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Tissue engineering for meniscus regeneration

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1. Introduction

Menisci represent fundamental structures for the maintenance of knee homeostasis, playing a key role in knee biomechanics. Lesions of the meniscus are frequently observed in orthopedic practice. Injury to the meniscus is one of the most common problems in the knee joint, with a mean annual incidence of 60 to 70 per 100 000 knee injuries (Erkman et al. 1975; Hede et al 1990; Neilson et al 1991; Renstrom P 1990; Weinand et al 2006). Injury or loss of meniscal tissue leads to pain, knee dysfunction and osteoarthritis at long term (Cook et al 2005; Wyland 2002). Studies have demonstrated that knee degeneration is inversely related to the amount of meniscal tissue resected (McDermott and Amis 2006; Rijk 2004). Unfortunately, their intrinsic regenerative potential is poor. Healing is usually limited to the vascularized areas in the outer one third of the meniscus (Arnoczky and Warren 1983; Miller 1994). When a lesion involves the avascular portion of the meniscus, the reparative process cannot occur, since it emanates from bleeding and subsequent inflammation. As a result, the standard biological healing process produces limited results. Thus, a large proportion of meniscal tears observed at arthroscopy remain irreparable, and partial, subtotal or even total meniscectomy is often necessary, regardless of the recognized consequence. Even after an only partial meniscectomy, knee mechanics are subject to dramatic changes, leading often to the development of early osteoarthritis. Therefore, menisci should be repaired whenever possible.

In the last decades, tissue engineering approaches have been advocated to improve the reparative processes of joint tissues. The possibility to entirely reproduce the meniscus structure and function is highly attractive, and some new biomaterials have been studied and applied in preclinical and clinical studies.

2. Preclinical studies

In the last decade, replacement strategies have been applied to the menisci tissue in order to enhance their intrinsic reparative properties (Peretti et al. 1999; Peretti et al. 2004; Weinand et al. 2006) or even to replace them with an engineered tissue. Several materials have been

tested as partial meniscus substitutes in preclinical studies. Veth et al (Veth et al. 1986) used carbon fiber for meniscus repair in dogs, with poor results. Small intestine submucosa (SIS) was successfully used to repair posterior vascular meniscal defects (Cook et al. 2006), but not for complete substitution. Total meniscus substitution remains difficult and has been poorly described in the literature. A polyvinyl alcohol-hydrogel meniscus in rabbits showed interesting results in terms of chondroprotection, but certain unresolved problems persisted: durability of the polymer, fixation method, complete tissue regeneration in a material that does not adhere to tissue (De Groot et al. 1997; Kobayashi et al.2003). Van Tienen et al (Van Tienen et al 2006) studied two different porous polyester urethane polymers as meniscus substitutes in dogs. Despite the promising results in tissue formation, one of the materials (aromatic 4,4,-diphenylmethanediisocynate) is thought to degrade into toxic products. Other polymer implants did not show to prevent cartilage degeneration or were not suitable as meniscal substitute because of poor tissue ingrowth related to polymer degradation rate and poor mechanical properties (Van Tienen et al. 2002; Van Tienen et al 2003). Tissue engineering has recently been proposed as a possible solution for meniscal regeneration. A few animal studies investigated the possibility of using cells in combination with different scaffold biomaterials for a partial or total meniscal substitution (Van Tienen et al. 2002; Van Tienen et al 2003; Van Tienen et al 2006; Weinand et al 2006; Chiari et al. 2006; Kon et al 2008).

Several polymers, both natural and synthetic, have been tested for engineering meniscal and cartilage tissue in vitro or in vivo. Among the synthetic scaffolds, favorable polymers are open lattice structures with large pores into which cartilage matrix is permitted to form and new synthetic hydrogels are also good candidate scaffolds for generating meniscal tissue.

Collagen sponges have many desirable properties as a biological scaffold, including porosity, biodegradability, and biocompatibility. Multiple methods for preparing collagen and collagen-GAG scaffolds have been reported. Generally, collagen scaffolds are made from animal tissues such as type I collagen from bovine tendon. It is also possible to chemically modify the biomechanical and biological properties of the collagen scaffolds to enhance certain characteristics that promote tissue formation (Speer et al. 1979). Open lattice collagen scaffolds, some of which also include glycosaminoglycans, have been synthesized and used for generating new matrix. Following the list of the potential biological scaffolds for meniscus repair, hyaluronan could play an important role. A resorbable biomaterial consisting of hyaluronic acid and polycaprolactone was also tested for total meniscal substitution (Kon et al; 2008).

The other critical element for engineering meniscal tissue is finding the most suitable cell source. Various cell-sources have been evaluated in vitro to find the most suitable source for cell augmentation of tissue engineered meniscus. Meniscal cells can be isolated by enzymatic digestion of the tissue. Histological evidence suggests that the meniscal cells are capable of generating fibrocartilagenous tissue resembling meniscus. It is unlikely, however, that autologous meniscal fibrochondrocytes will be used to engineer one's own meniscus because of the lack of expendable donor sites. It is conceivable that allogeneic meniscal cells could be used as an option for overcoming the donor site related problems. The central issue involving the use of allogeneic chondrocytes is their potential for eliciting an immune response once the extracellular matrix is removed and MHC antigens are exposed (Tiku et al. 1985).

Different cell lines have been proposed as alternative solution for meniscal tissue engineering. Chondrocytes could be used to generate meniscal tissue or used to induce

meniscal repair. Articular chondrocytes showed the highest tissue regeneration capacity, with cellular and matrix phenotypes similar to those of both the inner and outer meniscus regions (Ibarra et al. 2000; Marsano et al. 2007).

Moreover, cartilage harvesting from a less loaded joint area would be less damaging to the knee biomechanics as compared to healthy meniscal tissue harvesting. Peretti et al (Peretti et al; 2004) have shown that articular chondrocytes seeded onto a devitalized allogenic meniscal slices or on a Vicryl mesh scaffold are capable of inducing a healing process in swine meniscus. Other alternative cartilage sources for obtaining cells include the cartilage portion of ribs or ear cartilage (Johonson et al 2004). MSCs are another valid candidate for meniscal bioengineering. Multiple experiments using human, chicken, dog, and rabbit MSCs have shown that, under controlled in vitro conditions, these cells can differentiate into bone, fat, tendon, muscle and cartilage-like tissues (Caplan et al. 2001; Pittenger et al. 1999; Conget et al. 1999).

All these cell sources could be used to engineer reparative meniscal tissue.

Walsh et al. (Wlash et al. 1999) used a collagenous sponge loaded with mesenchymal stem cells to treat a partial meniscus defect in rabbits. They reported that the presence of cells augmented the repair process but did not prevent knee degeneration. Martinek et al. (Martinek et al. 2006) documented better macroscopic and histological results in CMI implants seeded with meniscal fibrochondrocytes in comparison to cell-free implants in sheep. However, the tissue-engineered meniscus was biomechanically unstable and the implant size reduced during the three-month observation period. Therefore, the authors suggested that an improvement in scaffold and cell seeding procedure is required before human application.

Ibarra et al. also reported on a pilot study in sheep where they used autologous meniscal fibrochondrocytes and PGA polymer (Ibarra et al. 2000). The authors report that the constructs produced a new tissue with fibroblasts and chondrocytes. The presence of collagen fibers was observed histologically and the cells produced proteoglycans. The number of animals was small, but the authors demonstrated proof-of-principle for the technique. Polyurethane was initially developed and tested in animal studies for total meniscal replacement but is now being assessed as a scaffold for partial meniscal replacement as an alternative for the CMI (Van Tienen et al. 2003). Brophy and colleagues (Brophy et al. 2008) showed in sheep cadavers that the contact pressures after partial meniscectomy and replacement with a polyurethane scaffold were less than the contact pressures after partial meniscectomy only.

We have investigated the feasibility of using a new resorbable biomaterial consisting of hyaluronic acid and polycaprolactone for total meniscal substitution in a sheep model (Chiari et al. 2006; Kon et al. 2008). Twenty-four skeletally mature sheep were treated with total medial meniscus replacements while 2 meniscectomies served as empty controls. The animals were divided in two groups: cell free scaffold or scaffold seeded with autologous chondrocytes. Autologous chondrocytes were used as we have previously demonstrated that, both in vitro and following ectopic implantation, expanded articular chondrocytes were superior to meniscal cells, synovial or fat pad cells in their capacity to reach phenotypes typical of the inner and outer meniscus regions (Marsano et al. 2007). Two different surgical techniques were compared: in 12 animals the implant was sutured to the capsule and to the meniscal ligament and in the other 12 animals also a transtibial fixation of the horns was used. The animals were euthanized after 4 months. The specimens were

assessed by gross inspection and histology. All implants showed excellent capsular ingrowth at the periphery. Marcoscopically, no difference was observed between Cell Seeded and Cell Free group. Better implant appearance and integrity was observed in the group without trans-osseous horn fixation. Using the latter implantation technique, lower joint degeneration was observed in the cell-seeded group with respect to cell-free implants. The histological analysis indicated cellular infiltration and vascularisation throughout the implanted constructs. Cartilaginous tissue formation was significantly more frequent in the cell-seeded constructs. Our study supports the potential of the Hyaff/PCL scaffold for total meniscal substitution and seeding of the scaffolds with autologous chondrocytes provides some benefit in the extent of fibrocartilaginous tissue repair.
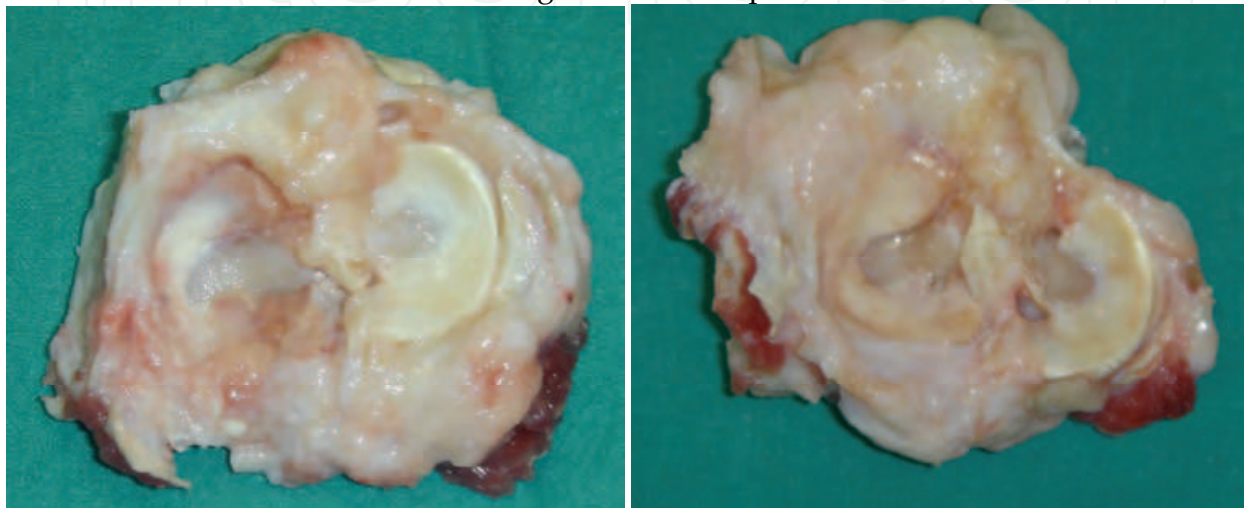

Fig. 1. Four month results after medial meniscus (left) replacement, (A) cell-seeded implants, (B) cell-free implants (left).

3. Clinical studies

Although the literature addresses merely the total replacement of the meniscus in animal studies, with or without cells, the current clinical studies only concern partial replacement of the resected meniscus with cell-free scaffolds. To the authors' knowledge, no clinical studies have been published in which the meniscus was replaced by a bioengineered meniscus.

Stone et al. (Stone et al. 1997) have developed a bioreadsorbable collagen matrix (CMI) which acts as a scaffold to restore the original medial meniscal. We studied (Zaffagnini et al. 2007) and prospectively evaluated the results of CMI implantation at a follow-up from a minimum of 6 to a maximum of 8 years. Eight patients (mean age 25) were evaluated at a final observation point from 6 to 8 years after CMI implantation. Inclusion criteria were an irreparable meniscal tear or a previous meniscectomy involving the medial meniscus. There were no complications related to the device. All patients were able to return to daily activities without limitations 3 months after surgery. Both subjective CKRS score and objective IKDC score showed improvement in all cases except one patient with an ACL re-injury. In two cases scores were slightly worse from 2 years after surgery to the final observation point. The other five cases obtained maximum score at final follow-up. In four cases the absence of pain remained until the final observation point, while in four cases a low entity of pain was described at long term follow-up. MRI showed in five cases mixoid degeneration signal, two had normal signal with reduced size, while one patient had no

recognizable implant. Six patients had preserved cartilage and articular space, with no changes compared to pre-op control. Arthroscopic second look evaluation has been performed in three cases, revealing in two cases the presence of the implant, although with a reduced size compared to the original one, while in one case the CMI was almost disappeared. Our small series of eight patients prospectively followed from 6 to 8 years of follow-up has shown highly satisfactory results. Although the aspect of the implant was mostly abnormal, the implant may have helped reduce the deterioration of the knee joint at final observation time.

Recently, a new polyurethane scaffold, presented by Brophy and colleagues (Brophy et al. 2008), has been introduced in clinical practice. The polyurethanes are believed to have better material properties For fixation to the remaining native meniscus tissue and to resist the extreme forces within the knee joint. A prospective clinical trial, which is being performed at this moment, should reveal if this hypothesis would hold. The preliminary results seem promising.

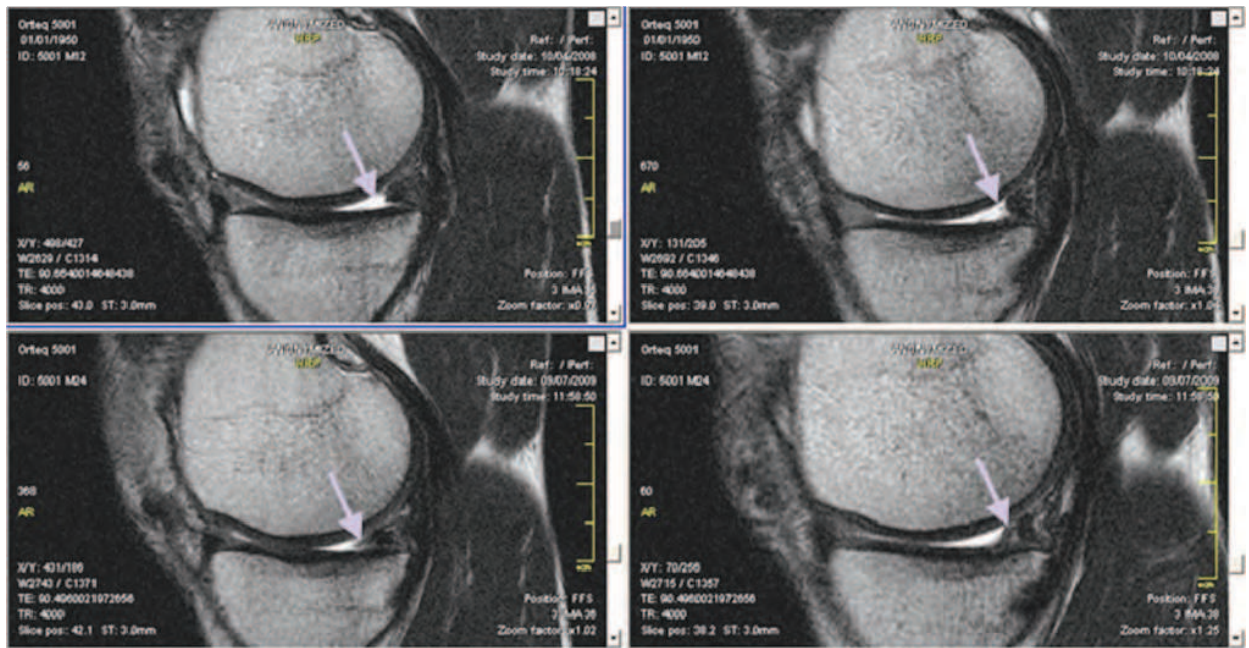Fig. 2. New polyurethane scaffold. (Courtesy of Orteq Bioengineering)

Fig. 3. At 24 months increased tissue can be seen anterior to the residual rim of the native meniscus when compared to the month 12 findings.

4. Conclusion

Subtotal or total meniscectomy is no longer considered a valid option for the treatment of meniscal tears, while preservation or regeneration of this tissue is now recommended, when possible, for the maintenance of a healthy knee. Menisci have poor intrinsic healing potential so that techniques based on fixation of the avascular regions of torn menisci often fail to allow for biological repair of the tissue. Until now, the properties of this tissue seemed hard to mimic. Tissue engineering for meniscal replacement represents an innovative and promising solution. Many materials have been proposed and tested in preclinical studies, but for the time being meniscus regeneration is still in its infancy, and further studies need to confirm the potential of the tissue engineering approach.

5. Acknowledgements

6. References

Arnoczky, S.P., and Warren, R.F. The microvasculature of the meniscus and its response to injury. An experimental study in the dog. Am J Sports Med 11, 131-41, 1983.

Brophy R, Cottrell J, Deng XH, et al. Dynamic contact mechanics of a scaffold for partial meniscal replacement [abstract 125]. In: Proceedings of the 75th Annual Meeting of the AAOS. San Francisco: American Academy of Orthopaedic Sur- geons; 2008.

Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. Review. Trends Mol Med 7(6):259-64, 2001.

Chiari, C., Koller, U., Dorotka, R., Eder, C., Plasenzotti, R., Lang, S., Ambrosio, L., Tognana, E., Kon, E., Salter, D., and Nehrer, S. A tissue engineering approach to meniscus regeneration in a sheep model. Osteoarthritis Cartilage 14, 1056-65, 2006.

Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 181(1):67-73, 1999.

Cook, J.L. The current status of treatment for large meniscal defects. Clin Orthop Relat Res , 88-95, 2005.

Cook, J.L., Fox, D.B., Malaviya, P., Tomlinson, J.L., Kuroki, K., Cook, C.R., and Kladakis, S. Long-term outcome for large meniscal defects treated with small intestinal submucosa in a dog model. Am J Sports Med 34, 32-42, 2006.

De Groot, J.H., Zijlstra, F.M., Kuipers, H.W., Pennings, A.J., Klompmaker, J., Veth, R.P., and Jansen, H.W. Meniscal tissue regeneration in porous 50/50 copoly(L-lactide/epsilon-caprolactone) implants. Biomaterials 18, 613-22, 1997.

Erkman MJ. The role of the menisci in force transmission across the knee. Clin Orthop Relat Res. 1975;109:184-192.

Hede A, Jensen DB, Blyme P, Sonne-Holm S. Epidemiology of meniscal lesions in the knee: 1,215 open operations in Copenhagen 1982-84. Acta Orthop Scand. 1990;61:435-437.

Ibarra, C., Koski, J.A., and Warren, R.F. Tissue engineering meniscus: cells and matrix. Orthop Clin North Am 31, 411-8, 2000.

Johnson TS, Xu JW, Zaporojan VV, Mesa JM, Weinand C, Randolph MA, Bonassar LJ, Winograd JM, Yaremchuk MJ. Integrative repair of cartilage with articular and non-articular chondrocytes. Tissue Eng 10(9-10):1308-15, 2004

Kobayashi, M., Toguchida, J., and Oka, M. Preliminary study of polyvinyl alcohol-hydrogel (PVA-H) artificial meniscus. Biomaterials 24, 639-47, 2003.

Kon E, Chiari C, Marcacci M, Delcogliano M, Salter DM, Martin I, Ambrosio L, Fini M, Tschon M, Tognana E, Plasenzotti R, Nehrer S. Tissue engineering for total meniscal substitution: animal study in sheep model. Tissue Eng Part A. 2008 Jun;14(6):1067-80

Martinek, V., Ueblacker, P., Bräun, K., Nitschke, S., Mannhardt, R., Specht, K., Gansbacher, B., and Imhoff, A.B. Second generation of meniscus transplantation: in-vivo study with tissue engineered meniscus replacement. Arch Orthop Trauma Surg 126, 228-34, 2006.

Marsano, A., Millward-Sadler, S.J., Salter, D.M., Adesida, A., Hardingham, T., Tognana, E., Kon, E., Chiari-Grisar, C., Nehrer, S., Jakob, M., and Martin, I. Differential cartilaginous tissue formation by human synovial membrane, fat pad, meniscus cells and articular chondrocytes. Osteoarthritis Cartilage 15, 48-58, 2007.

McDermott, I.D., and Amis, A.A. The consequences of meniscectomy. J Bone Joint Surg Br 88, 1549-56, 2006.

Miller MD, Warner JJ P, Harner CD . In: Fu FH, Harner CD, Vince KG Eds. Knee Surgery. Williams and Wilkins, 1994, .

Neilson AB, Yde J. Epidemiology of acute knee injuries: a prospective hospital investigation. J Trauma. 1991;31:1644-1648.

Peretti GM, Bonassar LJ, Caruso EM, Randolph MA, Zaleske DJ Biomechanical analysis of a cell-based model for articular cartilage repair. Tissue Eng 1999; 5(4):317–326

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(1):146–158

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143-7, 1999.

Renstrom P, Johnson RJ. Anatomy and biomechanics of the menisci. Clin Sports Med. 1990;9:523-538.

Rijk, P.C. Meniscal allograft transplantation--part II: alternative treatments, effects on articular cartilage, and future directions. Arthroscopy 20, 851-9, 2004.

Speer DP, Chvapil M, Volz RG, Holmes MD. Enhancement of healing in osteochondral defects by collagen sponge implants. Clin Orthop 144: 326-335, 1979.

Stone KR, Steadman JR, Rodkey WG, et al. Regeneration of meniscal cartilage with use of a collagen scaffold. Analysis of preliminary data. J Bone Joint Surg Am 1997;79:1770–7.

Tiku ML, Liu S, Weaver CW, Teodorescu M, Skosey JL. Class II histocompatibility antigen-mediated immunologic function of normal articular chondrocytes. J Immunol 135(5):2923-2928, 1985.

Van Tienen, T.G., Heijkants, R.G.J.C., Buma, P., De Groot, J.H., Pennings, A.J., and Veth, R.P.H. Tissue ingrowth and degradation of two biodegradable porous polymers with different porosities and pore sizes. Biomaterials 23, 1731-8, 2002.

Van Tienen, T.G., Heijkants, R.G.J.C., Buma, P., De Groot, J.H., Pennings, A.J., and Veth, R.P.H. A porous polymer scaffold for meniscal lesion repair--a study in dogs. Biomaterials 24, 2541-8, 2003.

Van Tienen, T.G., Heijkants, R.G.J.C., de Groot, J.H., Schouten, A.J., Pennings, A.J., Veth, R.P.H., and Buma, P. Meniscal replacement in dogs. Tissue regeneration in two different materials with similar properties. J Biomed Mater Res B Appl Biomater **76**, 389-96, 2006

Veth, R.P., Jansen, H.W., Leenslag, J.W., Pennings, A.J., Hartel, R.M., and Nielsen, H.K. Experimental meniscal lesions reconstructed with a carbon fiber-polyurethane-poly(L-lactide) graft. Clin Orthop Relat Res. 1986;286-93,

Weinand C, Peretti GM, Adams SB Jr, Bonassar LJ, Randolph MA, Gill TJ.An allogenic cell-based implant for meniscal lesions. Am J Sports Med. 2006 Nov;34(11):1779-89.

Wyland, D.J., Guilak, F., Elliott, D.M., Setton, L.A., and Vail, T.P. Chondropathy after meniscal tear or partial meniscectomy in a canine model. J Orthop Res **20**, 996-1002, 2002.

Walsh, C.J., Goodman, D., Caplan, A.I., and Goldberg, V.M. Meniscus regeneration in a rabbit partial meniscectomy model. Tissue Eng 5, 327-37, 1999.

Zaffagnini S, Giordano G, Vascellari A, et al. Arthroscopic collagen meniscus im- plant results at 6 to 8 years follow up. Knee Surg Sports Traumatol Arthrosc 2007; 15:175–83.
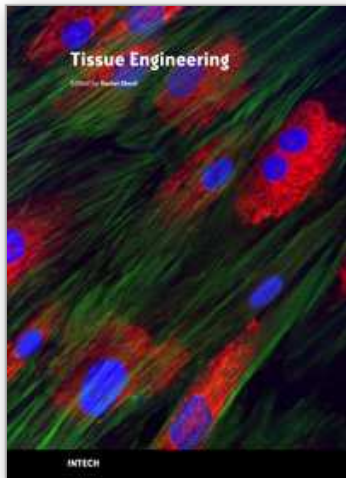
Tissue Engineering

Edited by Daniel Eberli

The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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