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An intramembranous ossification model for the *in silico* analysis of bone tissue formation in tooth extraction sites

Jennifer Paola Corredor-Gómez [a,b,*], Andrés Mauricio Rueda-Ramírez [a,b], Miguel Alejandro Gamboa-Márquez [a,b], Carolina Torres-Rodríguez [a,c], Carlos Julio Cortés-Rodríguez [a]

[a] Research Group on Biomechanics (GIBM-UN). Mechanical and Mechatronics Engineering. Universidad Nacional de Colombia, Sede Bogotá, Colombia
[b] Advanced Technologies Analysis Group (GATAcorp), Colombia
[c] Department of Dentistry. Universidad Nacional de Colombia, Sede Bogotá, Colombia

HIGHLIGHTS

- A new mathematical model for intramembranous ossification was developed.
- An error of 3.04% was obtained comparing model outcome with experimental data.
- Interactions among cells, extracellular matrices, and growth factors were modeled.
- The mathematical model includes growth factor-induced apoptosis of fibroblasts.
- The model also includes angiogenesis and oxygen-dependent effects.

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ABSTRACT

The accurate modeling of biological processes allows us to predict the spatiotemporal behavior of living tissues by computer-aided (*in silico*) testing, a useful tool for the development of medical strategies, avoiding the expenses and potential ethical implications of *in vivo* experimentation. A model for bone healing in mouth would be useful for selecting proper surgical techniques in dental procedures. In this paper, the formulation and implementation of a model for Intramembranous Ossification is presented aiming to describe the complex process of bone tissue formation in tooth extraction sites. The model consists in a mathematical description of the mechanisms in which different types of cells interact, synthesize and degrade extracellular matrices under the influence of biochemical factors. Special attention is given to angiogenesis, oxygen-dependent effects and growth factor-induced apoptosis of fibroblasts. Furthermore, considering the depth-dependent vascularization of mandibular bone and its influence on bone healing, a functional description of the cell distribution on the severed periodontal ligament (PDL) is proposed. The developed model was implemented using the finite element method (FEM) and successfully validated by simulating an animal *in vivo* experiment on dogs reported in the literature. A good fit between model outcome and experimental data was obtained with a mean absolute error of 3.04%. The mathematical framework presented here may represent an important tool for the design of future *in vitro* and *in vivo* tests, as well as a precedent for future *in silico* studies on osseointegration and mechanobiology.

1. Introduction

A deep understanding of the complex biological and biochemical processes in bone's metabolism and the consequent capacity to predict its behavior has contributed to the development of novel medical techniques such as the localized administration of growth factors for assisted bone healing (Luginbuehl et al., 2004), the ultrasound-mediated bone regeneration (Mitragotri, 2005), the careful and personalized selection of bone grafts, the development of scaffolds for regenerative medicine, among many others that still have to be considered. Such an understanding has been traditionally obtained from *in vitro* and *in vivo* experimentation involving high costs at several levels.

Abbreviation: BC, Blood clot; H-D, Hypoxia-dependent; IOM, Intramembranous ossification model

* Corresponding author at: Carrera 30 45-03, Edificio 407, Oficina 213, Colombia
E-mail address: jpcorredorg@unal.edu.co (J.P. Corredor-Gómez).

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However, the latest advancements in computational modeling and computer power have boosted a new field of study that can shed light on such matters: *in silico* testing.

It is possible to put forward mathematical models to describe the behavior of living tissues (Doblaré and Garcı, 2004). The *in silico* implementation of these models has a potential role in research and development of treatments, implants design and even in the prognosis of bone pathologies (Isaksson, 2012), significantly reducing the need for traditional testing techniques. Several mathematical models have been developed in order to understand the fundamentals of cell and tissue biology, and the behavior of biochemical factors in bone (Lacroix and Prendergast, 2002; Gomez-Benito et al., 2005; Bailon-Plaza and Van Der Meulen, 2001; Geris et al., 2010; Garcia-Aznar et al., 2007; Pivonka and Komarova, 2010; Komarova et al., 2003; Ribeiro et al., 2015; Geris et al., 2008; Peiffer et al., 2011; Carlier et al., 2013). However, while most of the efforts have focused on studying regeneration and remodeling in long bones, to the authors' knowledge, few *in silico* works emphasize bone formation in the mandible, specifically regarding to intramembranous ossification (IO).

This paper presents the formulation of a mathematical model for predicting the spatiotemporal behavior of bone healing via IO. The model was validated by comparing its results with an *in vivo* study of bone regeneration in tooth extraction sites. The proposed model builds on previous mathematical descriptions of fracture healing in long bones and incorporates modifications to account for the particular phenomena that take place in the mandibular bone. It also takes into account the findings by recent studies on the topic and introduces new biological and numerical considerations such as the growth factor-mediated apoptosis and a detailed description of the alveolar boundary conditions.

Our mathematical model can be used to predict the behavior of particular types of cells, the formation and degradation of important tissues and the action of growth factors during intramembranous bone healing, even in geometries that differ from the one used for validation. This makes possible to predict the events following a clinical intervention and to analyze some situations of compromised and assisted healing.

Due to the immense complexity of bone metabolism, and the lack of quantitative data and computational capacity (Zhang et al., 2012), it is currently impossible to reproduce all the interacting biological, biochemical and mechanical processes by means of *in silico* testing. This problem was tackled by making some simplifications and assumptions that helped the development of the current model. Furthermore, since a detailed histological examination of the alveolar bone regeneration in humans has many inherent difficulties, the validation of the model was performed by comparing the simulations outcome with an *in vivo* study conducted in dogs as it is normally done.

2. Theoretical background: non-pathological bone healing

Bone tissue formation (also termed as ossification or osteogenesis) following injury is a complex process that involves the action of several cells and molecules. The different types of cells migrate, proliferate, differentiate, interact, synthesize and degrade extracellular matrices (ECM), proteins and enzymes under the influence of mechanical stimuli (Gerstenfeld et al., 2003) and a variety of regulatory molecules. These molecules include insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), transforming growth factors (TGFs), and bone morphogenetic proteins (BMPs), among others (Barnes et al., 1999). This process has been described by various authors as a recapitulation of the different embryological processes of bone formation: inflammation, repair and remodeling (Sikavitsas et al., 2001; Ferguson et al., 1999; Vortkamp et al., 1998).

During bone formation, there are two distinct and interactive responses: endochondral and intramembranous ossification (Gerstenfeld et al., 2003). Endochondral ossification is characterized by chondrogenic growth factors and chondrocyte activity that allow the existence of cartilage; on the contrary, these aspects are absent in IO. Although the wound healing process in long bones (femur, tibia, ulna, etc.) has been clearly identified to occur via both responses (Reed et al., 2003; Kokubu et al., 2003; Harrison et al., 2003), there has been an active discussion about which response takes place during bone formation in the mandible (Frommer and Margolies, 1971).

Both intramembranous and endochondral ossification have been reported to occur in the mandible during fetal development (Shibata et al., 2014). Nevertheless, many studies report the absence of cartilage during bone regeneration, as will be seen later. De facto, three ossification centers operate in the mandible during fetal development: two predominant are intramembranous, whereas the third is an endochondral center within the Meckel's cartilage (Frommer and Margolies, 1971). Endochondral ossification occurs only where the cartilage bar is near to the primary intramembranous centers; the process is transient and dimmed by the IO. After the cartilage is calcified, it is quickly invaded and surrounded by membrane bone and the endochondral osteogenic zones rapidly lose their identity (Frommer and Margolies, 1971).

One of the most common processes of bone healing in the mandible is the one that occurs inside an alveolus left by the extraction of teeth. It has been the subject of multiple studies, both in animal and human specimens (Cardaropoli et al., 2003; Araujo and Lindhe, 2005; Kuboki et al., 1988; Simpson, 1960; Amler, 1969; Christopher, 1941). All authors describe the healing process in the extraction socket in the following sequence (note the absence of cartilage):

1. Formation and maturation of a blood clot (BC), which serves as initial construct for the healing process.
2. Replacement of the BC with a provisional matrix (PCT) synthesized by fibroblasts, which soon becomes the main construct for vascularization and cell migration.
3. Replacement of the PCT with newly formed woven bone.
4. Bone mineralization.
5. Bone remodeling.

At the time of tooth extraction, blood vessels are damaged resulting in bleeding and the consequent formation of a BC. The platelets contained in the blood release PDGF and TGF-β, together with vasoactive factors in a phenomenon called platelet degranulation (Davies, 2003), which concludes with vasoconstriction and the generation of a fibrin network (Davies, 2003). The newly formed network serves as a scaffold for the migration of fibroblasts and mesenchymal stem cells (MSCs) that are attracted by both PDGF and TGF-β (Chandrasekhar and Harvey, 1996) and are originally immersed inside the bone marrow stroma (Hernandez-Gil et al., 2006). BC is gradually degraded by the action of macrophages and plasmin (a protein contained in plasma), contributing to the migration of MSCs and fibroblasts (Li et al., 2003). This initiates fibroplasia: the replacement of the blood clot with PCT and granulation tissue (GT), collagen-rich ECMs synthesized by fibroblasts which serve as a construct for angiogenesis (Aukhil, 2000). While the fibrin clot forms within minutes (Vanegas-Acosta and Garzón-Alvarado, 2011), its degradation can take up to 7 days (Cardaropoli et al., 2003).

Angiogenesis is initiated from the severed blood vessel ends and is mainly mediated by the presence of vascular-endothelial growth factors (VEGFs) (Ferrara and Davis-Smyth, 1997), which are