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A computational model of clavicle bone formation: A mechano-biochemical hypothesis

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ABSTRACT

Clavicle development arises from mesenchymal cells condensed as a cord extending from the acromion towards the sternal primordium. First two primary ossification centers form, extending to develop the body of the clavicle through intramembranous ossification. However, at its ends this same bone also displays endochondral ossification. So how can the clavicle be formed by both types of ossification? Developmental events associated with clavicle formation have mainly used histological studies as supporting evidence. Nonetheless, mechanisms of biological events such as molecular and mechanical effects remain to be determined.
The objective of this work was to provide a mathematical explanation of embryological events based on two serial phases: first formation of an ossified matrix by intramembranous ossification based on three factors: systemic, local biochemical, and mechanical factors. After this initial phase expansion of the ossified matrix follows with mesenchymal cell differentiation into chondrocytes for posterior endochondral ossification. Our model provides strong evidence for clavicle formation integrating molecules and mechanical stimuli through partial differentiation equations using finite element analysis.

Introduction

Developmental studies have evidenced bone formation driven by mesenchymal cell differentiation through two distinct mechanisms: endochondral or intramembranous ossification [1]. For the first process a cartilage intermediate is required, i.e. mesenchymal cells differentiate into a cartilaginous mold that is later replaced by bone [2]. Intramembranous ossification describes a process where mesenchymal cells directly differentiate into osteoblasts [3]. Clavicle development is unique as this bone is the result of both types of ossification [4]. For one, the shaft is developed from intramembranous ossification by formation of two primitive nuclei that will later form the diaphysis. After these nuclei have fused, secondary cartilage develops by chondrogenesis for posterior endochondral ossification at both ends. Developmentally it is the first bone to start the process of ossification, and the last to complete it. Many biological studies have focused on molecular mechanisms leading to these events: growth factors, signaling cascades, transcription factors, molecules of the extracellular matrix, to mention few. On the other hand, engineers have centered on physical force simulation resulting in bone formation [5]. For example, hydrostatic pressures or shear stress [6]. Few studies have tried to integrate both aspects to build a bridge to explain bone development. By means of a mathematical model we were able to integrate molecular and mechanical stimuli leading to clavicle embryological development to simulate developmental and morphogenic events.

This model presents a hypothesis describing mesenchymal differentiation into osteoblasts. Our hypothesis on blastema cell differentiation resulting in ossification is based on three fundamental factors: systemic factors, localized biochemical factors, and mechanical factors. For our model we suppose two consecutive phases: first, we model two primary ossification nuclei, resulting from induction by these fundamental factors. Second, the nuclei expand and form and ossified bridge, giving rise to vascularization and ossification [4].

Materials and methods

Primary ossification center appearance

In humans during the fifth week of gestational development undifferentiated mesenchymal cells migrate to the site of future skeletal element formation, defining the boundaries of the future clavicle. The clavicle starts as a fibrocellular proliferation, known as a blastema, arising from mesenchymal cell condensation that begins from the

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neighborhood of the scapula downward. It then moves forward and medially to the midline region [7]. Two primary centers of ossification develop, lateral and medial, which are in proximity to each other [8]. Later they fuse to form the diaphysis. For this first phase we propose these hypotheses:

1. Systemic factors such as cell adhesion molecules, extracellular matrix molecules, growth factors, and others surround the blastema.
2. Morphogens such as BMP2 regulate mesenchymal cell differentiation [9]. Effects of BMP can be modulated by extracellular antagonists such as Noggin [10]. This interaction generates a feedback mechanism resulting in spatio-temporal patterns, with areas of higher BMP concentration, where cell differentiation will occur.
3. At the same time biochemical factors are inducing cell differentiation; mechanical factors influence the appearance of primary centers of ossification. In places with high values of octahedral shear stress exertion on the blastema, mesenchymal cell will differentiate into osteoblasts. In contrast, zones with hydrostatic stress mesenchymal cells will differentiate into chondrocytes [11].

Factors resulting in cell differentiation

Systemic factors

First we proposed existence of systemic factors: cell adhesion molecules, extracellular matrix molecules, growth factors, and others. We will refer to them collectively as systemic factor S_s. These factors prevent blastema ossification. To explain these events we propose a reaction–diffusion model by partial differential equations that take into account factor S_s half-life. Quantification of these changes establishing local changes in mesenchymal tissue can be described by the following equation:

$$\frac{\partial S_S}{\partial t} = D_S \nabla^2 S_S - \frac{\ln(2)}{\tau_{av}} S_S \tag{1}$$

where a local change in factor S_s concentration is due to its diffusion D_s, and degradation determined by its half life τ_{av}. Our model proposed initial conditions at a null concentration of systemic factor.

Local biochemical factors

Primary ossification nuclei in the clavicle arise from mesenchymal cell direct differentiation into osteoblast. The second aspect of our hypothesis includes morphogens such as BMP2 and its effect on these events [12]. BMP2 is critical for bone formation activating osteoblast related genes [13]. Even though osteoblast differentiation and bone formation are regulated by many local factors, our model identifies BMP2 as the most potent factor. However, its action can be inhibited at the extracellular level by Noggin [14]. Noggin binds with very high affinity to BMP2 preventing it to bind to its receptor and initiate the signaling cascade. Furthermore, BMP2 can also stimulate Noggin synthesis [9].

Again we propose a reaction–diffusion model by partial differential equations, where an activator (BMP2) and an inhibitor (Noggin) interact with each other, forming a periodic pattern from an initial spatial distribution of activator and inhibitor [15]. We used an initial BMP2-Noggin random distribution in a stable state with concentrations fluctuating approximately by 10% to account for concentration variability for each molecule in the defined domain. The regulatory process consists of an activator with ability to enhance its own production (Fig. 1). In addition the activator (BMP2) promotes the Noggin production, which inhibits the activator, and decays with time [9].

$$\frac{\partial S_N}{\partial t} = C\left(\alpha_1 - \mu S_N + \gamma_0 S_N^2 S_B\right) + D_N \nabla^2 S_N \tag{2}$$

$$\frac{\partial S_B}{\partial t} = C\left(\alpha_2 - \gamma_0 S_N^2 S_B\right) + D_B \nabla^2 S_B \tag{3}$$

where C is the mesenchymal cell concentration expressing BMP2 and Noggin. S_B and S_N are activator and inhibitor concentrations, respectively. α_1 and α_2 are terms quantifying each term production by mesenchymal tissue. μ is a constant quantifying Noggin's production inhibition, by negative feedback. γ_0 regulates non-linear interaction between BMP2 and Noggin concentration, quantifying activation or inhibition for each molecule. D_B and D_N are BMP2 and Noggin diffusion coefficients, respectively. For biological interpretation $\gamma_0 S_N^2 S_B$ represents non-lineal S_N activation (BMP2 promotion of Noggin) and non-lineal S_B consumption, due to Noggin's presence.

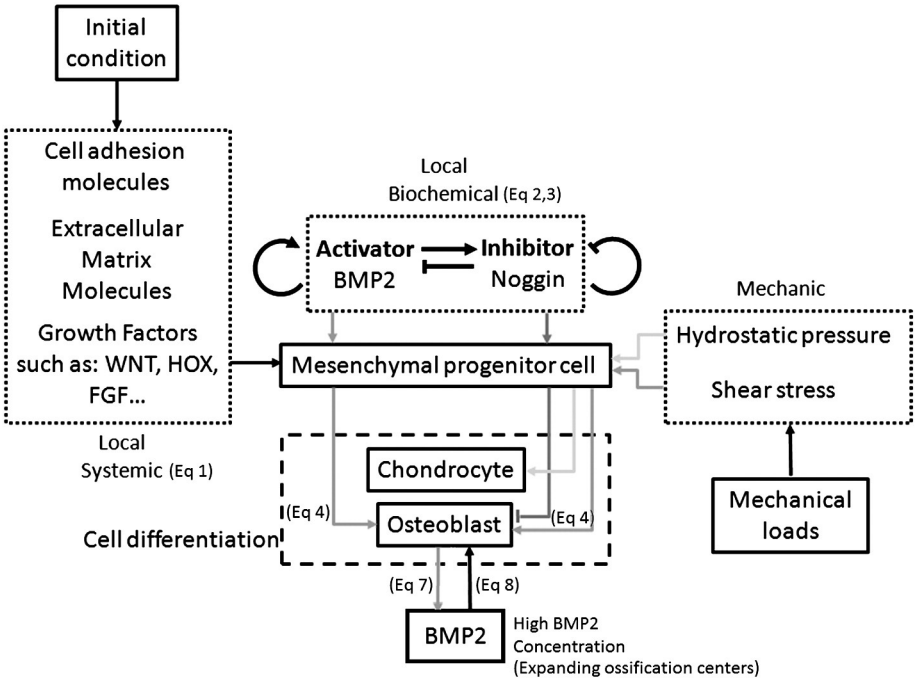

Fig. 1. Schematic representation of reaction–diffusion mechanism. Association between molecules can be quantified by equations that establish local changes in mesenchymal tissue.