Biomechanical model to simulate tissue differentiation and bone regeneration: application to fracture healing

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Abstract—*Bone regeneration is a common biological process occurring, for example, during fracture healing or osseo-integration of prostheses. Computer simulation of bone regeneration is difficult to carry out because it is a complex sequence of cell-mediated processes regulated by mechanobiological stimuli. An algorithm to predict the time-course of intramembranous and endochondral ossification has been developed. The algorithm assumes that there are precursor cells in the undifferentiated tissue and that these cells differentiate into either fibroblasts (to form fibrous connective tissue), chondrocytes (to form cartilaginous tissue) or osteoblasts (to form bone), based on a combination of biophysical stimuli derived from strain in the collagenous matrix and flow of the interstitial fluid. Both these stimuli are known to deform the precursor cells, and the authors hypothesise that this causes activation of cell differentiation pathways. The observation that precursor cells take time to spread throughout the fracture callus has been included in the algorithm. The algorithm was tested in an investigation of the fracture healing of a long bone using an axi-symmetric finite element model. The spatio-temporal sequence of tissue phenotypes that appear in the course of fracture healing was successfully simulated. Furthermore, the origin of the precursor cells (either surrounding muscle, bone marrow or periosteum) was predicted to have a fundamental effect on the healing pattern and on the rate of reduction of the interfragmentary strain (IFS). The initial IFS = 0.15 drops to 0.01 within seven iterations if cells originated from the surrounding soft tissue, but took more than 50% longer if cells originated in the inner cambium layer of the periosteum, and four times longer if precursor cells originated from the bone marrow only.*

Keywords—*Fracture healing, Mesenchymal stem cells, Mechanobiology, Computer simulation*

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List of symbols

$\sigma_{1,2,3}$	= principal stresses
D	= diffusion co-efficient
E	= average Young's modulus
$E_{granulation}$	= Young's modulus of granulation tissues
E_{tissue}	= Young's modulus of differentiated tissue
H	= hydrostatic stress
n	= cell density
n^{max}	= maximum cell density
OI	= osteogenic index
S	= octahedral shear stress

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1 Introduction

THE SUCCESS of many orthopaedic treatments relies on rapid bone regeneration, e.g. fracture healing or prosthesis osseo-integration. The process of fracture healing involves a sequence of several complex events that usually return a bone to an almost perfectly healed condition.

It has long been known that successful healing requires the maintenance of appropriate forces on the bone. For example, PAUWELS (1941) reported that the development of pseudo-arthroses occurs if a fractured bone is insufficiently stabilised. Later, he described the relationship between mechanical forces and tissue phenotype as follows: distortional stress is a specific stimulus for the development of fibrous connective tissue, and hydrostatic compression is a specific stimulus for cartilage formation; bone forms only after stabilisation of the mechanical environment by the soft tissues (PAUWELS, 1960).

Following on from Pauwels' ideas, CARTER *et al.* (1988) used two invariants of the stress tensor, hydrostatic stress H and octahedral shear stress S to characterise the mechano-regulatory

environment inhibiting or promoting ossification. They employed a linear elastic finite element model of a homogenous fracture callus to determine the pattern of mechanical stimuli at fixed time-points during fracture healing; see also BLENMAN *et al.* (1989). They combined the two stress variables into one parameter called the osteogenic index *OI*, as follows:

$$OI = \sum_{i=1}^{c} n_i(S_i + kH_i) \quad (1)$$

The subscript denotes the ith load case, c being the total number of specific load cases, and n_i being the number of loading cycles of the ith load case. They were able to correlate high *OI* with areas of cartilage-to-bone differentiation (*i.e.* endochondral ossification), whereas low values of *OI* inhibited bone formation and maintained cartilage.

In a more precise finite element model of a fracture callus. CLAES *et al.* (1998) and CLAES and HEIGELE (1999) proposed a quantified understanding of the stimuli governing the formation of fibrous connective tissue, fibro-cartilaginous tissue, cartilage or bone. They predicted direct bone formation if strains are lower than 5%; and cartilage formation under compressive hydrostatic pressures greater than 0.15 MPa and strains smaller than 15%. All other mechanical environments appeared to favour differentiation of fibrous connective tissue or fibrocartilage.

GARDNER *et al.* (2000) used finite element models with the shape of a callus whose compliance had been determined experimentally. They quantified the stress patterns within the callus and the relative displacement of the fracture fragments and predicted that stresses were sufficiently high for tissue damage to delay fracture healing.

These three studies (CARTER *et al.*, 1988; CLAES *et al.*, 1998; GARDNER *et al.*, 2000) modelled the regenerating tissue as an elastic material. However, tissues contain significant amounts of fluid and can be modelled as poro-elastic mixtures of solid and fluid constituents, better to characterise the biophysical stimuli acting within the tissues at a cellular level.

Taking this approach, PRENDERGAST *et al.* (1997) proposed a mechano-regulation concept (Fig. 1), where tissue differentiation depends on two biophysical stimuli: shear strain in the solid collagenous phase and fluid flow in the interstitial fluid phase. The total biomechanical stress acting on the cells is thus a combination of solid and fluid forces. KUIPER *et al.* (2000) presented a similar analysis using a biphasic model for a fracture callus and found that strain provides the dominant biophysical stimulus during bone healing.

The models described above do not simulate the time-course of bone regeneration by including either cell migration or proliferation in the simulations; see PRENDERGAST and VAN DER MEULEN (2001) for a detailed review. To develop the capability to analyse bone regeneration during orthopaedic treatments using computer simulation, an algorithm has been developed to include explicitly the distribution of the cells within the regenerating tissue. For a fracture callus in a long bone, MCKIBBIN (1978), EINHORN (1998), and YOO and JOHNSTONE (1998) have proposed that the stem cell origins are

(i) the bone marrow
(ii) the inner cambial layer of the periosteum
(iii) the external soft tissues.

Which cell origin is active may depend on the nature of the fracture fixation device used in a particular fracture treatment. For example, intramedullary nails remove part of the medullary tissue, and bone plates separate the fracture site from surrounding muscle (TENCER and JOHNSON, 1994). To investigate whether or not cell origin can make a difference to fracture healing patterns, the algorithm was used to predict the time-course of fracture healing for each of the three possible stem cell origin sites given above.

2 Methods

2.1 *Regulatory model*

Following PRENDERGAST *et al.* (1997), two biophysical stimuli (tissue shear strain and interstitial fluid flow) were used as regulators for the tissue differentiation process. Differentiation was effected according to the mechano-regulation concept presented in Fig. 1. The diagram, with limits to the various fields as quantified by HUISKES *et al.* (1997), was used to determine whether the precursor cells would differentiate into either fibroblasts, chondrocytes or osteoblasts, leading to the formation of fibrous tissue, cartilaginous tissue or osseous tissue, respectively. A temporal smoothing procedure was introduced to avoid any numerical instability that might occur owing to rapid changes in material properties; see LACROIX and PRENDERGAST (2000*a*).

The first step in the simulation involved calculation of the cell concentration in each element, using the procedure described in Section 2.2 below. Next, the material properties of that element were calculated. As one time step was unlikely to be sufficient time for a complete change of an element from one tissue type to another, the average of the elastic properties was calculated using the rule of mixtures, i.e. if n was the density of differentiated cells in an element, n^{max} was the maximum cell density in an element, and $E_{granulation}$ and E_{tissue} were the Young's moduli of the granulation tissue and the differentiated tissue, respectively, the average Young's modulus was calculated as

$$E = \frac{n^{max} - n}{n^{max}} E_{granulation} + \frac{n}{n^{max}} E_{tissue} \quad (2)$$

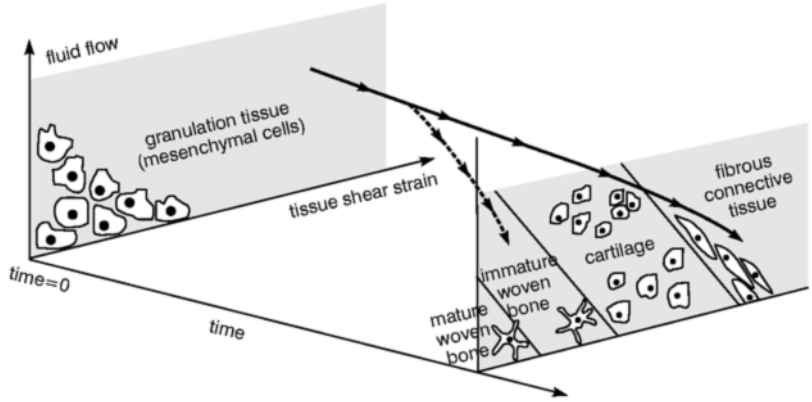

Fig. 1 *Mechano-regulation concept regulating cell differentiation to form fibrous connective tissue, fibro-cartilage or bone. Two mechanical stimuli used are tissue strain and interstitial fluid flow. Curves indicate possible changes in biophysical stimuli*

Next, the finite element analysis was iterated with the new material properties, and new biophysical stimuli were calculated at each element, thereby creating a simulation of the fracture healing process over time. This procedure was continued until an equilibrium was reached. The iterative procedure is illustrated in Fig. 2.

2.2 *Distribution of progenitor cells in callus*

The various mechanisms by which progenitor cells become distributed throughout the callus are complex and, as IWAKI *et al.* (1997) have observed, result in an early distribution of cells, although not a homogenous one, in a matter of days. Mesemchymal cells both migrate (ZOHAR *et al.*, 1998) and proliferate (IWAKI *et al.*, 1997); migration can involve crawling and cell convection in the fluid as the callus deforms. Proliferation is by cell division (mitosis). If it is assumed that the net effect of these processes is that precursor cells advance into areas of lower cell density, then the distribution of cells can be modelled using the diffusion equation

$$\frac{dn}{dt} = D\nabla^2 n \tag{3}$$

where D is a diffusion co-efficient in m^2 per day. To illustrate the use of this equation, the cell density was calculated in one dimension. Obviously, cell density would be predicted to increase with time (see Fig. 3*a*). In addition, there would be a time when the rate of increase of n at a position was maximum, and, in effect, a cell front was progressing down the channel (Fig. 3*b*).

2.3 *Application to fracture healing*

A structural model of a bone fracture was developed using an axi-symmetric finite element model. The bone diaphysis was

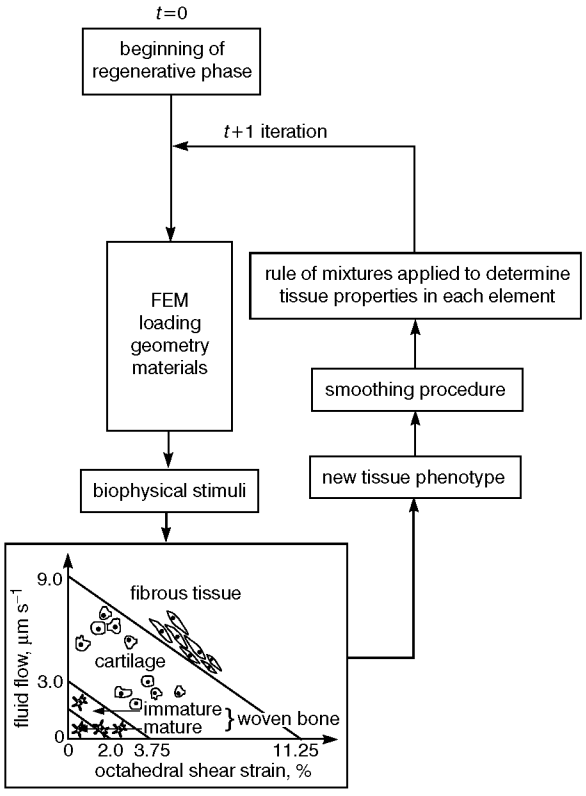

Fig. 2 *Flow chart predicting tissue differentiation over time. Calculated biophysical stimuli regulates cell differentiation through use of mechano-regulation rule. New tissue phenotype having been determined, material properties are updated using rule of mixtures*

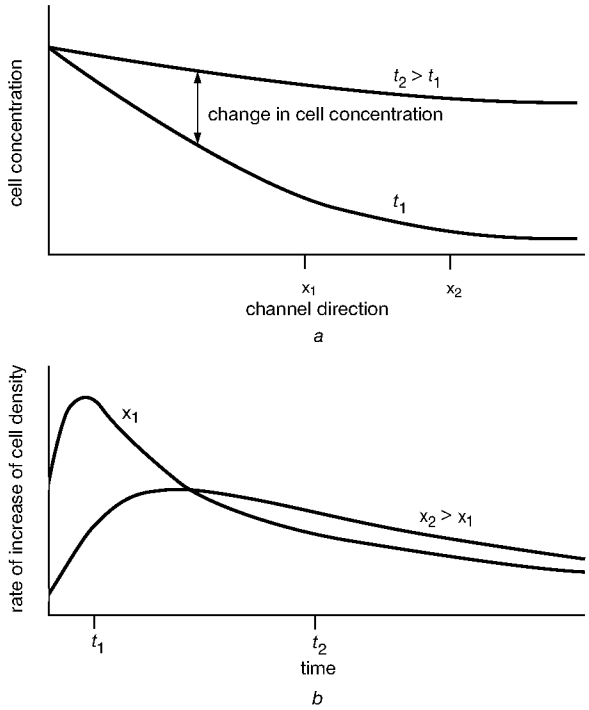

Fig. 3 *Cell concentration along channel of width equal to unity. (a) Cell concentration is calculated along channel at two times t_1 and t_2. Difference in cell concentration between t_1 and t_2 corresponds to change in cell concentration. (b) Rate of increase of cell density against time at two positions x_1 and x_2*

cylindrical, with a 30 mm outer diameter and an 18 mm inner diameter, representing a human tibia. A 10 mm fracture gap was modelled, and the external callus had a maximum diameter of 48 mm (Fig. 4). The mesh had 2536 quadrilateral elements. All tissues were modelled as biphasic poro-elastic mixtures of solid collagenous and interstitial fluid constituents, following the constitutive model proposed by MOW *et al.* (1980). The material properties were obtained by an extensive review of the literature and are listed in Table 1.

A 500 N axial ramp loading of 0.5 s was applied on the cortical shaft, and mechanical stimuli were calculated at the maximum load. Nodes in the transverse plane through the centre of the fracture were constrained in the longitudinal direction, and nodes on the centre line of the medullary canal were constrained radially (Fig. 5). The tissues on the external boundary were modelled as impermeable to fluid flow. Furthermore, a total Lagrange formulation was used to account for large strain. The Newton–Raphson iteration procedure, with a Euler backward time integration procedure, was used.

The finite element calculations were performed using DIANA*, after a validation of the code against experimental results (PRENDERGAST *et al.*, 1996). Octahedral shear strain and maximum resultant fluid velocity

$$v = \sqrt{v_r^2 - v_z^2}$$

were calculated at each iteration at the maximum loading.

In the analysis of the fracture callus, the origin of the progenitor cells was modelled by a fixed cell density being defined on either

(*a*) the external soft tissues
(*b*) the inner cambial layer of periosteum

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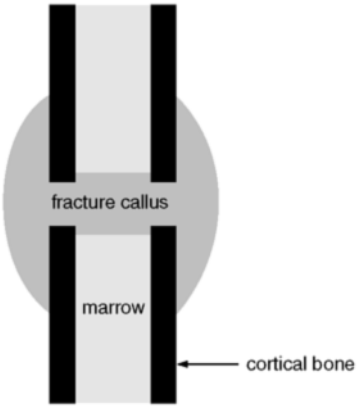

Fig. 4 *Axi-symmetric representation of a fractured long bone*

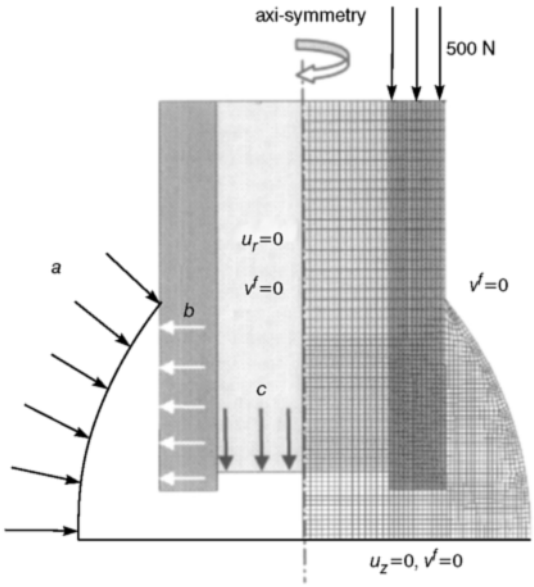

Fig. 5 *Axi-symmetric quarter model of fractured long bone. Right: biphasic model with loading and boundary conditions. Radial displacement is u_r, axial displacement is u_z, and fluid velocity is v^f. Left: cell origins, where arrows indicate three origins of progenitor cells: (a) surrounding tissues: (b) inner cambial layer of periosteum: (c) bone marrow*

(*c*) or the bone marrow interface

as shown in Fig. 5. For comparison, an equal combination of the three sources was also investigated.

The diffusion coefficient was estimated as the one that would give a steady-state cell concentration at the end of a 16 week healing period. This gave a diffusion coefficient of 2.37 mm^2 per day when cells originated from one site, with a maximum cell density obtained when cells originated from the surrounding tissues, and a coefficient of 0.34 mm^2 per day when cells originated from all three sites. Each iteration represented one day of cell propagation. To establish how inclusion of the spread of cells would make the model's predictions differ from other studies, such as that by KUIPER *et al.* (2000), the situation where the cells are immediately distributed in the callus (i.e. where $n = n^{max}$) was also analysed.

3 Results

The results showed that bone regeneration during fracture healing can be simulated using the iterative procedure proposed in Fig. 2. The various tissue differentiation sequences occurred in the order that they were observed experimentally, i.e., beginning from the early healing phase when the callus was composed mainly of granulation tissue, the simulation predicted both direct bone formation (called intramembranous ossification) and bone formation via a cartilage phase (called endochondral ossification). The spatial variation of tissue differentiation is discussed in below, and it is shown that this may be a consequence of the variation in biophysical stimuli. Finally, the clinically relevant variable of 'inter-fragmentary strain' is reported.

3.1 *Healing pattern*

The origin of the cells was predicted to have a significant influence on the healing pattern and on the healing rate. When progenitor cells originated from the surrounding tissues, it was predicted that direct (intramembranous) bone formation would occur along the periosteum some distance from the fracture, site; see the dark green region in Fig. 6*a* (note that lighter green indicates a lower amount of bone in these Figures). At iteration 2, some cartilaginous tissue was predicted in the external callus, and fibrous tissue was predicted in the fracture gap. In subsequent iterations, ossification of the external callus occurred, leading to a bridging of the fracture gap through endochondral ossification at iteration 11; only islands of cartilage remained at this time. As bridging caused the strain to reduce considerably, the remaining soft tissue was rapidly replaced by bone. This sequence is shown in Fig. 6*a*.

When cells originated from the inner cambial layer of the periosteum (see the sequence shown in Fig. 6*b*), a quite similar healing pattern occurred, except that the region of intramembranous bone formation was somewhat larger (compare iteration 2 in Figs 6*a* and *b*). However, the progress of endochondral ossification was slower, so that there were still islands of cartilage at iteration 15. Eventually, complete ossification and bridging of the external callus occurred.

When cells originated from the bone marrow only, the prediction was that bridging could also occur in the medullary canal, but only at a very slow rate; see the sequence shown in Fig. 6*c*. Cartilage was first predicted in the medullary cavity, until sufficient stability had been achieved to allow ossification to proceed. However, few cells reached the external callus, and thus no significant external callus seemed to be needed for successful fracture healing in this case.

When cells originated from the three sources, it was predicted that healing would occur through formation of both an external

Table 1 Material properties of tissues

	Granulation tissue	Fibrous tissue	Cartilage	Marrow	Immature bone	Mature bone	Cortical bone
Young's modulus, MPa	1	2*	10*	2	1000	6000†	20 000†
Permeability, $m^4(Ns)^{-1}$	10^{-14}	10^{-14*}	$5\times10^{-15*}$	10^{-14}	10^{-13}	3.7×10^{-13}‡	10^{-17**}
Poisson's ratio	0.17	0.17	0.17	0.17	0.30	0.30	0.30

* HORI and LEWIS (1982)
† CLAES and HEIGELE (1999)
‡ OCHOA and HILLBERRY (1992)
** COWIN (1999)

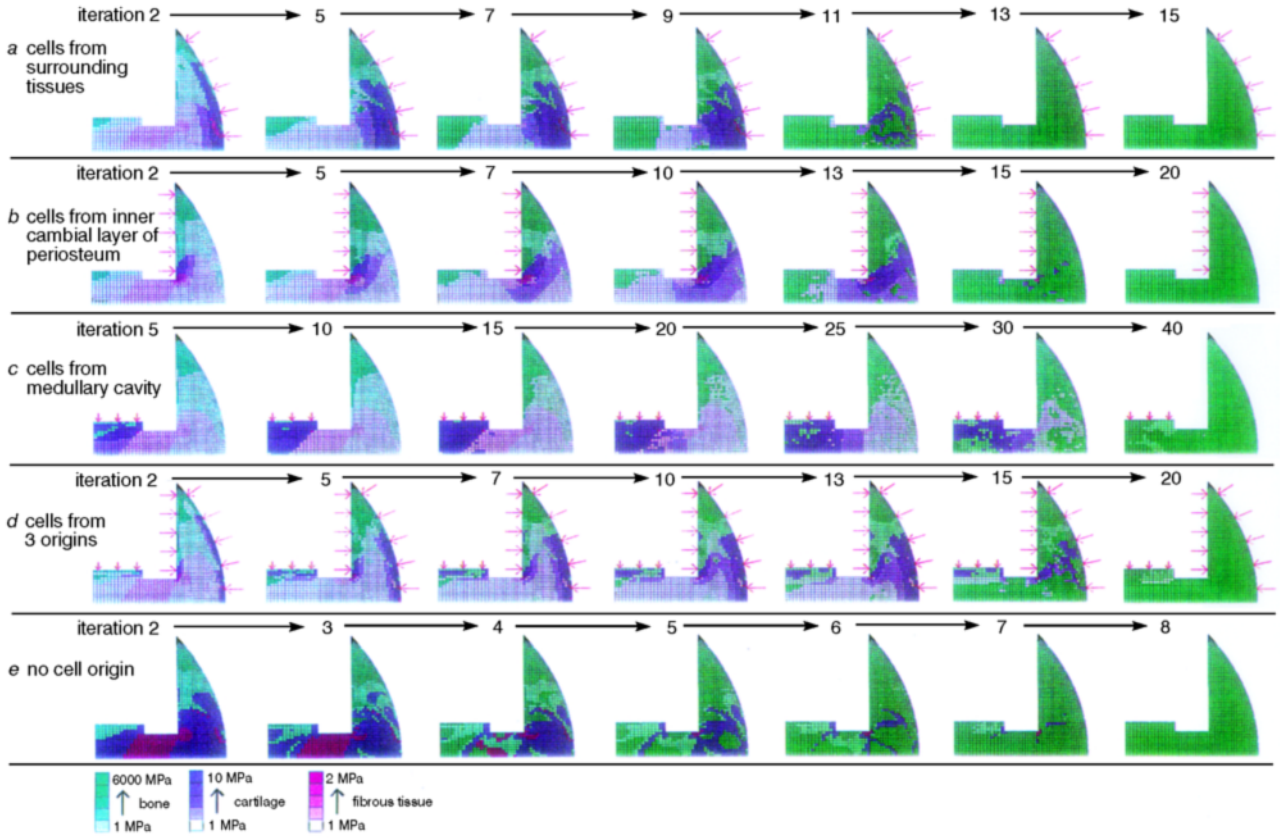

Fig. 6 *Tissue differentiation when cells are coming either from (a) surrounding tissues; (b) inner cambial layer of periosteum; (c) medullary cavity; (d) three origins; or (e) no cell origin. Arrows indicate origin of precoursor cells*

callus and an internal callus; see the sequence shown in Fig. 6*d*. In effect, the model predicted two independent ossification fronts progressing at different rates. In this model, stabilisation was mainly achieved by bone formation in the external callus. Therefore, if it is true that the cells spread through the callus in a way that can be modelled by the diffusion equation, then these results indicate the importance of knowing precursor cell origins to simulate fracture healing.

The situation of cells being instantaneously distributed in the callus was analysed. Relative to the cell proliferation simulations, the region of direct (intramembranous) bone formation was larger, and the fracture gap contained highly differentiated fibrous tissue. A very rapid ossification of the callus was predicted, leading to bridging of the fracture gap; see the sequence shown in Fig. 6(*e*).

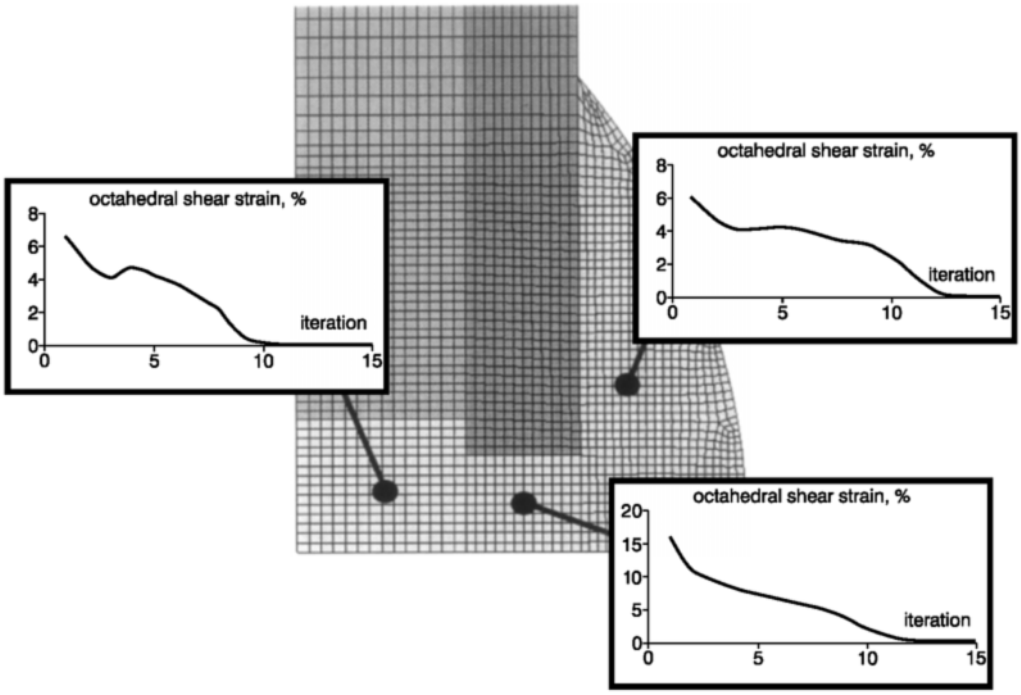

Fig. 7 *Prediction of octahedral shear strain when cells originate from three origins. Three characteristic locations were chosen in external callus, fracture gap and medullary cavity*

3.2 *Mechanical stimuli*

The change in biophysical stimuli was considered only for the case where the cells originated from all three locations, i.e. the sequence shown in Fig. 6*d*. Octahedral shear strain was highest in the inter-fragmentary gap, intermediate in the internal callus and lowest in the external callus. In each case, it reduced over time, as shown in Fig. 7. It was the high strain in the fracture gap that was responsible for creating fibrous tissue there, which was quickly replaced by cartilage and eventually differentiated to bone when the strain became low enough: this happened after bridging had occurred in the external callus, because bridging shielded the inter-fragmentary tissue from the applied load, reducing the strain in the inter-fragmentary tissue and allowing bone formation to occur there.

On the other hand, the fluid flow pattern during the reparative phase was rather complicated; see Fig. 8. In the early stages of healing, the highest fluid flow was generated in the fracture gap and it reduced as cartilage formed; when bone began to differentiate (iteration 15/16; see Fig. 6*d*), the fluid flow increased, because woven bone is more permeable than cartilage. In the external callus, fluid flow was generally low, except when tissue differentiated into bone, and fluid flow suddenly increased. Thereafter, the increased stiffening of the solid phase reduced the ability of the fluid to flow; this is shown after iteration 15 in the graph on Fig. 8. Fluid flow was predicted to be very low initially in the medullary cavity, but to increase rapidly until stabilisation was sufficient. Even if the values of the fluid flow calculated here are not precisely what occurs in reality (owing to the difficulty of defining the permeability for the differentiating tissues), the predictions do support the conclusion that a very heterogeneous flow distribution occurs within the callus.

3.3 *Interfragmentary strain*

PERREN (1979) proposed that the strain in the regenerating tissues should reduce as new tissues differentiate in the fracture gap during healing. The numerical simulations of this study

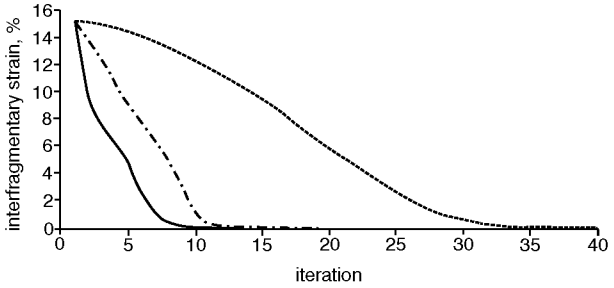

Fig. 9 *Interfragmentary strain within fracture gap. (—) Cells from surrounding tissues; (-·-·-) cells from periosteum; (. . .) cells from marrow*

corroborate the inter-fragmentary strain theory (as shown in Fig. 7). In addition, the simulations presented here predict that the reduction in interfragmentary strain depends considerably on the source of cells, with the most rapid reduction occurring when cells originate from the outside, i.e. muscle, and the slowest when cells originate from the marrow; see Fig. 9.

4 Discussion

The capability to predict bone regeneration after orthopaedic treatments would allow computer simulation to be used alongside animal experiments in the preclinical testing of new implants and other devices. In this paper, a method that can simulate bone regeneration has been developed and applied to the problem of fracture healing simulation. To our knowledge, it is the first reported simulation of mechanically mediated bone regeneration. Furthermore, it has been shown that the predicted tissue differentiation patterns accord with those observed histologically.

One key attribute of the simulation algorithm was the inclusion of the process of osteochondral progenitor cell diffusion in the callus. It was predicted that this has a considerable

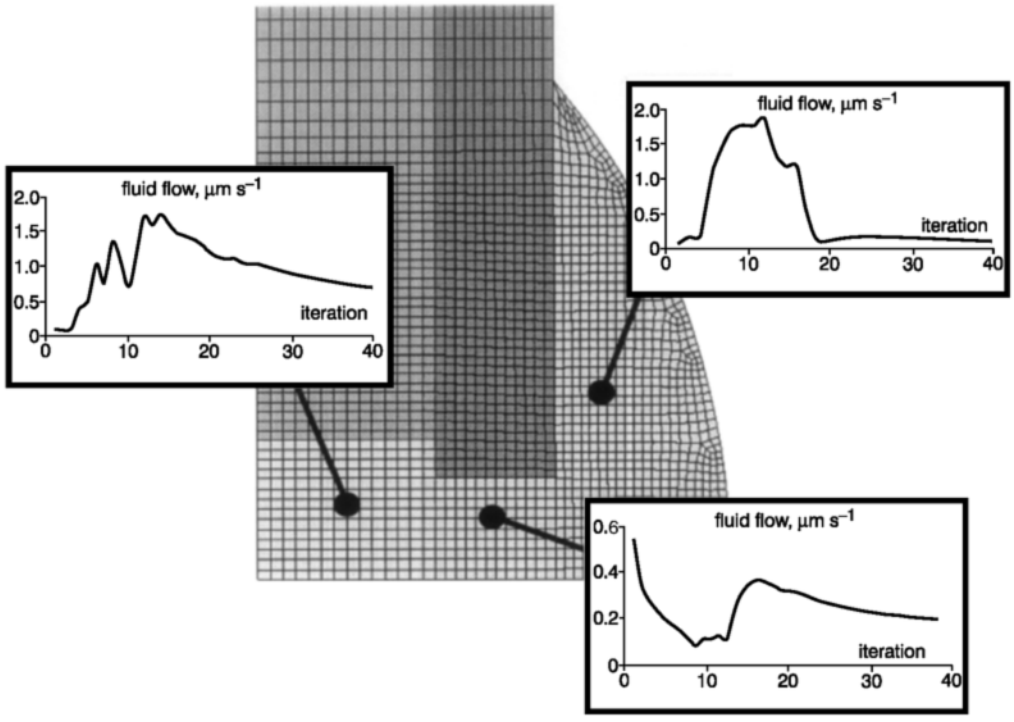

Fig. 8 *Prediction of fluid flow when cells originate from three origins. Three characteristic locations were chosen in external callus, fracture gap and medullary cavity*

effect on the healing pattern (Fig. 6) and the healing rate (Fig. 9). However, these predictions should be considered carefully, as cells will also proliferate, and explicit inclusion of proliferation requires the rate of increase of cell density n at a site to be given as

$$\frac{dn}{dt} = D\nabla^2 n + ns(c) - kn \tag{4}$$

The first term on the right-hand side of (4) describes cell migration by simple linear diffusion (compare (3)); the second term describes cell mitosis, where $c(\boldsymbol{x},t)$ is the chemical concentration of a mitosis-inducing factor; $s(c)$ is a function describing the mitosis rate per cell; and k is a constant describing the cell death or removal rate; see SHERRATT *et al.* (1992). As our solution neglected the final two terms in (4), only cell migration was modelled. If proliferation had been included, the rate of tissue differentiation would have increased everywhere in proportion to the number of cells there (as the second term in (4) is proportional to n). As proliferation would be linear in cell density, it is not expected to alter fundamentally the tissue differentiation patterns predicted in Fig. 6. Future simulations should take account of cell proliferation and apoptosis, and also of the observation that the cell origin moves as the callus resorbs during the healing process. Finally, to keep the model as simple as possible, it was assumed that cells were not committed to a specific lineage when they entered the callus.

The analysis has other limitations that should be mentioned. First, the structural finite element analysis is limited, depending on the assumptions regarding geometry, loading and definition of material properties (PRENDERGAST, 1997). In this study, an axi-symmetric finite element model was used to simulate a fully symmetric transverse fracture with a constant external callus and fracture gap size. Only axial loading was taken into consideration, meaning that the model was most representative of well-fixed fractures. Following PRENDERGAST *et al.* (1997), the tissues were modelled as compressible biphasic materials. However, the material properties were not measured by us but were taken from a thorough survey of the literature (Table 1). Finally, (2) is the simplest method to determine the aggregate Young's modulus; clearly, further advances in modelling the aggregate modulus would allow a more accurate determination of matrix stiffness.

This study opens the possibility of being able to simulate fracture healing when fixation devices that affect the source of precursor cells are used. According to RHINELANDER (1974), intramedullary nailing disrupts the medullary artery and, consequently, the source of cells coming from the marrow. Therefore it can be assumed that, with intramedullary nailing, tissue differentiation during fracture healing is dependent on cells originating from the surrounding tissues. On the other hand, if fracture-fixation plates are used to heal tibial fractures, the source from the surrounding tissues is more or less disrupted, depending on the tightness of the plates (RHINELANDER, 1974). Thus, healing by internal callus could become the dominant mode of healing: the biomechanical consequences of this are predicted from the present study in the case when cells originate from the marrow; see Fig. 9.

Three-dimensional models will be required if these methods are to be applied to patient-specific orthopaedic treatments. This has so far proven technically difficult (LACROIX and PRENDERGAST, 2000*b*), but will be the subject of further work.

5 Conclusions

A computational procedure to simulate tissue differentiation has been developed. It was tested by attempting to simulate fracture healing, and the results show tissue formation patterns similar to those observed.

The algorithm accounts for the non-homogenous distribution of precursor cells in the differentiating tissue. Besides showing that the time-course of healing can be simulated, it predicts that the site of origin of the cells has an important influence on the healing pattern. More sophisticated modelling of the mechanism of cell spreading to include explicitly cell proliferation and apoptosis in response to growth factors is required, using models already developed for wound healing (SHERRATT *et al.*, 1992; OLSEN *et al.*, 1996).

Acknowledgments

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