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Designing optimal calcium phosphate scaffold–cell combinations using an integrative model-based approach

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ABSTRACT

Bone formation is a very complex physiological process, involving the participation of many different cell types and regulated by countless biochemical, physical and mechanical factors, including naturally occurring or synthetic biomaterials. For the latter, calcium phosphate (CaP)-based scaffolds have proven to stimulate bone formation, but at present still result in a wide range of in vivo outcomes, which is partly related to the suboptimal use and combination with osteogenic cells. To optimize CaP scaffold selection and make their use in combination with cells more clinically relevant, this study uses an integrative approach in which mathematical modeling is combined with experimental research. This paper describes the development and implementation of 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 amount of bone formation predicted by the mathematical model corresponds to the amount measured experimentally under similar conditions. Moreover, the model is also able to qualitatively predict the experimentally observed impaired bone formation under conditions such as insufficient cell seeding and scaffold decalcification. A strategy was designed in silico to overcome the negative influence of a low initial cell density on the bone formation process. Finally, the model was applied to design optimal combinations of calcium-based biomaterials and cell culture conditions with the aim of maximizing the amount of bone formation. This work illustrates the potential of mathematical models as research tools to design more efficient and cell-customized CaP scaffolds for bone tissue engineering applications.

1. Introduction

It has been shown repeatedly that calcium phosphate (CaP) scaffolds induce bone formation, but at present still result in a wide range of in vivo outcomes. Despite the enormous research efforts that were devoted to optimizing (CaP) bone tissue engineering scaffolds, the answer still remains undiscovered [1]. Bohner et al. nicely summarize the main difficulties in defining the optimal scaffold architecture and also propose a new strategy to tackle this multidisciplinary problem: an integrative approach in which mathematical modeling is used to explain a mechanism of biomaterial–cell interactions, combined with experimental research to provide data for the determination of the model parameters as well as the validation of the model [1]. This process requires both a careful design and extensive characterization of the scaffold. Moreover, it is inherently an iterative process in which new experimental results can be fed to the model and thorough model analysis can lead to new research hypotheses.

This study applies the proposed approach to further elucidate the in vivo bone formation capacity of CaP biomaterials: more specifically, the influence of calcium ions on osteogenic cells. Therefore, semi-quantitative mathematical modeling was combined with dedicated experimental work. The mathematical tool which was built according to the proposed strategy, allows the design and testing of possible culture strategies in silico before they are tested in vitro or in vivo, thereby reducing the trial and error in experimental work. Moreover, it is a first step towards predicting the in vivo bone formation capacity of CaP scaffolds based on specific biomaterial characteristics.

CaP-based scaffolds have been proved to stimulate bone formation, but the underlying mechanism is still largely unknown [2–8].

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One possible hypothesis is that a high, local concentration of soluble factors, such as growth factors, can be achieved by adsorption on the biomaterial substrate, thereby creating a favorable microenvironment for bone formation [3,7]. Another mechanism through which CaP scaffolds can contribute to osteoinduction is the effect of surface topography on osteoblastic guidance and attachment, and the asymmetrical division of mesenchymal stem cells (MSCs) [6,9]. The chemical precipitation of a bioapatite layer, which is recognized by MSCs, is another mechanism proposed in the literature [6,10].

The release of calcium (Ca^{2+}) and phosphate (P_i) ions by dissolution is, however, believed to be the main origin of the bioactivity of CaP biomaterials [4,6,9,10]. Experimental evidence clearly indicates the key role of Ca^{2+} and P_i in osteoinduction. First, more ectopic and orthotopic bone formation has been observed in scaffolds made up of biphasic CaP than in those made of hydroxyapatite, the latter having a lower dissolution rate, after implantation without cells in dogs [3]. Secondly, Hanawa et al. investigated the effect of Ca^{2+} implantation in titanium on orthotopic bone formation in rat tibia, and a larger amount of new bone was found on the Ca^{2+}-treated side than on the untreated side [5]. Thirdly, differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs) towards osteoblasts is accompanied by the expression of Ca^{2+} binding-proteins and the incorporation of Ca^{2+} into the extracellular matrix [11]. Furthermore, osteoblasts sense and respond to the extracellular Ca^{2+} concentration independently of systemic calciotropic factors in a concentration-dependent manner, as shown by Dvorak et al. in an in vitro model system [12]. Finally, the extracellular Ca^{2+} concentration could control the frequency of the intracellular calcium spiking [13], which encodes specific cellular information [14]. Based on the above information, it is hypothesized that the primary condition for inducing ectopic bone formation is a critical level of free extracellular Ca^{2+}.

Mathematical models can be applied to unravel the role of CaP biomaterials in bone formation, which is a very complex physiological process, and this knowledge can be used to develop clinically relevant cell carriers. However, current computational models of bone formation and regeneration in general (reviewed in Ref. [15]), or even in (CaP) scaffolds specifically [16–18], do not include this influence of the local Ca^{2+} concentration on cellular activities and MSC-driven bone formation. In addition, models [19] describing CaP scaffold resorption in vivo exclusively look at geometrical scaffold properties and do not include biological variables such as cells or matrix densities. In this study, a semi-quantitative mathematical model was developed, incorporating the results of dedicated in vitro experiments to investigate the role of Ca^{2+} on in vivo ectopic bone formation in CaP scaffolds. Overall performance was corroborated by means of comparison with experimental results reported in the literature for normal [20] and impaired [2] cases of bone formation in ectopically implanted CaP scaffolds. Finally, the design of the CaP scaffold (in terms of Ca^{2+} release rate) was studied in silico by investigating the interaction between initial cell seeding density and the required calcium release rate in order to customize cell carriers and maximize the predicted amount of bone formation.

2. Materials and methods

In order to optimize CaP scaffold selection and make their use in combination with cells more clinically relevant, this study used an integrative model-based approached to study the effect of Ca^{2+} on MSC-driven ectopic bone formation. Since Ca^{2+} appears to be the most important dissolution product [21], as discussed above, this semi-quantitative model neglects the effects of P_i. Fig. 1A shows a conceptual overview of the research presented in this study. The development of the mathematical model and the execution of dedicated experiments are discussed in more detail below.

2.1. Mathematical framework

The semi-quantitative calcium model presented is inspired by the bioregulatory model of Geris et al. [22]. This starting point was chosen because the model of Geris et al. [22] is a mathematical framework of bone formation which was successfully applied to the set-up of fracture healing [22], peri-implant bone healing [23] and bone formation in a rabbit bone chamber [24]. The calcium model consists of six delay differential equations (DDEs) and 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. Fig. 1B shows a schematic overview of the model, its variables and their interactions.

A short definition of all the model parameters, as well as the parameter values, is given in Table 1. Owing to space limitation, a thorough discussion of the parameter values can be found in the supplementary material.

2.1.1. Calcium

The dissolution kinetics of the CaP scaffold are modeled by a general empirical equation:

$$\frac{dc}{dt} = ks(c_\infty - c)^n \tag{1}$$

where dc/dt is the rate of dissolution, k is the rate constant for dissolution, s represents the specific area, c_∞ stands for the maximal concentration at which artificial precipitation does not yet occur (the solubility limit), c is the calcium concentration in the extracellular space, and n is the effective order of the reaction [25]. For the specific case modeled here, the effective order of the reaction is considered to be 1, owing to lack of experimental data. The parameters k and s are combined in a single model parameter σ. During dissolution, both parameter s and parameter k will change, since the geometry and the microstructure, respectively, of the CaP will alter as the dissolution proceeds. The time-dependence of the parameter σ was, however, neglected owing to the lack of experimental data. Remark that $c(t)$ represents the time-dependent calcium concentration due to the dissolution of the CaP biomaterial. $Ca(t)$ represents the time-dependent calcium concentration due to the dissolution of the CaP biomaterial and the uptake of calcium by the cells.

Biological mineralization is a complex process resulting in the deposition of hydroxyapatite on the mature collagen matrix. There are two general approaches described in the literature: (1) matrix vesicle-mediated mineral initiation and (2) heterogeneous nucleation of bone mineral crystals on collagen fibrils [26]. The formation of intracellular vesicles containing bioapatite is a metabolic process which requires the uptake of calcium [26]. The calcium flux from the extracellular space towards the cytosol is modeled as a flux, inspired by work from Maurya and Subramanian [27]:

$$J(t) = J_{in} \cdot \frac{Ca(t)}{H_{Ca4} + Ca(t)}. \tag{2}$$

The calcium uptake for the metabolic activities of both osteoblasts and MSCs, and hence its removal from the extracellular space, is included in the mathematical model by a constant decay function (d_{Ca}).

In an in vivo situation there will be interstitial fluid flow, as well as blood flow in the scaffold. Moreover, calcium ions will diffuse in