REVIEW ARTICLE

Repair of Articular Cartilage Defects: Review and Perspectives

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Articular cartilage defects heal poorly and lead to catastrophic degenerative arthritis. Clinical experience has indicated that no existing medication substantially promotes the healing process and the cartilage defect requires surgical replacement, preferably with an autograft. However, there is a shortage of articular cartilage that can be donated for autografting. A review of previous unsuccessful experiences reveals the reason for the current strategy to graft cartilage defects with regenerated cartilage. Autologous cartilage regeneration is a cell-based therapy in which autogenous chondrocytes or other chondrogenic cells are cultured to constitute cartilaginous tissue according to the principles of tissue engineering. Current studies are concentrating on improving such techniques from the three elements of tissue engineering, namely the cells, biomaterial scaffolds, and culture conditions. Some models of articular cartilage regeneration have yielded good repair of cartilage defects, in animal models and clinical settings, but the overall results suggest that there is room for improvement of this technique before its routine clinical application. Autologous cartilage regeneration remains the mainstay for repairing articular cartilage defects but more studies are required to optimize the efficacy of regeneration. A more abundant supply of more stable cells, i.e. capable of maintaining the phenotype of chondrogenesis, has to be identified. Porous scaffolds of biocompatible, biodegradable materials that maintain and support the presentation of the chondrogenic cells need to be fabricated. If the cells are not implanted early to allow their *in vivo* constitution of cartilage, a suitable *in vitro* cultivation method has to be devised for a consistent yield of regenerative cartilage. [*J Formos Med Assoc* 2009;108(2):87–101]

Key Words: cartilage repair

Articular cartilage defects may result from injury or osteochondral pathology, such as osteonecrosis and osteochondritis dissecans. In adults, these defects heal poorly and progress to catastrophic degenerative arthritis. Articular cartilage is a thin viscoelastic layer, usually less than 3 mm thick, which covers the articulating surface of the bone in a diarthrodial joint, and permits a smooth motion with minimal friction against the opposite contacting cartilage. Cartilage is constituted by a unique extracellular matrix (ECM) produced and maintained by a limited number of chondrocytes, which are distributed predominantly in the deep layer near the osteochondral junction, and are trapped by the ECM, with much limited ability of migration. The ECM has a structural function, contributes to the mechanical property of cartilage, has a feedback regulatory role on chondrocyte activities,[1] and also characterizes the phenotype of the chondrocytes. The whole structure

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lacks blood or lymphatic vessels, therefore, the cell infiltration in the normal inflammation process after injury is unlikely to occur and injury does not heal.

The clinical finding that articular cartilage "once destroyed, is not repaired," has not been changed since it was first observed by Hunter in 1743.[2] Conservative treatment with various medications gives only temporary relief of symptoms rather than cure, and clinicians have sought a surgical strategy. Initial surgical interventions aim to stimulate the natural repair process for rebuilding cartilage tissue, and include abrasion arthroplasty to evoke local chondrocytes, and multiple drilling or microfracture to release the subchondral progenitor cells, which in turn might generate new cartilage.[3,4] The unfavorable outcome of debridement and abrasion chondroplasty has confirmed the low intrinsic activity of human chondrocytes, and has led to subsequent serial trials of marrow stimulation techniques.[5] Among the latter, Gridie's multiple drilling was first introduced in the 1980s, but the original idea was the revascularization of the defect site instead of recruitment of marrow progenitor cells. The clot cannot remain in the defect, and only sparse scar tissue can be expected. Steadman's microfracture of subchondral bone may allow more bone marrow to enter the site of cartilage defect, where more variety of marrow content can accumulate. This technique largely replaced multiple drilling in the 1990s. However, the reparative tissue is fibrocartilage, which has different biomechanical properties from the native hyaline cartilage.[2]

More recent surgical strategies aim to replace the defect with patches or grafts. Cartilage allograft has problems with preparation and storage, and chondrocytes expire during the process.[6–8] If implanted freshly, cartilage allograft is challenged by immune reaction from the immersing synovial fluid, which was once considered insignificant for the avascular nature of articular cartilage.[9] Autografting is more promising.[10] Mosaicplasty with autologous osteochondral graft has yielded better clinical outcomes than other surgical modalities, including abrasion arthroplasty, Pridie drilling, and microfracture.[11–14] This technique is easily applied and the grafts self-secure to the subchondral bone without additional fixing procedures or devices.[15] This is a major advantage because cartilage-only grafts are difficult to fix to the recipient site. Usually, the graft is secured to the surround native cartilage by sutures, which is a technically demanding and time-consuming procedure and may further damage the native tissue. In addition, the thin patch of graft without a secure osteochondral adhesion may easily detach from the underlying bone as a result of the shearing force during joint motion. The mosaicplastic grafts are firmly implanted and can be applied using a minimally invasive arthroscopic procedure.[16] Finally, such osteochondral transplantation concurrently replaces the pathological subchondral bone that frequently exists with cartilage defects, such as those of the osteochondritis dissecans and osteonecrosis. Unfortunately, the utility of mosaicplasty has been largely limited by the extreme shortage of autogenous donor sources in the human body.

The human body has little spare mature articular cartilage to serve as autografts, and mosaicplasty is valid only for smaller-sized cartilage defects.[17] Artificially constituted extra cartilage is needed to repair larger defects. Periosteum or perichondrium has been considered a potential tissue to generate articular cartilage and to patch cartilage defects.[18,19] The progenitor cells residing on the cambium layer of the periosteum are induced by environmental factors at the recipient site to present as chondrocytes, while the periosteum itself serves as a scaffold to accommodate these cells.[20,21] Some success has been reported but the results are less favorable than for mosaicplasty.[18,22,23] Periosteal patching grafts cartilage defects with progenitor cells rather than mature chondrocytes, and the mechanism and efficiency of transformation of progenitor cells to chondrogenic cells remain unclear. Success is enhanced by knee motion after transplantation of the periosteum,[24] but the abrasion force during motion may cause early suture failure and a subsequent unsuccessful outcome.

As with most conventional medicine, all these historical efforts were clinical observations that were based on trials and experience, and were not deduced from evidence-based medicine. The inadequate basic knowledge about cartilage repair makes the evolution of these experience-based techniques difficult and less efficient. Solutions to the clinical problem of articular cartilage repair may need multidisciplinary collaboration from biotechnology.

Nevertheless, these clinical experiences have led to the development of a new technique that transplants laboratory-expanded chondrocytes in an attempt to overcome the inadequate supply of autogenous cartilage. Autologous chondrocyte implantation (ACI)[25] was first introduced in the late 1900s. This technique harvests a minimum amount of autogenous cartilage to retrieve chondrocytes, which are cultured *in vitro* to expand the population, and seeded onto a biodegradable porous sheet to constitute cartilage. The regenerated cartilage is in turn used for grafting. Although they are not yet being used widely, surgical procedures specifically developed using selected biomaterials have been introduced in North America and Europe (Carticel; Genzyme Biosurgery, Cambridge, MA, USA). ACI combines the concepts of cell therapy and tissue engineering to regenerate articular cartilage as a patch for the repair of chondral defects,[26,27] and remains the mainstay of treating articular cartilage defects. The elements of ACI have been improved continually to regenerate cartilage of better quality. The next section reviews the recent progress of such cartilage regeneration.

Articular Cartilage Regeneration

Articular cartilage regeneration develops new cartilage as an autograft to overcome the shortage of donor material. In principle, chondrocytes or chondrogenic cells, with or without preceding culture to multiply their number, are implanted into the cartilage defect, where these cells deposit ECM to constitute cartilage repair. This cell-based technology has evolved over generations to improve the efficiency of tissue regeneration and surgical outcome (Table 1).[15,28–32] The mainstay of current practice is known as the third generation, in which the three elements of tissue engineering are applied, namely the cells, the scaffold that bears these cells, and a suitable cultivation environment (Figure 1). Currently, a fourth generation technique is on the horizon, with the introduction of stem cells and various growth factors, but the principles of tissue engineering remain fundamental to cartilage regeneration. In general, autologous chondrogenic cells are inserted onto a biodegradable scaffold that supports their growth and chondrogenesis. The cell-laden scaffold is cultivated with environmental factors appropriate for enhancing cell presentation. Adopting the concept of tissue engineering to cartilage regeneration has made the related research more systematic and evidence-based, and by using control studies. Recent evolution of the cartilage regeneration technique can be categorized by the three above elements of tissue engineering, and we review the modern modifications in the following discussion (Table 2).

When constituting a regenerated cartilage, two strategies have been proposed: (1) *in vitro* constitution of complete chondral or osteochondral grafts ready for implantation; and (2) cell-based repair for *in vivo* development of regenerated cartilage. Although the former seems more straightforward, the optimal environmental parameters for the constitution of cartilaginous tissue have not yet been defined. Modern designs of bioreactors may largely improve the yield of such laboratory work, but the biological and mechanical properties of the regenerated cartilage are currently inferior for clinical application. The second strategy regards the intra-articular environment as a naturally suitable condition for the cultivation of regenerated cartilage. Instead of well-constituted tissue, cells are implanted to repair the cartilage defect. This is similar to other models of cell therapy in current clinical practice, in which chondrogenic cells work *in vivo* to produce cartilaginous substances and repair chondral defects.[33] With this strategy, we need to: (1) provide a sufficient number of

Table 1. Evolution of articular cartilage regeneration, and analysis of discovered information in each generation

Generation	Description	Strength	Weakness	Examples
First generation	A piece of periosteal patch is sutured to the cartilage defect. Culture-expanded chondrocytes suspended in aqueous culture media are injected to the space beneath the patch.	1. Introducing the concept of cell-based therapy to replace the conventional, simple tissue grafting. 2. Open the subsequent evidence-based studies on cartilage repair.	1. Need to harvest periosteal patch, and complex suture technique. 2. Water-seal around patch may not maintain the chondrocytes in defect site. 3. Chondrocytes dedifferentiate during culture process. 4. Need arthrotomy and time-consuming surgery. Subsequent stiffness of joint is common.	ACI[15]
Second generation	Chondrocytes are seeded onto a porous, absorbable scaffold that supports the chondrocytes during the culturing process and early post-implantation stage.	1. Introducing biodegradable scaffolds to ensure cellular habitation at defect site and support cellular presentation. 2. Some models can be applied through minimally invasive surgery or arthroscopy to decrease postoperative joint stiffness.	1. The safety of scaffold has to be tested. Few biomaterials have currently been approved by the FDA in the United States. 2. Clinical reports lack long-term follow-up, some are case reports.	Collagen membrane[28] Hyalograft C[29] Fibrin glue[30] Atelocollagen gel[31]
Third generation	Improvement from the previous generation, including: 1. Cells of more promising source and chondrogenesis. 2. Scaffold of chondro-inductive and/or chondro-conductive materials. 3. Culture conditions including mechanical stimulation to improve the physical properties of cell-scaffold construct before implantation. 4. Simpler surgical techniques to reduce repeats of operations to the joint.	1. Introducing various improvements on culture conditions that better preserve the chondrogenic ability of chondrocytes. 2. All three elements of the "tissue engineering triad" are considered for the cartilage regeneration, making further improvements more systematic.	1. Very limited clinical information to date. 2. Information derives largely from the companies that provide the materials, lack independent studies.	DeNovo ET™ (Zimmer) NeoCart™ (Histogenics)

Fourth generation	Biotechnology is introduced to tissue engineering, including: 1. Chondrocytes are treated with gene therapy to improve their chondrogenesis. 2. Stem cells from various origins are used to replace chondrocytes. 3. Various growth factors are used on chondrocytes and/or stem cells to enhance their chondrogenesis. 4. More improvements on biomaterials, including their biocompatibility and cell affinity.	1. More potential cells can give more promising regeneration of cartilaginous tissue. 2. Information is derived from evidence-based control studies.	1. Safety of cell manipulation by gene therapy and growth factors is unclear. 2. All findings are from *in vitro* or animal studies, no human information available to date. 3. Ethical concerns are raised when more potential stem cells, such as those of embryonic origin, are used.	Hyaff-11[32]

chondrogenic cells, which can properly produce ECM and constitute the regenerated cartilage; and (2) ensure that these cells remain in the grafted cartilage defect, for the obvious reason that their production can accumulate locally. ACI is a typical example of a technique that uses this strategy. The original attempt at ACI was a combination of the two strategies: the chondrocytes were expanded *in vitro* to reach a sufficient number and were implanted as cells before constitution of the tissue. The recently modified ACI processes are closer to the second strategy.

Chondrogenic cells

Cartilage is relatively a hypocellular tissue, which contains approximately 100×10^6 cells/cm^3 on average, throughout the full thickness of mature cartilage. These cells display a unique palisade architectural pattern with round, single or columnar cells within lacunae. Absence of this character is indicative of degenerative changes or less differentiation.[33,34] Properly presenting chondrocytes produce and organize the ECM that is composed of type II collagen and glycosaminoglycans.

The limited number of autologous chondrocytes from spare cartilage may hardly be adequate for the high demand of cells to constitute engineered cartilage. The seeding density on biomaterials to develop cartilage has been reported as $10–130 \times 10^6$ cells/cm^3, and is optimized at 60×10^6 for the best mechanical properties of the yielded tissue.[34–38] To collect adequate cells for such density, the number of harvested chondrocytes has to expand *in vitro* before seeding. Chondrocytes expanded in monolayer culture easily lose their phenotype and transform to more fibroblast-like cells, which possess type I instead of type II collagen.[39] This problem has been improved by recently renovated culture methods; for example, chondrocytes cultured in type I collagen gel may preserve their phenotype, and those that have dedifferentiated may redifferentiate.[40]

Chondrogenic cells that are in more abundant supply can be used for cartilage tissue engineering. Bone-marrow-derived mesenchymal stem cells (MSCs) are more plentiful, and can be induced

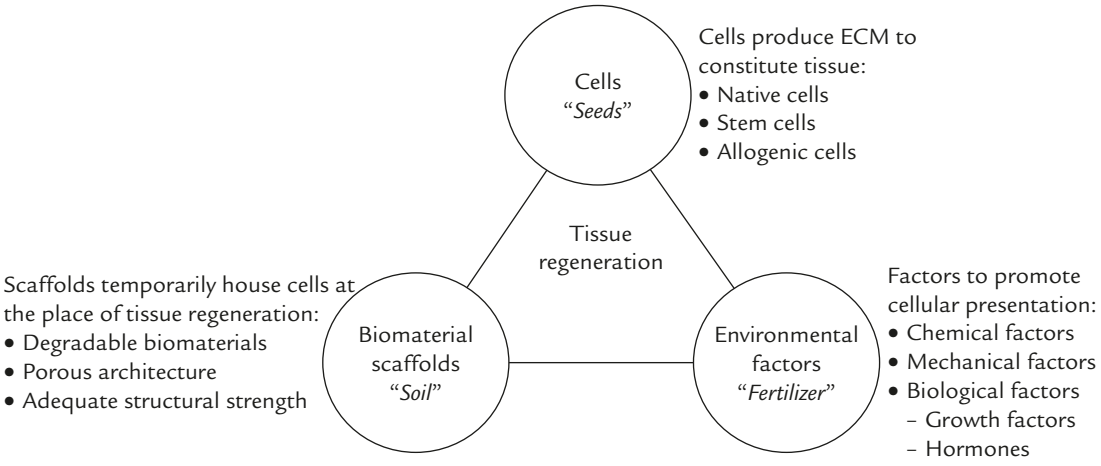

Figure 1. The "tissue engineering triad". The cell is the main character to generate tissue. Porous scaffolds made from absorbable biomaterials provide the habitation for the cells. Cell behavior is directed by the environmental factors. The environmental factors are the chemical, physical and biological variables and substances in the culture system.

to form chondrogenic cells in chemically specified culture media supplemented with transforming growth factor-β (TGF-β).[41,42] These multipotential cells can also be induced to form osteogenic cells under different culture conditions, and both types of induction together may constitute a biphasic osteochondral construct graft from a single cell source. Some culture systems add mechanical conditions to enhance the production of cartilaginous tissue by MSCs.[43] MSCs can also be collected from other origins, including the periosteum, perichondrium, adipose tissue, placenta, and fetal tissues. However, the bone marrow-derived MSCs exhibit better chondrogenesis than MSCs of other origin, under presently defined culture and induction conditions.[44]

Aging of the cells is an issue when these autogenous cell-based techniques are applied to aged people. Old chondrocytes have much lower ability to build cartilage than young ones.[45] For cartilage repair in aged people, using cell type with greater potential, such as bone marrow stromal progenitor cells, may be more promising.[42]

The behavior of chondrogenic cells is affected in many ways, such as: (1) the conditions under which they are incubated, primarily the substance that contains the cells; and (2) the physical, chemical and biological factors applied to the cells. These variables affect the cells' ability to survive, multiply, present the proper chondrogenic phenotype, and constitute the cartilage tissue. The influence of various chemicals, pharmaceutical preparations, and biological factors on the cells has been investigated extensively in recent laboratory studies.[46] However, reports about the *in vivo* conditions, primarily from animal experiments, are relatively few. There have been even fewer human studies, which means that cartilage regeneration has a long way to go before it reaches routine clinical application.

Biomaterial scaffolds

Biomaterial scaffolds provide the chondrogenic cells with a temporary habitation, where they survive, multiply, and produce ECM to constitute regenerated cartilage. Although the cellular products are expected to replace the degradable biomaterial, the process is usually time-consuming and the scaffold should be implanted before completion of the process. The biomaterials thus play the role of a vehicle to transfer cells and therefore should be compatible with the native tissue around the recipient site.[47]

Many natural substances are suitable as the cell-carrying scaffold for cartilage engineering, including fibrin, agarose, alginate, collagen, chitosan and hyaluronan. Many of these are hydrogels and can be designed as injectable in their liquid form, which blends well with chondrogenic cells.[48] After being injected into the recipient site, they

Table 2. Analysis of strength and weakness of current information on cartilage regeneration by the three elements of tissue engineering

Elements	Strength	Weakness	Authors' preference
Cells	1. Stem cells are induced to chondrogenic cells, largely solves the donor shortage of chondrocytes. 2. With improved handling of allogeneic chondrocytes, these cells can be an alterative cell source for cartilage regeneration.	1. Chondrocytes expanded with conventional monolayer culture dedifferentiate easily and lose their chondrogenic phenotype. 2. Induction of stem cells requires chemically defined culture medium supplemented with specific growth factors, which may result in side-effects in human use.	Autologous chondrocytes are effective and safe for cartilage regeneration based on current information.
Scaffolds	1. More varieties of scaffolds are developed, with various novel biomaterials and scaffold micro-architecture. 2. Some biomaterials can induce stem cells seeded on the scaffold to become chondrogenic. 3. Combining with specially designed surgical instruments, new scaffolds help to develop single, less-demanding surgical procedures for cartilage repair.	1. Few of these biomaterials have been proved safe in human use. 2. Very limited clinical information about these new developments to date.	Biphasic osteochondral scaffold can be securely implanted without additional fixation, and repair subchondral pathology concomitantly.
Culture conditions	1. New culture techniques such as the 3-D system can better preserve the chondrogenic ability of chondrocytes. 2. Serum-free culture technique can eliminate the risk of zoonoses. 3. When stem cells are used as the chondrogenic cells, various growth factors have been researched to promote the cellular transformation.	1. The molecular biological effects of most growth factors are unclear. Cells may be affected in uncertain ways. 2. The laboratory facilities are extremely expensive to meet the criteria of Good Tissue Practice.	1. Minimizing the *in vitro* culture process can reduce the cost and complexity of cartilage regeneration. 2. *In vivo* intra-articular environment may be optimal for the constitution of cartilaginous tissue by chondrocytes.

set by gelation to fill in any shape and size of cartilage defect.

Fibrin is a major component of blood clots. It can be used to adhere other engineered cartilage onto the recipient site, as a stand-alone scaffold, or as a growth factor.[49,50] Its utility is much limited by its inferior mechanical properties, the possibility of evoking immune and inflammatory responses, and its inability to allow immigration of host cells. Agarose and alginate have better mechanical strength than fibrin, and have been reported to support the regeneration of cartilage in a rabbit model.[51] However, they are not sufficiently strong to survive the friction of joint motion in larger animals and are not absorbed well by the body.

Some native components of joint tissues that are considered to have the best biocompatibility evoke the least immune response. Collagen sponge has been used in many studies that have loaded chondrocytes or MSCs to build cartilage *in vitro* or *in vivo* in various animals.[52–56] It has also been used with other materials and techniques such as gene treatment to enhance cartilage regeneration.[57,58] However, collagen is only available from living creatures, which means that it is expensive and has the possibility of transmitting prion-induced diseases, especially with collagen of bovine origin. Furthermore, human cells cultured in contact with such animal-derived protein may express molecules that induce the host immune response, which would be harmful for the implanted cells.[59] Hyaluronan is a native component of synovial fluid and ECM of cartilage, and has been used for cartilage engineering.[60–62] Although not a native substance in the human body, chitosan is a polysaccharide and evokes minimal inflammatory reaction, at least theoretically. It has been prepared as a thermally sensitive product that is injectable as a liquid that sets to gel at body temperature.[63]

Synthetic polymeric scaffolds also have potential for tissue engineering, with the advantages of reliable sources and flexibility by manipulating the fabrication process. The most widely used are the poly-α-hydroxy esters, especially polylactic acid (PLA) and polyglycolic acid. These polymers have been approved for clinical use in the USA and are manufactured for routine hospital or surgical use. They are readily made into scaffolds for tissue engineering, in the form of foam or woven or nonwoven fiber mesh. Products of these polymers have much better mechanical strength than those of natural substances, which makes it easier for them to be fixed to the recipient site, and makes them more resistant to the friction of joint motion.[64] Copolymers of these two substances allow adjustment of the degradation rate of the scaffold. This is important because the residence time of the implanted polymer must be sufficient to serve its scaffold purpose, but not so long as to impede tissue regeneration. If MSCs are seeded onto a PLA scaffold, they display chondrocyte differentiation in culture medium supplemented with TGF-β.[65] Other polymers of interest include poly(ethylene glycol)-terephthalate, poly(butylene terephthalate), poly(ethylene glycol) fumarate, poly(N-isopropylacrylamide), and carbon fiber scaffolds.[66–69]

Beside the biomaterial content, the design of the scaffold architecture may also affect the seeded cells. Because the ECM of natural cartilage distributes nonhomogeneously, with the chondrocytes present predominantly near the osteochondral junction and collagen fibers along the articular surface, a scaffold architecture that mimics the natural environment may facilitate the growth of seeded chondrocytes. A layered agarose scaffold with such depth-dependent nonhomogeneity has been designed for good *in vitro* regeneration of cartilage from chondrocytes.[70]

The clinical success of mosaicplasty brings the idea of engineering biphasic osteochondral composites for cartilage repair.[47,71,72] Osteochondral repair has several advantages over cartilage-only repair. A uniform, predefined tidemark at the osteochondral junction can prevent the detachment of cartilage from the subchondral bone during joint motion. The osseous phase of the engineered osteochondral composite is a rigid support of the overlying chondral phase, and can self-secure to the recipient site by press-fit. Many studies use tricalcium phosphate (TCP), a major component of bone minerals, to fabricate this osseous phase.

When the marrow stromal cells at the recipient site make contact with the TCP content of the implant, they become osteogenic and build bony replacement within the scaffold, such that the implant integrates well with the host bone.[73–76] Alternatively, osteogenic and chondrogenic cells can be preseeded concurrently and respectively to their corresponding phases. The chondrogenic cells preferentially stay in the chondral phase of the construct.[71,72]

Culture conditions

Chondrocytes or other induced chondrogenic cells should be able to produce ECM that constitutes the regenerated cartilage. In both of the aforementioned strategies, chemical, physical and biological factors may be applied to promote cellular presentation when culturing the construct. Bioreactors are designed to adjust environmental factors for the optimal performance of the chondrocytes.[77,78] Factors of interest include the friction caused by surface motion, compressive stress, oxygen tension, hydrostatic force, and dynamic mechanical stimulation.[79–83]

MSCs have been considered to substitute for insufficient autogenous chondrocytes, therefore, more factors are required to efficiently induce these cells to become chondrogenic. Various cytokines and growth factors are added to the chemically defined culture media to promote chondrogenesis, including various isoforms of TGF-β, bone morphogenic protein (BMP), activin, osteogenic protein-1, fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), prolactin, interleukin-1β, Cyr-61, and growth hormone.[84–100] The most well known is the superfamily of TGF-β, which consists of more than 40 polypeptides that share high homology and affect the cells through similar transmembranous receptor complexes and intracellular pathways.[101,102] The cascade triggered by TGF-β can cross-talk with the Wnt pathway.[103] Some other nonproteinaceous chemical factors have also been shown to promote chondrogenesis, including prostaglandin E2, thyroxin, 1.25-dihydroxy vitamin D, ascorbic acid, dexamethasone, ethanol, staurosporine, dibutyryl cAMP, concanavalin A, and vanadate.[104–116] These chemicals are less labile, with a longer half-life than the protein-based factors, and are thus advantageous for prolonged *in vitro* culture over several weeks.

Regardless of the nature of the cells, standard culture conditions require the presence of serum, basically of bovine origin. The risk of undesired pathogen transmission has been debated when the cells are implanted to humans. Autologous serum-supplemented culture medium has become the state of the art for ACI, but serum-free culture is more attractive.[117] The avascular condition of natural cartilage does not suggest that serum is needed to support the chondrocytes. One study has even indicated that serum hinders the chondrogenic ability of chondrocytes.[118] Serum-free culture is worthy of further development to develop regenerated cartilage for clinical application.

Needs and Perspectives

Safety of engineered cartilage

Either chondrocytes or MSCs are used to constitute engineered cartilage, and *in vitro* manipulation of the cells is necessary in most of the currently available systems. When the constructed cartilage tissue is considered for clinical use, the safety of the whole process has been debated and the cost is high. The entire process has to be conducted with expensive laboratory facilities that meet the high standard of good tissue practice. In addition, all reagents involved in the process should be proven as safe for human use. More complicated manipulation of the cells will arouse more concern that the cells may be affected in unknown ways. When developing a system to regenerate cartilage for clinical application, we should always consider its safety and simplicity, even if it is necessary to compromise the quality of the regenerated tissue.

Surgical applicability

The ultimate goal of cartilage engineering is the surgical application of the product in humans.

Animal experiments have testified to the surgical applicability and efficiency of cartilage defect repairs, and this is necessary before clinical application of the models.[119] The *in vivo* environment in animal experiments can be a naturally optimized "bioactive chamber" to construct cartilage tissue without any artificial bioreactor.[26] An ideal animal model has similar articular anatomy and physiology to those in humans, with suitable dimensions for surgical operation. Porcine and canine models are good examples in the literature for cartilage repair.

A well-designed model of cartilage repair should be easily applicable in the operating room, so that the operative time is shortened and surgical invasion minimized. For example, the biphasic osteochondral construct can self-secure to the prepared recipient site within seconds. We have previously developed a system that implants freshly harvested chondrocytes directly to the cartilage defect, using a specially designed biphasic biodegradable scaffold.[76,120] The cylindrical scaffold is a porous construction with two phases: a thin, spongy chondral phase of PLA on the top, and a more rigid osseous phase of PLA–TCP as the base (Figure 2). With a low seeding density of 2×10^6 chondrocytes/cm^3 to the chondral phase, the subsequent *in vivo* growth yields good cartilage tissue at the grafted site in porcine knees (Figure 3). This avoids the complex culture process and finishes the harvest of autogenous chondrocytes and their implantation in one surgical procedure.

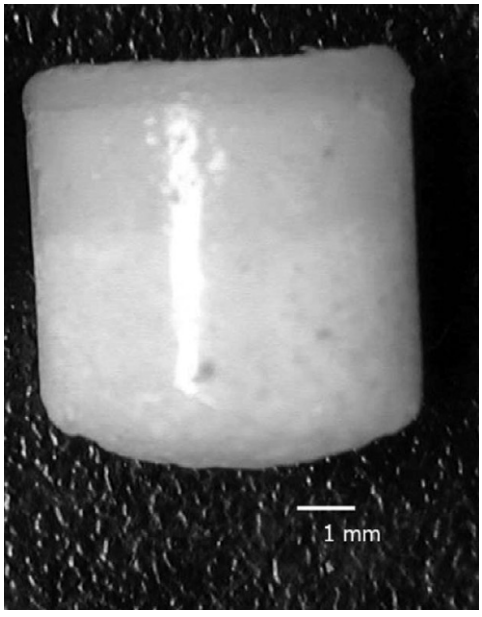

Figure 2. The cylindrical biphasic osteochondral scaffold. The diameter and height of this cylinder are 8 mm.

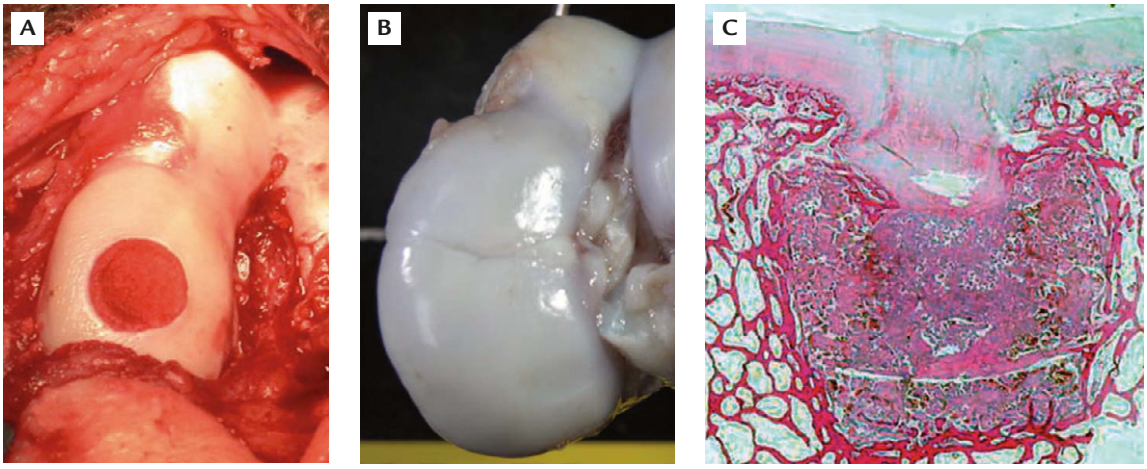

Figure 3. Demonstration of osteochondral repair with a biphasic scaffold in a porcine model. Autogenous cartilage was harvested, pulverized, and enzymatically treated to retrieve chondrocytes, which were then seeded onto the chondral phase of the scaffold. (A) An osteochondral defect of the identical dimension to the prepared scaffold was created artificially on the femoral condyle in the knee joint. The prepared scaffold was press-fit installed. (B) Six months after surgery, the defect was repaired with regenerated cartilage. (C) Microsection of the recipient site shows the histology of the regenerated tissue (hematoxylin & eosin, 1×). Without preseeding cells to the osseous phase, the bone marrow stromal cells migrated into the porous scaffold and mineralized the space. The regenerated tissue, both chondral and osseous, integrated well with the surrounding native tissue.

From laboratory to clinic

As a result of the unfavorable clinical experiences of cartilage repair in the past, biotechnology has been introduced to this field for evidence-based development of a solution. The knowledge to date supports that articular cartilage is best repaired with autologous engineered cartilage, and a lot of research has been carried out to improve cartilage regeneration. Although the efficacy of regeneration has much improved in the laboratory and animal studies, most findings have not been investigated for their clinical safety and performance. Further studies should highlight their clinical relevance to facilitate the development of products applicable to humans.

We need to organize currently available knowledge to develop clinically applicable models of cartilage repair, on the basis of autogenous chondrogenic cell implantation. A clinically applicable model of cartilage regeneration should be safe, efficient, and as simple as possible. Our model described in the previous section may be an example. It can be finished in a single seed-and-implant surgery procedure, which decreases the surgical risks and complications from repetitive operations of conventional ACI. If the site of repair allows an arthroscopic approach, the surgery can be done in a minimally invasive manner within a short time, estimated at 1 hour. By avoiding the complex treatment of the autogenous cells *in vitro*, the safety of the procedure can be improved and the cost reduced.

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