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MECHANO-REGULATION OF TISSUE DIFFERENTIATION DURING FRACTURE HEALING BY DEVIATORIC STRAIN ALONE IS INSUFFICIENT

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INTRODUCTION: For the regulation of tissue differentiation during secondary fracture healing several local mechanical parameters have been proposed. Mechano-regulation algorithms tend to use one deviatoric (strain) and one volumetric component, e.g. hydrostatic pressure.[1, 2] It was demonstrated, however, that tissue differentiation during normal fracture healing with idealized *axial loading* could equally well be guided by deviatoric strain alone[3]. We hypothesized that a mechano-regulation algorithm based on deviatoric strain alone will also correctly predict healing for other loading modalities. To validate our computational analyses we characterized *in vivo* healing of a gap osteotomy under combined displacement- and load-controlled interfragmentary axial motions.[4]

METHODS: The *in vivo* experiment used ovine tibial, 2.4 mm gap osteotomies, subject to 0.5 Hz sinusoidal, axial displacements of 0.6 mm (maximum), and a load limit of 360N. When the fracture healed and the tissue in the gap became stiffer, the reaction force reached its 360 N limit prior to maximum displacement. The displacement was then truncated at the value that generated a reaction force of 360 N. This allowed for full tissue differentiation of the callus into mature woven bone. The same loading regime was applied in the computational model. A 3D, rotationally symmetric, mechano-regulatory, adaptive FEA model (ABAQUS) of an ovine tibia was used. A healing, transverse fracture gap and external callus were included, based on an earlier 2D model.[3] Fracture healing was simulated (MATLAB) by using the biophysical stimuli calculated from the FEA, at maximal displacement or when the cut-off load was reached. New element material properties were predicted, according to the mechano-regulation rules. The callus initially consisted of granulation tissue into which precursor cells could migrate, according to a diffusive process from the surrounding soft tissues, callus, periosteum and marrow.[3] The cells within the callus were allowed to differentiate into fibroblasts, chondrocytes or osteoblasts and to produce their respective matrices. A rule-of-mixtures was used to calculate element material properties, based on the stimulated tissue types in the last ten days and on cell density. All materials were assumed linear poroelastic, with the latest available material properties.[3]

In addition to the algorithm regulated by deviatoric strain alone,[3] fracture healing was also predicted using the mechano-regulation algorithms proposed by Carter *et al.,*[1] and Lacroix and Prendergast.[2] The computational predictions were compared with the *in vivo* experimental results, by correlating simulated tissue types to histological findings.

RESULTS: The histological analysis of the healing osteotomy at 4 weeks, showed new woven bone formation in the external callus, away from the gap. The gap was filled with fibrous-connective and small islands of cartilaginous tissues. There was no bridging of the external callus or gap. At 8 weeks, there was bony bridging, limited to the periphery of the external callus. The intra-cortical gap was still filled with soft tissue, rich in proteoglycans.

The mechano-regulation algorithm based on deviatoric strain alone predicted complete fracture healing, but dissimilar to that observed with histological. Initially, intra-membranous bone formation occurred at the callus tip and along the periosteum, but bone formation was also predicted along the external surface of the callus, with immediate bridging of immature bone. Endochondral ossification and creeping substitution towards the gap, as was seen in histology at 8 weeks, then followed.

The algorithms regulated by volumetric, in addition to deviatoric components also predicted bony healing, but not completely. With the algorithm regulated by tensile strain and hydrostatic pressure[1] the initial healing pattern was similar to that regulated by deviatoric strain alone. The histological findings at 8 weeks were also corroborated, but after external bridging occurred, no further creeping substitution of bone into the gap was predicted. High hydrostatic pressures within the gap inhibited ossification (Fig 1a). Deviatoric strain and fluid flow[2] in combination correctly predicted initial intra-membranous bone

formation at only the callus tip and the periosteum, which grew into the callus during the process of endochondral ossification, similar to what was seen in histology after 4 weeks. However, no bridging occurred thereafter. High fluid flow in the callus and the gap predicted a non-union, with a cartilage filled gap (Fig 1b).
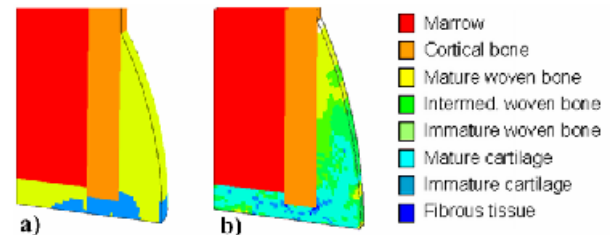
Figure 1 Tissue types after healing as computed with the algorithms regulated by a) principal tensile strain and hydrostatic stress[1], and b) deviatoric strain and fluid flow.[2]

DISCUSSION: Although all of these mechano-regulation algorithms were previously shown to predict temporal and spatial tissue distributions in normal fracture healing,[3] when applying the conditions from this experimental study, the outcomes were quite different. The algorithm regulated by deviatoric strain alone predicted much more robust healing than observed *in vivo*. It also did not differ in its predictions of healing from earlier simulations with significantly different loads. Even under large interfragmentary displacements, the deviatoric strain at the callus tips, along the periosteum, and its external surface was very low stimulating osteogenic differentiation of the cells. Furthermore, this algorithm, without an inhibiting stimulus, may be unable to simulate difficulties in union.

In contrast, the algorithms regulated by both a deviatoric and a volumetric component better corroborated early and intermediate phases of healing. Initially, the low deviatoric component stimulated healing of the callus, but it was too strong and required inhibition. In early healing, when intra-membranous ossification dominates, this modulation is provided by fluid flow. As healing progresses and the dominant mode of healing became endochondral ossification of the callus, modulation by hydrostatic pressure agreed better with *in vivo* healing. However both volumetric stimuli finally inhibited endochondral ossification of the intra-cortical gap, which is always observed *in vivo* once external callus bridging has occurred.

In conclusion, tissue differentiation during fracture healing, as predicted by mechano-regulation based on deviatoric strain alone, was not confirmed by what was observed in our *in vivo* experiment. An inhibiting signal is needed to balance the stimulation of healing. However, healing predicted by the addition of fluid flow or hydrostatic pressure was not confirmed for all phases of callus healing, either. Hence, these algorithms require further refinement. Carter *et al.* proposed that the modulating effect of hydrostatic pressure could be described by a constant,[1] but perhaps the influence of these modulating stimuli are not constant and are in fact a function of the tissue composition itself; hence, their influence might change while healing proceeds. To try and understand this effect, we believe that modeling at a finer scale of the cell, and its interaction with the extracellular matrix, may provide a better base to describe the process of fracture healing.

REFERENCES: [1] Carter *et al.,* CORR 1998; [2] Lacroix and Prendergast, J Biomech, 2002; [3] Ito *et al.*, 50th ORS, 2004; [4] Bishop *et al.,* 49th ORS 2003;

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