Tissue Engineering for the Meniscus: A Review of the Literature

Bethan Tucker, Wasim Khan, Mamun Al-Rashid and Hesham Al-Khateeb*

University College London Institute of Orthopaedics and Musculoskeletal Sciences, Royal National Orthopaedic Hospital, Stanmore, Middlesex, HA7 4LP, UK

Abstract: The menisci disperse the load at the knee joint. Removal of the menisci can lead to osteoarthritis due to the higher load placed on the underlying cartilage. If they become injured it is therefore important to replace or regenerate the meniscus to prevent the progression of osteoarthritis. Many materials have been trialled to find a scaffold that can withstand the stresses and strains across the joint without causing any adverse effects. This review looks at these materials further to clarify the current position of tissue engineering for the meniscus and to highlight the areas where further research is needed. A scaffold which can produce high quality *in vivo* results in everyone has not yet been found.

Keywords: Meniscus, scaffold, regeneration, engineering.

INTRODUCTION

The menisci in the knee primarily function to disperse the load across the knee joint. As the femoral and tibial condyles only meet at one point, without the menisci the forces transmitted would be large and unevenly distributed which could lead to premature osteoarthritis. The lateral meniscus carries up to 70% of the load going across the lateral compartment; and the medial meniscus as much as 50% of the medial load [1]. The medial meniscus also helps prevent anterior displacement of the tibia relative to the femur which is of particular relevance in patients without an anterior cruciate ligament (ACL). Other proposed functions of the menisci include lubrication and nutrition of the articular cartilage by helping to spread the synovial fluid over the articulating surfaces, proprioception (due to nerve fibres found within the menisci) and increased joint stability [2].

MENISCAL INJURY

Meniscal tears can occur due to trauma commonly in young people (below 40 years) such as twisting on a flexed, loaded knee for example during football or skiing at an incidence of 61 per 100 000 per year; or in older people due to degeneration, with an approximate incidence of 60% in patients over 65 years [3]. With age, the mensisci become stiffer making them more prone to tearing. 50% of degenerative tears occur spontaneously and many of the remaining 50% from minor trauma such as rising from squatting.

SYMPTOMS AND EFFECTS

Meniscal tears can cause pain, swelling, clicking, catching, giving way or locking of the knee [4]. In a menisectomised knee, the contact area is reduced by about 50% which greatly increases the load-per-unit area on the articular cartilage leading to damage and degeneration. Even partial menisectomy can greatly affect the knee biomechanics and increase the contact pressure on the soft tissues. Meniscal tears are associated with cartilage defect, loss of cartilage volume, alteration in bone size and prevalence of radiographic osteoarthritis in a non-osteoarthritis cohort [5]. It is therefore vital to encourage meniscal healing to decrease the occurrence of osteoarthritis in later life and to relieve the patient of the immediate symptoms.

HEALING

The menisci are vascularised in the peripheral 10-30%; the remainder receives nutrition *via* passive diffusion and by mechanical pumping [3]. It is accepted that meniscal lesions in the outer vascularised portion heal and lesions in the central non-vascularised portion do not, particularly the large, complex tears especially if there is knee instability [6]. Various approaches to repair and replace the meniscus have been trialled with limited success. Arthroscopic partial menisectomy has become very common as it relieves the immediate short-term symptoms, however, the risk of osteoarthritis is still applicable as meniscal tissue is being removed, reducing its load bearing function. Tissue engineering is becoming more popular as a method to instigate meniscal repair and hence reducing both the short-term and long-term symptoms by using a combination of scaffolds, cells and growth factors. Growing interest in using biocompatible and biodegradable biomaterials to regenerate the damaged meniscal tissue has lead to many materials being trialled [7].

ALLOGRAFT MENISCAL TRANSPLANTATION

Allograft meniscus transplantation, in which a menisci from a cadaver is inserted to replace a meniscus previously removed during a total menisectomy, has become a very common operation. Wirth *et al.* [8] studied 23 medial allograft meniscus transplantations combined with ACL reconstructions in 20 men and 3 women whose meniscal rims were still intact. 17 patients of which received a lyophilized, γ-sterilized homologous meniscal allograft and 6

*Address correspondence to this author at the University College London Institute of Orthopaedics and Musculoskeletal Sciences, Royal National Orthopaedic Hospital, Stanmore, Middlesex, HA7 4LP, UK;
Tel: 00447906261414; Fax: 00442073945333;
E-mail: drheshamalkhateeb@msn.com

1874-3250/12

patients received a deep-frozen homologous meniscal allograft. 2 control groups existed: one of patients with intact menisci who have undergone ACL reconstruction; and a second group undergoing both parital menisectomy and ACL reconstruction but no transplantation. In the treatment groups the pre-operative Lysholm function score was 59, at 3 years post-operative 84, and at 14 years was 75. At each of these time points the patients with deep-frozen meniscal transplants scored better to those with lyophilized meniscal transplants. The deep-frozen group and the intact group scored similar results which were significantly different to the lyophilized and menisectomised groups which scored similarly. The Tegner scores followed a similar time pattern: 1.0 pre-operative, 5.1 at 3 years and 4.6 at 14 years. Any deterioration seen radiographically was in the lyophilized group. In all 5 second look arthroscopies in the deep frozen group, at an average 3.8 years post-op, was complete healing of the meniscus. 2 of the 14 second look arthroscopies in the lyophilized group were detached and in all but one there was a reduction in size between one third and three thirds. The cartilage damage in this group was worse than at the time of surgery. These results show the deep frozen meniscal transplant performed better than the lyophilized transplant in terms of cartilage production, pain and function scores. The implant produced good results in the initial 3 years which decreased over time but still remain high. Allograft meniscal transplantation has, therefore, been shown to successfully last for up to 15 years, improving physical ability and pain relief.

Allogenic meniscus transplantation is contraindicated in people with chondral defects as this puts excessive load onto the defect, and autologous chondrocyte implantation has not been given to people with meniscus defects. A study by Bhosale *et al.* [9] has shown that allogenic meniscus transplantation can be combined with autologous chondrocyte implantation. 7 men and 1 woman with both a previous total menisectomy and chondral defects underwent both procedures. The pre-operative lysholm score was 49 which increased to 66 at 1 year post-operative with 6 out of 8 patients showing improved pain and function after 1 year with continued improvement.

Although some studies have shown allograft meniscus transplantation does not cause immunoreactions [10, 11] other studies have suggested slow immunoreactions do occur against the foreign material leading to failure of the transplant. Other problems include the transmission of infectious diseases, slow graft remodelling, availability, sizing and poor incorporation into the host tissues highlighting the need for an alternative.

ACELLULARIZATION

Acellularization of the meniscus could reduce the antigenicity while preserving the extracellular matrix (ECM) and the original strength. It is debated if the scaffold should then be seeded with autologous cells or left acellular. The hypothesis for seeding with autologous cells is that when this is implanted these cells will remain and maintain the matrix until recipient cells infiltrate and can go on to regenerate the matrix. An acellular scaffold on the other hand is hypothesised to encourage the movement of recipient cells into the scaffold which can then help to regenerate the matrix.

Rabbit, rat, pig, sheep and dog menisci can be decelluarized and seeded with bone marrow-derived mesenchymal stem cells or fibrochondrocytes, which will then imitate the mechanical and histological properties of a normal meniscus [12-14]. Sandmann *et al.* has shown complete accellularization of human meniscus is possible using sodium dodecyl sulphate. No difference was observed between normal and acellular menisci for stiffness, compression force, residual force or labelling patterns for collagen types I, II and VI. With repetition, the viscoelastic properties show increased stiffness, compression force and residual force but this increase is smaller in the treated group than the control group, illustrating the decellularization has no adverse effects on the mechanical properties of the menisci. One of the major reasons for the failure of previously implanted scaffolds is biomechanical failure. Acellular human cadaveric meniscal scaffolds have similar biomechanical properties and are thus a promising option; further *in vivo* studies should now be investigated.

Porcine acellularized menisci have been used *in vivo* in mice to assess biocompatibility and cell infiltration and attachment. 6 groups existed: fresh menisci transplants, decellularized, and α-galactosidase-treated (a negative control) into vaccinated and non-vaccinated mice against porcine red blood cells. There was a native rigid-like constituency, no obvious signs of tissue shrinkage, and ECM in a good condition showing no signs of damage in any of the groups. Capsules surrounding the implants were more pronounced in the fresh group compared to the acellular group. There was no specific immune response to any of the implants. The acellular porcine menisci were capable of supporting the attachment and infiltration of primary human fibroblasts and primary porcine meniscal cells illustrating acellular porcine meniscal tissue has potential for cellular regeneration and shows good immunocompatability [15]. Therefore the use of acellularization of cadaveric menisci is still very much in the experimental stages but appears a promising option. However, the disadvantage of availability and sizing still exists.

COLLAGEN MENISCUS IMPLANT

The collagen meniscus implant (CMI) consists of collagen type I fibres from bovine achilles tendon and glycosaminoglycans sterilised *via* γ-irradiation. This scaffold would eliminate the drawbacks of availability and sizing seen with transplantation. It can be inserted arthroscopically decreasing the recovery time and infection risk and it eliminates the need to harvest endogenic tissue. However, the CMI also has disadvantages: a risk of infection from the bovine tissue and a high cost of the implant. The CMI has received positive *in vitro* results showing the collagen scaffold promotes the migration of fibrochondrocytes into the scaffold and fibronectin can enhance this migration [16].

When CMI went on to be used *in vivo*, it received variable results. Rodkey *et al.* [17] carried out a 16-centre, randomised trial comparing the 5 year effect of CMI versus partial medial menisectomy (PMM) as a control. The patients were split into acute cases in which 75 received CMI and 82 received PMM and chronic cases of which 85

received CMI and 69 PMM. The different treatments received different rehabilitation regimes however after 5 years post-op this is not thought to have an effect. The CMI patients underwent second look arthroscopy at 1 year post-operative which found the new tissue generated by the CMI looked grossly meniscus-like, well integrated, was stable with no shrinkage and significantly increased the total surface area. In most of the CMI cases, maturing fibrous connective tissue was within the indentations of the CMI and differentiating towards meniscus-like fibrochondrocytic tissue. The final mean pain score after 5 years, Lysholm function score and patient self-assessment scores showed no significant difference between groups. The Tegner function scores were significantly increased in the CMI group compared to the control group; however the patients were not blinded to their allocation which may introduce bias here. The similar pain scores may be due to a compensatory decrease in function in the PMM group. CMI was successful in 97% of the chronic group and 70% of the acute group. A serious or clinically relevant complication was found in 7.5% of patients receiving CMI and 7.3% in the control group. Patients were significantly more likely to need a reoperation in the control group than the CMI group. This 5 year study can examine the effects of treatment on the immediate short term symptoms however longer follow-up may be needed to look at the probability of arthrosis.

Zaffagnini *et al.* [18] also studied the effect of CMI compared to partial medial menisectomy (PMM) over a 10-year period. 36 male patients with both acute and chronic meniscal injuries were enrolled. The patients were not randomised but chose which group they wanted to be in, this did not lead to any significant differences in demographics between groups at baseline however they therefore were not blinded which could have biased the subjective scores. 18 patients underwent medial CMI implantation and 18 patients PMM. Compared to the pre-operative scores, the CMI group showed significantly improved clinical, general health and activity levels and the medial joint space narrowing, compared to the contralateral knee, was not significantly altered which was not the case in the PMM group with a significant difference between groups. At 10 years, the CMI group compared to the PMM group showed significantly improved VAS pain score, International Knee Documentation Committee (IKDC) and Tegner index. However there were no significant differences between groups for the Lysholm scores. The results of this trial showed a long-term survival rate of the CMI of about 85% which is higher than the 75% reported by Verdonk *et al.* for an open meniscal allograft transplantation.

A study by Linke *et al.* [19] compared the results of a high tibial osteotomy alone in patients with subtotal loss of the medial meniscus with osteotomy combined with CMI replacement. With 30 patients in each group, but only 23 evaluated 8-18 months post-operatively in the CMI group with 8 completely healed, 7 good and partially healed, 1 was resected due to luxation and 7 had poor results with limited CMI remaining. Subjective pain data at 24 months showed no significant differences for 23 of the CMI patients and 16 of the osteotomy only patients.

CMI therefore produces variable results when introduced into patients. It has many of the properties which are needed in a scaffold such as being resorbed at the same rate as new tissue is being deposited. It has high mechanical strength and allows the ingrowth of host tissue so the wear rate and functions of the menisci will be regained. CMI is a good option for people who have lost only a partial amount of meniscus as an intact meniscal rim is vital. Allograft meniscal transplantation however can be used in people with total meniscal loss. Both allograft meniscal transplantation and CMI have positive results in a lot of studies however some studies with negative results have also been carried out. A scaffold which can produce high quality results every time in every patient is therefore needed.

TISSUE ENGINEERED SCAFFOLDS

Polymeric scaffolds can eliminate the problem of availability, sizing and the transmission of infectious diseases. It would need to have good mechanical stability, non-toxic degradation products, degrade at a similar rate to the deposition of the new tissue and have good blood compatability.

FIBROUS SILK PROTEIN

Fibrous silk protein has been trialled *in vitro* by Mandal *et al.* [20] as the biopolymer, due to its good mechanical properties, biocompatibility and versatility into many forms. Its controlled degradation gives time for new tissue integration, maintaining transport and mechanical load during the regeneration process. The authors predicted the failure of previous implants was due to the unnatural alignment of the fibres within the scaffold and have therefore produced a scaffold to promote alignment of the new collagen fibres in hope to provide high intrinsic tensile and compressive properties. The aqueous-derived silk scaffolds were made of 3 individual layers with different pore sizes and orientations. Adult human articular chondrocytes were seeded into the centre of the scaffold and primary human dermal fibroblasts at periphery to mimic spatial distributions in native meniscal tissue. Accumulation of glycosaminoglycans (GAGs) and collagen type I and II appeared within the scaffold pores in all 3 layers which mimicked the morphology and arrangement seen in native menisci. Cell attachment and distribution was observed in each scaffold layer suggesting growth and proliferation. Collagen type I was abundantly deposited by both cell types by day 28 and type II was produced by the chondrocytes alone. The authors do not know of any studies using a silk scaffold *in vivo*, but the positive results of this study suggest it would be beneficial to continue on to *in vivo* studies.

POLY-L-LACTIC ACID

Another recently trialled scaffold is a bioadsorbable poly-l-lactic acid (PLLA) cylinder [21]. 2 tears in the avascular portion of one medial meniscus in 25 dogs were made. The anterior and posterior tear in 21 dogs was randomised to one receiving the implant and the other trephination. The remaining 4 dogs received another implant. At 12 weeks there was complete or partial healing within the PLLA scaffold knee however there was no evidence of healing in any of the trephinated knees. At 24 weeks the PLLA knees had 56% strength of a normal meniscus (results were compared to the contralateral untreated leg) and the trephine treated knees were 5%. A quantitative assessment of the

repair tissue was not carried out and the degradation products of PLLA were not assessed.

HYALURONAN AND GELATINE

Sponge scaffolds made from 70% completely derivitalized hyaluronan ester and 30% gelatine have also been studied *in vivo* [7]. The previous *in vitro* studies using this scaffold have shown stem cells can become chondrogenic when cultured in chondrogenic medium and will produce abundant ECM throughout the scaffold. Bone marrow was taken from rabbits 5 weeks prior to the meniscal surgery and the BM-MSCs were isolated and expanded. These cells were then seeded onto the scaffold and incubated in chondrogenic medium. In 6 rabbits the empty scaffold was inserted and the contralateral defect was left empty. In 12 rabbits the cell-seeded scaffolds were inserted into the pars intermedia of the same rabbit the bone marrow was taken from. These rabbits received an empty scaffold in the contralateral defect. The final 6 rabbits received no surgical treatment. The rabbits receiving no treatment showed limited healing consisting of a thin, fibrous-like band next to the surrounding tissue. No type II collagen was present with high fibroblastic cellularity. The mean cross-sectional width was 1204μm. The knees receiving the empty scaffold showed more complete healing with good integration of the repair tissue. However there were surface irregularities between the repair and preserved tissue. Again, no type II collagen was present and the mean cross-sectional width was 1844μm. The cell seeded scaffold showed near-complete filling of the defects and integration was seen in all rabbits compared to only 6 of 11 knees receiving the scaffold alone. Meniscus-like fibrocartilage with hyaline cartilage-like areas was seen in 8 of 11 knees. The mean cross-sectional width here was 2194μm which is about 85% of the width of a normal meniscus (2562μm) compared to 68% seen in the scaffold alone group. No giant cell, foreign body reaction or other adverse effects due to the scaffold were detected.

CONCLUSION

There are many new scaffold types being assessed in order to find a scaffold which can perform highly in every patient however most of these are still at the early experimental stages. Allograft meniscus transplantation and the collagen meniscal implant remain good options for treatment in the meantime but both patients and doctors must be aware of the potential drawbacks of these.

ACKNOWLEDGEMENT

Declared none.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

REFERENCES

[1] Cameron J, Saha S. Meniscal allograft transplantation for unicompartmental arthritis of the knee. Clin Orthop Relat Res 1997; (337): 164-71.

[2] Lee J, Fu F. The meniscus: Basic science and clinical applications. Oper Tech Orthop 2000; 10(3): 162-8.

[3] Baker B, Young C. Meniscus Injuries. Medscape Reference 2011. Ref Type: Online Source. Available from: emedicine.medscape.com/article/90661-overview

[4] Hamberg P, Gillquist J. Knee function after arthroscopic meniscectomy: A prospective study. Acta Orthop 1984; 55(2): 172-5.

[5] Ding C, Martel-Pelletier J, Pelletier J, *et al*. Meniscal tear as an osteoarthritis risk factor in a largely non-osteoarthritic cohort: a cross-sectional study. J Rheumatol 2007; 34(4): 776-84.

[6] Buma P, Ramrattan NN, van Tienen TG, Veth RPH. Tissue engineering of the meniscus. Biomaterials 2004; 25(9): 1523-32.

[7] Angele P, Johnstone B, Kujat R, *et al*. Stem cell based tissue engineering for meniscus repair. J Biomed Mater Res 2008; 85A(2): 445-55.

[8] Wirth C, Peters G, Milachowski K, Weismeier K, Kohn D. Long-Term Results of Meniscal Allograft Transplantation. Am J Sports Med 2002; 30(2): 174-81.

[9] Bhosale A, Myint P, Roberts S, *et al*. Combined autologous chondrocyte implantation and allogenic meniscus transplantation: A biological knee replacement. Knee 2007; 14(5): 361-8.

[10] Kuhn J, Wojtys E. Allograft meniscus transplantation. Clin Sports Med 1996; 15(3): 536-7.

[11] Xing X, Jiakuo Y, Jiying Z. Type Ⅰ, II, III & X collagen expression in meniscus and articular cartilage and immunoreaction study after xenogenic and allogenic meniscus transplantation in rabbits. Chinese J Sports Med 2005; 1: 4-8.

[12] Maier D, Braeun K, Steinhauser E, *et al*. *In vitro* analysis of an allogenic scaffold for tissue-engineered meniscus replacement. J Orthop Res 2007; 25(12): 1598-608.

[13] Peretti G, Gill T, Xu J, Randolph M, Morse K, Zaleske D. Cell-Based Therapy for Meniscal Repair. Am J Sports Med 2004; 32(1): 146-58.

[14] Yamasaki T, Deie M, Shinomiya R, *et al*. Meniscal regeneration using tissue engineering with a scaffold derived from a rat meniscus and mesenchymal stromal cells derived from rat bone marrow. J Biomed Mater Res 2005; 75A(1): 23-30.

[15] Stapleton T, Ingram J, Fisher J, Ingham E. Investigation of the regenerative capacity of an acellular porcine medial meniscus for tissue engineering applications. Tissue Eng Part A 2011; 17(1): 231-42.

[16] Stone K, Rodkey W, Webber R, McKinney L, Steadman J. Meniscal regeneration with copolymeric collagen scaffolds. Am J Sports Med 1992; 20(2): 104-11.

[17] Rodkey W, DeHaven K, Montgomery W, *et al*. Comparison of the Collagen Meniscus Implant with Partial Menisectomy. A Prospective Randomised Trial. J Bone Joint Surg 2008; 90: 1413-26.

[18] Zaffagnini S, Marcheggiani M, Lopomo N, *et al*. Prospective Long-Term Outcomes of the Medial Collagen Meniscus Implant Versus Partial Medial Meniscectomy. Am J Sports Med 2011; 39(5): 977-85.

[19] Linke R, Ulmer M, Imhoff A. Replacement of the Meniscus with a Collagen Implant (CMI). Oper Orthop Traumatol 2006; 18(5-6): 453-62.

[20] Mandal B, Park S, Gil E, Kaplan D. Multilayered silk scaffolds for meniscus tissue engineering. Biomaterials 2011; 32(2): 639-51.

[21] Cook J, Fox D. A Novel bioabsorbable conduit augments healing of avascular meniscal tears in a dog model. Am J Sports Med 2007; 35(11): 1877-87.

Received: December 27, 2011 Revised: February 12, 2012 Accepted: March 12, 2012