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Relationships between tissue dilatation and differentiation in distraction osteogenesis

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Abstract

Mechanical factors modulate the morphogenesis and regeneration of mesenchymally derived tissues via processes mediated by the extracellular matrix (ECM). In distraction osteogenesis, large volumes of new bone are created through discrete applications of tensile displacement across an osteotomy gap. Although many studies have characterized the matrix, cellular and molecular biology of distraction osteogenesis, little is known about relationships between these biological phenomena and the local physical cues generated by distraction. Accordingly, the goal of this study was to characterize the local physical environment created within the osteotomy gap during long bone distraction osteogenesis. Using a computational approach, we quantified spatial and temporal profiles of three previously identified mechanical stimuli for tissue differentiation—pressure, tensile strain and fluid flow—as well as another candidate stimulus—tissue dilatation (volumetric strain). Whereas pressure and fluid velocity throughout the regenerate decayed to less than 31% of initial values within 20 min following distraction, tissue dilatation *increased* with time, reaching steady state values as high as 43% strain. This dilatation created large reductions and large gradients in cell and ECM densities. When combined with previous findings regarding the effects of strain and of cell and ECM densities on cell migration, proliferation and differentiation, these results indicate two mechanisms by which tissue dilatation may be a key stimulus for bone regeneration: (1) stretching of cells and (2) altering cell and ECM densities. These results are used to suggest experiments that can provide a more mechanistic understanding of the role of tissue dilatation in bone regeneration.

Keywords

Mechanobiology; Dilatation; Density; Mesenchymal tissue; Extracellular matrix; Poroelasticity

1. Introduction

Mechanical factors modulate the morphogenesis and regeneration of mesenchymally derived tissues. Forces arising from muscle contraction, contact at joint surfaces and different growth rates in adjacent tissues produce a variety of local mechanical stimuli in skeletal tissues, including pressures, strains and fluid flow. Using the concept that specific combinations of these stimuli regulate the differentiation of multipotent mesenchymal tissue, previous studies have investigated the mechanobiology of fibrocartilaginous metaplasia (Giori et al., 1993; Wren et al., 1998), implant integration (Prendergast et al., 1997), the development of pseudarthroses (Loboa et al., 2001), fracture healing (Blenman et al., 1989; Carter et al.,

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1998;Claes and Heigele, 1999;Gardner et al., 2004,2000;Lacroix and Prendergast, 2002;Smith-Adaline et al., 2004) and distraction osteogenesis (Carter et al., 1998;Loboa et al., in press). Results from these studies suggest the possibility of using the physical environment as a factor that can be controlled in order to promote a specific healing or development outcome. Consequently, elucidating the role of certain mechanical stimuli in influencing mesenchymal tissue differentiation will impact the fields of developmental biology, orthopaedics and tissue engineering.

Mechanical regulation of tissue morphogenesis and regeneration is mediated by the extracellular matrix (ECM). It is well known that integrins and their ECM ligands function as physical links that transfer tissue-level forces and deformations to cells (Burridge et al., 1988;Ingber, 1991) However, the role of the ECM in mechanotransduction is not limited to this capacity. Deformation of the ECM directly alters the structural arrangement of the matrix; therefore, such deformation alters local concentrations and gradients of matrix-bound growth factors and adhesion sites. In this manner, tissue dilatation (local deformation occurring equally in all directions) may act as an important mechanical stimulus, because it represents local expansion or local compaction of the tissue (Fig. 1). Modulation by the ECM of growth factor availability has been described as the “instructive role” of the insoluble matrix (Aumailley and Gayraud, 1998;Ramirez and Rifkin, 2003). Finally, in an extreme case, large forces and displacements can cause damage to the extracellular matrix, thereby initiating an inflammatory cascade involving a host of cellular responses. From these examples, it is clear that enormous potential exists in using knowledge of cell–ECM interactions to provide insight into mechanisms involved in the mechanobiology of multipotent mesenchymal tissue differentiation.

A promising model for investigating such mechanisms is distraction osteogenesis (DO), or “limb-lengthening”, where large volumes of new bone result from successive application of a tensile displacement across an osteotomy gap. Clinical protocols involve a latency phase after the osteotomy, followed by a lengthening phase during which an external fixator is used to pull the osteotomized ends apart at a rate of approximately 1 mm/day. In the final phase, consolidation, bone continues to form and mineralize within the lengthened osteotomy gap, or the distraction gap. As compared to the latency and consolidation phases, the lengthening phase is characterized by numerous osteogenic phenomena. These include sustained proliferation of osteoblast progenitor cells in the center of the distraction gap (Aronson et al., 1997;Li et al., 1997), marked increases in blood flow and vascular proliferation in the region (Aronson, 1994;Choi et al., 2000;Rowe et al., 1999), and upregulation within the gap of growth factors and matrix proteins involved in bone formation (e.g. TGF β -1, BMP-2, BMP-4, IGF I, bFGF, OPN) (Fang et al., 2004;Liu et al., 1999;Mehrara et al., 1999;Rauch et al., 2000;Sato et al., 1999;Sato et al., 1998;Weiss et al., 2002). Similar results have been reported for experiments in which the lengthening phase of DO is compared to healing following a simple osteotomy (no distraction) (Aronson et al., 1997;Lammens et al., 1998;Weiss et al., 2002). While many of these studies have suggested that these osteogenic phenomena are associated with the “tension-stress” created by the distraction process, little formal quantification of the physical environment within the distraction gap has been performed.

The goal of this study was to characterize, both spatially and temporally, the physical environment within the distraction gap, using distraction osteogenesis in a long bone as a model system. A computational approach was used to quantify distributions of pressure, tensile strain, tissue dilatation and fluid flow velocity within and surrounding the distraction gap. Previous studies have indicated the importance of these stimuli in influencing mesenchymal stem cell fate (Angele et al., 2004;Angele et al., 2003;Carter et al., 1998;Prendergast et al., 1997;Takahashi et al., 1998), in regulating synthesis of ECM molecules (Hall et al., 1991;Smith et al., 1995;Smith et al., 1996) and in regulating reorganization of cytoskeletal components

(Begg et al., 1983; Pavalko et al., 1998) in multiple cell types. Accordingly, the spatiotemporal distributions of these stimuli are interpreted in the context of the current understanding of cell–ECM interactions and of the mechanobiology of regenerating skeletal tissues.

2. Experimental procedures

A finite element model of a long bone diaphysis with a transverse osteotomy and regenerate, or callus, in the mid-diaphyseal region was created (Fig. 2, TrueGrid, XYZ Scientific Applications, Livermore, CA). The geometry and scale of the model were based on a pre-existing finite element model (Carter et al., 1998). The diaphysis and regenerate were idealized as being cylindrical in shape, with the regenerate having a slightly enlarged outer diameter across the osteotomy gap. The dimensions of the diaphysis were representative of those of the human tibia (endosteal diameter=12.8 mm, periosteal diameter=20 mm). Due to symmetry about the diaphyseal axis and about the midline of the gap, the analysis could be reduced to two dimensions, with only one-quarter of the longitudinal cross-section modeled (Fig. 2b and c). The model consisted of 5964 nodes and 5264 axisymmetric, poroelastic, first order quadrilateral elements (CAX4P, Abaqus 6.2, Abaqus, Inc., Rising Sun, RI).

All tissues (cortical bone, bone marrow, callus and mesenchymal tissue) were modeled as poroelastic materials. Poroelastic materials, which include nearly all biological tissues, are those that consist of a porous solid matrix (the ECM) saturated with interstitial fluid. The fluid flows through the solid matrix in response to gradients in pressure (a local force intensity that acts equally in all directions, resulting in either a pressurized or vacuum-like state). The velocity of the fluid flow depends on both the magnitude of the local pressure gradient and the permeability of the tissue. High pressure gradients and high tissue permeability result in high fluid velocities. Due to the occurrence of fluid flow, poroelastic materials exhibit time-dependent behavior such that pressures and strains (local deformations) can change over time even when the applied forces or displacements are constant. In addition, tissue permeability can change as the tissue deforms. For example, changes in the pore size of the solid matrix due to tissue dilatation will alter the permeability. Thus, the overall mechanical properties of poroelastic materials depend on the mechanical properties of, and the interaction between, the solid matrix and interstitial fluid. The poroelastic material properties for the tissues used in this model of distraction osteogenesis were assigned based on values in the literature (Table 1). A commonly used relationship for granular materials was used to describe the dependence of the permeability of the soft tissues on porosity (Simon, 1992).

The finite element analysis simulated the first 12-h period of a distraction protocol involving a rate of 2 mm per 12 h and a rhythm of one distraction every 12 h (i.e. only one 2-mm distraction was simulated). A tensile displacement of 1 mm was applied to the diaphysis on each side of the osteotomy gap over a time interval of 1 s and was then held constant for 12 h (Fig. 2c). Thus, the osteotomy gap, which was initially 5 mm wide, was increased to 7 mm wide. Due to the large difference in stiffness between the cortical bone and mesenchymal tissue, large strain gradients and excessive distortion of the finite elements occurred in the mesenchymal tissue near the corners of the osteotomized ends at applied tensile displacements greater than 0.375 mm. Accordingly, a remeshing algorithm (Espinosa et al., 1998) was employed at five separate times (at 0.375 mm and at every 0.125 mm afterwards) during the displacement ramp to 1 mm in order to relieve the mesh distortion and allow the analysis to continue.

Distributions of pressure, tensile strain, tissue dilatation and fluid velocity within and surrounding the distraction gap were analyzed over the 12-h period following the 2-mm distraction. Pressures in the fluid and solid phases were added to yield the total pressure. Tensile strain was defined as the maximum principal tensile strain (the component of strain

representing the largest amount of elongation), and tissue dilatation was defined as the change in volume of a given region per unit volume, i.e. $\Delta V / V_0$ where V_0 is the original volume.

3. Results

Pressure, tensile strain, tissue dilatation and fluid velocity varied substantially throughout the regenerate (Fig. 3). Magnitudes of all of these stimuli were highest in the soft tissue adjacent to the osteotomized cortex. In this region, maximum values of total pressure, tensile strain, tissue dilatation and fluid velocity were 0.27 MPa, 99% strain, 43% strain and 40 $\mu\text{m/s}$, respectively. Regions within the distraction gap experienced mild negative (tensile) pressures, substantial tissue dilatation, large tensile strains along the direction the distraction vector and fluid influx. Endosteal regions adjacent to the osteotomized cortex also experienced negative pressures and tissue dilatation, but with only moderate tensile strains and minimal fluid flow. In contrast, periosteal regions adjacent to the osteotomy site experienced moderate positive (compressive) pressures, tissue compaction (negative dilatation), high tensile strains and fluid efflux.

All four mechanical stimuli also changed markedly over time. Maximum values of total pressure, tensile strain and fluid velocity were achieved immediately after the 2-mm distraction was applied, whereas the maximum dilatation was achieved at the end of the subsequent 12-h period. Within the first 20 min, total pressures decayed to less than 31% of the initial values (Fig. 4). Fluid velocities decreased even more rapidly, reaching zero everywhere within 10 min. Tensile strains decreased to 57% of the initial values near the periosteal edge of the distraction gap but exhibited much smaller changes with time in all other regions. In marked contrast to the temporal decay observed in pressures, fluid velocities and tensile strains, tissue dilatation *increased* from near zero to as much as 43% strain over the first 20 min following distraction and remained nearly constant with respect to time for the remainder of the 12-h period.

The net effect of these temporal changes in tissue dilatation and tensile strain can be seen by examining the overall changes in the shape of the regenerate over time (Fig. 5). The net lengthening of the regenerate caused by the applied distraction was initially accompanied by contraction of the regenerate in the transverse plane. Over time, this contraction dissipated, resulting in pure elongation of the regenerate along the direction of the distraction vector.

4. Discussion

In light of the growing interest in quantifying the role of mechanical cues in modulating skeletal tissue differentiation, the overall goal of this study was to characterize the physical environment created within the regenerate during long bone distraction osteogenesis. The results indicate that overall distraction creates complex and dynamic distributions of mechanical stimuli within and surrounding the distraction gap. Large gradients in pressure, tensile strain, tissue dilatation and fluid velocity were found throughout the regenerate, and values of these stimuli also varied substantially over time. In the context of bone formation, perhaps the most notable result is that stretch, in the form of tensile strain and tissue dilatation, is large and essentially static within the gap over much of the period of time between one distraction and the next. This is in sharp contrast to the highly transient nature of the pressures and fluid velocities. Thus, the distraction gap, which is the site of robust bone formation, is exposed to prolonged stretch-related stimuli but very short-lived pressure-related and flow-related stimuli.

This study has provided the first estimates of the time-dependent, local physical environment present during the lengthening phase of distraction osteogenesis. These estimates constitute an important step for elucidating quantitative relationships between mechanical stimuli and

osteogenesis that could be applied to improve clinical outcomes in distraction osteogenesis. More generally, such relationships could be applied to augment bone formation in a broad range of clinical scenarios.

There are several limitations of the analyses presented here. Due to the relatively high distraction rate (2 mm per 12 h as compared to approximately 1 mm per 24 h in clinical practice), it is likely that the present estimates of the magnitudes of the mechanical stimuli are higher than those that occur clinically. However, due to the use of linear material properties for the tissues, any overestimation will be uniform. Consequently, the temporal changes and the spatial distributions, or patterns, of these stimuli are largely independent of the distraction rate. Additional limitations of this study relate to three sets of simplifications or assumptions made in the modeling process. First, the model did not account for any irregularities in shape of the diaphysis and regenerate and instead idealized these as cylindrical. This simplification is expected to affect somewhat the distributions of the four physical stimuli investigated in this study. However, the shape of the osteotomy, which is formed via a transverse, full-thickness cut through the long bone diaphysis, is represented well in the model. Thus, we expect that the distributions of pressures, strains and fluid flow are most accurate within the gap as compared to in the peripheral regions and that these distributions are reasonable estimates of the actual physical environment within the gap. Second, the model did not include the presence of any peripheral soft tissues, most notably muscle. Active and resting tension in the muscles adjacent to the regenerate and diaphysis create forces that oppose the distraction and may consequently alter the distributions of mechanical stimuli throughout the regenerate in ways that are not captured in the present analysis.

The third set of assumptions relates to the material properties of the regenerate and medullary tissues. Due to the paucity of experimental data available on these properties, we assigned typical values from the literature and we verified through an ancillary parameter study that the conclusions drawn from our results are not specific to the particular set of material properties used in the present analysis (Appendix A). Further, only one set of material properties was used for both the regenerate and medullary tissue. This rendered the analysis most applicable to the early stages of the distraction phase, when only granular and loose connective tissues, rather than mineralized tissues, are present in the regenerate (Rauch et al., 2000; Tay et al., 1998). We also assumed that the tissues did not become damaged during distraction. The tensile strains in some regions are sufficiently high such tissue damage likely occurs (Fig. 3); this is consistent with recent experimental evidence that each bi-daily distraction does induce damage (Loboa et al., 2004). It is clear that more experimental data regarding the poroelastic material properties and failure properties of regenerate tissue are needed.

These findings raise two key questions regarding the effect of the duration of a given physical stimulus on the differentiation of skeletal tissues. The first is what duration is *sufficient* to influence differentiation. Within minutes following each distraction event, pressure and fluid velocity decay to zero or near zero throughout all regions within and surrounding the distraction gap. The tissue compaction (negative tissue dilatation) observed in the periosteal regions is similarly short-lived. As noted in previous studies (Carter et al., 1998; Loboa et al., in press), these periosteal regions are of interest in that they are the only locations within the callus and gap that experience both positive pressures and tissue compaction (Fig. 3), and they are also the only locations in which cartilage formation predominates (Tay et al., 1998). This cartilage subsequently undergoes endochondral ossification. Based on the temporal profiles presented in Fig. 4, these findings suggest that very brief periods of pressure and compaction may be sufficient to shunt mesenchymal tissue down a chondrogenic pathway. While it has been shown previously that commitment of mesenchymal stem cells to a certain cell fate can occur within several days (Le et al., 2001), no experimental studies to our knowledge have investigated the

effects of periods of mechanical stimulation that last only a fraction of an hour. Consequently, the impact of such short periods of stimulation on bone and cartilage formation is not known.

The second question raised by the present results is whether skeletal tissue differentiation is influenced differently by static, as opposed to cyclic, mechanical stimuli. The effects of cyclic stimuli have been investigated *in vivo* and *in vitro* in various skeletal tissues and cell types, motivated by the observation that many activities of daily living such as walking create oscillatory mechanical stimuli. In contrast, the findings presented here indicate that the temporal nature of the physical environment created by distraction is markedly different. The tissue within the distraction gap experiences brief, intermittently imposed pressures and fluid flow, but near static tensile stretch. The osteogenic effect of static, equibiaxial tensile stretch applied to osteoblasts *in vitro* has been shown previously (Fong et al., 2003). These results are consistent with the spatial correspondence observed between the regions experiencing static tensile stretch and those in which membranous bone formation occurs (Choi et al., 2002; Tay et al., 1998; Yasui et al., 1997; Yeung et al., 2002). At present, however, it is not known if it is the static nature of the physical stimulus *per se* that promotes these osteogenic phenomena. Although static compression is known to affect chondrocyte metabolism (Hunter et al., 2004; Kim et al., 1994; Quinn et al., 1998) and growth plate morphogenesis (Robling et al., 2001) differently than cyclic compression, it remains to be seen if this is also the case for mesenchymal precursor cells and osteoblasts. A distraction experiment that compares the effect of a cyclic displacement oscillating between 0 and 2 mm to that of the constant displacement used typically in DO would provide valuable insight into this matter.

In the context of cell–ECM interactions, the large amounts of static tissue dilatation that occur within the distraction gap are notable because they create large changes in local tissue density. During a period of time in which the rates of ECM production and degradation are such that the mass of a given region of tissue remains constant, the change in tissue density, $\Delta\rho(=m/\Delta V)$, is directly related to the tissue dilatation, $e(=\Delta V/V_0)$, by

$$\Delta\rho = -e / (1 + e) \quad (1)$$

where m is the tissue mass, V_0 the original tissue volume, ρ the tissue density and e the tissue dilatation. Thus, the positive dilatation observed throughout the distraction gap corresponds to large reductions in tissue density in these regions (Fig. 6). Moreover, these reductions in tissue density are not uniform throughout the gap but instead differ by as much as 23% over distances of less than 500 μm . Numerous studies on various cell types have demonstrated that cell and ECM densities and density gradients affect rates of cell proliferation, differentiation, migration and apoptosis (Harris et al., 1984, 1981; Ingber and Folkman, 1989; Vailhe et al., 1997). Such evidence has formed the basis for development of “mechanochemical models”, which have been used to study problems as diverse as mesenchymal condensation during formation of the skeletal anlage (Oster et al., 1983) and tissue revascularization during wound healing (Olsen et al., 1997). These collective findings suggest that tissue dilatation may serve as a key stimulus for bone formation via altering cell and ECM densities as well as stretching of cells and their matrix. Further, the occurrence of tissue dilatation after the tissue is damaged by the distraction event may provide a particularly conducive environment for osteogenesis due to potential coupling between stretch, density and inflammatory effects.

As such, the results of the present study provide a strong link between the mechanobiological phenomena observed in distraction osteogenesis and what is continuing to emerge regarding the role of the extracellular matrix in regulating cellular activity. While the complexity of the extracellular milieu presents a large number of candidate mechanisms and pathways involved in mechanically stimulated bone regeneration, these results strongly motivate further investigation of integrin-mediated and inflammation-induced signaling in DO. In addition, they

suggest the potential of using an in vitro approach to examine the independent and possibly synergistic effects of stretch and cell and ECM densities on mesenchymal tissue differentiation.

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Appendix A

An ancillary parameter study was conducted in order to assess the sensitivity of the spatial and temporal distributions of mechanical stimuli throughout the regenerate to the particular values of material properties used for the regenerate tissue and to the size of the regenerate. Six alternate sets of material properties and two alternate diameters of the regenerate were investigated (Table 2). Each combination of material properties and regenerate size was formed by changing the value of only one parameter (a material property or the regenerate diameter) with respect to the combination used in the main study (the “baseline” combination). In all cases, a distraction rate and rhythm of 2 mm every 12 h was simulated, as illustrated in Fig. 2.

Regardless of the values of material properties and size of the regenerate, pressures and fluid velocities decayed rapidly, while tissue dilatation increased to large steady state values over the 12-h time period (Fig. 7). In all cases, pressures throughout the regenerate decreased to less than 39% of the initial values within 25 min and fluid velocities decayed to zero within 11 min (data not shown). As expected, increasing the tissue permeability resulted in more rapid temporal changes in the stimuli.

The spatial distributions of the mechanical stimuli were only moderately affected by the choice of material properties and regenerate size. Increasing the elastic modulus and decreasing the regenerate size resulted in more uniform distributions of tensile strain and tissue dilatation (Fig. 7). However, the locations experiencing the most extreme values of each of the four mechanical stimuli remained the same for all combinations of material properties and regenerate diameters. Taken together, these ancillary results indicate that, for a broad range of reasonable estimates of tissue material properties and regenerate sizes, the conclusions made in the main study are largely unaffected by the choice of material properties and regenerate size.

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Fig 1.

Tissue dilatation (also known as volumetric strain) represents equal deformation of a region of tissue in all directions; this deformation results in a change in the volume that the tissue occupies. Positive dilatation corresponds to an increase in the occupied volume (A), while negative dilatation corresponds to a decrease in the occupied volume (B).

Fig 2.

(A) The diaphysis of a long bone undergoing distraction osteogenesis is idealized as a hollow cylinder with a transverse osteotomy gap. The regenerate is also represented as cylindrical in shape with an enlarged diameter across the osteotomy gap. (B) A longitudinal cross-section of osteotomized diaphysis shows the locations of cortical bone, osteotomy gap, and regenerate and medullary tissues. Idealizing the diaphysis and regenerate as cylindrical renders this cross-section symmetric about the diaphyseal axis and about the midline of the gap (shown with dotted lines). (C) As a result of this symmetry, a finite element model of only one-quarter of the cross-section is sufficient to capture the behavior of the entire section of the diaphysis shown at the bottom of (A). A 1-mm tensile displacement is applied to the diaphysis on each side of the gap, resulting in a 2-mm distraction. An enlarged view of the quarter of the cross-section that is explicitly modeled shows the finite element mesh as well as the boundary conditions that enforce the symmetry of the problem: no displacement or fluid flow in the transverse direction is allowed across the diaphyseal axis; and no displacement or fluid flow in the longitudinal direction is allowed across the midline of the gap. However, fluid may flow throughout other regions of the regenerate, the cortical bone and the surrounding medullary tissues. The cortex is outlined with a heavy line.

Fig 3.

Magnitudes of total pressure, tensile strain, tissue dilatation and fluid velocity vary substantially throughout the regenerate. For most of these mechanical stimuli (total pressure, tensile strain and fluid velocity), these gradients are large immediately after the 2-mm distraction (1 s), whereas the gradients in tissue dilatation are highest 12 h after distraction.

Fig 4.

Temporal profiles of (A) total pressure, (B) tensile strain, (C) tissue dilatation and (D) fluid velocity at six locations within and surrounding the osteotomy gap in the 12 h following the 2-mm distraction.

Fig 5.

The 2-mm distraction initially causes contraction of the regenerate in the transverse anatomical plane (perpendicular to the distraction vector). The magnitude of this contraction is depicted by the arrows. Over the subsequent 12-h period, the contraction completely dissipates, leaving pure elongation of the regenerate, as illustrated with the aid of the vertical lines.

Fig 6.
Temporal profiles of changes in tissue density at the locations depicted in Fig. 4.

Fig 7.

Tissue dilatation throughout the regenerate as a function of time for the different combinations of poroelastic material properties and different diameters of the regenerate listed in Table 2. (A)–(F) represent the six locations, A–F, shown in Fig. 4, respectively. Results corresponding to the combination of properties and regenerate size used for the main study (the “baseline” combination) are represented with the open triangles and a heavier line. Labels in the legend indicate how the combination of properties and regenerate size differ from the baseline combination.

Table 1
Poroelastic material properties used in the finite element model

	Modulus (MPa)	Poisson's ratio	Permeability ^a (m ⁴ /N s)	Initial porosity
Cortical bone ^b	14,580	0.325	1×10^{-17}	0.04
Callus/medullary/ mesenchymal tissue ^c	0.2	0.0	1×10^{-13}	0.80

^aThe soft tissue permeability, k , is a function of current porosity, p , initial porosity, $p_0=0.8$, and initial permeability, $k_0=1 \times 10^{-13}$ m⁴/N s:

$$k = k_0 \left(\frac{p}{p_0} \right)^3 \left[\frac{1 - p_0}{1 - p} \right]^2 \quad (\text{Simon, 1992}).$$

^bCowin (1999).

^cLacroix and Prendergast (2002) and Perren and Cordey (1980).

Table 2

Combinations of the poroelastic material properties and size that were investigated for the regenerate

Modulus (MPa)	Poisson's ratio	Permeability ($\text{m}^4/\text{N s}$)	Initial porosity	Diameter (mm)
0.2	0.0	1×10^{-13}	0.80	34
0.1	0.0	1×10^{-13}	0.80	34
0.4	0.0	1×10^{-13}	0.80	34
0.2	0.0	1×10^{-12}	0.80	34
0.2	0.0	1×10^{-14}	0.80	34
0.2	0.0	1×10^{-13}	0.70	34
0.2	0.0	1×10^{-13}	0.90	34
0.2	0.0	1×10^{-13}	0.80	30
0.2	0.0	1×10^{-13}	0.80	38

"Diameter" is the diameter of the regenerate at its widest point (at the midline of the osteotomy gap). The first row represents the combination of material properties and diameter used in the main study (Figs. 3 - 6).