In Vivo Quantification and Mathematical Description of Osteogenesis in Tissue Engineering Scaffold

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INTRODUCTION

Developing a successful bone tissue engineering strategy entails translation of experimental findings to clinical needs. A major leap forward towards this goal is a quantitative tool to predict spatial and temporal bone formation in scaffold. We hypothesized that bone formation in an osteoconductive scaffold follows diffusion phenomenon. In order to identify the proposed model, we implanted PLA/β-TCP scaffolds in distal femur of rats and measured bone formation using longitudinal micro-CT imaging. We then validated the proposed model using two other published in vivo models.

METHODS

Animal model and measurement of bone formation

Left distal femurs of 7 female Wistar rats were drilled (Veterinary Authority from the Canton of Vaud, authorizations No. 2140) and scaffolds of 3 mm in diameter and height made of PLA/β-TCP [1] were implanted inside the holes (Figure 1a). All animals were scanned at 7 time points (2, 4, 7, 11, 15, 22 and 35 weeks after surgery) using SkyScan 1076 In Vivo Scanner (SkyScan, Belgium).

The region of interest (ROI) was a cylinder fitting the defect in the bone. The ROI was divided into 16 axisymmetric regions, such that the height and thickness of the regions were equal, resulting in 16 concentric regions (Figure 1b). The corresponding bone volume fraction (BVF) in each region was quantified.

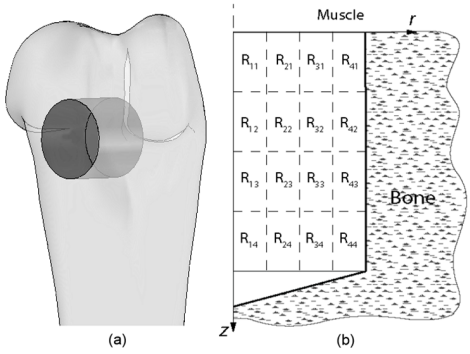

Figure 1. (a) Anterior-posterior view of distal femur with an implanted scaffold, (b) the axisymmetric cross-section of bone and scaffold.

Mathematical model

Assuming that the continuum model governing bone formation inside the scaffold was the diffusion equation, we have

$$\frac{\partial c}{\partial t} = \alpha \Delta c \qquad (1)$$

where c denoted the BVF, t was time in days, α (mm^2/day) was defined as the so-called *scaffold osteoconduction coefficient*, and Δ was the Laplacian. We assumed that a bone flux exists from surrounding bone to scaffold, but that there was no flux at the scaffold-muscle interface. This hypothesis was translated in the following boundary conditions

$$-\alpha \left.\frac{\partial c}{\partial r}\right|_{r=R} = h[C - c(r,z,t)]_{r=R}, \quad \left.\frac{\partial c}{\partial r}\right|_{r=0} = 0 \qquad (2)$$

$$-\alpha \left.\frac{\partial c}{\partial z}\right|_{z=H} = h[C - c(r,z,t)]_{z=H}, \quad \left.\frac{\partial c}{\partial z}\right|_{z=0} = 0 \qquad (3)$$

where R and H were the radius and height of the scaffold, respectively. We defined h (mm/day) as the so-called *peri-scaffold osteoinduction coefficient*, and C as the *final BVF*. Equation 1 was solved analytically in the axisymmetric geometry of scaffold (Figure 1b). Nonlinear regression was employed to fit the *in vivo* data to the model and the three unknown parameters, $C, h,$ and α, were estimated.

The proposed model with estimated parameters, i.e. $C, h,$ and α obtained from the present *in vivo* study, was used to predict the bone formation obtained from two other published *in vivo* experiments. The first *in vivo* experiment studied was a segmental bone defect in the rat femur [2], and the second one was cortical bone perforations in the mice femur [3].

RESULTS

The identification of the three parameters of our model allowed us to obtain a good fit of the *in vivo* data, both temporally and spatially (line red, Figure 2). Not only was the estimated model within the confidence interval, but it also follows the pattern of BVF evolution over time in different regions.

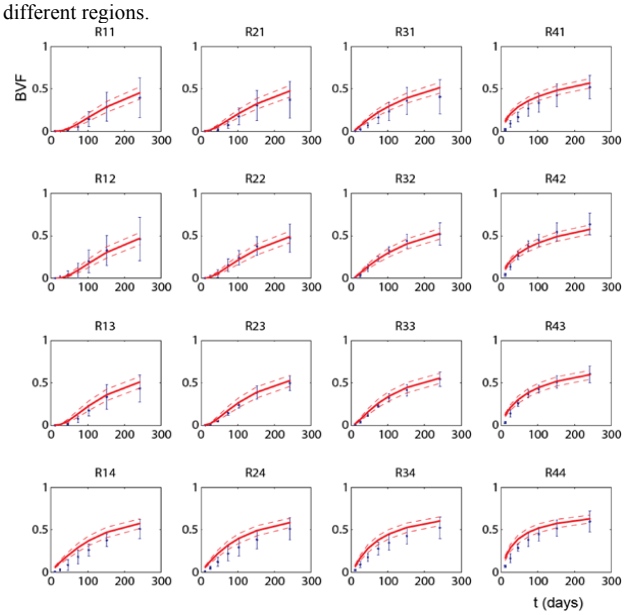

Figure 2. The mean and its 95% confidence interval for BVF are shown as thin error bars for all regions inside the scaffold. The calculated value and the 95% confidence interval are shown as thick and dashed curves.

The results of the case studies are shown in Table 1, comparing the experimental and predicted values for BVF. Although we used the same identified values of $C, \alpha,$ and h obtained from our rat experiment, the predicted values for BVF are close to the experimental values of the two published *in vivo* studies.

In vivo model	Time points	Experimental BVF (%)	Predicted BVF (%)
This study	7 Weeks	19±2	24
	22 Weeks	41±7	47
Reference [2]	3 Weeks	1.4±0.2	1.9
	12 Weeks	4.2±1.0	5.7
Reference [3]	14 Days	45±13	36
	28 Days	58±8	52

Table 1. Comparison between the experimental and predicted BVF for the three cases.

DISCUSSION

Diffusion equation not only could describe bone formation in our vivo model spatially and temporally, but it also could predict the results of two other in vivo models. The advantage of the proposed model is its simplicity and use of only 3 unknown parameters that can be estimated using in vivo data.

SIGNIFICANCE

The proposed model can estimate how bone forms in tissue engineering scaffold. It can be used to translate the findings of animal studies into a clinical application.

REFERENCES

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