In Silico Biology of Bone Regeneration Inside Calcium Phosphate Scaffolds

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Abstract Bone tissue engineering plays a key role in finding better solutions for the healing of large bone defects and non-unions. Despite extensive experimental research, many of the mechanisms of the bone regeneration process still remain to be elucidated. As such, mathematical modeling is a useful tool to further investigate the different influential factors and their interactions *in silico*. This chapter starts with a description of the biological processes that take place during bone regeneration in calcium phosphate (CaP) scaffolds. The second section gives an overview of the most recent mathematical models of bone regeneration in (CaP) scaffolds. One model is explained in more detail and used to illustrate the potential of mathematical modeling in the bone tissue engineering field. Finally, the drawbacks of the current modeling techniques and the need for more quantitative experimental research, together with possible solutions are presented.

1 Introduction

The need for bone tissue regeneration is continuously increasing due to the improvement of the quality of life and the increase in life expectancy. In the United States alone approximately 6 million fractures occur yearly, of which 5–10 % result in a delayed union or in a non-union. An extrapolation of these numbers to the Indian population results in 240 million fractures a year, of which 12 million non-unions [4]. Bone tissue engineering aims at finding a better solution for the healing of large bone defects and non-unions. This interdisciplinary research field applies principles of engineering and life sciences to create an *in vivo* micro-environment that

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promotes local bone repair or regeneration [14, 19]. Bone formation is a very complex physiological process, involving the participation of many different cell types and regulated by countless biochemical and mechanical factors. Therefore, mathematical models can make a significant contribution in further unraveling the interactions between the different influential factors. Thus, *in silico* experimentation seeks to explain and understand the underlying principles of the biological phenomenon. Moreover, mathematical models can be used to design and test possible experimental and therapeutic strategies *in silico* before they are tested *in vitro* or *in vivo*. These experimental results will, in turn, guide further model building.

This book chapter will start with an overview of the biology of bone regeneration inside calcium phosphate (CaP) scaffolds. Then some mathematical models of bone regeneration inside (CaP) scaffolds will be discussed, indicating clearly the opportunities of an integrative approach that combines mathematical modeling with experimental research. Finally, some future prospects are presented.

2 Biology of Bone Regeneration Inside CaP Scaffolds

Tissue engineering aims to develop biological substitutes that restore, maintain or improve tissue function. Two main strategies have been developed to regenerate bone tissue: the use of biomaterials to induce bone formation chemically and the construction of hybrid implants composed of a biomaterial scaffold seeded with osteogenic cells [19, 25]. Delayed and non-unions are characterized by an *in vivo* micro-environment that fails to support bone repair or tissue regeneration. Hence, the micro-environment found at a non-union could be considered as an ectopic site [14]. Consequently, the tissue engineering constructs should display osteoinductive properties. CaP bioceramics are then interesting candidates, because of their biocompatibility, bioactivity and osteoinductive characteristics. It has been clearly shown that CaP induces bone formation, but the exact mechanism is still largely unknown [2, 10, 14, 20, 35, 46]. There are, however, several mechanisms proposed in literature to explain the influence of CaP particles on bone formation as observed in many experiments.

It has been stated that a high local concentration of growth factors and proteins can be achieved by adsorption on the biomaterial substrate, thereby creating a favorable micro-environment for bone formation [28, 35, 46]. Another explanation for the osteoinductive properties of CaP biomaterials is given by the surface topography, since it influences the osteoblastic guidance and attachment and can cause the asymmetrical division of MSCs [1, 2]. Barrère et al. [2] also suggest that the surface charge of the substrate can play a key role by triggering cell differentiation. Furthermore, negative charges distributed on the surface of the biomaterial can be an obstacle for cell-material adhesion, because the cell surface is also negatively charged [40, 47]. The bioapatite layer, formed *in vivo*, might also be recognized by MSCs [19]. A low oxygen tension in the central region of the biomaterial, which triggers the pericytes of microvessels to differentiate in osteoblasts, is another mechanism

proposed in literature [2]. However, the release of calcium and phosphate ions by dissolution, is believed to be the main origin of the bioactivity of CaP biomaterials [1, 2, 10, 19]. The dissolution properties of CaP biomaterials are influenced by the exposed surface area, the composition and the pH. Pioletti et al. [34] showed that small CaP particles (< 10 μm) can induce phagocytosis. This process could then, in turn, produce an accumulation of Ca^{2+} in the mitochondria, which can cause lysis of the mitochondria and cell death. Phagocytosis also alters the pH of the surrounding body fluids. This pH-change subsequently alters the dissolution properties of the CaP particles. The size of the particles is not only critical because it can induce phagocytosis, it also determines the reactivity of the particles. The smaller the particles, the larger the exposed surface to the environment and the faster the biomaterial will dissolve. The dissolution rate will increase, simply because larger quantities of exchange can take place [1]. The composition of the calcium phosphate biomaterials is another important characteristic that determines the dissolution properties. A change in the calcium to phosphate ratio means a change in phase composition, which directly affects the ionic exchange mechanisms [1].

Experimental evidence clearly indicates the key role of calcium. Yuan et al. [53] observe more bone formation in scaffolds made up of biphasic CaP than of hydroxyapatite, the latter having a lower dissolution rate. The effect of calcium ion implantation in titanium on bone formation was investigated by Hanawa et al. [20]. They found a larger amount of new bone on the Ca^{2+}-treated side than on the untreated side. Eyckmans et al. [14] noticed that the CaP granule remnants in a decalcified scaffold serve as anchoring points for cell attachment. Titorencu et al. [44] report that osteoblasts respond to changes in Ca^{2+} concentration in the bone microenvironment. Moreover, differentiation of MSCs towards osteoblasts is accompanied by the expression of Ca^{2+} binding-proteins and the incorporation of Ca^{2+} into the extracellular matrix [44]. Chai et al. [9] observed a significant Ca^{2+}-induced cell proliferation and upregulation of osteogenic gene expression in a dose- and time-dependent manner. It also appears that osteoblasts sense and respond to the extracellular Ca^{2+} concentration independently of systemic calciotropic factors in a concentration-dependent manner [13]. Bootman et al. [7] report that the extracellular calcium concentration could control the frequency of the intracellular calcium spiking, which encodes specific cellular information according to Sun et al. [43].

The release of PO_4^{3-} also plays a key role by regulating the cell cycle and proliferation rate, influencing gene expression [3] and the secretion of bone-related proteins [23]. However, several *in vitro* studies showed that the addition of high levels of exogenous PO_4^{3-} (5–7 mM) induced osteoblast apoptosis and non-physiological mineral deposition [27]. Nevertheless, PO_4^{3-} is believed to play a critical role in bone matrix mineralization [32].

Despite of the vast *in vitro* research findings, the influences of Ca^{2+} and PO_4^{3-} differ from cell type to cell type. This implies that there will be not one optimal Ca^{2+} and P_i concentration that could universally drive all cell types towards successful osteogenesis. Moreover, the optimal concentration may vary according to the cellular stage, e.g. proliferation and differentiation. Therefore, specific windows

of ion concentration need to be determined and optimized for a specific *in vitro* and *in vivo* response.

3 Mathematical Models of Bone Regeneration Inside (CaP) Scaffolds

3.1 From Models...

Improvements in computer capacity now enable an increased model realism and complexity (e.g. 3D calculations, complex geometries, multi-scale, multi-physics, ...) [45]. As a consequence of this technological revolution, there has been an enormous increase in the use of mathematical models in biology and medicine. These mathematical models can propose and test possible biological mechanisms, contributing to the unraveling of the complex nature of biological systems. Moreover, they can be used to design and test possible experimental strategies *in silico* before they are tested *in vitro* or *in vivo*. Finally, all this knowledge can be used to develop clinically relevant cell carriers.

Currently, many computational models of bone formation and regeneration in general (reviewed in Geris et al. [16–18]), or even in (CaP) scaffolds specifically (reviewed in Sengers et al. [39]) exist. Bohner et al. [6] propose a theoretical approach to determine the effect of geometrical factors on the resorption rate of CaP scaffolds. The theoretical model was based on five assumptions: (i) the pores are spherical, (ii) the pores follow a face-centered cubic packing, (iii) the resorption is surface-controlled, (iv) the resorption requires the presence of blood vessels (50 μm in diameter) and (v) the resorption time is proportional to the net amount of material [6]. Based on these assumptions the model calculations show that the resorption time of a macroporous block depends on the pore radius which is dependent on the size of the bone substitute and the interpore distance [6]. The model was also used to optimize the pore size of CaP scaffolds and validated with experimental data. The theoretical model looks, however, exclusively at geometrical scaffold properties and does not include biological variables such as cells or matrix densities. Byrne et al. [8] developed a 3D mechanoregulatory model of bone regeneration in a regular scaffold to investigate the effect of porosity, Young's modulus and dissolution rate on bone regeneration in different loading conditions. They model the scaffold degradation in a linear, load-independent fashion, i.e. the porosity will be increased by a 0 %, 0.5 %, 1 % per iteration for low, intermediate and high dissolution rates respectively [8]. Consequently, the size of all scaffold elements decreases uniformly resulting in an overall volumetric reduction while the scaffold geometry remains unaltered [8]. Their calculations show that as scaffold degradation progresses, the regenerating tissue must take over the mechanical function of the bone-scaffold system which would otherwise collapse due to a lack of mechanical strength [8]. Moreover, all three variables (i.e. porosity, Young's modulus and dissolution rate) appear to influence the amount of bone formation in a non-intuitive way, demonstrating

the need to optimize scaffolds for site-specific loading requirements [8]. This model was improved by including blood vessel growth thereby establishing a framework to investigate the effect of vascularization on bone formation [12].

Other studies have modeled the bone regeneration process inside biodegradable polymer-based scaffolds. Stops et al. [42] further investigated the influence of mechanical strain and perfusive fluid flow on cell differentiation and proliferation within a collagen-glycosaminoglycan scaffold. Sanz-Herrera et al. [37] presented a multi-scale model of bone regeneration inside a porous scaffold. The biodegradable polymer scaffold degrades hydrolytically, i.e. the water content in the polymer chemically reacts and breaks down the material, which was modelled accordingly [37]. The mechanical properties of the polymer were assumed to relate linearly to its molecular weight [37]. The evolution of the bone formation process in a scaffold implanted in the femoral condyle of a rabbit was simulated with the model. They found a good qualitative agreement between the obtained computational and experimental results [37]. Although further validation is necessary, the proposed multi-scale model is a useful tool to investigate the complex phenomena that occur at different length and time scales, i.e. the bone formation and scaffold resorption at the microscopic scale and the change of mechanical properties at the macroscopic scale [37]. Lacroix et al. [24] nicely review the current techniques used for scaffold development: from scaffold optimization of scaffolds by mathematical models (e.g. FEM) to scaffold design using computer aided design (CAD) and scaffold characterization by computed tomography (CT).

Although the above models can be used to optimize some (mechanical) properties of scaffolds, e.g. the porosity, the micro-architecture, the Young's modulus and dissolution rate, they neglect the influence of growth factors and other biochemical signals on the bone formation process. Moreover, the dissolution process is only crudely modeled, neglecting the influence of the degradation products (e.g. Ca^{2+} and P_i) on the cellular activities and bone formation process. Carlier et al. [11] developed and implemented an experimentally informed bioregulatory model of the effect of calcium ions released from CaP-based biomaterials on the activity of osteogenic cells and mesenchymal stem cell driven ectopic bone formation. The model describes the effect of CaP biomaterials on the activity of osteogenic cells as a temporal variation of six variables: free extracellular Ca^{2+} concentration (Ca), MSC density (c_m), osteoblast density (c_b), mineral matrix density (b), collagen matrix density (m) and a generic, osteogenic growth factor concentration (g_b). The sum of the mineral matrix and the collagen matrix represents the total bone density. The evolution of each of these continuous variables is described by the following set of delay differential equations (DDEs) (see Fig. 1):

$$\frac{\partial c_m(t)}{\partial t} = \overbrace{A_m(t).c_m(t).\big(1-\alpha_m.c_m(t)\big).\beta_{cm}(t)}^{proliferation} - \overbrace{F_1(t).c_m(t-t_1)}^{differentiation} - \overbrace{d(t)}^{removal}$$

$$\frac{\partial c_b(t)}{\partial t} = \overbrace{A_b(t).c_b(t).\big(1-\alpha_b.c_b(t)\big).\beta_{cb}(t)}^{proliferation} + \overbrace{F_1(t).c_m(t-t_1)}^{differentiation} - \overbrace{d_b.c_b(t)}^{removal}$$

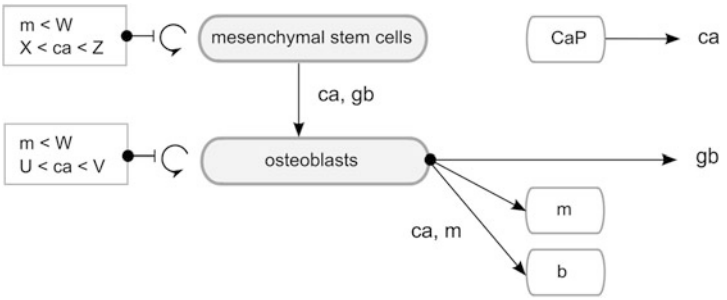

Fig. 1 Schematic overview of the calcium model. W = maximum tissue density for proliferation, X = minimum calcium concentration for proliferation of MSCs, Z = maximum calcium concentration for proliferation of MSCs, U = minimum calcium concentration for proliferation of osteoblasts, V = maximum calcium concentration for proliferation of osteoblasts, $ca = Ca^{2+}$, gb = growth factor, m = osteoid, b = mineral matrix. The participation of a variable in a subprocess is indicated by showing the name of that variable next to the *arrow* representing that subprocess, e.g. calcium modulates differentiation and bone formation (adapted from Carlier et al. [11])

$$\frac{\partial m(t)}{\partial t} = \overbrace{P_{bs}.\big(1-\kappa_b.m(t)\big).c_b(t-t_2)}^{production}$$

$$\frac{\partial b(t)}{\partial t} = \overbrace{P_{bb}.\big(1-\delta-\kappa_{bb}.b(t)\big)^6.c_b(t)}^{production}$$

$$\frac{\partial g_b(t)}{\partial t} = \overbrace{E_{gb}(t).c_b(t)}^{production} - \overbrace{d_{gb}g_b(t)}^{decay} - \overbrace{R(t)}^{consumption}$$

$$\frac{\partial Ca(t)}{\partial t} = \overbrace{\sigma.\big(Ca_\infty - Ca(t)\big)}^{release} - \overbrace{J(t).c_b(t)}^{consumption\ (HA)} - \overbrace{d_{Ca}.Ca(t).\big(c_b(t)+c_m(t)\big)}^{consumption\ (metabolism)}$$

In short, cell differentiation is controlled by the presence of growth factors and calcium. The local cell and matrix densities, as well as the calcium concentration influence the proliferation of both MSCs and osteoblasts. Matrix synthesis is controlled by the local cell and matrix densities. The local cell and growth factor concentrations influence the growth factor production, whereas the calcium concentration depends on the dissolution rate of the CaP biomaterial and uptake by the osteogenic cells. The model equations are implemented in Matlab (The MathWorks, Inc.) using delay differential equations routines. Additional information, including an extensive discussion on the processes described by these equations, the boundary and initial conditions, parameter values and implementation details can be found in the Appendix (Tables 1 and 2) and in Carlier et al. [11].

Mathematical models have several advantages with respect to experimental research. The act of developing a model consists out of translating the biological processes into mathematical equations, which can be continuous, discrete or a hybrid variant. This process of translation is in itself very valuable since it requires a thor-

ough understanding of the biological process under study. The modeler will need to decide which variables are most important to answer the research question at hand and how the underlying biological processes will be represented. Often this is done in close collaboration with experimental biologists who might have a different view and strategy of tackling problems. These very fundamental discussions and questions often lead to new insights and research tracks and are therefore a noteworthy advantage of mathematical modeling.

Mathematical models can also be used to compute information that would be impossible to obtain experimentally. For example, Milan et al. [31] use a finite element analysis to calculate the shear strain, fluid flow and pore pressures inside a porous polymeric scaffold. Also Byrne et al. [8] use a finite element analysis to compute the strain and fluid flow which are then used as input for the mechanoregulatory model of tissue differentiation. It is clear that these biophysical stimuli play a key role in the bone regeneration process. Besides the mechanoregulatory variables, also bioregulatory variables are difficult to measure in an *in vitro* or *in vivo* setting. Carlier et al. [11] calculate the amount of calcium that is released by the CaP scaffold and taken up by the osteogenic cells. Although this model is only one-dimensional, an extension with spatial dimensions would allow the determination of the calcium distribution inside the scaffold and developing tissue. This is important since calcium influences many cellular processes as was shown in the previous section.

The experimental difficulties mentioned above entail however problems for model validation. The results of a model have indeed no meaning if they are not corroborated by real *in vitro* or *in vivo* data. This problem is often solved by measuring related quantities and seeing how they correspond to the model predictions. Byrne et al. [8] suggest for example to implant a scaffold into a bone defect in an animal model and making histological measurements of tissue phenotype at several time points which could then be compared to the simulation results. Another technique is to use the model framework in a different application and determining whether the predictions also fit in this new setting. The model of Carlier et al. [11] was validated by comparison with experimental data. Firstly, it was found that the model of Carlier et al. [11] is able to reproduce the sequential events observed experimentally during intramembranous healing: (1) proliferation, (2) differentiation, (3) collagen production and (4) mineralization [29, 41]. Secondly, the model results were compared to the experimentally determined amount of bone formation by Hartman et al. [21]. It was found that the results of the simulation and the experiment correspond qualitatively. Thirdly, the modeling platform successfully predicted the absence of bone in the impaired healing situations of scaffold decalcification and insufficient cell seeding. However, the results of the model should be interpreted in a qualitative way due to some simplifications and parameter value estimations. The current tool would have much more potential if it could be made more quantitative. A major problem in that respect is the lack of extensive characterization and quantification of the scaffold properties. As such it is difficult to match the experimental conditions found in literature with the modeled ones. Currently, specific *in vivo* and *in vitro* testing procedures are being set up to determine the calcium release rate and relate it to the *in vivo* bone forming capacity of different CaP scaffolds.

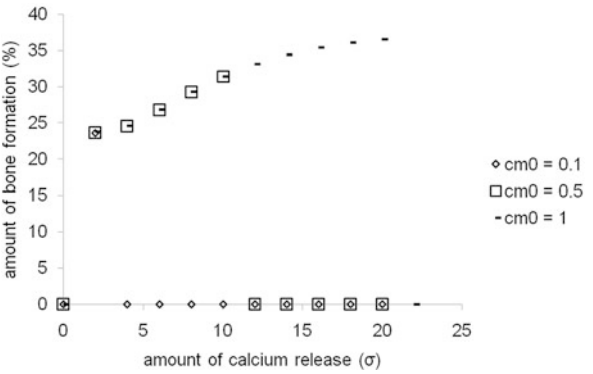

Fig. 2 Amount of bone formation at day 90 as a function of the calcium release rate (σ) and initial MSC concentration (c_{m0}) according to the mathematical model

After the development and validation of the model, the model needs to be further analyzed and can be used for optimization. The analysis phase allows determining which factors are the most important ones and how they interact. This is another major advantage of modeling. Since biological systems are so complex, it is often difficult to intuitively predict what will happen if a specific factor is changed. To determine the most influential factors of the mathematical model, Carlier et al. [11] performed a sensitivity analysis by "Design of Experiments" (DOE). DOE is a statistical tool that enables the determination of an efficient design for (in this case) a multi-parameter sensitivity analysis. Carlier et al. [11] used the JMP statistical software (8.0.1. SAS Institute Inc.) to generate the array of combinations of different parameter values within a pre-defined parameter space. The sensitivity analysis showed that the bone formation rate P_{bb}, the initial MSC density c_{m0} and the initial osteoblast density c_{b0} are the most important factors influencing the amount of bone formation at day 21 and 42 [11]. The model outcome also largely depends on the initial conditions, which therefore should be realistically defined. The sensitivity analysis indicated a significant interaction between the calcium release and the initial MSC seeding density which was subsequently further investigated (see Fig. 2). The model indicates that a low initial MSC density requires a low calcium release rate, while a high initial MSC density requires a high calcium release rate in order to maximize the amount of bone formation. The amount of bone formation for low initial MSC concentrations is also very sensitive to calcium, whereas high initial MSC concentrations produce similar amounts of bone for a range of calcium release. For tissue engineering strategies it is interesting to start with a low initial cell density but since the margin is very small, the optimization is critical for these types of constructs. The high MSC concentrations entail a larger window of allowable calcium release rates which allows for more optimization and potentially higher benefits. The *in vivo* bone formation capacity of different CaP scaffolds seeded with a fixed concentration of hPDCs was studied by Roberts et al. [36]. They found that the calcium release rate is a strong determinant in discriminating bone-forming scaffolds from scaffolds that did not lead to any bone formation thereby confirming our initial hypothesis. This integrative research shows that mathematical models can be

used to determine whether a certain mechanism, proposed in literature, can indeed explain the experimental findings.

Mathematical models are also a practical tool for the optimization of the properties of (CaP) scaffolds and the tissue engineering process in general. Byrne et al. [8] showed that scaffolds could be tailored for the site of implantation, which is characterized by specific loading conditions. In a low loading environment, a highly porous and stiff scaffold with medium dissolution rate would give the greatest amount of bone. In a high loading environment, however, a low initial porosity and rate of dissolution are necessary to maintain the mechanical and structural integrity of the bone-scaffold system [8]. Checa et al. [12] investigated the effect of cell seeding density on the bone formation process. They found that a reduction in the initial MSC seeding density increased the amount of bone formation since the vessels could more easily penetrate the construct ensuring the supply of oxygen and nutrients. The model of Carlier et al. [11] is able to define the optimal scaffold properties (in terms of Ca^{2+} release) for different initial cell seeding conditions. Moreover, the model can be used to determine the optimal scaffold properties for different cell types (e.g. hPDC, hBMSC) since these cell types are characterized by different proliferation and differentiation parameters. This optimal dissolution rate could be an input parameter for the model of Bohner et al. [6] to determine the optimized microstructural properties of the CaP scaffold.

Besides the optimization of scaffold properties or seeding protocols, mathematical modeling allows for an *in silico* screening of novel biomaterials based on biomaterial characteristics encompassed in the model (such as calcium release rate in Carlier et al. [11]) thereby increasing the initial quality of the biomaterials selected for *in vivo* experimentation and reducing the number of false positive or negative results. Moreover, mathematical modeling can help to design and test possible treatment strategies. Peiffer et al. [33] used a hybrid bioregulatory model of angiogenesis during fracture healing to investigate the effect of vascular endothelial growth factor (VEGF) on the healing of MMP9 deficient fractures. They convincingly show that a treatment comprising daily bolus injections of VEGF is not as efficient as using a slow-release VEGF carrier. Similarly, Geris et al. [15] used an *in silico* technique to investigate the effect of MSC injection in an atrophic non-union model. They show that after the injection of the cell transplant in the callus region, the amount of bone was predicted to increase whereas the amount of fibrous tissue was predicted to decrease. The amount of soft tissue was however strongly dependent on the exact location of injection of the cell transplant with excentral injection leading to unicortical bridging. The model of Carlier et al. [11] shows that insufficient cell seeding on CaP scaffolds may lead to impaired bone formation due to the very high Ca^{2+} concentration that negatively influences cellular proliferation and differentiation. The *in silico* treatment strategy of exponentially reducing the calcium release after implantation was found to lead to bone formation. The controlled ad hoc reduction of calcium release after implantation is not feasible in reality. However, owing to cellular attachment and protein adsorption, the theoretical release rates which are generally determined in acellular dissolution experiments most likely do not correspond to the actual release rates *in vivo*.

Mathematical models are often less time consuming than experimental research. Due to the enormous increase in computational power, both on a personal workstation as in high performance computing facilities, computer simulations can be used to model and optimize biological processes. In the model of Carlier et al. [11] this time gain is quite dramatic, simulating the process of bone formation that occurs over 90 days in only a few minutes. Also, experimental research is often much more expensive than mathematical modeling. Simple mathematical models can be easily simulated on a personal workstation whereas wet lab facilities require specific laboratories and equipment. Moreover, the cost of specific biological components and transgenic animals can be very significant.

3.2 . . . to Experiments . . .

Although computational modeling has many advantages, it will never fully replace experimental research. As already mentioned, mathematical models can help experimental research in several ways. Firstly, mathematical models ask fundamental questions and provoke discussion. Secondly, mathematical models contribute to the general knowledge of the biological processes. Thirdly, mathematical models can be used to guide experimental design. But the experimental research is also invaluable for mathematical modeling. Experimental research is necessary to establish the fundamental knowledge on the biological processes and allows determining the important parameters and their respective parameter values. Experimental research also plays a key role in the validation of mathematical models.

As already mentioned in the previous section, there is a strong need for more quantitative data. Extensive quantification of *in vitro* and *in vivo* measurements and characterization of (CaP) scaffold properties would not only allow a more accurate determination of the model parameter values but also a more complete validation and enhancement of the model predictions. However, experimental research is often still qualitative. Moreover, the experimental measurements do not necessarily correspond to the data that are needed in the modeling framework. It is therefore imperative that *in vivo* and *in vitro* experiments are specifically designed and set-up to match to modeling conditions in order to further validate and improve the model's predictions.

As an essential part of the integrative approach that Carlier et al. [11] use to investigate the effect of Ca^{2+} on the bone forming capacity of CaP scaffolds, specific *in vitro* experiments were performed to determine the effect of Ca^{2+} on the proliferation of MSCs and osteoblasts. In short, cells were expanded in a monolayer using growth medium. Upon confluence, human periosteal derived cells (hPDCs) were replated and synchronized. Freshly prepared Ca^{2+} supplemented growth media were then added to the cell cultures and incubated for 1, 3, 7, 14, 21 and 28 days before being harvested for analysis. At each time point the DNA content was quantified. The data measured at 7 days ware assumed to be representative of MSC proliferation, whereas the data measured at 28 days were assumed to be representative of

cells further down the osteoblastic lineage. A least-square fitting through the experimental data determined the parameters that characterize the influence of calcium on the proliferation of MSCs and osteoblasts.

Another example of this challenge in experimental research is the determination of the dissolution rate of CaP scaffolds. As shown by Carlier et al. [11], this property has an important effect on the final amount of bone formation and should as such be thoroughly characterized. However, the degradation rate is influenced by many scaffold characteristics (e.g. micro- and macrostructure, the material composition, specific surface area) and testing conditions (e.g. temperature, composition of dissolution medium, pH, specific surface area to medium ratio). Currently dissolution tests still lack standardization [22] and often the (CaP) scaffolds are not completely characterized, making it very difficult to compare the results of different studies found in literature. Impens et al. [22] show for example that perfusion tests result in a higher dissolution rate when compared to bath shaking tests due to the easier entrance of the fluid flow inside these scaffold. Moreover, these *in vitro* tests do not take the influence of protein adsorption or osteoclastic activity after implantation into account. From the above it is clear that there is still a long way to go and that specific experiments should be designed that resemble the *in vivo* conditions as close as possible.

3.3 . . . and Back

The multidisciplinary problem of optimizing scaffold architecture and seeding protocols for bone tissue engineering strategies requires an integrative approach. This strategy uses mathematical modeling to explain a mechanism of biomaterial-cell interactions in combination with experimental research to provide data for the determination of the model parameters as well as the validation of the model [5]. Moreover, this process is inherently iterative, where new experimental results can be fed to the model and thorough model analysis can lead to new research hypotheses.

4 Prospects

Most of the current models look either at mechanoregulatory or bioregulatory stimuli, depending on the specific research question that is being answered. In the future, however, these models could be combined to further improve the predictive capabilities of the model. Another issue is the specific scale at which most models are created. Some models look in more detail at a small scale (e.g. [11]) while others look at a larger scale [8, 15]. The problem of bone regeneration inside (CaP) scaffolds is however regulated by countless biochemical and mechanical factors across multiple organizational scales. The time scales of these individual events range from seconds

for phosphorylation events to hours for mRNA transcription to weeks for tissue formation and remodeling processes [26]. The spatial scales vary from nanometers at the molecular level to millimeters at the tissue level and meters at the level of the organism [26, 30]. As such, one can conclude that the bone regeneration process is a multiscale problem and should be studied and modeled accordingly. Some attempts have already been made like Sanz-Herrera et al. [38] who use a multiscale modeling approach to determine the role of the scaffold microarchitecture in bone tissue regeneration. Besides some localized activities, coordinated efforts should also focus on the integration of models at different biological scales [26]. Liu et al. [26] propose for example an object-oriented module-based computational integration strategy to link currently available models of different methodologies (algebraic equations, PDEs, AB). In this way the computational infrastructure effectively integrates multiple modules by coordinating their connectivity and data exchange. Not only does such a platform allow the straightforward combination of existing mathematical models, it is also intrinsically a multiscale modeling environment thereby approaching the true multiscale nature of biological processes.

As already pinpointed in the previous section, quantitative data are crucial for mathematical models to reach their true potential. Thus *in vitro* or *in vivo* experiments should be designed so that they enable quantification. The highly controllable and quantifiable environment is a major advantage of *in vitro* set-ups [18]. However, the conclusions should be carefully translated to the actual *in vivo* environment since the cells and tissues are isolated from their natural environment. The use of *in vivo* models has the advantage of resembling the reality but quantification will be more challenging. Moreover, as mathematical models predict the dynamics at different scales (e.g. molecular, cellular and tissue) as a function of time and space, there is a need for temporal and spatial experimental data. A possible strategy is the use of imaging techniques (e.g. micro-computed tomography) that allow non-invasively monitoring and quantification of the *in vivo* dynamics.

5 Conclusion

This chapter discussed the biology of bone regeneration in CaP scaffolds and the related modeling efforts. A number of advantages of mathematical modeling were indicated and illustrated by examples of the bone tissue engineering field. It is clear that only a true integrative approach, that combines mathematical modeling with experimental research will help to further elucidate the biological process of bone regeneration inside CaP scaffolds. The integrative strategy is necessary during both the development of the model (determination of parameter values) and the model validation phase (comparison of the model predictions to experimental findings). Building this bridge between different disciplines requires a lot of effort but it is the only way to truly obtain predictive models that can be used to advance the research in the bone tissue engineering field.

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Appendix

The equations contain the following model parameters:

$$J(t) = J_{in}.\frac{Ca(t)}{H_{Ca4} + Ca(t)}$$

$$A_m(t) = \frac{A_{m0}.m(t)}{K_m^2 + m(t)^2}$$

$$\beta_{cm}(t) = \frac{a_{cm}}{c_{cm}}.\exp\left(-\frac{1}{2}.\left(\frac{Ca(t) - b_{cm}}{c_{cm}}\right)^2\right)$$

$$F_1(t) = \frac{Y_{11}.g_b(t)^6}{H_{11}^6 + g_b(t)^6}.F_{11}.\exp\left(-\frac{1}{2}.\left(Ca(t) - F_{12}\right)^2\right)$$

$$A_b(t) = \frac{A_{b0}.m(t)}{K_b^2 + m(t)^2}$$

$$\beta_{cb}(t) = \frac{a_{cb}}{c_{cb}}.\exp\left(-\frac{1}{2}.\left(\frac{Ca(t) - b_{cb}}{c_{cb}}\right)^2\right)$$

$$E_{gb}(t) = \frac{G_{gb}.g_b(t)}{H_{gb} + g_b(t)}$$

$$R(t) = \left|c_m(t) - c_m(t - t_3)\right|.\frac{G_{con}.g_b(t)}{H_{con} + g_b(t)}$$

The following scaling factors were chosen for the non-dimensionalization of the model variables:

$$\widetilde{t} = \frac{t}{T}, \qquad \widetilde{c_m} = \frac{c_m}{c_0}, \qquad \widetilde{c_b} = \frac{c_b}{c_0}, \qquad \widetilde{m} = \frac{m}{m_0}$$
$$\widetilde{b} = \frac{b}{m_0}, \qquad \widetilde{g_b} = \frac{g_b}{g_0}, \qquad \widetilde{Ca} = \frac{Ca}{Ca_0}$$

The time $T = 1$ day was considered to be a representative unit time for the process under study (similar to fracture healing models e.g. Geris et al. [50]). Representative concentrations for the collagen content ($m_0 = 0.1$ g/ml) and growth factors ($g_0 = 100$ ng/ml) are adopted from Geris et al. [50]. A typical value for the cell density ($c_0 = 10^6$ cells/ml) is derived from Bailón-Plaza and van der Meulen [48]. The

Table 1 Non-dimensionalized parameter values of the presented model (tildes are omitted for simplicity)

Parameter	Nominal value	Source
MSCs		
α_m	1	Bailón-Plaza et al. [48]
A_{m0}	0.85	Bailón-Plaza et al. [48]
K_m	0.1	Bailón-Plaza et al. [48]
a_{cm}	5.98	measured
b_{cm}	1.67	measured
c_{cm}	3.33	measured
Y_{11}	10	Bailón-Plaza et al. [48]
H_{11}	14	Bailón-Plaza et al. [48]
F_{11}	8	Dvorak et al. [13]
F_{12}	1.5	Dvorak et al. [13]
d_{cm}	1.5	estimated
Osteoblasts		
A_{b0}	0.202	Bailón-Plaza et al. [48]
K_b	0.1	Bailón-Plaza et al. [48]
α_b	1	Bailón-Plaza et al. [48]
a_{cb}	41.82	measured
b_{cb}	5.06	measured
c_{cb}	1.9	measured
d_b	0.1	Bailón-Plaza et al. [48]
γ	7	measured
Collagen matrix		
P_{bs}	0.18	Yuan et al. [53]
κ_b	1	Bailón-Plaza et al. [48]
Mineral matrix		
δ	0.05	estimated
P_{bb}	0.0398	Yuan et al. [53]
κ_{bb}	1	Bailón-Plaza et al. [48]
m_{thres}	0.85	estimated

scaling factor for the calcium concentration was assumed to be equal to the extracellular calcium concentration ($Ca_0 = 1$ mM). An overview of the model parameter values and the initial variable values is given in Table 1 and Table 2 respectively.

The model parameters were non-dimensionalized as follows (the tildes represent the non-dimensional parameters):

Table 1 (Continued)

Parameter	Nominal value	Source
Calcium		
σ	10	estimated
Ca_∞	50	Maeno et al. [51]
J_{in}	750	estimated
H_{Ca4}	0.01	estimated
d_{ca}	100	estimated
Growth factors		
G_{gb}	350	Geris et al. [50]
H_{gb}	1	Geris et al. [50]
G_{con}	1	estimated
H_{con}	0.001	estimated
d_{gb}	75	Geris et al. [50]

Table 2
Non-dimensionalized initial variable values of the presented model

Variable	Nominal value	Source
c_{m0}	1	Eyckmans et al. [49]
c_{b0}	0	estimated
m_0	0.01	Liu et al. [52]
b_0	0	estimated
g_{b0}	15	Liu et al. [27]
ca_0	1	estimated

$$\widetilde{P_{bs}} = \frac{P_{bs}.c_0.T}{m_0}, \qquad \widetilde{\kappa_b} = \kappa_b.m_0, \qquad \widetilde{A_{m0}} = \frac{A_{m0}.T}{m_0},$$

$$\widetilde{K_m} = \frac{K_m}{m_0}, \qquad \widetilde{a_{cm}} = \frac{a_{cm}}{Ca_0}, \qquad \widetilde{b_{cm}} = \frac{b_{cm}}{Ca_0},$$

$$\widetilde{c_{cm}} = \frac{c_{cm}}{Ca_0}, \qquad \widetilde{\alpha_m} = \alpha_m.c_0, \qquad \widetilde{H_{11}} = \frac{H_{11}}{g_0},$$

$$\widetilde{Y_{11}} = Y_{11}.T, \qquad \widetilde{G_{gb}} = \frac{G_{gb}.T.c_0}{g_0}, \qquad \widetilde{H_{gb}} = \frac{H_{gb}}{g_0},$$

$$\widetilde{d_{gb}} = d_{gb}.T, \qquad \widetilde{A_{b0}} = \frac{A_{b0}.T}{m_0}, \qquad \widetilde{K_b} = \frac{K_b}{m_0}, \qquad \widetilde{a_{cb}} = \frac{a_{cb}}{Ca_0},$$

$$\widetilde{b_{cb}} = \frac{b_{cb}}{Ca_0}, \qquad \widetilde{c_{cb}} = \frac{c_{cb}}{Ca_0}, \qquad \widetilde{\alpha_b} = \alpha_b.c_0,$$

$$\widetilde{d_b} = d_b.T, \qquad \widetilde{P_{bb}} = \frac{P_{bb}.c_0.T}{m_0}, \qquad \widetilde{\kappa_{bb}} = \kappa_{bb}.m_0,$$

$$\widetilde{\sigma} = \sigma.T, \qquad \widetilde{Ca_\infty} = \frac{Ca_\infty}{Ca_0}, \qquad \widetilde{J_{leaky}} = \frac{J_{leaky}.T.c_0}{Ca_0},$$

$$\widetilde{H_{ca4}} = \frac{H_{ca4}}{Ca_0}, \qquad \widetilde{d_{Ca}} = d_{Ca}.T.c_0, \qquad \widetilde{F_{11}} = F_{11}, \qquad \widetilde{F_{12}} = F_{12},$$

$$\widetilde{G_{con}} = G_{con}.c_0, \qquad \widetilde{H_{con}} = \frac{H_{con}}{g_0}, \qquad \widetilde{d_{cm}} = d_{cm}.T.m_0$$

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