Original Articles

Different Effects of PLGA and Chitosan Scaffolds on Human Cartilage Tissue Engineering

Young Hoon Jeon, MD,* Jin Hyun Choi, PhD,† Joo Kyung Sung, MD, PhD,‡ Taek Kyun Kim, MD,§ Byung Chae Cho, MD, PhD,§ Ho Yun Chung, MD, PhD§

Daegu, Korea

Clinical application of the cartilage formed by tissue engineering is not practical due to the failure to maintain long-term tissue structural integrity. One of the important factors for maintaining integrity is the biomaterial for a scaffold. The purpose of the current study was to evaluate the difference between poly-lactic glycolic acid (PLGA) and chitosan as scaffolds. Human auricular chondrocytes were used. Chondrocyte-scaffold complexes were implanted in nude mice and analyzed at 4, 8, 12, 16, and 24 weeks after implantation. The volume of chondrocyte-PLGA complexes decreased rapidly. The volume of chondrocyte-chitosan complexes was well maintained with a slow decrease rate. In histological findings, mature cartilage was formed by 4 weeks in the PLGA group. However, cartilage structure was hardly found after 16 weeks. In the chitosan group, mature cartilage was detected at 8 weeks and cartilage formation became more marked with time. The expression of type II collagen protein and mRNA became weaker with time in the PLGA group. However, the expression in the chitosan group was strong for the whole period. These results suggest that chitosan is a superior scaffold for cartilage tissue engineering in terms of the maintenance of structural integrity. It is expected that after some modification for more rapid chondrogenesis, chitosan scaffolds may become one of the most useful scaffolds for cartilage tissue engineering.

Key Words: Poly-lactic-co-glycolic acid (PLGA), chitosan, cartilage-scaffold, structural integrity, tissue engineering

A number of reconstruction methods for tissue defects arising from various causes have been developed, and yet there remain many problems to overcome. Cartilage is a unique tissue without any blood vessels in parenchymal tissue, and regeneration hardly occurs after being injured. Reconstruction methods for cartilage defects applied so far are homologous or autologous cartilage grafts, and use a synthetic prosthetic device. Among them, autologous tissue grafts are the most ideal. However, autologous cartilage grafts have disadvantages such as donor morbidity, difficulty in trimming and grafting for the desired shape, and different qualities between the donor and recipient sites.[1]

In an effort to overcome these shortcomings, studies of the cartilage formation using tissue engineering have been performed since the late 1980s. Some studies had shown the actual possibility of cartilage tissue reconstruction using tissue engineering by successfully forming the tissue in a desired shape by grafting chondrocytes on three-dimensional, high molecular scaffolds.[2–4] Three important factors of tissue engineering are the biological characteristics of cells, choosing biocompatible scaffolds, and adapting to in vivo environments. In contrast with other organs, it is especially hard to use clinically the cartilage tissue formed by tissue engineering due to the problems of resorption and deformation. Choosing biocompatible scaffolds is one of the important points for solving serious problems, and thus many studies are focusing on this

From the Department of *Anesthesiology, †Natural Fiber Science, ‡Neurosurgery, and §Plastic and Reconstructive Surgery School of Medicine, Kyungpook National University, Daegu, Korea.

Address correspondence and reprint requests to Dr. Ho Yun Chung, Department of Plastic and Reconstructive Surgery, School of Medicine, Kyungpook National University, 50 Samduk 2 ga, Joong-gu, Daegu, 700-721, Korea; E-mail: hy-chung@mail.knu.ac.kr

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problem. Poly-lactic glycolic acid (PLGA), a synthetic prosthetic material that was widely used for scaffolds in early tissue engineering studies and that showed a distinct ability for tissue regeneration, has been reported as excellent in biocompatibility and biodegradability.[5,6] Chitosan, a substance extracted from natural sources and which exhibits distinct biocompatibility, has wound healing properties and is thus used more and more in tissue engineering. Because chitosan is able to maintain structures in vivo, it is suitable to be used as a scaffold for cartilage tissue engineering.

In this study, PLGA and chitosan were used as scaffold materials, and cultured human auricular chondricytes were seeded into the scaffolds. Cartilage tissues so formed were compared in terms of their formation of shape and resorption.

Materials and Methods

Isolation and Culture of Chondrocytes

Elastic cartilages were taken from human auricle from subjects under 20 years of age, and chondrocytes were isolated and cultured through Klagsbrun's method. Cartilage tissues were completely dissolved in 0.2% of type II collagenase (Gibco/BRL, Carlsbad, CA). After enzymatic digestion, the cells were suspended in a culture media with 100 μg/mL of streptomycin, 100 unit/mL of penicillin G, 25 μg/mL of ascorbic acid, 4.5 g/L glucose-including DMEM (Dulbeccós Modified Eagle Media, Gibco/BRL, Carlsbad, CA) plus 10% fetal calf serum (HyClone, South Logan, UT). The fluid was filtered through a 150 μm sieve. A monolayer culture was performed at 37°C in a 5% CO_2. Cultured cells were confirmed to be chondrocytes, through Alcian blue staining, before being grafted on scaffolds.

Fabrication of a High Molecular Scaffold

After dissolving 10% PLGA (polyglycolic acid 35%, polylactic acid 65%, molecular weight: 110,000 g/mol: Boehringer Ingelheim, Ingelheim, Germany) in dichloromethane, 75–105 μm of ammonium bicarbonate was mixed to be 5%. Three types of disc-shaped scaffolds, 7 mm in diameter and 3 mm in thickness, were manufactured: mixed, dried, and gel-like. Any dichloromethane remaining inside of the scaffolds was dried in a hood and completely removed. Then, ammonium bicarbonate was to be yielded out of 70°C of water in bubbles. When no more bubbles remained, the scaffolds were washed in water at a normal temperature, dried, and sonicated for 10 minutes.

Chitosan was fabricated in two different concentrations of 2.5% and 5%. The manufacturing processes were as follows: After putting 2.5 g and 5 g, respectively, of chitosan (poly(1→4)-β-D-glucosamine, Sigma, St. Louis, MO), whose molecular weight was 340.3 g/mol(M), in 100 ml of 1 M acetic acid solution, the mixtures were stirred at room temperature for about 20 hours and neutralized with 1 M sodium hydroxide. To make the neutralized chitosan solution into a gel of the same size as PLGA, it was put in a disc-shaped container, 7 mm in diameter and 3 mm in thickness, frozen to −70°C, and freeze-dried in a freeze dryer (Il-shin, Seoul, Korea) for about 12 hours; disk-shaped chitosan scaffolds were fabricated.

We observed the surface and structure of the scaffolds with a scanning electron microscope (SEM, S-800, Hitachi, Tokyo, Japan) and measured the pore size. The porosity was determined by an improved liquid replacement method.[7] The scaffolds were sterilized with ultraviolet light for 6 hours before chondrocyte-scaffold complex formation.

Chondrocyte-Scaffold Complex Formation

Cell suspension was made in 1×10^8 cells/mL, and seeded 200 μL, respectively, and the final number of cells was maintained as 2×10^7 cells per scaffold. Cells were adhered to the scaffolds for 4 hours at 37°C in a 5% CO_2. They were then put carefully into the culture media and cultured in vitro for one week. After this process, the chondrocyte-scaffold complex was implanted into the experimental animals.

Experimental Animals and the Implantation of the Chondrocyte Scaffold Complex

40 male nude mice (Balb/c-nu, SLC, Shizuoka, Japan), 5 weeks of age, were used as experimental animals. The mice were anesthetized with an intraperitoneal injection of 0.3–0.5 mg/g of Avertin® (tribromoethanol, Aldrich, MO) and the dorsal region was sterilized with a betadine solution. A transverse incision was performed on the mouse's back which dissected extensively between the subcutaneous layer and fascia layer. Then two chondrocyte-scaffold complexes were inserted into each cranial and caudal part. Finally, the skin was sutured. After grafting the chondrocyte-scaffold complex formed by chitosan on the cranial part of the incision line, we compared the two sides. Five percent of the chitosan scaffold was used for the left side and 2.5% for the right. The chondrocyte-scaffold complex was implanted in the caudal part

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using PLGA as the scaffold on both the left and right sides. An antibiotic (Cefazolin, 0.1 mg/kg, Yuhan Co., Seoul, Korea) and an analgesic (Buprenorphine, 0.1 mg/kg, Hanlim Pharm Co., Seoul, Korea) were administered for 5 days after transplantation. The mice were housed singly after surgery and received humane care in compliance with the guidelines of NIH for the care and use of laboratory animals.

Experimental Examination

After the chondrocyte-scaffold complexes were implanted, eight animals were sacrificed in each time with the use of an excess amount of anesthetic. Chondrocyte-scaffold complexes were retrieved and analyzed at 4, 8, 12, 16, and 24 weeks.

Gross Examination

After observing the retrieved chondrocyte-scaffold complexes in their entirety, their diameters and thicknesses were measured with a minute ruler, and the volume of the remaining complexes was calculated. In absorbed or deformed complexes, the longest and shortest diameters were measured, and using the average value, the diameter of the discs was calculated. By multiplying the thickness measured, the value of the volume was determined. The measured values were analyzed statistically by comparing each complex over time using ANOVA and Scheffe's tests.

Histological Examination

A histological examination with a light microscope confirmed the extent and the nature of cartilage formation. Those tissues including fibrous capsules that covered chondrocyte-scaffold complexes were taken as a unit, then fixed in 10% buffer formalin, and embedded in paraffin. After that, each unit was sectioned serially into 6 μm thick sections, and stained with hematoxylin-eosin, Masson's trichrome, and alcian blue.

Protein Analysis

Western blotting was performed to analyze for collagen type II protein, which is a cartilage specific protein among the extracellular matrix of cartilage. Tissues were put in a lysis buffer solution (Invitrogen, Carlsbad, CA), ground with a homogenizer, and completely dissolved. The same amount of protein was dissolved in a loading buffer, differentiated in 12% of SDS (sodium dodecyl sulfate) polyacrylamide gel (Invitrogen, Carlsbad, CA), and transferred to a nitrocellulose membrane (Amersham, Roosendaal, Netherlands). A binding of monoclonal antibody to type II collagen (Santa Cruz Biotech, Santa Cruz, CA) at a dilution of 1:1000 and horseradish peroxidase conjugated anti-rabbit IgG were detected using enhanced chemiluminescence reagents on Hyperfilm-ECL.

mRNA Analysis

To analyze the expression of mRNA for the genes of collagen type II protein, a reverse transcription-polymerase chain reaction (RT-PCR) was carried out. Total RNA was isolated from a pellet using a RNeasy Mini Kit (Qiagen, Valencia, CA). The isolated RNA samples were converted to cDNA using reverse transcriptase (SuperScript II, Invitrogen, Carlsbad, CA) and oligo (dT) primers, and then amplified by PCR using a PCR kit (GeneAmp RNA PCR kit, Perkin Elmer, Norwalk, CT). The amplification condition of RT-PCR was as follows. For the amplification of GAPDH, 5′-GGGCTGCTTTTAACTCTGGT-3′ as a forward primer and 5′-TGGCAGGTTTTTCTAGACGG-3′ as a reverse primer were used. We repeated the amplification the first 35 times at 94°C for 30 seconds, then at 60°C for 30 seconds, then at 72°C for 30 seconds and finally at 72°C for 7 minutes. For the amplification of COL2A1, 5′-CCAGGACCAAAGGGCAGAAAG-3′ as a forward primer, and 5′-TTCACCAGGTTCACCAGGATTG-3′ as a reverse primer were used. The amplification was repeated first 35 times at 94°C for 30 seconds, then at 56°C for 30 seconds, then at 72°C for 1 minute, and finally at 72°C for 7 minutes.

Following the PCR, 5 L of the samples was loaded onto a 1.5% agarose gel containing ethidium bromide, electrophoresed, and visualized under ultraviolet light.

RESULTS

Electron Microscopic Findings of Pregraft Scaffolds

As a result of examining the scaffolds with a scanning electron microscope, they were determined to be suitable for use in tissue engineering because the PLGA and chitosan scaffolds generally showed pore-well formed structures. The interfiber distance in the PLGA scaffolds was 100~200 μm, and the porosity was 93~96% (Fig 1A).

In the chitosan scaffolds, the distance between each fiber was 50~100 μm, and the porosity was 83~87% with smaller pores and a higher density than in the PLGA scaffolds. In the 5% chitosan,

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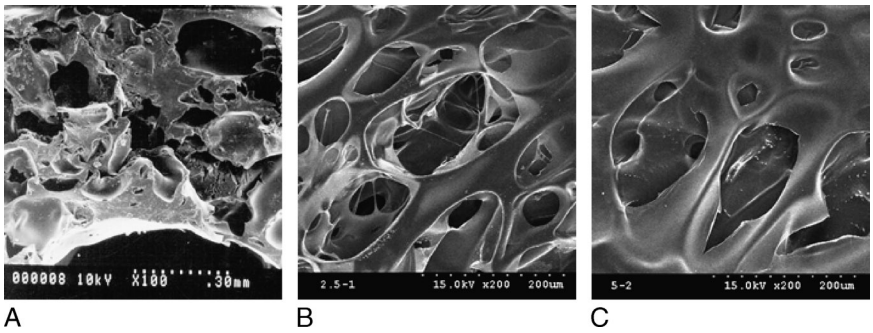

Fig 1 Scanning electron microscopical finding of scaffolds. (A) PLGA scaffold. (B) 2.5% chitosan scaffold. (C) 5% chitosan scaffold. PLGA scaffold has longer interfiber distance and higher porosity than 2.5% and 5% chitosan scaffolds.

porosity was 80~83%; that is, the pores were smaller and denser than in the 2.5% chitosan. The distances between the fibers did not show any difference between the 2.5% and 5% chitosans (Fig 1B, C).

Gross Examination

Chondrocyte-scaffold complexes implanted in each of the eight animals were retrieved at 4, 8, 12, 16, and 24 weeks after implantation. All complexes were easily separated from the surrounding tissues. At 4 weeks after implantation, milk-colored cartilage tissue formation was observed more in the PLGA-scaffold complex than in the chitosan-scaffold complexes at the same period, but the volume of the PLGA-scaffold complex was 109.3 ± 7.1 mm^3, which had no significant differences from the chitosan group, and which was rather similar to the volume at the time of the graft. At 8 weeks after implantation, a significant decrease in volume was observed in the PLGA-scaffold complex. The average decreased to 52.1 ± 10.1 mm^3, which was about half of 115.4 mm^3, the original volumed of the disc-shaped scaffolds. Chondrogenesis increased in the chitosan-scaffold complexes at 8 weeks after implantation, and milk-colored cartilages were observed. The volumes, 112.7 ± 7.1 mm^3 in the 5% chitosan and 110.2 ± 5.9 mm^3 in the 2.5% chitosan, were similar to those of the original volume of the disc-shaped scaffolds (Fig 2A). At 12 weeks, the mean volume of the PLGA-scaffold complex was 16.7 ± 9.7 mm^3, an 86% value of the decreased volume. However, compared to this, the mean volume of the 5% chitosan-scaffold complex was 107.1 ± 5.3 mm^3 and that of the 2.5% complex was 101.7 ± 8.1 mm^3, thus indicating that the volume of the original form was relatively well maintained (Fig 2B). In the PLGA-scaffold complex, absorption occurred at 16 weeks and many of them almost lost their original form. At 24 weeks,

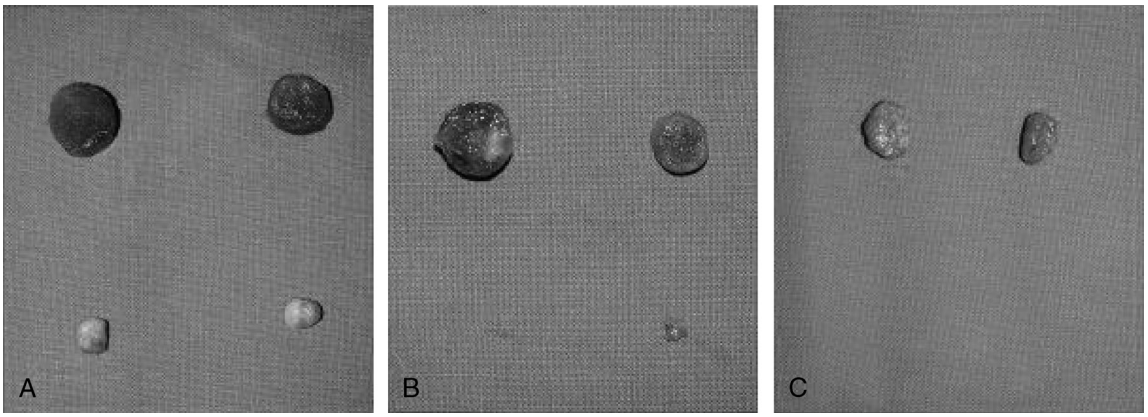

Fig 2 Volume changes of the different chondrocyte-scaffold complexes. (A) Chondrocyte-scaffold complexes 8 weeks after implantation. (B) Chondrocyte-scaffold complexes 12 weeks after implantation. (C) Chondrocyte-scaffold complexes 24 weeks after implantation. Upper left complex on the upper right and both lower pictures is a chondrocyte chitosan complex with 5% chitosan. Upper right complex is a chondrocyte-chitosan complex with 2.5% chitosan. Both lower complexes are the chondrocyte- PLGA complexes. Most of the chondrocyte-PLGA complexes on lower pictures were absorbed.

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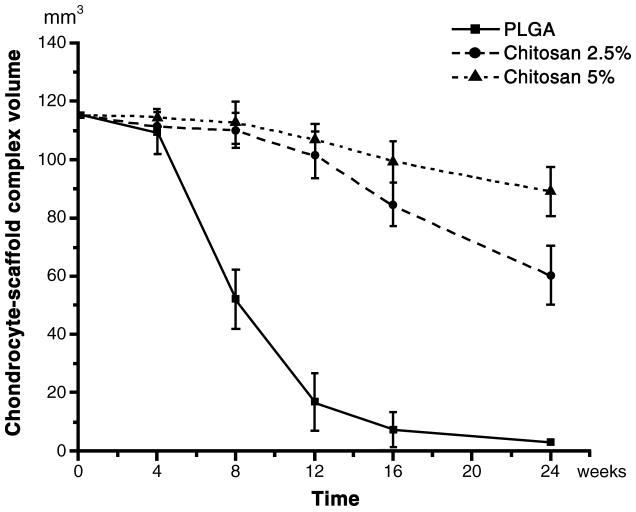

Fig 3 Changes of chondrocyte-scaffold complex volume following the period of implantation. The volume decrease rate in chondrocyte- PLGA complexes is rapid than in chondrocyte-chitosan complexes.

complexes were observed in 2 out of the 8 mice, and the mean volume was as small as 3.1 ± 1.3 mm^3, indicating that absorption was almost complete (Fig 2C). At 16 weeks, the volume of the 2.5% chitosan-scaffold complex was 84.5 ± 7.5 mm^3, which showed more absorption than the 99.4 ± 7.1 mm^3 in the 5% chitosan-scaffold; however, there was no statistical significance. At 24 weeks, the chitosan still was not fully biodegraded, and the decreased rates in volume were 60.3 ± 10.1 mm^3 in the 2.5% chitosan and 89.1 ± 8.5 mm^3 in the 5% chitosan, which meant that the volume decreased in the 2.5% chitosan more than in the 5% chitosan with a statistical significance (Fig 3).

Histological Examination

At 4 weeks, in the PLGA-scaffold complex, there appeared to be normal cartilage tissue; that is, the structure of cartilage tissues with chondrocytes was dispersed in the myxoid extracellular matrix and there were some undissolved PLGA fibers. Around those fibers, polymorphonuclear leukocytes and giant cells, which are evidence of an inflammatory response, were observed (Fig 4A). At 8 weeks, the distances between cells increased, the cell density decreased, and thus the existence of lacuna was confirmed, indicating mature chondrocytes (Fig 4B). After 16 weeks, histological observation was difficult because the scaffolds were almost absorbed and the form was not maintained. On trichrome staining, abundantly formed collagen fibers were observed at 4 and 8 weeks, and on alcian blue staining, which is used to stain glycosaminoglycan, positive reactions were observed at 4 and 8 weeks of the initial experimental period. That is, at 4 and 8 weeks, chondrocytes were confirmed to have formed cartilage structures by synthesizing collagen and glycosaminoglycan, the main substance of the extracellular matrix (Fig 4C, D). At 12 weeks, they showed similar characteristics to those at 8 weeks, however, the amount of cartilage tissue was generally decreased. The cartilage tissues were almost absorbed after 16 weeks from implantation indicating that histological observation would be difficult.

The formation of mature cartilage tissues at 4 weeks in the chitosan-scaffold complexes was not observed histologically. However, although round chondrocytes were seen, they hadn't made any extracellular matrices yet. Strong inflammatory responses were due to the infiltration of polymorphonuclear leukocyte and giant cells around the retiform structure of the chitosan. At 8 weeks, cartilage structures were observed in some parts, but no mature cartilage structures were found (Fig 5A). With the degradation of the chitosan at 12 and 16 weeks, it was found to be displaced with mature cartilage structure (Fig 5B), and positive reactions to the trichrome staining and alcian blue staining grew gradually stronger, indicating that chondrocytes form extracellular matrices and cartilage structures with time (Fig 5C, D). However, undissolved chitosan structures were still observed even after 24 weeks, and inflammatory responses persisted, although fewer than before. In histological findings, there was not any difference in the formation of cartilage structure between the concentrations of 2.5% and 5%.

Protein Analysis

In the western blotting examination to detect collagen type II protein, chondrocyte-scaffold complexes formed with PLGA as a scaffold, showed the expression of collagen type II protein from 4 weeks after implantation, and the strongest expression at 8 weeks, which gradually decreased later on.

Chondrocyte-scaffold complexes using 2.5% chitosan as a scaffold showed the expression of collagen type II protein at 4 weeks, but the intensity of the expression was quite weak. It began to strongly be expressed after 8 weeks, and the strong expression was maintained through 12, 16, and 24 weeks. Chondrocyte-scaffold complexes using 5% chitosan showed a relatively strong expression of collagen

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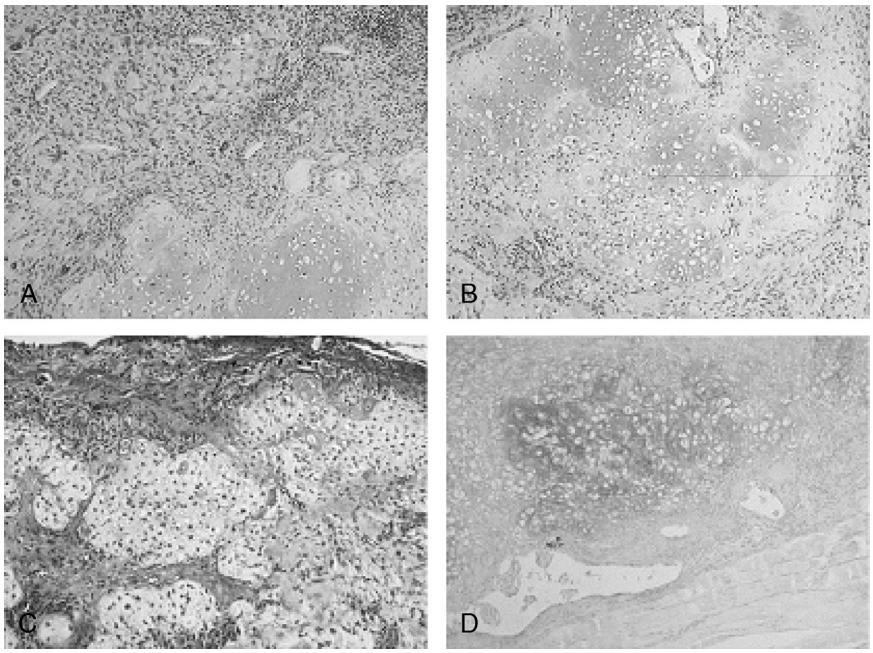

Fig 4 Histological findings of chondrocyte-PLGA complexes. (A) 4 weeks after implantation (H&E, ×100). (B) 8 weeks after implantation (H&E, ×100). (C) 8 weeks after implantation (Masson's trichrome stain, ×100). (D) 4 weeks after implantation (alcian blue stain, ×100). The mature cartilage tissues with abundant extracellular matrix are found on 4 and 8 weeks after implantation.

type II protein at 4 weeks, which was maintained continuously for 16 weeks with the strongest level at 24 weeks (Fig 6).

mRNA Analysis

As a result of observing the mRNA expression for collagen type II genes, chondrocyte-scaffold complexes using PLGA as scaffolds, showed a strong expression at 4 and 8 weeks, which tended to decrease with time. On the other hand, in chondrocyte-scaffold complexes using 2.5% and 5% chitosan as scaffolds, the expression of mRNA of chondrocytes for collagen type II genes was strongly expressed after 4 weeks of the experimental period. Those two experimental groups with different concentrations did not show any differences of expression, and the extent of expression was observed to increase with time (Fig 7).

Discussion

Tissue regeneration and reconstruction by tissue engineering have been recently spotlighted through the formation of desirable tissues and organs from a small amount of autologous tissues using biocompatible scaffolds created from a culture procedure. Since 1988, when cartilage tissue engineering first started to be used for the regeneration of hyaline cartilage,[8] many studies and good results have been reported from the early stage.[4] However, clinical applications of the cartilage tissue formed by tissue engineering have not been successful due to the failure to maintain long-term tissue structural integrity.[1,3–5] To develop clinical applications, obtaining the most suitable scaffold through the comparison and analysis of biomaterials for tissue regeneration, is an important factor. Generally, scaffolds for cartilage regeneration need to be maintained for two to three weeks during which time they should compose sufficient extracellular matrixes so that cells can support the physical structure, and thereafter when the tissues needed are regenerated, the scaffolds should be completely degradable. Polyglycolic acid polymer (PGA) with a diameter of 15 m has been most widely used as scaffolds for cartilage regeneration and is known to be a suitable scaffold for the cartilage formation.[6] Polylactic acid (PLA) and PLGA have also been widely used as synthetic, high molecular materials for scaffolds for cartilage formation.[4–6] They are biodegradable in vivo. A hydrolysis process, which is a process of degradation, occurs more easily in the case of PGA because it has more hydrophilicity than

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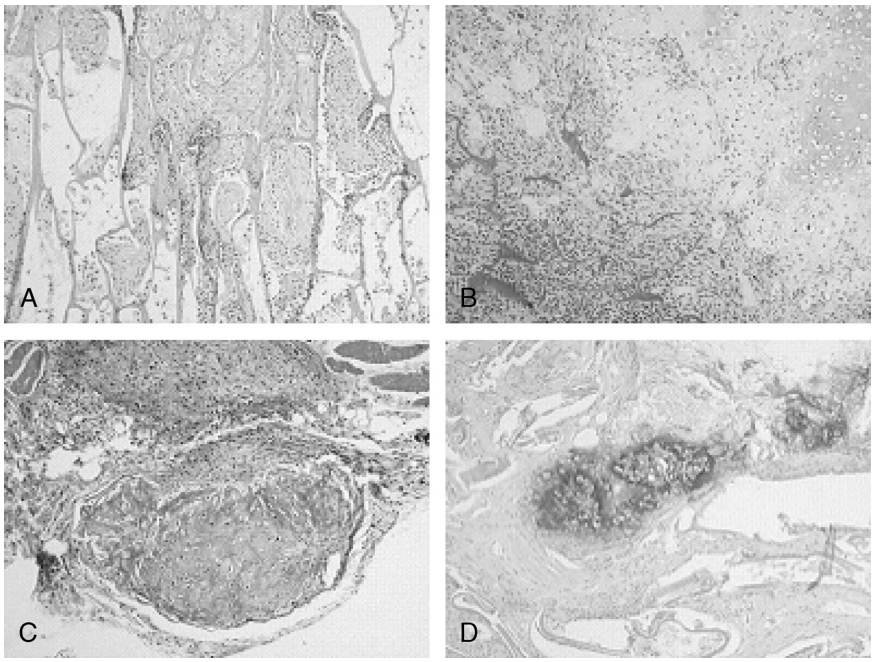

Fig 5 Histological findings of chondrocyte-chitosan complexes. (A) 8 weeks after implantation of the chondrocyte-chitosan complex using 5% chitosan (H&E, ×100). The cartilage tissues can be found between chitosan fibers. (B) 16 weeks after implantation of the chondrocyte-chitosan complex using 5% chitosan (H&E, ×100). (C) 16 weeks after implantation of the chondrocyte-chitosan complex using 2.5% chitosan (Masson's trichrome stain, ×100). (D) 16 weeks after implantation of the chondrocyte-chitosan complex using 5% chitosan (alcian blue stain, ×100). Mature cartilage tissues are formed with time.

PLA, and the degradation rate of PGA is fast enough to be 99% dissolved in four weeks.[6] However, more hydrophilicity is considered to be useful to make cartilage matrices because it is conductive to the adhesion and growth of the cells. In comparison, PLA has a slower speed of hydrolysis because its hydrophobicity is strong and the invasion of water is slow, so that it is easier to maintain the structure but harder for cells to adhere due to its hydrophobic property. PLGA is a scaffold to supplement both merits and demerits. PGA and PLA are usually mixed at ratios from 30:70–50:50 according to the purpose of the experiments. In this experiment, we used a ratio of 35:65, which is most widely used, and it has been reported that they took about two to three months to be completely dissolved.[9]

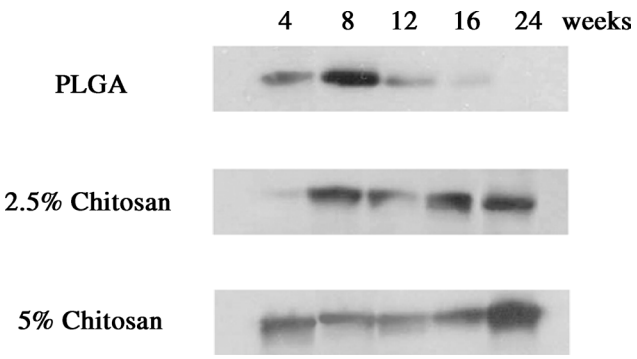

Fig 6 The expression pattern of collagen type II protein using western blots. The expression of type II collagen becomes weaker with time in chondrocyte-PLGA complexes. The expression of type II collagen is strong for whole experimental period in chondrocyte-chitosan complexes using 2.5% and 5% chitosan scaffold.

Many kinds of high molecular biomaterials extracted from natural substances have been used as scaffolds for cartilage tissue engineering because of better biocompatibility and a higher cell adhesion. Among those are proteins such as collagen, gelatin, kelatin, and fibronectin, and polysaccharides such as cellulose, dextran, alginate, hyaluronic acid, chitosan, and GAG. Most of the natural high molecular materials tend to be weaker in intensity than synthetic ones, and yet there has been much reinforcement recently with the development of biomaterials. Among them, chitosan is obtained by deacetylating chitin, which is a natural high molecular material abundantly present in the shells of crustaceans, insects, mollusks, molds, yeasts, mushrooms, etc.[10]

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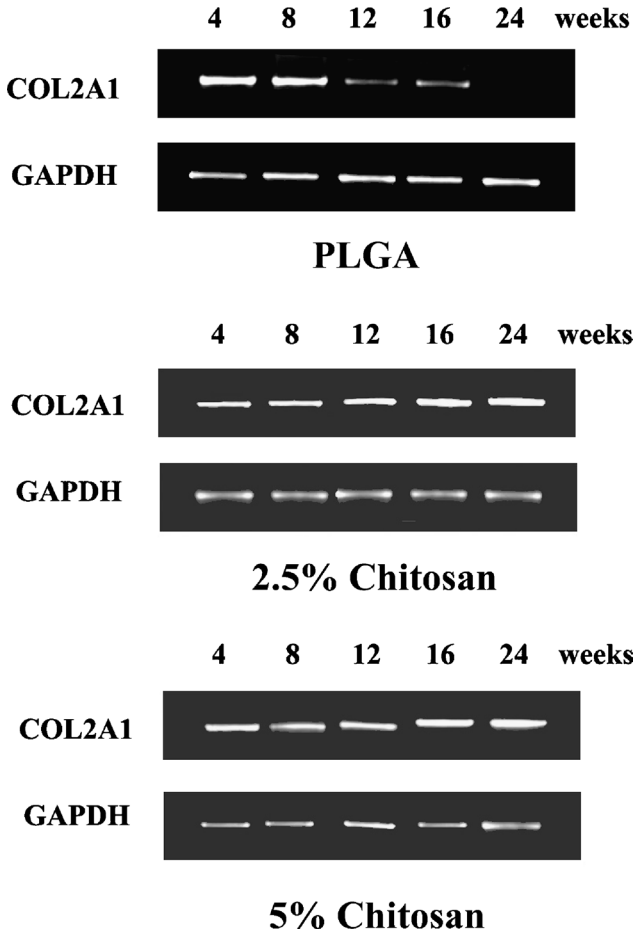

Fig 7 The expression pattern of collagen type II gene mRNA using RT-PCR. The experession of COL2A1 becomes weaker with time in chondrocyte-PLGA complexes. The expression of COL2A1 is strong for whole experimental period in Chondrocyte-chitosan complexes using 2.5% and 5% chitosan scaffold. COL2A1: collagen type II gene. GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Chitosan has been widely used for many purposes, such as an anionic reagent to manage pollutants, agricultural materials, food additives, anticoagulants, antithrombotics, cosmetic materials, materials of crops, papers, films, sponges, materials of analytical reagents, or in chromatography.[11] Many experimental and clinical studies have been reported since Hoffmeister et al[12] first demonstrated the wound-healing effects of chitosan.[13] Although the specific mechanism has not yet been elucidated, there have been many studies of chitosan as a good wound-healing material with an antimicrobial character, biocompatibility, resorption of permeators, etc.[14–16] Chitosan has a high possibility of being used for tissue engineering because it has an excellent biocompatibility, the possibility of being decomposed into oligosaccharides, and the ability to form insoluble complexes in vivo with such connective tissues as collagen and GAG, etc.[9,17] Especially, it has a high utility value in that its mechanical intensity is controllable with the concentration of chitosan, and it is possible to get as strong an intensity as with synthetic high molecular materials.

In the current study, PLGA was considered to be superior to chitosan in the formation of the structure because it forms cartilage early. However, after two to three months during which the PLGA was dissolved, the cartilage tissue structure was not maintained. In comparison, the cartilage-scaffold complexes lasted for a long time so that the volume was also maintained, and the functions of the human chondrocytes inside were found not to have decreased with the absorption of scaffolds.

In tissue engineering, cell-matrix interaction and cell-biopolymer interaction still have to be verified. It is necessary to apply this concept of in vivo experiments, such as in this study, to interpret the results. Naturally, the original properties of biomaterials used as scaffolds affect the results; however, the kind of interaction between cells and the environment in vivo should be considered to evaluate the results. There has not been any definite report on the interaction of PLGA with cells in vivo, nor with the extracellular matrix produced by the cells. But there have been some reports that when PLGA was cultured adhering chondrocytes in vitro, chondrocytes in a circular shape were proliferated in three dimensional structures which maintained their character well, and formed extracellular matrices. The chondrocytes were differentiated and proliferated to be matured through signal transmission between extracellular matrices and ligands of membranes.[18–21] The results of this in vivo study do not maintain such a mature structure. It is considered that PLGA tends to accompany many inflammatory responses as foreign substances in vivo, and that the products from the hydrolysis process of PLGA have acidity and make a low pH environment.[9,22] Accordingly, chondrocyte maturation is blocked and inflammatory responses accompanied by extracellular matrix degradation are intensified such that the resorption of cartilage tissues seems to be accelerated. Moreover it is interpreted to be an opposite result that with in vitro, acidification is neutralized by culture fluid, and inflammatory reactions do not exist. The degradation process of chitosan is different from than that of PLGA because the former is depolymerized by lysozyme. Chitosan is polycationic, but

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different from other polycationic materials which have cytotoxicity in that the amino group is dissociated in vivo and has low cytotoxicity.[23] It seems to have a better result than PLGA in terms of the chondrocyte survival, maintenance of character and functions, extracellular matrix formation, and cartilage structure maintenance.

As observed from the gross and histological investigations of this study, cartilage formation seemed to be lower in the chitosan-used complexes than in the PLGA complexes for the initial period of implantation, which can be attributed to the porosity differences. There seemed to be no significant differences between them, because both had hydrophilicity, excellent adhesion, proliferation in accordance, and a differentiation capacity. The PLGA used in this study had a significant difference from chitosan in cartilage formation at an early period after implantation because it had more than 95% porosity. Porosity is an important factor in tissue engineering. By securing porosity, cell movement and proliferation are possible and the space to make an extracellular matrix is created. Generally, if a cell is 10 μm in size, the average interfiber distance should be 67 μm for cells to move and proliferate; more than 95% of porosity is required for cells to spread through biomaterials to get well supplied with various serum materials.[22] In this study, the interfiber distance of chitosan was 50–100 μm, narrower than that of PLGA which had 100–200 μm. As well, the porosity was 85% in the 2.5% chitosan and 80% in the 5% chitosan, and therefore smaller than the 95% for PLGA. This seems to explain why the cartilage formation did not occur well enough in the early stage. The exact time when the chitosan was completely dissolved in vivo was not clear, although Tomihata and Ikada[23] has observed that less than 10% is dissolved 50 hours after soaking 84% deacetylated chitosan film in a lysozyme solution in vitro. They mentioned that even after 12 weeks of injection in the subcutaneous tissue, 80% of the original weight was maintained. At 12 weeks after implantation, 88% of the volume was maintained in the case of the 2.5% chitosan complexes, 93% in the case of the 5% complexes, and a considerable amount of the chitosan seemed to be left without being dissolved. Generally in cell adhesion, the degradation of the cells by lysis enzyme, which is a biologically active product, is faster than that of biomaterials that have no cell adhesion, and the speed is expected to be very slow which seems to be a great advantage in terms of the structural maintenance. On the other hand, in the aspect of desired tissue regeneration, continuing inflammatory responses to the foreign material and fibrosis might be caused and thus this should be considered.

CONCLUSIONS

With this study using PLGA and chitosan as scaffolds, the formation and maintenance of cartilage tissues, the character maintenance of accompanying chondrocytes, and the formation of extracellular matrices, were compared and analyzed. As a result, it was found that it is important to choose a good biomaterial which has a superior biocompatibility and a more lasting mechanical intensity in the view of resorption and deformation for clinical application. In the future, good results from clinical applications can be expected with the effort of verifying the precise character of these kinds of biomaterials.

REFERENCES

1. Rodriguez A, Cao YL, Ibarra C, et al. Characteristics of cartilage engineered from human pediatric auricular cartilage. Plast Reconstr Surg 1999;103:1111–1119
2. Kim WS, Vacanti JP, Cima L, et al. Cartilage engineered in predetermined shapes employing cell transplantation on synthetic biodegradable polymers. Plast Reconstr Surg 1994; 94:233–237
3. Raghunath J, Salacinski HJ, Sales KM, et al. Advancing cartilage tissue engineering: the application of stem cell technology. Curr Opin Biotechnol 2005;16:503–509
4. Risbud MV, Sittinger M. Tissue engineering: advances in vitro cartilage generation. Trends Biotechnol 2002;20:351–356
5. Ma PX, Schloo B, Mooney D, et al. Development of biomechanical properties and morphogenesis of in vitro tissue engineered cartilage. J Biomed Mater Res 1995;29:1587–1595
6. Hubbell JA. Biomaterials in tissue engineering. Biotechnology(NY) 1995;13:565–576
7. Zhang R, Ma PX. Poly(α-hydroxyl acids)/hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology. J Biomed Mater Res 1999;44:446–455
8. Vacanti JP. Beyond transplantation. Arch Surg 1998;123: 545–549
9. Pachence JM, Kohn J. Biodegradable polymer. In: Lanza RP, Langer R, Vacanti JP, eds. Principles of Tissue Engineering. San Diego: Academic press, 2000:263–277
10. Kumar MN, Muzzarelli RA, Muzzarelli C, et al. Chitosan chemistry and pharmaceutical perspectives. Chem Rev 2004; 104:6017–6084
11. Hirano S. Chitin biotechnology application. Biotechnol Annu Rev 1996;2:237
12. Hoffmeister FS, Wenner C, Wilkens HJ, et al. Effect of N-acetyl-D-glucosamine on wound healing of surgical wounds and on mitochondrial phosphorylation uncoupled by dinitrophenol. Surgery 1964;56:1129–1133
13. Shigemasa Y, Minami S. Applications of chitin and chitosan for biomaterials. Biotechnol Genet Eng Rev 1996;13:383–420
14. Muzzarelli R. Role of lysozyme and N-acety1-β-D-glucosaminidase in the resorption of wound healing. In: Brine CJ, Sandford PA, Zikakis JP, eds. Advances in Chitin and Chitosan, 1st Ed. New York: Elsevier Applied Sci, 1992:25–33

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15. Okamoto Y, Shibazaki K, Minami S, et al. Evaluation of chitin and chitosan on open wound healing in dogs. J Vet Med Sci 1995;57:851–854
16. Koyano T, Minoura N, Nagura M. Attachment and growth of cultured fibroblast cells on PVA/chitosan blended hydrogels. J Biomed Mater Res 1998;39:486–490
17. Nettles DL, Elder SH, Gilbert JA. Potential use of chitosan as a cell scaffold material for cartilage tissue engineering. Tissue Eng 2002;8:1009–1016
18. Dessau W, Sasse J, Timpl R, et al. Synthesis and extracellular deposition of fibronectin in chondrocyte cultures. Response to the removal of extracellular cartilage matrix. J Cell Biol 1978;79: 342–355
19. Hynes RO. Integrins: versatility, modulation and signal in cell adhesion. Cell 1992;69:11–25
20. Glowacki J, Trepman E, Folkman J. Cell shape and phenotypic expression in chondrocytes. Proc Soc Exp Biol Med 1983;172:93–98
21. Baek CH, Lee JC, Jung YG, et al. Tissue-engineered cartilage on biodegradable macroporous scaffolds: cell shape and phenotypic expression. Laryngoscope 2002;112:1050–1055
22. Kang IG. Biomaterials for tissue engineering applications. In: Yoo JJ, Lee IW, eds. Tissue engineering and regenerative medicine. Seoul: Koonja Pub Inc, 2002:205–226
23. Tomihata K, Ikada Y. In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. Biomaterials 1997;18: 567–575

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