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A COMPUTATION STUDY OF GROWTH FACTOR SIGNALING IN THE GROWTH PLATE

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INTRODUCTION:

Endochondral ossification is the differentiation process of cartilaginous into osseous tissue. It is the regulatory mechanism in skeletogenesis, growth plate development and fracture healing. In a fetal cartilage anlage, the first differentiation of chondrocytes into hypertrophic cells starts in the center at approximately 8 weeks of gestation. These cells are then mineralized and replaced into bone by invading osteoclasts. The anlage continues to grow, while the front of newly formed bone tissue moves up towards both ends of the bone. A large variety of locally produced signaling molecules play a role in controlling this process of skeletogenesis. However, data from the literature suggest that PTHrP, Ihh and VEGF are the most important growth factors (GFs).

Our aim was to quantitatively evaluate whether these three growth factors together can control the initiation and development of the fetal growth plate, starting from a cartilage anlage at Streeter's stage 22. For this purpose, we employed a new, temporo-spatial finite element model.

METHODS:

Once initial hypertrophiation has started in the center of the anlage, organized columns of proliferating (PZ), prehypertrophic (pHZ) and hypertrophic zones (HZ) emerge. As the front of bone approaches the ends of the anlage, these zones move along. PTHrP, Ihh and VEGF are produced in the articular perichondrium (PC), pHZ and HZ, respectively (Fig 1). These GFs need to be transported through the bone, as they have their effects on cells in a distinct zone.

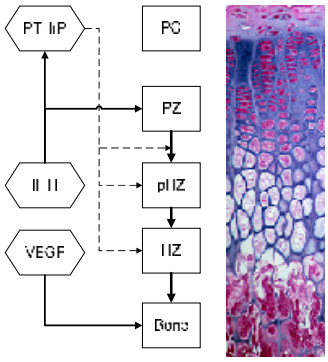

Fig 1.
Right: PZ, pHZ, HZ and bone in the growth plate column.
Left: Regulatory effects of GFs, depicted with solid (stimulatory) or dotted (inhibitory) lines. Arrows between zones indicate cell differentiation.

A 1-D FE model was developed representing half a cartilage anlage of a long bone from center to one end. All interactions of GFs as depicted in Figure 1 were incorporated. All cells with surrounding extracellular matrix were represented by groups of elements. As cells hypertrophied in the pHZ and HZ, the number of elements representing one cell increased.

Synthesis (s), diffusion (t) and decay (d) of all GFs determined the change in local GF concentration (c) per element:

$$\frac{dc}{dt} = s - t - d = s - \nabla.(D\nabla c) - \frac{\ln 2}{\tau}c$$

in which half-life time (τ) was assumed to be 10 minutes for all GFs [4]. The diffusion coefficient (D) was 63$\mu m^2/s$ for the PZ, based on FRAP measurements in porcine growth plate. Assuming that diffusion occurs in the extracellular matrix and not in the cells, D for pHZ and HZ was derived from matrix/cell ratios [3].

The proliferation rate in PZ was assumed to be Ihh dependent, in addition to a basal rate of one cell per 40 hours. This rate was doubled in the presence of normal Ihh levels. All other processes, i.e. cell differentiation rate and ossification, were controlled by local GF concentration.

All effects of GFs were concentration dependent following a Michaelis-Menten equation. Lengths of cells and zones were taken from human data [1,2].

RESULTS:

Differentiation of cells started with one cell in the center and continued towards the end of the bone, solely controlled by signaling of growth factors, according to the scheme in figure 1. Initially, the rate of mineralization was higher than the proliferation rate. The mineralization rate then decreased until it was equal to the proliferation rate, leading to stability within the growth plate. This is similar to the physiological processes observed in the development of an anlage into a bone.

The stable growth plate consisted of zones in which the number of cells varied cyclically within a small, physiological range (Fig 2). At this stage, bone growth was found to be constant and self-regulating by growth factors synthesized by the cells for 200 cycles (167 days), which was the maximal simulation number. After this period, secondary ossification starts, which was not considered in this model.

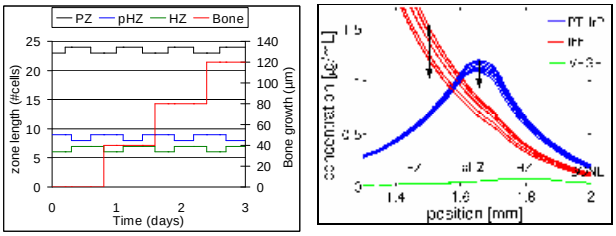

Fig 2. Left: Nr of cells per zone and bone growth during the simulation, once the growth plate has formed. Right: PTHrP and Ihh profiles as a result of the transition of a cell from pHZ to HZ. The subsequent PTHrP and Ihh decreases lead to transition of a cell from PZ to pHZ.

DISCUSSION:

The growth factors PTHrP, Ihh and VEGF together were able to control the initiation and development of the growth plate in a computational model, simulating the development of the cartilage anlagen at Streeter's stage 22 to a mineralized bone at three months after birth.

Enhanced or decreased GF synthesis resulted in new steady states, which corresponded with changes seen in transgenic mice and with human growth plate pathologies (data not shown in this abstract).

Stevens et al [5] developed a mechanobiological model of endochondral ossification in a long bone, Bailon-Plaza et al [4] developed a model of fracture healing in which growth factor effects were accounted for. The approach to model endochondral ossification in a long bone based on growth factor effects, however, is new. It is a promising addition to earlier computational models, which generally focussed on the mechanical environment in the tissue as a trigger for endochondral ossification.

Future developments enable extensions to later developmental stages, 2-D and 3-D representations, and incorporation of the interactions of mechanical loading and synthesis, transport and receptors of growth factors.

REFERENCES:

[1] Fazzalari, et al., 1997, The Anat. Record 248:1-12
[2] Kember, et al., 1976, JBJS 58-B: 426-35
[3] Hunziker, et al., 1987, JBJS 69:162-72
[4] Bailon-Plaza, et al., 2001, J. Theor. Biol. 212:191-209
[5] Stevens, et al., 1999, JBJS 17:646-653