Published Online April 17, 2008

Clinical applications of musculoskeletal tissue engineering

Scott J. Roberts[†‡], Daniel Howard[†], Lee D. Buttery[†], and **Kevin M. Shakesheff[*†]**

[†]Wolfson Centre for Stem Cells, Tissue Engineering and Modelling, [‡]Division of Medicinal Chemistry and Structural Biology, Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, NG7 2RD, UK

Background: Current surgical techniques for the repair of the musculoskeletal system can be often limited by the availability, quality and quantity of materials, such as grafts to effect repair. This has led to the exploration and development of novel methods of intervention based on tissue engineering and regenerative medicine.

Source of data: This review summarizes the successes and investigations which are happening to date in the field of musculoskeletal tissue engineering. This is based on an extensive literature search and through basic research being performed by the authors.

Areas of agreement: Due to the constraints surrounding certain surgical techniques and restrictions on their use, novel procedures are required for the repair and regeneration of damaged tissues.

Areas of controversy: The choice of cell type has caused much debate within the tissue-engineering field. However it is widely accepted that currently only autologous primary/adult stem cells are fit for transplantation, until such times that optimized differentiation and selection protocols exist for embryonic stem cells.

Growing points: The current results of the clinical cases utilizing tissue engineered constructs for bone and cartilage repair provide insights for improvement of these techniques thus allowing treatments to become increasingly viable.

Areas timely for developing research: There is a need to better understand the integration of scaffolds and cell populations into the target tissue. This should provide vital information influencing scaffold manufacturing procedures and cell selection.

Keywords: Tissue engineering/regenerative medicine/musculoskeletal/embryonic stem cells/mesenchymal stem cells/scaffolds

Accepted: March 27, 2008

**Correspondence to: Prof. Kevin Shakesheff, STEM CBS School of Pharmacy, University of Nottingham NG7 2RD, UK. E-mail: kevin.shakesheff@nottingham.ac.uk*

DOI:10.1093/bmb/ldn016

Introduction

The ultimate aim of the tissue-engineering field concerns 'understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use'.[1] This process, depicted by the schematic diagram in Figure 1, uses combinations of cells and/or scaffold matrices which have the ability to form tissues within the body upon transplantation. However, this has not always been the only meaning of the term; historically it has been associated with the use of artificial devices, which replace tissues at sites of trauma—such as prosthetic limbs—and has also been associated with manipulation of body tissues.

Perhaps the first report of tissue engineering, as viewed today, occurred in the late 1970s by Howard Green at MIT. Dr Green successfully grew colonies of epidermal keratonicytes into sheets of epithelium, which resembled the epidermis. This cultured epithelium could be successfully introduced onto a wound of athymic mouse in nearly all cases studied.[2] This technique was further used for the coverage of burn wounds in humans in 1984 by Gallico *et al.*[3] The use of this technique of *in vitro* tissue regeneration has created much excitement within the medical community and subsequently a great deal of research has occurred, particularly into the regeneration of tissues from the musculoskeletal system.

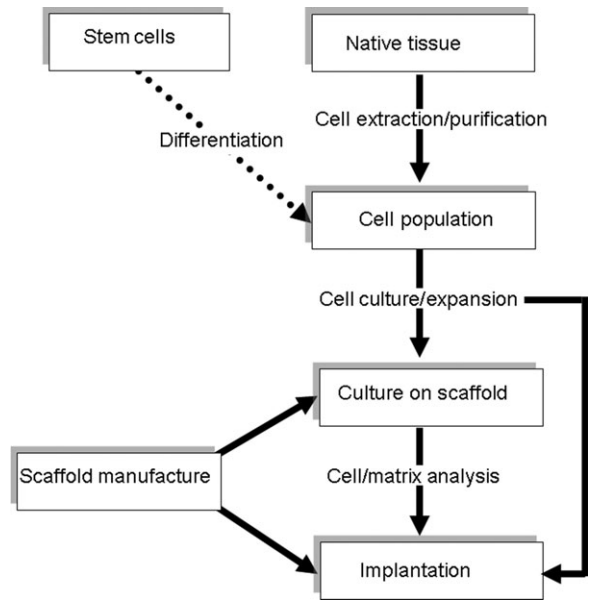

Fig. 1 Schematic diagram demonstrating the theory behind tissue engineering. Cells can be derived from tissue or from stem cell sources and combined with scaffold materials.

When considering musculoskeletal tissue engineering the primary uses of this technique would be concerning the replacement of lost or damaged bone, cartilage, skeletal muscle and tendon/ligament. Many factors would influence the use of this technique for tissue replacement ranging from the type and manufacture of scaffold materials to the source of cells used for scaffold seeding. The primary function of the scaffold is to deliver growth factors and/or cells to the site of tissue trauma to aid the repair/regeneration of the injury. The scaffold should mimic the structural properties of the native tissue, i.e. scaffolds for cartilage tissue engineering should be able to withstand the forces felt at load bearing surfaces whereas scaffolds for muscle tissue engineering should be able to flex and stretch. Similarly the source of cells needs to be tissue matched, i.e. cartilage cells (chondrocytes) for cartilage. Stem cells, both adult and embryonic are also being investigated for their use in tissue-engineering protocols.

Clinical need for tissue engineering

Current gold standards for the repair of bone, cartilage and skeletal muscle entail intense surgical processes for reparative or replacement therapy. Perhaps the area most suitable for tissue engineering is that of bone and cartilage defects. Current therapies which are utilized today for cartilage defects include autologous chondrocyte implantation (ACI),[4] osteochondral autografts[5] and allografts[6] and in extreme cases total joint replacement.

The current gold standard for cell based repair of articular cartilage is implantation of autologous chondrocytes in the site of cartilage damage. Treatment using this method requires keyhole surgery to remove several slivers of undamaged cartilage from a non-load bearing region, cells are extracted from the tissue and expanded *in vitro*. The cartilage defect is debrided and a patch of periosteum is stitched over the defect to provide a cell source and a cover for the cell suspension, which is injected under the patch. The repair will typically produce a new tissue to fill the defect, with function restored in ~82% of patients at 2 years.[7] There have been many modifications to this procedure, although the periosteum helps with the repair process other materials have been used such as hyaluronic acid sponges to fill the defect and provide shielding and support the cells, this procedure unlike artificial knee replacements which degenerate over time actually may regenerate over time. The process of *in vitro* expansion has been commercialized with companies such as Genzyme providing services to expand autologous chondrocytes (Carticel®).

Complications relating to ACIs are rare however from the American patient registry's 6286 patients, 5.8% reported side effects or adverse events. The most frequent of these, with an incidence of 1.6% was that of adhesions or fibroarthrosis, complete treatment failure accounted for 1.3%, with hypertrophic changes to the implant site at 1.1%. Of all patients, 4.8% reported reoperations following autologous chondrocytes implantation (Washington State Department of Labour and Industries, 2002). However, a report by Minas (1999) states that 26/70 patients (37%) reported complications, which required surgical intervention. This was primarily due to the loss of full motion in the affected joint followed by pain accompanied by effusion due to hypertrophic changes at the site of implantation. A failure rate of 5/70 (7%) was also reported, however, only two were due to the implantation, with the others being a result of trauma during the recovery period.[8]

In comparison the main treatments for non-union bone defects are autograft and allografts, distraction osteogenesis and implantation of strengthening devices. Bone autografts are the only graft material that has osteogenic, osteoinductive and osteoconductive properties thus making for a highly effective form of non-union treatment.[9] In addition to autografts and allografts bone graft substitutes can be used to promote the ability of non-union defects to fuse, however many of these products are not widely used due to lack of knowledge on their efficacy for bone healing.[10] This leaves the current gold standard as the iliac crest autologous graft. This process involves the removal of bone segments from either the posterior or anterior iliac crest with osteotomes and gouges. The bone segments collected contain both compact dense cortical bone and trabecullar bone. Due to the properties of autografts, upon transplantation into a non-union defect, new bone is formed allowing fusion of the bone. Although this technique is the standard for non-unions there are multiple complications associated with it. These include reported cases of arterial lacerations (superior gluteal, fourth lumbar, iliolumbar and deep iliac circumflex arteries),[11] enterocutaneous fistula[12] and arteriovenous fistula[13] and femoral cutaneous nerve injury.[14] In addition to this post-operative pelvic instability,[15] persistent pain has been reported. Another study reported that out of a cohort of 192, 2.4% of patients had major complications, 21.8% had minor complications and 37.9% of patients reported pain from the graft site 6 months post-operation.[16]

Although these techniques have efficacy in retaining the structure and function of the relevant tissue the surgical manipulation involved in each can cause its own intrinsic problems. These complications are testament that current gold standard therapies for musculoskeletal repair

have unavoidable side effects, which can have an impact on the patient's ability to make a full recovery from surgery. Tissue engineering could be a technique that would allow the production of functional tissue without the need for grafting. This technique would involve production of a scaffold from a biodegradable structure, which could be seeded with cells and/or growth factors to promote tissue regeneration. The source of cells for this technique may be either primary or stem cell populations, including both adult and embryonic sources.

Sources of cells for tissue-engineering strategies

The production of an engineered tissue *in vitro* requires the use of cells to populate a scaffold and produce matrix resembling that of the native tissue. The main successes in this field have come from the use of primary cells, taken from the patient and used, in conjunction with scaffolds to produce tissue for re-implantation. However this strategy has limitations, mainly the invasive nature of cell collection and the potential for cells to be in a diseased state. Due to this much attention has been focused on the use of stem cells for tissue-engineering protocols. These include embryonic stem (ES) cells, bone marrow mesenchymal stem cells (MSCs) and umbilical cord derived MSCs.

Primary cells

The use of autologous cells from the individuals' tissue has shown promise in the field of tissue engineering with perhaps the largest breakthrough coming from this source. Atala *et al.*[17] have utilized cells from bladder biopsies seeded onto collagen scaffolds to treat patients with end-stage bladder disease. Further investigations are being carried out in animal models using autologous cells for musculoskeletal tissue engineering such as skeletal muscle repair using myoblasts[18] and tendon repair using tenocytes.[19]

The use of cells from this source would involve the removal of tissue from the individual, an *in vitro* isolation and expansion before re implantation to the site of intervention. Although from an immunological stance the replacement of tissue with autologous cells is an ideal situation there are problems associated with this method. Firstly, the harvest of tissue to allow cell production requires surgical intervention which would be on par with the grafting processes described previously. This has the potential to cause pain and discomfort at a distant graft site. In addition to this some cell populations from primary sources have a low propensity for division thus the expansion of such cells may prove

problematic. This is sometimes compounded with cellular senescence – a phenomenon when primary cells cease cellular division, usually caused by a shortening of telomere length. Although these limitations do exist autologous, cell-based therapy is used for tissue repair for the treatment of cartilage defects and skin burns. These limitations have made the search for cell populations, which can be expanded in culture before implantation, of the utmost importance. Perhaps hope can be drawn from the level of research being carried out on stem cell populations, which would potentially overcome some of the drawbacks of autologous primary cells.

Bone marrow derived mesenchymal stem cells

Adult stem cells from autologous sources perhaps provide the greatest hope for tissue engineering. These cells provide all of the benefits that a primary cell does, however have the ability to undergo multi-lineage differentiation and a higher propensity for cell division. These cells can be isolated from bone marrow (stromal cells) and purified to a generic marrow cell population. Bone Marrow MSCs have been shown to be able to differentiate into the osteogenic,[20] myogenic,[21] chondrogenic[22] and neurogenic[23] lineages. In addition, these cells have already been used to augment the repair of bone.[24] The MSC cell population can be isolated as adherent bone marrow colony forming units – fibroblastic (CFU-F).[25]

MSCs can be purified from the complex mixture of cell subsets, found in bone marrow, to a more defined starting cell population through the use of antibodies, which recognize specific markers on the surface of stem cell populations. Cells can be sorted on the basis of epitope expression such as Endoglin[26] and STRO-1[27] antibody selection procedures. A possible clinical application for these cells is to enhance materials such as the filler used for stabilizing artificial hip joints or for joining critical sized defects in bone that would not otherwise heal.[28] In addition this cell type can be transplanted in an undifferentiated state and in most cases will assume the phenotype of the neighbouring cells, this has already been shown with tissues such as skeletal muscle[29] and bone. A potential drawback of this cell type is its propensity to loose differentiating potential with age[30] thus an attempt to find novel populations of adult stem cells is underway.

Cord-derived mesenchymal stem cells

Since the discovery that umbilical cord blood contains MSCs, which can undergo multi-lineage differentiation, much research has been

focused on determining their applications. The analysis of their gene expression profile reveals similarities to bone marrow MSCs,[31] with an ability to differentiate into chondrocytes, osteoblasts,[32] hepatocytes[33] and neuronal like cells.[34]

Indeed if this type of stem cell does function as the classical bone marrow MSC it would greatly improve the availability of matched tissues for treatments. With 669 531 births in 2006 in the UK alone (Office for National Statistics) this source of stem cells would provide a large pool of material, which could be purified using non-invasive techniques and could be recipient matched.

Embryonic stem cells

ES cells have the ability to be maintained for long (theoretically indefinite) culture periods, therefore, potentially providing large amounts of cells for tissues that could not be derived directly from a tissue source. Proof of the true pluripotent nature of ES cells is teratoma formation. This property demonstrates the ability of stem cells to tissue engineer multiple tissue types but also highlights the importance of using a terminally differentiated cell stock without latent cells with ES cell like properties. This scenario would cause unchecked tissue growth. The use of stem cells will therefore require a method to ensure differentiation, either by demonstration of selection of only non-stem cells or removal of all stem cells[35] and by *in vivo* demonstration of an absence of teratoma formation.

One of the critical steps of stem cell usage for regenerative medicine is therefore the ability to control the differentiation of the cells to the desired tissue lineages. ES cells have been shown to have the propensity to differentiate into lineages of the musculoskeletal system. Differentiation into osteoblasts,[36] and chondrocytes[37] have previously been demonstrated.

Scaffold materials for use in musculoskeletal tissue engineering

Materials used for tissue-engineering processes require properties similar to the tissue being reproduced. Furthermore the ability to be biocompatible preferably actively integrating and inducing the formation of the appropriate tissue are strong considerations. When considering bone tissue engineering the primary source would typically be morcilized autologous bone taken from the same patient as it would inevitably be a good match for the patient, however, the site from

which it is recovered has to be able to sustain the loss and provide appropriate volume and shape of material, e.g. – illiac crest from the pelvis for volume and split ribs for longer structural pieces. The use of autologous material has the drawback that there would be the need for a secondary operation site, increasing the complexity, time and pain of surgery. To reduce the need for second operations a range of alternatives has been developed such as 'off-the-shelf' allogenic or xenogenic de-cellularized bone material that has been extensively processed and treated to sterilize and remove potential immunogens and pathogens.

Bone material alternatives include synthetic minerals, Bioglass® (a calcium and phosphate containing silica glass),[38] coral like materials (calcium carbonates), tricalcium phosphates (βTCP) and hydroxyapatite (HA). HA and TCP are often combined to take advantage of the differing properties of the two materials as HA takes longer to integrate but is more strongly osteo-inductive whereas TCP is quicker to degrade (dependant upon the specifics of usage) therefore combinations of the two materials allows good integration and enhanced remodelling. These materials are formatted typically into granules as a filler material or shapeable/shaped blocks for larger defects. These materials can also be combined with aspirates of bone marrow, which contain cell populations able to enhance bone growth. To enhance repair using bone marrow aspirates, a ceramic material-based system has been developed specifically to adhere osteoprogenitor cells through selective retention and enriched osteogenic cell population (DePuy, Cellect). Where a mouldable ceramic is required, a range of cements has been developed typically calcium phosphates, carbonates and or sulphates combined with setting and handling agents, setting without raising the temperature and generating fewer bubbles to reduce mechanical instabilities.

The use of bioabsorbable polymeric scaffolds is being investigated for use in bone tissue engineering as their properties can be tailored to allow them to dissolve and integrate at optimal rates so the bone re-modelling process is able to complete. Where integration and persistence of implants is an issue, a range of supports and pins has been produced from biodegradable polymeric materials. These implants and many of the polymers used for tissue engineering have been derived from biodegradable suture development and drug delivery devices and are FDA approved for specific usage.[39] Polymers can be processed/formatted using a variety of techniques based upon melting, dissolving and polymerization, therefore can be shaped into a large range of structural architectures. Polymers used primarily include the poly hydroxyl acids; poly lactic acid (PLA), polyglycolic acid (PGA) and the co-polymer poly lactic co glycolic acid (PLGA). Both lactic acid and glycolic acid can be dealt with by the body's metabolism, and by

adjusting molecular weight, ratios and block design of monomers the rate of degradation can be profiled. Degradation occurs through the entire polymer structure as the process is not enzyme limited but degrades by hydrolysis. A further advantage of using materials that were designed for drug delivery is the ability to incorporate controlled growth factor release from the cell support matrices and the ability to produce different release profiles of different growth factors from the same material.[40] Controlled release of different growth factors from polymeric scaffolds is advantageous as the event associated with bone mineral maturation is the invasion with blood vessels, therefore both vascular endothelial growth factor and bone morphogenic protein-2 have been incorporated into PLA scaffolds to provide a controlled release of signals which can initiate vascularization and osteogenesis.[40]

The combination of ceramics and polymers into composite scaffold matrices can provide advantages over those produced from neat ceramic or polymer in many situations. Enhanced compressive strength and stiffness of polymers can be achieved with addition of ceramics, while the resultant construct still retains the ability to easily incorporate and release bio-molecules. Composites can also allow simpler scaffold manufacture and be designed to more closely mimic the physical properties of the tissues with the desired degradation profile (for a review of composite materials see Rezwan *et al.*[41]).

Degradable polymer custom designed implants can be produced for individual patients via custom 3D printing techniques using surface selective laser sintering (SSLS) of PLA particles (Fig. 2). This allows a custom scaffold to be built from computed 3D information derived from patient scans and computer simulations of the components needed for a successful operation.[42] The process is similar to rapid prototyping procedures whereby layers of small particles are selectively sintered using a directed laser; these fused particles are further layered and sintered until several hundred layers have been bonded together producing the individual scaffold in the desired shape.

Cartilage repair requires different structural properties of bone and increasingly repair of cartilage defects are being performed using biologically based treatments as opposed to debridement, or in severe cases total knee replacement. Increasingly popular treatments for focal defects are mosaicplasty and the ACI technique[4] where a sample of cartilage is cultured to supply a large number of cells to fill a defect. If a support material is used for repair they tend to be flexible materials that can withstand compression while providing a supporting environment for chondrocytes. For cartilage repair hyaluronic acid and collagen scaffold supports have been used, these materials are able to integrate into the cartilage material and are already components of mature hyaline cartilage. Delivery of chondrocytes can also be

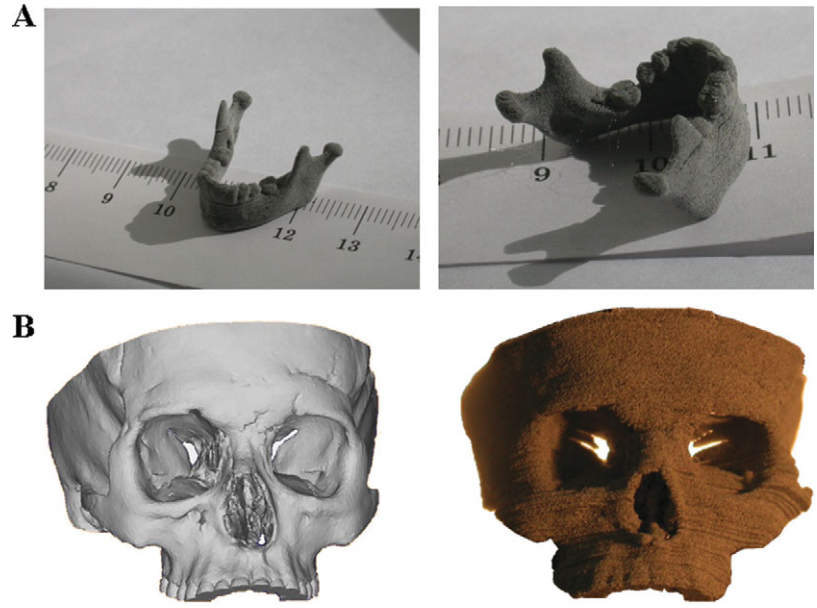

Fig. 2 Use of the SSLS scaffold manufacturing procedure to produce custom scaffold shapes. Jaw structure (A) and skull structure (B) produced from corresponding MRI scan. Figure kindly provided by Dr V. K. Popov, Institute of Laser and Information Technologies, Russian Academy of Sciences.

performed using a range of gels including alginate, collagen and even synthetic self-gelling peptides.[43] As articular cartilage is composed of a matrix of collagen fibrils containing proteoglycan a combined approach has also been proposed, using a woven fibrous PGA material containing an agarose/fibrin gel support to more closely replicate the mechanical composition of native hyaline cartilage.[44]

Another important property for certain scaffolds, especially those for muscle tissue engineering, is flexibility. Flexible scaffolds are also needed, as this tissue requires movement as a fundamental part of its mode of action. To address this issue, modification of a flexible polymer Poly (1,8-octanediol-co-citric acid) to make it more suitable for culture of muscle cells has been made.[45] Further research is ongoing to assess the efficacy of other scaffold types such as glass fibres[46] for muscle tissue engineering.

The materials introduced here are only a few key examples, however, as polymers can be designed to have a wide range of properties and are easily modified to incorporate different moieties and growth factors, therefore there are many more in development. As there are many polymers of potential use for clinical situations some groups have set out to examine the influence of series of novel polymers on cell phenotype[47] to isolate particular characteristics of polymers. There is also

considerable interest in the properties and use of metal foams and composites of mineral and polymer as they are able to combine a range of qualities.

Current clinical studies for musculoskeletal repair

Presently a substantial amount of research is being undertaken to investigate the efficiency of scaffold and cell/growth factor combinations to support tissue growth in an *in vivo* system. This is usually in an animal model, however, certain techniques have already made it to restricted clinical trials, with most documented cases being concerned with the repair of bone and cartilage. Perhaps one of the first reports of tissue engineered bone implantation in humans was in 2001 when Quarto *et al.* utilized a HA scaffold system, seeded with bone marrow stromal cells for the repair of non-union bone defects. This was trialled in patients that had not responded to other surgical interventions. All patients displayed a recovery in bone function between 6 and 12 months post-operation. Good integration of the graft was also displayed, with no apparent adverse side effects.[48] The follow-up to this study investigated the bone repair of the aforementioned patients at 6–7 years post-operation, good implant integration was observed, one of which is shown in Figure 3.[49] This pilot study proved what had already been investigated in large animal models – that implants could be used in place of conventional bone grafting for tissue repair. More recently this technique was used, along with autologous MSCs – differentiated to the osteoblast lineage on HA scaffolds, to fill bone voids following tumour curettage.[50]

Similar results have been seen when scaffolds seeded with bone marrow MSCs are used for reconstruction of articular cartilage. A 2 year study using autologous chondrocytes embedded in a three-dimensional (3D) bioresorbable two-component gel–polymer scaffold (BioSeed-C) for the treatment of post-traumatic and osteoarthritic defects.[51] Clinical outcome scores demonstrated an improvement in overall knee related quality. Similar results were found when using a hyaluronan-based scaffold seeded with autologous chondrocytes for the treatment of deep chondral lesions.[52] In addition to the use of autologous chondrocytes, a recent study has investigated the use of autologous bone marrow stromal cells in conjunction with collagen scaffolds for the repair of a full-thickness articular cartilage defect in the medial femoral condyle.[53] In this study bone marrow stromal cells were expanded in culture and embedded within a collagen gel, which was subsequently implanted to the large articular cartilage defect and covered with an autologous periosteal flap. This resulted in the defect being filled with hyaline-like

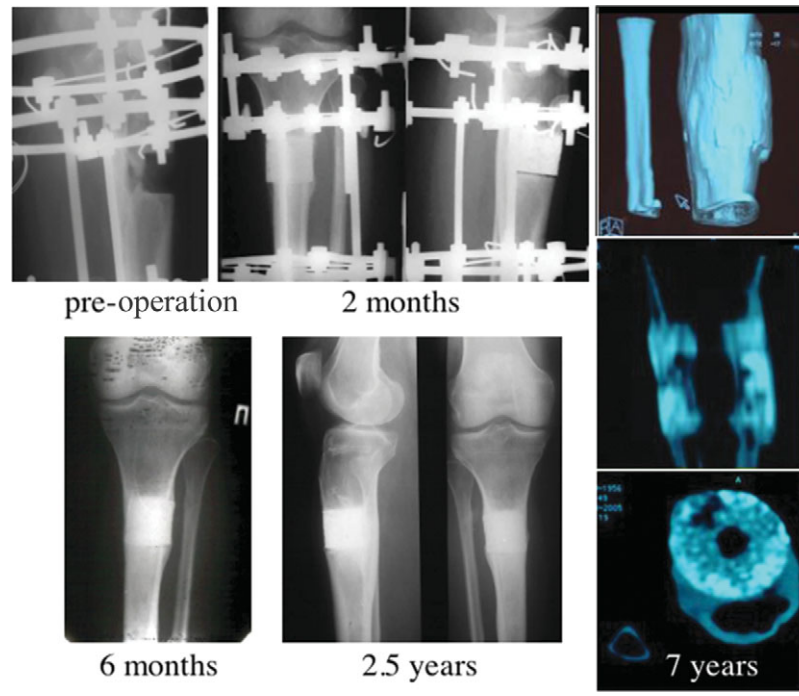

Fig. 3 Repair of non-union bone defects using hydroxyapatite scaffolds associated with bone marrow stromal cells. As seen on the pre-operative radiograph a 4 cm gap is observed in the proximal tibia. Following implantation of the scaffold/cell structure bone callus formation is evident; this further progressed at 6 months with bone-implant integration evident. Complete bone-implant integration was observed at 2.5 years post-operation. CT scans at 7 years showed the complete healing of the non-union with the presence of a medullary channel and new bone within the HA scaffold pores (HA scaffold still present 7 years post-operation). Figure kindly provided by Dr E. Kon, Istituti Ortopedici Rizzoli, Bologna, Italy. This figure was published in Tissue Engineering, Volume 13, 2007[48] by Mary Ann Liebert, Inc., Publishers.

cartilage and a concordant improvement in clinical symptoms after 7 months (Fig. 4). This indicates that autologous bone-marrow stromal cells can also be utilized for the repair of cartilage defects.

Repair of other tissues of the musculoskeletal system is still at the pre-clinical, animal model stage. Tendon repair has been shown in a porcine model using autologous dermal fibroblasts and tenocytes.[54] In addition, skeletal muscle repair has been shown to occur in a murine model when murine satellite cells are implanted along with a scaffold structure to skeletal muscle defects.[55]

In addition, various products for the treatment of musculoskeletal defects have been approved for clinical trials/use. One such product, Matrix-Induced Autologous Chondrocyte Implantation (MACI®), from Genzyme Biosurgery utilizes autologous chondrocytes, which are seeded onto collagen membranes; these are then implanted to the site

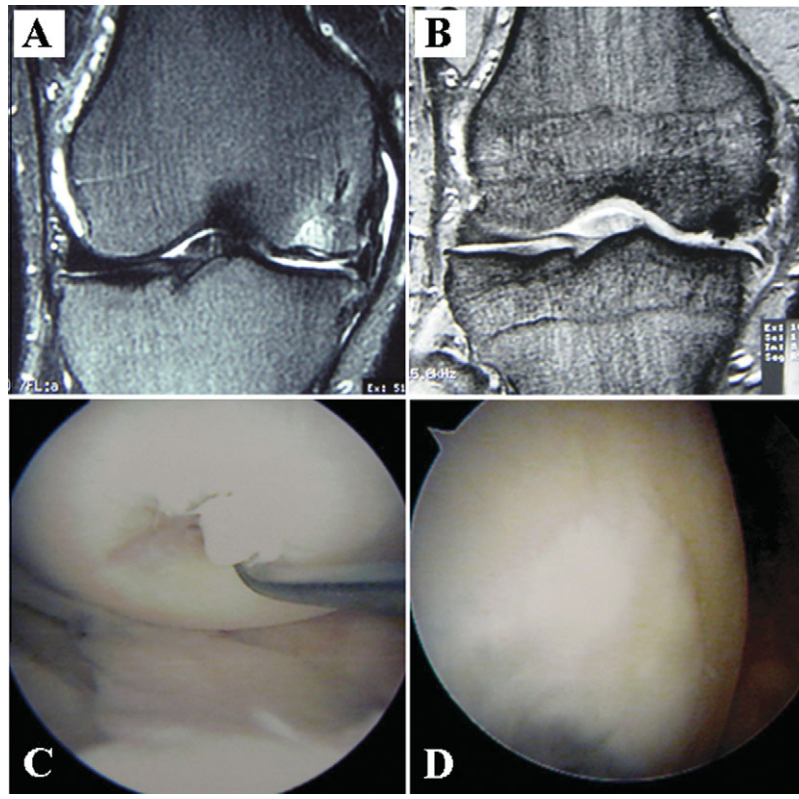

Fig. 4 Treatment of a full-thickness articular cartilage defect with autologous bone-marrow stromal cells embedded within a collagen gel. Magnetic resonance images before (A) and 1 year after surgery (B). Arthroscopic findings before (C) and 7 months after surgery (D). A 20 × 30-mm full-thickness cartilage defect is apparent within the weight-bearing area of the medial femoral condyle. The repaired defect is completely covered with tissue, which is a little softer than the surrounding articular cartilage. Figures kindly provided by Dr R. Kuroda, Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Japan. This figure was published in Osteoarthritis and Cartilage, Volume 15, 2007[52]. Copyright Elsevier 2007.

of cartilage defect. This particular product is approved for use in Europe and Australia. An example of a product currently in clinical trials is Chondrogen™ from Osiris Therapeutics, which combines allogenic bone marrow MSCs suspended in hyaluronic acid for meniscal fibrocartilage regeneration. Although these products are in use or in clinical trials there are many others at similar stages of development.

Summary

Since the inception of the field of tissue engineering much excitement has been displayed with respect to the possible clinical applications that this field could be applied to. Of course the field is in its relative

infancy, however much has been discovered with respect to scaffold manufacture and modification along with the ongoing research into stem and primary cell populations. However, further hurdles must be overcome before tissue engineering becomes a common practice. Investigations into possible immune reactions, stem cell differentiation, growth factor incorporation and scaffold design will ultimately lead us to the knowledge required to construct living tissue implants. This—albeit the holy grail of the tissue-engineering field—would mark a revolutionary point in the world of regenerative medicine.

Acknowledgements

Thanks go to Dr Clare Upton, Dr David Chau and Dr Glen Kirkham for help in preparing this manuscript.

Funding

Many thanks to the BBSRC, EPSRC, EU (Osteocord) and MRC for funding.

References

1 MacArthur BD, Oreffo RO (2005) Bridging the gap. *Nature* **433**, 19.
2 Green H, Kehinde O, Thomas J (1979) Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. *Proc Natl Acad Sci USA* **76**, 5665–5668.
3 Gallico GG 3rd, O'Connor NE, Compton CC, Kehinde O, Green H (1984) Permanent coverage of large burn wounds with autologous cultured human epithelium. *N Engl J Med* **311**, 448–451.
4 Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* **331**, 889–895.
5 Boulas HJ, Herren A, Buchler U (1993) Osteochondral metatarsophalangeal autografts for traumatic articular metacarpophalangeal defects: a preliminary report. *J Hand Surg [Am]* **18**, 1086–1092.
6 Meyers MH (1985) Resurfacing of the femoral head with fresh osteochondral allografts. Long-term results. *Clin Orthop Relat Res* **197**, 111–114.
7 Peterson L, Brittberg M, Kiviranta I, Akerlund EL, Lindahl A (2002) Autologous chondrocyte transplantation. Biomechanics and long-term durability. *Am J Sports Med* **30**, 2–12.
8 Minas T (1999) The role of cartilage repair techniques, including chondrocyte transplantation, in focal chondral knee damage. *Instr Course Lect* **48**, 629–643.
9 Sen MK, Miclau T (2007) Autologous iliac crest bone graft: should it still be the gold standard for treating nonunions? *Injury* **38** (Suppl 1), S75–S80.
10 De Long WG Jr, Einhorn TA, Koval K, McKee M, Smith W, Sanders R, Watson T (2007) Bone grafts and bone graft substitutes in orthopaedic trauma surgery. A critical analysis. *J Bone Joint Surg Am* **89**, 649–658.

11 Velchuru VR, Satish SG, Petri GJ, Sturzaker HG (2006) Hernia through an iliac crest bone graft site: report of a case and review of the literature. *Bull Hosp Jt Dis* **63**, 166–168.
12 Dosoglu M, Orakdogen M, Tevruz M, Gogusgeren MA, Mutlu F (1998) Enterocutaneous fistula: a complication of posterior iliac bone graft harvesting not previously described. *Acta Neurochir (Wien)* **140**, 1089–1092.
13 Sieunarine K, Lawrence-Brown MM (1993) Arteriovenous fistula: a rare complication after iliac bone graft. *Cardiovasc Surg* **1**, 734–735.
14 Murata Y, Takahashi K, Yamagata M, Sameda H, Moriya H (2002) Injury to the lateral femoral cutaneous nerve during harvest of iliac bone graft, with reference to the size of the graft. *J Bone Joint Surg Br* **84**, 798–801.
15 Chan K, Resnick D, Pathria M, Jacobson J (2001) Pelvic instability after bone graft harvesting from posterior iliac crest: report of nine patients. *Skeletal Radiol* **30**, 278–281.
16 Goulet JA, Senunas LE, DeSilva GL, Greenfield ML (1997) Autogenous iliac crest bone graft. Complications and functional assessment. *Clin Orthop Relat Res* **339**, 76–81.
17 Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB (2006) Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* **367**, 1241–1246.
18 Beier JP, Stern-Straeter J, Foerster VT, Kneser U, Stark GB, Bach AD (2006) Tissue engineering of injectable muscle: three-dimensional myoblast-fibrin injection in the syngeneic rat animal model. *Plast Reconstr Surg* **118**, 1113–1121; discussion 1122–1124.
19 Cao D, Liu W, Wei X, Xu F, Cui L, Cao Y (2006) In vitro tendon engineering with avian tenocytes and polyglycolic acids: a preliminary report. *Tissue Eng* **12**, 1369–1377.
20 Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem* **64**, 295–312.
21 Wakitani S, Saito T, Caplan AI (1995) Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* **18**, 1417–1426.
22 Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU (1998) In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* **238**, 265–272.
23 Woodbury D, Schwarz EJ, Prockop DJ, Black IB (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* **61**, 364–370.
24 Bruder SP, Jaiswal N, Ricalton NS, Mosca JD, Kraus KH, Kadiyala S (1998) Mesenchymal stem cells in osteobiology and applied bone regeneration. *Clin Orthop Relat Res* S247–S256.
25 Friedenstein AJ, Piatetzky S II, Petrakova KV (1966) Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* **16**, 381–390.
26 Haynesworth SE, Baber MA, Caplan AI (1992) Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone* **13**, 69–80.
27 Simmons PJ, Torok-Storb B (1991) Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* **78**, 55–62.
28 Bolland BJ, New AM, Madabhushi SP, Oreffo RO, Dunlop DG (2007) Vibration-assisted bone-graft compaction in impaction bone grafting of the femur. *J Bone Joint Surg Br* **89**, 686–692.
29 Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* **279**, 1528–1530.
30 D'Ippolito G, Schiller PC, Ricordi C, Roos BA, Howard GA (1999) Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. *J Bone Miner Res* **14**, 1115–1122.
31 Jeong JA, Hong SH, Gang EJ, Ahn C, Hwang SH, Yang IH, Han H, Kim H (2005) Differential gene expression profiling of human umbilical cord blood-derived mesenchymal stem cells by DNA microarray. *Stem Cells* **23**, 584–593.
32 Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH (2004) Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* **103**, 1669–1675.
33 Kang XQ, Zang WJ, Bao LJ, Li DL, Xu XL, Yu XH (2006) Differentiating characterization of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Cell Biol Int* **30**, 569–575.
34 Hou L, Cao H, Wang D, Wei G, Bai C, Zhang Y, Pei X (2003) Induction of umbilical cord blood mesenchymal stem cells into neuron-like cells in vitro. *Int J Hematol* **78**, 256–261.

35 Hewitt Z, Priddle H, Thomson AJ, Wojtacha D, McWhir J (2007) Ablation of undifferentiated human embryonic stem cells: exploiting innate immunity against the Gal alpha1-3 Gal beta1-4GlcNAc-R (alpha-Gal) epitope. *Stem Cells* **25**, 10–18.

36 Buttery LD, Bourne S, Xynos JD, Wood H, Hughes FJ, Hughes SP, Episkopou V, Polak JM (2001) Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. *Tissue Eng* **7**, 89–99.

37 Kramer J, Hegert C, Guan K, Wobus AM, Muller PK, Rohwedel J (2000) Embryonic stem cell-derived chondrogenic differentiation in vitro: activation by BMP-2 and BMP-4. *Mech Dev* **92**, 193–205.

38 Hench LL (2006) The story of bioglass. *J Mater Sci Mater Med* **17**, 967–978.

39 Langer R, Folkman J (1976) Polymers for the sustained release of proteins and other macromolecules. *Nature* **263**, 797–800.

40 Kanczler JM, Barry J, Ginty P, Howdle SM, Shakesheff KM, Oreffo RO (2007) Supercritical carbon dioxide generated vascular endothelial growth factor encapsulated poly (DL-lactic acid) scaffolds induce angiogenesis in vitro. *Biochem Biophys Res Commun* **352**, 135–141.

41 Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR (2006) Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* **27**, 3413–3431.

42 Antonov EN, Bagratashvili VN, Whitaker MJ, Barry JJ, Shakesheff KM, Konovalov AN, Popov VK, Howdle SM (2004) Three-dimensional bioactive and biodegradable scaffolds fabricated by surface-selective laser sintering. *Adv Mater Deerfield* **17**, 327–330.

43 Nagai Y, Unsworth LD, Koutsopoulos S, Zhang S (2006) Slow release of molecules in self-assembling peptide nanofiber scaffold. *J Control Release* **115**, 18–25.

44 Moutos FT, Freed LE, Guilak F (2007) A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. *Nat Mater* **6**, 162–167.

45 Hidalgo-Bastida LA, Barry JJ, Everitt NM, Rose FR, Buttery LD, Hall IP, Claycomb WC, Shakesheff KM (2007) Cell adhesion and mechanical properties of a flexible scaffold for cardiac tissue engineering. *Acta Biomater* **3**, 457–462.

46 Shah R, Sinanan AC, Knowles JC, Hunt NP, Lewis MP (2005) Craniofacial muscle engineering using a 3-dimensional phosphate glass fibre construct. *Biomaterials* **26**, 1497–1505.

47 Anderson DG, Levenberg S, Langer R (2004) Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nat Biotechnol* **22**, 863–866.

48 Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M (2001) Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* **344**, 385–386.

49 Marcacci M, Kon E, Moukhachev V, Lavroukov A, Kutepov S, Quarto R, Mastrogiacomo M, Cancedda R (2007) Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Eng* **13**, 947–955.

50 Morishita T, Honoki K, Ohgushi H, Kotobuki N, Matsushima A, Takakura Y (2006) Tissue engineering approach to the treatment of bone tumors: three cases of cultured bone grafts derived from patients' mesenchymal stem cells. *Artif Organs* **30**, 115–118.

51 Ossendorf C, Kaps C, Kreuz PC, Burmester GR, Sittinger M, Erggelet C (2007) Treatment of posttraumatic and focal osteoarthritic cartilage defects of the knee with autologous polymer-based three-dimensional chondrocyte grafts: 2-year clinical results. *Arthritis Res Ther* **9**, R41.

52 Podskubka A, Povysil C, Kubes R, Sprindrich J, Sedlacek R (2006) [Treatment of deep cartilage defects of the knee with autologous chondrocyte transplantation on a hyaluronic Acid ester scaffolds (Hyalograft C)]. *Acta Chir Orthop Traumatol Cech* **73**, 251–263.

53 Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, Ohgushi H, Wakitani S, Kurosaka M (2007) Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage* **15**, 226–231.

54 Liu W, Chen B, Deng D, Xu F, Cui L, Cao Y (2006) Repair of tendon defect with dermal fibroblast engineered tendon in a porcine model. *Tissue Eng* **12**, 775–788.

55 Boldrin L, Elvassore N, Malerba A, Flaibani M, Cimetta E, Piccoli M, Baroni MD, Gazzola MV, Messina C, Gamba P, Vitiello L, De Coppi P (2007) Satellite cells delivered by micro-patterned scaffolds: a new strategy for cell transplantation in muscle diseases. *Tissue Eng* **13**, 253–262.