Determining relative importance of cellular processes in a model of bone healing

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Introduction: The complete sequence of cellular and molecular events during bone healing is not yet known. To explore these complex interrelated processes experimentally is difficult. We have developed a novel mechanistic mechanobiological model of bone healing, which includes a thorough description of biological events during tissue differentiation, combining a detailed description of cell-phenotype specific activities and rates during fracture repair, i.e. migration, proliferation, differentiation, and apoptosis etc. It can correctly simulate normal and abnormal tissue-specific activities and rates during fracture repair, i.e. migration, proliferation, differentiation, and apoptosis etc. It can correctly simulate normal and abnormal (altered mechanical and biological environments) bone healing [1].

This study aimed to reveal which cellular parameters have the greatest influence on sequential spatial and temporal tissue differentiation events, bone formation rate and time until complete healing. For this purpose, a statistical approach developed by Taguchi [2] was used. It utilizes fractional factorial design (orthogonal arrays) to account for variations in parameter data and quantify the importance of each cellular parameter. The outcome of this study can be useful for optimizing treatment protocols of pathologic fracture healing.

Materials and Methods: Description of individual cell processes was used as part of a mechanobiological tissue-differentiation model [1]. Briefly, the entire calculus was initially assumed to consist of granulation tissue. Cells could differentiate into fibroblasts, chondrocytes or osteoblasts. Depending on cell phenotype, cells differentially responded to the magnitude of deviatoric strain and fluid velocity [3] by: proliferation, differentiation, migration or apoptosis. Additionally, the cells could synthesize or degrade extracellular matrix (ECM) at phenotype-specific rates. A 2D adaptive finite element model of a long bone with a 3 mm transverse fracture gap was used for all analyses [1]. The non-linear equations were implemented as user-defined FE in ABAQUS, and the variables were coupled individually to permit cell phenotype specific differentiation pathways. A 1 Hz cyclic load of 300 N was applied.

Parameter values were based on literature [1]. To assess their respective importance, all factors in the cell model were examined in the parametric analysis. These are: initial cell concentrations of MSC; rates of proliferation, differentiation, migration, and apoptosis for each cell type (MSC's, fibroblasts, chondrocytes and osteoblasts); rate of ECM synthesis and degradation for each tissue type (fibrous tissue, cartilage and bone). First, a two-level fractional factorial design (resolution IV L64) was used as a screening experiment to identify the ten most significant factors. Thereafter, a three-level array (L27) was used to study the curvature (non-linearity) of the most important parameters. Three outcome responses were quantified: 1) the ability to predict sequential spatial healing events; 2) bone formation at early, mid and late stages of healing; and 3) total time until complete healing. Statistical analysis of variance (ANOVA) was performed. The percentage of the total sum of squares was used as a measure of ‘importance’ of the factors [2].

Results: Throughout all analyses, the parameters that were most influential were related to synthesis and degradation of cartilage and ECM production of bone (Fig 1). The ability to predict the sequences of normal healing was most influenced by the rate of matrix production of bone. In the early stages, ECM production of bone was most influential on the amount of bone formation. However, during mid and late phases, the ECM production rate of cartilage was most important. The time to complete healing was primarily dependent on the rate of cartilage degradation during endochondral replacement.

The statistical analysis revealed that higher values of parameters related to bone formation and osteoblasts were more beneficial to healing. In contrast, parameters related to fibrous tissue and cartilage showed optimum levels (Fig 2). The normal (mid) levels of soft tissue matrix production resulted in more bone formation relative to both the high and the low rates.

Discussion: This study evaluated the importance of several cell parameters on cellular processes during bone healing. The outcome analyses were chosen to capture different aspects of the complex mechanobiological processes. Overall, the most important parameters were related to ECM of bone and cartilage. As expected, ECM production was a major factor in the healing process, but it was also shown that cartilage degradation or lack thereof can have a significant effect on healing. Surprisingly, parameters related to cartilage were often more influential than parameters related to bone, even for the measure of the amount of bone (Fig 1). This highlights the importance of cartilage formation and endochondral ossification during bone healing. It also relates well to in vivo studies that have identified this step as crucial for successful healing. ECM production of fibrous tissue and cartilage had optimum levels (Fig 2). Some soft tissue formation was beneficial to the amount of bone formed, but too much delayed healing. This is also consistent with literature, where formation of these tissues stabilizes the gap and subsequently allows for external bone bridging, creeping substitution and complete healing. Similar to our findings, in vivo bone healing studies have shown that too much cartilage formation delays healing.

This study clearly identified and quantified the relative importance of the most critical parameters during tissue differentiation and bone healing. The outcome is similar to what have been suggested crucial steps, from a biological standpoint. Our study further suggested that future experimental efforts should focus at understanding the processes and rates of cartilage synthesis and replacement better.


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