BIOPHYSICAL STIMULI ON CELLS DURING TISSUE DIFFERENTIATION AT IMPLANT INTERFACES

P. J. Prendergast,* R. Huiskes* and K. Soballe†

*Biomechanics Section, Institute of Orthopaedics, University of Nijmegen, Nijmegen, The Netherlands; †Department of Mechanical Engineering, Trinity College, Dublin, Ireland; ‡Department of Orthopaedics and Institute of Experimental Clinical Research, University Hospital of Aarhus, Denmark

ABSTRACT—If musculoskeletal tissues are indeed efficient for their mechanical function, it is most reasonable to assume that this is achieved because the mechanical environment in the tissue influences cell differentiation and expression. Although mechanical stimuli can influence the transport of bioactive factors, cell deformation and cytoskeletal strain, the question of whether or not they have the potential to regulate tissue differentiation sequences (for example, during fracture healing or embryogenesis) has not been answered.

To assess the feasibility of biophysical stimuli as mediators of tissue differentiation, we analysed interfacial tissue formation adjacent to a micromotion device implanted into the condyles of dogs. A biphasic finite element model was used and the mechanical environment in the tissue was characterised in terms of (i) forces opposing implant motion, (ii) relative velocity between constituents, (iii) fluid pressure, (iv) deformation of the tissue and (v) strain in cells on the interfacial tissue. Specifically, as the forces opposing motion increase, the implant changes from being controlled by the maximum-allowable displacement (motion-control) to being controlled by the maximum-available load (force-control). This causes a decrease in the velocity of the fluid phase relative to the solid phase and a drop in interstitial fluid pressure accompanied by a reduction in peri-prosthetic tissue strains. The variation of biophysical stimuli within the tissue can be plotted as a 'mechano-regulatory pathway', which identifies the transition from motion-control to force-control as a branching event in the tissue differentiation sequence.

KEYWORDS: Tissue differentiation; Cell stimuli; Morphogenesis; Micromotion; Implant stability.

INTRODUCTION

It is an axiom of biomechanics that load-bearing tissues of the adult skeleton have reached a certain 'efficiency' with respect to their mechanical function (Carter et al., 1996; Mow et al., 1992). This could be achieved if the mechanical function generates appropriate patterns of biophysical stimuli in the tissue and if these biophysical stimuli could, in turn, be sensed by cells as part of a regulatory process. Cell sensitivity to biophysical stimuli has been studied intensively and mechanisms have been proposed—for example, biophysical stimuli alter cell shape which influences the cellular interaction with the biochemical environment (Stein and Bronner, 1989). It is presumed that the relationship between the mechanical forces on cells and biochemical environment generated by cell expressions, ultimately generates a harmonious relationship between the tissue and its mechanical function.

The events during the tissue formation processes of skeletal regeneration have been characterized (Pan and Einhorn, 1992) as (i) pooling of mesenchymal cells, (ii) mesenchymal cell differentiation and expression of collagen, (iii) calcification and angiogenesis accompanied by remodelling and (iv) osteoblast proliferation and bone formation. Wakitani et al. (1994) described chondral defect repair as a sequence starting with invasion of bioactive factors into the defect site, followed by differentiation of mesenchymal cells into chondrocytes. Collagen is then expressed interstitially to form a specialised collagenous network of water and ionised proteoglycan constituents. In both cases, the first event is the conglomeration of mesenchymal cells within the granulation tissue. The mesenchymal cells must then differentiate to form those cells which are capable of manufacturing the collagenous constituents of the intermediate and final load-bearing tissues. Fibrous connective tissues are generated by fibroblasts and cartilaginous tissues are generated by chondrocytes (Caplan, 1994). Cells committed to becoming osteoblasts and forming bone (osteoprogenitor cells) originate in the pluripotent cell pool of the stroma and they eventually lay down bony matrix (Owen, 1980).

Regarding the bone/implant interface, it is believed that the skeletal regeneration required for implant integration and stability is determined by micromotion at the bone/implant interface (Boye and Jones, 1985; Weinans...
et al., 1993). Sometimes a fibrous tissue layer develops, leading to loosening of the prosthesis, sometimes fibrocartilage develops, and sometimes tissue differentiation occurs to form bone (Bechtold et al., 1995; Rose et al., 1984). Control of fibrous tissue differentiation at the bony implant bed is therefore important for implant/bone integration and long-term implant stability (Carter and Giori, 1991; Rubin and McLeod, 1994).

In pursuit of a further understanding of tissue differentiation, we calculated biophysical stimuli within regenerating tissues and attempted to relate the results with the survival of cells and the differentiation of new cell populations. We analysed an experiment previously reported by Soballe (1993a) by modelling the tissue as a biphasic material of both solid and fluid constituents (Mow et al., 1980). We hypothesise that the mechanical environment is changed in a sufficiently systematic way that biophysical stimuli could stimulate the replacement of one cell population by another. If this is true then the regeneration of musculoskeletal tissues could be viewed as a tissue differentiation sequence that continues until a tissue type is formed which transfers appropriate biophysical stimuli, through the ECM, to the cells under functional loading.

METHODS

Review of the biological experiment

Soballe (1993a) reported an investigation of tissue regeneration during gap healing around a specially designed micromotion device implanted into the condyles of dogs (weight 21–35 kg), see Fig. 1.

Different tissues were found in the peri-implant gap as a function of (i) time after implantation, (ii) magnitude of micromotion, and (iii) implant-coating characteristics. Two levels of micromovement were used: 150 μm (Soballe et al., 1992a) and 500 μm (Soballe et al., 1992b). Two types of implant coating were used for each level of micromotion: plasma-sprayed titanium alloy implants and plasma-sprayed hydroxyapatite-coated implants. In Soballe et al. (1992a, b), all implants were subjected to micromotion for 4 weeks and the results were compared to stable controls. In a further study (Soballe et al., 1993b), the 150 μm micromotion results were extended to 16 weeks, using two loading regimes: (i) 16 weeks of loading and (ii) four weeks of loading followed by 12 weeks of immobilisation by cutting the polyethylene plug.

Push-out tests of the interfacial gap tissue were carried out for every experiment. Slices were cut of the implant/gap/bone system and placed on a rigid surface in which a 3.5 mm radius hole had been drilled. Given an implant radius of 3 mm, this meant that the implants were supported to within 500 μm of the tissue/implant interface. The implants were then preloaded to 2 N and held for approximately 10 s before application of a ramp displacement rate of 5 mm/min. The force/deflection curves were recorded. We have collected the data and plotted interfacial strength against time (Fig. 2). It is then easily seen that interfacial tissue growth is a process whose rate is retarded by greater micromotion and whose (initial) rate is increased by hydroxyapatite coating.

Interface tissues were also submitted to histological analysis. For the initial low strength phase, the tissue was described as predominantly 'fibrous connective tissue'; see Fig. 2. This included all the four-week experiments for the Ti-coated implants and only the 500 μm four-week experiment for the HA-coated implants. In the next phase, which is characterized by somewhat superior strength, the tissue included fibrocartilage. This included the four-week results for those HA implants which were stable and those subjected to the lower micromotion level of 150 μm, and the 16-week results for the 150 μm Ti implants. The third and the final phase was characterized by high strength. This included all the 16-week results, except the 16-week results for the 150 μm Ti-coated implants which are only in the second phase. The histological analysis showed that this final phase may be described as 'various amounts of bone' with some fibrocartilage.

From Jayes and Alexander (1978), it can be calculated that the maximum force generated at the canine knee is of the order of 300 N. Therefore, it is possible that, as the interfacial tissue stiffens, the implant will no longer displace the full amount of motion. Under such circumstances, motion-control would give way to force-control; a maximum load acts rather than a maximum displacement.

A mathematical description of the problem using the biphasic theory

Mow et al. (1980) present the biphasic theory as a development of the theory of mixtures. The principle of equipresence can be used to assert that each constituent of the mixture is present at each material point. Hence, a volume containing v constituents is given as

$$dV = dV^1 + dV^2 + \cdots + dV^v = \sum_{i=1}^{v} dV^i.$$  (1)
where z denotes the zth constituent. An apparent density ρ*, and a true density ρT can be defined for each constituent as

\[ ρ^* = \frac{dm^*}{dV}, \tag{2a} \]

\[ ρ^T = \frac{dm^*}{dV^*}, \tag{2b} \]

where \( dm^* \) denotes mass of the zth constituent. The volume fraction of the zth constituent is given by \( ϕ^z = dV^z/dV \) and the particles of each constituent combine so as the apparent densities sum to the true density and the volume fractions sum to one. In a coordinate frame attached to the material point (material description) we write

\[ \frac{D}{DT}(ρ^* dV^z) = \frac{D}{DT}(ρ^T ϕ^z dV) = 0. \tag{3} \]

Note that the apparent density, \( ρ^* \), changes, whereas, assuming incompressible constituents, the true density does not change, i.e. \( Dρ^T/DT = 0 \). Describing the rate of volume change in terms of the local velocity components and converting to spatial coordinates, we get

\[ \frac{∂ϕ^z}{∂t} + V \cdot (ϕ^z v^z) = c^z, \tag{4} \]

where V is the gradient operator. Following Kelly (1964), the quantity \( c^z \) is included to describe the rate of supply of the zth constituent from all other constituents due to the reactive nature of the mixture. Cowin and Hegedus (1976) write a similar mass balance for the solid constituent only. The sum of the first and last terms of equation (4) is zero because, for the first term, if one constituent is displaced out of the differential volume, the space must be filled by another constituent, and for the second term, the reactions between constituents cannot create new matter. This gives the continuity equation for the mixture as

\[ ∑_{z=1}^{n} V \cdot (ϕ^z v^z) = 0. \tag{5} \]

Conservation of linear momentum gives the equation of motion for the zth constituent as

\[ V \cdot σ^z + ρ^* q^z + π^z + ρ^z c^z v^z - ρ^z \frac{Dv^z}{DT} = 0, \tag{6} \]

where \( σ^z \) is the partial stress, \( q^z \) is the body force per unit mass and \( π^z \) is the rate of momentum supply to the zth constituent and \( ρ^z c^z v^z \) is the momentum supply from biochemical reactions (Kelly, 1964). The internal forces resulting from such reactions can contribute to the partial stresses. This approach assumes that the particles coming into the zth constituent are kinematically indistinguishable from any pre-existing z constituent. The balance of linear momentum for the mixture as a whole requires that the sum of the momentum supplies is zero.

The constitutive relationships must satisfy thermodynamic constraints (i.e. the energy balance and the entropy inequality), as described by Mow et al. (1980). For a biphasic material in which the fluid is inviscid and each
Fig. 3. Force/displacement curves recorded during push-out tests for stable titanium, unstable titanium, stable hydroxyapatite and unstable hydroxyapatite. The thickness of the push-out slice was different in each case, being equal to 2.69, 2.47, 2.62 and 2.75 mm, respectively.

constituent is isotropic, and where the infinitesimal strain–displacement relationship is assumed, we can write, following Mow et al. (1980);

\[ \sigma^s = \phi p^t I + \lambda^s \varepsilon^t I + 2\mu^s \varepsilon^s, \]  
\[ \varepsilon = \phi p^t I, \]  

Where \( \varepsilon \) denotes strain and \( \varepsilon^s \) denotes the dilatational strain, \( \phi \) and \( \varepsilon^t \) denote solid-phase and fluid-phase quantities, \( p \) is the apparent pressure and \( \lambda^s \) and \( \mu^s \) are the Lamé constants. Based on equation (7b) we could conclude that the divergence of the fluid stress is

\[ V \cdot \sigma = -\nabla p + \phi \nabla \cdot \varepsilon + \phi \nabla \cdot \varepsilon. \]  

The first term in equation (8), called the 'buoyancy force', is due to the resultant of the fluid pressure acting on the solid phase. For a medium with homogeneous porosity, this force is zero. Comparing with equation (6) and, given Darcy’s law (Atkin and Craine, 1976), an expression for \( \sigma^s \) in a mixture can be deduced as \( \sigma^s = K (\varepsilon - \varepsilon^s) \). The momentum supply is due to the drag of the fluid phase against the solid phase at the fluid/solid interface. \( K \) is the diffusive drag coefficient and is related to permeability (Lai and Mow, 1980). To calculate the momentum supply from mass exchanges between the phases as a result of biochemical reactions we need an expression for one of either \( c^s(t) \) or \( c^t(t) \). In this study, the term was calculated using an equation of the form \( c^s(t) = Ct^n \) where \( C \) and \( n \) are empirical constants.

Finite element model of peri-implant tissue

A finite element model was used to analyse the tissues, implemented using the soil mechanics capability of MARC (Palo Alto, U.S.A.; see Prendergast et al. (1996). The approach used eight-noded isoparametric elements which have pressure degrees-of-freedom at the corner nodes only. Thus, pressure is linearly interpolated within the element. The solution was iterated using a backward Euler time-stepping scheme.

Push-out tests were used to determine Young’s modulus of the interface tissues. Using axisymmetric finite element models for each push-out test, Young’s modulus was estimated which gave the experimental force/deflection relationships for four different stages of interfacial fibrous tissue formation (see Fig. 3): Tissue 1—fibrous connective tissue, Tissue 2—inclusion of fibrocartilage, Tissue 3—fibrous tissue with small amounts of bone and Tissue 4—fibrous tissue with greater amounts of bone. A significant problem arises because the mechanical properties of the soft fibrous gap tissues have not been fully determined in mechanical tests. The permeability of the gap tissue had very little effect on the push-out analyses so it could not be identified. For the final analyses, estimates of permeability were made based on values reported for similar tissues (Armstrong and Mow, 1980; Levick, 1987) and from the fact that permeability decreases as solid fraction increases (Meijer, 1984; Simon, 1992). The cancellous bone was modelled as a biphasic material of Young’s modulus equal to 4590 MPa (Choi et al., 1990). The measured permeability for cancellous bone varies widely. Ochoa and Hillbery (1992) present 3.7 x 10^{-13} m^4/N s as the average value in the proximal tibia and this value was used in the analyses.

An axisymmetric finite element mesh was used (Fig. 4). A prescribed axial displacement which increased to 150 \( \mu \text{m} \) in 0.5 s and reduced to zero in 0.5 s followed by 1 s at zero was applied to the implant/gap boundary. For force-control, motion continued until a maximum force of 300 N was obtained and held. The cancellous border was mechanically restrained and no fluid flow was permitted across the boundary. A zero pressure was prescribed at the distal gap.

The momentum transfer due to the reactivity of the gap tissue was expected to be small compared with the momentum transfer due to Stokes’ drag. If it is assumed that a maximum solidity \( \phi_{\text{max}} \) develops in a time \( T \), then \( C = (n + 1)\phi_{\text{max}} / T \) (see the Appendix). Assuming, as a first approximation, that the solid-phase formation rate to be given by \( n = 0 \), and by using the observation of Seballe (1993a) that encapsulation by a fibrous phase \( (\phi_{\text{max}} \approx 0.2) \) was attained by four weeks, the reactivity term can be very roughly calculated and compared with the Stoke’s drag term.

RESULTS

The Young’s moduli of the interfacial tissues increased as tissue differentiation progressed; see Table 1. These results were obtained from the finite element models of the push-out tests.
The reaction forces calculated at 150 μm micromotion show that the forces opposing implant micromotion increase during interfacial tissue regeneration; this result holds true for the full permutation of tissue properties given in Table 1 (see Fig. 5). The effect of decreasing the permeability is to further increase the reaction force, but only slightly. Given an estimated available force of 300 N at the knee of dogs, the calculations reported in Fig. 5 imply that the implant will no longer displace the full 150 μm for tissue states 3 and 4. The transition will be 'blurred' somewhat due to the randomness of the loading. Nevertheless, we can focus on two different loading types. The first is motion-control where a certain micromotion is maintained during tissue formation. The second is force-control where a certain maximum reaction force is carried whatever the displacement.

**Peri-implant mechanical stimuli**

As tissue regeneration proceeds, the maximum cyclic pressure was predicted to increase during motion-control and decrease during force-control (Fig. 6), whereas the velocity (Fig. 7) was predicted to decrease whether motion-control or force-control operated, though the decrease is larger under force-control. Hence, we can see that the mechanical effect of greater reaction force is paralleled by more subtle changes of the mechanical environment within the gap. Pressure and velocity were sampled at a position mid-way down the implant and half-way across the gap (Fig. 4), a similar position from where histological samples were taken by Serballe et al. (1992a, b; 1993) and Soballe (1993). Pressure and velocity did not vary substantially across the gap but there was a definite variation in the vertical direction indicating the importance of taking the histological samples from the same vertical position.

The need for a biphasic analysis is highlighted by the extent of the deflection of the interfacial tissue from a straight line (i.e. the linear elastic solution) which occurs because the micromotion device acts like a 'piston', forcing fluid to flow first outwards through the distal gap and then back in through the distal gap; see inset of Fig. 7.

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**Table 1. Young’s moduli and permeability of the tissue used in the finite element analysis**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Young’s modulus (MPa)</th>
<th>Permeability (m⁴/N s)</th>
</tr>
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<tbody>
<tr>
<td>Tissue 1</td>
<td>2</td>
<td>1 x 10⁻³³</td>
</tr>
<tr>
<td>Tissue 2</td>
<td>10</td>
<td>5 x 10⁻¹⁵</td>
</tr>
<tr>
<td>Tissue 3</td>
<td>30</td>
<td>1 x 10⁻¹⁵</td>
</tr>
<tr>
<td>Tissue 4</td>
<td>70</td>
<td>5 x 10⁻¹⁰</td>
</tr>
</tbody>
</table>

Note: Compare Young’s moduli of the following tissues:
(i) Bovine miniscal cartilage $0.410 \pm 0.088$ MPa (Proctor et al., 1989).
(ii) Fibrous connective tissue $1.18 - 2.09$ MPa at $0.45$ MPa (Hori and Lewis, 1982).
(iii) Calcified cartilage $320 \pm 250$ MPa (Mente and Lewis, 1994).
(iv) Subchondral bone $1150 \pm 370$ MPa (Choi et al., 1990).
(v) Tibial bone tissue $4590 \pm 160$ MPa (Choi et al., 1990).

Compare the permeability of the following tissues:
(i) Meniscal cartilage $1.26 \times 10^{-13} \text{m}^4/\text{N s}$ (Spilker et al., 1992).
(ii) Human articular cartilage $4.7 \times 10^{-13} \pm 3.6 \times 10^{-12} \text{m}^4/\text{N s}$ (Armstrong and Mow, 1982).
(iii) Compact bone $1.0 \times 10^{-17} \text{m}^4/\text{N s}$ (Johnson et al., 1982).
(iv) Young canine tibial compact bone $3.53 \times 10^{-16} \pm 0.93 \times 10^{-16} \text{m}^4/\text{N s}$ (Li et al., 1987).
Fig. 6. The change of maximum cyclic pressure during tissue differentiation. The pressures generated when a full 150 μm was applied (motion-control) and when a maximum of 300 N was applied (force-control) are shown. The dark bars indicate the most likely combination of Young’s modulus and permeability: see Table 1. The sampling position given in Fig. 5.

Fig. 7. The change of maximum cyclic velocity during tissue differentiation. The pressures generated when a full 150 μm was applied (motion-control) and when a maximum of 300 N was applied (force-control) are shown. The dark bars indicate the most likely combination of Young’s modulus and permeability: see Table 1. The sampling position given in Fig. 5. The inset shows the cyclic velocity for Tissue 2 (i.e. with Young’s modulus of 10 MPa and permeability of 5 × 10⁻¹³ m⁴/Ns).

The result is a ‘pumping’ that generates a drag force in the tissue in the direction opposite to the motion of the implant. This effect is predicted to be dominant the early stages of tissue regeneration; see Fig. 8(a). As tissue differentiation progresses, the drag force is reduced relative to the forces generated by elastic stretching; see Fig. 9(a).

The Stokes’ drag force has a substantial effect on the shear strain in the gap tissue in the initial stages of interfacial tissue regeneration; see Fig. 8(b). The effect is
Biophysical stimuli on cells during tissue differentiation

Fig. 8. For Tissue 1 (Young's modulus = 2 MPa and permeability = $1 \times 10^{-14}$ m$^4$/N s); (a) deflection of the regenerating tissue during a loading cycle is nonlinear across the gap; (b) strain in the tissue during the loading cycle.

Fig. 9. For Tissue 4 (Young's modulus = 70 MPa and permeability = $5 \times 10^{-16}$ m$^4$/N s); (a) maximum deflection of the regenerating tissue, which is linear across the gap and (b) maximum strain in the tissue. Results are given for both motion-control and force-control emphasizing the fact that the transition to force-control allows the increase in collagen content in the gap to reduce the strains in the regenerating tissue.

to increase the shear strain near the surfaces and reduce it to zero somewhere within the gap (where the solid phase is under dilatational strain). This effect disappears as tissue regeneration progresses [Fig. 9(b)].

These predictions confirm a posteriori that, as a first approximation, it may be possible to neglect reactivity in the solution of the gap tissue partial stresses. The predicted average fluid velocity for Tissue 1, at the sampling point (see Fig. 4), is $6.6 \times 10^{-6}$ m s$^{-1}$ giving the Stokes' drag force [third term in equation (6)] of approximately $6.6 \times 10^8$ kg m$^{-2}$ s$^{-2}$. This is very much larger than the momentum transfer due to reactivity [fourth term in equation (6)], of approximately $1.5 \times 10^{-10}$ kg m$^{-2}$ s$^{-2}$.

**DISCUSSION**

Roux (1912) hypothesized that there is a 'competition to attract the functional stimulus' within the tissues of living organisms. The functional stimulus, according to this viewpoint, is different for different tissues: tension forms fibrous connective tissue, shearing forms cartilage and compression forms bone. Pauwels (1941, 1980) later proposed

"the distortion of the shape (elongation) . . . is . . . the specific stimulus for the formation of collagenous fibrils, the compression from every side (hydrostatic pressure) . . . [is the stimulus] . . . for the development of cartilaginous tissue. There is no specific mechanical stimulus for the formation of bony tissue".

According to this theory, therefore, bone does not form directly but through intermediate fibrous constituents. Interpreting Pauwels' theory for interface tissue formation: interfacial integration is a process which has ossification as its final outcome, but only if soft-tissue formation sufficiently stabilizes the mechanical environment to facilitate the required osteogenic pathway. The question of this paper is whether or not tissue differentiation in regenerating tissues could be related to biophysical stimuli in a systematic way. If this can be answered affirmative then biophysical stimuli could potentially have a regulatory influence on tissue
fluid velocities are very dependent on permeability. How-
able at the knee during gait, the predictions of the finite
and Soballe (1993) find eventual osteoblast proliferation.
predict to be a high strain, high fluid velocity mechanical
element model suggest that
state at this stage. However, given the limited force avail-
arise at which time the implant will not bottom-out. If
this is true, then the mechanical environment becomes
decreases. In this environment, Soballe (1993) and
matrix and align it, causing stiffening of the solid phase
later chondrocytes. The fibroblasts generate collagenous
environment in which Soballe et
increases during tissue differentiation, as in Table 1. It was predicted that the cyclic pressure
increases with decreased tissue permeability, but that the
effect did not overlap with the effect caused by Young’s
modulus. On the other hand, the predictions regarding fluid velocities are very dependent on permeability. How-
ever, since it is likely that the permeability decreases as collagen concentration increases. Based on these results, we
may safely assume some decrease in permeability as Young’s modulus increases during tissue differentiation,
as in Table 1. It was predicted that the cyclic pressure
The effect of momentum change due to reactivity has
been predicted to be small. However, the reason for this is
that we did assume a linear tissue formation rate. It is
worth noting that, in reality, tissue may form by locally
rapid or ‘explosive’ reactions which would generate large
local partial stresses which could have an influence on
cell-level stimuli.
The mechanical stimuli in the gap can be summarized.
In the beginning, the mechanical milieu is motion-control-
led. The cyclic pressure is lowest, the relative velocity
between the phases is highest and the shear strains near
the implant and bone surfaces are high. This is the
environment in which Soballe et al. (1992a, b; 1993) and
Soballe (1993) find extensive presence of fibroblasts and
later chondrocytes. The fibroblasts generate collagenous
matrix and align it, causing stiffening of the solid phase
and reducing the permeability (Levick, 1987). This causes
a further fluid pressure increase and a further relative
velocity decrease; the shear strain near the surface also
decreases. In this environment, Soballe (1993) and
Soballe et al. (1992a, b; 1993) find chondrocytes more so
than fibroblasts. If motion-control were to persist, then it
is likely that tissue development would reach a steady
state at this stage. However, given the limited force avail-
able at the knee during gait, the predictions of the finite
element model suggest that force-control will eventually
arise at which time the implant will not bottom-out. If
this is true, then the mechanical environment becomes
one of even lower velocity between fluid and solid, de-
creased fluid pressure and, most significantly, lower shear
strains. In this environment, Soballe et al. (1992a, b; 1993)
and Soballe (1993) find eventual osteoblast proliferation.
The question to be answered is whether or not cells in
the gap tissue are actually responding to the changed
biophysical stimuli. The cell pool available for tissue
regeneration consists of mesenchymal cells. Differenti-
ation to fibroblasts is possible (Owen, 1980), and prolif-
eration and migration of fibroblasts around the gap is the
first cellular event reported by Soballe et al. (1992b).
This leads to strengthening of the interfascial tissues in what we
predict to be a high strain, high fluid velocity mechanical
environment. Perhaps fibroblasts can maintain tractive
contact with the collagen in this environment (Stopak
and Harris, 1982). The next phase identified by Soballe
et al. is the development of fibrocartilage in which chon-
drocytes are present. Chondrocytes can differentiate from
the mesenchymal cell pool (Caplan, 1991). That this did
not happen directly could be because mesenchymal cells
will not differentiate into chondrocytes until a suitable
mechanical environment is present. According to Caplan
(1991)

“the key factor in the conversion of a mesenchymal cell
to a chondrocyte is maintaining the progenitor cell in
a round, unspread confirmation”.

This suggests that a reduction in the shearing (flow) of the
fluid is needed—and this is indeed provided due to syn-
thesis of collagen by fibroblasts and the attendant reduc-
tion of fluid velocity in the precursor cell pool. It fits with
the evidence of these analyses that fibroblasts create a
new mechanical environment (lower-flow, higher-pres-
sure, lower-shear strain) in which they are no longer
sustained. Furthermore, it is known that mechanical
stimuli regulate chondrocyte cell metabolism and syn-
thesis rates, and it is believed that chondrocytes have
baroreceptors to sense the pressure (Stockwell, 1987).
Therefore, the absence of a suited biophysical environ-
ment may be the reason why the chondrocyte cell popu-
lation is not maintained. Rather osteoblast proliferation
occurs and ossification proceeds. The present analysis
predicts that pressure reduces as the tissue becomes
stiffer when force-control loading is present. Reduced
pressure is then accompanied by a substantial reduction
in the relative velocity between the fluid and solid phases.
Therefore, a reduction in fluid/solid velocity in the
precursor cell pool, accompanied by a reduction in shear
strain in the solid phase, would seem to be the circum-
stances favouring osteoblast proliferation, and hence in-
terfacial ossification. It would therefore seem that cells
synthesize an extracellular matrix in which a biophysical
environment is set up which may or may not be suited to
survival of that cell when competing against the other cell
types capable of differentiating from the mesenchymal
cell pool; see Weinans and Prendergast (1996) for a more
general discussion of this point.
The hypothesised regulatory influence of mechanical
factors on interfacial tissue development can be repre-
sented graphically as a mechano-regulatory pathway.
Consider the mechanical environment to be represented
by, say, two biophysical stimuli, the shear strain (x-axis)
and cyclic fluid velocity (y-axis). The shear strain is a
measure of the mechanical stimulus in the solid and the
fluid velocity is a measure of the agitation in the precursor
cell pool. As time progresses, cells will enter pre-
programmed differentiation sequences and synthesize
collagenous matrices. Collagen synthesis will automati-
cally change the biophysical stimuli in the tissue. A tra-
jectory of the time course of change of shear strain and
cyclic fluid velocity can be drawn for any element in
regenerating tissue as follows. At the start, the mechani-
cal environment is one of the high surface shear strain
and high fluid velocity. This is represented by a point on
the t = 0 plane; see Fig. 10. Next, fibroblasts begin to
force-control

ing tissue. This suggests that there may be boundaries between mechanical states in the tissue such that, when the boundary is crossed, cell-driven biochemical reactions are initiated which switch the tissue from one type to another by tissue differentiation. Therefore, the results support the hypothesis that the mechanical environment in the tissue has a controlling influence on tissue differentiation. To prove the generality of this proposition, similar mechanical stimuli changes would need to be found at some stage the implant no longer ‘bottoms out’, at which point the mechanical stimuli changes to reduced fluid velocity and a reduction in the shear strain in the tissue would reduce and ossification will occur—but intermediate tissue types (tissue differentiation) may be required.

In conclusion, systematic changes in biophysical stimuli are predicted to occur in peri-implant regenerating tissue. This suggests that there may be boundaries between mechanical states in the tissue such that, when the boundary is crossed, cell-driven biochemical reactions are initiated which switch the tissue from one type to another by tissue differentiation. Therefore, the results support the hypothesis that the mechanical environment in the tissue has a controlling influence on tissue differentiation. To prove the generality of this proposition, similar mechanical stimuli changes would need to be found in other differentiating systems, such as during fracture healing or embryogenesis.

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In conclusion, systematic changes in biophysical stimuli are predicted to occur in peri-implant regenerating tissue. This suggests that there may be boundaries between mechanical states in the tissue such that, when the boundary is crossed, cell-driven biochemical reactions are initiated which switch the tissue from one type to another by tissue differentiation. Therefore, the results support the hypothesis that the mechanical environment in the tissue has a controlling influence on tissue differentiation. To prove the generality of this proposition, similar mechanical stimuli changes would need to be found in other differentiating systems, such as during fracture healing or embryogenesis.

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**APPENDIX**

\[ c^e = \text{rate of supply of the solid constituent, i.e.} \]

\[ \frac{dc^e}{dt} = c^e \]

\[ \text{substituting} \]

\[ c^e = \int c^e \, dt = \frac{C_{e+1}}{(n + 1)} \]

At time \( T \), \( C = \frac{C_{e+1}(n+1)}{T^{n+1}} \).