www.vbripress.com, www.amlett.com, DOI: 10.5185/amlett.2011.1211 Published online by the VBRI press in 2011

Cartilage tissue engineering: current scenario and challenges

Chhavi Sharma[1], Sneh Gautam[1], Amit K. Dinda[2], Narayan C. Mishra[1,*]

[1]*Department of Paper Technology, Indian Institute of Technology Roorkee, Saharanpur Campus, Saharanpur 247001, U.P, India*

[2]*Department of Pathology, All India Institute of Medical Sciences, New Delhi 110029, India*

[*]Corresponding author. Tel: (+91)132 2714352; Fax: (+91) 132 2714310; E-mail: mishrawise@gmail.com

Received: 13 Jan 2011, Revised: 23 Feb 2011 and Accepted: 5 March 2011

ABSTRACT

Cartilage is an avascular connective tissue found in many locations in the body, such as, in the joints between the bones, rib cage, ear, nose and intervertebral discs. Cartilage plays a vital role in our body by working as a cushion between joints so that rubbing of bones against each other is prevented. It also holds some bones together, for instance, rib cartilage, and makes the area shock-proof. Cartilage is composed of single type of cells called chondrocytes. There are several diseases associated with cartilage, e.g., osteoarthritis, traumatic rupture of cartilage. These defects are not easy to repair as cartilage possesses limited self repair capacity due to the lack of a sufficient supply of healthy chondrocytes to the defective sites. Tissue engineered cartilage can serve as a lifelong treatment to such problems. Reconstruction of the cartilage can be achieved by use of appropriate cell source, scaffold, and growth factors. Development of a 3D cartilaginous skeleton have challenged the researchers for decades as the pursuit for suitable cell source, biomaterials and growth factor combination is not yet over. Various composite biomaterials and multiple growth factor approach are applied nowadays to regenerate cartilage. Stem cell has emerged as a potent source of cells for cartilage regeneration. This review highlightens the advances in cartilage tissue engineering by throwing light on cell sources, scaffold materials as well as on growth factors used so far in cartilage tissue engineering. It also reflects a range of problems and future perspectives to overcome the existing hurdles in cartilage regeneration.

Keywords: Cartilage; chondrocytes; tissue engineering; biomaterials.

Chhavi Sharma obtained her B.Sc. from C.C.S University, Meerut, India. She has completed her post-graduation (M.Sc. and M. Phil.) in Microbiology from C.C.S, University Campus, Meerut, India. At present, she is pursing Ph.D. in "Tissue engineering" field, from Department of Paper Technology, IIT Roorkee, India. Her main research interests are biomaterials and their application in biomedical area.

Amit Kumar Dinda is Additional Professor of Pathology in All India Institute of Medical Sciences, New Delhi. He did his MBBS from Calcutta Medical College and completed his postgraduation in Pathology at All India Institute of Medical Sciences. He did his PhD in the area of Cancer Biology at All India Institute of Medical Sciences. His area of work includes Renal & Immunopathology, Cell Biology, Biomaterials and Nanomedicine. He is in the editorial committee of 14 National-International journals. He is a Visiting Professor of Institute of Molecular Nephrology, Long Island Jews Medical Center & Elbert Einstein Medical College, New York, USA and Center for Biomedical Engineering, University of New South Wales, Sydney, Australia. He has published 200 articles in indexed journals, edited 4 books, written 14 chapters in books and acquired 4 patents.

Narayan Chandra Mishra is presently working as an assistant professor in the department of paper technology, IIT Roorkee, India. He did my B.Tech. (Chem. Tech) from University College of Science & Technology, Calcutta, M.Tech. (Chem. Engg.) from IIT Kanpur and PhD (Nanothin films) from Max – Planck Institute Colloids and Interfaces, Germany. He worked as a postdoctoral fellow in the area of tissue engineering and biomimetic membrane in Academia Sinica, Taiwan and Max –Planck Institute Colloids and Interfaces, Germany. He had also worked as visiting professor in Acdemia Sinica. His present areas of research are: "tissue engineering", "nanofibers" and "biofuels".

Contents

1. Introduction

Cartilage is an aneural, avascular flexible connective tissue. Cartilage functions in holding some bones together (e.g., rib cartilage) and make the area shock-proof. It also prevents the rubbing of bones against each other. Cartilage is composed of specialized type of cells called chondrocytes. The chondrocytes produce and maintain the cartilaginous matrix, which consists of collagen and proteoglycans, mainly. Cartilage grows and repairs slowly. This is because the chondrocytes are bound in a small space called lacunae, and thus they cannot migrate to the damaged areas. Besides, as there are no blood vessels, chondrocytes are supplied by diffusion with the help of pumping action generated by compression of the cartilage. Therefore, if cartilage is damaged, it is difficult to heal. Improper functioning or loss of cartilage results in certain disease like osteoarthritis, achondroplasia, etc. These disorders can be partially repaired through cartilage replacement therapy or other surgical interventions. But these treatments are often less than satisfactory, and seldom renovate full function or return the tissue to its native normal state [**1**]. Therefore, there is a tremendous need to find the ways to circumvent these problems.

Tissue engineering approach is emerging as a potential solution, by which the tissue that fails to heal spontaneously, can be repaired or regenerated fast. The major advantage of this approach is that tissue can be reconstructed in such a way, that they closely match the patient's requirements by using appropriate cells harvested from patient or a donor. Scaffolds, cells and growth factors together form the building blocks of tissue engineering, and thus these three are called tissue-engineering-triad. The basic principle is to utilize a scaffold (3-D supporting polymeric matrix) that is seeded with an appropriate cell source and laden with bioactive molecules to promote cellular growth, differentiation and maturation. Cartilage possesses some characteristics which make its regeneration feasible by tissue engineering. Firstly, it is a relatively simple tissue, consisting of only one type of cell, i.e., chondrocytes. Secondly, in vivo, the cartilage relies on diffusion rather than on a vascular network for its nutrition and excretion of waste products, and therefore the neovascularization of cell-scaffold constructs will probably be needless [**2**]. There have been a number of approaches to engineer cartilage, by using composite polymeric scaffold chondroprogenitor cells and multiple chondroinductive growth factors. The purpose of this review is to provide an update on various chondrogenic cell sources, biomaterials, growth factors as well as the current challenges and recent progress in the field of cartilage tissue engineering.

2. Cartilage: types, anatomy and morphology

Cartilage is classified into three types: hyaline cartilage (e.g., tracheal and articular), elastic cartilage (e.g., ear) and fibrocartilage (e.g., meniscus and intervertebral disc) [**3**]. The type of cartilage differs in the various locations of the body (at the articular surface of bones, in the trachea, bronchi, nose, ears, larynx, and in intervertebral disks) (**Table 1**). Hyaline cartilage lines the bones in joints, assisting them to articulate smoothly. Hyaline cartilage is made up of mostly type II collagen fibers. Spoiled hyaline cartilage is generally replaced with fibrocartilage, which unfortunately cannot bear weight due to its rigidity. Elastic cartilage is the most flexible cartilage because it contains more elastin fibers. The perfect balance of structure and flexibility provided by this cartilage helps keep tubular structures open. It is present in the outer ear, the larynx and the Eustachian tubes. Fibrocartilage is the strongest and most rigid type of cartilage, since it contains more collagen than other types. Collagen in fibrocartilage is more of type I collagen, which is tougher than type II. Fibrocartilage is found in the intervertebral discs. It helps connect tendons and ligaments to bones. It is present in other high-stress areas and protects the joints from shocks.

3. Cells used for cartilage tissue engineering

The cell type which is highly responsible for cartilage regeneration is chondrocytes. Chondrocytes can be isolated from patients/donors'organs containing cartilaginous tissues, e.g., menisci of knee joint, trachea, and nose. But these cells have limited availability and further they have the intrinsic tendency to lose their phenotype during expansion. Stem cells, because of their capacity to self-replicate, and their ability to differentiate into multiple cell lineages, have become an alternative cell source and good choice for cartilage tissue engineering.

Embryonic stem cells (ESC), pluripotent to differentiate into multiple cell lineages are a potential source of cells for cartilage tissue engineering, [**4, 5**] and are derived from pre-implantation embryo. There is a difficulty of directing ESCs'differentiation along a specific lineage because three embryonic germ layers of ESC (ectoderm, mesoderm and endoderm) give rise to different cell types, from which the specific cell type (e.g. chondrocytes for cartilage) is to be sorted out [**4**].

Adult mesenchymal stem cells (MSCs) can be easily isolated from various tissues including bone marrow, adipose tissue, periosteum, synovial membrane, muscle, trabecular bone, articular cartilage, and deciduous teeth (**Fig. 1**) and it has the potential to differentiate, upon stimulation by specific signalling molecules, into cell types of multiple lineages. Because of their ease of isolation and expansion, and their multipotential differentiation into cells of connective tissue lineages, adult stem cells are increasingly being considered as a promising alternative to differentiated chondrocytes for use in cell-based cartilage repair strategies [**5**].

Table 1. Anatomy and morphology of cartilaginous tissues [**3**].

Features	Articular cartilage	Meniscal Fibrocartilage	Intervertebral disc
Cell type	chondrocytes	Fibrochondrocytes	Fibrochondrocytes and chondrocyte
Cell density	1.4-1.7x10^4 cells/mm^3	Fusiform superficial cells ovoid cells in deeper zone	5.8x 10^4 cells/mm^3
Collagen type	Collagen II 65-80% w/w	CollagenI 55-65% dry wt Collagen II,V,VI 5-10% dry wt	Nucleus: collagenII Annulus: CollagenI, II, III,V
Fibre orientation	Superficial: Parallel Deep:orthogonal	Circumferential	CollagenI/II: Radially opposite
Proteoglycans	Aggrecan, 4-7% w/w	Aggrecan 4-7%	Large aggregating proteoglycan, but monomers are smaller.
Glycosaminoglycans	Chondrotin 6-sulphate Keratin sulphate	Chondrotin-6-sulpahate (40%) Chondrotin-4-sulphate (10-20%) Dermatan sulphate (20-30%) Keratin sulphate (15%)	Chondrotin6- sulphate Keratin suphate
Intrinsic repair capacity	Low	Low	Low
Synthetic activity	Low	Low	Low
Mitotic activity	Low	Low	Low

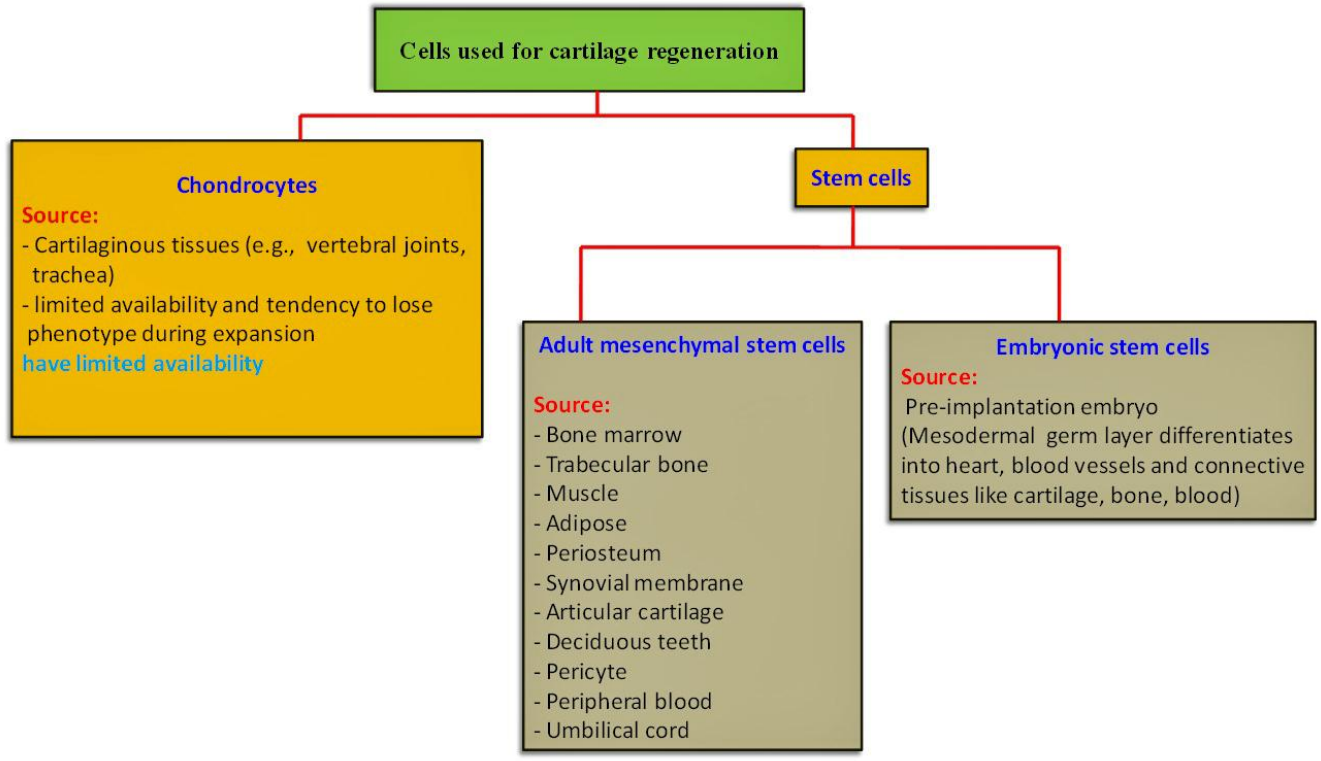

Fig. 1. Cell sources for cartilage tissue engineering.

Human umbilical cord-derived MSCs (hUCMSCs) are foetus-derived stem cells collected from discarded tissue (Wharton's jelly) after birth [**6**], and compared with human bone marrow-derived MSCs (hBMSCs), hUCMSCs have the advantages of abundant supply and also there is no donor site morbidity. These (hUCMSCs) have been successfully used for fibrocartilage tissue engineering and might also be most suitable alternative source to

chondrocytes [**6**]. Various cell sources used in cartilage tissue engineering, are summarized in **Fig. 1**.

4. Characteristics of 'scaffold and biomaterials' used for making scaffold, for cartilage regeneration

Scaffold is a 3-dimensional structure (house for cells) where upon the cells can attach favorably, and grow potentially. Materials used for building the scaffold are often called as biomaterials. An ideal biomaterial must be biocompatible, and it should possess some special characteristics that assist efficient cell adhesion, proliferation and differentiation into specific phenotype e.g., cartilage. Furthermore, the materials should be biodegradable and assist in remodeling, as the new cartilage forms and replaces the original construct. In this regard, the material should be non-toxic, non-attractive and non-stimulatory of inflammatory cells, and also non-immunogenic. In addition to this, the material should not corrode at physiological pH and at body temperature.

Table 2. Important characteristics of a tissue scaffold [**7**].

Characteristics	Explanation
3D structure	To assist cellular ingrowth and transport of nutrition and oxygen
Porosity	To maximize the space for cellular adhesion, growth, ECM secretion
Interconnected pores	To get adequate nutrition and oxygen supply, and to aid in cell migration
Vascular supportive	Should provide channels for angiogenesis for fast and healthy tissue regeneration
Nano-scale topography	To promote cell adhesion and better cell-matrix interactions
Mechanical strength	To withstand *in vivo* stresses
Biocompatible	Biologically compatible to the host tissue i.e. should not provide any rejection, inflammation or immune response
Biodegradable	The rate of degradation must perfectly match the rate of tissue regeneration and the degraded product(s) should not harm the living cells
Non-toxic	Should not evoke toxicity to tissues
Non-immunogenic	Should not evoke immunogenic response to tissues
Non-corrosive	Should not corrode at physiological pH and at body temperature
Surface modifiable	To functionalize chemical or biomolecular groups to improve tissue adhesion
Adequate mechanical strength	To withstand *in vivo* stimuli
Sterilizable	To avoid toxic contamination

A scaffold should also bear some unique characteristics, so that it can mimic the physiological functions of the natural extracellular matrix of the cells. The important characteristics of a scaffold for cartilage regeneration are summarized in **Table 2**. For cartilage tissue engineering, an ideal scaffold must possess high porosity and pore-to-pore interconnectivity. High porosity, usually above 90%, would allow sufficient space for *in vitro* cell adhesion, ingrowth and reorganization of cells [**7**]. Interconnected porous structure aids in cell migration and directly influences the diffusion of physiological nutrients and gases to the cells. Interconnected pores also aids in removal of metabolic waste and by-products from cells.

Nanoscale topography, another important property, having larger surface area compared to micro-scale and macro-scale surface structures, creates biomimmetic cellular environments that encourage the cellular growth considerably. The scaffold should also have enough mechanical strength to protect the cells contained within it, withstanding *in vivo* forces during joint movement. Finally, the scaffolds should be easily handled under clinical conditions, enabling fixation of the materials into the implanted site [**8**].

5. Scaffold materials for cartilage tissue engineering

A wide variety of materials based on both natural and synthetic polymers have been used to fabricate scaffolds for cartilage repair in a variety of forms, including fibrous structures, porous sponges, woven or non-woven meshes and hydrogels. Synthetic polymers have several advantages including their flexibility in tailoring the physical, mechanical and chemical properties, and easy processability of scaffold into desired shape and size. There is a huge array of synthetic polymers that have already been used successfully in cartilage tissue engineering (**Table 3**). Few synthetic polymers are under current clinical investigation for their potential use in cartilage repair e.g., poly-hydroxyalkanoates (PHAs). PHAs have been investigated to possess broad range of mechanical and biodegradation properties and thus, have a good scope in fabricating scaffold for cartilage repair [**9**].

Natural polymers are cost effective and eco-friendly, and have good biodegradability, low toxicity, low manufacture costs, low disposal costs and renewability [**7**]. Moreover, they have the properties of biological signaling, cell adhesion, cell responsive degradation and re-modeling, which are the most important controlling factors for cartilage tissue regeneration. A great number of natural materials have also been studied to fabricate scaffold for cartilage repair (**Table 3**).

Despite several advantages of natural and synthetic polymers, both of them offer some disadvantages also. The drawbacks associated with natural polymers are that, they undergo rapid degradation and there is a possibility of losing their biological properties during scaffold fabrication processes. Furthermore, the risk of immunorejection and disease transmission imposes the necessity of proper screening and purification of the natural polymer [**7**] The major disadvantages associated with synthetic polymers, include adverse tissue reactions caused by acidic degradation products, and lack of cellular adhesion and interaction.

Table 3. Scaffold materials used for cartilage tissue engineering.

Synthetic materials	Natural Materials
Polyvinyl alcohol [**18**]	Cellulose [**10**]
Poly (L-lactide-co-3-caprolactone) (PLCL) [**19**]	Collagen [**11**]
Polyglycolic acid (PGA) [**20**]	Hyaluronic acid [**12**]
Polylactic acid(PLLA) [**5**]	Dextrans [**7**]
Polylactic-co-glycolic acid(PLGA) [**21**]	Fibrin [**13**]
Polyurethane [**22**]	Chitosan [**14**]
Polybutyric acid [**23**]	Carboxymethyl chitosan [**15**]
Polytetrafluorethylene [**24**]	Alginate [**16**]
Polyethyleneterephtalate [**24**]	Agarose [**17**]
Poly(N-isopropylacrylamide) [**25**]	
Polyethylene glycol fumerate [**26**]	

The disadvantages associated with synthetic and natural polymers are generally overcome by using composite scaffolds made of two or more polymers, and by functionalization of the polymers, which can create suitable environment for cartilage regeneration. Composites combine various properties of different polymers, for controlling biodegradation, cell adhesion, proliferation and differentiation. Currently, various composite materials are being used to fabricate scaffold for cartilage tissue regeneration [**27**]. Composite scaffold made of gelatin, hyaluronic acid, chondroitin-6-sulfate, and fibrin was reported to be used to enhance chondrogenesis [**28**]. Composite scaffold of hydroxyapatite combined with chitosan, was used for the treatment of osteochondral defects [**27**].

Functionalization can introduce various functional groups in the polymer, which might provide specific cues to the cells for cartilage regeneration, and nowadays, functionalized polymers are extensively used to overcome the problems associated with natural and synthetic polymers. The integrin binding activity of adhesion proteins can be reproduced by introducing short synthetic peptides, containing the Arg-Gly-Asp (RGD) or other similar adhesion sequences in the polymer, which enhances cell adhesion [**29**]. A wide array of materials had been modified with peptide ligands to promote chondrogenesis [**30**]. For example, the addition of RGD sequences in polyethylene glycol (PEG) hydrogels that are normally devoid of cell-matrix interactions, leads to an increase in human MSCs viability [**31**]. Hwang *et al.*, described that encapsulation of human embryonic stem-cell-derived cells in RGD-modified hydrogels led to increased cartilage formation [**32**]. In another study, chitosan-alginate-hyaluronate complexes modified with RGD-containing proteins were used to increase *in vitro* cartilage formation by rabbit chondrocytes [**33**]. RGD-coupled alginate hydrogels in which osteoblasts and chondrocytes were co-transplanted led to the formation of growing tissues that structurally and functionally resembled a growth plate cartilage [**34**].

Besides this, a variety of materials were developed which are available in injectable form, microspheres and thermoreversible hydrogels, which are mainly used for *in situ* tissue-regeneration [**25**]. An injectable and *in situ* gelable poly(*N*-isopropylacrylamide)-grafted gelatin scaffold was developed, that might serve to fully fill the space of cartilaginous defects of complex shapes [**35**]. In another study, injectable biodegradable chitosan-hyaluronic acid based hydrogels were used for in situ cartilage tissue engineering [**36**].

6. Growth factors for cartilage regeneration

Growth factors are basically the signaling molecules that coax the cells to differentiate into specific phenotype. The most influential factors that favours chondrocyte regeneration includes polypeptide growth factor, transforming growth factor β (TGF-β), insulin-growth factor I (IGF-I), basic fibroblast growth factor (FGF-2), bone morphogenetic growth factors (BMPs), Hedgehog (hh), wingless (Wnt) proteins (**Table 4**).These growth factors are used individually or in combination to enhance chondrogenesis.

Polypeptide growth factors play a major role in the regulation of cell behaviour, including that of chondrocytes [**3**]. However, it was also found to inhibit the transcription of cartilage specific matrix genes in long term cultures [**37**]. Platelet derived growth factor (PDGF) play a role in chondrocyte-differentiation and matrix synthesis. TGF-β1, TGF-β2 and TGF-β3 are another class of growth factor that are found to promote in vitro differentiation of chondrocytes and thus support matrix synthesis [**5**].

Another family of growth factor includes insulin growth factors, composed of two ligands (IGF-1 and IGF-2), two cell surface receptors (IGF1R and IGF2R), at least six different IGF binding proteins (IGFBP-1 to IGFBP- 6), and multiple IGFBP proteases, which regulate IGF activity in several tissues. IGF-1 is the most studied form with respect to cartilage repair [**38**]. FGF-2 is a potent mitogen for articular chondrocytes, and it also supports chondrocytes to be in differentiated state within a 3D culture system [**39**]. FGF-18 was also involved in cartilage repair.

Besides this, BMPs is a group of growth factors which plays a vital role in cartilage repair. They are also known as cytokines or metabologens [**40**]. Several BMPs are also named as 'cartilage-derived morphogenetic proteins' (CDMPs) [**41**]. There are 20 known BMPs till now. Out of these, the main BMPs involved in cartilage repair are BMP-1, BMP-2, BMP-4, BMP-5, BMP-7, BMP-8a, BMP-9 and BMP-12. BMP activity is a requisite for correct cartilage formation [**42**]. BMPs interact with specific receptors on the cell surface, referred to as bone morphogenetic protein receptors (BMPRs). BMPs are involved in all phases of chondrogenesis and directly regulate the expression of several chondrocyte specific genes. Thus, this class of signaling molecules has a strong effect on chondrocyte proliferation and matrix synthesis. The BMP-induced chondrogenic differentiation appears to be mediated through gap junction-mediated intercellular communication [**43**]. BMP-1 is a metalloprotease that acts on procollagen I, II, and III. It is involved in cartilage development. BMP-2 was reported to play an important role in the early stages of cartilage repair by recruiting local sources of skeletal progenitors within periosteum and endosteum, and by determining their differentiation towards the chondrogenic and osteogenic lineages [**44**]. BMP-2, -4 and -5 have been reported to up-regulated cell proliferation and matrix production in growth plate chondrocytes [**45**]. In cultured human normal articular ankle chondrocytes, BMP-2, -4, and -7 stimulated aggrecan synthesis [**46**]. BMP-5 and BMP-8a performs functions in cartilage development. BMPs are also used in combination with TGF-β3 and TGF-β1 to promote chondrogenesis [**25**]. The combination of BMP-2 and TGF-β3 induced the chondrogenic phenotype in cultured bone-marrow derived human MSC pellets. For synovium-derived human MSCs, it was necessary to combine BMP-2 with TGF-β3 and dexamethasone for optimal chondrogenic differentiation [**47**]. BMP-2 and BMP-7 have received FDA (food and drug administration, U.S.) approval for human clinical uses [**40**].

Various Wnt members are involved both in early and late skeletal development, and play a role in the control of chondrogenesis [**38**]. Although there are varieties of growth

factors, but till now it is difficult to recommend a single growth factor or a cocktail of different growth factors to promote cartilage repair either during *in vitro* or *in vivo* cartilage tissue engineering. This is because the actions of growth factors are not yet completely understood or sometimes even contradictory. Some studies have shown the synergistic effects of TGF-β and FGF-2, in combination [**48**]. They interact to modulate their respective action, creating effector cascades and feedback loops of intercellular and intracellular events that control articular chondrocyte functions. However, some growth factors may elicit seemingly opposite effects under different experimental conditions [**49**]. **Table 4** summarizes some of the important growth factors that enhance chondrogenesis.

Table 4. Growth factors evaluated for their effects on chondrocyte growth and matrix production.

Growth factors	Chondrocyte growth	Matrix production
TGF-β	Promotes differentiation	Proteoglycan synthesis
FGF-α	Mitogenic differentiation	Matrix synthesis
IGF-1	Mitogenic differentiation	Matrix synthesis
PDGF(platelet derived growth factors)	Mitogenic differentiation	Matrix synthesis
BMP-1	Cartilage proliferation	Collagen synthesis
BMP-2	Promotes cartilage formation by inducing production of cartilage matrix.	Collagen synthesis
BMP-4	Promotes cartilage formation by inducing MSCs to become chondroprogenitors and chondrocyte maturation.	Matrix synthesis
BMP-5	Chondrocyte proliferation	Matrix synthesis
BMP-9	Potent anabolic factor for juvenile cartilage	Matrix synthesis
BMP-12 (GDF7)	Modulates in vitro cartilage formation in a similar fashion as BMP-2 does	Collagen synthesis

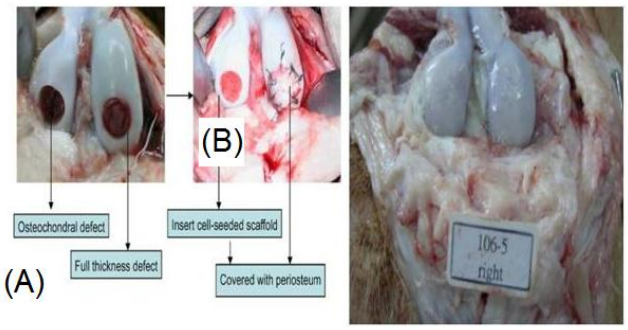

Fig. 2. (A) Surgical procedure in the study (tissue engineering) group. (B) Result with good gross appearance [**50**].

5. Recent advances in cartilage tissue engineering

Currently, a lot of scientists are focussing on research in the area of cartilage tissue engineering, and great advances have been made in cartilage regeneration. A combination of allogenous chondrocytes and gelatin–chondroitin–hyaluronan tri-copolymer scaffold was found to be successful for cartilage repair in a porcine model (**Fig. 2**) [**50**]. Human polymer-based cartilage tissue engineering grafts made of human autologous chondrocytes, human fibrin and PGA, are found to be clinically suitable for the regeneration of articular cartilage defects (**Fig. 3**) [**20**].

Gene modified cartilage was regenerated by introducing the TGF-β1 gene into chitosan scaffolds [**51**] and implanted into the full-thickness articular cartilage defects of rabbits' knees. To the surprise, twelve weeks after implantation, the defects were found to fill with regenerated hyaline like cartilage tissue (**Fig. 4**) [**51**]. Rabbit knee cartilage regeneration was also possible by using highly-elastic three-dimensional PLCL scaffold and chondrocytes (**Fig. 5**) [**5**]. In another study, a sheep articular cartilage defect had been repaired by using chitosan hydrogels (**Fig. 6**) [**52**].
Recently, Cui et al., 2011, showed that cartilage defect in pigs, can be repaired, by using osteochondral composite scaffold of PLGA and tricalcium phosphate, and chondrocyte- PLGA construct (**Fig. 7**), but cartilage regeneration by using osteochondral composite scaffold of PLGA and tricalcium phosphate, was found to perform better [**21**].

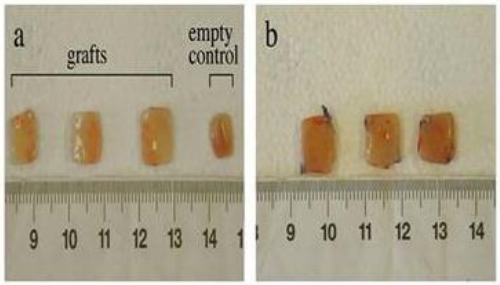

Fig. 3. Polymer-based cartilage grafts 6 weeks after implantation into nude mice. Single layer transplants showed good form stability, a smooth surface and marginal size reduction compared to the control (a). Double layer implants with residual sutures at the edge of each construct (b). These double layer constructs showed good form stability, a smooth surface and marginal size reduction comparable to the single layer constructs [**20**].

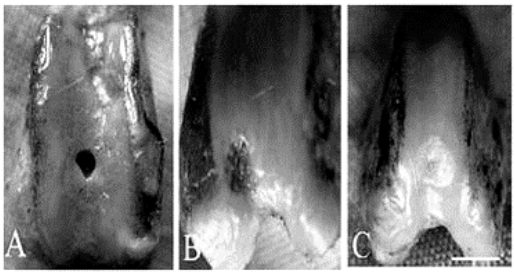

Fig. 4. Macroscopic appearance of defects 12 weeks after operation. (A) Defect filled with chitosan only. (B) Defect filled with chitosan and nontransfected MSCs. (C) Defect filled with chitosan and TGF-β1-transfected MSCs [**51**]. Bar: 7.0 mm .

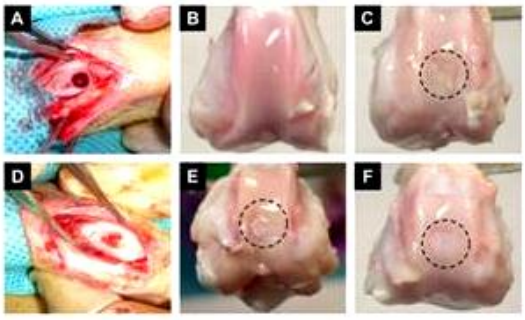

Fig. 5. Photographs of rabbit knee articular cartilage defects immediately after creation (A), and after treatment with the scaffolds (D). The images are of the positive controls (B) and the defects at 12 weeks without treatment (C), with PLCL scaffold treatment (E), and with PLGA scaffold treatment (F) [**5**].

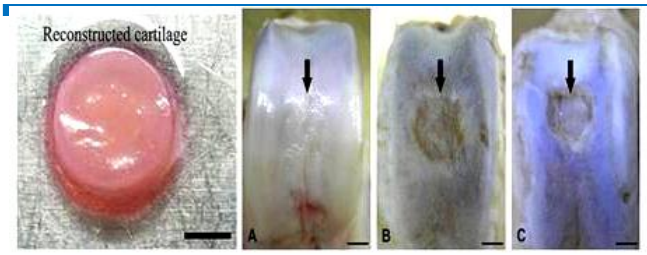

Fig. 6. Gross observation of the articular cartilage repair at 24 weeks post-operation. A, the defect part of the cartilage in the experimental group was covered by the smooth, consistent, glistening white hyaline tissue nearly indistinguishable from the surrounding normal cartilage. No clear signs of margin with normal cartilage could be spotted on the surface of the regenerated areas; B, The defects in control group 1 were partially repaired with fiber-like tissue, leaving a small depression in the defect areas; C, The defects in control group 2 detected a thin and irregular surface tissue, with obvious defects and cracks surrounding the normal cartilage. Arrow: the defect; Bar ¼ 0.5 cm [**52**]. (Scale bar = 0.5cm).

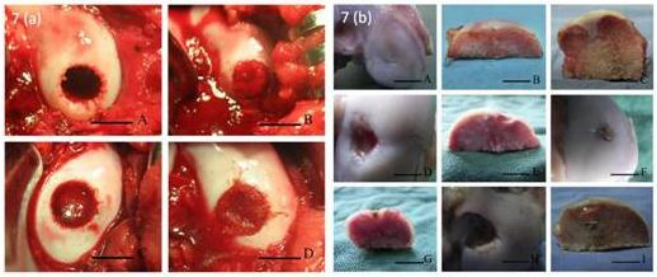

Fig. 7 (a). Implantation surgery. (A) An osteochondral defect (diameter, 8 mm; depth, 8 mm) was generated in the distal weightbearing surface of the medial condyle of the femur. (B) The biphasic construct was manually "press-fit"-inserted into the defect. (C) A cartilage defect (diameter, 8 mm; depth, 2 mm) was generated in the distal weightbearing surface of the medial condyle of the femur. (D) The PLGA construct was inserted into the defect and sutured to the surrounding native cartilage with interrupted stitches. (b): Gross and cross-sectional appearance of the repaired cartilage at 6 months postoperatively after surgery. From the gross (A) and cross-sectional (B) appearance, most of the osteochondral defects were repaired with neo-cartilage and neo-bone in the composite group. Subchondral bone in some defects was partly replaced by neocartilage (C). From the appearance of (D) and (E) appearance, defects were occupied with soft tissue with little cartilage regeneration in the TE group. Another defect in the TE group were repaired with engineered cartilage into a relatively smooth surface (F and G). Defects in the control group show no obvious repair in the defects; only a small amount of fibrous tissue was found within the defect site (H and I) (bar scales: 8000 μm) [**21**].

Since last few years, many scientists have been attempting to use polymeric nanofibers for cartilage regeneration [**8**]. Various types of synthetic and natural polymers, have been processed into nanofibrous scaffolds for the application in cartilage tissue engineering [**53**]. It had been possible to regenerate a superficial zone of articular cartilage by seedng mesenchymal stem cells on oriented nanofibrous scaffold made of collagen (**Fig. 8**) [**53**]. Chen et al., 2011, modified electrospun PLLA nanofibers by plasma treatment and then cationized by gelatin immobilization, for its application in cartilage tissue engineering. This modified scaffold was seeded with chondrocytes and implanted subcutaneously in a rabbits' backbone which resulted in the cartilage formation after 28 days of implantation (**Fig. 9**) [**54**].

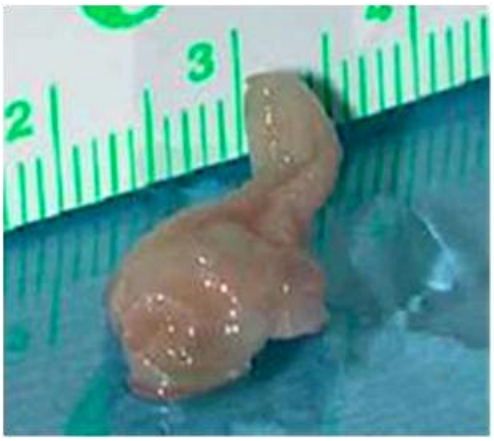

Fig. 8. Articular cartilage sample prepared from normal human chondrocytes using electrospun collagen II scaffold [**53**].

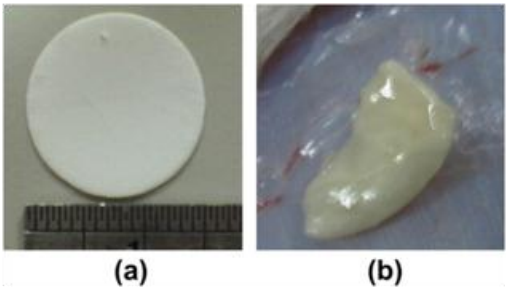

Fig. 9. Bulk appearances of (a) Cationized gelatine-PLLA- nanofibreous membrane (CG-PLLA NFM) before implantation and (b) chondrocyte–NFM constructs 4 weeks post-implantation [**54**].

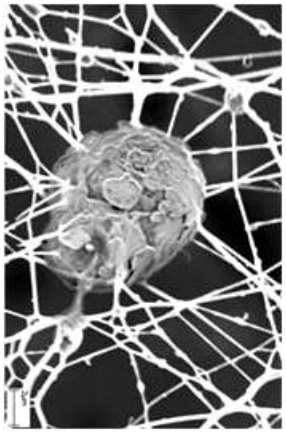

Fig. 10. Growing cartilage: A cartilage cell grows on a textured surface coated with carbon nanotubes [**55**].

Recently, a group of scientists are focussing on the use of carbon nanotubes to engineer a cartilage tissue. The researchers mixed the nanotubes into sheets of polycarbonate urethane, an FDA-approved polymer, which have a rough surface and also readily conduct electricity [**55**]. When they cultured chondrocytes on these sheets, the cells grew more densely on the roughened surface compared to a smooth polycarbonate surface (**Fig. 10**). The researchers' team believes that the nanostructures alter the surface properties of a material, thus promoting it to attract

more proteins that can stick to cells to enhance cell-scaffold interaction.

8. Obstacles and future perspectives in cartilage regeneration

A number of obstacles in the way of optimal cartilage repair remain to be surmounted. These hurdles are associated with the three cornerstones of cartilage tissue engineering: cells, scaffold and growth factors. The major challenging issues associated with cartilage tissue engineering are summarised in **Fig. 11**. The key cell related issue is how to enhance chondrogenesis, and how biophysical, chemical and mechanical stimuli can be introduced within the cells to promote chondrogenesis. chondrogenesis in MSCs [**57**]. Thus, there is a need to focus on the study of releasing multiple growth factors at a time, to favour the production of more natural cartilage tissue.

Furthermore, it should be kept in mind that most of the studies in cartilage tissue engineering were performed using mostly young adult and even fetal animal cells, and not with cells from elderly osteoarthritis patients. Therefore, extensive research on using the cells from elderly osteoarthritis patients will be needed to extend the results for treating human cartilage defects.

The final and probably most difficult problem is how to translate the results of *in vitro* and animal studies into clinical application and thereby introduction to market. To date, a large number of new cartilage products and growth factor carrier materials have been developed, but only few

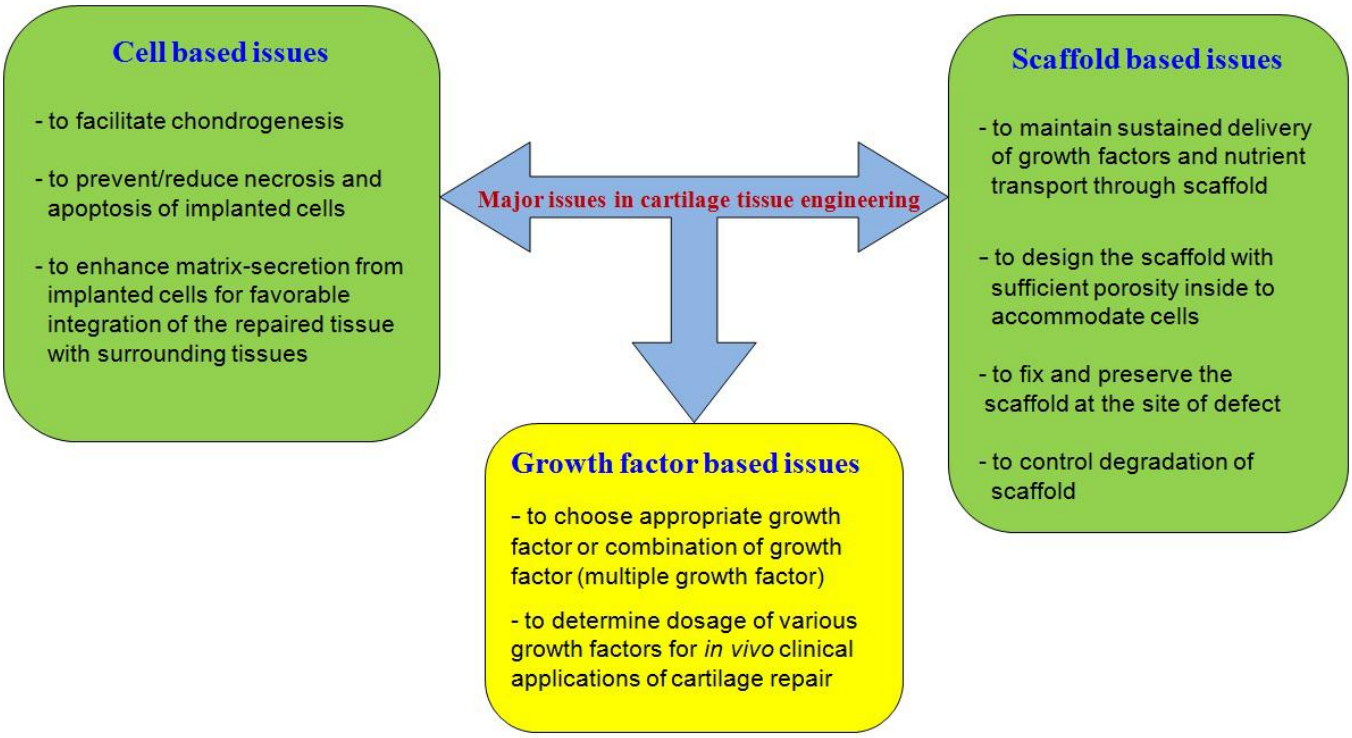

Fig. 11. Major challenging issues in cartilage tissue engineering.

Major scaffold related issue includes the fabrication of scaffold in such a way that can exactly mimic the natural environment of the tissue. As far as growth factors are concerned, very little is known about the sequences and concentrations of growth factors for which cartilage regeneration is optimized.

Although cartilage regeneration is complex and affected by multiple growth factors which are released in a well-orchestrated manner, but most of the studies involved use of single growth factor. A little number of systems have been developed been developed that allow the biphasic release of dual growth factors. An example is the encapsulation of IGF-1 in gelatin spheres that are then suspended in oligo[poly(ethylene glycol) fumarate] scaffolds containing TGF-β1.This system provides a fast release of TGF-β1 and a more sustained delivery of IGF-1 to promote cartilage repair [**56**]. Dual growth factor releasing alginate based nanoparticle/hydrogel system was recently used to deliver BMP-7 and TGFβ-2 to promote

of them are approved for use in patients. Some of the major reasons for the small numbers of approved products include, hurdles posed by the cost of development, cost of goods, manufacturing scale-up, sterility and patent issues. In addition, there are many regulatory hurdles, including quality control and quality assurance for consistent manufacturing, comparability studies required for component and process changes, establishment of shipping and storage conditions, and appropriate shelf life [**58**]. Already existing as well as new cartilage engineering products should be approved by the government organizations so that they can be easily available for clinical applications. Most importantly, major efforts are needed to move the obtained results in laboratory towards the application for treating human cartilage defects [**58**]. This can be solved, to certain extent, by making the cost effective tissue engineered products. Moreover, the tissue engineered solution for cartilage repair must involve minimal donor site morbidity and should be free of any

peri-operative complications. This will attract the patients suffering from cartilage defects towards tissue engineering solution rather than opting for other surgical interventions and prosthetics. Thus, it is clear that despite progress, further advances in cartilage tissue engineering are required to find optimal conditions for cartilage regeneration economically.

9. Conclusion

Cartilage tissue engineering serves as a thriving area that has revolutionized the treatment of disease and damaged cartilage. It is an alternative to currently used techniques that are full of limitations and do not serve as a permanent lifetime therapy. Although, there is progress in orthopaedic surgery, but the lack of efficient modalities in treatment of large chondral defects has prompted research on tissue engineering. Several points that still required more directed research includes: i) defining the best cell candidates among chondrocytes and multipotent progenitor cells (e.g., multipotent mesenchymal stromal cells), in terms of readily available sources for isolation, expansion and repair potential; ii) engineering biocompatible and biodegradable scaffolds for enhancing growth and proliferation of cells; iii) identifying highly specific growth factors and the appropriate scheme of their application that will promote chondrogenesis and iv) there is a need to study more on simultaneous release of multiple growth factors to produce more natural cartilage tissue. Besides these, efforts must be made to avail the approved cartilage tissue engineering products from preclinical trials to the market. We hope that the development in this field will find more tremendous applications in our aging population by overcoming all the major roadblocks in cartilage regeneration in near future.

10. References

1. Tuli, R.; Li, J.;Tuan, R.S. *Arthritis Res Ther.* **2003**, *5*, 235. **DOI:** 10.1186/ar991
2. Jallali, N.; James, S.; Elmiyeh, B.; Searle, A.; Ghattaura, A.; Dwivedi, RC.; Kazi, R.; Harris, P. *Indian J. Cancer.* **2010**, *47*, 274. **DOI:** 10.4103/0019-509X.64722
3. Kinner, B.; Spector, M. *J. Orthop. Res.* **2001**, *19*, 233. **DOI:** 10.1016/S0736-0266(00)00081-4
4. Junying, Yu.; Thomsom, J. A. *Regenerative medicine*; Terese Winslow, **2006** ,pp 1-2
5. Chen, F.H., Rousche, K. T., Tuan, R.S. *Nature clinical practice rheumatology*. **2006,** *2 (7)*, 373. **DOI:** 10.1038/ncprheum0216
6. Editorial, *Joint Bone Spine.* **2010**, *77*, 283. **DOI:** 10.1016/j.jbspin.2010.02.029
7. Puppi, D.; Chiellini, F.; Piras, A.M.; Chiellini, E. *Progress in Polymer Science*. **2010**, *35*,403. **DOI:** 10.1016/j.progpolymsci.2010.01.006
8. Stoop, R. *Int. J. Care Injured.* **2008**, *39(1)*, 77. **DOI:** 10.1016/j.injury.2008.01.036
9. Vinatier, C.; Mrugala, D.; Jorgensen, C.; Guicheux, J., Noël, D. *Trends Biotechnol.* **2009**, *27*, 307. **DOI:** 10.1016/j.tibtech.2009.02.005
10. Pulkkinen, H., Tiitu, V., Lammentausta, E. et al. *Biomed Mater Eng.* **2006**,16(4),S29.
11. Misch,C.E.; Suzuki,J.B. *Tooth extraction, socket grafting, and barrier membrane bone regeneration***.** In: Contemporary Implant Dentistry. 3rd ed. St Louis, MO: Mosby. **2007**, pp. 870-904
12. Fan, H.; Hu, Y.; Li, X. *Int. J. Artif. Organs.* **2006**, *29(6)*, 602. **PMID:** 16841290
13. Wang, Y.; Kim, H.J.; Vunjak-Novakovic, G., et al. *Biomaterials*. **2006**, *27(36)*, 6064. **DOI:** 10.3390/ijms10041514
14. Shi, C.; Zhu, Y.; Ran, X.; Wang, M.; Su, Y.; Cheng, T.; *J Surg Res.* **2006**, *133(2)*, 185. **DOI:** 10.1016/j.jss.2005.12.013
15. Suh, J.K.F.; Matthew, H.W.T. *Biomaterials*, **2000**, *21*, 2589. **DOI:** 10.1016/S0142-9612(00)00126-5
16. Gutowska, A.; Jeong, B.; Jasionowski, M. *Anat Rec.* **2001,** *263(4)*, 342. **DOI:** 10.1002/ar.1115
17. Salcedo, S.; Nieto, A.; Vallet-Reg, A. *Chemical Engineering Journal*. **2008**, *137*,62. **DOI:** 10.1016/j.cej.2007.09.011
18. Abbas, A.A.; Lee, S.Y.; Selvaratnam, L.; Yusof, N.; Kamarul, T. *European Cells and Materials.* **2008**, *16(2)*, 50. **DOI:** 10.1002/jbm.a.33005
19. Gong, Y.; He, L.; Li, J.; Zhou, Q.; Ma, Z.; Gao, C.; Shen, J. *J Biomed Mater Res B Appl Biomater.* **2007**, *82(1)*,192. **DOI:** 10.1002/jbm.b.30721
20. Endres, M.; Neumannb, K.; Schr¨oder, S.E.A. et al., *Tissue and Cell* **2007,** *293*, 39. **DOI:** 10.1016/j.tice.2007.05.002
21. Cui, W.; Wang, Q.; Chen, G, et al., *Journal of Bioscience and Bioengineering*, **2011,** *xx (xx)*, xxx. **DOI:** 10.1016/j.jbiosc.2010.11.023
22. Lee, C.R.; Grad, S.; Gorna, K. *Tissue Eng*. **2005**, *11*, 1562. **DOI:** 10.1089/ten.2005.11.1562
23. Larno, S.; Calvet, M.C.; Calvet, J., et al. *Life Sci.* **1985**, *36(21)*, 2069. **DOI:** 10.1016/0024-3205(85)90458-8
24. Messner, K.; Gillquist, J. *Biomaterials* **1993**, *14***,** 513. **DOI:** 10.1016/0142-9612(93)90240-3
25. Chen, J.P.; Cheng, T.H. *Macromol. Biosci*. **2006**, *6(12)*, 1026. **DOI:** 10.1002/mabi.200600142
26. Holland,T.A.; Tabata, Y.; Mikos, A.G. *J. Control Release.* **2005**, *101(1−3)*, 111. **DOI:** 10.1016/j.jconrel.2004.07.004
27. Oliveira, J.M.; Rodrigues, M.T.; Silva, S.S. *Biomaterials.* **2006**, *27(36)*, 6123. **DOI:** 10.1016/j.biomaterials.2006.07.034
28. Chou, C.H.; Cheng, W.T.; Kuo, T.F. *J. Biomed. Mater. Res. A.* **2007**, *8(3)*, 757. **DOI:** 10.1002/jbm.a.31186
29. Garcia, A.; Collard, D.; Keselowsky, B. *Biomimetic materials and designs*, New York: Marcel Dekker, Inc. **2002**, 29.
30. Harbers, G.; Barber, T.; and Stile, R. *Biomimetic materials and design*. New York: Marcel Dekker, Inc. **2002**, 55.
31. Nuttelman, C.R.; Tripodi, M.C; Anseth, K.S. *Matrix Biol.* **2005**, *24(3)*, 208. **PMID:** 15896949
32. Hwang, N.S.; Varghese, S.; Zhang, Z. *Tissue Eng.* **2006**, *12(9)*, 2695. **PMID:** 16995803
33. Hsu, S.H.; Chang, S.H.; Yen, H.J. *Artif. Organs*. **2006**, *30(1)*, 42. **PMID:** 16409397
34. Alsberg, E.; Anderson, K.W.; Albeiruti, A. *Proc. Natl. Acad. Sci.*, U S A. **2002**, *99(19)*, 12025.
35. Ibusuki, S.; Fujii, Y.; Iwamoto,Y.; Matsuda, T. *Tissue Engineering*. **2003**, *9(2)*, 371. **PMID:** 12740100
36. Tan, H.; Chu, C.R.; Payne, K.A.; Marra, K.G. *Biomaterials*. **2009,** *30(13)*, 2499. **PMID:** 19167750
37. Sandell, L.J.; Daniel, J.C. *Tissue res*.**1988**, *17*, 11. **PMID:** 3383569
38. Vinatier, C.; Bouffi, C.; Merceron, C et al., *Current Stem Cell Research & Therapy.* **2009,** *4*, 318. **DOI:** 1574-888X/09 $55.00+.00
39. Martin, I.; vunjak-Novakovic, G.; Yang, J., Langer, R. and Freed, L. E. *Exp. Cell Res*. **1999**, *253*, 681. **DOI:** 10.1006/excr.1999.4708
40. Reddi, A.H.; Reddi, A. *Cytokine & growth factor reviews.* **2009**, *20(5-6)*, 341. **DOI:** 10.1016/j.cytogfr.2009.10.015
41. Bleuming, S.A.; He, X.C.; Kodach, L.L.; Hardwick, J.C.; Koopman, F.A.; TenKate, F.J.; Van Deventer, S.J.; Hommes, D.W.;

Peppelenbosch, M.P.; Offerhaus, G.J.; Li, L.; van den Brink, G.R." *Cancer Research*. **2007**, *67 (17)*, 8149.
DOI: 10.1158/0008-5472.CAN-06-4659
42. Pan, Q.; Wu, Y.; Lin, T.; Yao, H.; Yang, Z.; Gao, G. et al. *Biochem Biophys Res Commun*. **2009**, *379(2)*, 356.
DOI: 10.1016/j.bbrc.2008.12.062
43. Zhang, W.; Green, C.; Stott, N.S. *J Cell Physiol* **2002**, *193(2)*, 233.
DOI: 10.1002/jcp.10168
44. Yan, Y.Y.; Shirley, L.; Chuanyong, L.; Colnot, C. Bone. **2010**, *47*, 65.
DOI: 10.1016/j.bone.2010.03.012
45. Mailhot, G.; Yang, M.; Mason-Savas, A.; Mackay, C.A.; Leav, I.; Odgren, P.R. *J Cell Physiol* **2008**, *214(1)*, 56.
DOI: 10.1002/jcp.21164
46. Chubinskaya, S.; Segalite, D.; Pikovsky, D. Hakimiyan ,A.A; Rueger, D.C. *Growth Factors* **2008**, *26(5)*, 275.
DOI: 10.1080/08977190802291733
47. Shirasawa, S. *et al. J Cell Biochem* **2005**, *97*, 84.
DOI: 10.1002/jcb.20546
48. Mattey, D.L.; Dawes, P.T.; Nixon, N.B.; Salter, S. *Ann. Rheum. Dis*. **1997**, *56*, 426.
DOI: 10.1136/ard.56.7.426
49. Trippel, S.B. *J. Rheumatol*. **1995**, *43*, 129.
PMID: 7752116
50. Chang, C.H.;Tzong-Fu B.; Lina,C.C.; Choua, C.H.; Chene,K.H.; et al., Biomaterials **2006**, *27* 1876.
DOI: 10.1016/j.biomaterials.2005.10.014
51. Guo, C.; Liu,X.; Huo,J.Z.; Jiang, C.; et al., *Journal of bioscience and bioengineering* **2007**, *103(6)*,547.
DOI: 10.1263/jbb.103.547
52. Hao, T.; Wenza, N.; Cao, J.-K. et al., *Osteoarthritis and Cartilage* **2010**, *18*, 257.
DOI: 10.1016/j.joca.2009.08.007
53. Sell, S.; Barnes, C.; Smith, M.; McClure, M.; Madurantakam,P.; Grant,J.; Mcmanus, M.; Bowlin, G. *Polym Int*. **2007**, *56*, 1349.
DOI: 10.1002/pi.2344
54. Chen, J.P.; Su, C.H. *Acta Biomaterialia* **2011**, *7*, 234.
DOI: 10.1016/j.actbio.2010.08.015
55. www.technologyreview.com/biotech/20893/
56. Holland,T.A.; Tabata, Y.; Mikos, A.G. *J. Control Release.* **2005**, *101(1−3),* 111.
DOI: 10.1016/j.jconrel.2004.07.004
57. Lim, S.M.; Oh, S.H.; Lee, H.H.; Yuk, S.H.; Im, G.I.; Lee, J.H. J. *Mater Sci Mater Med*. 2010, *21(9)*, 2593.
DOI: 10.1007/s10856-010-4118-1
58. Langer, R. and Peppas, N. *AIChE Journal*. **2003**, *49(12)*, 2990**.**
DOI: 10.1002/aic.690491202

