Ortopedia *Traumatologia* **Rehabilitacja**
© MEDSPORTPRESS, 2011; 4(6); Vol. 13, 319-326

Advances in Meniscal Tissue Engineering

Nnaemeka Eli[1(A,B,D,E,F)], Emeka Oragui[2(A,B,D,E,F)], Wasim Khan[3(A,B,D,E,F)]

[1] Clinical Fellow, Department of Trauma & Orthopaedics, Queen's Hospital, Barking, Havering & Redbridge NHS Trust, London, United Kingdom
[2] Specialty Registrar, Department of Trauma & Orthopaedics, Queen's Hospital, Barking, Havering & Redbridge NHS Trust, London, United Kingdom
[3] University College London Institute of Orthopaedic and Musculoskeletal Sciences, Royal National Orthopaedic Hospital, Stanmore, Middlesex, United Kingdom

SUMMARY

Injuries and lesions to the meniscal cartilage of the knee joint are common. As a result of its limited regenerative capacity, early degenerative changes to the articular surface frequently occur, resulting in pain and poor function. Currently available surgical interventions include repair of tears, and partial and total meniscectomy but the results are inconsistent and often poor. Interest in the field of meniscal tissue engineering with the possibilities of better treatment outcomes has grown in recent times. Current research has focused on the use of mesenchymal stem cells, fibrochondrocytes, meniscal derived cells and fibroblast-like synoviocytes in tissue engineering. Mesenchymal stem cells are multipotent cells that have been identified in a number of tissues including bone marrow and synovium. Current research is aimed at defining the correct combination of cytokines and growth factors necessary to induce specific tissue formation and includes transforming growth factor-β (TGF-β), Platelet Derived Growth Factor (PDGF) and Fibroblast Growth Factor 2 (FGF2). Scaffolds provide mechanical stability and integrity, and supply a template for three-dimensional organization of the developing tissue. A number of experimental and animal models have been used to investigate the ideal scaffolds for meniscal tissue engineering. The ideal scaffold for meniscal tissue engineering has not been identified but biodegradable scaffolds have shown the most promising results. In addition to poly-glycolic acid (PGA) and poly-lactic acid (PLLA) scaffolds, new synthetic hydrogels and collagen sponges are also being explored. There are two synthetic meniscal implants currently in clinical use and there are a number of clinical trials in the literature with good short- and medium-term results. Both products are indicated for segmental tissue loss and not for complete meniscal replacement. The long-term results of these implants are unknown and we wait to see whether they will be proved to have benefits in delaying arthritic change and chondral damage.

Key words: meniscus, tissue engineering, mesenchymal stem cells, scaffolds, animal models, implants

BACKGROUND

Injury to the knee joint meniscal cartilage is common and the results of surgical treatment are inconsistent. Because of their poor vascular supply and limited healing potential, injuries of the inner white zone are largely treated by resection. This intervention initially gives good clinical results but finally leads to osteoarthritis of the knee as a result of altered biomechanical force distributions [1]. Attempts at meniscal tissue engineering pose particular challenges considering the variety of cell types, growth factors and appropriate tissue scaffolds and the optimal bioreactor conditions needed to produce a suitable tissue construct that can successfully replace a torn meniscus [2]. In this review, we detail the important anatomical, biochemical and histological features of the meniscus that should be considered when engineering tissues to replace the damaged meniscus. We discuss the cells that have been used in meniscal tissue engineering and evaluate the growth factors and scaffolds that have shown the most promising results. We look at animal models that have added to the body of knowledge of meniscal engineering and describe the only two implants that are currently authorized for clinical use.

OVERVIEW OF THE MENISCUS

The human knee joint menisci are a pair of wedge shaped semi-lunar cartilages that are positioned between the femoral condyles and tibial plateau and attached by ligaments to structures around the knee joint. The menisci function mainly to make the flat surface of the tibial plateau and the curved surface of the femoral condyle congruent. They also stabilize the knee and transmit biomechanical forces across the knee joint [3].

Human meniscus is composed of water, extracellular matrix and cells. The extracellular matrix consists of collagen and proteoglycans. The predominant cells in the meniscus have been termed fibrochondrocytes because they exhibit characteristics of both chondrocytes and fibroblasts [4]. These cells function to produce collagen, proteoglycans and all that is necessary to maintain the microstructure and biochemistry of the meniscal tissue [5]. Aggrecan is a major proteoglycan and minor glycosaminoglycans include decorin, biglycan and fibromodulin. The organization and architecture of collagen fibers and proteoglycans is complex, providing the meniscus with its tissue-specific biomechanical characteristics. This structure is different from articular cartilage. A longitudinal section through the meniscus shows collagen to be arranged in three zones; superficial, lamellar and deep. Orientations of collagen fibers are of three types; circumferential, radial and random. Circumferential fibers are predominant and occupy the deep zone. Radial and random fibers are found in the lamellar zone while random fibers are scattered all over the superficial zone.

IN VITRO MODELS OF MENISCAL TISSUE ENGINEERING

Mesenchymal Stem Cells

Mesenchymal stem cells are multipotent cells that eventually give rise to cartilage, tendon and bone tissue amongst others and have been identified in the bone marrow, periosteum, muscle, liver, and blood. Depending on the prevailing biochemical and biophysical environment, mesenchymal stem cells can differentiate into mature cells of different lineage such as osteoblasts, chondrocytes and fibroblasts. Being intermediate between embryonic and adult tissue, mesenchymal stem cells may provide an in situ source of healing cells throughout an adult's lifetime. In culture, they have remarkable viability and proliferative capacity and these characteristics make them a favourable cell source for meniscal tissue engineering [6].

The majority of modern culture techniques are based on the colony forming unit fibroblastic (CFU-f) approach of Friedenstein [7,8], and raw unpurified bone marrow or ficoll-purified bone marrow mononuclears are plated directly into cell culture plates or flasks. Mesenchymal stem cells are adherent to tissue culture plastic within 24 to 48 hours. Adherent cells are detached by trypsinization and can be maintained by subculturing, Dulbecco's modified Eagle's medium (DMEM) is frequently used for the culturing of mesenchymal stem cells.

Mesenchymal stem cells can be enriched by immunodepletion of haematopoietic contaminants [9]. Mesenchymal stem cells can be made to expand for at least 40 population doublings [10]. Cell seeding density may also inversely affect the population doublings and low-density human mesenchymal stem cells have higher population doublings [11]. To promote chondrogenic differentiation, mesenchymal stem cells are centrifuged to a pelleted micromass or cell aggregate, and cultured in the presence of transforming growth factor-β (TGF-β). Mackay et al. cultured human mesenchymal stem cells in micromass pellets in the presence of a medium that included 100nM dexamethasone and 10ng/ml TGF-β [12]. Within 14 days they were able to show that cells secreted an extracellular matrix incorporating type II collagen, aggrecan and anionic proteoglycans. They were able

to further differentiate human mesenchymal stem cells to the hypertrophic state by the addition of 50nM thyroxine, the withdrawal of TGF-β and the reduction of dexamethasone concentration to 1nM.

Pittenger et al. have isolated human mesenchymal stem cells from bone marrow aspirates and shown that in the absence of serum these cells displayed a stable phenotype and remained as a monolayer in vitro but TGF-β3 in serum free medium can induce differentiation into the chondrocytic lineage [13]. In a later study Im et al. have shown that an effective proliferation and chondrogenesis is possible using mesenchymal stem cells from older individuals [14]. Tan et al. have investigated the feasibility of co-culturing meniscus cells and synovium-derived stem cells on small intestine submucosa and found that the co-culture with synovium-derived stem cells yielded tissue constructs with greater survivability and differentiation and a concomitant increase in equilibrium modulus or stiffness [15].

Current research is aimed at defining the correct combination of cytokines and growth factors necessary to induce specific tissue formation. The pharmacology of mesenchymal stem cells also needs further study as it may be possible to employ the molecules secreted by mesenchymal stem cells as therapeutic agents in animal models or use molecules to modify the natural behaviors of mesenchymal stem cells in vivo.

Fibrochondrocytes

Histological evidence suggests that the fibrochondrocytes are capable of generating fibrocartilaginous tissue resembling meniscus, and these cells have been isolated using a variety of methods for tissue culture applications. Webber et al. treated minced tissue with 0.05% hyaluronidase, 0.2% trypsin, and 0.2% clostridial collagenase, in conjunction with mechanical stirring, to release the cells, followed by filtering and washing to remove the debris [16]. Tanaka et al. used 0.8% pronase followed by digestion with 0.4% collagenase to isolate fibrochondrocytes from the meniscus [17]. Nakata et al. concluded that digestion with simple collagenase provided the most consistent results in terms of cell number and phenotype [18].

Webber et al. examined the effect of age and gender on fibrochondrocyte quantity, proliferation, and proteoglycan synthesis [16,19]. They showed that female menisci contain more fibrochondrocytes than male menisci, and that in-growth of fibrochondrocytes into a fibrin clot is significantly quicker in skeletally immature individuals. Tanaka et al. showed that fibrochondrocyte cells harvested from the inner part of the menisci exhibited greater chondrocytic phenotype compared to cells from the outer meniscus grown in the same culture [19]. The authors also demonstrated that certain peptides enhance the attachment of fibrochondrocytes to a chondroitin sulphate coated surface. The effect of numerous growth factors on fibrochondrocyte proliferation and differentiation in tissue and cell cultures has been examined. Collier et al. and Tanaka et al. have shown that TGF increases proteoglycan synthesis in fibrochondrocytes in a dose-dependent manner from all different regions of the meniscus [19,20].

Human Meniscal Derived Cells

The ability of human meniscal derived cells to produce tissue favorable for meniscal replacement has been tested in vitro with promising results. Gruber et al. used a collagen sponge microenvironment, without added growth factors to culture human meniscal derived cells [21]. They also tested the responsiveness of cells cultured in this manner to TGF-β and found that cells produced a favorable extracellular matrix. Baker et al. recently isolated human meniscal derived cells from the surgical waste of human donors of varying ages with differing disease status [22]. Meniscal derived cells were seeded onto scaffolds and cultured in a chemically-defined, pro-fibrocartilaginous medium over ten weeks. Tensile properties, biochemical contents, and histological features were shown to improve with time elapsed. From the results of these studies and others, early results suggest that native human meniscal derived cells from surgical debris are a potent cell source for the fabrication of mechanically functional engineered meniscal constructs.

Fibroblast-Like Synoviocytes

The influence of growth factors on fibroblast-like synoviocytes was assessed in vitro to determine the potential of developing a novel cell-based repair strategy. Results showed that despite suboptimal extracellular matrix production, fibroblast-like synoviocytes can exhibit fibrochondral characteristics and may have potential for cell-based tissue engineering for avascular meniscal regeneration [23]. Another study similarly demonstrated that fibroblast-like synoviocytes cultured in a monolayer and treated with chondrogenic growth factors had a higher fibrocartilage extracellular matrix gene expression, matrix production, and expression of fetal chondrogenic genes. The authors concluded that growth factor induced transcription of embryonic chondrogenic genes may be involved in the process of in vitro chondrogenesis, and these genes may be targets for future fibrocartilage engineering [24].

Growth Factors

Growth factors uniquely affect the behavior of cells in vitro and in vivo, and studies suggest a benefit in the modulation of cells used in meniscal tissue engineering. TGF-β has been shown to induce in vitro differentiation of bone marrow mesenchymal stem cells into cells with chondrocytic phenotype and character whereas Kuznetsov et al. have also used Platelet Derived Growth Factor (PDGF) to increase the rates of proliferation of meniscal derived cells in vitro [25,26].

The authors have investigated the effects of Fibroblast Growth Factor 2 (FGF2) on human meniscal cells in a chondrogenic medium and shown that FGF2 increased the re-expression of type II collagen and chondrocytic differentiation [27]. This effect was further enhanced by hypoxia. More work will have to be done to precisely define the combination of growth factors needed for optimal differentiation and proliferation of mesenchymal and chondrocytic stem cells into mature meniscal tissue.

TISSUE SCAFFOLD

Scaffolds provide mechanical stability and integrity to the construct and supply a template for three-dimensional organization of the developing tissue. Scaffolds may be biodegradable or non-biodegradable and may be implanted with seeded cells or cultivated tissue into the defective site or used to engineer tissue to maturity which is then harvested from the scaffold and inserted into the defective site. They are selected with mechanical properties that resemble the native tissue, thus polymers that are more compliant are often chosen as scaffolds for cartilage, tendon and ligament [28].

The ideal scaffold for meniscal tissue engineering has not been identified but biodegradable scaffolds have shown the most promising results. Veth et al. used carbon fiber-polyurethane graft on inflicted meniscal injuries in dogs and demonstrated evidence of healing and few structural changes similar to hyaline cartilage after a period of eight weeks [29].

Poly-glycolic acid (PGA) scaffolds are frequently used for cartilage tissue engineering, as cartilage implants grown on PGA have a morphology, cellularity and matrix composition comparable to normal cartilage and the rate of proliferation of chondrocytes seeded on these scaffolds has been shown to be twice as high compared to that of cells seeded on porous poly-lactic acid (PLLA) scaffolds [30]. Solid Free-Form Fabrication technologies have been employed to fabricate anatomical 3D scaffolds from computer tomography or magnetic resonance imaging patients' dataset. A study using pigs showed a beneficial outcome following grafts of these engineered constructs on injured meniscus when compared to partial or total meniscectomy [31]. New synthetic hydrogels and collagen sponges have many desirable properties as a biological scaffold, including porosity, biodegradability and incompatibility, and are likely to have an important role in the future of meniscal engineering. Mandal et al. have recently reported on the use of a multilayered silk scaffold seeded with fibroblasts and chondrocytes in vitro [32]. Histology and immunohistochemistry results have shown the maintenance of chondrocytic phenotype with higher levels of sulfated glycosaminoglycans and collagen types I and II.

ANIMAL MODELS OF MENISCAL TISSUE ENGINEERING

Autologous Chondrocyte Cells

A study involving the use of autologous chondrocyte cells to engineer meniscal tissue healing was performed in pigs [33]. Identical tears were produced in the avascular portion of the left medial meniscus of 16 pigs and treated with autologous chondrocyte cells. The experimental group, consisting of autologous chondrocyte cells seeded onto devitalized allogenic meniscal slices and secured with two sutures, was compared to three control groups, consisting of unseeded scaffold, sutured meniscus and untreated meniscus. Meniscal samples were collected after nine weeks and analyzed grossly, histologically and histomorphometrically. Results showed bonding of the lesion margins in the specimens of the experimental group, whereas no repair was noted in any of the control group specimens. Histological analyses showed multiple areas of healing in the specimens of the experimental group

Mesenchymal Stem Cells

Izuta et al. applied bone marrow derived mesenchymal stem cells to injured meniscus in rats and were able to show evidence of some healing at eight weeks [34]. In another study on pigs, the authors were able to show substantial healing of torn avascular menisal tissue impregnated with mesenchymal stem cells compared to a control model where no healing was observed [35]. However, they noted that the mechanical properties of the healed meniscus were inferior to the normal native tissue.

Angele et al. similarly showed a more effective healing response in medial meniscal defects in rabbits treated with a hyaluronan/gelatin composite scaf-

fold loaded with autologous mesenchymal stem cells compared to the muted response of untreated defects and those treated with cell-free implants [36].

More recently Zellner et al. have examined the role of mesenchymal stem cells in meniscal tissue repair in rabbits [37]. Artificial punch defects in the avascular zone of rabbit menisci were left empty or filled with hyaluronan-collagen composite matrices, platelet-rich plasma, autologous bone marrow or autologous mesenchymal stem cells. Another group consisted of matrices with stem cells were precultured in chondrogenic medium for two weeks before implantation. Post-surgery rabbits were allowed free cage movement for up to 12 weeks. Poor healing responses were observed in untreated defects, defects treated with cell-free implants, and matrices loaded with bone marrow or platelet-rich plasma. Precultured chondrogenic stem cell-matrix constructs resulted in partially integrated fibrocartilage-like repair tissue. Non-precultured mesenchymal stem cells in hyaluronan-collagen composite matrices stimulated the development of completely integrated meniscus-like repair tissue.

The role of bone marrow-derived mesenchymal stromal cells has also been studied and Yamasaki et al. have compared the effect of transplanting allogenic menisci and allogenic menisci seeded with bone-marrow-derived mesenchymal stromal cells onto artificially created meniscal defects [38]. Four weeks after surgery they studied the defective menisci and observed that articular cartilage in the cell-free group was greater than that in the cell-seeded group or the meniscectomy group.

Growth Factors

Other studies have been performed to check the direct effects of growth factors on the healing of open defects and tears in the meniscus. Hyaluronic acid, hyaluronan and endothelial cell growth factor (ECGF) have been studied in both tear healing and defect repair. Suzuki et al. created a cylindrical defect in the anterior lateral horn of rabbit menisci and demonstrated increased healing rates following weekly injections of hyaluronic acid [39]. Sonoda et al. found that hyaluronan stimulated collagen remodelling in the peripheral zone of torn rabbit menisci and inhibited swelling in the avascular zone [40]. Another study tested the effect of ECGF on the healing of an allograft and found that although ECGF increased short-term healing rate, over the long-term there was no observable difference [41].

Hashimoto et al. studied the effect of ECGF on the healing of a cylindrical full-thickness defect placed in the meniscus of a dog [42]. The defects that contained fibrin sealant and ECGF showed the best healing results at 24 weeks with 90% of the defect filled by the end of this study.

Genetic Enhancement

The role of genetic enhancement of engineered meniscal tissue has yielded promising results. Steinert et al. isolated bovine meniscal and bone marrow-derived mesenchymal stem cells and transduced them with adenoviral vectors encoding fluorescent protein, luciferase or TGF-β1 [43]. They found that recombinant adenovirus readily transduced meniscal cells and mesenchymal stem cells, and in-particular the transfer of TGF-β1 increased cellularity and led to stronger staining for proteoglycans and type II collagen and enhanced expression of meniscal genes. These early results suggest that genetic enhancement with growth factors may potentiate the effects of tissue engineering in vivo.

Hypoxia

The authors have compared the gene expression analysis, and the responses of cells isolated from the inner and outer meniscus to lowered oxygen, and compared it with the response of articular chondrocytes [44]. We found that hypoxia increased the expression of type II collagen and SOX9 in outer meniscus cells and inner meniscal cells and articular chondrocytes from a similar joint were much less sensitive to hypoxia. There results suggest that altering the oxygen tension may affect the formation of cartilage-like matrix. More work needs to be undertaken to understand how the physiological environment affects the development of engineering tissue.

MENISCAL TISSUE ENGINEERING IN CLINICAL PRACTICE

The Menaflex™ collagen meniscus implant, previously collagen meniscal implant, is a biocompatible and biodegradable surgical scaffold composed of purified type I collagen from bovine Achilles tendon. It was developed in the early 1990s and early work suggested that the collagen meniscal implant would support ingrowth and maturation of meniscus fibrochondrocytes, and the development of a mature and functional new tissue. The arthroscopic technique for insertion was described by Stone et al. and the clinical results reported through 2005-2008 as part of a US Food and Drug Association (FDA) study [45].

Steadman and Rodkey [46] and Zaffagnini et al. have both reported improved clinical results and enhanced regeneration in the medium-term [47]. The most convincing results have been reported by Rodkey et

al., who prospectively randomized 311 patients with an irreparable injury of the medial meniscus or a previous partial medial meniscectomy, either to receive the collagen meniscus implant or to serve as a control subject treated with a partial meniscectomy only [48]. Patients underwent frequent clinical follow-up examinations over two years and completed validated outcomes questionnaires over seven years. The patients who received a collagen meniscus implant had a second-look arthroscopy at one year to determine the amount of new tissue growth and to perform a biopsy to assess tissue quality. The authors showed that the collagen meniscus implants resulted in significantly ($p = 0.001$) increased meniscal tissue compared with that seen after the original index partial meniscectomy. In the chronic group, the patients who had received an implant regained significantly more of their lost activity than did the controls and underwent significantly fewer reoperations. However, the authors note that no differences were detected between the two treatment groups in the acute arm of the study.

Actifit™ Polymer Meniscal Implant is the other synthetic product currently in clinical use. It is a composite scaffold made from polyurethane stiff segments linked by soft flexible. The manufacturers, Orteq, conducted a prospective, non-randomized, single-arm, multi-centre, pilot clinical investigation to assess the safety and efficacy of Actifit™. Fifty-two patients enrolled in the trial, 34 for medial meniscus and 18 for lateral meniscus pathology. Clinical efficacy results showed that the Visual Analogue Score (VAS), Lysholm score, International Knee Documentation Committee (IKDC) score and the Knee Injury and Osteoarthritis Outcome Score (KOOS) all significantly improved after 12 months, and relook arthroscopy at one year showed regenerated tissue.

Both products are indicated for segmental tissue loss, and not for complete meniscal replacement, and compliance with the postoperative rehabilitation program is essential. The long-term results of these implants are unknown and we wait to see whether they will be proved to have benefits in delaying arthritic change and chondral damage.

SUMMARY

The micro and macro structure of the knee meniscus adapts it to the important biomechanical role it plays in the knee joint. Resection of meniscal tears disrupts knee biomechanics and leads to early degenerative changes in the joint. Attempts have been made to bioengineer a tissue construct suitable for partial or total meniscal transplant; however, a consensus has yet to be reached regarding optimal strategies, including the ideal cell source and growth factors for ensuring mechanical stability and matrix deposition. Research in this field has largely been subclinical with only two synthetic products currently licensed for clinical use. Very limited data is available regarding the clinical effectiveness of these implants. However, available evidence suggests that outcomes may be better than partial meniscectomy in the short-term.

REFERENCES

1. Rockborn P, Gillquist J. Outcome of arthroscopic meniscectomy: a 13 year physical and radiographic follow-up of 43 patients under 23 years of age. Acta Orthop Scand 1995;66:113-7.
2. Buma P, Ramrattan NN, van Tienen TG, Veth RP. Tissue engineering of the meniscus. Biomaterials 2004;25:1523-32.
3. Messner K, Gao J. The menisci of the knee joint. Anatomical and functional characteristics and a rationale for clinical treatment. J Anat 1998;193:161-78.
4. Ghadially FN, Thomas I, Yong N, Lalonde JMA. Ultrastructure of rabbit semilunar cartilages. J Anat 1978;125:499-517.
5. Shin GS, Fermor B, Weinberg JB, Pisetsky DS, Guilak F. Regulation of matrix turnover in meniscal explants: role of mechanical stress, interleukin-1, and nitric oxide. J Appl Physiol 2003;95:308-13.
6. Bianco P, Riminucci P, Gronthos S, Robey P. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 2001;19:180-92.
7. Friedenstein AJ. Determined and inducible osteogenic precursor cells. In Hard Tissue Growth, Repair and Remineralisation, Ciba Fdn Symp 1973;11:169-185. North-Holland, Amsterdam: Elsevier-Excerpta Medica.
8. Friedenstein, A. J. Precursor cells of mechanocytes. Int Rev Cytol 1976;47:327-55.
9. Baddoo M, Hill K, Wilkinson R et al. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. J Cell Biochem 2003;89:1235-49.
10. Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem 1997;64:278-94.
11. Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci U S A 2000; 97:3213-8.
12. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. Tissue Eng 1998;4:415-28.
13. Pittenger MF,Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284:143-7.

14. Im GI, Jung NH, Tae SK. Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. Tissue Engineering 2006;12; 527-36.
15. Tan Y, Zhang Y, Pei M. Meniscus reconstruction through coculturing meniscus cells with synovium-derived stem cells on small intestine submucosa--a pilot study to engineer meniscus tissue constructs. Tissue Eng Part A 2010;16:67-79.
16. Webber RJ, Zitaglio T, Hough AJ Jr. In vitro cell proliferation and proteoglycan synthesis of rabbit meniscal fibrochondrocytes as a function of age and sex. Arthritis Rheum 1986;29:1010-6.
17. Tanaka T, Fujii K, Kumagae Y. Comparison of biochemical characteristics of cultured fibrochondrocytes isolated from the inner and outer regions of human meniscus. Knee Surg Sports Traumatol Arthrosc 1999;7:75-80.
18. Nakata K, Shino K, Hamada M et al. Human meniscus cell: characterization of the primary culture and use for tissue engineering. Clin Orthop Relat Res 2001;391;208-18.
19. Webber RJ. In vitro culture of meniscal tissue. Clin Orthop 1990;252:114-20.
20. Collier S, Ghosh P. Effects of transforming growth factor beta on proteoglycan synthesis by cell and explant cultures derived from the knee joint meniscus. Osteoarthritis Cartilage 1995;3:127-38.
21. Gruber H, Mauerhan D, Chow Y et al. Three-dimensional culture of human meniscal cells: Extracellular matrix and proteoglycan production. BMC Biotechnology 2008; 8:54.
22. Baker B, Nathan A, Huffman G, Mauck RL. Tissue engineering with meniscus cells derived from surgical debris. Osteoarthritis Cartilage 2009;17(3): 336-45.
23. Fox DB, Warnock JJ, Stoker AM, Luther JK, Cockrell M. Effects of growth factors on equine synovial fibroblasts seeded on synthetic scaffolds for avascular meniscal tissue engineering. Res Vet Sci 2010;88: 326-32.
24. Warnock JJ, Stoker A, Fox DB, Cook JL. In vitro chondrogenesis for meniscal tissue engineering using fibroblast-like synoviocytes from normal versus osteoarthritic joints; available from www.orthovetsupersite.org (accessed 02/01/11).
25. Kuznetsov SA, Friedenstein AJ, Robey PG. Factors required for bone marrow stromal fibroblast colony formation in vitro. Br J Haematol 1997;97:561-70.
26. Roelen BA, Dijke P. Controlling mesenchymal stem cell differentiation by TGF beta family members. J Orthop Sci 2003; 8:740-8.
27. Adesida A, Grady L, Khan W, Hardingham TE. The matrix-forming phenotype of cultured human meniscus cells is enhanced after culture with fibroblast growth factor 2 and is further stimulated by hypoxia. Arthritis Res Ther 2006;8:61.
28. Wendt D, Marsano A, Jakob M, Heberer M, Martin I. Oscillating perfusion of cell suspensions through three-dimensional scaffolds enhances cell seeding efficiency and uniformity, Biotechnol Bioeng 2003;84;205-14.
29. Veth RP, Jansen HW, Leenslag JW, Pennings AJ, Hartel RM, Nielsen HK. Experimental meniscal lesions reconstructed with a carbon fiber-polyurethanepoly (L-lactide) graft. Clin Orthop Relat Res 1986;286-93.
30. Vunjak-Novakovic, et al., Microgravity studies of cells and tissues, Ann N Y Acad Sci 2002;974;504-17.
31. Moroni L, Lambersb FM, Wilson W et al. Finite Element Analysis of Meniscal Anatomical 3D Scaffolds: Implications for Tissue Engineering. Open Biomed Eng J 2007;1:23-34.
32. Mandal BB, Park SH, Gil ES, Kaplan DL. Multilayered silk scaffolds for meniscus tissue engineering. Biomaterials 2011; 32:639-51.
33. Peretti GM, Gill TJ, Xu JW, Randolph MA, Morse KR, Zaleske DJ. Cell-based therapy for meniscal repair: a large animal study. Am J Sports Med 2004;32:146-58.
34. Izuta Y, Ochi M, Adachi N, Deie M, Yamasaki T, Shinomiya R. Meniscal repair using bone marrow-derived mesenchymal stem cells: experimental study using green fluorescent protein transgenic rats. Knee 2005: 217-23.
35. Dutton AQ, Choong PF, Goh JC, Lee EH, Ui JH. Enhancement of meniscal repair in the avascular zone using mesenchymal stem cells in a porcine model. J Bone Joint Surg Br 2010;92:169-75.
36. Angele P, Johnstone B, Kujat R et al. Stem cell based tissue engineering for meniscus repair. J Biomed Mater Res A 2008; 85:445-55.
37. Zellner J, Mueller M, Berner A et al. J Biomed Mater Res A 2010;94:1150-61.
38. Yamasaki T, Deie M, Shinomiya R, Yasunaga Y, Yanada S, Ochi M. Transplantation of meniscus regenerated by tissue engineering with a scaffold derived from a rat meniscus and mesenchymal stromal cells derived from rat bone marrow. Artif Organs 2008;32:519-24.
39. Suzuki Y, Takeuchi N, Sagehashi Y, Yamaguchi T, Itoh H, Iwata H. Effects of hyaluronic acid on meniscal injury in rabbits. Arch Orthop Trauma Surg 1998;117:303-6.
40. Sonoda M, Harwood FL, Amiel ME et al. The effects of hyaluronan on tissue healing after meniscus injury and repair in a rabbit model. Am J Sports Med 2000;28:90-7.
41. Nabeshima Y, Kurosaka M, Yoshiya S, Mizuno K. Effect of fibrin glue and endothelial cell growth factor on the early healing response of the transplanted allogenic meniscus: a pilot study. Knee Surg Sports Traumatol Arthrosc 1995;3:34-8.
42. Hashimoto J, Kurosaka M, Yoshiya S, Hirohata K. Meniscal repair using fibrin sealant and endothelial cell growth factor. An experimental study in dogs. Am J Sports Med 1992;20:537-41.
43. Steinert AF, Palmer GD, Capito R et al. Genetically enhanced engineering of meniscus tissue using ex vivo delivery of transforming growth factor-beta 1 complementary deoxyribonucleic acid. Tissue Eng 2007;13:2227-37.
44. Adesida AB, Grady LM, Khan WS, Millward-Sadler SJ, Salter DM, Hardingham TE. Human meniscus cells express hypoxia inducible factor-1alpha and increased SOX9 in response to low oxygen tension in cell aggregate culture. Arthritis Res Ther 2007;9:R69.

45. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with use of a collagen scaffold. Analysis of preliminary data. J Bone Joint Surg Am 1997;79:1770-7.
46. Steadman JR, Rodkey WG. Tissue-engineered collagen meniscus implants: 5- to 6-year feasibility study results. Arthroscopy 2005; 21:515-25.
47. Zaffagnini S, Giordano G, Vascellari A et al. Arthroscopic collagen meniscus implant results at 6 to 8 years follow up. Knee Surg Sports Traumatol Arthrosc 2007;15:175-83.
48. Rodkey W, Kenneth D, DeHaven E, et al. Comparison of the Collagen Meniscus Implant with Partial Meniscectomy – A Prospective Randomized Trial. J Bone Joint Surg 2008;90:1413-26.

Liczba słów/Word count: 5000 **Tabele/Tables:** 0 **Ryciny/Figures:** 0 **Piśmiennictwo/References:** 48

Adres do korespondencji / Address for correspondence
Wasim S Khan, Clinical Lecturer, University College London Institute of Orthopaedics and Musculoskeletal Science, Royal National Orthopaedic Hospital, Stanmore, Middlesex, London, HA7 4LP, UK, tel/fax: +44 (0) 7791 025554, e-mail address: wasimkhan@doctors.org.uk

Otrzymano / Received *21.01.2011 r.*
Zaakceptowano / Accepted *12.04.2011 r.*