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MINIREVIEWS

Use of bone marrow derived stem cells in trauma and orthopaedics: A review of current concepts

Philip S Pastides, Matthew J Welck, Wasim S Khan

Philip S Pastides, Wasim S Khan, University College London Institute of Orthopaedics and Musculoskeletal Sciences, Royal National Orthopaedic Hospital, Stanmore, Middlesex HA7 4LP, United Kingdom

Matthew J Welck, Wexham Park Hospital, Slough, West Berkshire SL2 4HL, United Kingdom

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Correspondence to: Wasim S Khan, MBChB, MSc, PhD, FRCS (Tr and Orth), Orthopaedic Registrar, Clinical Lecturer, University College London Institute of Orthopaedics and Musculoskeletal Sciences, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex HA7 4LP, United Kingdom. wasimkhan@doctors.org.uk
Telephone: +44-20-889542300
Fax: +44-20-7679200

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Abstract

There is a considerable amount of interest in the future role of bone marrow-derived stem cells (BMDSCs) and tissue engineering techniques to manage conditions within the musculoskeletal system. Repair of soft tissue and bone defects, in the early stages of injury, may lead to a reduction in progression of symptoms. Furthermore, troublesome soft tissue injuries that are notoriously fraught with problems either in healing or function, could be augmented with such techniques. The aim of this review paper is to look at the advances in such strategies to tackle these problems and assess how BMDSCs, with the aid of growth factors and scaffolds, are being used *in vitro*, animal and even human models to treat problems within the field of trauma and orthopaedics. There is plenty of evidence that the results are encouraging and thus gaining momentum toward their use in human studies.

Key words: Trauma; Orthopaedics; Bone marrow-derived stem cells; Scaffolds; Growth factors

Core tip: Tissue engineering techniques using bone marrow-derived stem cells is an attractive, promising and growing area of research within the field of trauma and orthopaedics. There are plenty of *in vitro* and animal studies showing the benefits of such treatments with a slow and steady growth of human *in vivo* studies emerging.

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INTRODUCTION

The advances in modern medicine over the last century

have been dramatic. Life expectancy has risen as has patient expectation and demands. This has now led to a new target, that of not solely survival until an elderly age, but of a pain free, mobile and reduced co morbidity survival.

Tissue engineering strategies, in the context of musculoskeletal medicine, focus on repair and prevention of soft tissue and osseous structures. For successful tissue regeneration, it is necessary to have cells that are capable of high proliferation but also differentiation. These must be placed in a suitably created environment to allow for such regeneration to occur. In recent years, regenerative medicine has emerged as an attractive field for new cellular and non cellular approaches to tissue repair. Bone marrow-derived stem cells (BMDSCs) can be influenced by external factors and cause them to differentiate down a desired path. Growth factors are peptide signaling molecules whose role includes the regulation of several pathways regulating metabolism at a cellular level including extra-cellular matrix production growth and production. Another obstacle to overcome is how to adequately deliver and keep the BMDSCs at the injured or repaired site. This has led to the further interest in the development of appropriate scaffolds to act as a mould to keep the cells *in situ*. As such, the ideal scaffold must be of appropriate size, shape and porosity in order to allow the cells to move from the scaffold to the injured area and potentially proliferate and grow.

Musculoskeletal injury can involve tendons and ligament, bone, meniscus and cartilage. More long term complications can include large bone defects and non unions. All such injuries are painful, troublesome, limiting to patients and costly to society. The high incidence of such injuries highlights the need for novel, more effective treatments. Currently a lot of research is being carried out into this area. The use of BMDSCs is one such option[1] and the aim of this review is to present current studies within the field.

ROTATOR CUFF

The rotator cuff muscles comprise of a group of four muscles around the shoulder girdle that contribute to both stability and movement of the joint. Tears within the rotator cuff are associated with muscle pathology, such as weakness or impingement[2]. Injuries to the rotator cuff can be managed operatively, with either open or arthroscopic surgery with satisfactory outcomes, but are associated high re-rupture rates[3]. This is partly due to the poor healing capabilities of tendon. Supraspinatus biopsies, obtained from 24 patients who underwent an arthroscopic repair of partial or full-thickness supraspinatus tendon tears, were analysed at a cellular level. Those with full-thickness tears were found to have a reduction in the density of satellite cells, atrophy of MHC1+ and MHC2+ (major histocompatibility complex) myofibers and reduced MHC1 content. Histological analysis revealed that the tendons did not heal by the regeneration of normal fibrocartilage, but by forming scar tissue with a high content of type III collagen[4,5]. As a result, tissue engineering techniques could have a huge role in the augmentation of rotator cuff tears and is undergoing constant evaluation.

Yokoya *et al*[6] surgically created defects within the infraspinatus tendons of rabbits. They used two different materials to repair the defects; a polyglycolic acid (PGA) sheet alone (PGA group) and a PGA sheet seeded with autologously cultured BMDSCs. Performing a tendon defect with no graft created a control group. At 8 wk, the layers of fibrocartilage and Sharpey fibers in the BDMSCs group were regularly identified at the supraspinatus footprint compared with the PGA group. In the control group, thin membranes with many fibroblasts arranged in an irregular pattern were identified at the tendon-bone interface, lacking any evidence of Sharpey fibers or type Ⅰ collagen. An abundance of type Ⅰ collagen relative to type Ⅲ collagen was seen at 16 wk in the BDMSCs group, whereas type Ⅲ collagen was more prevalent than type Ⅰ in the PGA group. The tendon maturing score was the highest in the BMDSCs group at both 8 and 16 wk, with a statistically significant better tensile strength than in the PGA and control groups. Funakoshi *et al*[7] showed similar tendon regeneration and mechanical properties in rabbit infraspinatus defects using fibroblast seeded scaffold.

There is early evidence that this technology can be translated into humans. Mazzocca *et al*[8] showed that BMDSCs could safely be aspirated and cultured from the proximal humerus in 23 patients during arthroscopic rotator cuff repair. They later showed in a follow up study[9] that exposure of the harvested cells to a one-time physiologic dose of insulin is capable of differentiating BMDSCs into tenocytes. Another group of researches found that the implantation of BMDSCs, harvested from the iliac crest at the time of surgery, and injected into the repaired rotator cuff, led to a 100% radiological (MRI) intergrity of the rotator cuff at 12 mo[10].

However, there is also evidence to suggest that the use of tissue engineering strategies in rotator cuff defects is not always successful. Gulotta *et al*[11] used three groups of Lewis rats to investigate whether BMDSCs that with a fibrin carrier, no carrier or a non-augment repair altered the histological or biomechanical outcomes following rotator cuff repair. At no point in time, did they notice any significant differences in the amount of new cartilage formed, the collagen fibre organization or mechanical properties between the groups.

The potential benefits, or not, of biological approaches involving BMDSCs to improve the outcome of rotator cuff therapies and reduce rates of re injury as still very unclear. In fact, a recent systematic review focusing on such techniques found only 3 papers in their

initial literature review, forcing the authors to expand their search criteria[12]. This highlights the needs for further high level and targeted studies to evaluate the efficacy in human subjects.

TENDONS AND LIGAMENTS

Tendons and ligaments are critical to the musculoskeletal system in order to attach the force generating muscles to the solid skeleton of the body[13,14]. Tendon repair is a slow process that often results in structurally weaker and less functional properties compared to undamaged tissue[15]. The hypothesis at the centre of many researchers is that it may be possible to improve the reparative potential of tendons by implementing biological techniques.

An animal study to assess this was conducted by Adams *et al*[16] using 54 rat specimens. The 108 bilateral hind limbs underwent a transection of the Achilles tendon. Randomisation to repair with suture only (SO), suture plus injection (SI) of BDMSCs at the repair site or sutures loaded with BMDSCs (suture with stem cells SCS) was performed. At 14 and 28 d post surgery, 54 specimens were humanely killed and the tendons harvested and subsequently underwent a blinded histological examination and mechanical testing. Ultimate failure strength was significantly higher in the SI and SCS groups *vs* the SO group. Histology scores were best in the SCS group.

Biologically culturing of the BMDSCs can modify the outcome of such techniques. A study by Yao *et al*[17] used BMDSCs harvested from Sprague-Dawley rat femurs. Coated sutures (CS) with intercellular cell adhesion molecule 1 and poly-L-lysine and seeded with labelled BMDSCs formed the intervention group. Control (substrate-only) coated group sutures were coated with intercellular cell adhesion molecule 1 and poly-L-lysine only. The CS suture repairs were statistically stronger than SO repairs at 7 and 10 d, without any significant difference in strength 4, 14 and 28 d. Their findings suggest that suture repair augmented with biological substrates may kick start the repair process. Improved early strength might, in turn allow earlier unprotected mobilization and thus reduce the rate of early re-rupture rates. However in a similar study using the same animal model, but using recombinant human growth differentiation factor-5 (rhGDF-5) to culture the cells instead, Dines *et al*[18] came to a different outcome. Histological assessment at 3 wk showed improved healing in tendons repaired with coated suture *vs* a control group. By 6 wk, there were no significant differences in any mechanical property tested. At 3 wk, tendons repaired with rhGDF-5-coated sutures were found to have a significantly higher ultimate tensile load and stiffness.

The true benefits of augmentation in tendon and ligament repair with BMDSCs remains unclear. What is evident it that the stem cells can be cultures under various stimuli to produce a more beneficial outcome. Further studies, including human trials need to be conducted[15].

CARTILAGE

Undoubtedly, joint arthroplasty is a triumph of modern day orthopaedics. Osteoarthritis, the loss of articular cartilage, is a chronic disease effecting an increasingly aging population. Joint replacement arthroplasty has been a tremendous success in restoring independence to an otherwise frail group of patients. Cartilage loss, or damage, in the younger, more active patient still remains a challenge. Damage of cartilage is often asymptomatic and related to sporting activities. The decision to treat such lesions is related to the extent of symptoms the patient expresses, but growingly there is a trend to prophylactically address these defects because once damaged cartilage becomes vulnerable to further degradation due to its poor ability to heal[19]. Thus even small defects may degenerate over time, ultimately causing osteoarthritis[20]. While arthroplasty remains a successful treatment option, performing such procedures in this population group will mean further revision surgery in the future[21,22]. It is this area that tissue engineering is focusing its attention[23,24].

Current treatments such as arthroscopic debridement and microfracture, autologous osteochondral transfer and autologous chondrocyte implantation, all of which have been shown to produce positive results[25]. BMDSCs are a good cell source for regeneration of cartilage as they can migrate directly to the site of cartilage injury and differentiate into articular chondrocytes[26,27]. There is a plethora of publications showing how under different stimulation, scaffolds and gene therapy, BMDSCs can lead to regeneration and/or an increase rate of regeneration of damaged articular cartilage[28]. The vast majority of these studies are either *in vitro* or make use of animal studies. Zhu *et al*[29] reported on a combined technique of articular cartilage repair, consisting of BMDSCs transfected with connective tissue growth factor (*CTGF*) gene and NaOH-treated poly(lactic-co-glycolic) acid (PLGA) scaffolds. Full-thickness cartilage defects were created unilaterally in the patellar grooves of rabbits. Defects were either left empty, implanted with BMDSCs/PLGA, BMDSCs/NaOH-treated PLGA or CTGF-modified BMDSCs/NaOH-treated PLGA. Overall, the CTGF-modified BMDSCs/NaOH-treated PLGA group showed successful hyaline-like cartilage regeneration similar to normal cartilage, which was superior to the other groups in all histological and mechanical assessments.

The effect of other growth factors on chondrocyte differentiation is also being investigated. Reyes *et al*[30] showed that the addition of bone morphogenetic protein (BMP) 2 to BMDSCs with a alginate/PLGA osteochondral scaffold was just as efficient at repairing an osteochondral defect in rabbit knees. Equally good results have been reported by Guo *et al*[31] who investigated the effects of transforming growth factor

(*TGF*)-*β(1*) gene modified BMDSCs and a biodegradable poly-L-lysine coated polylactide biomimetic scaffolds, cultured *in vitro*, and then allografted into full-thickness articular cartilage defects in 18 New Zealand rabbits. They found that hyaline cartilage began to infill within the chondral defects, whilst at 24 wk, the subchondral region contained a mix of both compact and trabecular bone.

Likewise, the choice of scaffold to further augment repair has been the subject of many investigations. For example, Deng *et al*[32] showed that the addition of a silk fibrion/chitosan scaffold in combination with BMDSCs augmented osteochondral defects in rabbit knee better than no scaffold at all. They found that the scaffold resulted in near complete repair of the defect and scaffold degradation at 12 wk.

Significantly, there is a slow and steady growth in the body of evidence of such studies involving human patients. A systematic review was conducted by the authors looking at the outcome of studies reporting on BMDSCs treatment in human subjects. Our findings were that there is early and promising data but more high level studies, with extensive and robust validated reporting methods, should be conducted to evaluate the true effect of such techniques in human cartilage defect repairs as well as the effects of scaffolds and growth factors to improve the quality and timing to repair[33].

MENISCUS

Meniscal injuries are a very frequent sport related injuries. Removal of an extensive area of meniscus can alter the knee biomechanics and thus predispose patients to osteoarthritis. Thus tissue engineering poses an attractive reparative option to attempt meniscal tissue repair and avoid the long-term sequelae[34,35].

Studies have shown that growth factor differentiation and the use of scaffolds can result in good outcomes in animal models. Steinert *et al*[36] investigated the use of a scaffold seeded with genetically modified meniscal cells or BMDSCs isolated from bovine calves were transduced with adenoviral vectors encoding green fluorescent protein, luciferase or TGF-β1 complementary deoxyribonucleic acid (cDNA). These cells were then germinated within type Ⅰ collagen-glycosaminoglycan matrices and transplanted into the avascular zone of injured bovine menisci. At 3 wk, recombinant adenovirus readily transduced meniscal cells and MSCs, and transgene expression remained high after the cells were incorporated into collagen-glycosaminoglycan matrices. Transfer of TGF-β1 cDNA resulted in an increased cellularity and cell synthesis.

Yamasaki *et al*[37] assessed the transplantation of regenerated menisci using scaffolds from normal allogeneic menisci and BMDSCs in rats. After 4 wk, the tissues were transplanted to a defect within the menisci. Repopulation of BMDSCs and expression of extracellular matrices were observed in the transplanted tissues at 4 wk after surgery. At 8 wk, articular cartilage in the cell-free group appeared to be more damaged compared to the other groups.

Hatsushika *et al*[38] showed a very promising study that may be useful for the management of acute, massive meniscal injuries which tend to affect young patients. They investigated how repetitive intraarticular injections of synovial BMDSCs effected meniscal regeneration in porcine knees that two weeks prior had undergone partial anterior menisectomies. BMDSCs were injected into the right knee at 0, 2, and 4 wk and assessed prospectively with serial MRI. Regeneration was significantly better both histologically and radiologically in the BMDSCs group compared to the control group. Macroscopically, the meniscal defect already appeared to be filled with synovial tissue at 2 wk.

Although promising, the use of BMDSCs and tissue engineering strategies for meniscal repair are still in their infancy and require further evaluation to establish the benefits or not of such methods[39].

BONE DEFECTS

Reconstruction of bony defects remains a challenge in modern day trauma and orthopaedic cases. Treatment options such as the Masquelet[40,41] technique are gaining in popularity. Henrich *et al*[42] investigated the cellular, histological, growth factor expression and biochemical make-up of the membranes induced around femoral defects during this technique. They found that the membranes formed around bone defects were similar to those formed in subcutaneous pockets; however, both were significantly different from periosteum with regard to structural characteristics, location of blood vessels and overall thickness. Membranes induced at the femoral defect at 2 wk and in periosteum contain mesenchymal stem cells (MSCs; STRO-1^{+}) which were not found in membranes induced subcutaneously. BMP-2, TGFβ and vascular endothelial growth factor were significantly elevated in membranes induced around femur defects. This raises the question of whether BMDSCs can be used to repair bone defects.

A recent systematic review and metaanalysis was conducted by Liao *et al*[43] to assess the treatment outcomes for bone repair using BMDSCs. The combined findings of the 20 included preclinical studies showed statistically significant beneficial effect of stem cell therapy by increasing new bone formation and bone mineral density. Stratified analysis showed that predictors of new bone formation included the number of cells and that the addition of a scaffold was more effective than isolated direct cell injection. The results appeared to be sustainable at 12 wk.

Furthermore there is evidence that augmenting bone allograft with BMDSCs has beneficial outcomes in revision surgery. In a case-control study, Hernigou *et al*[44] treated 60 patients with aseptic failure of a cemented acetabular implant with bone allograft with

or without BDMSCs incorporation. Both groups of 30 patients were matched for the size of the periacetabular osteolytic areas. They compared the evolution of the allografts and evaluated cup migration and revision of the hips as end points at a minimum of 12 years or until failure. Better radiographic graft union rates and less allograft resorption were observed with allografts loaded with stem cells. Allograft resorption was significantly decreased in the group with allograft loaded with BMDSCs. The rate of mechanical failure was highest (P = 0.01) among the 30 patients with allograft without stem cells (9/30; 30%) compared with no failures for patients with allograft loaded with stem cells. Revision of the cup was necessary in nine patients in the control group. No revision was performed in the 30 patients of the study group with BMDSCs. This leads to an encouraging hypothesis that the addition of BMDSCs to these bone graft may restore the osteogenic capacity of an allogenic dead bone and therefore enhance incorporation of allografts with the host bone and decrease the number of failures related to the allograft.

OSSEOUS NON-UNIONS

Osseous non-unions represent a significant and troublesome problem with a high patient morbidity rate, despite surgical advances[45]. As such, tissue engineering could be an attractive addition to the traditional approaches implemented in the treatment of fracture non-unions[46-48].

Giannotti *et al*[49] investigated the long-term outcomes of *in vitro* expanded BMDSCs, embedded in autologous fibrin clots, for the healing of atrophic pseudarthrosis of the upper limb. Tissue-engineered constructs designed to embed the BMDSCs from 8 patients in autologous fibrin clots were locally implanted with bone grafts. Radiographic healing was evaluated at a mean of 6.7 and 76.0 mo. All patients recovered limb function, with no evidence of tissue overgrowth or tumour formation. Successful results have also been reported in lower limb non-unions. Fernandez-Bances *et al*[50] successfully treated 7 patients with long bone non-unions with autologous BMDSCs from iliac crest combined with frozen allogenic cancellous bone graft. All patients showed complete bone consolidation at a mean of around 5 mo. Moreover, limb pain disappeared in all of them. At a mean follow-up of 36 mo there was no recurrence of pain or limitations of function. Bajada *et al*[51] successfully treated a nine-year old tibial non union, that had undergone six previous operative attempts to treat it, using BMDSCs and a calcium sulphate scaffold. Applying the concept of growth factor stimulation, Grgurevic *et al*[52] showed that exposure of BMDSCs to growth factor such as BMP1-3, increased the expression of collagen type I and osteocalcin in MC3T3-E(1) osteoblast like cells, and enhanced the formation of mineralised bone nodules in rat long bone non unions.

CONCLUSION

There has been a remarkable progression during the past two decades in the development of tissue engineering techniques and strategies. Large amounts of attention are being focussed on the development of suitable scaffolds to deliver the cells, as well as the positive influence of growth factors on isolated BMDSCs. A huge obstacle in the application of such techniques is the ethical issues surrounding the trials of such products in humans. There is an ever increasing move to perform studies within the human population but more work and resources are needed to assess the safety and efficacy of treatments. Although in the infancy, there is no doubt that the use of BMDSCs and tissue engineering techniques represents an attractive, feasible and exciting prospect that may hold to future key to repairing rather than replacing within the Trauma and Orthopaedic setting.

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