Endogenous musculoskeletal tissue engineering – a focussed perspective

Adam CJ

QUT/Mater Hospitals Paediatric Spine Research Group, Faculty of Built Environment and Engineering, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

e-mail: c.adam@qut.edu.au

Abstract

Two major difficulties facing widespread clinical implementation of existing Tissue Engineering (TE) strategies for the treatment of musculoskeletal disorders are (1) the cost, space and time required for ex vivo culture of a patient's autologous cells prior to re-implantation as part of a TE construct, and (2) the potential risks and availability constraints associated with transplanting exogenous (foreign) cells. These hurdles have led to recent interest in endogenous TE strategies, in which the regenerative potential of a patient's own cells is harnessed to promote tissue regrowth without ex vivo cell culture. This article provides a focused perspective on key issues in the development of endogenous TE strategies, progress to date, and suggested future research directions toward endogenous repair and regeneration of musculoskeletal tissues and organs.

Keywords

Tissue engineering, Musculoskeletal system, Endogenous, Regeneration, Cell homing

Introduction

Diseases and disorders of the musculoskeletal system occur at all stages of life. During childhood and adolescence, deformities during skeletal growth can arise due to genetic defects, infectious disease, and other growth imbalances[1,2]. In adulthood, osteoarthritis and back pain are major contributors to immobility, pain, and lost productivity[3,4]. In the elderly, skeletal thinning due to osteoporosis causes increased susceptibility to fracture[5]. At all ages, cancer, trauma and infectious diseases can lead to large scale bone, joint and soft tissue destruction[6,7,8].

Musculoskeletal disorders are a major cause of disability worldwide. In the United States, musculoskeletal diseases are reported more than any other health condition. In 2004, the estimated direct cost of treatment for musculoskeletal conditions in the US was $510 billion, with a further $339 billion in indirect costs, in total comprising 7.7 percent of Gross Domestic Product[9]. Table 1 below shows the estimated global burden of musculoskeletal disease in 2001, comprising just under 30 million Disability Adjusted Life Years (DALYs), of which 21 million DALYS were in developing countries, and nine million were in developed countries. It is important to note that the already high proportion of musculoskeletal disease in developing regions is expected to grow further due to rapidly increasing life expectancy in these countries. Note also that the data in Table 1 do not include osteoporotic fractures, which are separately classified[10].

Table 1. Global burden of musculoskeletal disease in 2001 (DALYS = Disability Adjusted Life Years)[10]

Condition	Developing countries (DALYS)	Developed countries (DALYS)	Total (DALYS)
Rheumatoid arthritis	3,238,000	1,520,000	4,757,000
Osteoarthritis	11,049,000	5,323,000	16,372,000
Other musculoskeletal diseases	6,789,000	1,880,000	8,699,000
All musculoskeletal diseases	21,076,000	8,723,000	29,798,000

Derived from the embryonic mesoderm, the human musculoskeletal system is a complex structure of bones and joints connected by ligaments. Articulating movement between adjacent bones occurs between the smooth sliding surfaces of articular cartilage, and the muscles which power movement are attached to bone via tough, flexible tendons. The role of the musculoskeletal system is predominantly mechanical, exerting and withstanding the forces associated with physical function and mobility. It follows that each of the tissues in the musculoskeletal system are highly adapted to a particular mechanical role. Bone achieves lightweight stiffness and strength (fracture resistance) using a hierarchical assembly of mineralised collagen fibres, built from the nanoscale up[11]. Ligaments are tough, collagenous tension-bearing structures with non-linear force versus stretch characteristics, and low elastic energy storage capacity[12]. Articular cartilage is comprised of graduated zones of collagen fibre network pre-strained by the physico-chemical swelling of hydrophilic proteoglycan molecules within the network[13]. Skeletal muscle is comprised of a repeated hierarchical assembly of actin and myosin fibres again held in place by a collagen network (the endomysium)[14]. The intervertebral discs of the spine are the largest avascular structures in the body, resisting forces of up to nine times bodyweight during strenuous activity, and are comprised of a series of annular rings of collagenous fibres criss-crossed in alternating orientations, surrounding a jelly-like inner nucleus which is pressurised within the annular outer layers[15]. Tendon is a tough and resilient parallel-fibred collagenous tissue which allows transmission of high forces from muscles to their bony attachment points[16].

The defining characteristic of the musculoskeletal tissues is their primary load-bearing function, which structurally requires a dense, tough extra-cellular matrix (ECM) comprised of hierarchically assembled fibrous proteins. These ECM structures are built and maintained by a network of living cells both on and within the ECM. Cell activity is in turn controlled by soluble signalling molecules, as well as by signals from the ECM itself, so that the ECM acts as both a substrate for cell attachment and a chemical and mechanical signalling source to control cell activity[17]. Within this general framework, musculoskeletal tissues exhibit a diverse range of morphologies and mechanical properties specifically adapted for their structural role.

The individual tissues of the musculoskeletal system exhibit a strong ability to adapt their composition and structure in response to alterations in external loading conditions. For example, it is well known that rapid bone loss occurs during extended bed rest, space flight, or on a local scale adjacent to a stiff,

metallic load bearing implant after surgical implantation[18,19,20,21]. The mechanobiological response of musculoskeletal tissues strengthens them (hypertrophy) in response to increased loading, and removes tissue (atrophy) in response to reduced loading. This remodelling ability is successful in adapting the musculoskeletal system to altering demands throughout life, and there is strong evidence that remodelling in both mineralised and soft tissues is induced in response to microscope damage of the ECM[22].

In the case of large scale macroscopic insult to a musculoskeletal organ however, repair is not guaranteed, and there is a limit to the size of defect which the body can spontaneously repair. The concept of the critical sized bone defect was introduced by Schmitz & Hollinger in 1986[23], defined as the smallest defect which will not spontaneously heal. Similarly in non-mineralised musculoskeletal tissues such as cartilage and intervertebral disc, focal defects of a given size lead to further degeneration (and eventual complete destruction of the organ in question) rather than spontaneous repair[24,25]. These critical sized defects are a key target of Tissue Engineering strategies for musculoskeletal regeneration. We note however that Cooper et al[26] have recently recommended that the term "critical size defect" be discontinued due to the difficulties surrounding the clinical applicability of the concept.

The overarching goal of the rapidly developing fields of Tissue Engineering (TE) and Regenerative Medicine is the controlled growth of new tissues to replace diseased, damaged and degenerate native tissues and organs. Continuing interest in tissue regeneration has been driven by the promising outcomes of research in the field to date, combined with strong growth in clinical demand due to increasing numbers of patients surviving severe trauma, burns, cancer, degeneration and chronic disease, treatments for which often require replacement of lost or damaged tissue.

Research in musculoskeletal TE to date has yielded genuine advances in harnessing the regenerative ability of precursor cells when seeded on biomaterial scaffolds together with growth factors in musculoskeletal defects, demonstrating that clinically significant results can be achieved[27]. However, major challenges remain in the development of effective TE approaches suitable for widespread clinical use. In particular, there are key difficulties around (i) the requirement for multiple surgical procedures if autologous cells are harvested, expanded *ex vivo* and then re-implanted later, (ii) the cost, space and time required for *ex vivo* culture of a patient's own cells, and (iii) the risks associated with implanting exogenous progenitor cells. Logistical concerns are particularly important given the high burden of musculoskeletal disease in developing countries (Table 1), therefore treatments should be simple, effective, and as economical as possible.

For these reasons, endogenous approaches to tissue regeneration have been proposed. These approaches are attractive in principle, because they do not require *ex vivo* cell culture, require only a single surgical procedure, and do not involve exogenous cell implantation, thus potentially avoiding key barriers to clinical adoption. In the context of Tissue Engineering approaches, recent work has demonstrated exciting possibilities for the regeneration of load-bearing musculoskeletal tissues by endogenous cells guided by synthetic scaffolds[28], but there is much further work to be done in elucidating the interaction between cells, host extra-cellular matrix, signalling molecules, and biomaterial scaffolds to effectively facilitate endogenous cell migration and repair of a defect site. With this in mind, the aim of this paper is to highlight emerging approaches to endogenous Tissue Engineering of the musculoskeletal system and discuss current progress, challenges and future research directions. The overview given here is not intended to be an exhaustive review, but to provide an introduction to key issues in this newly emerging area of research.

Definition and classification of endogenous TE strategies

We define the term 'endogenous' from the perspective of the origin of all cells involved in the regeneration process. From the perspective of cell origin, all cells involved in regenerating the damaged or diseased tissue must be endogenous to the patient. We note that others have suggested a broader definition which would include *ex vivo* cultured autologous cells[29], however because it is known that *ex vivo* culture changes cellular signalling responses[30], and due to the aforementioned cost and time barriers associated with *ex vivo* culture, we use the term 'endogenous' in its strict sense. Note also that this nomenclature does not preclude the use of exogenous synthetic scaffolds or exogenous signalling molecules, so long as all cells are endogenous to the patient. It follows that progenitor cells which populate the defect site must come from one of three possible sources; (i) auto-transplantation from another anatomical site at the time of surgery, (ii) via cell homing from the bloodstream or neighbouring tissues, or (iii) via de-differentiation of committed cells at the defect site. Each of these possibilities is discussed further below, and Table 2 identifies these three possible endogenous TE strategies within the broader spectrum of TE strategies. For an overview of endogenous approaches to regenerative medicine (including, but not limited to, musculoskeletal applications) the reader is directed to several recent review papers[29,31-34]. Our focus here is specifically on Tissue Engineering strategies in which a biomaterial scaffold assists and modulates endogenous cells to regenerate load bearing musculoskeletal tissues.

Table 2. Classification of TE strategies according to cell source, signalling molecule source, and scaffold source. The three endogenous strategies (from a cellular perspective) are highlighted in grey.

	C1: *Endogenous (autotransplantation)*	**C2: *Endogenous (homing)***	**C3 : Endogenous (*de-differentiation*)**	**C3: *Expanded autologous***	**C4: *Exogenous***
Cell source(C)	Progenitor cells transplanted from other anatomical sites (e.g. bone marrow, fat, muscle biopsy - MSCs, adipose-derived stromal cells)	Cell migration from bone marrow via bloodstream, or from neighbouring tissue niche	Previously committed cells induced to de-differentiate. Mammalian skeletal capacity unknown?	Autologous cells expanded via ex vivo culture (e.g. Autologous Chondrocyte Implantation)	Cells derived from sources external to the patient (e.g. cord-derived mesenchymal stem cells, embryonic stem cells)
Growth factor source (G)	**G1: *Endogenous***			**G2: *Exogenous***	
	e.g. platelet derived growth factor (PDGF) from autologous blood			e.g. recombinant human bone morphogenetic protein-2 (rhBMP-2)	
Scaffold source (S)	***S1: Endogenous tissue scaffold***			***S2: Exogenous tissue scaffold***	***S3: Synthetic scaffold***
	e.g. fibrin clot, autologous bone graft			e.g. allogeneic bone graft, xenograft bone	e.g. polycaprolactone + tricalcium phosphate

In this schema, it is possible to define a particular TE strategy in terms of its cell (C), growth factor (G), and scaffold source (S), for example $C_2G_1S_1$. Then according to the definition given above, there are 18 possible 'endogenous' strategies (i.e. $C_1G_xS_x$, $C_2G_xS_x$ and $C_3G_xS_x$) from a cellular perspective, even though

exogenous scaffolds or growth factors may be involved. The $C_4G_xS_x$ and $C_5G_xS_x$ groups of strategies represent the widely used TE approaches involving cultured autologous cells, and exogenous cells respectively, on which the majority of research attention has been focussed to date.

Regenerative biology and the potential for cellular de-differentiation

The defining characteristic of an endogenous TE strategy as defined above is that the patient's own stem/progenitor cells must either be attracted to, auto-transplanted to, or de-differentiated at, the regeneration site. Furthermore, the biochemical and biophysical micro-environment in the regeneration site must promote appropriate processes of epimorphic cellular proliferation, differentiation, and ECM synthesis to facilitate the controlled regeneration of viable, vascularised tissue. Valuable lessons can be learnt in this regard by considering the native regenerative potential of the mature vertebrate musculoskeletal system as studied in the field of regenerative biology[35]. Gillers has pointed out that existing studies on limb regeneration have received little attention in the TE literature to date[36].

Urodeles such as newts and salamanders have the capacity to spontaneously regenerate lost body parts and injured organs. Following limb amputation, various cell types (including connective tissue cells) de-differentiate and migrate to form a mass of undifferentiated cells (blastema) at the end of the stump. The key aspect of this regenerative ability is the de-differentiation of fully differentiated cells in the wound region, followed by proliferation and re-differentiation into the appropriate cell types for regeneration[37-40]. The signalling pathways that control this regeneration process are not fully known, however it appears that they only invoke embryonic organogenesis pathways to a limited extent[41], and various signalling molecules have been identified during limb regeneration. For example, Maden[42] has discussed the importance of retinoids (vitamin A derivatives) in limb and tail regeneration, such that in cases where retinoic acid synthesis is inhibited, limb and tail regenerative ability is inhibited as well. Kumar et al[43] have shown how the nerve dependence of limb regeneration (denervated limbs do not spontaneously regenerate) is related to expression of the anterior gradient protein nAG in the regenerating nerve. nAG is a growth factor for blastemal cells and a ligand for the surface protein Prod 1.

The regenerative capacity of mammals is much less than that of Urodeles however. Mammals (including humans) have the ability to repair small defects and regions of damaged tissue, but are not able to spontaneously repair large tissue defects. This difference seems to be mainly related to the ability of amphibians such as salamanders to produce progenitor cells by de-differentiation of differentiated cells back to precursor cells, whereas mammalian regeneration relies on recruitment of existing progenitor cells. Against this background of the limited innate regenerative capacity of mammals, what is the potential of endogenous strategies to enhance the regenerative ability of the human musculoskeletal system?

Firstly, several key studies appear to show that mammalian myotubes[44] and cardiomyocytes[45] can be induced to de-differentiate, proliferate, and re-differentiate into adipocytes, chondrocytes, and osteogenic cells. This work was subsequently extended to demonstrate therapeutic regeneration of rat hearts following surgically induced myocardial infarction using FGF1/p38 MAP kinase inhibitor therapy[46]. A significant focus of attention with regard to mammalian regeneration is the MRL mouse, which demonstrates exceptional healing ability in an ear punch wound model[47], and has also been claimed to demonstrate myocardial regenerative ability. However a recent study compared infarct sizes, levels of fibrosis, and subsequent cardiac function between MRL and C57BL/6 mice and found no difference

between the two strains[48]. To the author's knowledge there are no studies of regeneration in mammals invoking de-differentiation, proliferation and re-differentiation of mammalian bone, joint or skeletal muscle cells as was achieved for the rat cardiac muscle study described above. However the rat myocardial infarction results indicate a potentially beneficial and so far unexplored potential research direction for de-differentiation based strategies in musculoskeletal regeneration.

Auto-transplantation and homing of endogenous cells

Another option for delivering cells to the defect site is auto-transplantation. Several clinical studies have demonstrated the healing ability of bone marrow aspirate re-injected percutaneously into fracture non-unions[49,50]. In a related approach, the 'microfracture' technique for cartilage regeneration[51,52] uses perforations of the subchondral bone to enable localised transport of marrow and blood into contact with degenerate cartilage[a]. Fong et al[53] have discussed the logistical difficulties associated with direct marrow transplantation approaches, pointing out that only 7-10 MSCs per million mono-nucleated cells can be isolated from bone marrow aspirates, therefore several millilitres of bone marrow would yield less than 100 MSCs[51,53]. Hernigou et al[54] found that concentrating bone marrow aspirate to increase cell density improved the healing rate when treating tibial non-unions, and suggested that a cell density >1000 cells/cm^3 was required, which is substantially higher than the 600 cells/cm^3 found in un-concentrated iliac crest bone marrow[53].

Aside from MSCs in bone marrow, progenitor cells have been reported in other tissues[55]. For instance, Jackson et al[56] report multipotent cells (MPCs) in traumatically injured muscle tissue capable of differentiation into osteoblasts, adipocytes and chondrocytes, but with limited lineage commitment compared to bone marrow derived MSCs. The MPCs also demonstrated trophic (immunoregulatory and pro-angiogenic) properties comparable to MSCs. The authors suggest that the ready availability of these cells following orthopaedic injuries (involving traumatized muscle tissue) makes them an attractive possible cell source for regenerative medicine approaches. Adipose tissues also provide a readily available source of stromal cells, and adipose derived stromal cells have been used to successfully regenerate bone[57,58].

Even if sufficient numbers of cells can be aspired, concentrated, and re-injected into a defect site, auto-transplantation strategies still suffer from the same key difficulty as any Tissue Engineering approach which uses cell implantation, namely that a population of cells are instantaneously placed into a micro-environment which has not yet developed the necessary microvasculature, and any cells located more than a few hundred micrometres from the nearest blood supply will not receive an adequate nutrient supply to survive. In principle however, this problem is avoided using the third possibility for endogenous cell recruitment - cell homing. Under the influence of molecular cues, migrating MSCs can exit the marrow space, enter the circulation, and migrate to a defect site using cellular adhesion, rolling, and transmigration through the epithelium[34,59] in an analogous manner to the more well defined leukocyte transport mechanisms[60]. Or progenitor cells already existing in joint tissues can be mobilised[55,56,61]. Tissue niche progenitor cells have even been reported in large avascular structures such as the intervertebral disc. Risbud et al[62] analysed cells from intervertebral disc samples of five patients with degenerate discs as well as adult rat lumbar spine cells, and found that the degenerate human disc

[a]We note that this is not strictly auto-transplantation, because the marrow is not removed and re-implanted, but the microfracture technique is mentioned here for completeness since it is closely related to marrow auto-transplantation approaches as a method for inducing localised marrow transport.

contained populations of skeletal progenitor cells which the authors suggested could be used to orchestrate the repair of the intervertebral disc. Cell homing strategies exhibit exciting potential to utilise a patient's existing progenitor cells, however to the extent that they rely on recruitment of bone marrow progenitors through the bloodstream, defects in poorly vascularised tissues such as cartilage and intervertebral disc may prove problematic and require a focus on signalling stem cells in the surrounding tissues rather than trafficking via the bloodstream. With regard to poorly vascularised tissues, we note the recent pilot study by Brown et al[63] in which fibrocartilage of the canine temporomandibular joint meniscus was regenerated using a porcine urinary bladder extracellular matrix scaffold, which acted as an inductive template for cell infiltration and new matrix formation.

Molecular signalling

Whichever approach is used to locate endogenous cells at the defect site, appropriate modulation of cellular activity to induce tissue regeneration is critical. Since the initial discovery of nerve growth stimulating factor in 1954[64], a large body of research has explored the spatiotemporal characteristics of autocrine and paracrine cell signalling via growth factors during wound healing, and attempts have been made to mimic the healing cascade through the controlled administration of growth factors. Growth factors are polypeptide molecules that bind to membrane-bound receptors[35], and those involved in musculoskeletal tissue regeneration include; vascular epithelial growth factor (VEGF) which plays a key role in angiogenesis, transforming growth factor beta (TGF-β) which is involved in thrombogenesis during wound healing and is also a potent inhibitor of epithelial cell proliferation, bone morphogenetic protein (BMP), especially BMP-2 and BMP-7 which are important for bone formation, epithelial growth factor (EGF) which is responsible for proliferation of epithelial cells, platelet derived growth factor (PDGF) which modulates growth of mesenchymal cells (thrombocytes release large amounts of PDGF for the initiation of thrombogenesis in wound healing), and fibroblast growth factor (FGF) which is a ubiquitous factor in the musculoskeletal system controlling growth & survival of fibroblasts, chondrocytes, osteoblasts and smooth muscle cells.

A key challenge in endogenous regeneration is the controlled spatiotemporal delivery of these molecular cues at a regeneration site[65]. The majority of TE studies involving growth factor delivery to date have administered a single growth factor, and the growth factors currently in clinical use (e.g. BMP-2 and BMP-7) are often delivered in a single supra-physiological dose which is dissipated shortly after delivery. This single dose, single factor approach is a gross approximation to the tightly controlled sustained delivery of multiple growth factors which occurs in endogenous wound healing, and a recent paper by Chen et al[66] has reviewed more sophisticated attempts to deliver two or more growth factors in a controlled manner.

Parekkadan et al[67] recently re-conceptualised the role of MSCs in repair and regeneration by suggesting that MSCs promote regeneration by acting as molecular cues at a defect site, rather than engraftment and proliferation themselves. This reductionist approach (i.e. MSCs are effectively molecular therapeutics) is attractive in principle, because it suggests that if MSCs essentially act as molecular cues, their role could be recapitulated by an appropriately designed system of synthetic biochemical cues. However the replacement of a closed loop signalling system in which MSCs both sense and signal their environment, with an open loop system in which MSC-mimicking cues are released in a pre-determined fashion risks losing the tightly integrated control exhibited by MSCs as trophic mediators[68].

Role of the scaffold in endogenous TE

We have so far discussed sources of endogenous cells and the importance of signalling molecules in biochemical modulation of cell behaviour. What is the role of the scaffold in endogenous regeneration? Here we conceptualise the scaffold as a bi-domain cell signalling device, which provides both biochemical and biophysical cues to endogenous cells by acting as;

(i) a vector for encapsulation and controlled delivery of signalling molecules to modulate the regeneration process, and,

(ii) a deformable porous structure providing biophysical (substrate strain and interstitial fluid flow) cues for migration, attachment, proliferation, angiogenesis and patterned tissue formation by cells in the vicinity of the defect site.

With respect to the scaffold's role in delivery of biochemical cues, numerous approaches for controlled spatiotemporal release of signalling molecules are currently under development[66]. As one example, hepatocyte growth factor (HPC) is a powerful chemoattractant to MSCs, however it is rapidly proteolysed in vivo, so strategies to deliver a sustained release of HPC by encapsulation in a semi-synthetic ECM-like hydrogel are under development[69]. As another example, in exploring the inductive potential of scaffolds composed of decellularised ECM, Badylak's group have recently documented the progenitor cell recruitment potential of a single cryptic peptide derived from the α subunit of the collagen III molecule[70]. To this end, future research efforts specifically targeted at C_2 endogenous TE strategies (see Table 2) should focus on incorporation and controlled release of cues to attract bone marrow and tissue niche precursor cells via cell homing. If C_3 strategies prove viable in musculoskeletal tissues, scaffold research efforts could focus on controlled signalling to promote de-differentiation, proliferation, and re-differentiation of endogenous cells. An alternative conception of the scaffold as a biochemical signalling device is reviewed by Evans et al, in which the scaffold is conceived as a 'gene activated matrix' such that a scaffold impregnated with plasmid DNA transfects endogenous progenitor cells which then express the desired gene[32].

With respect to the scaffold's role as a biophysical signalling device, the scaffold plays a complex mechanical role which requires it to provide a degree of structural support to the neighbouring ECM in a load-bearing musculoskeletal tissue, while at the same time providing appropriate micro-scale signals to cells through substrate strain and interstitial fluid flow, and also degrading in a controlled manner for creeping substitution by newly forming tissue. This requirement for mechanical integrity is a major challenge in musculoskeletal applications of TE, and a wide range of biomaterials and fabrication techniques are currently under development. Macro-scale assessments of scaffold integrity are often limited to basic quasi-static compression tests, and in many cases no attempt is made to define or design the biophysical micro-environment experienced by cells in the scaffold from the perspective of substrate strain, interstitial fluid flow, or oxygen diffusion[71]. There is much potential for improved, quantitative biophysical assessment of the cellular environment in and around scaffolds[72], and a small body of studies using structural analysis techniques such as the Finite Element Method provide a valuable starting point in this regard[73-77]. An improved understanding of scaffold biophysical signalling is essential for the viable development of endogenous TE strategies, since whether cells are auto-transplanted, attracted via homing, or de-differentiated, the biomechanical micro-environment must promote cell viability and new tissue growth. In addition to biomechanical aspects (solid deformation and fluid flow), the generation of endogenous electric fields may be an important but thus far little

considered topic in scaffold biophysics, as it is known that endogenous electric fields play an important role in guiding native tissue growth during both development and regeneration[78-81].

While developments in scaffold research for endogenous TE would be expected to parallel those for use with exogenous or cultured cells to some extent, in an endogenous strategy the scaffold may be promoting quite different cellular processes (homing or even de-differentiation) and so future scaffold designs for biochemical and biophysical signalling in endogenous TE may diverge from those used for cultured autologous or exogenous cells. Chen et al[34] provide an excellent discussion of bioscaffold considerations for cell homing. The overarching philosophy is that the scaffold acts as an artificial ECM which modulates cellular activity and enhances the body's innate regenerative potential[82]. Note that the term 'artificial' is used here in contrast to the functioning native tissue ECM at the regeneration site, and does not preclude the use of 'natural' ECM from other sources as the scaffold material[83]. Figure 1 shows a schematic realisation of this philosophy, in which a scaffold encapsulating growth factors provides both biophysical and biochemical cues to direct stem cell activity through one of the three possible endogenous strategies.

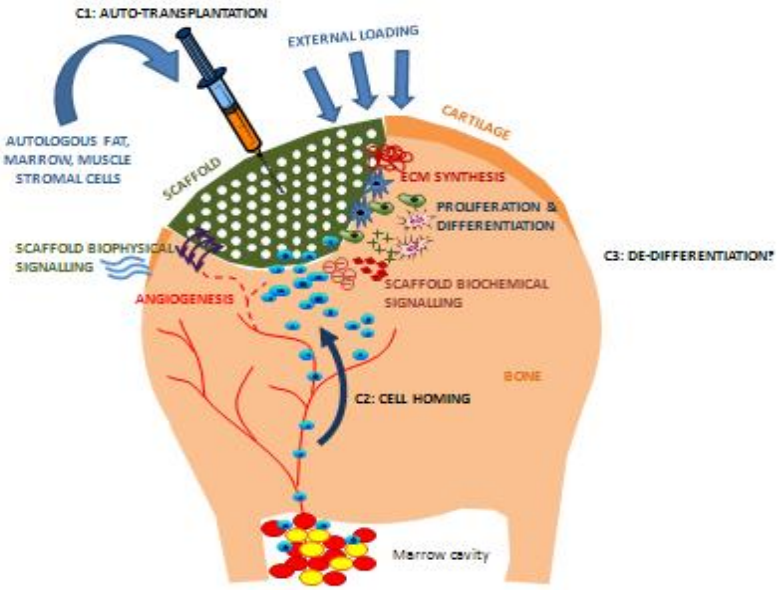

Figure 1. Schematic showing three potential endogenous Tissue Engineering strategies for regeneration of an osteochondral defect: C1:Auto-transplantation of progenitor cells, C2: Stem cell homing from bone marrow via bloodstream and from tissue niche, C3: De-differentiation of previously committed cells . In each case, the scaffold is a biophysical and biochemical signalling source to modulate the regenerative process.

Toward clinical impact

What clinical impact have endogenous TE approaches had to date? Ingber et al[84] give a compelling overview of the challenges which have hindered attempts at clinical translation of TE to date. However, there have also been successes. Firstly, there are several 'traditional' orthopaedic surgical techniques

which are essentially endogenous TE approaches, even if not named as such. Long bone fracture repair, in which autologous bone graft is placed in the defect site, is a clinically proven technique in which auto-transplanted autologous bone promotes successful fracture healing. Similarly, distraction osteogenesis achieves rapid bone formation without the use of exogenous cells, scaffolds or growth factors through the application of a mechanical strain signal to the native tissue ECM. Both of these orthopaedic interventions are separately covered in another paper in this special edition (Berner et al). Another widely used orthopaedic procedure involves fusing of two adjacent vertebrae in the spine to treat joint pain or instability. Spine fusion is also effectively an endogenous TE strategy, in which the joint is held immobile with an implant (after intervertebral disc removal), and new bone formation by endogenous cells is encouraged through grafting with a biomaterial scaffold and application of growth factors to fuse the joint space.

Although these orthopaedic procedures are effectively endogenous TE strategies, there are significant problems with autologous bone grafting with regard to donor site morbidity and availability. In the case of large bone defects, sufficient quantities of autologous bone may not be available. Furthermore, defects in other musculoskeletal tissues (ligaments, skeletal muscle, tendon, cartilage and intervertebral disc) do not generally have a source of autogenous tissue available for use as a scaffold, therefore synthetic scaffolds are required. An example of successful spine fusion using a synthetic scaffold is given by Abbah et al[85] who used 15x12x4mm^3 porous scaffolds to successfully induce lumbar interbody spinal fusion in a pig study. The scaffolds were 70% porosity with 100% pore interconnectivity and 350-500μm pore size, composed of 80% polycaprolactone (PCL) and 20% β-tricalcium phosphate (TCP). Prior to implantation the scaffolds were impregnated with recombinant human bone morphogenetic protein-2 (rhBMP-2). Compared to a control group which received autogenous bone graft in the excised disc space, the scaffold+rhBMP-2 group showed greater bone formation earlier than in the control group. This study demonstrates that rapid bony fusion is possible in the spine using a synthetic scaffold and growth factor combination without cell transplantation.

One of the most promising studies to date in terms of demonstrating the potential of endogenous TE strategies for large scale complex musculoskeletal defect regeneration is that of Lee et al[28], who successfully regenerated the entire articular surface of the rabbit synovial joint using a composite (80% polycaprolactone and 20% hydroxyapatite) scaffold, impregnated with TGFβ3 growth factor absorbed in a collagen hydrogel. A 12x10x17mm^3 scaffold of pore size 200-400μm was used, and the authors noted that endogenous cell recruitment rather than transplantation was a key factor in their approach, modulated by providing an appropriate biological cue in an anatomically correct bio-scaffold. The authors noted that a similar study which did not use growth factors was unsuccessful[86]. Taken together, these two studies again highlight the importance of appropriate molecular signalling for successful regeneration.

Another approach which appears to have achieved a degree of clinical success in regeneration of musculoskeletal defects is so-called 'Endoret' (endogenous regenerative technology) which focuses on the use of autologous platelets encapsulated within autologous fibrin scaffolds[31,87,88]. Anitua et al reported extensive bone regeneration using Endoret in twenty patients who underwent tooth extraction for fractures or periodontal disease[89]. Another study comparing Endoret with controls for bilateral sinus floor augmentation in a series of five patients reported typical findings of 20-30% new bone formation in the treated area versus 8% new bone formation in the control area. Based on immunohistochemical analysis, the authors reported 116 blood vessels per mm^2 in the treated area, compared with only 7 per mm^2 in the control area[90].

Summary and Conclusions

Endogenous regeneration of the musculoskeletal system is a potentially attractive alternative to other Tissue Engineering strategies requiring *ex vivo* cell culture or implantation of exogenous cells. Whilst several widely used orthopaedic procedures are effectively endogenous TE approaches because they regenerate defects without cell implantation, endogenous TE is still a nascent research field because relatively little is known about the molecular signalling processes governing endogenous cell homing from marrow and tissue niches, the de-differentiation potential of mammalian musculoskeletal cells is unknown, and critical sized defects in musculoskeletal tissues often do not have a sufficient source of autologous graft tissue.

Endogenous cell homing strategies using the 'body as bioreactor' approach have the advantage of attracting cells to a defect site because the microenvironment and blood supply are conducive to regeneration, rather than implantation of cells into a scaffold where their nutrient status and viability is uncertain. Several recent studies have demonstrated the capability of cell homing approaches with synthetic scaffolds to regenerate large defects in musculoskeletal tissues, and further investigation in this field is strongly warranted.

The scaffold is a crucial component in designing Tissue Engineering constructs for load-bearing musculoskeletal applications, and in endogenous strategies the scaffold should be considered as both a biochemical and biophysical signalling device, modulating and orchestrating cellular activity in the defect microenvironment to harness and direct a patient's own regenerative potential. There are many challenges in furthering this concept, firstly in developing a proper understanding of the native regenerative capacity of musculoskeletal organs and tissues, and then in engineering clinically feasible interventions to optimise this capacity.

References

1. **Dixon MJ, Marazita ML, Beaty TH, Murray JC.** Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet*. 2011;12:167-78.
2. **Trobisch P, Suess O, Schwab F.** Idiopathic scoliosis. *Dtsch Arztebl Int*. 2010;107:875-83.
3. **Lories RJ, Luyten FP.** The bone-cartilage unit in osteoarthritis. *Nat Rev Rheumatol*. 2011;7:43-9.
4. **Walker BF, Muller R, Grant WD.** Low back pain in Australian adults: the economic burden. *Asia Pac J Public Health*. 2003;15:79-87.
5. **Cauley JA**. Defining Ethnic and Racial Differences in Osteoporosis and Fragility Fractures. *Clin Orthop Relat Res*. 2011 Mar 23. [Epub ahead of print].
6. **Godley K, Watts AC, Robb JE**. Pathological femoral fracture caused by primary bone tumour: a population-based study. *Scott Med J*. 2011;56:5-9.

7. **Schett G, Smolen JS**. New insights in the mechanism of bone loss in arthritis. *Curr Pharm Des*. 2005;11:3039-49.

8. **McKee MD**. Management of segmental bony defects: the role of osteoconductive orthobiologics. *J Am Acad Orthop Surg*. 2006;14:S163-7.

9. **Jacobs JJ, Andersson GBJ, Bell JE, Weinstein S, Dormans JP, Gnatz SM, Lane N, Puzas JE, St Clair EW, Yelin EH.** The burden of musculoskeletal disease in the United States. 2008 Report produced by the American Academy of Orthopaedic Surgeons, American Academy of Physical Medicine and Rehabilitation, American College of Rheumatology, American Society for Bone and Mineral Research, Arthritis Foundation, National University of Health Sciences, Orthopaedic Research Society, Scoliosis Research Society, and the United States Bone and Joint Decade. www.boneandjointburden.org (access date 7th April 2011).

10. **Brooks PM.** The burden of musculoskeletal disease – a global perspective. *Clin Rheumatol* 2006;25:778-71.

11. **Paris O, Zizak I, Lichtenegger H, Roschger P, Klaushofer K, Fratzl P.** Analysis of the hierarchical structure of biological tissues by scanning X-ray scattering using a micro-beam. *Cell Mol Biol*. 2000;46:993-1004.

12. **Mandel U, Dalgaard P, Viidik A.** A biomechanical study of the human periodontal ligament. *J Biomech*. 1986;19:637-45.

13. **Maroudas A**. Balance between swelling pressure and collagen tension in normal and degenerate cartilage. *Nature* 1976;260:808 –809.

14. **Borg TK, Caulfield JB.** Morphology of connective tissue in skeletal muscle. *Tissue Cell*. 1980;12(1):197-207.

15. **Marchand F, Ahmed AM**. Investigation of the laminate structure of lumbar disc anulus fibrosus. *Spine* 1990;15:402-10.

16. **Rigozzi S, Müller R, Snedeker JG.** Collagen fibril morphology and mechanical properties of the Achilles tendon in two inbred mouse strains. *J Anat.* 2010;216:724-31.

17. **Kim SH, Turnbull J, Guimond S.** Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol*. 2011;209:139-51.

18. **Chen JH, Liu C, You L, Simmons CA.** Boning up on Wolff's Law: mechanical regulation of the cells that make and maintain bone. *J Biomech*. 2010;43:108-18.

19. **Sievänen H.** Immobilization and bone structure in humans. *Arch Biochem Biophys*. 2010;503:146-52.

20. **LeBlanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, Voronin L.** Bone mineral and lean tissue loss after long duration space flight. *J Musculoskelet Neuronal Interact*. 2000;1:157-60.

21. **Merle C, Streit MR, Volz C, Pritsch M, Gotterbarm T, Aldinger PR.** Bone remodeling around stable uncemented titanium stems during the second decade after total hip arthroplasty: a DXA study at 12 and 17 years. *Osteoporos Int*. 2010 (epub ahead of print).

22. **Burr DB.** Remodeling and the repair of fatigue damage. *Calcif Tissue Int*. 1993;53 Suppl 1:S75-80.

23. **Schmitz JP, Hollinger JO**. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop Relat Res*. 1986;205:299-308.

24. **Gratz KR, Wong BL, Bae WC, Sah RL.** The effects of focal articular defects on cartilage contact mechanics. *J Orthop Res*. 2009;27:584-92.

25. **Michalek AJ, Buckley MR, Bonassar LJ, Cohen I, Iatridis JC.** The effects of needle puncture injury on microscale shear strain in the intervertebral disc annulus fibrosus. *Spine J*. 2010;10:1098-105.

26. **Cooper GM, Mooney MP, Gosain AK, Campbell PG, Losee JE, Huard J.** Testing the critical size in calvarial bone defects: revisiting the concept of a critical-size defect. *Plastic and Reconstructive Surgery* 2010;125:1685-92.

27. **Roberts SJ, Howard D, Buttery LD, Shakesheff KM**. Clinical applications of musculoskeletal tissue engineering. *British Medical Bulletin* 2008; 86:7-22.

28. **Lee CH, Cook JL, Mendelson A, Moioli E, Yao H, Mao JJ.** Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet* 2010;376:440-8.

29. **Chen FM, Zhang J, Zhang M, An Y, Chen F, Wu ZF**. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* 2010;31:7892-7927.

30. **Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE**. Human bone marrow stem cells express a distinct set of biologically functional chemokine receptors. *Stem Cells* 2006;24:1030-41.

31. **Anitua E, Sanchez M, Orive G**. Potential of endogenous regenerative technology for in situ regenerative medicine. *Adv Drug Del Rev* 2010;62:741-52.

32. **Evans CH, Palmer GD, Pascher A, Porter R, Kwong FN, Gouze E, Gouze JN, Liu F, Steinert A, Betz O, Betz V, Vrahas M, Ghivizzani SC**. Facilitated endogenous repair: Making Tissue Engineering simple, practical and economical. *Tissue Engineering* 2007;13:1987-93.

33. **Löwenheim H**. Regenerative medicine for diseases of the head and neck: Principles of in vivo regeneration. *DNA and cell biology* 2003;22:571-92.

34. **Chen F, Wu L, Zhang M, Zhang R, Sun H**. Homing of endogenous stem/progenitor cells for in situ tissue regeneration: Promises, strategies and translational perspectives. *Biomaterials* 2011;32:3189-3209.

35. **Guntias-Lichius O, Wittekindt C**. The role of growth factors for disease and therapy in diseases of the head and neck. *DNA and Cell Biology* 2003;22:593-606.

36. **Gillers, BS**. The promises and problems of induced endogenous regeneration. *Journal of Undergraduate Life Sciences* 2007;1:18-23.

37. **Odelberg SJ**. Inducing cellular dedifferentiation: a potential method for enhancing endogenous regeneration in mammals. *Semin Cell Dev Biol* 2002;13-5:335-43.

38. **Thornton CS**. The histogenesis of muscle in the regenerating fore limb of larval Amblystoma punctatum. *J Morphol* 1938;62:17–47.

39. **Thornton CS.** The histogenesis of the regenerating forelimb of larval Amblystoma after exarticulation of the humerus*. J Morphol* 1938;62:219–235.

40. **Chalkley DT.** A quantitative histological analysis of forelimb regeneration in Triturus viridescens. *J Morphol* 1954;94:21–70.

41. **Geyer M, Borchardt T, Schreiyäck C, Wietelmann A, Müller-Schrobsdorff F, Müller C, Müller-Ladner U, Dinser R.** Endogenous regeneration after collagenase-induced knee joint damage in the adult newt Notophthalmus viridescens. *Ann Rheum Dis* 2011;70-1:214-20.

42. **Maden M.** Retinoids as endogenous components of the regenerating limb and tail. *Wound Repair Regen* 1998;6-4:358-65.

43. **Kumar A, Godwin JW, Gates PB, Garza-Garcia AA, Brockes JP**. Molecular basis for the nerve dependence of limb regeneration in an adult vertebrate. *Science* 2007;318:772-7.

44. **Odelberg SJ, Kollhoff A, Keating MT**. Dedifferentiation of mammalian myotubes induced by msx1. *Cell* 2000;103:1099-109.

45. **Engel FB, Schebesta M, Duong MT, Lu G, Ren S, Madwed JB, Jiang H, Wang Y, Keating MT**. p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. *Genes Dev.* 2005;19:1175-87.

46. **Engel FB, Hsieh PC, Lee RT, Keating MT.** FGF1/p38 MAP kinase inhibitor therapy induces cardiomyocyte mitosis, reduces scarring, and rescues function after myocardial infarction. *Proc Natl Acad Sci USA.* 2006;103:15546-51.

47. **Heber-Katz E, Gourevitch D**. The relationship between inflammation and regeneration in the MRL mouse: potential relevance for putative human regenerative (scarless wound healing) capacities? *Ann N Y Acad Sci*. 2009;1172:110-4.

48. **Moseley FL, Faircloth ME, Lockwood W, Marber MS, Bicknell KA, Valasek P, Brooks G**. Limitations of the MRL mouse as a model for cardiac regeneration*. J Pharm Pharmacol*. 2011;63:648-56.

49. **Connolly JF, Guse R, Tiedeman J, Dehne R**. Autologous marrow injection as a substitute for operative grafting of tibial nonunions. Clin Orthop Related Res 1991;266:259-70.

50. **Hernigou P, Poignard A, Manicom O, Mathieu G, Rouard H**. The use of percutaneous autologous bone marrow transplantation in non-union and avascular necrosis of bone. *J Bone Joint Surg Br* 2005;87:896-902.

51. **Richter W**. Mesenchymal stem cells and cartilage in situ regeneration. *J Intern Med* 2009;266:390-405.

52. **Steadman JR, Rodkey WG, Briggs KK, Rodrigo JJ**. The microfracture technique in the management of complete cartilage defects in the knee joint. *Orthopade* 1999;28:26-32.

53. **Fong ELS, Chan CK, Goodman SB.** Stem cell homing in musculoskeletal injury. *Biomaterials* 2011;32:395-409.

54. **Hernigou P, Poignard A, Beaujean F, Rouard H.** Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am* 2005;87:1430-7.

55. **Chen CW, Montelatici E, Crisan M, Corselli M, Huard J, Lazzari L, Peault B.** Perivascular multi-lineage progenitor cells in human organs: regenerative units, cytokine sources or both? *Cytokine and Growth Factor Reviews* 2009;20:429-34.

56. **Jackson WM, Lozito T, Djouad F, Kuhn NZ, Nesti LJ, Tuan RS**. Differentiation and regeneration potential of mesenchymal progenitor cells derived from traumatized muscle tissue. *J Cell Mol Med* 2010 (epub ahead of print).

57. **Parrilla C, Saulnier N, Bernardini C, Patti R, Tartaglione T, Fetoni AR, Pola E, Paludetti G, Michetti F, Lattanzi W.** Undifferentiated human adipose tissue-derived stromal cells induce mandibular bone healing in rats. *Arch Otolaryngol Head Neck Surg*. 2011;137:463-70.

58. **Levi B, James AW, Nelson ER, Vistnes D, Wu B, Lee M, Gupta A, Longaker MT.** Human adipose derived stromal cells heal critical sized mouse calvarial defects. PLoS One 2010;5:e11177.

59. **Liu ZJ, Zhuge Y, Velaquez OC.** Trafficking and differentiation of mesenchymal stem cells. *J Cell Biochem* 2009;106:984-91.

60. **Luster AD, Alon R, von Andrian UH.** Immune cell migration in inflammation: present and future therapeutic targets. *Nature Immunol* 2005;6:1182-90.

61. **De Bari C, Dell'accio F**. Mesenchymal stem cells in rheumatology: a regenerative approach to joint repair. *Clin Sci*. 2007;113:339-48

62. **Risbud MV, Guttapalli A, Tsai TT, Lee JY, Danielson KG, Vaccaro AR, Albert TJ, Gazit Z, Gazit D, Shapiro IM**. Evidence for skeletal progenitor cells in the degenerate human intervertebral disc. *Spine* 2007;32:2537-44.

63. **Brown BN, Chung WL, Pavlick M, Reppas S, Ochs MW, Russell AJ, Badylak SF**. Extracellular matrix as an inductive template for temporomandibular joint meniscus reconstruction: A pilot study. *J Oral Maxillofac Surg* 2011 (epub ahead of print).

64. **Cohen S, Levi-Montalcini R, Hamburger V.** A nerve growth stimulating factor isolated from sarcomas 37 and 180. *Proc Nat Acad Sci USA* 1954;40:1014-8.

65. **Kolambkar YM, Boerckel JD, Dupont KM, Bajin M, Huebsch N, Mooney DJ, Hutmacher DW, Guldberg RE.** Spatiotemporal delivery of bone morphogenetic protein enhances functional repair of segmental bone defects. *Bone* 2011 (epub ahead of print).

66. **Chen F, Zhang M, Wu Z.** Toward delivery of multiple growth factors in tissue engineering. *Biomaterials* 2010;31:6279-6308.

67. **Parekkadan B, Milwid JM.** Mesenchymal stem cells as therapeutics. *Ann Rev Biomed Eng*. 2010;12:87-117.

68. **Caplan AI, Dennis JE**. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98:1076-84.

69. **Zhao J, Zhang N, Prestwich GD, Wen X**. Recruitment of endogenous stem cells for tissue repair. *Macromol Biosci.* 2008;8:836-42.

70. **Agrawal V, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF**. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. *Tissue Eng Part A* 2011 (epub ahead of print).

71. **Androjna C, Gatica JE, Belovich JM, Derwin KA.** Oxygen diffusion through natural extracellular matrices: implications for estimating "critical thickness" values in tendon tissue engineering. *Tissue Eng Part A* 2008;14:559-69.

72. **Lutton C, Goss B.** Caring about microenvironments. *Nat Biotech* 2008;26:613-4.

73. **Jaecques SVN, Van Oosterwyck H, Muraru L, Van Cleynenbreugel T, De Smet E, Wevers M, Naert I, Vander Sloten J**. Individualised, micro CT-based finite element modelling as a tool for biomechanical analysis related to tissue engineering of bone. *Biomaterials* 2004;25:1683–1696.

74. **Tuan HS, Hutmacher DW**. Application of micro CT and computation modelling in bone tissue engineering. *Comput Aid Design* 2005;37:1151-1161.

75. **Lacroix D, Chateau A, Ginebra MP, Planell JA**. Micro-finite element models of bone tissue-engineering scaffolds. *Biomaterials* 2006;27:5326–5334.

76. **Lacroix D, Planell JA and Prendergast PJ.** Computer-aided design and finite-element modelling of biomaterial scaffolds for bone tissue engineering. *Phil. Trans. R. Soc. A* 2009;367:1993-2009.

77. **Butler DL, Goldstein SA, Guldberg RE, Guo XE, Kamm R, Laurencin CT, McIntire LV, Mow VC, Nerem RN, Sah RL, Soslowsky LJ, Spilker RL, Tranquillo RT**. The impact of biomechanics in tissue engineering and regenerative medicine. *Tissue Eng B* 2009;15:477-84.

78. **Altizer AM, Moriarty LJ, Bell SM, Schreiner CM, Scott WJ, Borgens RB.** Endogenous Electric Current Is Associated With Normal Development of the Vertebrate Limb. *Dev Dynamics* 2001;221:391–401.

79. **Nuccitelli R.** Endogenous electric fields in embryos during development, regeneration and wound healing. *Radiation Protection Dosimetry* 2003;106:375–383.

80. **Robinson KR, Messerli MA**. Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion. *BioEssays* 2003;25:759–766.

81. **Fassina L, Saino E, Sbarra MS, Visai L, De Angelis MG, Magenes G, Benazzo F**. In vitro electromagnetically stimulated SAOS-2 osteoblasts inside porous hydroxyapatite. *J Biomed Mater Res A*. 2010;93:1272-9.

82. **Lutolf MP, Gilbert PM, Blau HM.** Designing materials to direct stem-cell fate. *Nature* 2009;462:433-41.

83. **Badylak SF, Taylor D, Uygun K**. Whole-organ tissue engineering: Decellularization and recellularization of three-dimensional matrix scaffolds. *Ann Rev Biomed Eng* 2010 (epub ahead of print).

84. **Ingber DE, Mow VC, Butler D, Niklason L, Huard J, Mao J, Yannas I, Kaplan D, Vunjak-Novakovic G.** Tissue engineering and developmental biology: going biomimetic. *Tissue Engineering* 2006;12:3265-83.

85. **Abbah SA, Lam CXL, Hutmacher DW, Goh JCH, Wong HK.** Biological performance of a polycaprolactone-based scaffold used as fusion cage device in a large animal model of spinal reconstructive surgery. *Biomaterials* 2009;30:5086-93.

86. **Woodfield TB, Guggenheim M, von Rechenberg B, Reisle J, van Blitterswijk CA, Wedler V.** Rapid prototyping of anatomically shaped, tissue-engineered implants for restoring congruent articulating surfaces in small joints. *Cell Prolif* 2009;42:485-97.

87. **Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, Wolff LF, Yoshie H.** Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-beta and modulates the proliferation of periodontally-related cells in vitro. *J. Periodontol* 2003;74:849-57.

88. **Kanno T, Takahashi T, Tsujisawa T, Ariyoshi W, Nishihara T.** Platelet-rich plasma enhances human osteoblast-like cell proliferation and differentiation. *J Oral Maxillofacial Surg* 2005;63:362-9.

89. **Anitua E.** Plasma rich in growth factors: preliminary results of use in the preparation of sites for implants. *Int J Oral Maxillofac Impl* 1999;14:529-35.

90. **Anitua E, Prado R, Orive G**. Bilateral sinus elevation evaluating PRGF technology: a report of five cases. *Clin Implant Dent Relat Res B.* 2010 (epub ahead of print).