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Effect of Short-Term Enzymatic Treatment on Cell Migration and Cartilage Regeneration: *In Vitro* Organ Culture of Bovine Articular Cartilage

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Depending on the damage extent and adjacent tissue condition in traumatic cartilage injury, it is possible to heal the tissue by resident cells. Unlike autologous chondrocyte implantation, short-term enzymatic treatment is an effective single-step procedure without extra cell expansion. Moreover, this method has been shown to significantly increase cellularity in lesion edges, resulting in enhanced integration and interfacial strength. We hypothesize that the locally digested extracellular matrix by treatment allows effortless cell migration from the adjacent tissue. Full-thickness cartilage discs and osteochondral explants were prepared from mature bovine stifle joints. These specimens were treated with collagenase in a culture medium. Two concentrations, 0.25 and 0.5 mg/mL, were used with various treating time of 10, 30, and 180 min. The cartilages were subsequently washed and cultured with fibrin hydrogel. The effect of enzymatic treatment on cell migration was apparent in both experiments of the cartilage disc and full-thickness cartilage defect model. In the disc culture, the treatment resulted in an approximately three to four times higher number of migrated cells than nontreated control. In short-term collagenase-treated groups, the proteoglycan (PG) loss was localized in the edge of tissue with minimal cell death. The treatment also accelerated cell migration in the full-thickness cartilage defects and some cells differentiated into chondrocytes with the deposit of PG. Gene expression results could support the characteristics of migrated cells, which had migratory ability and chondrogenic differentiation potential with overexpression of collagen type I and II, respectively. Based on these results, short-term enzymatic treatment, which can accelerate cell migration into traumatically injured cartilage, has great potential for clinical application.

Introduction

Articular cartilage injuries can be caused by either traumatic mechanical destruction like sport accident or progressive mechanical degeneration such as wear and tear. The injuries may result in pain, swelling, and subsequent loss of joint function, finally leading to osteoarthritis. In mature articular cartilage, chondrocytes mainly receive their nutrition through diffusion from the synovial fluid and this limits their intrinsic capacity for cartilage healing.

Decisions about whether and how to treat damaged cartilage remain a challenge to an orthopedic surgeon.[1] Autologous chondrocyte implantation (ACI) is one of the promising techniques for repair of articular cartilage defects. Brittberg *et al*.[2] and Brittberg[3] have reported a good clinical outcome in their long-term study of ACI. On the other hand, ACI has resulted in hypertrophic differentiation with subsequent ossification and poor integration to host tissue.[4] Not only autologous chondrocytes, but also mesenchymal stem cells (MSCs),[5] periostal cells,[6] skeletal muscle,[7] adipocytes,[8] and synovial fibroblasts[4,9,10] represent possible cell sources for cell-based cartilage repair. However, cell-based therapy requires two surgical procedures, which are isolating of articular cartilage biopsy for chondrocyte isolation and implanting of the cells. Furthermore, cell expansion and differentiation require coordination and maintenance of the regular chondrogenic differentiation and may be possible to induce unsolved problems at present.[11]

Although stable regeneration of hyaline cartilage is still challenging, it is possible to heal articular cartilage by resident cells depending on the injury extent and location. Short-term enzymatic treatment is an effective single-step procedure without extra cell expansion for inducing the migration of resident cells. Several studies have reported a

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significantly increased chondrocyte density in lesion edges by enzymatic treatment. Lee *et al.*[12] and Hunziker and Kapfinger[13] used chondroitinase ABC to remove proteoglycans (PGs) in superficial zone of the cartilage. The chondroitinase ABC can selectively degrade the PGs without affecting the collagen matrix.[14] The treatment also showed increased adhesion force and cell population after treatment. Treatment with hyaluronidase (0.1%–0.3%) and collagenase (type VII, 10–30 U/mL) showed significant chondrocyte density in lesion edges and enhanced integration and interfacial strength.[15–17] However, there is no current study focusing on the effect of enzymatic treatment for cell migration. Thus, increased cell density has a strong potential to allow active cell migration in the lesion edges, eventually leading enhanced cartilage regeneration.

Unfortunately, none of enzymes have taken U.S. Food and Drug Administration (FDA) approval to use in clinic except collagenase. Collagenase isolated from *Clostridium histolyticum* was clinically proven for treatment of Depuytren's disease.[18] It acts as a collagenolytic enzyme, which digests triple-helical collagens into small peptides. Since the collagenase contains both type I and II, it is also applicable to digest the extracellular matrix of articular cartilage, which has mainly type II collagen. Therefore, we determine to use collagenase as a tissue enzyme in this study and hypothesize that the short-term treatment of collagenase can break down collagen and PG network around the injured site without cell damage and accelerate cell migration from the host tissues to injured cartilage. The aims of the present work were to evaluate the effect of short-term enzymatic treatment on cell migration and determine the optimal concentration and treated duration of collagenase using bovine cartilage discs. Moreover, the selected optimal condition tested for a potential of cartilage regeneration using full-thickness cartilage defect model in bovine osteochondral explant.

Materials and Methods

Harvesting of bovine articular cartilage discs and explants

Ten bovine stifle joints from skeletally mature animals were obtained after slaughter from a local abattoir (Bud's Custom Meats, Riverside, IA). Full-thickness cartilage discs ($\sim$2-mm thickness) were prepared using a 2-mm-diameter biopsy punch (Miltex, Inc., York, PA) from the femoral trochlea, and osteochondral explants were prepared by manually sawing an $\sim 25 \times 12.5 \times 10$ mm (thickness) from the outer bovine tibial plateau, which was underneath the meniscus and created two 4-mm-diameter defects with 2-mm depth in an explant. The 55 discs and 32 explants were rinsed in Hank's balanced salt solution and cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL fungizone. All explants were cultured under hypoxic culture condition (5% O_2/CO_2 at 37°C).

Enzymatic treatment of cartilage discs and explants

Isolated cartilage discs and explants were treated with collagenase from *C. histolyticum* (C0130; Sigma-Aldrich, St. Louis, MO) in the culture medium. Two concentrations, 0.25 and 0.5 mg/mL, were used with various treated time periods of 10, 30, and 180 min. The cartilages were subsequently washed and cultured with TISSEEL™ fibrin hydrogel (Baxter Healthcare Corp., Westlake Village, CA). One cartilage disc was placed in a 96-well culture plate and added with the 1:1 mixture of 50 mg/mL fibrinogen and 10 U/mL thrombin. The discs were cultured for 12 days and the medium was changed every 2 days. In an explant model, the defects were locally treated by collagenase (0.25 mg/mL for 30 min) and filled with the fibrin hydrogel. The explants were cultured for 3 weeks and the medium was changed every 3 days.

Safranin-O/fast green stain

The samples were fixed in 10% neutral buffered formalin and embedded in a tissue freezing medium. Ten micrometer-thick cryosections were stained with Safranin-O/fast green and imaged in a transmitted light mode on an Olympus BX-60 microscope.

Dimethylmethylene blue assay

To quantify PG, cartilage discs were digested with the papain digestion buffer containing 1 mg/mL papain (Sigma-Aldrich), 5 mM L-cysteine HCl, 100 mM Na_2HPO_4, and 5 mM Na_2-EDTA in a 65°C oven for 2 h. GAG content was determined by dimethylmethylene blue (DMMB) dye-binding assay. Briefly, serially diluted samples were prepared and the DMMB solution was added. The absorbance was measured at 530 nm using the VMax Kinetic ELISA microplate reader (Molecular Devices, Inc., Sunnyvale, CA). The PG standard was established using chondroitin-6-sulfate (Sigma-Aldrich).

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

To identify cell death after collagenase treatment, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed using the *In Situ* Cell Death Detection Kit (Roche, Mannheim, Germany). Cartilage disc cryosections were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and incubated in a permeabilization solution (0.1% Triton X-100 and 0.1% sodium citrate). Finally, the TUNEL reaction mixture was added and imaged on the Olympus BX-60 microscope. DNase I recombinant was treated for positive control.

DNA quantification assay

The fibrin hydrogel isolated from disc culture was digested in the papain buffer to quantify the amount of migrated cells from a cartilage disc and the fluorometric DNA quantitation method was performed using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Molecular Probes, Inc., Eugene, OR). DNA standard was also loaded to allow the conversion of selective fluorescent units to cell contents. Digested fibrin hydrogel without cells was prepared as a blank. Fluorescence was measured on the SpectraMax M5 multidetection microplate reader (Molecular Devices, Inc.) set to 480 nm excitation and 520 nm emission.

Confocal examination

To assess the cell migration on the surface of a fibrin filler, the explants were stained with 1 μg/mL calcein AM

and 1 μM ethidium homodimer (Invitrogen) and imaged on a Bio-Rad 1024 confocal microscope. The sites were scanned to an average depth of 330 μm at 40-μm intervals. Z-axis projections of confocal images were made using ImageJ (rsb.info.nih.gov/ij). After taking images of the explants, cartilage was separated from the bone and embedded in the tissue freezing medium for collagen type VI (Col VI) immunostaining. Cryosections were digested by 1600 U/mL testicular hyaluronidase (Sigma-Aldrich). After washing in PBS with 0.3% Tween 20 (PBST), the sections were overlaid by the Col VI antibody (Abcam, Inc., Cambridge, MA) and incubated overnight at 4°C. Then, the samples were incubated in the goat anti-mouse secondary antibody (Alexa Fluor 568) and observed under the Zeiss 710 confocal microscope.

Gene expression analysis

For gene expression analysis, bovine osteochondral explants with full-thickness cartilage defects were treated with 0.25 mg/mL collagenase for 30 min or without collagenase. Fibrin hydrogels filled in the defects were harvested and total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The primer information of target and reference genes is summarized in Table 1. Quantitative real-time PCR (qRT-PCR) was performed with the SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR kit (Invitrogen) following our previous method.[19] Final quantitation of qRT-PCR was calculated using the comparative C_T method with the following equation:

$$\text{Fold change} = 2^{-\Delta\Delta C_T}, \text{where } -\Delta\Delta C_T = (C_{T\,\text{target}} - C_{T\,\beta\text{-actin}})_{\text{nontreated}} - (C_{T\,\text{target}} - C_{T\,\beta\text{-actin}})_{\text{treated}}.$$

Statistical analysis

All data from TUNEL ($n=4$), DMMB ($n=5$), and DNA ($n=4$) assays were processed for statistical analysis using SPSS software (Ver. 19; SPSS, Inc., Chicago, IL) with one-way ANOVA and *post hoc* pairwise comparison. All the results are expressed as mean ± standard deviation. Statistical significance was set at $p<0.05$ and minimum acceptable power set at 0.9.

Results

We examined the effect of short-term collagenase treatment on cell migration using full-thickness cartilage discs. After short-term (10 and 30 min) collagenase treatment, the Safranin-O/fast green stain showed PG loss in only edge of tissues (Fig. 1B–D) compared with nontreated control (Fig. 1A). On the other hand, most of the PG was depleted in long-term treatment (180 min, Fig. 1E). After 12-day culture, all treated groups showed no apparent PG loss, but the stain intensity was weak. In particular, the tissue structure was instable with gaps in groups of 0.5 mg/mL for 10 min (Fig. 1I) and 0.25 mg/mL for 180 min (Fig. 1J). The total PG content was quantified using DMMB assay (Fig. 1U). Similar to the Safranin-O/fast green stain, short-term treatment induced a partial PG loss at day 0 and gradually increased the PG loss during the 12-day culture. Compared with control (no treatment), most of the groups showed a significant PG loss except the group of 0.25 mg/mL for 10 min. In the individual group, the PG loss in the group of 0.25 mg/mL for 30 min was only significant at day 12 ($p=0.005$). Long-term treatment showed severe PG loss even at day 0 with ~30% cell death (Fig. 1O, T, and V). In contrast, only few cells were positively stained in both the control and treated groups.

The effect of enzymatic treatment on cell migration was also apparent in the full-thickness cartilage defect model. In light microscopic examination at day 12, a number of elongated cells were migrated out from a 2-mm cartilage disc into the fibrin hydrogel after short-term enzymatic treatment (Fig. 2B–D). Long-term collagenase treatment stimulated more active cell migration, but round-shaped cells, which were damaged by overdigestion, were observed in the edge of tissue (Fig. 2E). On the other hand, it was hard to find migrated cells in the nontreated control (Fig. 2A). These migrated cells were quantified by DNA quantification assay at day 7 and 12 (Fig. 2F). All treated groups showed a significantly increased DNA content compared with control at day 7. In particular, the group of 0.25 mg/mL collagenase treatment for 30 min stimulated a three to four times higher cell migration than nontreatment.

In the full-thickness cartilage defect model, we could observe active cell migration on the surface of host tissue. Pericellular matrices were positively stained around chondrocytes in normal cartilage (Fig. 3A). These matrices were dramatically changed with an irregular shape in injured tissue (Fig. 3B). Migrated cells out of the cartilage surface also stained Col VI and showed a morphologically elongated shape, which was distinct with round chondrocytes (Fig. 3C). The distribution of these migrated cells on the surface was enhanced by short-term collagenase treatment. In the group of 0.25 mg/mL for 10 min, cells migrated toward the center of cartilage defect with ~25% coverage of the defect surface (Fig. 3E). The defects were repopulated by 80%–90% of migrated cells in longer treatment and higher

Table 1. Primer Information of Target and Reference Genes for qRT-PCR

Name	*Gene ID*	*Forward*	*Reverse*	*Size (bp)*
Sox-9	353115	CGGTGGTGTTTGGCCATGTAATGA	GAGAGAGGGGAGTCCTATCCTGGT	110
RunX-2	536911	CGCACCGACAGCCCCAACTT	CTTGAAGGCCACGGGCAGGG	94
Col I	282187	CCCACCCCAGCCGCAAAGAG	GAGCAGGAGCCGGAGGTCCA	101
Col II	407142	AAGACGCAGAGCGCTGCTGG	GGGTCTCTACCGCGCCCTCA	80
Col VI	511422	GTGCACGGGGCCCTAGAGGA	GACGGGGCCTTCTCGTCCCT	71
β-Actin	280979	TCGACACCGCAACCAGTTCGC	CATGCCGGAGCCGTTGTCGA	70

qRT-PCR, quantitative real-time PCR.

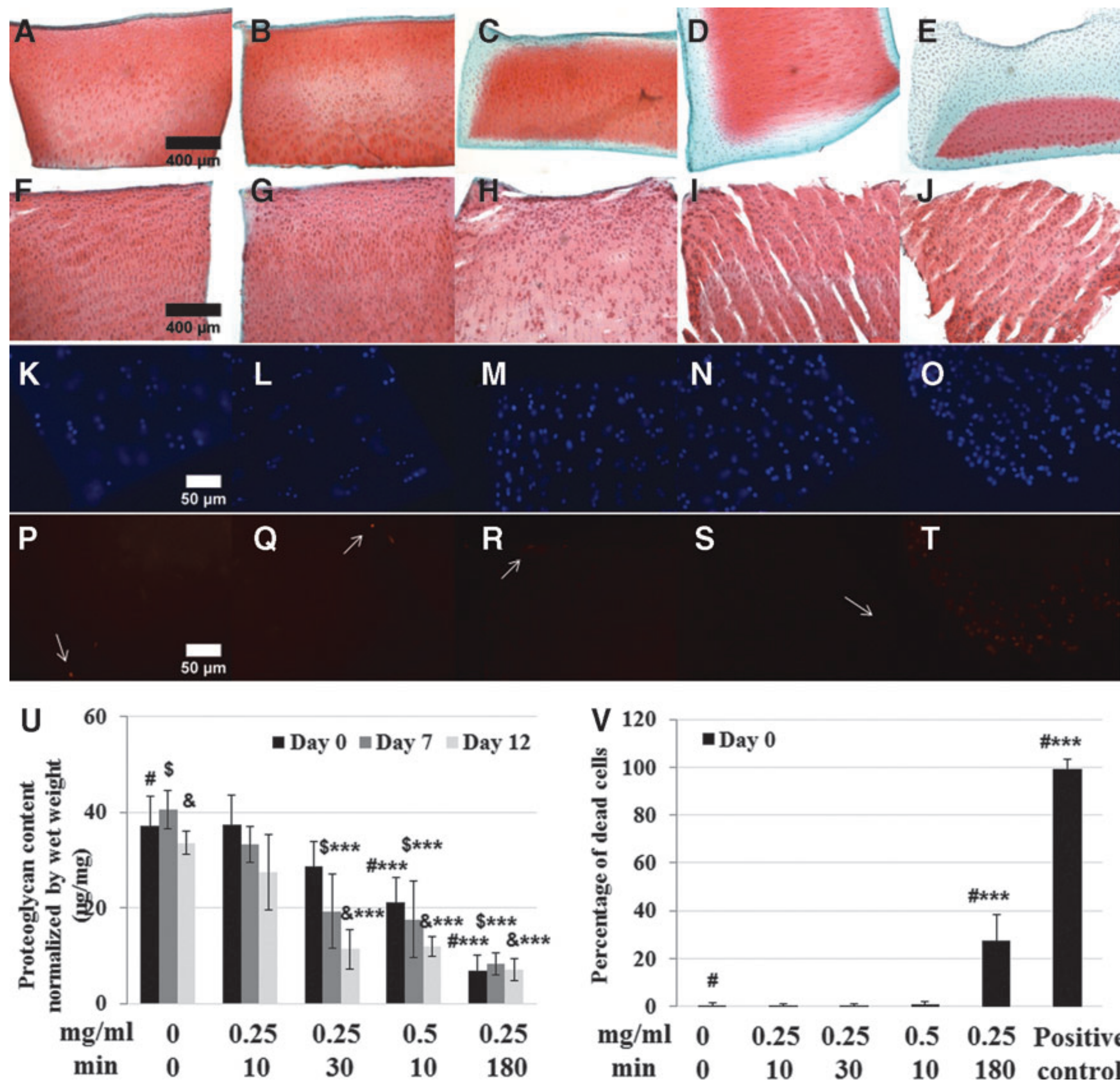

FIG. 1. Effect of collagenase treatment on proteoglycan (PG) loss and cell death. **(A–E)** PG loss was dependent on the treating concentration and time of collagenase in Safranin-O/fast green stain at post-treatment. Groups of 0.25 mg/mL for 10 min **(C)** and 0.5 mg/mL for 10 min **(D)** showed partial PG loss in the edge of 2-mm-diameter cylinderic cartilage. After 12 days, partially depleted PG deposited newly synthesized PG in 0.25 mg/mL for 30-min group **(H)**, but the color was brighter than the no treated group. Groups of 0.5 mg/mL for 10 min **(I)** and 0.25 mg/mL for 180 min **(J)** showed unstable structure with lots of partial gaps. In terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, short-term collagenase treatment showed almost no cell death **(Q–S)**, however, chondrocyte death was apparent in long-term treatment **(T)**. The number of postive-stained cells (dead cells) was counted and showed no significant difference in short-term-treated groups **(V)**. DNase I recombinant was used for positive control. PG content was quantified using dimethylmethylene blue assay **(U)** and showed a gradual decrease in all treated groups except 0.25 mg/mL for 10-min group. **(A, F, K, P)** No treatment; **(B, G, L, Q)** 0.25 mg/mL for 10 min; **(C, H, M, R)** 0.25 mg/mL for 30 min; **(D, I, N, S)** 0.5 mg/mL for 10 min; **(E, J, O, T)**; 0.25 mg/mL for 180 min; **(A–E, K–T)** day 0 after treatment; **(F–J)** day 12; **(K–O)** DAPI; **(P–T)** TUNEL. Arrows indicate dead cell. Error bars are mean ± standard deviation (SD) [n = 4–6 **(U)**, n = 4–12 **(V)**, $***p < 0.001$, #, no treatment at day 0; $, no treatment at day 7; &, no treatment at day 12].

concentrations of collagenase (Fig. 3F, G). In contrast, no elongated cells were observed in the control (Fig. 3D).

After 3 weeks, cartilage explants were fixed and stained with Safranin-O/fast green (Fig. 4). Compared with nontreated control that had almost no migrated cells (Fig. 4A), enzymatic treatment accelerated cell migration. In the edge of host cartilage, the cell population was dramatically increased by collagenase treatment (Fig. 4E, F). These cells migrated into a defect-embedded fibrin hydrogel and covered almost 80% of a defect embedded with hydrogel in the group of 0.25 mg/mL for 10 min (Fig. 4B). In contrast, the migrated cells were evenly distributed in the entire area in groups of 0.25 mg/mL for 30 min and 0.5 mg/mL for 10 min (Fig. 4C, D, G, and H) and some cells began to differentiate into chondrocytes with abundant deposit of PG (Fig. 4G, arrowhead).

The explants were also prepared for gene expression to identify the characteristics of migrated cells (Fig. 5). qRT-PCR analysis revealed similar expression of chondrogenic-specific genes, Sox-9 and Col VI, and osteogenic-specific gene, RunX-2. Although there was no statistically significant

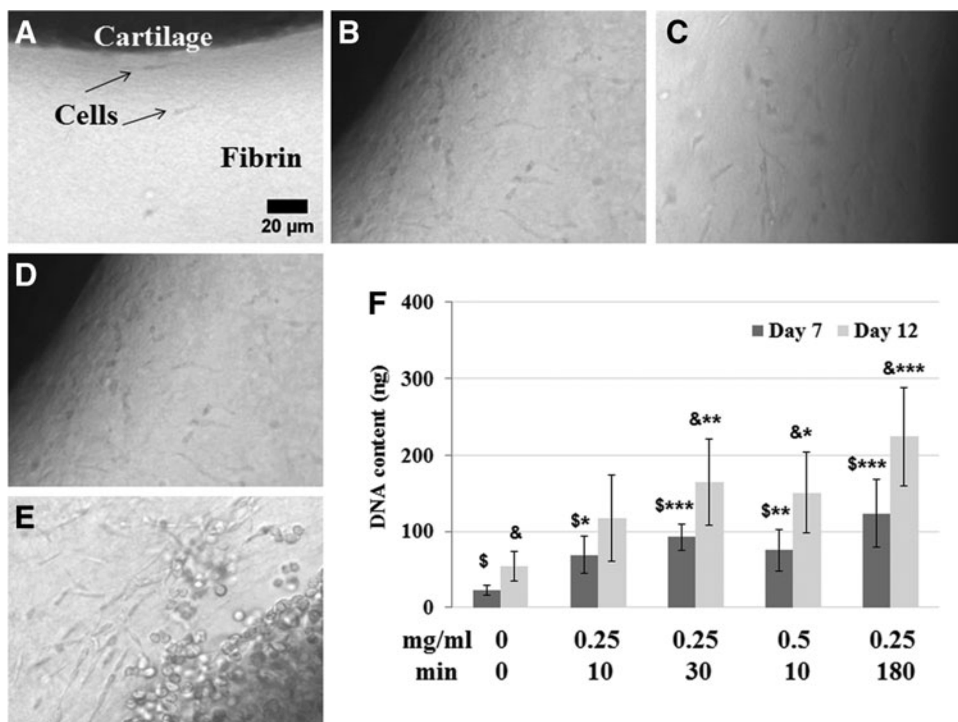

FIG. 2. Effect of collagenase treatment on cell migration. **(A–E)** Cell migration was examined using a light microscope. At day 12, only few cells were migrated from 2-mm-diameter cylindric cartilage **(A)**. On the other hand, abundant cells were migrated with elongated shape in collagenase-treated groups **(B–E)**. The number of migrated cells was quantified using DNA quantification assay at day 7 and 12 **(F)**. Collagenase treatment showed a significantly higher cell number than no treated control. **(A)** no treatment; **(B)** 0.25 mg/mL for 10 min; **(C)** 0.25 mg/mL for 30 min; **(D)** 0.5 mg/mL for 10 min; **(E)** 0.25 mg/mL for 180 min. Error bars are mean ± SD (n = 4–7, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $, no treatment at day 7; &, no treatment at day 12).

difference in collagen type I and II, some collagenase-treated samples showed higher expression with maximum 5-fold (Col I) and 8.8-fold (Col II).

Discussion

In traumatic cartilage injury, cell migration from the host tissues to the injury site plays an important role in tissue healing. However, since cartilage cells are surrounded by the PG-rich pericellular matrix and by the presence of a network of fibrillar collagen, their migration is extremely challenging. Although the damaged matrix during the injury allows a permissive environment for cell migration,[20] the proteolytic degradation of the matrix is still a long process. In this study, we used a simple method using enzymatic treatment to accelerate the process of matrix degradation around damage tissues. A disrupted collagen matrix by short-term-treated collagenase can provide an effortless cell migration into the injured site. Our results showed the obvious effect of short-term enzymatic treatment on cell migration in both experiments of cartilage disc and full-thickness cartilage defect model. In the disc culture, the treatment resulted in an approximately three to four times higher number of migrated cells than nontreated control. Moreover, the short-term treatment could locally damage collagen and PG in only the edge of cartilage tissue without cell death. The effect of collagenase was similar in the defect explant model. A number of cells were migrated into the fibrin hydrogel in treating groups and the degree of migrated cells was dependent on the dosage and treating time of collagenase.

Cells that migrated into fibrin mainly originated from the cartilage surface and subchondral bone. They showed different characteristics in terms of migratory and chondrogenic activity. The cells from the surface of cartilage had highly migratory ability. In our previous study, we identified the characteristics of these chondrogenic progenitor cells (CPCs) isolated from injured articular cartilage.[21] These cells showed notable chemotatic activity, clonogenicity, and side populations like MSCs. On the other hand, they underexpressed cartilage-specific genes, such as type II, IX, and X collagens and aggrecan, compared with normal chondrocytes. A few of explants showed active cell migration from subchondral bone and/or deep zone cartilage (Fig. 4D). The cells produced highly dense PG around cells compared with cells from the surface. Koelling *et al.*[22] have also found these progenitor cells from repair tissue during the late stages of human osteoarthritis. The cells exhibited a high migratory ability through the tidemark and a chondrogenic potential. Thus, the short-term enzymatic treatment accelerated the migration of these two different progenitor cells for cartilage repair.

Safranin-O/fast green stain showed cells that invaded the fibrin filler in the defect deposited by a PG-rich pericellular matrix. This signature chondrogenic activity involves coordinated expression of numerous structural proteins (e.g., aggrecan, hyaluronan, collagens, and fibronectin) and processing enzymes (e.g., MMPs, lysyl oxidase, and prolyl hydroylase). Staining immediately around the cells (50–100 μm) was often as intense as in a normal cartilage matrix, but in only one of the four cases did the entire fibrin structure contain normal PG levels. The result of gene expression was also similar. Two samples showed overexpression of collagen type II (4.4- and 8.8-fold). On the other hand, the DNA content of the fibrin gels embedded in defects was modestly greater than normal cartilage, an indication of near normal cellularity. It remains to be seen if all of these cells will eventually engage in high levels of matrix production spontaneously, or will require intervention with chondrogenic growth factors, which we saw drive 90% of

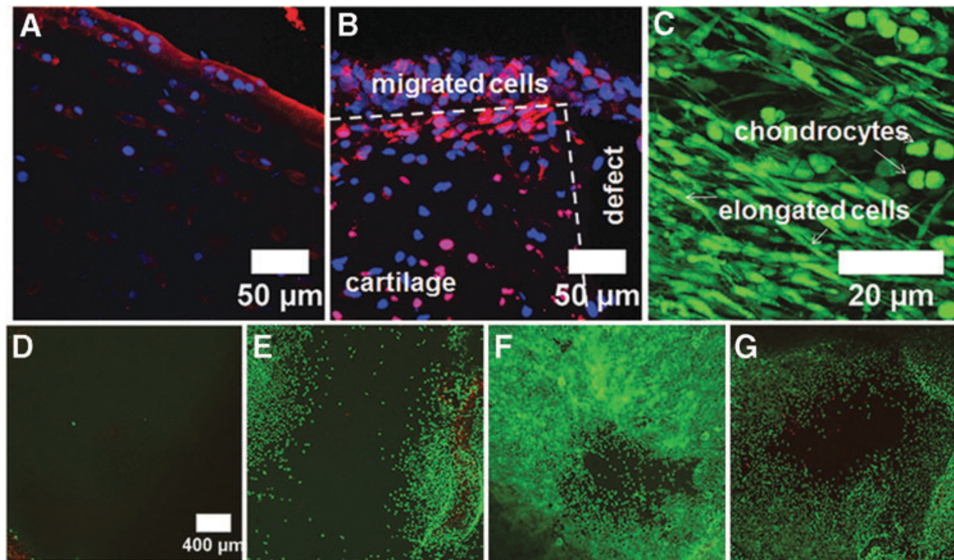

FIG. 3. Migrating cells in full-thickness cartilage defects. Defect-injured explants were cultured for 1 week and performed confocal examination. Collagen type VI stained the pericellular matrix (red) around a chondrocyte (blue) in intact cartilage **(A)**. In a full-thickness cartilage defect, chondrogenic progenitor cells (CPCs) were migrated out of cartilage surface through the pericellular matrix **(B)** and they were morphologically elongated **(C)**. Short-term collagenase treatment enhanced accelerated cell migration into the surface of defect area **(E–G)** compared with no treatment **(D)**. **(D)** No treatment of collagenase, **(E)** 0.25 mg/mL collagenase for 10 min, **(F)** 0.25 mg/mL collagenase for 30 min, and **(G)** 0.5 mg/mL for 10 min.

CPCs in pellet cultures to vigorously synthesize PGs.[21] The control cartilage in our explant model begins to deteriorate after ~3 weeks, due possibly to the lack of mechanical stimulation in culture. This may not have been long enough to fully evaluate the potential for matrix regeneration in defects. Multiple *in vivo* joint injury models offer solutions to this problem.

Several studies have reported enzymatic digestions to increase the initial adhesion of tissue by enhancing cell density. They usually used hyaluronidase, chondroitinase,

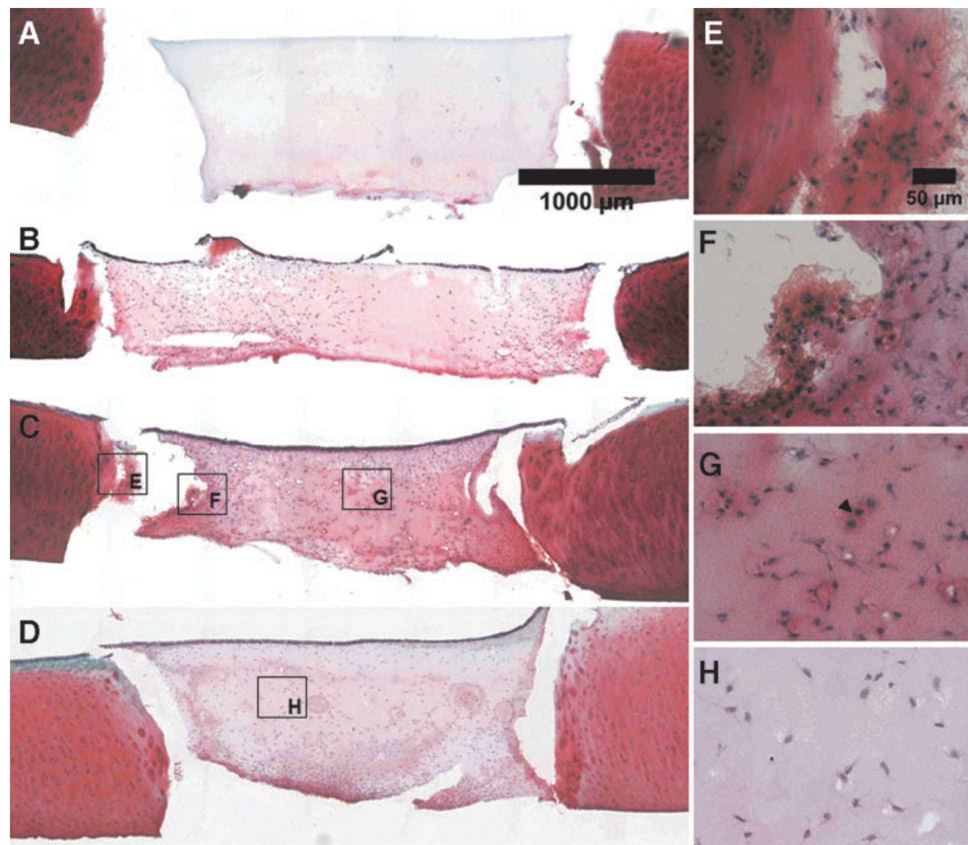

FIG. 4. Effect of collagenase treatment on cell migration in full-thickness cartilage defects. Defect-injured explants were cultured for 3 weeks and stained Safranin-O/fast green; **(A)** no treatment of collagenase, **(B)** 0.25 mg/mL collagenase for 10 min, **(C)** 0.25 mg/mL collagenase for 30 min, and **(D)** 0.5 mg/mL collagenase for 10 min. There was no cell migration in the non-collagenase treatment group **(A)**. On the other hand, abundant cells were migrated into fibrin in collagenase-treated groups **(B–D)**. CPCs were highly dense in the edge of host tissue **(E, F)** and evenly distributed inside hydrogel **(G, H)** in collagenase-treat groups. Some migrated cells were differentiating to chondrocyte-like cells with abundant PG deposit around cells (**G**, arrowhead).

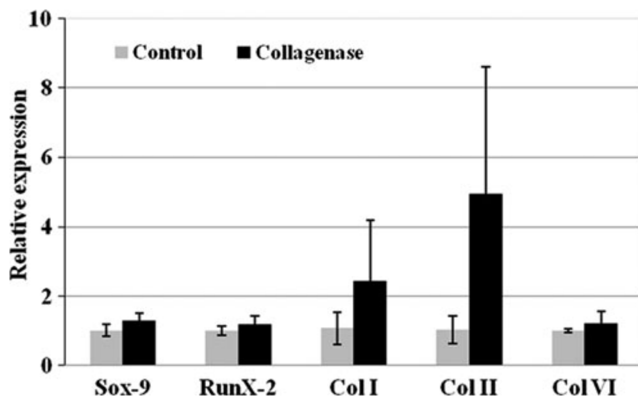

FIG. 5. Gene expression. Full-thickness cartilage explants were treated with or without collagenase (0.25 mg/mL for 30 min) and cultured with implanted fibrin for 3 weeks. Collagen (Col) type I and II were overexpressed, but there was no significant difference. Error bars are mean ± SD ($n=4$).

and/or collagenase as an enzyme. Although we did not compare with others, collagenase was selected in this study since it is ready for use in clinic. Collagenase isolated from *C. histolyticum* is commonly used for cartilage dissociation to obtain single chondrocyte[23] and is the only FDA-approved nonsurgical treatment for Dupuytren's disease (XIAFLEX®, Auxilium Pharmaceuticals, Inc., Lake Oswego, OR), that is, abnormal thickening of the tissue just beneath the skin known as fascia.[24] On the other hand, hyaluronidase and chondroitinase failed in clinical trials for pharmacologic vitreolysis, which is a new treatment modality to potentially eliminate untoward effects of vitreous upon the retina.[25] This is due to an insufficient understanding of the molecular effect mechanism of the agents.

Most of the protocols for chondrocyte isolation use collagenase with slightly different concentrations and digestion duration. Two studies have shown the optimal condition of collagenase digestion. Hayman *et al.*[26] have found that the treatment of 4.5 mg/mL for 6 h indicated the fewest gene expression changes compared with native chondrocytes. Similarly, isolated cells using 2 mg/mL collagenase for 10 h maintained their expression of chondrocyte-specific markers.[27] Based on these results, our short-term enzymatic treatment might not influence the cell's original function since the concentration of collagenase used in this study was very mild ranging from 0.25 to 0.5 mg/mL with maximum 30 min of treatment.

In this study, we introduced a simple and promising strategy for repairing of cartilage regeneration. Our strategy was not isolating the cells, but accelerating the cell migration into the defect. For this purpose, we used the short-term enzymatic method using collagenase to loosen cartilage tissue. Surprisingly, numerous CPCs migrated into fibrin defect and differentiated into chondrocyte-like cells with abundant deposit of PGs. This result strongly supports that migrated progenitor cells, which are activated in traumatic cartilage injury, have great potential for cartilage repair.

Acknowledgments

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Disclosure Statement

No competing financial interests exist.

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