CARTILAGE TISSUE ENGINEERING

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ABSTRACT

Tissue engineering is a new approach for articular cartilage repair. The aim of the present article was to review the current status of cartilage tissue engineering researches. The scaffold materials used for cartilage tissue engineering, the in vitro, in vivo studies and the clinical trials were all reviewed. Our researches about in vitro cartilage tissue engineering with new type bioactive scaffold and preliminary animal studies results will also be described. The scaffold was tricopolymer made from gelatin, hyaluronan and chondroitin. Chondrocytes seeded in tricopolymer showed in vitro engineered cartilage formation. The engineered cartilage constructs were implanted into knee joints of miniature pigs for animal study.

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1. TISSUE ENGINEERING: NEW APPROACH FOR CARTILAGE REPAIR

Damage to and subsequent degeneration of articular cartilage as well as its relationship to osteoarthritis is a significant health problem. Approximately 70% of the population aged 65 years and older is affected in some way by osteoarthritis [1]. Currently, there is no cure for osteoarthritis and no

treatments that have been proven to retard disease progression.

Before discussion of this review article, we have to differentiate focal articular injury from diffuse degenerative joint disease. The former means a focal chondral or osteochondral lesion in the weight bearing region of the distal femur in which the surrounding articular cartilage is normal or near normal. If left untreated, this focal lesion will progress to secondary degeneration of articular cartilage. The focal lesion is different from the primary aging and degenerative process in primary osteoarthritis. However, we still need to treat this lesion that will at least delay the need for total joint replacement especially in active young patients. Although articular cartilage is a metabolically active tissue, the chondrocytes in the matrix have a relatively slow state of turnover. The tissue itself lacks a blood supply to support repair and remodeling. Because of the limited capacity for spontaneous repair, minor injury to articular cartilage may lead to

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progressive damage and degeneration. In older patients with diffuse articular cartilage injury, total joint arthroplasty is the treatment of choice. In young patients with focal articular cartilage injury, autogenous chondrocytes implantation, mosaicplasty, or marrow stimulating techniques, such as microfracture or multiple drilling, are better alternatives than joint replacement therapy, but the results are variable and the techniques have some limitations [2]. Autogenous chondrocytes implantation with a periosteal graft has shown encouraging results, but the predictability and reliability of hyaline or fibrocartilage formation are still questionable [3]. Marrow stimulating techniques result in fibrocartilage formation, which has a lower mechanical strength than hyaline cartilage and only limited repair capacity [4]. For mosaicplasty, the major problems are limited availability of autogenous tissue and donor site morbidity; this procedure also involves destroying healthy non-weight-bearing tissue to treat diseased tissue, and both the donor site and treated area would be expected to degenerate [5]. The integration of subchondral bone into the host tissue is excellent; however, integration to host cartilage is always a problem [6].

Recently, tissue engineering has emerged as a new method involving the combining of cells, scaffold, and bioactive factors to fabricate functional new tissue to replace damaged tissue [7]. The advantages of incorporating scaffold into this approach are that the scaffold matrix can provide the initial structural support and retain cells in the defective area and is then degraded when the cells secrete their own matrix, and scaffold matrix can act as a delivery system for bioactive agents, such as growth factors. The following sections will discuss about the current status in cartilage tissue engineering, including the scaffold used, in vitro study, in vivo study, and clinical trials.

2. SCAFFOLD MATERIAL FOR CARTILAGE TISSUE ENGINEERING

Ideally, a scaffold should be porous to permit the migration of seeded cells or the infiltration of native cells from the host. The surface properties should promote cell adhesion, which can be enhanced by coupling the scaffold material with specific cell adhesion-promoting peptides (such as Arg-Gly-Asp). The scaffold material must be biocompatible which induce only minimal adverse immunological reactions. The material must also be biodegradable and be capable of replacement by physiological extracellular components. The degradation products should have less cytotoxicity, tumorigenicity, nephrotoxicity or

other undesirable effects as possible. The mechanical properties to support cell in-growth and extracellular matrix production must also be satisfied by the scaffold. In the following sections, we will discuss about the biomaterial currently used in cartilage researches and the new scaffold we intend to design.

2.1 Scaffold Made from Synthetic Polymer

The poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) copolymers, have frequently used in the cartilage - issue engineering literature [8-10], because FDA had proved their use in biodegradable sutures for more than 20 years.

These materials degrade through hydrolysis of the ester bond in the polymer backbone. Controls of the physical characteristics of the scaffolds, such as fiber diameter, pore size, and polymer crystallinity can regulate the scaffold degradation rates, which can range from 6-8 weeks in the case of highly porous PGA fibrous mesh to 6-18 months in the case of a highly crystalline PLA [11]. Similarly, the mechanical properties of these scaffolds can be regulated and have been shown to range from 5 kPa [12] to 1 GPa [13].

The most common material used for cartilage tissue engineering has been non-woven PGA mesh [14]. PLA and PLGA sponges and foams are inherently stiffer than PGA scaffolds and are in general easier to process than PGA owing to their solubility in common organic solvents.

However, there are some shortcomings of these materials. These polymers have relatively poor cell-adhesion and tissue-integration properties and their potentially poor biocompatibility. Further, there is a significant foreign body giant cell reactions associated with these systems [15]. This is particularly true in applications for reconstructive surgery, where acute reactions to subcutaneous implants involve a significant inflammatory response [16]. Inflammatory agents such as interleukin-1 (IL-1) have been shown to inhibit cartilage formation and degrade cartilage ECM. Even in a non-inflammatory environment, the natural breakdown products of these materials are acidic and will lower local pH, which are cytotoxic. Chondrocytes are known to be acutely sensitive to changes in environmental pH [17].

The Pluronics or polaxomers are polymers composed of blocks of poly(ethylene oxide)(PEO) and poly(propylene oxide)(PPO) that have the property of thermal gelation [18]. Gelation at temperatures near body temperature makes Pluronic an ideal candidate for in vivo cell delivery. The use of Pluronic to deliver articular and auricular chondrocytes has been successful in promoting cartilage formation [16]. The main shortcomings of these systems are in the mechanical properties of such gels, which are normally

quite weak. Besides, the rapid degradation rates of these polymers make this a suboptimal delivery choice for cells with low biosynthetic rates.

2.2 Scaffold Made from Natural Substance

Many naturally derived polymers had been used to support chondrocytes growth, the results seems appealing. There are two kinds of naturally derived polymers. The first is carbohydrate-based polymers, such as alginate, agarose, hyaluronan and chitosan. The other is protein-based matrices, such as collagen and fibrin.

Collagen: Type I collagen have generally been utilized as carriers for chondrocytes or mesenchymal stem cells [19-21]. Many preparations of collagen type I are soluble under acidic conditions. The neutralization of collagen solutions results in the formation of a hydrated collagen gel. When cells are included in the neutralizing solution, they are effectively encapsulated in the collagen gel. Collagen is a natural bodily constituent whose fibrils have cell adhesion surface and carry the required biological information for their activity. Furthermore, the degradation products of collagen are physiological and nontoxic. One of the main shortcomings in using these systems in clinical practice is the poor mechanical strength. An additional concern associated with the use of type I collagen as a scaffold material is that most cartilages do not contain type I collagen. Some authors suggested type II collagen from native cartilage matrix may be better than type I collagen [22-24].

Fibrin: Fibrin clots or fibrin glues form from the reaction of fibrinogen and thrombin. Fibrin is pro-inflammatory and induces its own degradation and substitution by cellular components of the extravascular tissue spaces. Its degradation products are nontoxic physiological substances. Fibrin has been employed extensively not only as a scaffold material by incorporating chondrocytes into the exogenous fibrin clot, both *in vitro* [25] and *in vivo* [26-27]. However, some immunological reactions to exogenous fibrin have been observed in several animal studies [28-29].

Alginate: Alginate is an anionic polysaccharide derived from seaweed. In the presence of calcium cations, alginate chains are crosslinked by ionic bonding through guluronic acid segments [30]. Typical methods for *in vitro* chondrocyte culture in alginate involve encapsulation of cells in beads made by dropping cells suspended in 1-20% alginate solution into a bath of CaCl. If CaSO₄ were used as a crosslinking agent, the time required for crosslinking will be lengthened. This allows for injectable delivery of chondrocytes in alginate and results in *in situ* gelation [31]. Within three dimensional alginate

cultures, dedifferentiated chondrocytes can readily re-differentiate [32]. Likewise, bonemarrowderived mesenchymal stem cells can differentiate into chondrocytes when seeded within this matrix and under the appropriate nutritional and stimulatory conditions, both *in vitro* and *in vivo* [33]. However, *in vivo* applications of alginate matrices have yielded disappointing results. Alginatebased cartilage tissue engineered induces severe foreign body giant cell reactions and immunological responses when implanted within full thickness defects in experimental animals [34]. Hence, alginate matrices have not been employed in human patients for articular cartilage repair.

Agarose: Agarose is a modified form of the agar material used for bacterial cell culture which is isolated and purified from certain Asian seaweeds. Typically, agarose is heated in aqueous solution to maximize solubility, and then mixed with cold media containing cells [35]. The mixture forms a hydrogel when cooled, effectively encapsulating cells. *In vitro* cultures of chondrocytes in alginate have demonstrated the production of significant amounts of cartilage ECM and increases in the mechanical properties of the constructs. But it has poor biodegradability because there is no enzyme degradation system for agarose in mammalian tissues.

Hyaluronan: Hyaluronan is a physiological component of the articular cartilage matrix and can induce embryonic mesenchymal stem cells to differentiate into chondrocytes [36]. Theoretically, hyaluronan would be an ideal matrix to support articular cartilage repair. However, crosslinking by esterification or other chemical methods mean are usually necessary to fabricate hyaluronan-based scaffold, which will compromise its biocompatibility. Matrices composed of hyaluronan have been frequently used as carriers for chondrocytes or bonemarrowderived mesenchymal stem cells [37-38]. Hyaluronan matrices loaded with such cells have been shown to form a cartilagelike tissue both *in vitro* and *in vivo* [39]. Such constructs have likewise been shown to support cell differentiation processes". However, the degradation products of crosslinked hyaluronan had been reported may lead to chondrolysis [40].

Chitosan: Sechriest et al. have utilized a hydrogel scaffold comprised of ionically crosslinked chondroitin sulfate A and chitosan to support chondrogenesis [41]. Several *in vitro* studies yield evidence of its potential value as a matrix to facilitate articular cartilage repair. It efficiently supports not only chondrogenic activities [41-43], but also the *in vitro* expression of cartilage extracellular matrix proteins by human chondrocytes [44]. It can also serve as a carrier for growth factors. Chitosan has excellent biodegradability.

2.3 Tricopolymer Mimic Natural Matrix as Scaffold for Cartilage Tissue Engineering

Many kinds of scaffold, both natural and synthetic, have been proposed for use in cartilage tissue engineering [45]. Scaffold plays an important role as the extracellular matrix (ECM) during engineered tissue development, and scaffold with an informational function, e.g., material containing the Arg-Gly-Asp sequence which facilitates cell attachment, should be better than non-informational synthetic polymers [46-47]. The mechanism by which the cell synthesizes and secretes ECM and is then, in turn, regulated by the ECM is termed dynamic reciprocity. Inclusion of chondroitin sulfate in scaffold may promote the secretion of proteoglycan and type II collagen, but may also inhibit mitosis [41]. Hyaluronan is present in high amounts in the ECM during embryonic cartilage development, and also facilitates the integration of engineered cartilage. Since gelatin is basically denatured collagen and presumably retains informational signals, such as the Arg-Gly-Asp sequence, we hypothesized that a tri-copolymer formed from gelatin, chondroitin, and hyaluronan might mimic cartilage matrix composition (type II collagen, glycosaminoglycan and hyaluronan) and provide the necessary information for cell attachment to meet the requirement for dynamic reciprocity for cartilage tissue engineering. In our study, a tri-copolymer scaffold was produced from chondroitin-6-sulfate, hyaluronan, and gelatin to mimic cartilage matrix as a scaffold for cartilage tissue engineering [48]. The scaffold produced had a uniform pore size of about $180\ \mu\text{m}$ and adequate porosity of 75 % (Fig. 1). Porcine chondrocytes were seeded onto the tri-copolymer scaffold and cultured in Petri dishes or spinner flasks for 2, 3, 4, or 5 weeks. Chondrocytes were uniformly distributed in the scaffold in the spinner flask cultures, but less so in the Petri dish cultures. Secretion of extracellular matrix was found under histology examination. In spinner flask cultures, chondrocytes retained their phenotype for at least 5 weeks, as shown immunohistochemically, and synthesized type II collagen. These results show that gelatin/chondroitin sulfate/hyaluronan tri-copolymer has potential for use as a cartilage tissue engineering scaffold (Fig. 2 ~ 4).

3. IN VITRO STUDY OF CARTILAGE TISSUE ENGINEERING

The in vitro study of cartilage tissue engineering has been critical for determining the factors that modulate the proliferation and differentiation of chondrocytes and chondrocyte precursor cells, the retention of cells and growth factors within scaffolds,

Fig 1. Scanning electron microscopic examination of the gelatin/chondroitin-6-sulfate/hyaluronan tri-copolymer scaffold. The scaffold showed a porous structure with a uniform pore size of about $180\ \mu\text{m}$ and a porosity of 75 %.

and the interactions between scaffolds, cells, growth factors and the mechanical environment.

To evaluate of the scaffold property, to determine the biocompatibility of scaffold and to see if the scaffold is suitable for chondrocytes in-growth and matrix secretion, the in vitro study is the first step.

Gordana Vunjak-Novakovic and Freed LE et al had published series of studies about culturing engineered cartilage constructs in bioreactor. They cultured chondrocytes seeded PLGA scaffold in rotating bioreactors (slow turning lateral vessel, STLV) under microgravity environment. The constructs remained suspended due to a dynamic equilibrium between gravity, centrifugal and drag forces. The constructs contained type II represented more than 90% of the total collagen [49]. As compared to native bovine calf articular cartilage, 6-week constructs had comparable cellularities, 68% as much glycosaminoglycan, and 33% as much collagen type II per unit wet weight [49]. Construct mechanical properties (i.e., equilibrium modulus, hydraulic permeability, dynamic stiffness, and streaming potential) correlated with the wet weight fractions of GAG, collagen, and water [13]. Their studies provided a model for in vitro cartilage tissue engineering in bioreactor. The analysis methods they used were widely cited by other authors.

As the development of cell-polymer scaffold-bioreactor systems, many in vitro studies had been conducted under these systems. The third component of tissue engineering, the bioactive factors, can be

25 μ m

Fig. 2 After 4 weeks' cultivation in spinner flask, there is newly formed matrix with lacuna formation around chondrocytes as shown by Alcian Blue staining. The morphology of the lacunas is similar to those seen in natural cartilage.

25 μ m

Fig. 4 Phenotype retention by the chondrocytes after 5 weeks' cultivation in spinner flask. Chondrocytes retained their round shape and were stained with anti-S-100 protein antibody (200X). (From Chang CH et al: Biomaterial 24: 4853-4858, 2003)

25 μ m

Fig. 3 After 5 weeks' cultivation in spinner flask, Alcian Blue staining showing that the peri-cellular matrix around chondrocytes stains light blue, while the tri-copolymer scaffold stains darker, indicating that the scaffold contains more glycosaminoglycan. SC: Scaffold. SECM: Secreted extracellular matrix, CC: Chondrocytes (200X).

provided by bioreactors. They can provide controlled environments for pH, temperature, pressure waste removal and nutrition supply. They can also improve cell seeding efficiency on a 3D scaffold, increase mass transport and provide mechanical stimulation. As for detailed review, please refer to the authors' another article [50].

In vitro studies also offered models for testing the effects of growth factors. The process of chondrogenesis is regulated by a number of growth factors including insulin-like growth factor-1 (IGF-1), transforming growth factor beta (TGF- β) super family members, such as TGF- β , bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and cartilage derived morphogenetic protein (CDMP) [51-58]. In vitro studies have demonstrated that supplementation with at least some of these growth factors can enhance the production of cartilage in tissue-engineered constructs [59-61]. Blunk et al. [61] in their study examined the effects of IGF-1, interleukin-4 (IL-4), PDGF, and TGF- β on tissue-engineered cartilage constructs formed by poly(glycolic acid) (PGA) scaffolds seeded with bovine chondrocytes. The results showed that IGF-1, IL-4 and TGF- β increased construct growth, glycosaminoglycan (GAG) and collagen content, while PDGF decreased these parameters [61]. In another study using the same model, Gooch et al. [62] demonstrated that BMP-2, BMP-12 and BMP-13 also had the benefit effect on the engineered cartilage constructs. Actually, in the true in vivo environments, there are multiple growth factors released at different time sequence during chondrogenesis. Some investigators had tried to add different growth factors combinations at different time interval. For example, Pei et al. [63] had demonstrated that sequential treatment of constructs with TGF- β and FGF-2 followed by IGF-1 treatment yielded constructs with the greatest biochemical and mechanical properties.

4. IN VIVO STUDY OF CARTILAGE TISSUE ENGINEERING

Two different animal models had been used in cartilage tissue engineering researches. The first is implantation of the chondrocytes seeded scaffolds in dorsal subcutaneous tissue of immuno-compromised nude mice [8, 10, 64-67]. Actually the engineered constructs became an ectopic tissue, which is not identical to the real physiological environment. We can say this kind of animal model is actually using the nude mice as a living bioreactor which can provide nutrient and bioactive factors. This kind of model can be used in evaluation of the efficacy of xenogenic cells seeded scaffold for tissue engineering. However, because the nude mice lack the immune response, the results will be different from the constructs which were implanted in the joint defect of animals. So the logical thinking of the preclinical study for a tissue engineered therapy should be directly implantation the engineered constructs in the joint defect of animals.

There are many different experimental animals used in articular cartilage defect studies, including rabbits, goats, sheep, horses and miniature pigs [68]. The genomes of these species have been characterized that allowed species-specific DNA sequence determination for investigation of functional phenotypes [69]. However, there are large variation among these species and significant difference between human tissue in the thickness of cartilage, density and behavior of chondrocytes. We agreed that no single animal species can ideally represent all human clinical situations, but each may have its advantages or disadvantages according the question addressed. Thus animal models pay important roles in preclinical screening and assessment of the potential problems of new cartilage repair procedures, which may used to determine whether a specific method can restore an articular surface and to compare methods of restoring articular surface in vivo [70]

There are some problems faced in the commonly used experimental small animals. In the human knee, the thickness of hyaline articular cartilage is approximately 1.65 to 2.65 mm [71]. In the mature rabbit, a commonly used experimental animal, hyaline articular cartilage has a depth of only approximately 300 to 400 μ m in the corresponding location. For dogs, the thickness of the cartilage is about 0.6 to 1.3 mm and for ovine, is about 0.4 to 0.5 mm. In larger animals, such as the sheep or goat, the articular cartilage layer in the medial femoral condyle is about 0.7 to 1.5 mm thick. The articular cartilage thickness is about 1.5 to 2.0 mm in horse and 1 to 2 mm in miniature pigs (personal communication to Professor Tzong-Fu Kuo, National Taiwan University Veterinary

Hospital). The sizes of chondrocytes within human and rabbit articular cartilage do not differ significantly from one another. However the matrix domain sustained and remodeled by an individual cellular unit is, in the human, approximately 8 to 10 times larger than that in the rabbit, the difference being most striking in the radial zone. The overall cell volume density in human articular cartilage is approximately 1.7% in the medial femoral condyle [71] as opposed to 12.2% in the adult rabbit. Thus creating a defect, in an experimental animal, of comparable dimensions to those commonly encountered in human patients, does not simulate the conditions

The unit volume of cartilage matrix to be replaced by one cellular unit is also different from human being to experimental model. Diffusional and nutritional considerations, and their influence on chondrocytes viability, are not comparable. The intrinsic architecture of human articular cartilage also is different from that in experimental animals: the chondrocytes distribution pattern and quantitative cell domain relationships cannot be mimicked. Besides, the mechanical environment is different in each case, and this may play a key role in the attainment of optimal repair results. At present, there exists no optimal solution to these problems, but a closer approach to the ideal may be achieved by careful consideration of defect design and of appropriate environmental modifications [71].

The differences between animal and human articular cartilage and the limited ability to evaluate function and symptoms in animals make extrapolating from experimental studies to clinical practice difficult. Because of these limitations of small animal studies, developing a large animal defect model is necessary, which can mimic human conditions more than small animal model. Besides, a systemically set up of control experiments is required for unequivocal conclusions [3]. And once a method has been shown to predictably restore a functional articular surface in animals, it is reasonable to perform clinical trial.

In our preliminary animal study, two miniature pigs will be used as animal model, because this specie is a large animal, the body weight is similar to human being, which we believed will mimic human conditions more than small animal models. Two sexually matured Lee-Sung miniature pigs had received tissue engineering implantation with allogeneous chondrocytes seeded tri-copolymer scaffold covered periosteum for 8 mm diameter full thickness articular defects (2mm depth) in lateral femoral condyle and osteochondral defects (5 mm depth) in medial femoral condyle in the study knee. The periosteum was harvested from the proximal tibia in the same surgical approach, with the cambium layer faced the defect. Auto-transplantation in contralateral knee was performed with the donor side defect (5 mm

depth) served as control group (we harvest 5 mm long autogenous osteochondral graft in lateral condyle and transplanted to medial condyle in the control knee, and the defect after harvesting served as model for spontaneous repair). No perioperative antibiotic was used. The pigs were allowed for free movement after surgery. The pig was sacrificed 36 weeks after surgical implantation (Fig. 5 and 6).

The gross appearances of reliminary results are encouraging. The tissue engineered knee showed smooth surface filled with repaired tissue. The control knee showed unfilled defect in defect with spontaneous repair. The autogenous transplantation site showed smooth surface but with integration problem at margin (Fig. 7). The histology examination of the study results (H&E stain) also showed encouraging results (Fig. 8). Tissue engineering in osteochondral defect showed formation of hyaline cartilage with well integration to margin area. However, the subchondral bone plate was not restored. Tissue engineering in full thickness articular defect showed formation of hyaline cartilage with well integration to margin area. The engineered cartilage showed clusters of chondrocytes formation, which is different from normal cartilage organization. The subchondral bone plate was preserved. The margin of auto-transplantation site showed a gap formation. Cell clusters formation was noted, indicating the attempt of tissue to re-integrate. However, the integration was failed. The osteochondral defect for spontaneous repair ends up with collapsed adjacent cartilage with subchondral cyst formation. Under this encouraging result, we had conducted a large scale animal study with 21 miniature pigs.

5. CLINICAL TRIAL STUDY OF CARTILAGE TISSUE ENGINEERING

Currently there are no real commercialized “tissue engineering” therapy for cartilage repair. The popular F.D.A. approved autogenous chondrocytes implantation offered by Genzyme Company is actually a “cell therapy”, not “tissue engineering”, because this surgical procedure did not use any scaffold material. However, there are still some limitations in autogenous chondrocytes implantation. First, is the loss of chondrocytes phenotype after dedifferentiated cells in a prolonged monolayer culture expansion. Chondrocytes tend to dedifferentiate into fibroblastic morphology and lose their ability to form a matrix with synthesis of Type I collagen [72]. Dedifferentiated chondrocytes can re-express the chondrocyte phenotype after only a limited number of passages [73]. Therefore, it is great concerns about whether the transplanted dedifferentiated chondrocytes

Fig. 5 Study design: The sexually matured Lee-Sung miniature pigs had received tissue engineering implantation with allogeneous chondrocytes seeded tri-copolymer scaffold covered periosteum for 8 mm diameter full thickness articular defects (2mm depth) in lateral femoral condyle and osteochondral defects (5 mm depth) in medial femoral condyle in the study knee.

Fig. 6 Control group in contralateral knee: 5 mm long and 8 mm in diameter autogenous osteochondral graft in lateral condyle was harvested and then transplanted to medial condyle in the control knee, and the defect after harvesting in the lateral femoral condyle served as model for spontaneous repair.

can retain the chondrocyte phenotype after transplantation. The second question is the possibility of leakage of chondrocytes from the transplanted site, because they are injected beneath the periosteal flap into the cartilage defect and sealed with fibrin glue only, which is also biodegradable. The last concern is about an uneven distribution of chondrocytes. Because the chondrocytes were injected into the three dimensional defect in the form of cell suspension, the

Fig. 7 The gross appearance of the tissue engineering treated knee and control knee. (a) The tissue engineered knee showed smooth surface filled with repaired tissue. (b) The control knee showed unfilled defect in defect with spontaneous repair. The autogenous transplantation site showed smooth surface but with integration problem at margin.

injected chondrocytes may accumulate on the dependent side of the defect due to gravity, which will make a uneven cell distribution and leading to uneven cartilage regeneration after transplantation. Some animal studies have provided evidence that ACI may result in poor integration into the host cartilage and subchondral bone, especially in osteochondral injury. This poorly integrated area may be a stress riser [1], ultimately resulting in degeneration of the repaired tissue [6, 74].

To overcome these potential disadvantages, many investigators had employed new tissue-engineering technology with scaffold containing chondrocytes were introduced to improve maintenance of chondrocytes phenotype and more even cell distribution. Many of the studies had conducted in clinical trials. For example, Dr. Ochi Mitsuo in Japan had used atelocollagen as a scaffold to carry autogenous chondrocytes in the treatment of 51 patients with osteochondral lesions. The clinical results were promising [75]. In Italy, Professor Paolo Cherubino in University of Insubria had conducted a 13 patients' clinical trial with matrix-induced autologous chondrocytes implantation using a bilayer collagen membrane (MACI; Verigen, Leverkusen, Germany). Six patients with knee lesions showed normal or nearly normal results [76].

A collagen scaffold copolymerized with hyaluronan and chondroitin, had approved by F.D.A. for clinical trials in treatment of meniscus injury [77]. The components of this collagen scaffold (type I collagen, chondroitin and hyaluronan) are very similar to our tricopolymer scaffold. We believe, if the large scale animal studies had succeeded with our tricopolymer scaffold based tissue engineering, a clinical trial could be applied in the future.

Fig. 8 The histology examination of the study results (H&E stain): (a) Tissue engineering in osteochondral defect showed formation of hyaline cartilage with well integration to margin area. However, the subchondral bone plate was not restored. (b) Tissue engineering in full thickness articular defect showed formation of hyaline cartilage with well integration to margin area. The engineered cartilage showed clusters of chondrocytes formation, which is different from normal cartilage organization. The subchondral bone plate was preserved. (c) The margin of Autotransplantation site showed a gap formation. Cell clusters formation was noted, indicating the attempt of tissue to re-integrate. However, the integration was failed. (d) Osteochondral defect ends up with collapsed adjacent cartilage with subchondral cyst formation.

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