Minireview

The tissue engineering of articular cartilage: cells, scaffolds and stimulating factors

L'uboš Danišovič[1], Ivan Varga[2], Radoslav Zamborský[3] and Daniel Böhmer[1]

[1]Institute of Medical Biology, Genetics and Clinical Genetics; [2]Institute of Histology and Embryology, Faculty of Medicine, Comenius University in Bratislava, Sasinkova 4, 811 08 Bratislava, Slovak Republic; [3]Department of Traumatology, Faculty of Medicine, Slovak Medical University in Bratislava, Limbová 12, 833 03 Bratislava, Slovak Republic

Corresponding author: L'uboš Danišovič, Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University in Bratislava, Sasinkova 4, 811 08 Bratislava, Slovak Republic. Email: lubos.danisovic@fmed.uniba.sk

Abstract

Damage or loss of articular cartilage as a consequence of congenital anomaly, degenerative joint disease or injury leads to progressive debilitation, which has a negative impact on the quality of life of affected individuals in all age groups. Classical surgical techniques for hyaline cartilage reparation are frequently insufficient and in many cases it is not possible to obtain the expected results. For this reason, researchers and surgeons are forced to find a method to induce complete cartilage repair. Recently, the advent of tissue engineering has provided alternative possibilities for the treatment of these patients by application of cell-based therapy (e.g. chondrocytes and adult stem cells) combined with synthetic substitutes of the extracellular matrix and bioactive factors to prepare functional replacement of hyaline cartilage. This communication is aimed at a brief review of the current status of cartilage tissue engineering and recent advances in the field.

Keywords: tissue engineering, hyaline cartilage, cell therapy, biomaterials, chondrogenic stimuli

Experimental Biology and Medicine 2012; **237**: 10–17. DOI: 10.1258/ebm.2011.011229

Introduction

Damage of articular cartilage due to congenital anomalies, degenerative joint diseases and traumatic injuries is painful, debilitating and a costly medical problem which affects individuals in all age groups. Mature hyaline cartilage has a very low self-repair potential due to its intrinsic properties.[1] Hyaline cartilage is an avascular and aneural tissue with low cell density and rich extracellular matrix (ECM) (Figure 1). Another limiting factor is the low mitotic potential of chondrocytes.[2] Small defects are healed by the migration of chondrocytes, while large ones are healed by formation of biomechanically insufficient fibrillar cartilage.[3] However, in many cases these processes are inadequate and osteoarthritis develops. Recent techniques of articular cartilage defect repair involve autologous osteochondral cylinder, periosteum or perichondrium transplantation, fresh osteochondral allograft implantation and autologous chondrocyte implantation under periosteal flap.[4] Many of these strategies appear to be promising, but they involve invasive tissue collection and suffer from size restrictions, low mitotic potential and the senescence of chondrocytes expanded under *in vitro* conditions.[5] Moreover, cultured chondrocytes undergo a dedifferentiation process, gradually changing their morphology to a fibroblast-like shape, and the production of collagen type II is replaced by the production of collagen type I typical for fibrillar cartilage.[6] For this reason, researchers and surgeons are forced to find a method to induce complete cartilage repair.

Recently, tissue engineering strategies combining cell therapy (e.g. chondrocytes and adult stem cells [ASCs]) with proper biomaterials of natural or synthetic origin as scaffolds as well as various growth and differentiation stimuli (Figure 2) represent a promising new approach for the treatment of articular cartilage defects.[7] The main purpose of the present communication is to review the current status and advances of the cartilage tissue engineering with respect to their potential application in orthopedic surgery.

Sources of cells

Chondrocytes

The use of chondrocytes is a logical choice because they are present in the mature hyaline articular cartilage. Chondrocytes are responsible for the production of the basic ECM constituents – collagen type II and proteoglycans. Moreover, they play an important role in maintenance

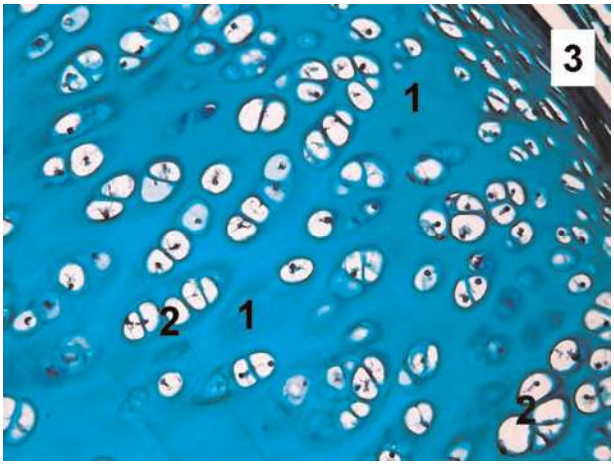

Figure 1 Hyaline cartilage is avascular and aneural compact tissue with abundant extracellular matrix (1) with collagenous microfibrils. Chondrocytes are present in lacunas as isogenous groups (2). The surface of hyaline cartilage is surrounded by perichondrium (3). (A color version of this figure is available in the online journal)

and remodeling of the cartilage matrix. Chondrocytes are easy to obtain by enzymatic digestion of cartilage and culture under proper *in vitro* conditions. The first human trial of cultured articular chondrocytes for the treatment of cartilage defects was reported by Brittberg *et al.*[8] They injected *in vitro* expanded autologous chondrocytes into the lesion covered by periosteal flap in 23 people with deep cartilage defects in the knee. The clinical results were more beneficial for defects on the femoral condyle than on the patella. Histological analysis of biopsies showed hyaline-like and fibrous tissue comprising the reparative tissue of asymptomatic patients. However, this technique had some disadvantages, such as reacquisition of phenotype when cultured *in vitro* and non-uniform distribution of cells as a result of gravitational force.[9,10]

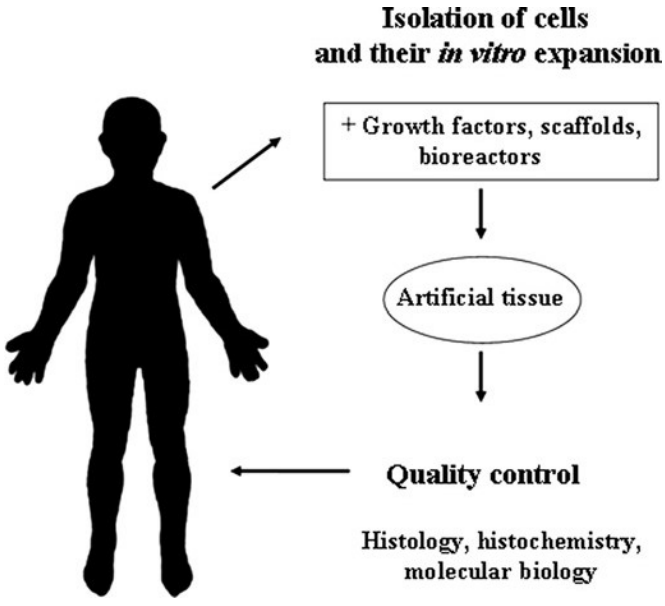

Figure 2 Basic concept of tissue engineering. Fabrication of artificial tissues suitable for clinical application starts with the procurement of tissue sample necessary for cell isolation and further expansion *in vitro* to obtain sufficient quantity of cells. Obtained cells are usually cultured within various types of scaffolds in bioreactors with addition of specific growth factors. Prepared artificial tissues have to be carefully tested for pathological events and potential microbiological contamination before application in human medicine

The main limitation of chondrocyte usage is their dedifferentiation process when cultured *in vitro*. Several attempts have been made to overcome this problem. It was shown that supplementation of culture media by growth factors, including transforming growth factor beta 1 (TGF-β1), fibroblast growth factor 2 (FGF-2) and insulin-like growth factor 1 (IGF-1) significantly retard this process, but they are not able to completely halt this cellular event.[11] Cultivation of chondrocytes in three-dimensional culture systems, such as agarose gel, alginate beads or fibrin glue, may also preserve the chondrocyte phenotype.[12] To date, numerous scaffolds have been used in conjunction with maintenance of chondrocyte phenotype, including collagen, hyaluronic acid, chitosan, etc. It was shown that these natural biopolymers may maintain the expression of aggrecan and collagen type II as well as positively influencing the production of sulfated glycosaminoglycans.[13,14] Similar effects may be achieved by cultivation of chondrocytes at high densities using micromass cultures without adding growth factors, which make them more suitable for clinical application. Obtained chondrocyte-based microtissue has a characteristic extracellular space comparable to the natural matrix of hyaline cartilage.[15]

Stem cells

Chondrocyte-based cell therapy represents a good approach for cartilage tissue engineering. However, it is difficult to obtain sufficient amounts of autologous chondrocytes and keep their original phenotype under *in vitro* conditions.[16] ASCs are generally characterized as undifferentiated cells with self-renewal capacity and plasticity (Figure 3). They occur in the tissues of all multicellular organisms throughout life. These cells are involved in the processes of embryogenesis and during adulthood they play a pivotal role in maintaining homeostasis and the integrity of organisms.[17] Over the past few years, ASCs have been derived from various types of tissues, including bone marrow, adipose tissue, skin, hair follicle, periosteum, dental pulp, etc.[18] ASCs are adherent and have a fibroblast-like morphology as well, being able to produce colony forming units-fibroblast when cultured *in vitro*.[19] These cells are heterogeneous and express a variety of surface markers including CD29, CD44, CD56, CD73, CD90, CD105, CD166, CD271, STRO-1 and MSCA-1. Moreover, they are usually negative for hematopoietic markers CD34, CD45 and for human leukocyte antigen class II.[20–22]

In a pioneer study, Johnstone *et al.*[23] reported that stem cells derived from bone marrow cultured as an aggregate with TGF-β1 undergo chondrogenic differentiation. Their results demonstrated over-expression of collagen type II and X typical for hyaline cartilage *in vivo*, while the expression of collagen type I was significantly decreased. More recently, Bosnakovski *et al.*[24] proved chondrogenic differentiation of bone marrow-derived stem cells in a

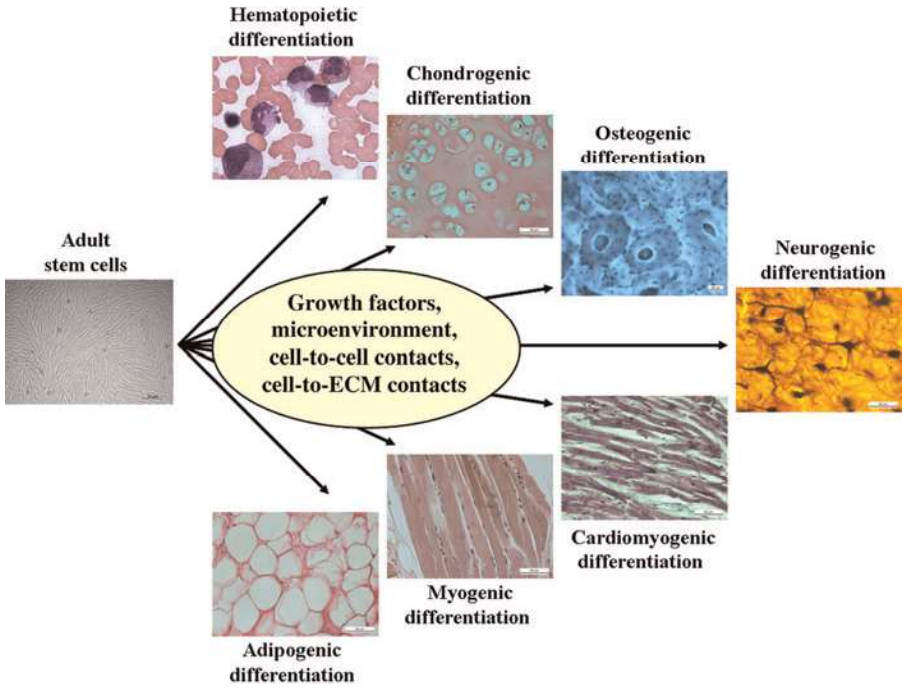

Figure 3 Schematic overview of stem cell plasticity. Stem cells have the ability to differentiate into other cell types under specific physiological (*in vivo*) or laboratory conditions (*in vitro*). Therefore, they represent a unique tool for regenerative medicine. ECM, extracellular matrix. (A color version of this figure is available in the online journal)

pellet culture system, without supplementation of growth factors.

Other sources of ASCs have also been studied with respect to cartilage tissue engineering. It has been reported that adipose tissue-derived stem cells obtained from lipoaspirates can undergo chondrogenic differentiation when cultured at a high density in medium supplemented by TGF-β, ascorbate and dexamethasone. These cells produced collagen type II and sulfated glycosaminoglycans.[25] Better results may be achieved when adipose-derived stem cells are cultured in the presence of FGF-2 and bone morphogenetic protein 6 (BMP-6). It was shown that FGF-2 increases cell proliferation and enhances chondrogenesis *in vitro*, while addition of BMP-6 significantly up-regulates the expression of aggrecan and collagen type II.[26,27] The potential for utilization of these cells is very attractive because subcutaneous fat is abundant in the human body and the liposuction procedure is minimally invasive for the patient. On the other hand, adipose-derived stem cells have lower chondrogenic potential than stem cells isolated from bone marrow.[28]

More recently, besides bone marrow and adipose tissue, skeletal muscle, synovial membranes and periosteum are other sources of ASCs being explored in the context of articular cartilage repair.[29–31] These stem cells were cultured predominantly in aggregates or micromass cultures with or without growth factors. In every case, chondrogenic differentiation was proved. Similar results were obtained when the stem cells were cultured within the structure of various ECM substitutes, including collagen, chitosan, polylactic acid, etc.[32] Obtained results indicate that ASCs derived from various tissues seem to be a promising tool for cartilage tissue engineering.

Scaffolding materials

Scaffolds belong to key components for tissue engineering. They are produced from natural or synthetic biomaterials (Table 1) and may be in the form of hydrogels, sponges, fibrous meshes and nanofibers. Materials for scaffolding have to be non-toxic, sterile, biodegradable and biocompatible.[7] The structure of the surface, pore size, porosity and structural strength are important characteristics, which influence their final utilization.[33,34] These scaffolds not only have a mechanical function, but also support cell attachment, migration, proliferation and differentiation for expression of desirable phenotypes.[35] Biodegradable polymers seem to be the most appropriate materials for scaffolding, because their interactions with cultured cells lead to incremental biological degradation. After their application, there is no need to undergo other surgery to remove foreign material from the patient's body.[7]

There are plenty of polymers, but only some of them are suitable for cartilage tissue engineering. Collagen is a

Table 1 Types of biomaterials used in cartilage tissue engineering

Natural polymers	Synthetic polymers
Agarose	Poly (α-hydroxy esters)
Alginate	Poly (ethylene glycol/oxide)
Cellulose	Poly (NiPAAm)
Collagen	Poly (propylene fumarate)
Chitosan	Poly (urethane)
Chondroitin sulfate	Poly (vinyl alcohol)
Fibrin glue	
Gelatin	
Hyaluronic acid	
Silk fibroin	

physiological component of various tissues, including cartilage, and is an intensively studied natural polymer with respect to tissue engineering.[14] The chemical, mechanical and biological properties of collagen offer wide possibilities to control its biodegradation. The problem of collagen antigenicity was solved by enzymatic removal of telopeptides from its molecule or by chemical cross-linking, so it is safe for clinical utilization.[36,37] It was found that chondrocytes cultured within collagen gels keep their phenotype and glycosaminoglycan production for almost six weeks in culture.[38] More recently, it was shown that collagen-based gel has a chondroinductive effect on ASCs derived from bone marrow.[39] Not only gels, but also collagen matrices and membranes stimulate cells to produce new collagen and preserve chondrocyte phenotype.[40]

Hyaluronan occurs at relatively high concentration in the ECM of articular cartilage. It is a biocompatible and biodegradable biopolymer, so it would be an ideal scaffolding material. However, in order to achieve proper characteristics, hyaluronan is chemically adjusted by cross-linking.[41] Grigolo *et al.*[42] demonstrated that chondrocytes cultured on a hyaluronan scaffold (HYAFF 11) express collagen type II and aggrecan and downregulate the production of collagen type I. More recently, it was also shown that hyaluronan creates an environment in which chondrocytes down-regulate the expression of catabolic factors and apoptosis. These results demonstrated a potential ability of hyaluronan to prevent cartilage against damage and may have benefit for the treatment of early osteoarthritic lesions.[43] Jakobsen *et al.*[44] cultured stem cells derived from bone marrow and adipose tissue in hyaluronan scaffolds. Their results showed that bone marrow-derived stem cells expressed 600-fold higher levels of collagen type II than chondrocytes after three weeks in the scaffold. Expression of collagen type II in adipose tissue-derived stem cells was lesser but similar to chondrocytes.

Another attractive polymer for cartilage tissue regeneration is fibrin glue. It is a natural polymer produced from the polymerization of fibrinogen with thrombin.[45] Scotti *et al.*[46] prepared an artificial substitute of cartilage by cultivation of chondrocytes within fibrin glue. Their results suggest that chondrocytes survived in the fibrin glue gel and enhanced their synthetic activity – DNA content remained stable, while all indices of cartilage matrix production increased. A similar effect was observed in experiments with ASCs.[47]

Other natural polymers are also used in the field of cartilage engineering, including alginate, agarose, chitosan, chondroitin sulfate, gelatine and silk fibroin. It was demonstrated that all of them in some extent potentiate the production of collagen type II and sulfated glycosaminoglycans by both chondrocytes and stem cells.[48]

Besides the above-mentioned natural biopolymers, a variety of synthetic polymers may be utilized with respect to cartilage defect healing. They can be modified in many ways such as mechanical properties, degradation rate and chemical modification. Moreover, they can be manufactured in unlimited scale.[49] The most widely used are polylactic acid (PLA), polyglycolic acid (PGA) (and their co-polymer) and polyethylene glycol (PEG).

PLA is a biodegradable polymer that can be produced from lactic acid, which can be fermented from crops. PGA is a biodegradable, thermoplastic polymer and the simplest linear, aliphatic polyester. It can be prepared starting from glycolic acid by means of polycondensation or ring-opening polymerization. In tissue engineering these two polymers are used for the preparation of scaffold alone, but most commonly they are used as a co-polymer. It was demonstrated that PLA and PGA increased chondrocyte proliferation and glycosaminoglycans.[50] More recently, Lee *et al.*[51] demonstrated that a co-polymer consisting of PLA and PGA is a better option for inducing cartilage tissue formation than both polymers individually. Histological and immunohistochemical results showed that chondrocytes retained their morphological phenotype to a greater extent than those seeded into PLA. Similar results were obtained in experiments with ASCs.[52,53]

PEG is a commonly used polymer for biomaterial applications due to its ability to resist protein absorption. Scaffolds made from PEG can be polymerized using either chemical or photoinitiators. These scaffolds can also be chemically modified to contain bioactive molecules, including peptides and heparin.[54] Hwang *et al.*[55] colonized PEG with primary chondrocytes. Their results showed that PEG supported attachment, viability, proliferation and biosynthetic activity of seeded chondrocytes. Moreover, it was also demonstrated that hydrogels prepared from modified PEG mimic the natural environment, which promoted chondrogenesis of stem cells derived from the bone marrow and enhanced the secretion of cartilage-specific ECM.[56]

Stimulating factors

Growth factors

Chondrogenic differentiation of stem cells should be induced by various intrinsic and extrinsic factors. Growth factors play the most important role in this process. They represent a group of biologically active polypeptides produced by the body, which can stimulate cell proliferation and differentiation. In the hyaline cartilage, growth factors regulate homeostasis and integrity, as well as development (Table 2).

Growth factors from the TGF-β superfamily probably belong to the most investigated biologically active substances within the field of cartilage tissue engineering. They play a pivotal role in cell proliferation, apoptosis and differentiation. Moreover, they are involved in regulation of the cell cycle and immune system.[57] TGF-β1 stimulates synthetic activity of chondrocytes, keeps their phenotype and acts against catabolic activity of inflammatory mediator interleukin 1 *in vivo*.[58] Experimental studies performed *in vitro* showed enhanced proliferation and chondrogenic differentiation of stem cells. Moreover, TGF-β1 treatment of stem cells leads to down-regulation of collagen type I gene expression and to increased expression of collagen type II and aggrecan, typical for hyaline cartilage formation.[28] TGF-β3 also induces cartilaginous ECM production by chondrocytes. Thorpe *et al.*[59] demonstrated that

Table 2 List of selected growth factors and their effect on stem cells

Growth factor	Effect on MSCs
TGF-β1	Increases proliferation and cartilaginous ECM production, downregulates collagen type I gene expression
TGF-β3	Increases cartilaginous ECM production
BMP-2	Increases proliferation and cartilaginous ECM production, downregulates collagen type I gene expression
BMP-4	Accelerates the progression of cartilage differentiation to maturation
BMP-7	Inhibits cell proliferation, induces chondrogenic differentiation, additive effect on chondrogenesis with TGF-β1 and IGF-1
GDF-5	Increases cartilaginous ECM production
IGF-1	Increases proliferation and cartilaginous ECM production, additive effect on chondrogenesis with TGF-β1 and BMP-7
FGF-2	Increases proliferation, increases proteoglycan production
FGF-18	Inhibits cell proliferation, induces chondrogenic differentiation

ECM, extracellular matrix; TGF, transforming growth factor; BMP, bone morphogenetic protein; GDF, growth differentiation factor; IGF, insulin-like growth factor; FGF, fibroblast growth factor

TGF-β3 treatment of stem cells led to enhanced synthesis of sulfated glycosaminoglycans.

BMPs belong to the TGF-β superfamily. They play crucial roles in the processes of chondrogenesis and osteogenesis *in vivo*.[60] BMP-2 treatment leads to an increase in cartilaginous ECM production and to a decrease in collagen type I gene expression. Moreover, BMP-2-treated stem cells produce more robust aggregates when compared with other ones and so it seems to be the most effective chondrogenic factor from the TGF-β superfamily.[61] BMP-4 is a critical signaling molecule involved in the processes of embryogenesis and exhibits osteogenic and chondrogenic potential *in vivo*. BMP-4 induces chondrogenic differentiation of ASCs toward a chondroprogenitor lineage and facilitates differentiation into mature chondrocytes. BMP-4 also enhances the production of cartilaginous matrix by stimulating the synthesis of collagen type II and aggrecan and suppresses expression of collagen type I and X.[62] BMP-7 is synthesized by chondrocytes and play an important role in articular cartilage regeneration. It has significant anabolic activity by which BMP-7 protects cartilage against damage.[63]

IGF-1 is a hormone similar to insulin. It plays an essential role in the growth of the organism and has a significant anabolic effect.[64] In the articular cartilage, IGF-1 is the main anabolic growth factor, which is necessary for cartilage homeostasis, proteoglycan synthesis and breakdown by the chondrocytes.[65] The effect of IGF-1 on chondrogenic differentiation of ASCs has been extensively investigated. It was demonstrated that IGF-1 modulates mesenchymal stem cell (MSC) chondrogenesis by stimulating proliferation, regulating cell apoptosis and inducing expression of the chondrocyte phenotype. This effect is independent from TGF-β signaling.[66]

The FGFs are heparin-binding polypeptides which are employed in various cellular events, such as proliferation, differentiation and motility. They also play a very important role during embryogenesis, angiogenesis and wound healing. FGF-2 occurs within the structure of the ECM of hyaline cartilage. This factor promotes the proliferation of chondrocytes *in vivo*. It was also shown that FGF-2 (with FGF-4 and FGF-8) plays a role in the process of anabolic pathway activation which leads to a decrease in aggrecanase effect after loading of cartilage. It protects cartilage against damage and osteoarthritis development.[67] Several studies were performed to assess the FGF-2 effect on ASC proliferation and chondrogenic differentiation. It was demonstrated that FGF-2 treatment of MSCs leads to acceleration of their proliferation and to enhancing of proteoglycan production. Furthermore, cultivation of ASCs in pellets with medium containing FGF-2 increases their chondrogenic potential. More recently, Park and Na,[68] in their work, confirmed increased production of glycosaminoglycans and collagen type II, which are typical for hyaline cartilage, in ASC-hydrogel constructs treated by FGF-2. However, fewer attempts were made to evaluate the effect of FGF-18, which is involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion *in vivo*. It was also shown that intra-articular administration of FGF-18 led to a decrease in articular damage in rats with developed osteoarthritis.[69] Yamaoka *et al.*[70] demonstrated its role in maintenance of chondrocyte properties, even though its expression was rather high in dedifferentiated chondrocytes. Moreover, results obtained from experiments with ASCs indicate that FGF-18 is a selective ligand for FGF receptor 3 which suppressed proliferation and promoted their differentiation and production of cartilage matrix.[71] However, further investigations must be conducted to clarify its potential for cartilage tissue engineering.

Hydrostatic pressure

Physiologically, articular cartilage exists in the microenvironment of reduced oxygen and irregular hydrostatic pressure. Imitation of these conditions may provide a way to improve chondrogenic differentiation *in vitro*. Low oxygen tension (5%) has potentiated cell proliferation and collagen type II expression, as well as increased cartilage-specific biosynthesis of chondrocytes.[72] Hydrostatic pressure applied within physiological levels has a positive effect on production of cartilage matrix. Chondrocytes loaded at 10 MPa and 1 Hz for 4 h/d, 5 d/week, for up to eight weeks showed an increase in collagen production.[73] The benefits of hydrostatic loading were also noted in experiments with stem cells, where 0.1 MPa of loading increased Sox 9 and aggrecan expression and 10 MPa of loading significantly increased the expression of collagen type II.[74]

Bioreactors

The ability of a monolayer culture system to promote generation of highly differentiated structures is limited because

cells are expanded on substrate, which is inappropriate and the metabolic condition is fluctuated *in vitro*.[75] To solve this problem, bioreactors that are able to imitate conditions *in vivo* have been constructed.

Spinner flasks are the simplest bioreactors, in which chondrocytes or ASCs are seeded on the scaffold fixed to the needles hanging from the stopper of the flask. The culture medium is added and permanently mixed. It has been shown that neocartilage prepared from chondrocytes in spinner flasks is larger and contains more cells and more robust cartilage matrix, compared with those grown in Petri dishes.[76] Wang *et al.*[77] cultured bone marrow-derived stem cells on the gelatin–hyaluronan scaffold in a spinner flask. After three weeks, they showed significant increasing of collagen type II, aggrecan and decorin expression.

Another simple design that can be utilized in cartilage tissue engineering is the perfusion culture system, which provides a constant flow of culture medium with differentiation factors through the chamber with scaffold and cells. The experiments with chondrocytes cultured at low density within alginate scaffold demonstrated cartilage ECM formation and a typical phenotype of chondrocytes after four weeks.[78] Enhancement of cell proliferation and collagen type II and glycosaminoglycan content were also recorded in artificial cartilage prepared by cultivation of ASCs on the various scaffolds using the perfusion culture system.[79]

In addition to the above bioreactors, a number of other systems were considered for application in cartilage engineering, including a parallel-plate bioreactor, a rotating wall bioreactor, a concentric cylinder bioreactor and a wavy-wall bioreactor.[12] The last one has been shown to increase chondrocyte and ASC proliferation and cartilage ECM deposition on scaffolds over the common spinner flask culture. Compared with a spinner flask, this novel bioreactor reduces fluid shear stresses and increases axial mixing, and seems to be more suitable and effective for cartilage engineering.[80]

Conclusion

Recently, tissue engineering has begun to provide alternative possibilities in clinical practice for healing patients with damaged articular cartilage. It combines cells, chondrogenic stimuli and appropriate scaffolds to prepare a biological substitute of hyaline cartilage tissue. Further investigations will be focused on producing and testing of new bioactive and biocompatible polymers with controlled biodegradability. Greater attention will be given to the use of layered scaffolds to recreate the zonal organization of hyaline cartilage. Considerable progress can be expected on the field of stem-cell research, especially in combination with gene therapy. The abovementioned will bring new approaches for cartilage tissue engineering and will be conducive in the treatment of various joint diseases, including osteoarthritis and rheumatoid arthritis.

Author contributions: All authors participated in the design, interpretation of the studies and review of the manuscript. LD and IV wrote the manuscript.

ACKNOWLEDGEMENTS

The present work was supported by the grant VEGA No. 1/0706/11 and partially by the Ministry of Health of the Slovak Republic under the project No. 2007/36-UK-07.

REFERENCES

1 Strehl R, Tallheden T, Sjogren-Jansson E, Minuth WW, Lindahl A. Long-term maintenance of human articular cartilage in culture for biomaterial testing. *Biomaterials* 2005;**26**:4540–9
2 Brown TD, Johnston RC, Saltzman CL, Marsh JL, Buckwalter JA. Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease. *J Orthop Trauma* 2006;**20**:739–44
3 Buckwalter JA. Articular cartilage injuries. *Clin Orthop Relat Res* 2002;**402**:21–37
4 Kellett CF, Boscainos PJ, Gross AE. Surgical options for articular defects of the knee. *Expert Rev Med Devices* 2006;**3**:585–93
5 Tew SR, Kwan AP, Hann A, Thomson BM, Archer CW. The reactions of articular cartilage to experimental wounding: role of apoptosis. *Arthritis Rheum* 2000;**43**:215–25
6 Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. *J Orthop Res* 2005;**23**:425–32
7 Vinatier C, Bouffi C, Merceron C, Gordeladze J, Brondello JM, Jorgensen C, Weiss P, Guicheux J, Noël D. Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. *Curr Stem Cell Res Ther* 2009;**4**:318–29
8 Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;**331**:889–95
9 Schnabel M, Marlovits S, Eckhoff G, Fichtel I, Gotzen L, Vecsei V, Schlegel J. Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. *Osteoarthritis Cartilage* 2002;**10**:62–70
10 Sohn DH, Lottman LM, Lum LY, Kim SG, Pedowitz RA, Coutts RD, Sah RL. Effect of gravity on localization of chondrocytes implanted in cartilage defects. *Clin Orthop Relat Res* 2002;**394**:254–62
11 Schulze-Tanzil G. Activation and dedifferentiation of chondrocytes: implications in cartilage injury and repair. *Ann Anat* 2009;**191**:325–38
12 Chung C, Burdick JA. Engineering cartilage tissue. *Adv Drug Deliv Rev* 2008;**60**:243–62
13 Di Martino A, Sittinger M, Risbud MV. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials* 2005;**26**:5983–90
14 Yates KE, Allemann F, Glowacki J. Phenotypic analysis of bovine chondrocytes cultured in 3D collagen sponges: effect of serum substitutes. *Cell Tissue Bank* 2005;**6**:45–54
15 Anderer U, Libera J. *In vitro* engineering of human autogenous cartilage. *J Bone Miner Res* 2002;**17**:1420–9
16 Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering. *Arthritis Res Ther* 2003;**5**:32–45
17 García-Gómez I, Elvira G, Zapata AG, Lamana ML, Ramírez M, Castro JG, Arranz MG, Vicente A, Bueren J, García-Olmo D. Mesenchymal stem cells: biological properties and clinical applications. *Expert Opin Biol Ther* 2010;**10**:1453–68
18 Shanti RM, Li WJ, Nesti LJ, Wang X, Tuan RS. Adult mesenchymal stem cells. Biological properties, characteristics, and applications in maxillofacial surgery. *J Oral Maxillofac Surg* 2007;**65**:1640–47
19 Caplan AI. Mesenchymal stem cells. *J Orthop Res* 1991;**9**:641–50
20 LeBlanc K, Pittenger M. Mesenchymal stem cells: progress toward promise. *Cytotherapy* 2005;**7**:36–45
21 Rojewski MT, Weber BM, Schrezenmeier H. Phenotypic characterization of mesenchymal stem cells from various tissues. *Transfus Med Hemother* 2008;**35**:168–84
22 Battula VL, Treml S, Bareiss MP, Gieseke F, Roelofs H, Zwart P, Muller I, Schewe B, Skutella T, Fibbe WE, Kanz L, Buhring HJ. Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1. *Haematologica* 2009;**94**:173–84
23 Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* 1998;**238**:265–72

24 Bosnakovski D, Mizuno M, Kim G, Ishiguro T, Okumura M, Iwanaga T, Kadosawa T, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells in pellet cultural system. *Exp Hematol* 2004;**32**:502–9

25 Huang JI, Zuk PA, Jones NF, Zhu M, Lorenz HP, Hedrick MH, Benhaim P. Chondrogenic potential of multipotential cells from human adipose tissue. *Plast Reconstruct Surg* 2004;**113**:585–94

26 Chiou M, Xu Y, Longaker MT. Mitogenic and chondrogenic effects of fibroblast growth factor-2 in adipose-derived mesenchymal cells. *Biochem Biophys Res Commun* 2006;**343**:644–52

27 Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adiposederived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum* 2006;**54**:1222–32

28 Havlas V, Kos P, Jendelová P, Lesný P, Trč T, Syková E. Comparison of chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells with cultured chondrocytes and bone marrow mesenchymal stem cells. *Acta Chir Orthop Traumatol Cech* 2011;**78**:138–44

29 Choi YS, Noh SE, Lim SM, Lee CW, Kim CS, Im MW, Lee MH, Kim DI. Multipotency and growth characteristic of periosteum-derived progenitor cells for chondrogenic, osteogenic, and adipogenic differentiation. *Biotechnol Lett* 2008;**30**:593–601

30 Arufe MC, De la Fuente A, Fuentes I, de Toro FJ, Blanco FJ. Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes. *J Cell Biochem* 2010;**111**:834–45

31 Wu X, Wang S, Chen B, An X. Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy. *Cell Tissue Res* 2010;**340**:549–67

32 Nesic D, Whiteside R, Brittberg M, Wendt D, Martin I, Mainil-Varlet P. Cartilage tissue engineering for degenerative joint disease. *Adv Drug Deliv Rev* 2006;**58**:300–22

33 Chung TW, Yang J, Akaike T, Cho KY, Nah JW, Kim SI, Cho CS. Preparation of alginate/galactosylated chitosan scaffold for hepatocyte attachment. *Biomaterials* 2002;**23**:2827–34

34 Liu L, Zhang L, Ren B, Wang F, Zhang Q. Preparation and characterization of collagen-hydroxyapatite composite used for bone tissue engineering scaffold. *Artif Cells Blood Substit Immobil Biotechnol* 2003;**31**:435–48

35 Yoo HS, Lee EA, Yoon JJ, Park TG. Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering. *Biomaterials* 2005;**26**:1925–33

36 Eriksson AE, Cousens LS, Weaver LH, Matthews BW. Three-dimensional structure of human basic fibroblast growth factor. *Proc Natl Acad Sci USA* 1991;**88**:3441–45

37 Ishihara M, Shaklee PN, Yang Z, Liang W, Wei Z, Stack RJ, Holme K. Structural features in heparin which modulate specific biological activities mediated by basic fibroblast growth factor. *Glycobiology* 1994;**4**:451–8

38 Kimura T, Yasui N, Ohsawa S, Ono K. Chondrocytes embedded in collagen gels maintain cartilage phenotype during long-term cultures. *Clin Orthop Relat Res* 1984;**186**:231–9

39 Zheng L, Fan HS, Sun J, Chen XN, Wang G, Zhang L, Fan YJ, Zhang XD. Chondrogenic differentiation of mesenchymal stem cells induced by collagen-based hydrogel: an *in vivo* study. *J Biomed Mater Res A* 2010;**93**:783–92

40 Zheng MH, Willers C, Kirilak L, Yates P, Xu J, Wood D, Shimmin A. Matrix-induced autologous chondrocyte implantation (MACI): biological and histological assessment. *Tissue Eng* 2007;**13**:737–46

41 Evanko SP, Tammi MI, Tammi RH, Wight TN. Hyaluronan-dependent pericellular matrix. *Adv Drug Deliv Rev* 2007;**59**:1351–65

42 Grigolo B, Lisignoli G, Piacentini A, Fiorini M, Gobbi P, Mazzotti G, Duca M, Pavesio A, Facchini A. Evidence for redifferentiation of human chondrocytes grown on a hyaluronan-based biomaterial (HYAff 11): molecular, immunohistochemical and ultrastructural analysis. *Biomaterials* 2002;**23**:1187–95

43 Grigolo B, Lisignoli G, Desando G, Cavallo C, Marconi E, Tschon M, Giavaresi G, Fini M, Giardino R, Facchini A. Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. *Tissue Eng Part C Methods* 2009;**15**:647–58

44 Jakobsen RB, Shahdadfar A, Reinholt FP, Brinchmann JE. Chondrogenesis in a hyaluronic acid scaffold: comparison between chondrocytes and MSC from bone marrow and adipose tissue. *Knee Surg Sports Traumatol Arthrosc* 2010;**18**:1407–16

45 Mosesson MW. Fibrinogen and fibrin structure and functions. *J Thromb Haemost* 2005;**3**:1894–904

46 Scotti C, Mangiavini L, Boschetti F, Vitari F, Domeneghini C, Fraschini G, Peretti GM. Effect of *in vitro* culture on a chondrocyte-fibrin glue hydrogel for cartilage repair. *Knee Surg Sports Traumatol Arthrosc* 2010;**18**:1400–6

47 Ahmed TA, Giulivi A, Griffith M, Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. *Tissue Eng Part A* 2011;**17**:323–35

48 Chung C, Burdick JA. Influence of three-dimensional hyaluronic acid microenvironments on mesenchymal stem cell chondrogenesis. *Tissue Eng Part A* 2008;**15**:243–54

49 Lee SH, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv Drug Deliv Rev* 2007;**59**:339–59

50 Hsu SH, Chang SH, Yen HJ, Whu SW, Tsai CL, Chen DC. Evaluation of biodegradable polyesters modified by type II collagen and Arg-Gly-Asp as tissue engineering scaffolding materials for cartilage regeneration. *Artif Organs* 2006;**30**:42–55

51 Lee NK, Oh HJ, Hong CM, Suh H, Hong SH. Comparison of the synthetic biodegradable polymers, polylactide (PLA), and polylactic-co-glycolic acid (PLGA) as scaffolds for artificial cartilage. *Biotechnol Bioprocess Eng* 2009;**14**:180–6

52 Mehlhorn AT, Zwingmann J, Finkenzeller G, Niemeyer P, Dauner M, Stark B, Südkamp NP, Schmal H. Chondrogenesis of adipose-derived adult stem cells in a poly-lactide-co-glycolide scaffold. *Tissue Eng Part A* 2009;**15**:1159–67

53 Wu SC, Chang JK, Wang CK, Wang GJ, Ho ML. Enhancement of chondrogenesis of human adipose derived stem cells in a hyaluronan-enriched microenvironment. *Biomaterials* 2010;**31**:631–40

54 Willerth SM, Sakiyama-Elbert SE. Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery. StemBook. Cambridge: Harvard Stem Cell Institute, 2008:1–18

55 Hwang Y, Sangaj N, Varghese S. Interconnected macroporous poly(ethylene glycol) cryogels as a cell scaffold for cartilage tissue engineering. *Tissue Eng Part A* 2010;**16**:3033–41

56 Liu SQ, Tian Q, Hedrick JL, Po Hui JH, Ee PL, Yang YY. Biomimetic hydrogels for chondrogenic differentiation of human mesenchymal stem cells to neocartilage. *Biomaterials* 2010;**31**:7298–307

57 Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor beta. *Biochim Biophys Acta* 1990;**1032**:79–87

58 Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. *Osteoarthritis Cartilage* 2007;**15**:597–604

59 Thorpe SD, Buckley CT, Vinardell T, O'Brien FJ, Campbell VA, Kelly DJ. The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. *Ann Biomed Eng* 2010;**38**:2896–909

60 Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). *J Tissue Eng Regen Med* 2008;**2**:81–96

61 Sekiya I, Larson BL, Vuoristo JT, Reger RL, Prockop DJ. Comparison of effect of BMP-2, -4, and -6 on *in vitro* cartilage formation of human adult stem cells from bone marrow stroma. *Cell Tissue Res* 2005;**320**:269–76

62 Miljkovic ND, Cooper GM, Marra KG. Chondrogenesis, bone morphogenetic protein-4 and mesenchymal stem cells. *Osteoarthritis Cartilage* 2008;**16**:1121–30

63 Chubinskaya S, Hurtig M, Rueger DC. OP-1/BMP-7 in cartilage repair. *Int Orthop* 2007;**31**:773–81

64 Gow DJ, Sester DP, Hume DA. CSF-1, IGF-1, and the control of postnatal growth and development. *J Leukoc Biol* 2010;**88**:475–81

65 Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on *in vivo* cartilage healing and repair. *Osteoarthritis Cartilage* 2006;**14**:403–12

66 Longobardi L, O'Rear L, Aakula S, Johnstone B, Shimer K, Chytil A, Horton WA, Moses HL, Spagnoli A. Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. *J Bone Miner Res* 2006;**21**:626–36

67 Ge Z, Hu Y, Heng BC, Yang Z, Ouyang H, Lee EH, Cao T. Osteoarthritis and therapy. *Arthritis Rheum* 2006;**55**:493–500

68 Park KH, Na K. Effect of growth factors on chondrogenic differentiation of rabbit mesenchymal cells embedded in injectable hydrogels. *J Biosci Bioeng* 2008;**106**:74–9

69 Moore EE, Bendele AM, Thompson DL, Littau A, Waggie KS, Reardon B, Ellsworth JL. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis Cartilage* 2005;**13**:623–31
70 Yamaoka H, Nishizawa S, Asawa Y, Fujihara Y, Ogasawara T, Yamaoka K, Nagata S, Takato T, Hoshi K. Involvement of fibroblast growth factor 18 in dedifferentiation of cultured human chondrocytes. *Cell Prolif* 2010;**43**:67–76
71 Davidson D, Blanc A, Filion D, Wang H, Plut P, Pfeffer G, Buschmann MD, Henderson JE. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. *J Biol Chem* 2005;**280**:20509–15
72 Scherer K, Schunke M, Sellckau R, Hassenpflug J, Kurz B. The influence of oxygen and hydrostatic pressure on articular chondrocytes and adherent bone marrow cells *in vitro*. *Biorheology* 2004;**41**:323–33
73 Hu JC, Athanasiou KA. The effects of intermittent hydrostatic pressure on self-assembled articular cartilage constructs. *Tissue Eng* 2006;**12**:1337–44
74 Miyanishi K, Trindade MCD, Lindsey DP, Beaupre GS, Carter DR, Goodman SB, Schurman DJ, Smith RL. Dose- and time-dependent effects of cyclic hydrostatic pressure on transforming growth factor-beta 3-induced chondrogenesis by adult human mesenchymal stem cells *in vitro*. *Tissue Eng* 2006;**12**:2253–62
75 Risbud MV, Sittinger M. Tissue engineering: advances in *in vitro* cartilage generation. *Trends Biotechnol* 2002;**20**:351–6
76 Vunjak-Novakovic G, Martin I, Obradovic B, Treppo S, Grodzinsky AJ, Langer R, Freed LE. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *J Orthop Res* 1999;**17**:130–8
77 Wang TW, Wu HC, Wang HY, Lin FH, Sun JS. Regulation of adult human mesenchymal stem cells into osteogenic and chondrogenic lineages by different bioreactor systems. *J Biomed Mater Res A* 2009;**88**:935–46
78 Lin YJ, Yen CN, Hu YC, Wu YC, Liao CJ, Chu IM. Chondrocytes culture in three-dimensional porous alginate scaffolds enhanced cell proliferation, matrix synthesis and gene expression. *J Biomed Mater Res A* 2009;**88**:23–33
79 Gonçalves A, Costa P, Rodrigues MT, Dias IR, Reis RL, Gomes ME. Effect of flow perfusion conditions in the chondrogenic differentiation of bone marrow stromal cells cultured onto starch based biodegradable scaffolds. *Acta Biomater* 2011;**7**:1644–52
80 Bueno EM, Laevsky G, Barabino GA. Enhancing cell seeding of scaffolds in tissue engineering through manipulation of hydrodynamic parameters. *J Biotechnol* 2007;**129**:516–31

(Received June 29, 2011, Accepted September 7, 2011)