Stem Cells, Tissue Engineering and the Mechanical Environment

C.E. Sarraf* and M. Eastwood

Summary

The human body might suffer damage after illness, accident or indeed the ravages of ageing. Healing is the natural response, but in severe examples (and in all ageing) powers of repair are insufficient to effect a cure. Awe-inspiring and technically prodigious surgical techniques already exist but when restoration of native tissues and organs is impossible, transplantation procedures are commonly called upon. There are two major problems for the outcome of transplantation surgery at the moment. One is that the requirement for new organs greatly exceeds the number of donor body parts available, and the second is the area of immunological discrepancy between donor and recipient. The potential for individuals to have undifferentiated stem cells extra-corporeally stored for them, in a tissue bank, exists. Upon necessity these can be stimulated to expand to a sufficient number and also to differentiate into the required cell type; tissue-engineered surrogate parts can then be constructed and used surgically for bodily repair. Ideally, it would seem that autologous implants would be preferable, but close match allogenic tissues could also be utilized. A wide range of biological/biochemical techniques is routinely used in vitro to govern the direction of stem cell differentiation to a required endpoint. However, in addition, tissues and organs of the body are ubiquitously influenced by innate mechanical forces. Stem cells also experience these forces and the mechanical contribution to determining pathways of stem cell differentiation should not be ignored.

Keywords: stem cells, tissues engineering, mechanical force, bioreactors

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Introduction

The properties of multipotential stem cells need to be understood to be able to control tissue assembly. They are central to natural embryological generation of tissues and organs, as well as to potential generation of artificial body parts for the purpose of repair. Such components will be of considerable size and complexity. Suitably differentiated autologous stem cells would be the perfect inhabitants of an engineered tissue surrogate. *In vitro*, empirical cell culture regimes are routinely used to differentiate stem cells down desired pathways, but in vivo, cell phenotype is also governed by mechanical forces. For example, in the cardiovascular system, the pulsatile nature of blood flow initiates both radial and longitudinal strain, while in addition steady state flow of blood past the vascular endothelium results in shear stress on luminal wall surfaces (1): atheroma tends to develop in different people, at anatomically common sites, partly as a result of this shear stress. As a second example, articular cartilage is exposed to tensile, compressive and shear forces; during body movement the forces on joints change, and synovial fluid is subject to this stress (2). The neighbouring extracellular matrix is sensitive to changes, and resting chondrocyte progenitor cells respond to their intimate environment. Hypothetically in all tissues, perfectly grafted cells of an engineered construct should have adaptive properties that not only allow them to be immunogenically acceptable to the host body, but also enable them to contribute to/withstand the body's local homeostatic and physical environment. It is of crucial importance that an artificially constructed neo-component, built *in vitro*, needs to mimic closely forces experienced (or initated by) the natural organ *in vivo*.

Tissue engineering, as we use the term today, implies the creation of specific, matching, living grafts. However, appropriately engineered totally mechanical devices have been used successfully for many years, in particular for such purposes as heart valve replacement (3) and joint repair (4). For a living graft, the fabric of a surrogate matrix is as crucial as the cells, and a wide variety of organic polymer matrices has been explored,

frequently compounds centered on polyglycolic acid or its chemical relatives. A matrix would need to be hydrolytically degradable after introduction into the host body – with the rate of degradation exquisitely matching the rate of anabolic substitution by graft cell-derived neo-extracellular components (5). The initial non-living matrix needs to be woven, meshed or cast as a honeycomb in order for seeded cells to have space in which to proliferate, achieve contact with each other and lay down native collagen, elastin, fibronectin, gycoproteins, proteoglycans, laminins, hyaluronan and further specific extracellular matrical molecules. Clearly, overall geometry of a proposed implant needs to be the same as any body part which a tissue surrogate is to replace. The focus of this chapter, however, is on the cells of a potential tissue-engineered biological device, in particular stem cells, and on how in addition to known chemical stimuli, they might differentiate in response to mechanical signals from their environment. Once seeded and ultimately engrafted, the cells must be able to proliferate (to the optimal level), differentiate and regenerate to replace the host defect in question.

Stem Cells and Tissue Engineering

Natural cell phenotypes in tissues and organs are the result of stem cell differentiation at the embryonic stage of development. Although *in vivo* many natural biochemical/molecular biological determinants that instruct the direction of stem cell differentiation are well known (6, 7), very many cell types are also subject to native mechanical forces which also play a role in their phenotypic outcome. The source of stem cells may be either embryonic or adult (umbilical cord blood stem cells are generally classified as 'adult' though clearly a more appropriate term is sought). They should be either pluripotent, able to differentiate into cell types characteristic of any of the three developmental germ layers, or at least multipotent, capable of differentiating into varieties of an individual cell lineage (8). Totipotent cells are those of the very early embryo that, in addition, have the potential to differentiate into trophectoderm and

placenta. The definition 'stem cells' also requires that they should be able to replicate through many cycles of cell division, albeit perhaps, intermittently.

Identifying stem cells

Stem cells can be identified in a number of ways, for example, anatomically, functionally or by their specific cell surface markers, characteristic proteins and transcription factor expression. Human embryonic stem (ES) cells express stage specific embryonic antigens SSEA-3 and SSEA-4 (9, 10), CD45 is typically expressed by hematopoietic stem cells and ES-derived endothelial cells express endothelial markers including CD31, Flk-1, VE-cadherin, PECAM, Tie-1 & Tie-2 (8, 11). Human bone marrow mesenchymal stem cells are $CD34^{-ve}$, and $CD45^{-ve}$, yet $CD73^{+ive}$, $CD105^{+ve}$ and express STRO-1 (12). Further endothelial progenitor cells and circulating hematpoietic stem cells express CD34, CD133 and VEGF (13). In the area of hematpoietic stem cells, expression of typical cell surface markers is characteristically linked to the ability to efflux Hoechst 33342 dye. Early work (on murine ES cells at least) has shown the following combinations, Thy-$1.1^{lo}Sca\text{-}1^{hi}Lin^{-/lo}$ ($CD34^{+}$), $Lin^{-/lo}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (with $CD34^{-}$), and $Lin^{-/lo}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (with $CD38^{+}$). Side population cells are $Lin^{-}Sca\text{-}1^{+}$ $CD34^{-}$ or $Sca\text{-}1^{+}$ $CD34^{low}$ $c\text{-}Kit^{+}$ (14-16). Hematopoetic stem cells expressing CD44 (E-/L-selectin ligand) specifically, experimentally have been reported to mediate homing after systemic delivery. Thus they are able to regenerate damaged organs (myocardial infarct, for example) and modulate generation of new blood vessels, an important point for an engineered repair (17). Additionally, reported stem cell surface markers have been CD29, CD49a, CD71, CD90, CD105, CD114, CD166, cell adhesion molecules and HLA class determinants. A wide range of receptors for circulating molecules has also been attributed to stem cells (6). Amongst characteristic proteins, bone marrow-derived mesenchymal stem cells have been shown to express troponin, myosin, actin and atrial naturetic factor, while in breast and skin stem cells the presence of cytokeratin-19 has been noted (18, 19). Enzymes such as alkaline phosphatase and telomerase can be involved; clearly however, expression of these molecules is not exclusive to stem cells. Similarly, appropriate glycoproteins TRA-

1-60 and TRA-1-81 have been demonstrated. Relevant transcription factors are GATA, MEF-2, Nkx2.5 (8) and Oct4 (20). Oct4 particularly demonstrates itself to be of growing importance; in human tissue it has been found to be expressed in adult stem cells, immortalized non-tumorigenic cells, tumor cells and in cell lines, but not, at the time of writing, in differentiated cells (21).

Will grafted stem cells be safe?

To ensure biological safety of living engineered implants, there are justified concerns with reference to extent and quality of proliferation in seeded cells. In developing grafts, cells should reach an appropriate limit to their proliferative potential and also should not pass on deleterious genetic mutations to their progeny. In this regard, stem cells may turn out to have phenotypic, molecular and/or mechanistic traits similar to those of malignant cells. Whether the undifferentiated state of the stem cell might render it vulnerable to neoplasia-inducing changes or whether deregulation of the normally tightly controlled molecular signals that govern cell proliferation may initiate neoplasia, remains to be seen. Concerning the mechanical environment, cell proliferation status in colon cancer for example, has been shown to be partially at least, modulated by extracellular hydrostatic pressure; here, physical forces have been shown to initiate a mechanism that *via* protein kinase C (PKC)/tyrosine signal transduction causes elevated cell replication levels (22). A further concern regarding overall biological safety of an extrinsically synthesized tissue surrogate is the use of (at least partially) animal-derived components grafted into the human body. Collagen from animal sources is a major component of many tissue-engineered constructs. Improvements in synthesis of recombinant collagen (23), mean that a pure and safe source of this human material can be produced from molecular first principles.

Embryonic stem cells

These are obtained from the inner cell mass of the blastocyst and for experimental purposes murine embryonic stem cells were first derived in 1981 (24, 25); a source of human embryonic stem cells was announced in 1998 (9). After dissociation from the blastocyst to grow successfully, human embryonic stem cells generally need a supply of basic fibroblast growth factor and a feeder layer - human fibroblast, mouse fibroblast, Matrigel™ or laminin (plus mouse fibroblast cell-free medium), or be feeder layer-free (26-29). Using the embryoid body technique, human embryonic stem cells can be induced to differentiate into mesodermal or ectodermal cell precursors within a few days, but require longer to differentiate into endodermal cell types (11). Current lack of global agreement on the ethical acceptance for use of human embryonic stem cells (in harvesting, the embryo is destroyed) for requisite experimental purposes, banishes consideration of their use for tissue engineering at the moment.

Cord blood cells

These are an alternative source of stem cells. During gestation they are produced in the embryo's bone marrow, but during pregnancy they circulate freely and cross the placenta. At birth, some are in the umbilical cord. Thus, a source of stem cells from the embryo exists which for harvesting are simply collected from the umbilical cord at birth, abrogating ethical difficulties associated with the use of embryonic stem cells. Cord blood cells already play a significant role as progenitors for hematopoietic reconstitution (30), and in direct bone marrow cell transplantation (31). The cells are CD133 positive and are a sub-set of CD34 positive hematopoietic stem/progenitor cells. They can be expanded in culture under the influence of flt3-ligand, thrombopoetin and c-Kit-ligand (13). Whether these cells can be shown to fulfill the whole cell repertoire of pluripotent stem cells remains to be seen, but they present an important potential reservoir for autologous human cells. To be able to benefit from engineered tissues seeded with their own umbilical cord progenitors, a person's cells need to have been gathered at birth and stored frozen until such a date as required. In 2005, even at this early stage in cord blood

cell technology, the number of parents is increasing who choose to have their baby's umbilical cord blood saved for potential future use. This is a cheap and simple procedure to potentially benefit their offspring thereafter.

Adult stem cells

Use of stem cells obtained from adults does not require destroying any embryo or the requisite forethought of parents for years ahead - of onset of disease. Controlled plasticity of these cells to differentiate into downstream competent phenotypes, is central to the hypothesis of stem cell/tissue engineering. In practice, adult stem cells have been found to have the ability to differentiate into tissues that during normal development might arise from the same or even a different germ layer. However, certain arguments have been proposed that suggest the intervention of myeloid cell intermediates (particularly of macrophage fusion) in the development of non-hematopoietic tissues from the hematopoietic stem cell compartment (32).

Adult bone marrow-derived stem cells may be considered in two categories, hematopoietic stem cells from the bone marrow and mesenchymal stem cells from the bone marrow stroma. Hierachically down-stream derivatives of each kind of bone marrow stem cell may be found in circulating blood (dendritic cells can be generated and expanded *ex vivo* from peripheral blood monocytes or from circulating bone marrow hematopoietic stem cells) or in local anatomical niches in the epithelium or mesenchyme. A niche is a local microenvironment which harbours clusters or individual stem cells and in which they can develop. It consists of the stem cells themselves plus their optimal supporting milieu, and may be lined with myofibroblastic cells (33). Whether these local niches are in place from embryological development, or whether cells travel from the bone marrow/circulation 'on demand' is not clear at present. Thus, functional cells of a tissue might differentiate directly from bone marrow progenitors, from circulating blood multipotent cells, or alternatively from specific tissue downstream niches. In practice, ethical difficulty of obtaining human multipotent stem

cells doesn't arise when using cells from bone marrow aspiration, from foreskin (epidermis), from breast and from abdominal reductions (adipose tissue), when the donor has undergone elective surgery and approves of its use, as the removed tissue would otherwise be destroyed. These post-surgical sources provide vast numbers of 'spare' stem cells which are easier to obtain and identify than cells from minute local niches. Even so, putative niches have been described in the gastrointestinal system (34), liver (35), lung (36), kidney (37), skin (epidermis (38)), dermal mesenchyme (39), muscle (40, 41), cardiovascular system (8), cartilage (42), bone (43), adipose tissue (7), nervous tissue (44) and others.

Adult bone marrow hematopoietic stem cells

Adult bone marrow is composed of two distinct tissues, hematopoietic stem cells that give rise to the blood cell lineages, and the bone marrow-derived stroma composed of mesenchymal cells (45); stem cells can be available from each. Hematopoietic precursor cells are responsible for derivation of all mature blood cell lineages. In the early embryo such cells flow through the whole organism, but during development this is superseded by the definitive mutilineage blood system (46). Apart from this, properties of hematopoietic stem cells are not dealt with in this chapter. On the other hand, multipotent bone marrow-derived stromal cells are hierarchical precursors of adult mesenchymal stem cells, and are considered in context, below.

Adult epithelial stem cells

In the adult, epithelial stem cells contribute to normal regeneration in a variety of cell lineages. The general hierarchy is between stem cells and the devolved amplifying compartment, however, stem cells are able to express a plasticity that allows them to be directed into heterotypic phenotypes (47). The parenchymal compartments of the digestive system, liver, lung and kidney are largely epithelial tissues from the endoderm. Epidermis is an ectodermal tissue with epithelial basal cells as the proliferative compartment (the dermis is largely populated by mesodermal

mesenchymal cells). The area of mechanical force contributing to differentiation of stem cells for tissue-engineering of epithelial cell-derived organ surrogates is largely unexplored, however, their natural biology and physiology strongly indicate the potential for such research.

In the *gastrointestinal* (GI) system, particularly the large (LI) and small intestine (SI), there is a migratory cellular hierarchy from the base of crypts to the tips of villi (SI). Under normal conditions cells are continuously derived, they move up the side of their crypt/villus and are lost at the top, into the gut lumen. The existance of a multipotential progenitor cell was established for columnar, mucous and endocrine cells in 1988 (48). The location of this (at least) tripotential stem cell is close to the base of the crypt (34). Some gastrointestinal diseases, such as the inflammatory bowel diseases, require intensive treatment and often surgery. The gut as an organ continuously engenders longitudinal and circular forces in peristalsis, and engineering potential grafts for GI disease surgery would require the new tissue to contribute to and to sustain a competent reaction with these forces. As ever, natural forces in the body must be closely mimicked in an engineered construct. Experimentally, in short bowel syndrome of the rat after massive surgical resection of a portion of the gut, anastomosis of tissue-engineered small intestine with the remnant has shown that animals significantly improved post-operatively in weight gain and metabolic processes for up to 40 days after the procedure (49). *In vitro*, embryonic stem cells have already been differentiated into a functional gut-like organ. The geometry of the culture was dome-like with a lumen, and the walls were organized into an inner layer of epithelium, covered by an outer muscular covering. The construct displayed rhythmic peristalsis-like contractions and cells in dense networks had immunoreactivity resembling that of interstitial cells of Cajal *in vivo*; also they expressed the neuronal marker PGP9.5 (50). In situations where a whole circumferential, cylindrical section of extracorporeally cultured surrogate would be needed, it would require the use of a specifically designed bioreactor, such as that described by McCulloch *et al.* (51), although this to date has only been used in vascular studies.

In natural *liver*, blood enters lobules at the portal area from the hepatic artery and from the hepatic portal vein, and leaves *via* the central vein. Hepatocytes near the portal tract have exquisitely different morphology and function from those near the central vein. Periportal cells express high levels of urea cycle enzymes and low levels of glutamine synthase, while central cells have opposite characteristics. Centrilobular cells primarily express enzymes of the cytochrome P450 family but their detoxification activities result in local oxidative stress. When hepatocytes are exposed to increasing oxygen tension, urea synthesis increases substantially but P450 activity decreases although albumin secretion is unchanged. Normally hepatocytes are proliferatively quiescent, but following liver damage they rapidly enter a replicative phase to replace missing tissue. When this activity is experimentally compromised, a previously cryptic stem cell population called oval cells undertakes repopulation of the liver, rapidly proliferating and differentiating into hepatocytes (35). Several approaches for engineering functional surrogate hepatic tissue are underway, some are extracorporeal, some for engraftment. The molecular adsorbent recirculating system (MARS) is acellular but other extracorporeal systems such as Promethius (52, 53) engage elegant combinations of hepatocytes in bioreactor constructs. To be useful in the clinic, such systems require very large numbers of functioning cells (54); at least 10^{10} are necessary for incorporation as micro-aggregates in a 3D matrix. In allogeneic transplantation of a liver substitute, to overcome immune system complications (55), hepatocytes can be (already expanded in number in culture) held to the walls of hollow fibre cartridges. Grafting autologous liver stem cells would clearly obviate this complexity. However, obtaining and expanding the number of potential liver stem cells has proved to be problematical. One factor that workers in liver transplantation have reported (33), is that grafted hepatocytes tend to fail to replicate unless innervated. By the same token, hepatocytes are sensitive to mechanical forces. There are considerable differences in blood pressure in the hepatic lobule. Entering the portal tract, pressure in the branch of the hepatic artery is around 30mm Hg, while that in the hepatic portal vein is only at 10mm Hg. However, the latter delivers two-thirds of the blood supply, and blood mixes to leave the lobule at the resulting pressure. Thus, periportal hepatocytes are subject to a higher level of

mechanical pressure than those proximal to the central vein. Hepatocyte metabolite production *in vitro* responds to increased fluid shear stress on cells; albumin and urea synthesis decrease significantly (55), while cytochrome P450-mediated detoxification increases (56). An appropriate mechanical environment reflecting these differences in blood pressure, would improve future liver bioreactor design for success in neo-liver fabrication.

In *lung* alveoli of the respiratory system, the principal parenchymal cells are type I and type II pneumocytes. Fetal cytokeratin- and surfactant C-expressing progenitors of type II pneumocytes have been shown to be competent to differentiate also into type I pneumocytes, mucus producing cells and possibly also pulmonary neuroendocrine cells. This demonstrates their stem cell properties. Also in the proximal airways, Clara cells have been shown to have stem cell properties (36). In normal conditions these cells provide a rapid response to injury. At present, management of respiratory failure is mainly with the aid of mechanical ventilation. Initiation of phenotypic differentiation and lung fibrotic processes as a result of mechanical stimulation, have been noted. Garcia *et al*. (57) have shown that lung epithelium is sensitive to forces and that there is activation of intracellular signal transduction. By RNA isolation followed by northern blot analysis and semi-quantitative reverse-transcription polymerase chain reaction they have demonstrated that cytosolic type III procollagen mRNA (a marker of lung parenchymal remodeling) expression occurs after force increases have been applied *in vitro*, although they noted no changes in extracellular collagen fibre synthesis. However, Breen observed that increased mechanical strain exerted on lung parenchyma has resulted in tissue remodeling and type I collagen expression (58).

The *kidney* is an anatomically, architecturally and developmentally complex organ. In normal embryological development, undifferentiated metanephric mesenchymal cells condense at the tips of branching ureteric buds, and differentiate into epithelium. That is, mesenchymal cells differentiate into epithelial cells as a normal event (59). [Interestingly, recent findings have shown that mesothelial cells can undergo epithelial

to mesenchymal transition and transform into myofibroblasts and smooth muscle cells. A proportion of mesothelial cells remains free-floating in the serosal fluid; in response to injury they can proliferate to regenerate lost tissue. The mesothelium is of mesodermal origin and the cells express characteristics of both epithelial and mesenchymal phenotypes; their origin, however, is at the moment unknown (60)]. Suggestions for the kidney's ability to self-repair have been widespread, including recruitment of hematopoietic stem cells to fulfill renal roles. Post-injury renal remodeling involves immigration of hematopoetic cells after which they differentiate into both tubular epithelium and glomerular phenotypes (61). Oliver *et al.* (37) have shown that long-term labeling of proliferating cells in rat and mouse kidney revealed stem cells in the renal papilla that not only had the ability to incorporate into the renal parenchyma, but also their phenotypic plasticity could be controlled by variations in oxygen tension. When cultured *in vitro*, the cells spontaneously formed spheres expressing mesenchymal, neuronal and/or epithelial markers, and were able to differentiate into myofibroblasts and other phenotypes, thus demonstrating their 'stemness'. Fluid volume passage and solute concentration are central in renal physiological function and kidney tubule cells, in particular, are sensitive to mechanotransductive events. Embryonic kidney cells respond to changes in fluid stress experienced across their surface and they are able to modulate calcium influx through stress-sensitive ion channels. Cytoskeletal changes (particularly in microfilaments and microtubules) eliminate fluid shear stress-induced increase of intracellular calcium, thus restoring the homeostatic *status quo*. Tissue engineering for the kidney has included: cell implants (renal primordial cells), uretic bud and mesenchymal cell grafts, transplantation and xenotransplantation of embryonic kidney, cells lining a bioartificial kidney and bioactive scaffolds. Success has followed a greater understanding of the branching morphogenesis of developing engineered kidney substitutes and the role played by mesenchymal cell differentiation (59). Greater success will be achieved when robust models are generated which take into consideration the forces of fluid volume passage and changes in fluid volume stress in the kidney.

Possibly because of its easy accessibility, the *epidermis* of the skin is the most successful tissue candidate for synthetic replacement, use of autologous keratinocytes being ideal. Mechanical forces experienced in normal skin are important because this organ covers the skeleton and all other tissues and organs. The latter are all in constant motion, thus skin needs always to be able to stretch and recover. Normally, epidermal skin cell turnover is endlessly dynamic and differentiated keratinocytes are replaced by the proliferation of differentiating epithelial progenitor/stem cells. The hair follicle has been described as having neural crest-derived multipotential stem cells (in the mouse, at least) living in a keratinocyte niche. Resuspended *in vitro* and grown in separate clonal cultures, these cells have been found to give rise to colonies of pigment cells, smooth muscle cells, neurons, Schwann cells, and chondrocytes. A further neural crest-derived cell differentiated from cells of the bulge area of the whisker follicle is the Merkel cell (62). Epidermal stem cell therapy is beginning to be used in conjunction with gene therapy in the treatment of burns, chronic wounds, leptin deficiency and diabetic skin conditions. Gene therapy to the general population of epidermal progenitor cells suffers from the normal continuous loss (by surface sloughing) of the genetically modified cells. Appropriately modifying the genome of permanent stem cells would provide a longer lasting option (63). In tissue engineering, epidermal substitutes largely consist of proliferating keratinocytes plus a feeder layer of stromal fibroblasts; in clinical practice, effective epidermis substitutes are derived from sub-culturing cells from patient biopsies (to ensure specific cell population components, keratin 19 expression may be used as a keratinocyte stem cell marker (19)). Cell number is then expanded on a suitable matrix prior to engraftment. Application of physical force to the proliferating keratinocytes has been found to enrich final epidermal cell number production. Such studies reporting *in vitro* results plus the following *in vivo* clinical outcome are rare. However, here, in the experimental study protocol, the initial cell source was human foreskin. Cells were digested and separated using trypsin-EDTA. A novel magnetic stirring technique was used which improved cell yield. Centrifugal force experienced by the cells cultured under these conditions resulted in increased levels of surface beta(1) integrin expression.´The hypothesis was that this cell adhesion molecule then initiated

expression of downstream second messengers across the cytoplasm to the nucleus, resulting in cell proliferation. Isolated keratinocytes were then grafted on to nude mice and fully differentiated epidermis, consisting of basal epidermal cells, transit amplifying cells and ultimately fully differentiated cells was formed. Clinically, biopsies of normal skin were taken from patients close to their wound sites. Using the magnetic stirring method *in vitro* cell number expansion time was shortened in which to achieve the required area of engineered skin, which was then grafted back to the patient *in vivo* (64). Mechanochemical force transduction processes initiated from the epidermis (for example, compression or tensional forces, frictional shear forces) or from the dermis (contraction of collagen fibres, resulting in an increase in tension; production of elastin, increasing elasticity and up-regulation of further structural proteins), complete the integral mechanical cohesion of the two skin compartments. Mechanochemical force transduction is the term used to describe how an area of a cell is able to react to mechanical force incident on it, and transduce this force into a signal that the genome in the nucleus can receive and interpret. On the cell membrane are integrins, particularly $\alpha 2\beta 1$ which by conformational change in the molecule initiates activation of intracellular second messengers. Also in the plasmamembrane are ion channels of both calcium and potassium (there are also calcium ion channels in gap junctions). The integrins and calcium and potassium conformations of ion channels stretch directly when mechanical force is applied. Standard second messenger systems are invoked, particularly *via* collagen twelve and tenacin mechanisms. Thus PKC activates the mitogen-activated protein (MAP) kinases that ultimately influence production of nuclear AP-1 which is a DNA transcription factor. Alternatively, the RAS system, *via* IKK or the MAP kinases can incur nuclear NF-κB which also results in DNA transcription. Changes at the genome level result in transcription of appropriate genes and then translation to their proteins. Molecules of both the cell's cytoskeleton and extracellular matrix for example, are generated and also molecules that govern control of cell proliferation and differentiation (65).

Plastic surgery to reconstruct skin lesions uses a wide range of tissue engineering techniques from simple local skin flaps to advanced microsurgery of free composite grafts (66). The grafted, engineered tissue cells need to be able to recapitulate mechanical properties of the lost area. They do this by being able to reconstruct the local extracellular matrix, by expression of matrix proteins including collagens and elastin. Stem cells can be applied to an anatomically acceptable matrix (67) - plasma copolymers of acrylic acid/octa-1,7-diene being successful in this respect (68). Vitiligo also is treated with keratinocyte-melanocyte co-cultures in such a copolymer matrix (69).

Adult mesenchymal stem cells

The normal *dermis* of the skin is largely populated by mesodermal mesenchymal cells such as fibroblasts, smooth muscle (vascular and lymphoid), skeletal muscle and adipose tissue. In normal skin, it is separated from the epithelial cells of the epidermis by the shared basement membrane, to which both cell types contribute components; mechanical continuity at the epidermal-dermal junction resides in this. In the dermis fibroblasts and myofibroblasts synthesise their extracellular matrix and at plasmamembrane fibronexi, extracellular fibronectin converges with cytoskeletal alpha-smooth muscle actin (70, 71) providing the stable link between mechanical contraction outside the cell and that of cytofilaments of the interior. Also here, stretch-induced changes that occur in plasmamembrane ion channels result in cell contraction which overall effects gross tissue contraction. Activation of membrane-initiated second messenger pathways that are able to influence cell synthetic functions, proliferation and differentiation occur, as described above (65). In a model for protein synthesis due to mechanical activation of human dermal fibroblast contraction (72), mechanical forces that cells derived have been measured in a culture force monitor (73). Similar samples were taken and external forces, in the physiological range, were applied to them in a tensioning-culture force monitor (74) (Fig. 1). The cells' phenotypes changed in a characteristic fashion under these conditions (Figs. 2 and 3), reflecting the synthesis of alpha-smooth muscle actin in the stressed cultures. Cells were then homogenised in Trizol™; biotinylated cRNA probes were generated and gene expression profiles were

assessed by hybridisation to gene chip arrays. Gene sequences found to be significantly elevated included those for proteases (for example serine protease) and structural proteins such as myosin and smoothelin. Vascular endothelial growth factor gene expression was seen to be elevated as were genes for transcription factors such as c-fos. Extracellular matrix modifying genes, inhibitors of metalloprotease, plasminogen activator inhibitor-1 and plasminogen activator receptor genes were also over-expressed. This extrapolated information from previous studies concerning application of mechanical force to dermal fibroblasts and the extracellular matrix (75).

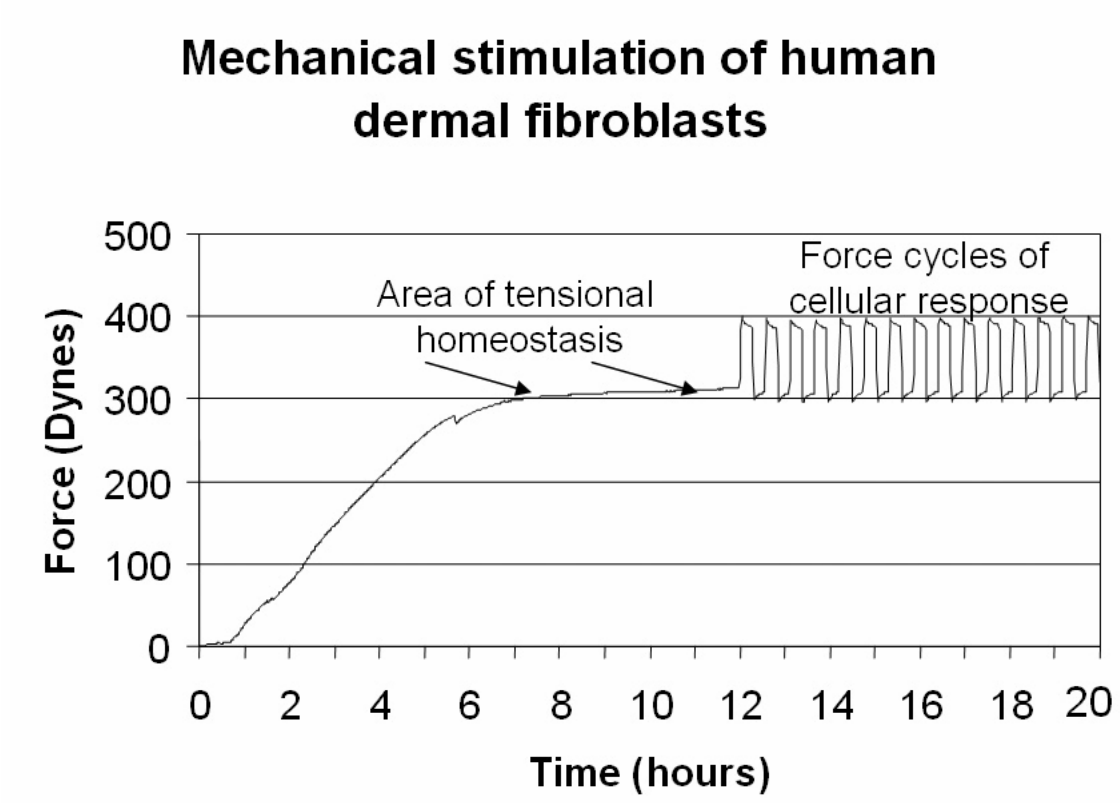

Fig. 1: Contractile curve generated by human dermal fibroblasts whilst residing in a 3D collagen matrix. Note the onset of tensional homeostasis at the 7 hour time point. Tensional homeostasis is a term that describes the value at which cells have reached their normal capacity to stretch. External mechanical stimulation commenced at 12 hours and the cellular response exerted to maintain tensional homeostasis is demonstrated.

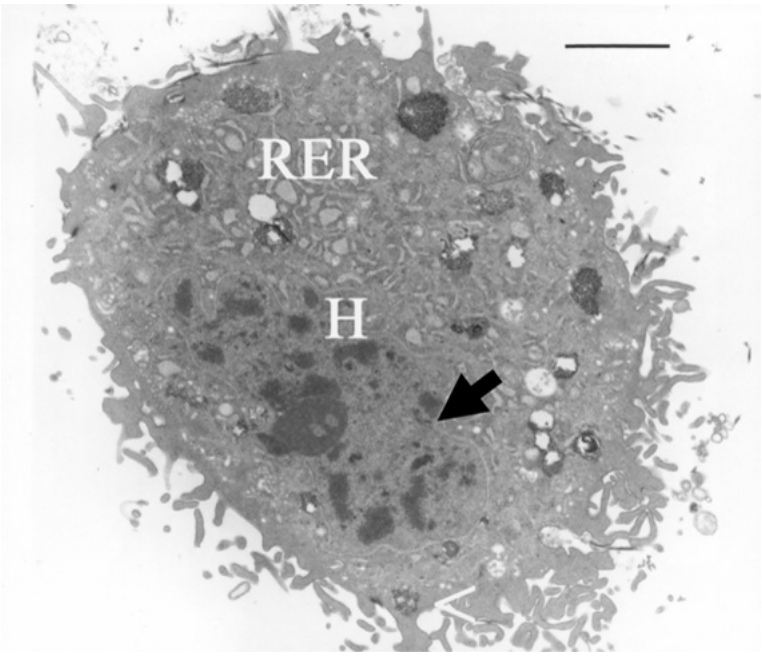

Fig. 2: Transmission electron micrograph of a dermal fibroblast, after harvesting and culturing *in vitro* then seeding into acid soluble type I collagen gel for 24hours. The plasmamembrane is highly folded and the nuclear membrane (large arrow) is indistinct and not associated with heterochromatin (H). Cytoplasm is rich in rough endoplasmic reticulum (RER) and there are dense vacuolar contents. Cells in this category of the experiment were the controls, neither attached nor subjected to external forces. Bar = 5microns.

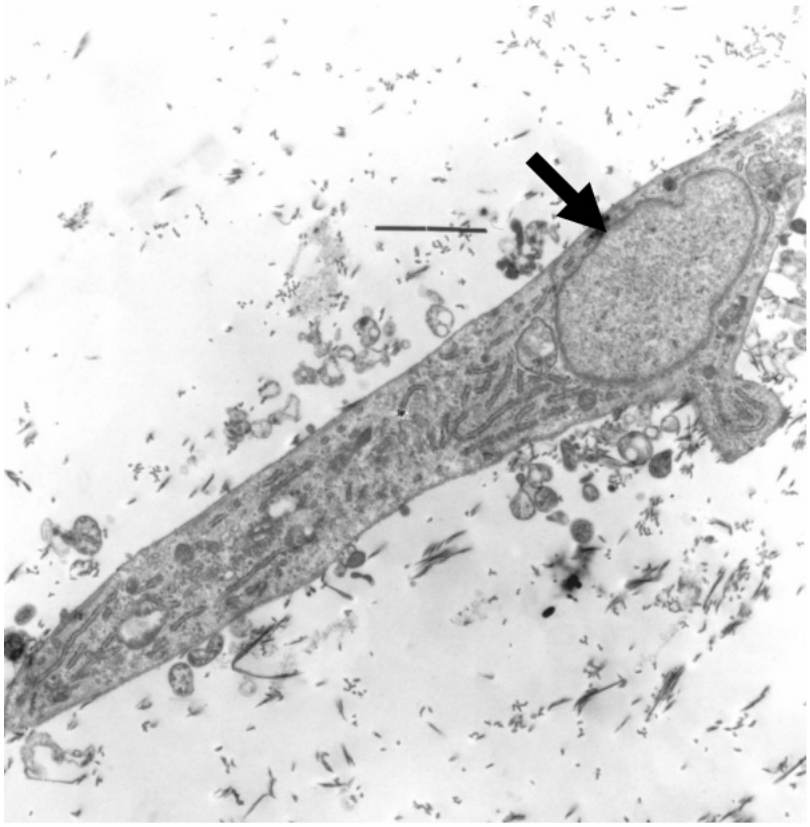

Fig. 3: Transmission electron micrograph of a dermal fibroblast, after harvesting and culture *in vitro* then seeding into acid soluble type I collagen gel for 24hours, attached in the culture force monitor. Changes in the cells' phenotype are related to attachment; no forces are applied in this bioreactor. The cell has an elongated phenotype in response to being tethered to the attachment, and has a smooth plasmamembrane. Nuclear characteristics are distinct from those of non-attached cells (cf. Fig. 2.), in particular the totally euchromatic nucleus with a well-formed nuclear membrane (large arrow). Bar = 10microns.

The *bone marrow stroma* is the source of bone marrow-derived mesenchymal stem cells (BM-DMSC)s. BM-DMSCs are multipotent and have been consistently reported to have the ability to differentiate into previously unanticipated tissues of both the epithelium and the mesenchyme. *In vitro*, as they differentiate under carefully controlled conditions, they express phenotypic characteristics of various end-point cell types such as endothelium, smooth muscle, skeletal myofibroblasts, cardiomyocytes and nerve (76). Early in the recent literature BM-DMSCs found in niches in skin, muscle (smooth, skeletal and cardiac), and adipose tissue have been shown to readily differentiate further into fibroblasts, myocytes, chondrocytes, osteocytes, adipocytes and nervous tissues (77). Such stem cells from local niches, as opposed to cells coming directly from the bone marrow, appear to have already progressed some way towards lineage commitment. They also are able to differentiate *in vivo* into endothelial cells, cardiomyocytes, and smooth muscle cells after direct injection into the heart (78), and they have migrated to areas of myocardial injury after systemic delivery (79). Anatomically, these multipotential cells tend to be very small, typically less than 3μ in diameter and in the laboratory they can be separated from whole bone marrow by simple filtration (80). Technically, to obtain stem/progenitor cells from aspirated bone marrow, it is anticipated that there would be around 2000 connective tissue stem cells per milliliter (~ one per 20,000 cells). Cartilage progenitors can be distinguished by their production of proteoglycans, early bone progenitor colonies by their expression of alkaline phosphatase (81), and later ones by presence of calcification stained with alizarin red, while lipid accumulation in differentiating adipocytes is demonstrated by staining with oil red O or sudan red. In our laboratory, we also have noted autonomous differentiation of human and mouse BM-DMSCs (82), into functional fibroblasts/myofibroblasts (Figs. 4 and 5) plus synthesis of extracellular matrix (Otto & Rao, Cancer Research UK, work in progress), in our case when the only stimulus was their spontaneous contraction against a collagen matrix. Forces derived by the cells were recorded (Fig. 6).

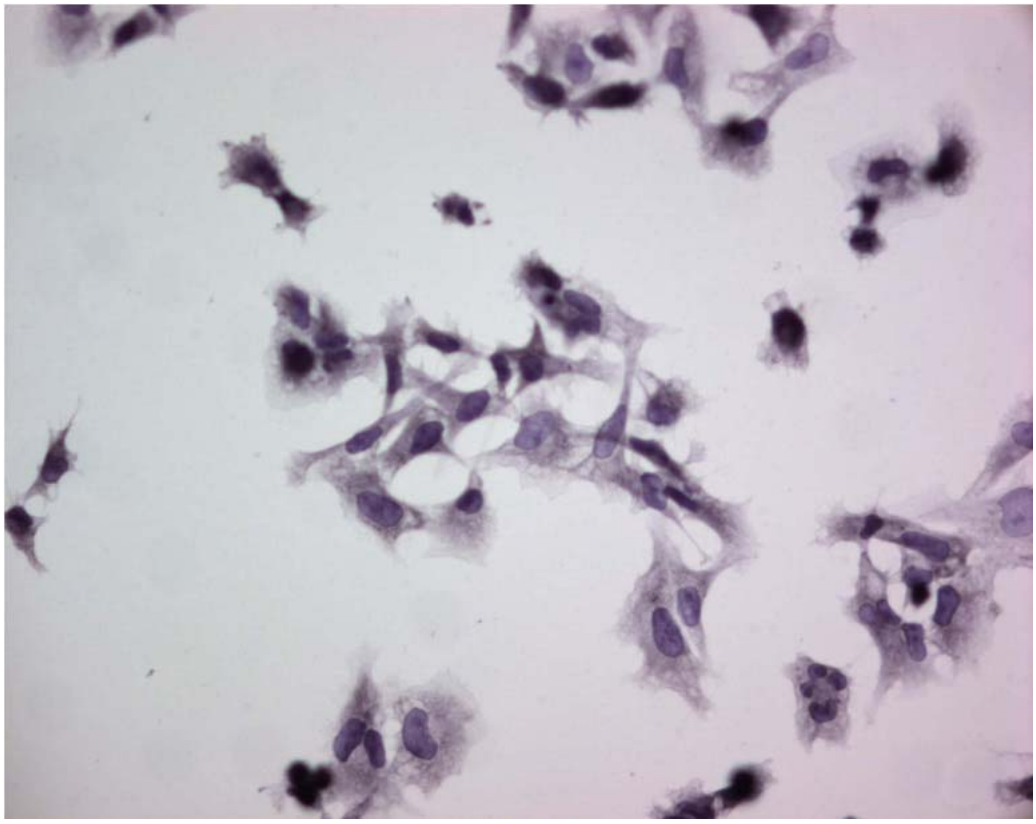

Fig. 4: Light micrograph of immunolabelling (DAB + nickel chloride) of alpha-smooth muscle actin in human stem cells after harvesting and culture *in vitro* then seeding into acid soluble type I collagen gel for 24hours. The cells have developed cell/cell junctions. Original magnification x40 objective lens.

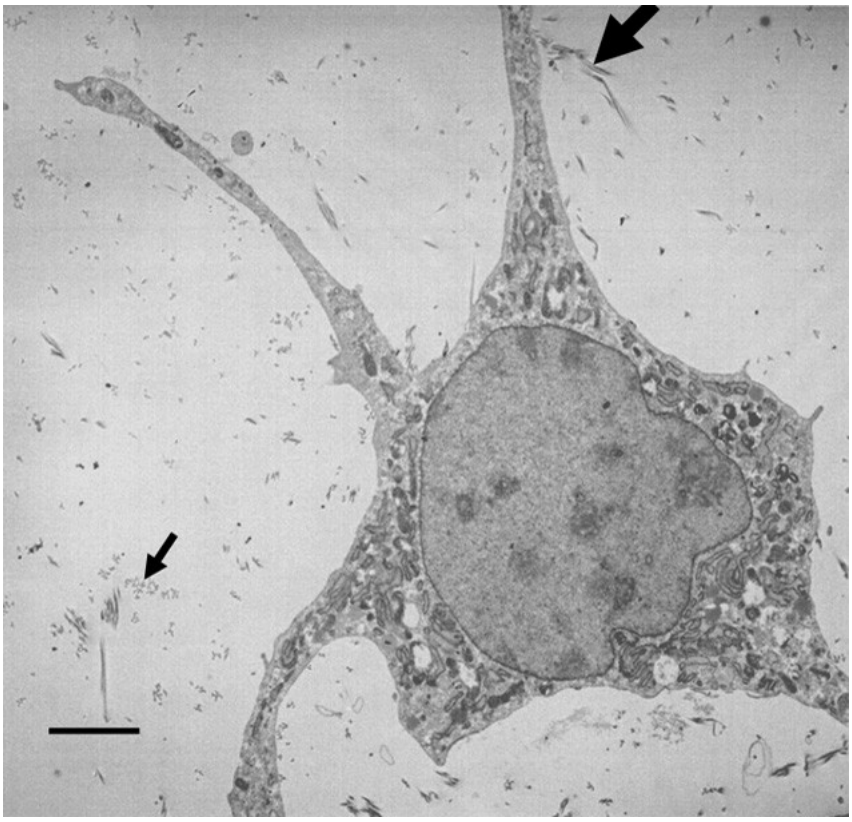

Fig. 5: Transmission electron micrograph of a bone marrow-derived mouse mesenchymal stem cell after contraction in acid soluble type I collagen gel for 24 hours. The cell has a stellate phenotype and synthesizes extracellular matrix components collagen (large arrow) and elastin (small arrow). Bar = 5microns.

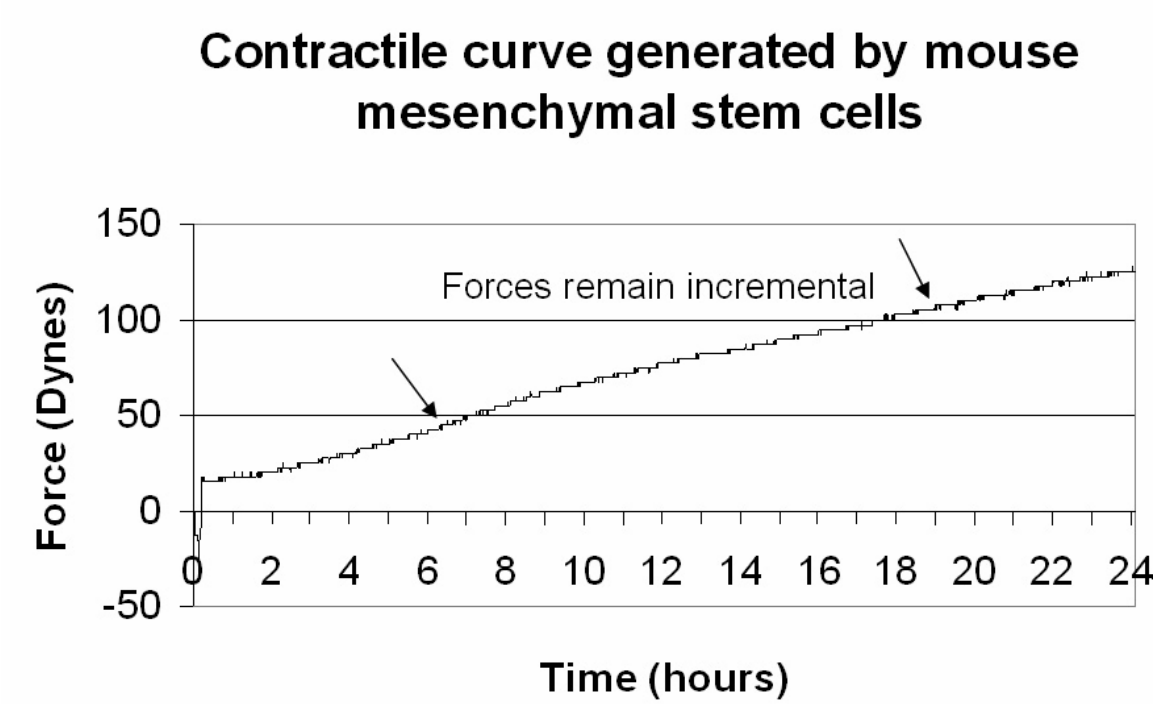

Fig. 6: Contractile curve generated by mouse mesenchymal stem cells whilst residing in a 3D collagen matrix. In comparison to Fig. 1. these cells have not reached tensional homeostasis.

Perhaps indicative of their hierarchical origin in the bone marrow, in the vicinity of hematopoietic precursors of immune system cells, BM-DMSCs have been shown to be able to the suppress the immune response in graft hosts (83), which would be a very favorable property for acceptance of a tissue-engineered construct. Dendritic cells, for example, are antigen presenting cells which acquire autologous characteristics which they present constitutively (84), although this immunosuppressive property of BM-DMSCs has been related to favor tumor growth in some animal models (85). A further unexpected property of BM-DMSCs has been the generation of fibrosis. Direkze *et al.* (86) have convincingly demonstrated in a mouse model that extra-tumoral fibrosis was initiated from a population of peri-tumoral bone marrow-derived mesenchymal stem cells. The ease with which BM-DMSCs can be stimulated to differentiate into fibroblasts

is an area in which caution is advised. The potential for development of fibrosis after surgical engraftment of a tissue-engineered construct would be seriously deleterious.

Smooth muscle is a more or less ubiquitous component of the connective tissues across the body, prominently in the gastrointestinal system, respiratory organs, urogenital tract, and crucially, the vasculature. Smooth muscle everywhere exhibits similar physiological contractile properties, yet displays multifunctionality depending on the anatomical site. The embryological origin of vascular smooth muscle is variously described as originating in the cardiac neural crest, or that it differentiates from local mesenchymal cells (87), in common with progenitors of endothelium, striated muscle, and nervous tissue. Hirschi & Majesky (41) are definitive in describing the origin of vascular smooth muscle as being from the cardiac neural crest, and proepicardial cells. In the adult, it has been shown to be able to differentiate directly from BM-DSMCs, circulating blood stem cells and from a local progenitor cell population (88), in response to injury and/or metabolic requirement. To differentiate *in vivo*, smooth muscle progenitor cells must spread and stretch. In mid-gestation they start to elongate and express characteristic smooth muscle proteins in response to the presence of certain extracellular matrix components. During myogenesis (at which time mechanotransduction pathways are set up) mechanical force is the most significant modulator of the tissue phenotype and hydrostatic pressure and/or shear stress are central to its morphological development in both the viscera and the vasculature. Mechanical strain regulates the expression of certain smooth muscle cell markers. *In vitro*, cyclic equiaxial strain down-regulates alpha-smooth muscle actin and alpha-smooth muscle actin-22 when the cells are grown on collagen or elastin-coated membranes, and decreases the proportion of alpha-actin stress fibres (89). However, cyclic uniaxial strain has the opposite effect. Uniaxial strain is the normal environment of mature smooth muscle cells, and as such is experimentally a more accurate mechanism by which to mechanically regulate the orientation of differentiating pro-smooth muscle BM-DMSCs (90). To spread and stretch on their basement membranes, the extracellular matrical molecules laminin-1 and laminin-2, serum response factor and

the guanine triphosphatase signaling protein RhoA (a GTPase) are necessary. A positively re-enforcing loop of events occurs in which the cells first produce the basement membrane protein laminin-1. Accumulation of this inhibits RhoA activity, allowing cells to elongate on the membrane. This, in addition to cell stretching leads to the synthesis of laminin-2, also an inhibitor of RhoA. Cytoplasmic serum response factor relocates to the cell nucleus, thus completing the signaling pathway. In bronchial myogenesis, undifferentiated mesenchymal stem cells expressing RhoA are able to differentiate towards the smooth muscle phenotype only upon contact with laminin-2 (91). The differentiation loop starts with the cells' response to their mechanical environment and is controlled with the level of RhoA being inversely proportional to differentiation status. High RhoA leads to the maintenance of undifferentiated mesenchymal cells.

In vascular smooth muscle, sensing and transduction of extracellular mechanical signals begins with phosphorylation of the platelet derived growth factor receptor. Integrin receptors, stretch-activated cations (calcium and potassium) and G proteins serve as mechanosensors followed by protein kinase C and the mitogen-activated protein kinases inducing activation of nuclear transcription factors (92). Expressing alpha-smooth muscle actin, platelet derived growth factor β and hepatocyte growth factor together during smooth muscle cell differentiation, indicates a possible hierarchical link to striated muscle satellite cells (93), see below. Tissue engineering with smooth muscle stem cells is anticipated to have an important impact, for example, in potential therapeutic intervention in vascular diseases. Atherosclerosis in particular arises in response to specific regions of hemodynamic and biomechanical stress in blood vessels (92), and vascular wall remodeling after injury in the presence of shear stress induces angiotensin converting enzyme expression, possibly with activity of basic fibroblast growth factor (94).

Mechanical contraction under voluntary control is the defining property of *skeletal muscle*. In the embryo, the initial phases of skeletal muscle organogenesis are directed by

elongation of bone to which the muscles are attached (95). Myogenic cells proliferate, differentiate and fuse into new myofibres (96). There is debate about the origin of putative skeletal muscle stem cells, whether they are derived from adult bone marrow (97), from circulating blood cells, from fusion between monocytes and myocytes (32), or their location, beneath the sarcolemma (satellite cells) dates from embryology. It is unclear whether these different kinds of undifferentiated cells represent populations which are distinct from each other or whether they are related by maturation state and/or hierarchy (98). *In vitro* they are undisputedly responsible for the generation of myogenic cell colonies (99). Satellite cells can be activated by mechanical stimuli, by signaling *via* nitric oxide, and *via* hepatocyte growth factor (100); also, expression of the c-met receptor rises rapidly after stretching newly formed myocytes (101). In the adult, increased workload and/or exercise change skeletal muscle morphology; there is both hypertrophy and hyperplasia of myocytes (102). Unstressed healthy adult striated muscle is generally non-proliferative, but when injured its response is activation of local stem cell populations to initiate rapid repair. Experimental work in adult mice on the rhabdosphincter (the striated muscle urethral sphincter) has shown that damage can be retrieved by activity of intrinsic satellite cells (103). In human clinical trials for the treatment of incontinence, applying autologous satellite cells (obtained from skeletal muscle of the forearm then grafted into the male urethra) has been successful (104). It has also been suggested, that striated muscle stem cells are of such multipotency that they themselves retain the ability to be progenitors, still demonstrating ability to differentiate into chrondrocytes, osteoblasts, and adipocytes (105), – with some reports showing them able to differentiate into hematopoietic cells (40). Experimental tissue engineering of striated muscle has already progressed considerably. In avian and rodent bioartifical muscles, unidirectional stretch followed by repetitive stretch lengthens, orientates and organizes muscle fibres (106, 107). Human bioartificial muscles have been produced by suspending progenitors (in a collagen/Matrigel™ matrix) cast in a silicone mold containing end attachment sites. For this study a mechanical cell stimulator was developed to apply appropriate forces directly to the engineered tissue (108), although other systems and matrices have been used (109). *De novo* production of striated muscle

was successful and phenotypically, specimens had parallel arrays of fibres which expressed sarcomeric contractile proteins; however, the fibres themselves were of narrow diameter and myofibre density was low, only 2-15% of the engineered tissue consisting of fibres. Also, in this set of experiments, the cells produced excessive amounts of extracellular matrix constituents. The authors (108) hypothesized that lack of innervation to the engineered tissue might be the cause of this low density of fibres.

In the adult, *cardiac muscle*, coronary vessel and cardiovascular system progenitor cells can be derived from certain bone marrow stem cells (76); their potential role in healing of cardiac infarcts is thus immense. *In vitro*, they may require the addition of 5-azacytidine to the culture medium to differentiate into cells of the required phenotype, which when observed, pleasingly express spontaneous contraction. However, some workers (110) find the addition of 5-azacytidine unreliable for differentiation of BM-DMSCs into cardiomyocytes. The differentiation capacity of non-identical (only a small percentage of this population will do so *in vitro* or *in vivo*) populations of bone marrow-derived stem cells into cardiomyocytes has been studied intensively, but the various results are not in agreement. Different isolation and identification protocols have been used between laboratories (111), thus it is difficult to make comparisons. Some workers find these cells impossible to derive in culture, while others claim to find it comparatively easy to differentiate them. In experimentally infarcted mouse myocardium, in 10 days, approximately 4.5 million biochemically and morphologically differentiated myocytes together with coronary arterioles and capillary structures have been reported to have been generated (112).

Intracardiac forces generated from blood flow are essential for natural cardiogenesis (113), and also are crucial in differentiation of progenitor cells. In our laboratory, a porcine model has been used. Fresh aortic wall was obtained and fibroblast-like progenitors (porcine aortic wall cells, PAWs) were collected and expanded in culture (Fig. 7). Endothelial cells were also harvested but as they require different culture conditions they were expanded separately. PAWs seeded on/in porous non-crosslinked

collagen sponges (Perous SARL, Lyon France) were infiltrative and proliferative. Cells inhabiting central sponge areas differentiated to synthesized both elastin and collagen extracellularly (Fig. 8). In a further experiment, a system of these myofibroblastic progenitor cells plus endothelial cells (Fig. 9) has been used to constitute an experimental proto-aorta (51). This time, PAWs were seeded on/in a matrix of Small Intestinal Sub-mucosa™, (Cook Biotech Inc., IN USA) and were left to equilibrate for four days. Then the endothelial cells were added to cover the surface. When the co-culture was stable, the two-dimensional construct was rolled into a tube to simulate an aorta, and was maintained attached to a force transduction apparatus, in culture. This proto-aorta was patent for more than 14 days in the multicue-bioreactor (51), designed specifically for culturing tubular specimens. The multicue- bioreactor provides an accurate computer-controlled pulsatile pump and strain induction mechanism and it applied physiological conditions naturally found at the aortic root, to samples throughout the experiment. Once more, cells proliferated, migrated and differentiated – in accord with their stem/progenitor cell nature.

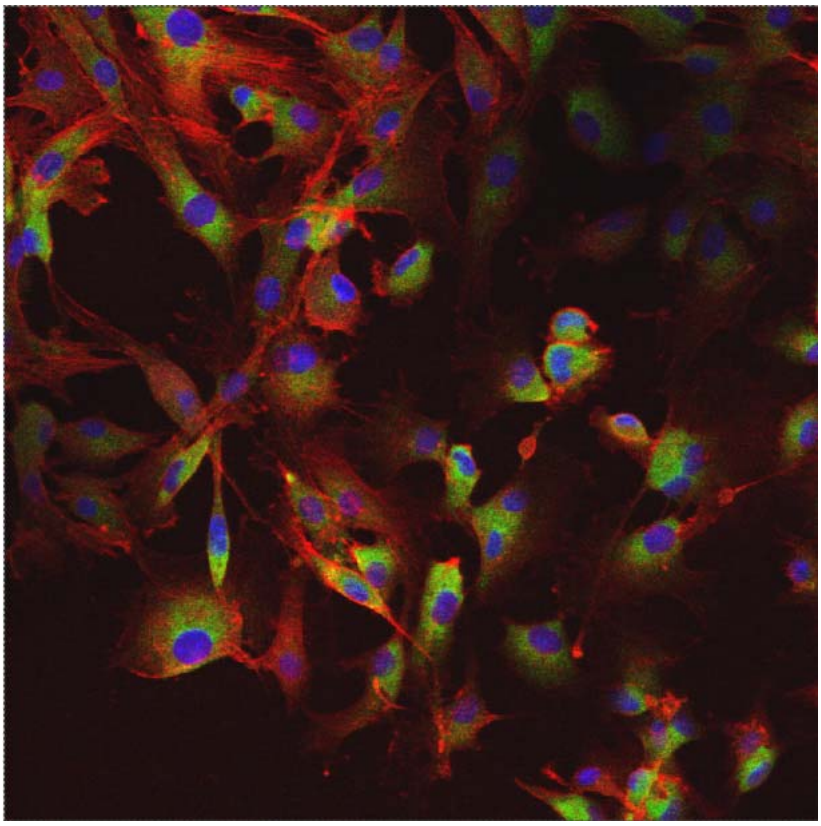

Fig. 7: Confocal micrograph of porcine aortic wall cells cultured and expanded in number in culture. Alpha-smooth muscle actin is stained green and filamentous actin is stained red (phalloidin). Original magnification x40 objective lens.

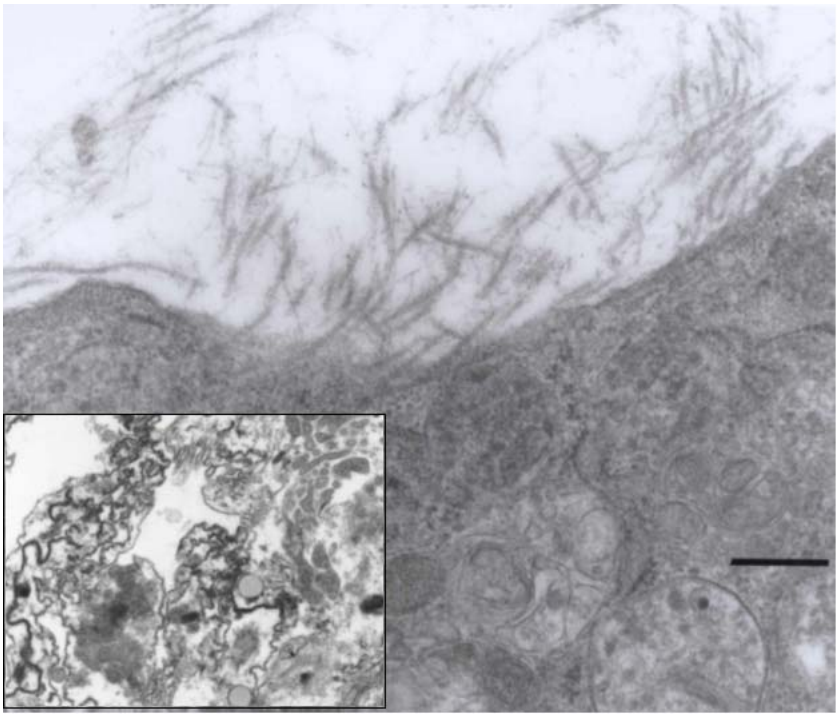

Fig. 8: Transmission electron micrograph of a porcine aortic wall cell showing the plasmamembrane with exiting collagen, synthesized by the cell; inset synthesised elastin fibres. Bar = 1micron.

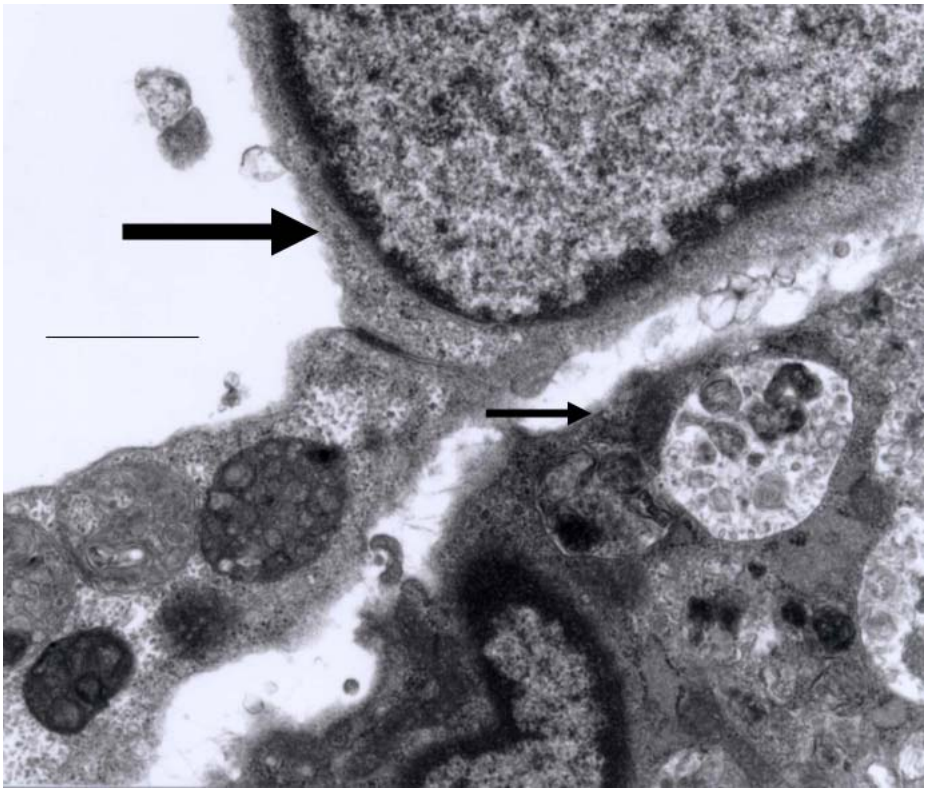

Fig. 9: Transmission electron micrograph of co-culture of an endothelial cell plus precursor porcine myofibroblasts. Typically, the endothelial cell (large arrow) lies superficial to the mesenchymal cell (small arrow). Bar = 5microns.

Amongst the orthopaedic tissues, bone marrow derived mesenchymal stem cells or progenitor cells from a local tissue niche, have been shown to be capable of reaching heterogeneous end-points, readily differentiating into a variety of downstream phenotypes, the result dependent on cell culture conditions, medium constitution and time in culture (6, 114), into chondroblastic, osteoblastic, and adipocytic cells.

Natural articular *cartilage* is avascular, aneural and alymphatic; it covers the articulating surface of bones allowing joints to move smoothly over each other, at the same time dissipating any biomechanical energy involved. The extracellular matrix of cartilage retains tissue fluid due to the intricate physical and spatial relations of

glycosaminoglycans, proteoglycans (aggrecan) and type II collagen. Successful response to repeated compressive load-bearing is due to hydrostatic forces exerted on this fluid, to which chondrocytes are sensitive. *In vivo*, cartilage is exposed to tensile, compressive and shear forces. Age-related or exercise-related injury can be treated by autologous cell grafting. Progenitor cells are obtained (by arthroscopy, for example) and expanded in culture before re-introduction into the defect. Mesenchymal stem cells have been used in cartilage repair, embedded in a type II collagen matrix (42). Cell number expansion has been shown to be accelerated under the effect of bone morphogenetic protein (BMP) - 2, and specimens retain the chondroblastic phenotype under the influence of basic fibroblast growth factor. TGF-β stimulates production of proteoglycans and further matrical components – however, it antagonizes retention of the chondrocyte phenotype. Addition of insulin-like growth factor 1 can modulate this. TGF-β expression can be upregulated by the use of mechanical forces such as fluid shear (4), yet bearing in mind the capacity of TGF-β to annul the chondrocytic phenotype. Cartilagenous biomaterials are constructed by seeding differentiated chondrocytes, progenitor or stem cells into a suitable matrix then expanding the cell number in a bioreactor for a number of weeks (115). By using a biodegradable polyglycolic acid scaffold and culturing the cells under centrifugal force and hydrodynamic conditions of a dynamic laminar flow field in rotating vessels, glycosaminoglycans and collagen have reached 75% and 39%, respectively, compared to levels measured in corresponding native cartilage (116). The requirement for application of physical forces for appropriate development of cartilage *in vitro* has been proven when comparative experiments were carried out on earth and in the Mir spacecraft. It was found that properties of cartilage cultured under zero gravity were poor compared to matched specimens grown on earth (117). Tissue engineering for articular cartilage surgery has been successful in clinical trials, as has meniscus repair and replacement (43), however, tendon and ligament repair are still at the experimental stage. Successful prolific replication of human cells from younger donors has been reported consistently to be more effective than those from older patients; in the future, this might prove to be a central observation in setting up stem cell banks for tissue engineering.

In natural *bone*, differentiation of local stem cells to mature osteocytes progresses through a number of stages. Stem cells give rise to bone progenitor cells, which then differentiate to pre-osteoblasts. This also is a transit stage after which the cells are osteocytes or else bone matrix lining cells. Progenitor cells can be directed towards an osteogenic phenotype by stimulation of intracellular pathways encompassing those of menin and sonic hedgehog (Shh) in which role Notch-1 and peroxisome proliferator activated receptor (PPARγ) are modulatory (6); bone morphogenic proteins stimulate osteogenic precursors. The ability to transplant bone-like constructs containing stem/progenitor cells in an appropriate matrix has a wide variety of clinical applications. Tissue engineering in orthopaedic surgery has become an added dimension of treatment for bone damage due to disease, ageing (such as vertebral degeneration), accidental injury and delayed healing/non-union fractures. In transplantation, maintenance of the substance of such a relatively dense construct requires adequate movement of metabolic molecules throughout the graft. Such mass transport depends upon physical conditions and restraints, such as pressure gradient driving fluid flow, mechanical loading (as a result of local muscular activity or arterial pulsation) and gravitational force. BMP treatment is used clinically to enhance and accelerate bone repair, and also in bone replacement. Human BMP is called OP-1 and this has been successful in promoting healing in cases of bone non-union, by recruiting osteogenic progenitor cells to repair the bone defect (43). Using ground coral (because of its highly interconnective, openly porous nature) as the extracellular matrix, mesenchymal stem cells have been used to become osteocyte substitutes in animal experimentation of tissue-engineered bone remodeling (118). The constructs were partially successful and materially stronger than samples with hydroxyapatite or hydroxyapatite-tricalcium phosphate ceramic matrices (119). In a clinical setting, a thumb distal phalanx has been reported to have been replaced with autologous osteogenic precursor cells inside an appropriate matrix (43). Adding the techniques of gene therapy to those of tissue engineering, bone has been formed *de novo* and has

regenerated in wounds when mesenchymal stem cells have been transfected with the gene for BMP-2 (120).

Human *adipose* tissue can be differentiated from BM-DMSCs (114), however amongst the general population to date, there has been no demand for replacement fat. On the other hand, adipose tissue harbours a relatively large proportion of local stem cells. The tissue is readily available from breast and abdominal elective reductions, so multipotential stem cells isolated from this source are an attractive candidate for directed differentiation into further cell types in related (or even seemingly unrelated) lineages. Undifferentiated human adipose tissue-derived stromal cells have been harvested and compared to human bone marrow-derived mesenchymal cells for differentiation potential and gene expression (7). From both bone marrow and adipose tissue, chondrogenic, osteogenic and adipocytic lineages were derived, depending on culture conditions. The two sources of cells exhibited a range of potential phenotypic and gene expression similarity. There was also variation between the two sources of cells in some parameters, but the study demonstrated that human adipose tissue, which otherwise would be destroyed, is a rich source of valuable stem cells.

Adult nervous system stem cells

Tissue-engineered constructs for treatment of diseases or damage to the nervous system face the problem that mature neurons do not naturally divide. Natural replacement in damaged neurological tissues compares poorly to that seen in the epithelia, although neuronal stem cells have been identified in many species including humans (121). They are a well-defined population of precursors that have been shown to be able to differentiate into both neurons and glial cells (122). This is an important area for treatment of many diseases, not only for surrogate neural components, but also because a tissue-engineered graft of any kind would be unable to function if it were not appropriately innervated, as has been surmised by Roskams *et al.* (33) in liver tissue engineering and by Powell *et al.* (108) for striated muscle. Adult nervous system stem cells have been derived from tissues previously not thought as having neuroectodermal

potential. Cells which were bone marrow-derived and/or from local niches have been shown to express neuronal or glial markers (123). In animal models of neurological disease, transplantation of stem cells has produced functional improvement, thus extending considerable hope for human sufferers.

Local niches of neural stem cells existing in the *central nervous systems* (CNS) have been cultured to differentiational end points of all the major cell types of the nervous system (124). However, their precise origin has been cause for dispute, so much so that it has been suggested that they make up a fifth element in the central nervous system, synantocytes, after neurons, astrocytes, oligodendrocytes and microglia (125). Development of techniques that indicate reparative cell proliferation in fully mature glial or neuronal cell populations is comparatively recent. In adult mice, after intravenous stem cell transplantation, recipient animals have been found to have donor hematopoietic cell-derived microglia and macroglia (126). In rats, bone marrow-derived stem cells have been shown to enhance remyelination in the spinal cord (127), while cerebral ischemia, Parkinson's disease and Huntingdon's disease have been shown to improve after grafting stem cells (128-131). Stem cell grafting has a potential role, not only in treatment of neurodegenerative disease, but also in neurological repair after traumatic CNS injury (132). In a mouse model of brain injury, after unilateral cortical damage, grafting neurospheres of progenitor cells has resulted in significant motor and cognitive improvement in the animals' responses, and at post mortem the cells were shown to have migrated into the injured hippocampus and adjacent cortical regions. In this case, the cells were shown to be NG2 (a chrondroitin sulphate proteoglycan, specific to certain oligodendrocyte progenitor cells) positive oligodendrocytically-derived cells (133). In general, NG2-expressing cells undergo physiological and phenotypic changes in response to a wide variety of central nervous system injuries, and seem to be particularly related to angiogenesis, cell proliferation and cell migration/invasion (134). Neural stem and progenitor cells (isolated from the embryonic rat cortical or subcortical neuroepithelium) seeded into a biologically derived polymer scaffold have been cultured with type I collagen in serum-free medium in the presence of basic fibroblast

growth factor. The culture expanded successfully and developed neuron-like cells which displayed neuronal polarity and excitability, expressed neuro-transmitters and had ion channels and receptors. These functional neurotransmitters had receptors which could be changed from cholinergic and purinergic to GABAergic and glutamatergic types. Spontaneous post-synaptic currents were recorded with GABAergic and glutamatergic synaptic activities. The matrix used allowed the cells to differentiate into a neural network (135). This work will have an exciting future for CNS differentiation, regeneration and tissue engineering *in vivo*.

In the *peripheral nervous system*, some diseases and/or injuries result in a patient's loss of functional recovery, for example in injuries where nerves cannot heal because direct apposition of ruptured nerves is not possible. Supporting cells have a capacity for regeneration, but this may not be sufficient to provide perfect repair. Response to provision of nerve growth factor, perhaps by implantation of nerve growth factor-producing cells, might improve the outcome (136). Schwann cells are able to participate in peripheral nerve regeneration. A niche for Schwann-like cell progenitors has been postulated within the rat fetal sciatic nerve, and amongst neurons of the dorsal root and autonomic ganglia (137, 138). Schwann-like cells also can differentiate from olfactory ensheathing cells that could be peripheral nerve progenitors. These are an intermediate glial cell type that in normal circumstances myelinate olfactory nerves, yet they retain the ability to self-renew and also to differentiate for repair. They have also been shown to stimulate axonal regeneration in the spinal cord (139). Success has been reported with transplantation of Schwann cells (140), neural progenitor cells (141), and BM-DMSCs into bioengineered conduits (142). Schwann cells provide mechanical support for potential neurite outgrowth and they release trophic factors and stimulatory antigens at the plasma membrane (143). In tissue engineering also, transplantation of progenitor Schwann cells has been shown to improve nerve regeneration, but for clinical purposes accessing, harvesting, purifying and expanding the numbers of these cells has proved to be difficult (144). Olfactory ensheathing cells though have been shown to be effective, and in rat, hippocampal progenitor cells seeded into a conduit have repaired divided

sections of the sciatic nerve. Stem cells at the wound site regenerated and characteristically differentiated into Schwann-like cells which integrated functionally (145, 140). Bridging nerve gaps is a major problem in peripheral nerve surgery. In animal models, local delivery of bone marrow-derived mesenchymal stem cells to the site of spinal cord injury has resulted in the formation of neurofilament bundles at the interface between scar tissue and graft. Axon bridging across rat sciatic nerve separation to close a wound, has been shown to depend mechanically on two competitive forces, axial forces generated by the outgrowth of axons and non-neuronal cells from the proximal stump, and constrictive circumferential forces imposed by the contractile tissue-emerging capsule. Rat bone marrow-derived stem cells have been transplanted into the proximal stump of dissected rat sciatic nerve; the cells migrated and differentiated to the site of sciatic nerve axotomy repair (146). Using tissue-engineered conduits containing collagen I and collagen III, Stang *et al.* (147) found that by seeding Schwann cells into the matrix they obtained revascularization into the wound site and successful directing of neurites. However, they determined that results were only encouraging when the Schwann cells were autologous. In tissue engineering of neural tissues, if re-innervation of wounds is to be achieved clinically, grafts must be designed that maintain the appropriate geometry, encourage cell adhesion and guide neurite out growth. In natural conditions of neural repair, Schwann cells self-orientate into linear arrays that provide guidance for neurite direction. This function of Schwann cells has been used in conjunction with microlithography to provide a three-dimensional lattice that holds information in its geometry that allows developing neurites to align in the desired pattern (143). Using chick dorsal root ganglia progenitor cells, a hydrogel matrix has been developed using co-polymerisation of 2-hydroxlethyl methacrylate with 2-aminoethyl methacrylate. In this study, cell phenotype development was successfully directed down longitudinal channels (148). Chitin and chitosan have both been used to direct developing neurites down biodegradable tissue-engineered tubes. Chitosan tubes have been found to be mechanically stronger than those made of chitin, and chitosan films have a higher amine content than chitin; developing neurites depend on amine concentration in their matrix for successful extension (149). Thus, neural stem cells, plus appropriate matrices hold

excellent promise for the future of nervous tissue engineering. Literature describing measurement of mechanical forces derived by and/or are incident on neural tissues is sparse. Alternatively, innervation by stem cell-derived nervous tissues would have a role in regeneration of disease ravaged tissues and organs. However, successful tissue-engineered grafts will need to be innervated for the part to be able to respond to the basic mechanical stresses of life. Body parts (and/or their substitutes) are in constant motion with respect to each other, and the force of gravity is universal.

Conclusion

The aim of this chapter has been to illuminate that stem cells associated with specific tissues and organs might be influenced to differentiate as much by their mechanical environment as by their biochemical milieu, and that this can be utilized in tissue engineering of living substitute body parts. Phenotypes of cells in the various natural tissues and organs are the result of embryonic stem cell differentiation, and recently, classically held assumptions of tissue specificity and lineage restriction of stem cells have been revised. There is a wealth of opportunity for demonstrating where tissue-engineering steps may be taken towards graft production incorporating stem cells, matrices and appropriate mechanical forces. In the largely epithelial organs, although they generate and also are subject to mechanical force, and their stem cell milieu is generally well known, these properties have scantly been linked to tissue engineering so far. Mesenchymal stem cells, perhaps due to their innate mechanical properties, have been more widely used in tissue engineering. There are however, a number of considerations to be incorporated in the study of tissue engineering with stem cells. First, as production of surrogate body parts develops, to be successful living grafts, they will need to be innervated; stem cell biology of the nervous tissues is well advanced to this end. Secondly, populating grafts by stem cells applied systemically can exploit their apparent ability to 'home' to a wounded area (in the cardiovascular system, at least). Thirdly, it is beginning to be reported that stem cells have the capacity to suppress an immune response from the host; this property is very interesting and potentially very

valuable, but it has not yet been utilized in tissue engineering with stem cells. In contrast, the potential of mesenchymal stem cells to differentiate into fibroblasts and lay down areas of fibrosis may be a serious obstacle to their use in a viable surrogate. Also, the biology of stem cells and any behavioral relationship they may share with malignant cells needs to be clarified, particularly with respect to these cells' control over the initiation/inhibition of cell proliferation. To tissue-engineer successful living grafts, heed must be paid to all aspects of the local anatomical environment of surrogate implants. The reality is that they will be subject to local anatomical forces *in vivo*. *In vitro* models must be designed to closely mimic *in vivo* parameters. Mechanical forces on, around and in body parts can not be ignored in the process of prosthesis construction using stem cells for tissue engineering.

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