastasis served as the microdomain and was used to predict the patterns of tissue differentiation based on fluid flow and shear strain [91] during the first 100 days after fracture. Equivalent material properties of the RVEs were then derived an passed up to their locations in the fracture gap. The predicted tissue differentiation patterns were consistent with those observed in vivo. The primary bone formation mode was endochondral ossication with new woven bone occupying most of the space within the fracture site about 7–8 weeks post fracture. The final stage predicted by the model was bone remodelling leading to the formation of a new trabecular architecture.

6 Conclusion

Computational engineering methods penetrate biology at many levels. Experimental and computational data is continuously being acquired at all levels from the molecular to the organism (or even population) level. Ultimately a coupling of the relevant time and length scales is envisioned but significant challenges remain at each individual level. Experimentation and simulation have to inform one another in an iterative and closely coupled fashion. Once confidence in a model for a certain phenomenon is established, the number of experiments needed can be reduced drastically to the level necessary for parameter identification. Furthermore, neural

network approaches that to a certain extent mimic learning processes can be used to reduce computational costs significantly by substituting complex simulations at one or more levels of a multiscale model. Since they can easily incorporate additional data as it becomes available from a variety of sources and adapt to them (via re-training) they present a powerful tool for the integration of information from mechanical, biological and other sources. Neural networks do not rely on any a-priori defined rules but "discover" them and as such may aid in the development of potent mechanobiological theories based on large amounts of diverse data, both experimental and theoretical, that may be difficult to condense analytically.

The advances made have provided and continue to provide insight into many aspects relevant for tissue engineering: Cellular models accounting for the dynamic nature of the cytoskeleton and focal adhesions improve our understanding of the cell's interaction with its immediate surrounding. This includes measurement tools so that these active models can help interpret experimental data. Models of regenerative processes in vivo allow the investigation of environmental factors on tissue differentiation processes. These frameworks help to uncouple individual mechanisms, such as the contribution of tissue structure to bulk mechanical properties, and test hypotheses related to these mechanisms that could not be tested directly experimentally. Computational techniques and engineering approaches to problem solving in general can benefit in particular processes such as scaffold design, where a large number of coupled criteria have to be balanced in order to achieve a desired product. Here, simulation techniques serve both purposes: Investigation and understanding of the biophysical environment and its biological consequences on the one hand, and, more traditionally yet not simpler, a robust quantification to facilitate decisions on design parameters on the other. The diversity of current modelling techniques reflects that of the length scales, questions, applications and challenges in the field of tissue engineering.

6.1 Outlook

Challenges in computational mechanobiology that offer opportunities for further development are abundant. From a basic research perspective, models need to be developed to advance our understanding of how cells and tissues perform in a mechanically challenging environment as well as why certain regenerative approaches work and others don't. In order to be able to provide such insights several challenges need to be addressed:

- Models should reflect individual aspects of a problem more accurately and mechanistically to provide quantitative understanding, predictive ability and allow the testing of hypotheses via e.g. numerical *knock-out* simulations.
- Models addressing problems in tissue engineering need to consider multiple concepts, such as transport, electromechanical stimulation, effects of the chemical environment and reactions occurring therein.

- These mechanisms need to be coupled, i.e. thermodynamical considerations (energy and mass exchange) affect growth and remodelling, which in turn alters the mechanical properties of the tissue and might affect the environment in which chemical reactions take place, etc.
- Time and length scales need to be bridged and knowledge from the individual models effectively linked.

When viewed from a clinical perspective other considerations enter the agenda. While in basic research the immediate goal is to gain knowledge, models are only justified and necessary in a clinical environment where there is a measurable patient benefit. Therefore, questions on whether a particular computational tool can aid in making treatment decisions, plan surgical interventions or choose an appropriate implant need to be answered under the pressure of health care budget constraints. Another major challenge lies before the realisation of computer-assisted patient specific regenerative medicine: Verification and validation. When the welfare of human beings is affected by decisions based on computational predictions, the underlying models will need to be rigorously tested and their creation, validation and application officially regulated.

Finally, the commercial sector can contribute to both the clinical and academic worlds. For example, one can envisage the development of adaptive bioreactors that feature the online integration of predictive models for the assessment of otherwise unknown state variables of the cultured tissue and the cells within it. Robustness, ease of use and validation are some of the principal requirements. The challenges and opportunities are huge and provide space for a large variety of modelling approaches and their development towards routine applications. It seems equally important to discover new approaches as it is to learn from established fields to avoid re-inventing the wheel. The enthusiasm in exploring these new developments needs to be paired with a critical assessment of their potential benefits.

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