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Modelling cartilage mechanobiology

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The growth, maintenance and ossification of cartilage are fundamental to skeletal development and are regulated throughout life by the mechanical cues that are imposed by physical activities. Finite element computer analyses have been used to study the role of local tissue mechanics on endochondral ossification patterns, skeletal morphology and articular cartilage thickness distributions. Using single-phase continuum material representations of cartilage, the results have indicated that local intermittent hydrostatic pressure promotes cartilage maintenance. Cyclic tensile strains (or shear), however, promote cartilage growth and ossification. Because single-phase material models cannot capture fluid exudation in articular cartilage, poroelastic (or biphasic) solid/fluid models are often implemented to study joint mechanics. In the middle and deep layers of articular cartilage where poroelastic analyses predict little fluid exudation, the cartilage phenotype is maintained by cyclic fluid pressure (consistent with the single-phase theory). In superficial articular layers the chondrocytes are exposed to tangential tensile strain in addition to the high fluid pressure. Furthermore, there is fluid exudation and matrix consolidation, leading to cell 'flattening'. As a result, the superficial layer assumes an altered, more fibrous phenotype. These computer model predictions of cartilage mechanobiology are consistent with results of *in vitro* cell and tissue and molecular biology experiments.

Keywords: cartilage; skeletal development; mechanobiology; biological models

1. INTRODUCTION

Cartilage first appears in the endoskeleton during early embryonic patterning events that are regulated by the expression of *Hox* genes, signalling molecules and embryonic growth factors. The limb buds develop at discrete locations that coincide with regions where specific *Hox* genes are expressed. The growth of the limb buds proceeds under the coordination of the apical endothelial ridge and the zone of polarizing activity where fibroblast growth factor-4 and sonic hedgehog are expressed, respectively. The expression of these two embryonic growth factors is crucial in specifying the proximal–distal and the anterior–posterior patterns of the cartilage condensations that are the templates, or anlagen, of the future skeleton. Wnt, another embryonic growth factor, similarly regulates the organizational pattern of the cartilage rudiments in the ventral–dorsal axis.

In humans, the cartilage rudiment of the femur begins to form in the blastema of the lower limb bud at *ca.* 38 foetal days. The appearance of other rudiments shortly follows. The muscles are developed and begin involuntary contractions at *ca.* 48 days, at which time the hip joint forms and ossification of the cartilage endoskeleton begins. It is by the growth and ossification of the cartilage rudiments that the development of the mature skeleton is realized. An important aspect of this growth and ossification, however, is that it is regulated locally in the cartilage tissue by the stresses and strains that are created by muscle contractions, prenatally, postnatally and throughout life.

In a developing cartilage rudiment, one can recognize the same endochondral growth and ossification processes both at the primary ossification front and around secondary ossification sites (figure 1). There are regions of quiescence that are characterized by the presence of resting chondrocytes. As growth proceeds, these cells proliferate and then mature as they begin to increase the production of ECM, which is characterized by important cartilage molecules, aggrecan and collagen II. As these mature chondrocytes begin to hypertrophy, they express Ihh an important growth factor that upregulates the expression of bone morphogenetic proteins. In the late stages of chondrocyte hypertrophy, the cartilage ECM septa between the columns of hypertrophic chondrocytes calcify. There is increased expression of MMPs (MMP9, MMP13), vascular endothelial growth factor, collagen X and CTGF, in preparation for angiogenesis and ossification. The hypertrophic chondrocytes then undergo apoptosis, and vascular invasion is initiated through their vacant lacuna. Chondroclasts are recruited to the site and begin to resorb the calcified cartilage, eventually destroying two-thirds of the calcified matrix. Perivascular mesenchymal cells differentiate into osteoblasts and begin to form new osteoid on the remaining calcified septa. The osteoid mineralizes to form primary bone trabeculae and the growth and ossification process is complete.

In the mature skeleton, endoskeletal cartilage remains primarily at the joints in the form of what has become

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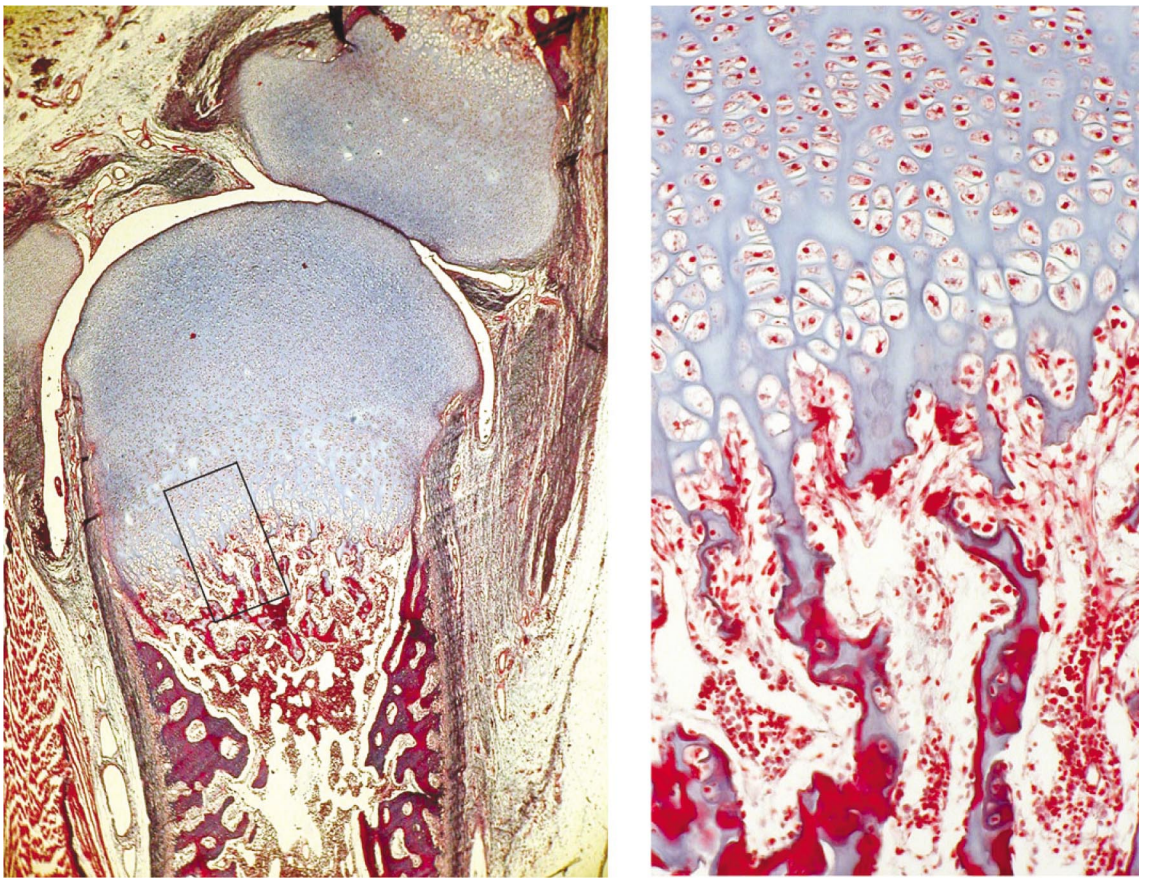

Figure 1. Histological sections illustrate the progression of cartilage growth and ossification at the primary growth front of a foetal cartilage rudiment.

mature articular cartilage. Although ossification of articular cartilage from the underlying subchondral growth front is greatly diminished, it is not entirely stopped (Trueta 1968; Lane *et al.* 1977). The cartilage gets thinner with age (Karvonen *et al.* 1994; Hudelmaier *et al.* 2001) as the subchondral growth front advances and can also wear mechanically by abrasion at the joint surface. It is by the surface wear, erosion and by the unrelenting process of cartilage growth and ossification that the eventual destruction of joint cartilage proceeds in ageing individuals (Trueta 1968; Carter & Beaupré 2001).

It is evident that basic biological processes in cartilage, and therefore in skeletal morphogenesis and ageing, are regulated in fundamental ways by the stresses and strains imposed by physical activity throughout life. To better understand this regulation and form hypotheses for further investigation, researchers have implemented analytic and computational stress analysis models for cartilage. Results from such analyses can be compared with biological processes and histomorphogenetic observations. Additionally, these models can be used to plan and interpret other *in vitro* investigations that seek to find relationships between imposed loading and the cellular and molecular biology of cartilage cell and tissue culture systems.

2. MODELS

(a) *Constitutive models*

Cartilage is composed of *ca*. 70% water, 20% type II collagen, 6% proteoglycans and 4% other organic molecules. Because the fluid permeability of cartilage is quite low, it is difficult to squeeze the water out. Consequently, for short static loading periods or for cyclic loading with moderate or high frequencies, the tissue behaves mechanically as a single-phase solid. In these conditions, the simplest constitutive model represents cartilage as a homogeneous, linear elastic, incompressible or nearly incompressible material. The nearly incompressible nature of cartilage at short loading times is related to its high water content. Only two material constants are needed to characterize the constitutive behaviour of homogeneous, isotropic, linear elastic materials. As a first approximation, one can simply measure the shear modulus (G) of a cartilage specimen *ex vivo* and specify the Poisson's ratio (ν) to be a number slightly less than or equal to 0.5, the Poisson's ratio of an incompressible material (Armstrong *et al.* 1984; Carter & Beaupré 1999; Wong *et al.* 2000). Typically, the dynamic shear modulus of normal bovine cartilage is measured to be *ca*. 2 or 3 MPa (Hayes & Bodine 1978). The elastic modulus (E) of cartilage for this linear elastic model can be calculated as

$$E = 2G(1 + \nu). \tag{2.1}$$

Amazingly, this simple linear elastic material representation of cartilage behaviour can be used with various stress analyses to provide many fundamental insights into the relationship between stresses and strains and cartilage biology. In these models, it would be appropriate to use Poisson ratio values of 0.47–0.50 and an elastic modulus of 6 MPa (corresponding to a shear modulus of 2 MPa).

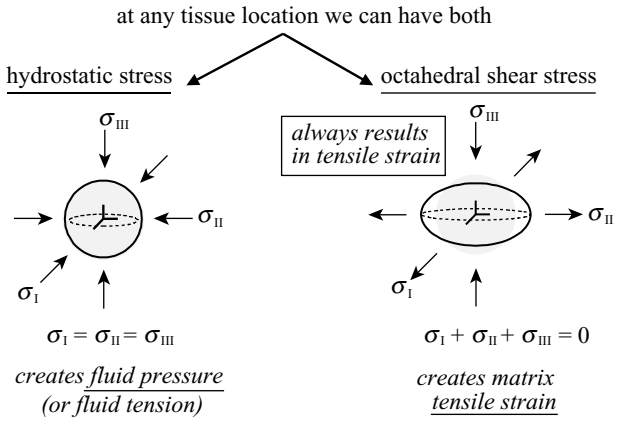

Figure 2. In a linear elastic constitutive model, two scalar components, the hydrostatic stress and the octahedral shear stress, can represent the full stress tensor at any location in cartilage.

Lower values of elastic modulus may be appropriate for theoretical models of immature cartilage, although few experimental data exist. Examples of such analyses will follow.

In some cartilage locations under some loading conditions, fluid exudation may be significant and the linear elastic, nearly incompressible representation of cartilage breaks down. An example of this is in the superficial zone of articular cartilage where cyclic joint forces and motion cause fluid exudation and matrix consolidation. In this case, considerable compressive strains are imposed on the cells and matrix from which the fluid is exuded. To represent this mechanical behaviour, researchers have turned to poroelastic (Biot 1941) or biphasic (Mow *et al.* 1980) constitutive models in which a solid phase and fluid phase are explicitly represented. Although poroelastic and biphasic constitutive models have a different conceptual basis, they have been shown to be mathematically identical when incompressibility is assumed, as is generally the case (Levenston *et al.* 1998).

Using the poroelastic or biphasic approaches, the fluid is allowed to be exuded from or imbibed into the permeable solid matrix in response to pressure gradients that are created by loading. Local fluid pressures and solid matrix stresses can be determined under specified loading conditions and the local consolidation strains associated with fluid exudation can also be calculated. If homogeneity, isotropy and similar tensile and compressive matrix behaviour are assumed, three material constants are required: the tissue fluid permeability, k, the aggregate modulus, H_a, and the Poisson's ratio of the solid matrix when the fluid is removed, ν_s. The fluid permeability is an experimentally determined constant that relates fluid flow in the cartilage to the fluid pressure gradient. The aggregate modulus is the 'equilibrium' modulus of a cartilage specimen in confined compression after all of the fluid has been exuded. The solid matrix Poisson's ratio is generally estimated by curve fitting cartilage specimen mechanical behaviour using the biphasic model that is assumed (Athanasiou *et al.* 1994).

Poroelastic or biphasic models capture quite well the mechanical behaviour of cartilage when fluid flow and matrix consolidation are of interest. One shortcoming of these models, however, is that they do not capture the fact that the solid matrix (with fluid exuded) is about 10 times stiffer in tension than in compression (Soulhat *et al.* 1999; Soltz & Ateshian 2000*a*). This difference is a result of the fact that the solid matrix comprises, in large part, a network of cross-linked collagen fibres. When this network is stretched, the fibres resist. When the network is compressed, however, the fibres simply collapse and offer little resistance to the imposed load. This behaviour is probably important at the superficial layer of the articular cartilage where there can be fluid exudation and imposed tensile stresses at the edge of the area of joint contact. The tensile stiffness of the fibre network will keep the tensile strains small and protect against mechanical failure at the cartilage surface.

The next step in elaborating the constitutive model of cartilage, therefore, is to consider a network fibre reinforced poroelastic model. This material model is similar to the poroelastic model but requires an additional constant, E_f, the fibre tensile elastic modulus, as well as the other three material constants (Soulhat *et al.* 1999).

Additional, more elaborate material models have been developed that seem to have utility to either: (i) account for additional material characteristic and thereby serve to 'fine tune' the analyses (e.g. anisotropy, inhomogeneity, poroviscoelasticity); or (ii) introduce additional compositional features that may prove useful in research on mechanotransduction pathways (e.g. triphasic models that incorporate osmotic pressures and poroelastokinetic models that can calculate electric charges associated with the flow of charged molecules). However, the linear elastic and poroelastic models above are sufficient to describe the basic mechanical behaviour of cartilage and are also sufficient to phenomenologically simulate how mechanical factors regulate the biology. Because the purpose of this paper is to address modelling the mechanical regulation of cartilage biology, we will confine ourselves here to the basic linear elastic and poroelastic (or biphasic) models.

(b) *Stress analysis models*

The determination of cartilage mechanical behaviour *in vivo* requires that the constitutive model be incorporated into a specific stress analysis model. In conducting the analysis, one must consider: (i) the stress analysis method to be used; (ii) the geometric representation to be employed; and (iii) the loading and boundary conditions that are incorporated.

Early analysis approaches to studying cartilage stresses employed elasticity or poroelastic (biphasic) approaches that yield closed-form solutions. A serious shortcoming of these approaches is that solutions can be found to only a relatively small group of idealized problems. Although analyses of this kind are still used, there has been a progressive shift towards the implementation of computational approaches using finite element models. Computational models are much more versatile and can be used to find solutions to problems that incorporate more complicated geometries, materials, loading and boundary conditions (Hughes 1987).

In constructing a finite element model, important considerations are the element type and the geometric representation of the structure to be analysed. Full representations of the 'exact' 3D geometry of a cartilage

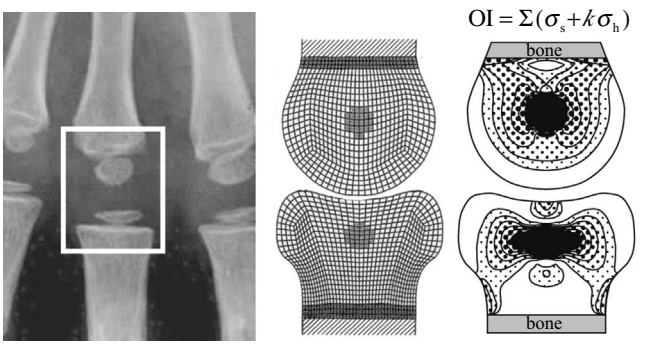

Figure 3. The location and the shape of secondary centres of ossification can be predicted from the distribution of hydrostatic and shear stresses calculated in finite element analyses. (Adapted from Carter & Wong (1988).)

structure or surface can be, and have been, implemented to study specific anatomical sites. However, to gain insights on fundamental relationships between cartilage mechanics and biology, it is often useful to employ a more idealized geometry that can be used to illustrate basic behaviour for a wide variety of cartilage structures. Results from these 'idealized models' can then be used to formulate hypotheses of cartilage mechanobiology.

Simplified geometric models can also open the door for analyses that use more complicated material models or loading conditions. (It is confusing when many complex modelling parameters are incorporated at the same time!) A danger of idealized geometric models is, of course, that they may misrepresent 'reality' as a consequence of their simplicity. Checks must be made so that the analyst is not misled. Appropriate simplified geometrical representations of cartilage structures include a 3D axisymmetric model geometry with non-axisymmetric loading, a 2D/3D axisymmetric model with axisymmetric loading, and 2D plane strain models.

Cartilage structures *in vivo* are exposed to extremely complicated loading histories throughout life and even over the period of a day. It is the cumulative influence of these loading histories that governs the biology of the tissue and determines its histomorphology. It is not possible or desirable to incorporate the entire loading history into an analysis, so substantial simplifications must be made. A simple approach for analysing a linear elastic cartilage model would be to impose a single force that is distributed as a pressure distribution over some assumed contact area. If a poroelastic constitutive model were used one would also identify the boundary flow conditions and the time of loading application or loading frequency. The model increases with complexity when one assumes that the load is moving and the pressure distribution changes in location, magnitude and distribution. Add to that the loading conditions caused by different physical activities over an extended period of time and the enormity of the problem of representing *in vivo* loading, even with the simplest constitutive and geometry representations, becomes clear. Nevertheless, the analyst should be aware of the simplifications of representing the loading history that are always made and interpret the results accordingly.

3. MODELLING CARTILAGE GROWTH AND OSSIFICATION

One of the most fruitful areas of modelling in cartilage mechanobiology has involved the use of linear elastic finite element models to study the role of intermittent tissue stresses and strains on endochondral growth and ossification. Early models that were used to investigate the pathogenesis of osteoarthritis (Carter *et al.* 1987*b*) and perinatal endochondral ossification of the femur (Carter *et al.* 1987*a*) led to the formation of a basic hypothesis that: (i) hydrostatic pressure maintains cartilage and; (ii) shear or tensile stresses damage and promote cartilage growth and ossification (figure 2). The results of numerous subsequent analysis models support the view that cartilage growth and ossification is inhibited by the application of local intermittent hydrostatic compressive stress and accelerated by intermittent octahedral shear stress. Octahedral shear stress always causes tensile strain in some direction. Therefore one could equivalently state that growth and ossification is accelerated by tensile strain and that cartilage tends to be maintained by hydrostatic compressive stress.

Models of the influence of mechanical stresses on growth have been used to simulate the changes that occur in the foetal joint surfaces as a result of the loading associated with muscle contractions and movements (Heegaard *et al.* 1999). Using 2D finite element models of two contacting rudiments at a developing joint, muscle and tendon loads were simulated to cause the large articular displacements that occur during joint flexion and extension. The distributions of hydrostatic and octahedral stress distributions in the rudiment articulating ends were calculated using a contact finite element algorithm for large displacements. In the computer simulations, a baseline growth was iteratively implemented as a simple dilatation of each element. The magnitude of this growth for successive time increments was modulated to incorporate the premise that local hydrostatic compressive stress slows growth. Owing to the local stresses imposed by joint movement, the simulations predicted the progressive changes in articulating surface geometry that are observed during embryonic development. The rudiment with tendon insertions close to the articulation experienced high compressive hydrostatic stresses that were concentrated at the apex at all flexion angles. This mechanical situation caused growth inhibition at the apex and the development of a concave surface geometry. The contact area of the opposing rudiment swept over a much broader area during joint flexion and the concentration of hydrostatic pressure in one area was avoided. Consequently the opposing rudiment developed a convex joint surface geometry, as well as other geometric aspects that are evident in development.

The modulation of cartilage growth by local stresses has also been simulated for the primary growth front and the progression of growth plates in late embryonic and foetal stages (Shefelbine 2002; Shefelbine *et al.* 2002). In these series of models, growth was simulated for only short time periods at the growth front, the major site of organized growth and ossification. Both 2D and 3D linear elastic models were analysed. It was assumed that there was an inherent baseline growth that was modulated by the local imposed stresses so that the growth was inhibited by

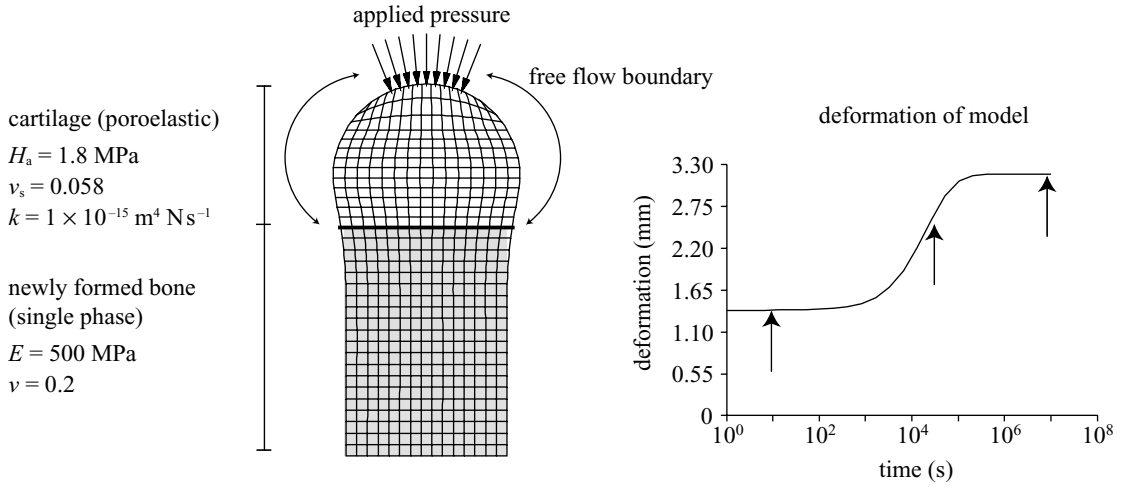

Figure 4. Poroelastic material models of foetal cartilage rudiments under static loading demonstrate time-dependent displacement due to the exudation of fluid. (Adapted from Shefelbine (2002).)

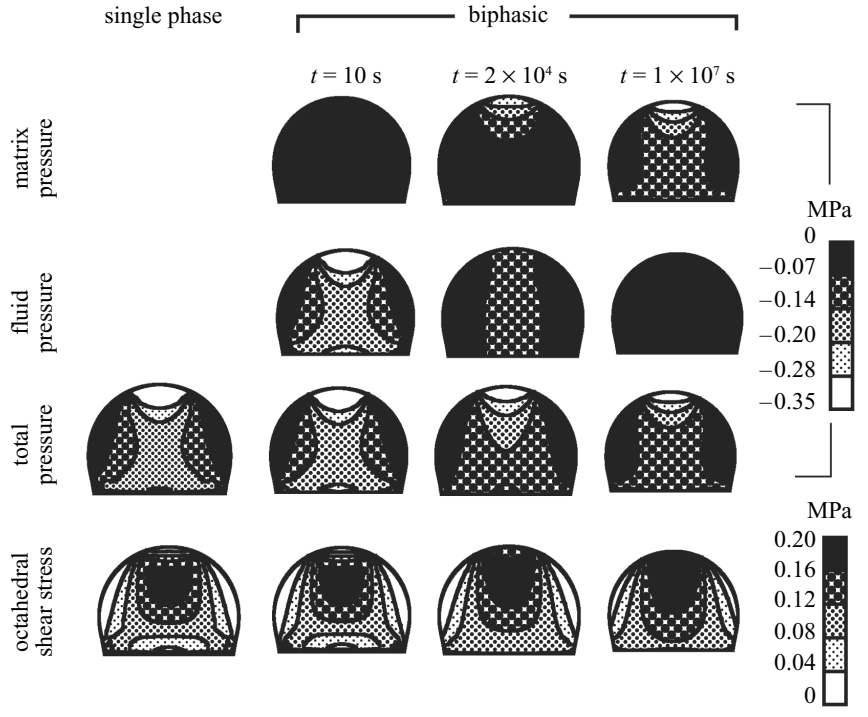

Figure 5. Progressive fluid exudation from the poroelastic cartilage rudiment model is associated with the progressive transfer of hydrostatic pressure from the fluid to the solid matrix after a very long time. (Adapted from Shefelbine (2002).)

hydrostatic compressive stresses and accelerated by intermittent octahedral shear stresses. Growth was then predicted using the thermal expansion capabilities of the elements in the finite element programme so that changes in growth front morphology could be predicted. Results of these simulations demonstrated that the normal developmental changes observed in growth front and growth plate morphology in the proximal and distal femur and in other bones could be emulated. Furthermore, the applications of altered loading conditions, such as those in developmental dysplasia of the hip, led to predicted morphological changes that are consistent with clinical findings. Specifically, when the head of the femur was modelled to be out of the socket, the laterally directed joint force accelerated cartilage growth medially, causing the femoral neck to grow into a valgus orientation.

Endochondral growth eventually leads to endochondral ossification. Ossification proceeds faster in some regions of cartilage than others during normal development. As a result, numerous secondary ossification sites appear throughout the skeleton and expand to form bone regions of various geometries. Numerous 2D, axisymmetric and full 3D linear elastic models have been used to emulate and predict the appearance of these secondary ossification patterns in long bones and in the sternum (Carter & Beaupré 2001). Most of the models that have been used to predict ossification patterns have not explicitly incorporated growth. However, the prediction of ossification patterns is based on the same fundamental assumptions that were introduced above; hydrostatic pressure inhibits cartilage growth and ossification while octahedral shear stress accelerates ossification. To calculate a single parameter

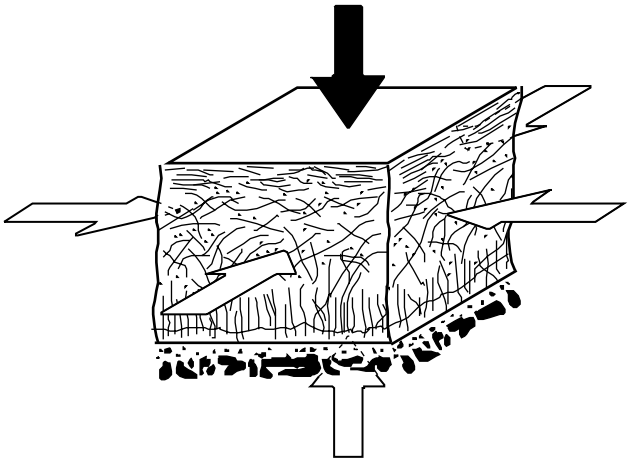

Figure 6. The simplest model of articular cartilage is that of an incompressible, linear elastic material that experiences nearly pure hydrostatic pressure when the joint surface is loaded. (From Carter & Beaupré (2001).)

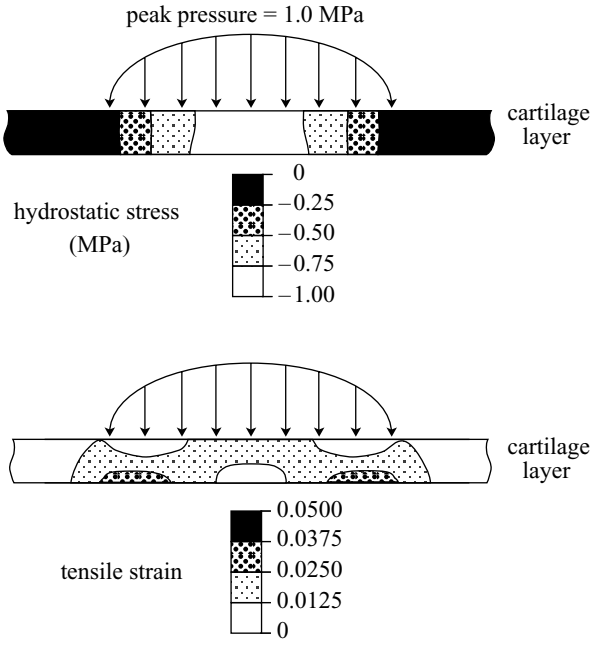

Figure 7. A linear elastic finite element model of articular cartilage on a rigid foundation calculates hydrostatic compressive stress directly under the joint contact pressure and also the distribution of relatively small tensile strains. (From Carter & Beaupré (2001).)

that embodies this concept we introduced (Carter & Wong 1988) the OI, which is calculated as

$$\mathrm{OI} = \sum(\sigma_s + k\sigma_h), \tag{3.1}$$

where σ_s is the octahedral shear stress and σ_h is the hydrostatic stress introduced during a specific loading cycle and k is an empirical constant that is found by trial and error to have a value between 0.3 and 1.0. The total value of the OI is determined by summing the contributions of each loading cycle over some period of time. Note that the octahedral shear stress is always positive, so shear will always increase the OI and hasten ossification. Compressive hydrostatic stresses are negative and tensile hydrostatic stresses are positive. Hydrostatic pressure, therefore, decreases the value of OI, signifying a delay in ossification. By applying a series of loading conditions to these models that simulate the *in vivo* loading history, the distribution of OI can be calculated and displayed as contour plots on the models. These plots can then be used to infer the distribution of ossification centres and give some indication of the geometric advance of ossification around these centres once they form.

The basic ossification characteristics of secondary centres near a diarthrodial joint can be illustrated by using the OI approach described (figure 3). The results of the analyses predict the appearance of secondary ossification centres at the end of the rudiments on both sides of the joint (Carter & Wong 1988). In the rudiment with a concave joint surface the secondary centre is predicted to appear closer to the surface that in the rudiment with a convex surface. When the material properties of the models are changed to represent the appearance of the secondary ossification centres, subsequent analyses predict that the bone epiphysis in the convex rudiment will assume a spherical shape while the bone epiphysis in the concave rudiment will take on a flatter, disc shape. This general finding predicted by the model is evident in a wide variety of joints throughout the body.

The predictions of cartilage growth and ossification using linear elastic models appear to be very robust and consistent for phenomenologically simulating a broad variety of events in normal and abnormal development. Some concerns about these models may be raised owing to the fact that they do not account for fluid flow and thus may not capture a potentially important aspect of the cartilage mechanics. However, similar analyses using poroelastic constitutive models have failed to provide any additional insights or change the basic conclusions drawn from the linear elastic models (Carter & Beaupré 1999; Sarin 2000; Shefelbine 2002).

To check the differences that might be predicted between the linear elastic and poroelastic model, one can analyse a plane stain 'generic' rudiment end with a cartilage cap on an ossified bone shaft (Shefelbine 2002; figure 4). An assumed contact pressure distribution is applied to cartilage surface and fluid is permitted to flow out of the free cartilage surfaces that are not in contact with the opposing joint cartilage. When a poroelastic cartilage representation is used, there is an initial deformation of the model that is due to elastic deformation. Although fluid can immediately flow out of the free surfaces, most of the fluid is deep in the tissue and is thus trapped by the surrounding cartilage, which has low permeability. Total fluid loss, therefore, proceeds quite slowly. After *ca.* 30 min (1800 s) enough fluid has been exuded that the deformation of the cartilage structure begins to increase significantly (figure 4). The cartilage matrix progressively consolidates as the fluid is forced out. At *ca.* 2 days (172 800 s) the deformation reaches a new plateau when the fluid flow diminishes and matrix consolidation stabilizes.

For the first 30 min of loading the stress fields calculated throughout most of the chondoepiphysis for the single-phase model are nearly identical to those calculated using the poroelastic (or biphasic) model (figure 5). During this time period, the hydrostatic compressive stress is carried almost entirely by the fluid, in the form of fluid

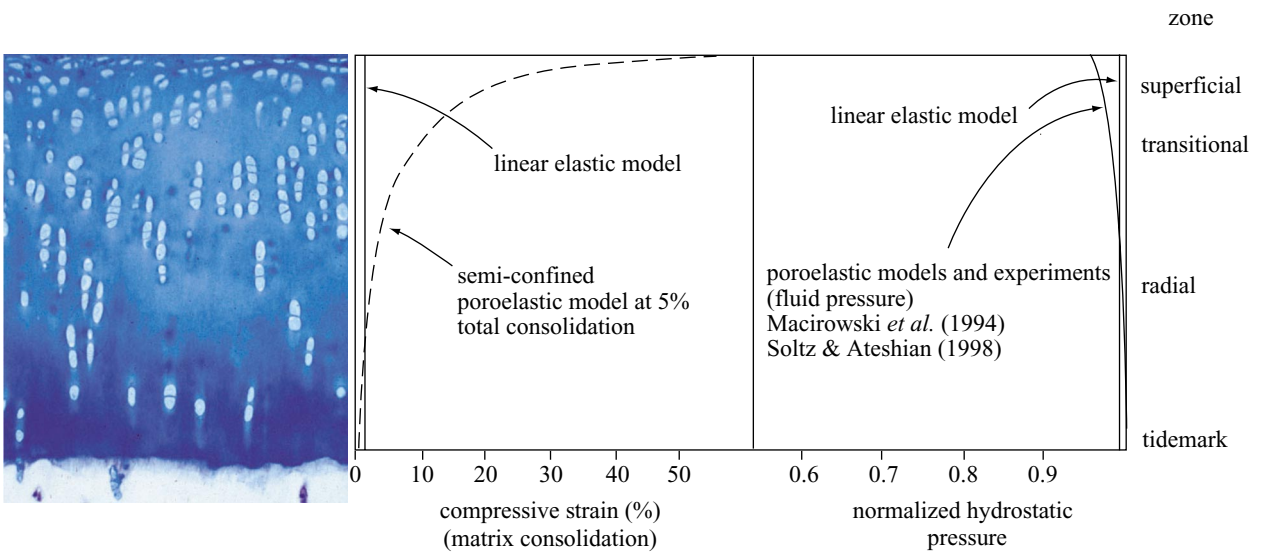

Figure 8. Poroelastic computational models demonstrate the high compressive strains in the superficial zone of articular cartilage that result from fluid exudation at the joint surface during exercise.

pressure (e.g. see figure 5, $t = 10$ s). The octahedral shear stress is carried entirely by the solid matrix. At *ca.* 5.5 h (19 800 s) there is a sharing of the hydrostatic stress between the fluid and solid phase. After equilibrium is achieved at 2 days, the fluid carries none of the hydrostatic stress (as considerable fluid has been squeezed out) and thus the solid matrix carries all of the hydrostatic and shear stresses.

The typical intervals of intermittent loading in developing rudiments are far less than 30 min and, in fact, most joint loads are applied at frequencies less that 1 Hz. It is clear, therefore, that single-phase models can be effectively used to study the mechanobiology of cartilage in the interior regions of the rudiments. Similar comparisons of results calculated from single-phase and biphasic models of the growth front and growth plate lead to a comparable conclusion (Shefelbine 2002). In cartilage regions near the free cartilage surfaces where significant fluid flow occurs even at short time periods, however, the advantages of biphasic models become evident. This is particularly true in studies of the mechanobiology of articular cartilage.

4. ARTICULAR CARTILAGE BIOLOGY AND MECHANICS

The articular cartilage is established as the subchondral growth front approaches the articulating surface of the joint. The speed at which this growth front advances decreases as it approaches the joint surface. Eventually, the ossification front stabilizes and the thickness of the overlying articular cartilage is established. Results from finite element models suggest that the slowing down and eventual stabilization of the growth front is related to the fact that as the front advances the local hydrostatic compressive stress increases and the octahedral shear stress decreases (Beaupré *et al.* 2000; Carter & Beaupré 2001). Eventually, a balance is reached. The distribution of articular cartilage thickness in a particular joint is thus related to the local stress history.

Correlations between the magnitude of cartilage contact stresses and cartilage thickness in various joints lead to the general conclusions that where the joint forces and pressures are high (e.g. the hip and knee) the cartilage is the thickest. Furthermore, there tends to be a good correlation between cartilage thickness and contact pressure distributions at particular joint surfaces such as the femoral head (Kurrat & Oberlander 1978; Rushfeldt *et al.* 1981*a*,*b*). *In vivo* imaging studies looking at the effect of increased activities on cartilage thickness in young individuals have not led to definitive conclusions. However, imaging studies of individuals with reducing activities have clearly shown cartilage thinning (Vanwanseele *et al.* 2002*a*,*b*), probably as a result of the activation and advance of the subchondral growth front (Smith *et al.* 1992).

The simplest model that one might present for articular cartilage mechanobiology would be as an incompressible tissue exposed to intermittent hydrostatic compressive stress that is carried in the form of fluid pressure (figure 6). This loading condition is created when joint contact pressures are imposed at the articulating surface. The subchondral bone reaction force balances the joint pressure with an oppositely directed, equal pressure. At the same time, there is a tendency for the tissue to bulge laterally, due to the Poisson effect. Lateral strain is prevented, however, as the cartilage is surrounded by adjacent, incompressible cartilage. Lateral pressures are thereby generated and a state of nearly pure hydrostatic compression is created in the cartilage.

This simple view of articular cartilage can be expanded using a 2D finite element model to represent a layer of cartilage as single-phase, linearly elastic tissue that is supported by rigid subchondral bone (figure 7). When a pressure distribution is applied at the surface, the tissue directly under the maximally loaded region experiences hydrostatic pressure through the full cartilage thickness that is comparable in magnitude to the surface contact pressure. At some regions in the cartilage, octahedral shear stresses of a relatively modest magnitude are created. These octahedral shear stresses are highest at the cartilage–bone interface where there is a rapid change in local material properties. The distribution of calculated

octahedral shear stresses is directly reflected in the distribution of the local tensile strains, which must be resisted by the solid fibre network of the collagen. If one imagines that the pressure distribution sweeps back and forth across the articular cartilage layer, it is clear that the entire layer is then exposed to high cyclic hydrostatic pressure throughout. Cyclic tensile strains are created in the superficial layer and also at the cartilage–bone interface.

The linear elastic model of articular cartilage with sweeping, time-varying loads does not capture any of the fluid flow and matrix consolidation that may occur at the articular cartilage surface. Although a consideration of flow is not necessary to phenomenologically simulate endochondral growth and ossification inside the rudiment and young bone, fluid flow and matrix consolidation at the joint surface is crucial in understanding joint lubrication and the mechanobiology of chondrocytes near the articular surface (Gray *et al.* 1988; Mow & Ateshian 1997; Prendergast *et al.* 1997; Wong *et al.* 1997; Yellowley *et al.* 1999). Poroelastic or biphasic models are particularly good for addressing this aspect of cartilage mechanobiology.

To assess how much fluid exudation and matrix consolidation occurs *in vivo*, researchers have performed magnetic resonance imaging of articular cartilage before and after various activities. Herberhold *et al.* (1999) found that *in vivo* continuous static loads of 150% body weight at the knee caused only a 3% femoral cartilage thickness loss after 1 min. Eckstein *et al.* (1999, 2000) found that young adult patella–femoral cartilage layers, with a thickness of over 5 mm, experienced a decrease in total thickness of *ca*. 5% after severe exercise. Measurements made under various other exercise conditions prompted them to suggest that '…*in vivo* only relatively few cycles may be necessary for the cartilage to reach a plateau-like deformation state, and that additional cycles cause no further deformation of the tissue' (Eckstein *et al*. 2000, p. 823).

Although the total consolidation of articular cartilage thickness may be *ca*. 5% *in vivo*, the amount of consolidation is not constant throughout. Poroelastic finite element models are therefore useful in estimating how the consolidation strains vary through the cartilage thickness. Rough estimates can be made using the solution of a problem wherein a plug of cartilage is confined from deformation and fluid flow from all sides while being loaded by a semi-permeable platen at the surface. The fluid must be exuded from the top surfaces of cartilage, much like the *in vivo* situation. This problem can be solved under an applied contact pressure of one 1 MPa and the consolidation strains examined at the time when the total cartilage thickness has decreased by 5%. Owing to the fact that the fluid is exuded only from the surface layer, the distribution of consolidation strains through the cartilage thickness is highly non-uniform (figure 8). Tissue consolidation results in compressive strains of over 50% in the superficial cartilage zone but these strains reduce to near zero in the middle and deep regions of cartilage. The linearly elastic model predicts almost no compressive strain through the cartilage thickness because it has the implicit assumption of no fluid flow (Carter & Beaupré 2001).

Although the 'free draining' poroelastic solution described can give reasonable estimates of consolidation strains through the cartilage thickness, it underestimates the fluid pressures that are generated and maintained *in vivo* near the surface. The fluid exuded *in vivo* gets 'trapped' between the two articulating cartilage surfaces and maintains a high pressure level, thereby preventing the generation of high stresses and friction between the solid matrix elements of the two articulating cartilage surfaces (figure 8). This pressurization has been demonstrated in both poroelastic (Macirowski *et al.* 1994) and biphasic models (Soltz & Ateshian 1998) and verified experimentally (Soltz & Ateshian 2000*b*). The linear elastic model also predicts a nearly constant level of hydrostatic compressive stress through the thickness, even though the consolidation strains cannot be determined (figure 8).

The results of poroelastic models of articular cartilage can be compared with the normal histomorphology and biology of the cartilage and also to experiments wherein controlled loading conditions are imposed and biological activities are measured. The superficial zone has been demonstrated to have low proteoglycan content, although there is generally an increased fibrous character, perhaps owing to either the matrix consolidation or the tensile strains imposed at the edges of joint contact. Additionally, tissue culture experiments of cartilage plugs that are loaded statically to cause fluid exudation and matrix consolidation have been shown to decrease their level of proteoglycan synthesis (Wong *et al.* 1997) and sulphate incorporation (Gray *et al.* 1988). This finding suggests that cartilage mechanobiological principles for *poroelastic* models can be expressed as follows.

(i) Hydrostatic fluid pressure inhibits cartilage growth and ossification, thereby maintaining the cartilage phenotype.
(ii) Tensile strain (or octahedral shear stress) accelerates cartilage growth, ossification and replacement by bone.
(iii) Matrix compressive consolidation, with or without fluid pressure, decreases cartilage proteoglycan synthesis and content and results in a more fibrous cartilage phenotype.

5. EXPERIMENTAL MECHANOBIOLOGY AND MODEL VALIDATION

Each cell interacts with its environment through cell–cell contacts, attachments to the ECM, receptors to diffusible factors and also to the mechanical pressures and deformations it perceives. No cell in a multicellular organism exists in stress-free state or in isolation of the cells around it. Mechanical loading, either externally applied, or by muscle contractions, or generated by growth gradients, is a key factor in guiding cell processes in skeletal morphogenesis (Henderson & Carter 2002).

The field of experimental mechanobiology has undergone enormous growth over the past decade. Numerous differentiated cell types, including smooth muscle cells, cardiocytes, myoblasts, fibroblasts, osteoblasts, chondrocytes and endothelial cells show changes in gene expression and proliferation in response to some kinds of mechanical loading. Our understanding of mechano-responsive proteins is also rapidly expanding and touches on almost every aspect of cell biology. Families of genes

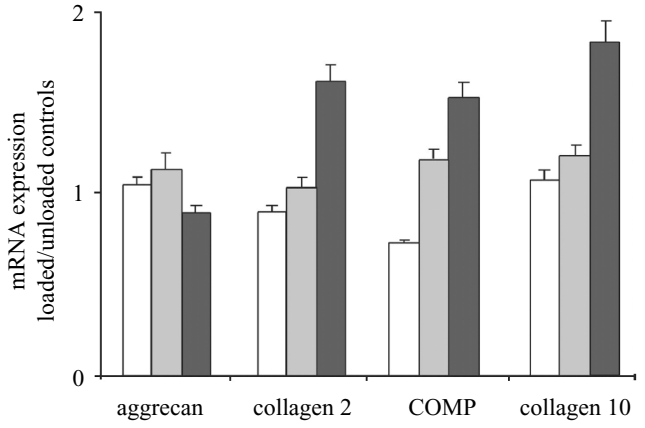

Figure 9. *In vitro* experiments of chondrocytes in alginate gels show the changes in gene expression when cyclic tensile strains are applied. COMP, cartilage oligomeric matrix protein. Open bars, 1%; light-grey bars, 2%; dark-grey bars, 3%. (Adapted from Wong *et al.* (2002).)

whose expression is sensitive to mechanical loading include ECM proteins, cell cycle regulators, cytokines, growth factors, cytoskeletal proteins, focal adhesion proteins, and proteases and their inhibitors (Kessler *et al.* 2001). Parallels in mechanisms of mechanotransduction in various cell types are beginning to emerge. The scope of the cells and proteins involved implies a much more fundamental role for mechanical loading in biology than has previously been assumed. The role of mechanics in the cellular processes associated with growth, remodelling, morphogenesis, angiogenesis and wound healing is progressively being established.

In this review, we have shown evidence that mechanical loading may regulate the morphogenesis of the skeleton, particularly the process of endochondral ossification. To reconcile predictions from theoretical models with biological phenomena, it is necessary to interpret experimental data in the context of these theories and discuss the mechanisms by which cells respond to mechanical loading. Endochondral ossification is a temporally and spatially complex process involving many cell types. The influences of mechanics may occur at several levels, but relevant experiments to these ideas are those studies that apply hydrostatic pressure and shear stress to chondrocytes *in vitro*. These studies show that physiological levels of cyclic hydrostatic pressure (2.8–10 MPa) increase expression of cartilage-specific genes such as aggrecan and type II collagen on an mRNA and protein level (Smith *et al.* 1996; Mizuno *et al.* 2002). Alternatively, shear stresses, in the form of uniaxial tension, have been shown to upregulate the expression of Ihh and type X collagen, a marker of the hypertrophic phenotype (Wu & Chen 2000; Wu *et al.* 2001). In our studies, articular cartilage chondrocytes were seeded into alginate hydrogels and subjected to intermittent tension, uniaxial compression or hydrostatic pressure (Wong *et al.* 2002). The expression of type X collagen was strongest in cells loaded in tension and the level of expression was dependent on the magnitude of the applied tensile strain (figure 9).

Mechanical loading exerts effects on the expression proteases and their inhibitors as well. The MMP family plays a particularly important role in bone development, particularly during vascular invasion into cartilage (Vu & Werb 2000). The regulation of MMP expression and activation may be related to whether the cell's natural state is perturbed by the load or not. Hydrostatic pressure maintains the shape of the chondrocyte and appears to have a chondroprotective effect (Trindade *et al.* 2000). By contrast, cyclic tension applied to chondrocytes increased the expression of MMP-13 (collagenase-3), a protease responsible for the removal and cleavage of type II collagen from the matrix before vascular invasion, and CTGF, an angiogenic factor found in hypertrophic chondrocytes. The upregulation of proinflammatory proteins in chondrocytes subject to high tensile strain, may result from the pathological spindle-shaped morphology the cells acquire during this kind of loading (Honda *et al.* 2000). Interestingly, many other cell types that are usually subjected to tension *in vivo* respond to applied tensile loading by *downregulating* synthesis of MMPs and proinflammatory substances (Long *et al.* 2001; Sun & Yokota 2001).

If we are to understand the morphogenesis of skeletal structures in the context of cell biology experiments, it is useful to consider the distributions of shear stresses and hydrostatic pressure that are imposed in the skeleton during physical activity. In articular cartilage, the deep and middle zones are primarily loaded under hydrostatic pressure, with little fluid flow or matrix consolidation. This is the region with the highest concentration of proteoglycans. At sites of endochondral ossification, the mismatch of material properties between cartilage and bone generates higher shear stresses than would otherwise be present. The work described in this section indicates that shear stress (or tensile strain) applied to chondrocytes drives the synthesis of those proteins that are responsible for angiogenesis and end-terminal differentiation, whereas hydrostatic pressure helps to preserve the cartilage phenotype from inflammation and vascular invasion.

6. CONCLUSIONS

Computer models of cartilage mechanobiology have led to the formulation of a fundamental framework for understanding cartilage mechanobiology from a phenomenological perspective. The results of these models help to form hypotheses that can be used to understand skeletal observations on the organ system level. Perhaps more importantly, however, these models have created a basis for current and future research into the molecular mechanisms that regulate cartilage biology.

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REFERENCES

Armstrong, C. G., Mow, V. C. & Lai, W. M. 1984 An analysis of the unconfined compression of articular cartilage. *J. Biomech. Engng* **106**, 165–173.

Athanasiou, K. A., Agarwal, A. & Dzida, F. J. 1994 Comparative study of the intrinsic mechanical properties of the human acetabular and femoral head cartilage. *J. Orthopaedic Res.* **12**, 340–349.

Beaupré, G. S., Stevens, S. S. & Carter, D. R. 2000 Mechanobiology in the development, maintenance, and degeneration of articular cartilage. *J. Rehabilitation Res. Dev.* **37**, 145–151.

Biot, M. A. 1941 General theory of three-dimensional consolidation. *J. Appl. Physics* **12**, 155–164.

Carter, D. R. & Beaupré, C. S. 1999 Linear elastic and poroelastic models of cartilage can produce comparable stress results: a comment on Tanck *et al*. (*J. Biomech*. **32**, 153–161, 1999, letter). *J. Biomech.* **32**, 1255–1257.

Carter, D. & Beaupré, G. 2001 *Skeletal function and form: mechanobiology of skeletal development, aging, and regeneration*. Cambridge University Press.

Carter, D. R. & Wong, M. 1988 The role of mechanical loading histories in the development of diarthrodial joints. *J. Orthopaedic Res.* **6**, 804–816.

Carter, D. R., Orr, T. E., Fyhrie, D. P. & Schurman, D. J. 1987*a* Influences of mechanical stress on prenatal and postnatal skeletal development. *Clin. Orthop.* **219**, 237–250.

Carter, D. R., Rapperport, D. J., Fyhrie, D. P. & Schurman, D. J. 1987*b* Relation of coxarthrosis to stresses and morphogenesis. A finite element analysis. *Acta Orthopaedica Scandinavica* **58**, 611–619.

Eckstein, F., Tieschky, M., Faber, S., Englmeier, K.-H. & Reiser, M. 1999 Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise *in vivo*. *Anat. Embryol.* **200**, 419–424.

Eckstein, F., Lemberger, B., Stammberger, T., Englmeier, K.-H. & Reiser, M. 2000 Patellar cartilage deformation *in vivo* after static versus dynamic loading. *J. Biomech.* **33**, 819–825.

Gray, M. L., Pizzanelli, A. M., Grodzinsky, A. J. & Lee, R. C. 1988 Mechanical and physicochemical determinants of the chondrocyte biosynthetic response. *J. Orthopaedic Res.* **6**, 777–792.

Hayes, W. C. & Bodine, A. J. 1978 Flow-independent viscoelastic properties of articular cartilage matrix. *J. Biomech.* **11**, 407–419.

Heegaard, J. H., Beaupré, G. S. & Carter, D. R. 1999 Mechanically modulated cartilage growth may regulate joint surface morphogenesis (in process citation). *J. Orthopaedic Res.* **17**, 509–517.

Henderson, J. & Carter, D. 2002 Mechanical induction in limb morphogenesis: the role of growth-generated strains and pressures. *Bone* **31**, 645–653.

Herberhold, C., Faber, S., Stammberger, T., Steinlechner, M., Putz, R., Englmeier, K.-H., Reiser, M. & Eckstein, F. 1999 *In situ* measurement of articular cartilage deformation in intact femoropatellar joints under static loading. *J. Biomech.* **32**, 1287–1295.

Honda, K., Ohno, S., Tanimoto, K., Ljuin, C., Tanaka, N., Doi, T., Kato, Y. & Tanne, K. 2000 The effects of high magnitude cyclic tensile load on cartilage matrix metabolism in cultured chondrocytes. *Eur. J. Cell Biol.* **79**, 601–609.

Hudelmaier, M., Glaser, C., Hohe, J., Englmeier, K.-H., Reiser, M., Putz, R. & Eckstein, F. 2001 Age-related changes in the morphology and deformational behavior of knee joint cartilage. *Arthritis and Rheumatism* **44**, 2556–2561.

Hughes, T. 1987 *The finite element method*. Englewood Cliffs, NJ: Prentice-Hall.

Karvonen, R. L., Negendank, W. G., Teitge, R. A., Reed, A. H., Miller, P. R. & Fernandez-Madrid, F. 1994 Factors affecting articular cartilage thickness in osteoarthritis and aging. *J. Rheumatol.* **21**, 1310–1318.

Kessler, D., Dethlefsen, S., Haase, I., Plomann, M., Hirche, F., Krieg, T. & Eckes, B. 2001 Fibroblasts in mechanically stressed collagen lattices assume a 'synthetic' phenotype. *J. Biol. Chem.* **276**, 36 575–36 585.

Kurrat, K. & Oberlander, W. 1978 The thickness of the cartilage in the hip joint. *J. Anat.* **126**, 145–155.

Lane, L. B., Villacin, A. & Bullough, P. G. 1977 The vascularity and remodelling of subchondrial bone and calcified cartilage in adult human femoral and humeral heads. An age- and stress- related phenomenon. *Br. J. Bone Joint Surg.* **59**, 272–278.

Levenston, M. E., Frank, E. H. & Grodzinsky, A. J. 1998 Variationally derived 3-field finite element formulations for quasistatic poroelastic analysis of hydrated biological tissues. *Computer Meth. Appl. Mech. Engng* **156**, 231–246.

Long, P., Hu, J., Piesco, N., Buckley, M. & Agarwal, S. 2001 Low magnitude of tensile strain inhibits IL-1beta-dependent induction of pro-inflammatory cytokines and induces synthesis of IL-10 in human periodontal ligament cells *in vitro*. *J. Dent. Res.* **80**, 1416–1420.

Macirowski, T., Tepic, S. & Mann, R. W. 1994 Cartilage stresses in the human hip joint. *J. Biomech. Engng* **116**, 10–18.

Mizuno, S., Tateischi, T., Ushida, T. & Glowacki, J. 2002 Hydrostatic fluid pressure enhances matrix synthesis and accumulation by bovine chondrocytes in three-dimensional culture. *J. Cell Physiol.* **193**, 319–327.

Mow, V. & Ateshian, G. 1997 Lubrication and wear of diarthrodial joints. In *Basic orthopaedic biomechanics* (ed. V. Mow & W. Hayes), pp. 275–315. Philadelphia, PA: Lippencott-Raven.

Mow, V. C., Kuei, S. C., Lai, W. M. & Armstrong, C. G. 1980 Biphasic creep and stress relaxation of articular cartilage in compression: theory and experiment. *J. Biomech. Engng* **102**, 73–84.

Prendergast, P. J., Huiskes, R. & Soballe, K. 1997 ESB research award 1996. Biophysical stimuli on cells during tissue differentiation at implant interfaces. *J. Biomech.* **30**, 539–548.

Rushfeldt, P. D., Mann, R. W. & Harris, W. H. 1981*a* Improved techniques for measuring *in vitro* the geometry and pressure distribution in the human acetabulum. II. Instrumented endoprosthesis measurement of articular surface pressure distribution. *J. Biomech.* **14**, 315–323.

Rushfeldt, P. D., Mann, R. W. & Harris, W. H. 1981*b* Improved techniques for measuring *in vitro* the geometry and pressure distribution in the human acetabulum. I. Ultrasonic measurement of acetabular surfaces, sphericity and cartilage thickness. *J. Biomech.* **14**, 253–260.

Sarin, V. 2000 Mechanobiology of sesamoid formation, development, and ossification. PhD thesis, Mechanical Engineering, Stanford University, Stanford, CA, USA.

Shefelbine, S. 2002 Mechanical regulation of bone growth fronts and growth plates. PhD thesis, Mechanical Engineering, Stanford University, Stanford, CA, USA.

Shefelbine, S. J., Tardieu, C. & Carter, D. R. 2002 Development of the femoral bichondylar angle in Hominid bipedalism. *Bone* **30**, 765–770.

Smith, R. L., Thomas, K. D., Schurman, D. J., Carter, D. R., Wong, M. & van der Meulen, M. C. 1992 Rabbit knee immobilization: bone remodeling precedes cartilage degradation. *J. Orthopaedic Res.* **10**, 88–95.

Smith, R. L., Rusk, S. F., Ellison, B. E., Wessells, P., Tsuchiya, K., Carter, D. R., Caler, W. E., Sandell, L. J. & Schurman, D. J. 1996 *In vitro* stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure. *J. Orthopaedic Res.* **14**, 53–60.

Soltz, M. A. & Ateshian, G. A. 1998 Experimental verification and theoretical prediction of cartilage interstitial fluid pressurization at an impermeable contact interface in confined compression. *J. Biomech.* **31**, 927–934.

Soltz, M. A. & Ateshian, G. A. 2000*a* A conewise linear elasticity mixture model for the analysis of tension-compression nonlinearity in articular cartilage. *J. Biomech. Engng* **122**, 576–586.

Soltz, M. A. & Ateshian, G. A. 2000*b* Interstitial fluid pressurization during confined compression cyclical loading of articular cartilage. *Ann. Biomed. Engng* **28**, 150–159.

Soulhat, J., Buschmann, M. D. & Shirazi-Adl, A. 1999 A fibril-network-reinforced biphasic model of cartilage in unconfined compression. *J. Biomech. Engng* **121**, 340–347.

Sun, H. B. & Yokota, H. 2001 Altered mRNA level of matrix metalloproteinase-13 in MH7A synovial cells under mechanical loading and unloading. *Bone* **28**, 399–403.

Trindade, M. C. D., Shida, J., Ikenoue, T., Yerby, S., Goodman, S. B., Schurman, D. J. & Smith, R. L. 2000 Intermittent hydrostatic pressure inhibits interleukin-6 and monocyte chemoattractant protein-1 expression by human osteoarthritic chondrocytes *in vitro*. In *Trans. 46th Ann. Mtg Orthopaedic Res. Soc., Orlando, FL, 2000*, vol. 25, p. 0175.

Trueta, J. 1968 *Studies of the development and decay of the human frame*. Philadelphia, PA: W. B. Saunders.

Vanwanseele, B., Eckstein, F., Knecht, H., Stussi, E. & Spaepen, A. 2002*a* Knee cartilage of spinal cord-injured patients displays progressive thinning in the absence of normal joint loading and movement. *Arthritis Rheumatism* **46**, 2073–2078.

Vanwanseele, B., Lucchinetti, B. & Stussi, E. 2002*b* The effects of immobilization on the characteristics of articular cartilage: current concepts and future directions. *Osteoarthritis Cartilage* **10**, 408–419.

Vu, T. H. & Werb, Z. 2000 Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev.* **14**, 2123–2133.

Wong, M., Wuethrich, P., Buschman, M. D., Eggli, P. & Hunziker, E. 1997 Chondrocyte biosynthesis correlates with local tissue strain in statically compressed adult articular cartilage. *J. Orthopaedic Res.* **15**, 189–196.

Wong, M., Ponticiello, M., Kovanen, V. & Jurvelin, J. S. 2000 Volumetric changes of articular cartilage during stress relaxation in unconfined compression. *J. Biomech.* **33**, 1049–1054.

Wong, M., Siegrist, M., Goodwin, K. & Park, Y. D. 2002 Hydrostatic pressure, tension and unconfined compression differentially regulate expression of cartilage matrix protein. In *Trans. 48th Ann. Mtg Orthopaedic Res. Soc., Dallas, TX, 2002*, vol. 27, p. 033.

Wu, Q. & Chen, Q. 2000 Mechanoregulation of chondrocyte proliferation, maturation, and hypertrophy: ion-channel dependent transduction of matrix deformation signals. *Exp. Cell Res.* **256**, 383–391.

Wu, Q., Zhang, Y. & Chen, Q. 2001 Indian hedgehog is an essential component of mechanotransduction complex to stimulate chondrocyte proliferation. *J. Biol. Chem.* **276**, 35 290–35 296.

Yellowley, C. E., Jacobs, C. R. & Donahue, H. J. 1999 Mechanisms contributing to fluid-flow-induced Ca2+ mobilization in articular chondrocytes. *J. Cellular Physiol.* **180**, 402–408.

GLOSSARY

CTGF: connective tissue growth factor
ECM: extracellular matrix
Ihh: Indian hedgehog
MMP: matrix metalloproteinase
OI: osteogenic index