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NEL-like molecule-1-modified bone marrow mesenchymal stem cells/poly lactic-co-glycolic acid composite improves repair of large osteochondral defects in mandibular condyle

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SUMMARY

Objective: Articular cartilage of the mandibular condyle has limited ability to regenerate itself after injury. This study was to investigate whether osteochondral defects in mandibular condyle could be repaired by NELL-1(NEL-like molecule-1)-modified autogenous bone marrow mesenchymal stem cells (BMMSCs) and poly lactic-co-glycolic acid (PLGA) composite.

Methods: Osteochondral defects of 3 mm-diameter × 5 mm-depth were created unilaterally in the central part of the condyle in 50 adult goats. The injury sites were treated with NELL-1-modified BMMSCs/PLGA, BMMSCs/PLGA, PLGA alone, or left empty. The defect area was monitored using gross examination, histology, immunohistochemistry, and micro-computed tomography (μ-CT). Implanted BMMSCs were tracked using Adeno-LacZ labeling.

Results: The NELL-1-modified BMMSCs/PLGA group showed vigorous and rapid repair leading to regeneration of fibrocartilage at 6 weeks and to complete repair of native articular cartilage and subchondral bone at 24 weeks. The BMMSCs/PLGA group also completely repaired the defect with fibrocartilage at 24 weeks, but the cartilage in the BMMSCs/PLGA group was less well-organized than the NELL-1-modified BMMSCs/PLGA. The osteochondral defects in the PLGA and empty defect groups were poorly repaired, and no cartilage in the empty defect group or only small portion of cartilage in the PLGA group was found. *In vivo* viability of implanted cells was demonstrated by the retention for 6 weeks in the defects.

Conclusion: These findings demonstrated that NELL-1-modified BMMSCs/PLGA composite can rapidly repair large osteochondral defect in the mandibular condyle with regeneration of native fibrocartilage and subchondral bone.

Introduction

Due to the avascular nature and limitation in cell movement, articular cartilage of the mandibular condyle has limited ability to regenerate itself after injury[1–3]. Although various methods have been developed for the treatment of articular cartilage lesions in the mandibular condyle, few of them yield satisfactory results[4–6]. Tissue engineering principles that combined living cells with scaffolds are promising techniques for osteochondral defect repair and have been studied in a variety of animal models[7,8].

Bone marrow mesenchymal stem cells (BMMSCs) have been considered as promising candidates in the field of bone and cartilage regeneration because of their rapid proliferation abilities, multiple differentiation potential, and easy harvest[9–11]. However, in many cases of tissue engineering applications, cell differentiation toward a specific phenotype is highly desirable. Local administration of growth factors that can enhance cartilage healing is a potentially powerful alternative approach to cartilage repair. NELL-1 (NEL-like molecule-1) is a secretary molecule containing a signal peptide sequence, an NH_2-terminal thrombospondin (TSP)-like module, five von Willebrand factor C domains, and six epidermal growth factor-like domains and expresses preferentially in cells of neural crest origin residing within the craniofacial complex and central nervous system[12–14]. It is a novel growth factor believed to specifically target cells committed to the osteochondral lineage. Previous studies had shown its ability to stimulate secondary cartilage and bone formation in a rat palatal suture

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distraction[12] model and rat calvarial defect regeneration model[13]. NELL-1 can also promote chondrocytes proliferation and deposition of cartilage-specific extracellular matrix materials *in vitro*[14]. These findings suggest potential therapeutic benefits of NELL-1 in the stem cell-based repair of osteochondral defect.

In the current study, we adopted autologous BMMSCs as a vector for control release of NELL-1 which is a differentiation factor used to induce the differentiation of BMMSCs into chondrocytes. Then, we prepared NELL-1-modified BMMSCs/Poly lactic-co-glycolic acid (PLGA) scaffold composite *in vitro* and aimed to explore the ability of NELL-1-modified BMMSCs/PLGA scaffold composite to repair large osteochondral defect in goat mandibular condyle.

Materials and methods

Scaffold fabrication

PLGA (LA:GA = 75:25 wt%, Mw: 120,000) copolymer was synthesized using a conventional method. PLGA scaffolds were fabricated by using a salt leaching method using a mixture ratio of 1:15 (Polymer:Salt). Briefly, 5 wt% PLGA solution was prepared by dissolving it in chloroform and then mixed with sodium chloride. The average pore diameter in the PLGA scaffold is 102.75 ± 32.37 μm. A Teflon mold (Ø 3×5 mm:3 mm in diameter and 5 mm in height) was then filled with the polymer/salt paste. The paste was subsequently removed from the mold and placed in distilled water for 2 days. Finally, samples were lyophilized for several days and examined under scanning electron microscope (S.E.M.) [Fig. 1(A)].

Cell culture and transfection

Autologous BMMSCs were obtained from the left iliac crest of all goats. The cells were isolated by density gradient centrifugation, then suspended in alpha-minimum essential medium with 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD, USA) and 100 mg/ml penicillin–streptomycin (Gibco BRL, Gaithersburg, MD, USA), and incubated at 37°C with 5% humid CO_2. Cells reached 90% confluence were suspended for passage. Cells of passage three were used for the gene transfection *in vitro* and transplantation *in vivo*.

Adenoviruses containing human rhNELL-1 (Ad-NELL-1) or control β-galactosidase (Ad-LacZ) were constructed by Vector Gene Technology Company Ltd., China. When BMMSCs were grown to 80% confluence, they were transfected with Ad-NELL-1/LacZ. After that, 10 ml of fresh culture medium was added to the cells and then subjected to 48 h incubation. On the third day, the infected cells were collected and seeded into PLGA scaffolds.

Preparation of Cell/PLGA scaffold composite

Fibrin glue (TISSEEL kit, Baxter AG, Vienna, Austria) was used as cell-loading carrier. BMMSCs or NELL-modified BMMSCs were collected and resuspended in thrombin solution. Forty microliters of thrombin solution containing 3×10^6 cells was seeded into a PLGA scaffold disc by micropipette. The scaffold was held with sterile forceps and turned upside-down several times during the seeding process so that the cells could be distributed throughout the scaffold. A 30-μL TISSEEL solution (500 IU thrombin/ml) was then added for fibrin gel formation in the scaffold. After seeding, the cell-scaffold constructs were kept in complete Dulbecco's

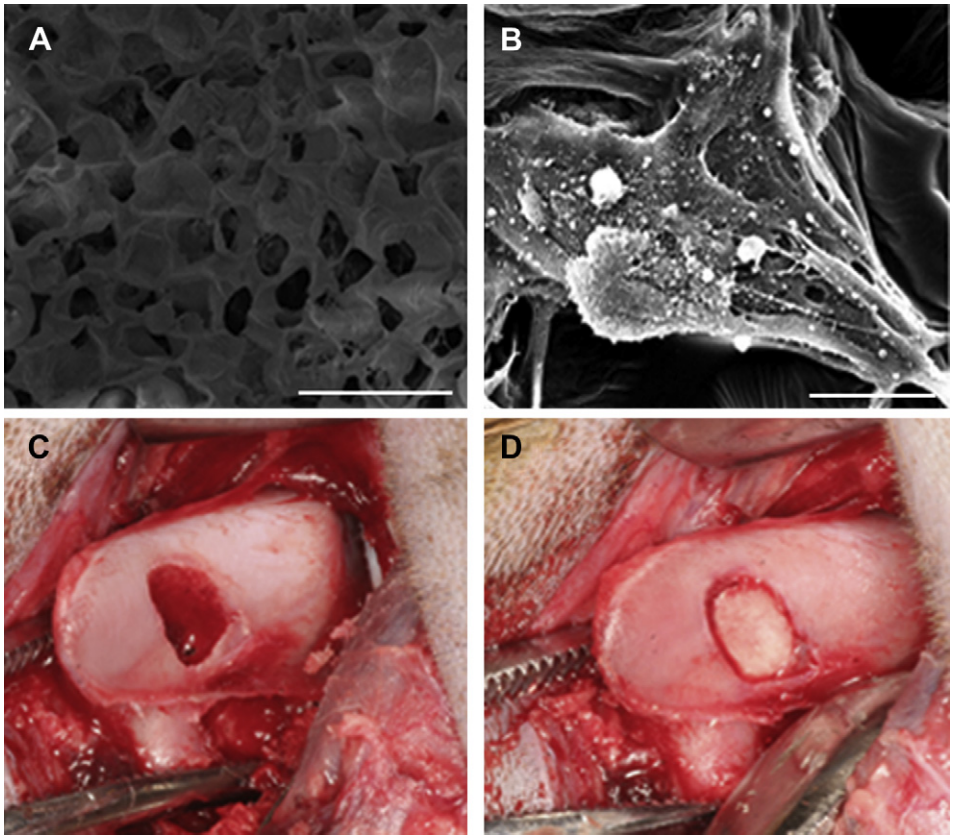

Fig. 1. (A) S.E.M. photographs of produced PLGA scaffold without cells. (B) The seeded BMMSCs attached on the PLGA scaffold. (C) Critical-size osteochondral defect created in the mandibular condyle. (D) Implantation of BMMSCs/PLGA composite in the defect. Bars = 1 mm in A and 50 μm in B.

Modified Eagle Medium (DMEM) for 1–2 h until subsequent implantation use [Fig. 1(B)]. For the PLGA group, the same volume fibrinogen and thrombin solutions without cells were seeded in PLGA scaffolds.

Surgical protocol

Adult male Eastern Cross goats (weighing 32–37 kg, ages 25–28 months; $n = 50$) were used in this study. The Institutional Animal Care and Use Committee approved all procedures used.

With the animals under general anesthesia, the left temporomandibular joint (TMJ) was exposed *via* a 3-cm pre-auricular skin incision. By lateral translation of the mandible, the condylar surface was exposed. Then an osteochondral defect, 3 mm in diameter and 5 mm in depth, was created in the central part of the condyle with a 3 mm diameter drill [Fig. 1(C)]. The articular disc was left intact. The PLGA scaffold with NELL-1-modified BMMSCs ($n = 12$) or BMMSCs ($n = 12$) was pressed fit into the defect [Fig. 1(D)]. In the PLGA group ($n = 12$), the defects were treated with cell-free PLGA scaffolds. No treatment was made in the empty defect group ($n = 12$). The contralateral joint remained intact as normal controls.

Gross morphology, histology and immunohistochemistry

Six goats in each group were sacrificed at 6 and 24 weeks postoperatively, respectively. The mandibular condyles were excised for gross examination and micro-computed tomography (μ-CT) examination. After that all samples were fixed in 4% formalin, decalcified in 30% formic acid, then embedded in paraffin and sectioned at 6-μm thickness. Sections were stained with Safranin-O. The specimens were graded blindly using a modified grading scale (Table I) from a previous study[15]. To detect expression of type II collagen, immunohistochemical evaluation was performed using the avidin–biotin immunoperoxidase method with an antibody raised against type II collagen (Santa Cruz Biotechnology, Inc. CA, USA).

Table I
Histological grading scale

Category	Score
Safranin-O staining of the matrix	
Normal	4
Slight increase or decrease	3
Moderate increase or decrease	2
Severe increase or decrease	1
None	0
Surface regularity	
Smooth and intact	3
Slight irregularity	2
Moderate irregularity	1
Severe irregularity and disruption	0
Structure integrity	
Normal	3
Slight disruption	2
Moderate disruption	1
Severe disruption	0
Bonding to the adjacent cartilage	
Bonded completely	2
Bonded partially	1
Not bonded	0
Cartilage thickness, %(Compared with adjacent normal cartilage thickness)	
Normal	4
Slight increase or decrease	3
Moderate increase or decrease	2
Severe increase or decrease	1
None	0

μ-CT examination

The mandibular condyles obtained at 6 and 24 weeks were evaluated with a μ-CT 80 scanner (Scanco Medical, Bassersdorf, Switzerland) for analysis of new bone formation within defects before histology examination. The system was set to 70 kV, 114 mA, 500 ms integration time. Two fixed, global thresholds were applied: an upper threshold that excluded the highly attenuating PLGA particles from the analysis, and a lower threshold that excluded unmineralized or poorly mineralized tissue from the analysis. The volume of interest (VOI) was a fixed cylinder of 5 mm in diameter and 7 mm in height encompassing the entire scanned volume of the defect. Bone volume to total volume ratio (BV/TV), indicating the portion of mineralized tissue, was calculated for comparison.

In vivo tracing of implanted cells

LacZ-BMMSCs/PLGA composite was implanted in two additional goats for *in vivo* tracing of implanted BMMSCs. The animals were sacrificed at 6 weeks postoperatively and the mandibular condyles were harvested and fixed in 4% formalin overnight. Following that, the condyle was stained in X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) substrate 1 mg/ml, 1 mmol/L $MgCl_2$, 10 mmol/L $K_4Fe(CN)_6$, and 10 mmol/L $K_3Fe(CN)_6$ in phosphate buffered saline (PBS) for 24 h. After X-gal staining, the samples were placed in 4% formalin again for complete fixation, and then the samples were decalcified and embedded in paraffin and sectioned in a sagittal plane. The sections were counterstained with Safranin-O.

Statistical analysis

All data were represented as means ± 95% confidence intervals (CI). Statistical significance of differences among means was determined by analysis of variance (ANOVA) with *post hoc* comparison of more than two means by the Bonferroni method (SPSS Inc., Chicago, IL, USA). A significance level of 0.05 was used for all analyses.

Results

Gross examination

At 6 weeks after transplantation, the defects in both NELL-1-modified BMMSCs/PLGA [Fig. 2(A)] and BMMSCs/PLGA [Fig. 2(B)] groups were filled with shiny, white, translucent tissue with sharply defined margins, although the surface of the defects in the BMMSCs/PLGA groups remained concave. The defects of the PLGA group [Fig. 2(C)] showed red, irregular surface with depression. In the empty defect group [Fig. 2(D)], the defects were filled with brown, opaque tissue with a concavity toward the center.

At 24 weeks after transplantation, the injury sites seeded with the NELL-1-modified BMMSCs/PLGA [Fig. 2(E)] and BMMSCs/PLGA [Fig. 2(F)] showed smooth, flush, and transparent cartilage. There was little depression in the defect and the margin with normal cartilage was indistinct. In the PLGA group [Fig. 2(G)], the defects showed an irregular surface with firm cartilage-like tissues of similar color to the surrounding cartilage. In the empty defect group [Fig. 2(H)], arthritic changes were evident and defects remained depressed and covered with fibrous tissue.

Histology and immunohistochemistry

At 6 weeks after transplantation, the repair tissue in the NELL-1-modified BMMSCs/PLGA [Fig. 3(A and E)] defect showed a well-repaired cartilage with a relatively smooth surface without depression, but the new cartilage was thicker and the cell arrangement

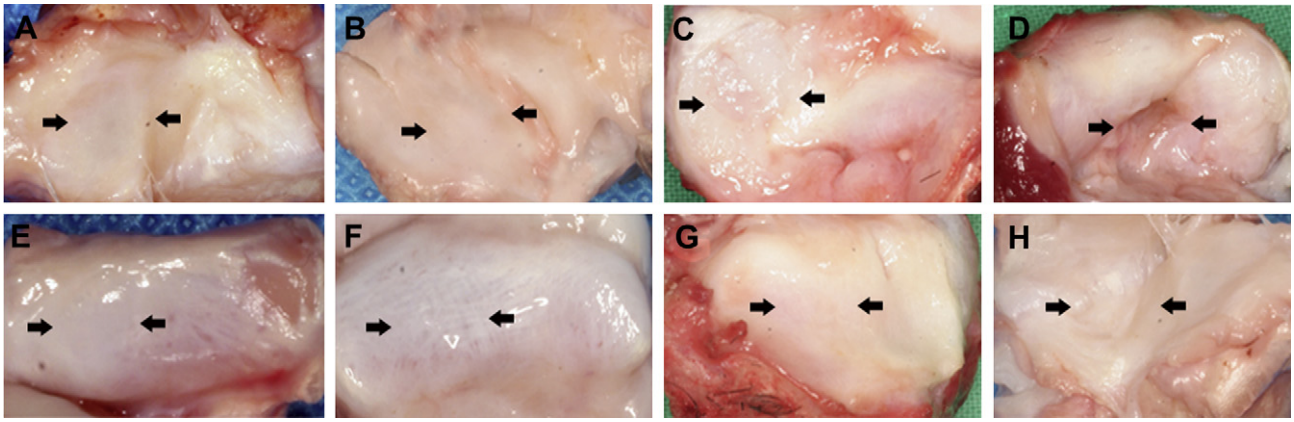

Fig. 2. Gross appearance of specimens. At 6 weeks, the defects in both NELL-1-modified BMMSCs/PLGA (A) and BMMSCs/PLGA (B) groups were filled with shiny, white, semi-transparent tissue, but the surface of the defects in the BMMSCs/PLGA-treated groups remained concave. The PLGA group (C) was rough with depression. In the empty defect group (D), the defects were filled with opaque tissue with a deep concavity. At 24 weeks, the defects in the NELL-1-modified BMMSCs/PLGA (E) and BMMSCs/PLGA (F) groups were substituted with smooth and translucent tissue just like host cartilage. In the PLGA group, the defects showed an irregular surface with firm cartilage-like tissues of similar color to the surrounding cartilage (G). In the empty defect group, arthritic changes were evident and defects remained depressed (H). Arrows showed the defect created in the mandibular condyle.

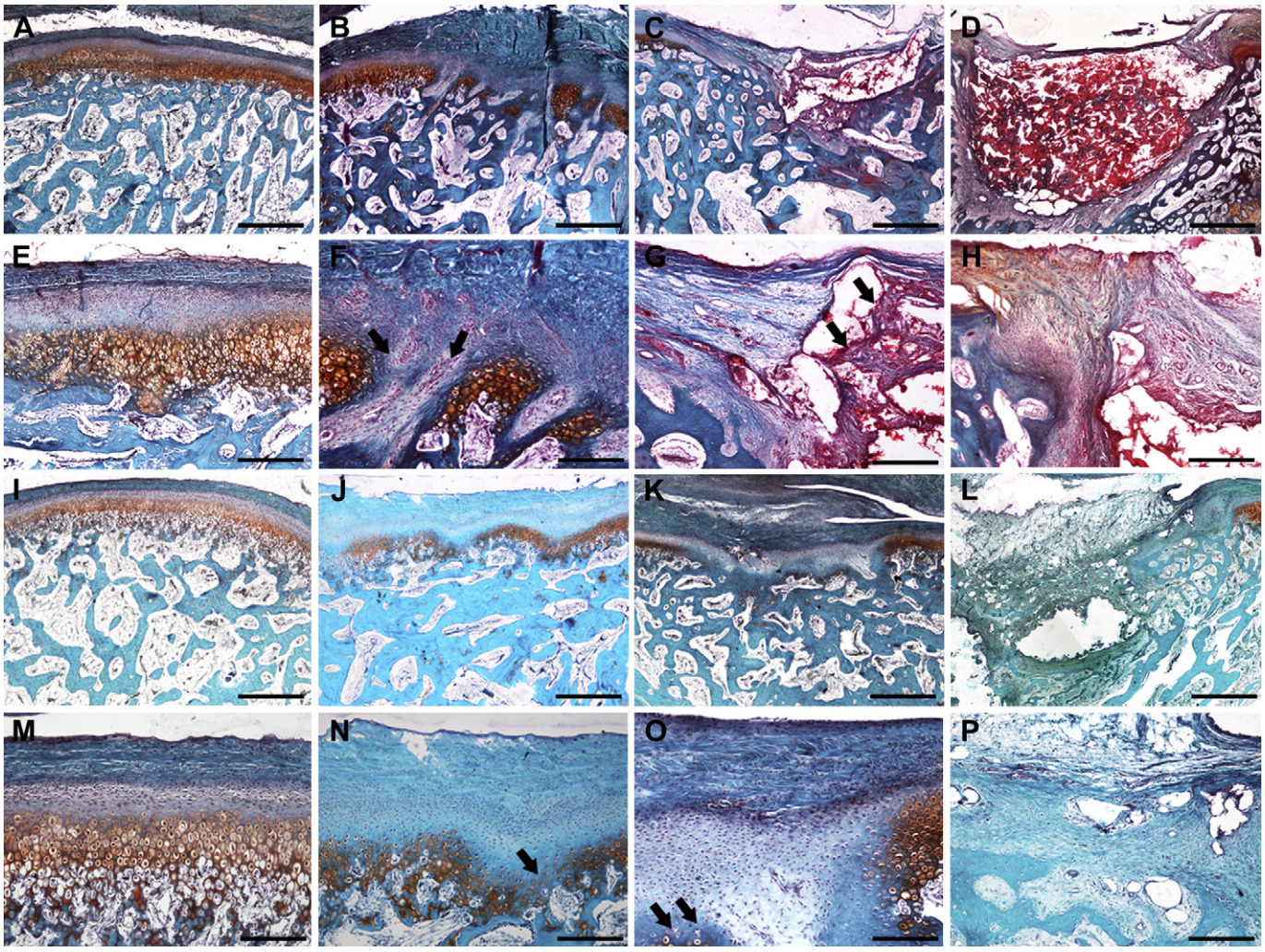

Fig. 3. Histological photomicrographs of specimens 6 (A–H) and 24 (I–P) weeks after operation (Safranin-O staining). At 6 weeks, fibrocartilage was regenerated completely in the NELL-1-modified BMMSCs/PLGA group, but was thicker and more cellular than normal (A and E). Partial cartilage regeneration was observed in the BMMSCs/PLGA group (B and F, arrows in F showed cartilage disruption). In the PLGA group, the defects were filled with concave fibrous tissues containing small bone islands (C and G, arrows in G showed fibrous tissues in the defect). In the empty defect group the surgical sites contained fibrous tissue (D and H). At 24 weeks, a layer of well-organized fibrocartilage covering the subchondral bone was observed in the NELL-1-modified BMMSCs/PLGA group (I and M); however, the regenerated cartilage in the BMMSCs/PLGA had incomplete integration (J and N, arrow in N showed incomplete integration of cartilage). The PLGA group showed small portions of cartilaginous tissue formed adjacent to host cartilage with an irregular surface (K and O, arrows in O showed cartilaginous tissue formed adjacent to host cartilage). The empty defect group showed non-cartilaginous structure and was almost filled with fibrous tissue and inflammatory changes (L and P). Bars = 500 μm in A–D and I–L, and 100 μm in E–H and M–P.

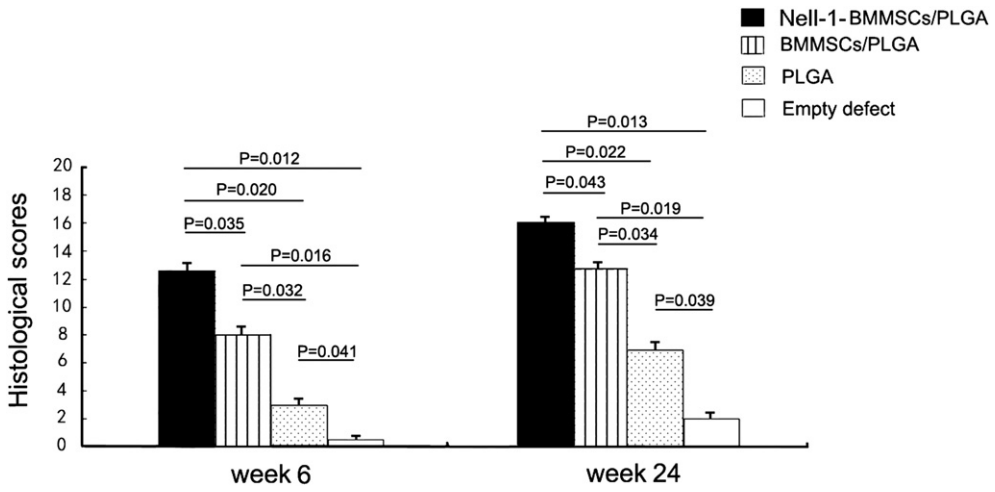

Fig. 4. Histological scores of three groups at 6 and 24 weeks after operation ($n = 6$). The data shown are the means ± 95% CI.

was more irregular than in surrounding articular cartilage. In the BMMSCs/PLGA group [Fig. 3(B and F)], large pieces of cartilage tissue appeared on portions of the defects near the surface, although cellular orientation in the neocartilage was disorganized. In the PLGA group [Fig. 3(C and G)], the defects were filled with concave fibrous tissues containing small bone islands and no cartilage was observed. In some of the samples some fissure or defects in the repaired tissue were observed. In the empty defect group [Fig. 3(D and H)] the surgical sites contained fibrous tissue and inflammatory changes.

At 24 weeks postoperatively, the cartilage repair of NELL-1-modified BMMSCs/PLGA group [Fig. 3(I and M)] demonstrated the thickness comparable to the normal one. The cell arrangement in the newly formed cartilage showed the typical characteristics of fibrocartilage. The newly formed cartilage in the BMMSCs/PLGA group was irregular and the surface was not as smooth as in the NELL-1-modified BMMSCs/PLGA group [Fig. 3(J and N)]. The PLGA group [Fig. 3(K and O)] showed small portions of cartilaginous tissue formed adjacent to host cartilage, and the defect surface was not completely filled. Empty defect group showed non-cartilaginous structure and was almost filled with fibrous tissue [Fig. 3(L and P)].

Histological grading scores are presented in Fig. 4. Within the corresponding time points, the NELL-1-modified BMMSCs/PLGA group (12.2 ± 0.65 at 6 weeks and 15.8 ± 0.42 at 24 weeks post-operatively, respectively $n = 6$) showed the highest score, followed by the BMMSCs/PLGA group (7.81 ± 0.45 and 12.8 ± 0.53, respectively), PLGA group (2.93 ± 0.25 and 6.28 ± 0.37, respectively) and empty defect group (0.62 ± 0.13 and 1.89 ± 0.17, respectively). No significant difference was observed between the NELL-1-modified BMMSCs/PLGA and normal groups at 24 weeks (data not shown).

At 6 weeks after operation, both the NELL-1-modified BMMSCs/PLGA [Fig. 5(A)] and BMMSCs/PLGA [Fig. 5(B)] groups were positive for type II collagen, but the matrix in the NELL-1-modified

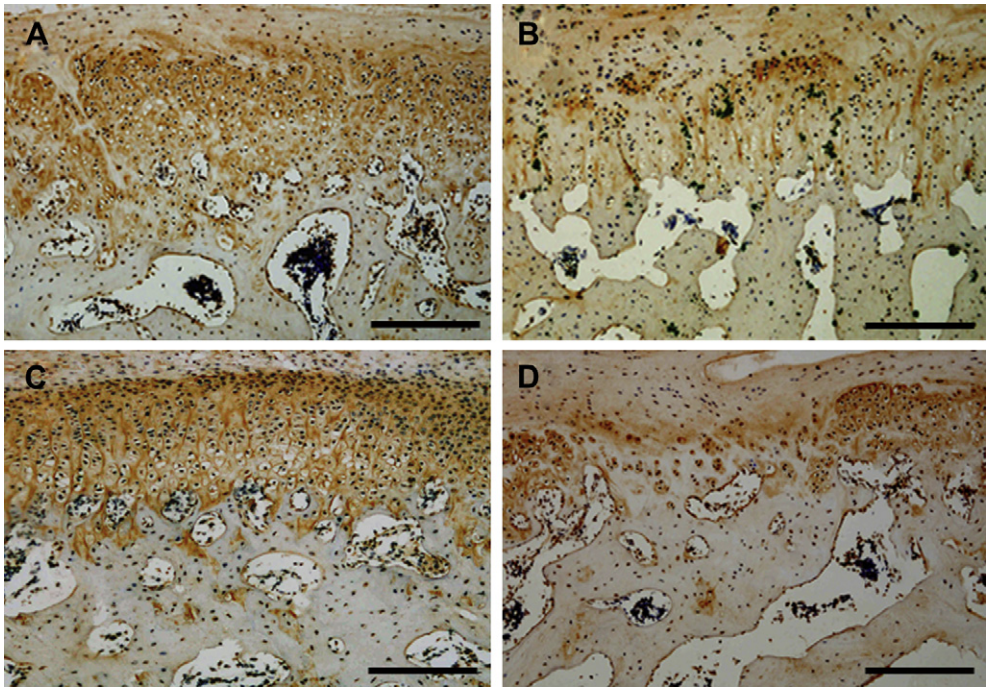

Fig. 5. Immunostaining for type II collagen in the NELL-1-modified BMMSCs/PLGA (A and C) and BMMSCs/PLGA (B and D) groups. The matrix in the NELL-1-modified BMMSCs/PLGA group had more intense type II collagen staining compared with the BMMSCs/PLGA group at 6 (A and B) and 24 (C and D) weeks after operation. Bar = 100 μm.

BMMSCs/PLGA group had more intense staining compared with the BMMSCs/PLGA group. At 24 weeks after operation, type II collagen staining was heterogeneous in regenerated cartilage of the BMMSCs/PLGA group [Fig. 5(D)] compared to the NELL-1-modified BMMSCs/PLGA group [Fig. 5(C)]. No positive immunostaining for type II collagen was observed in either of the PLGA group or empty defect groups (data not shown).

μ-CT examination

μ-CT analysis demonstrated increasing bone formation within defects throughout a 24-week time period for all animals (Fig. 6, $n = 6$). At 6 weeks, the NELL-1-modified BMMSCs/PLGA group [Fig. 6(A), $51.2\% \pm 7.1$] exhibited the highest mineralized volume fraction (BV/TV), followed by the BMMSCs/PLGA group [Fig. 6(B), $42.4\% \pm 6.3$] and PLGA group [Fig. 6(C), $31.7\% \pm 4.1$], and the empty defect group induced minimal bone formation [Fig. 6(D), $27.2\% \pm 2.5$]. At 24 weeks, BV/TV was higher in the NELL-1-modified BMMSCs/PLGA [Fig. 6(E), $60.7\% \pm 9.4$] and BMMSCs/PLGA [Fig. 6(F), $59.1\% \pm 9.5$] groups than in the PLGA [Fig. 6(G), $50.7\% \pm 6.8$] and empty defect [Fig. 6(H), $45.6\% \pm 5.8$] groups. However, no differences in BV/TV were found between the NELL-1-modified BMMSCs/PLGA and BMMSCs/PLGA groups.

In vivo tracing of implanted cells

BMMSCs transduced with Ad-NELL-1 were examined by X-gal staining under light microscopy [Fig. 7(A)]. In the 6-week repaired samples, blue color tissues filled within the scaffolds after X-gal staining [Fig. 7(B)]. Histological sections from sagittal plane with Safranin-O counterstaining also showed the presence of implanted cells in the defect. These blue cells stayed in the newly formed cartilage and bone [Fig. 7(C and D)].

Discussion

The present study evaluated the effects of NELL-1-modified BMMSCs/PLGA composite on repair of large osteochondral defects in the mandibular condyle. Our results demonstrate a therapeutic benefit of BMMSCs transplantation utilizing PLGA scaffolds to facilitate repair of osteochondral defects in the mandibular condyle, and the transfer of BMMSCs with NELL-1 gene can enhance the observed effects, as evidenced by better cartilage surfacing and subchondral bone formation.

Surgically created full-thickness articular cartilage defects have been studied in rabbits to evaluate the effect of new procedures designed to elicit articular cartilage repair. However, both the cartilage and the bone surrounding the defect have different

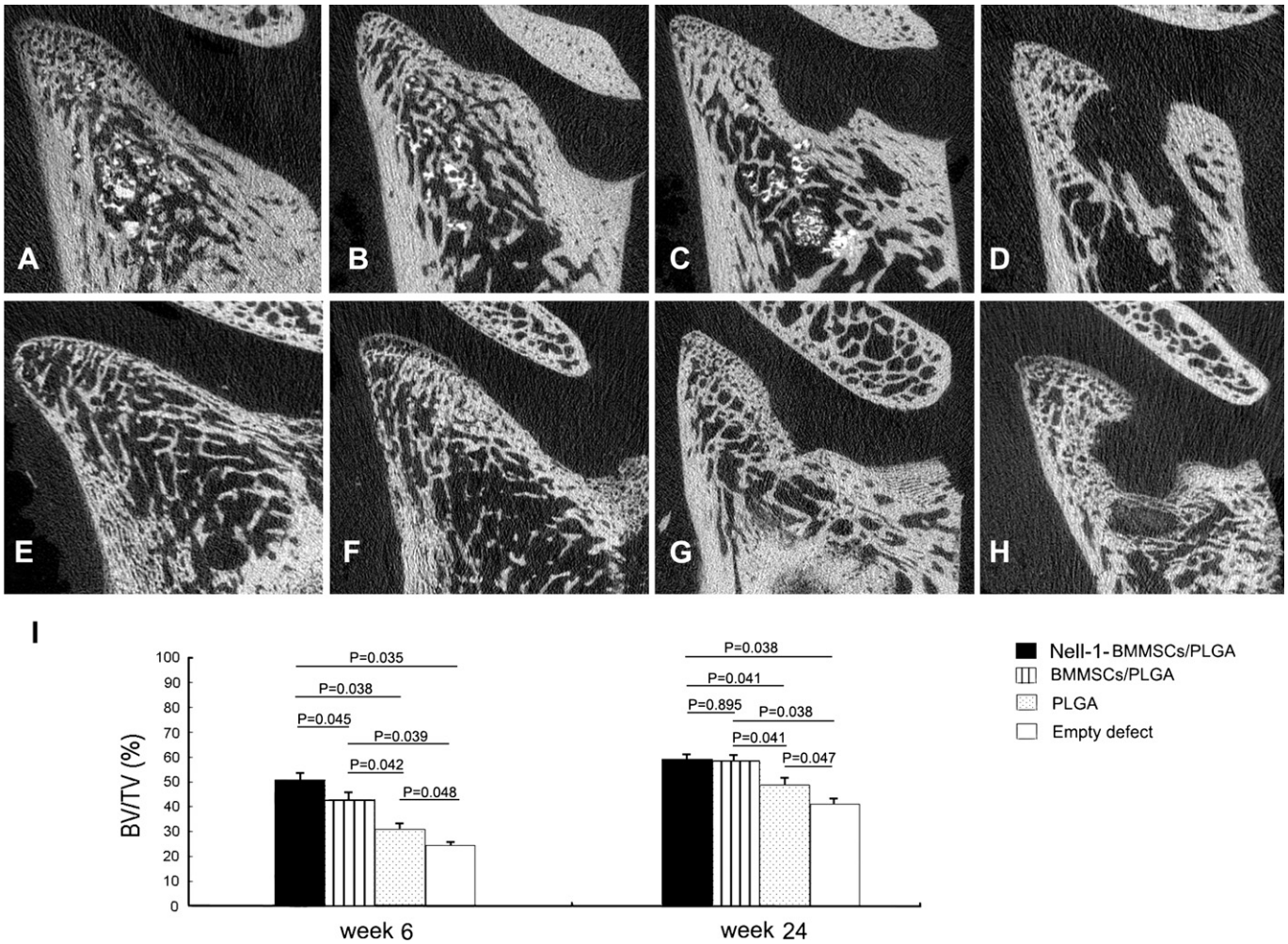

Fig. 6. Representative μ-CT images of the defects at 6 (A–D) and 24 (E–H) weeks after operation ($n = 6$). (A and E) NELL-1-modified BMMSCs/PLGA group. (B and F) BMMSCs/PLGA group. (C and G) PLGA group. (D and H) Empty defect group. (I) BV/TV of different groups at 6 and 24 weeks. The data shown are the means ± 95% CI.

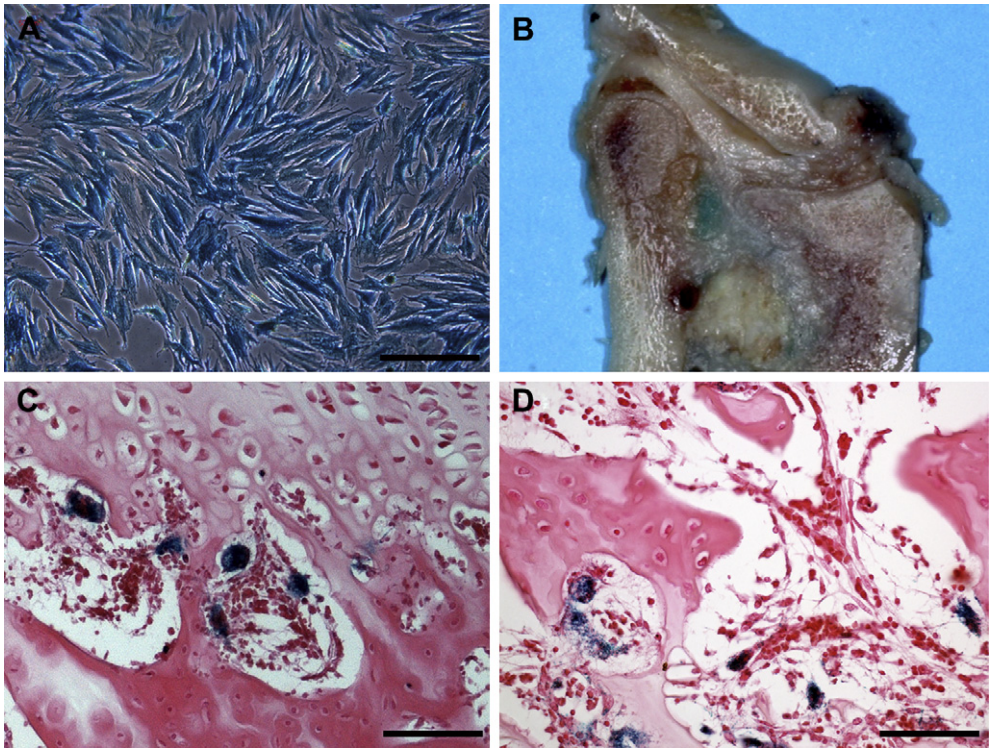

Fig. 7. X-gal staining for implanted cells tracing at 6 weeks after operation. (A) X-gal staining in Ad-NELL-1 transduced BMMSCs. (B) Blue color tissues filled within the defects. (C and D) Histological section showed the blue color tissues in the defect. Safranin-O counterstaining; bars = 100 μm in A, and 50 μm in C and D.

reparative responses depending on the size, shape, location, and depth of the lesion as well as on the animal model being used. To our knowledge, the repair of large osteochondral defects in mandibular condyle with tissue engineering has not been systematically studied. In this study, the goat was chosen because its TMJ resembles that of humans and the size of goat mandibular condyle is large enough to create a critical-sized osteochondral defect[16]. It has previously been reported that a 1-mm-diameter and 10-mm-deep defect in the sheep mandibular condyle was repaired with fibro-osseous connective tissue 3 months postoperatively[17]. In this study, the 3-mm-diameter and 5-mm-deep osteochondral defects presented a more challenging repair problem in mandibular condyle because such a defect exceeded a critical size for spontaneous repair.

Methods such as transplantation with chondrocytes[18] or local application of certain growth factors like rhBMP-2[19] or FGF-2[20] have been used to stimulate cartilage regeneration in rabbit mandibular condyle. In another study, it was found that a PLGA copolymer and gelatin sponge complex with or without rhBMP-2 could induce regeneration of new bone and cartilage-like tissue following condylectomy in the rabbits[21]. However, few of these studies had a sustained, reliable outcome. Since osteochondral injury involves damage to both bone and cartilage, addition of BMMSCs capable of differentiating into both chondrogenic and osteogenic lineages may help facilitate tissue regeneration. Several studies have suggested that combining BMMSCs with scaffold implantation might promote osteochondral repair in major synovial joints[7,8,10]. This study extends those findings and shows that the BMMSCs/PLGA group had significantly better cartilage repair of critically large defects in the mandibular condyle when compared with the goats received cell-free PLGA scaffolds.

On the other hand, cartilage repair using BMMSCs must solve some of the problems associated with differentiation into chondrocytes, remodeling and organization of a new extracellular matrix to form new cartilage tissue. Growth factors have shown promise in stimulating the differentiation into chondrocytes and the maintenance of lineage to chondrogenesis *in vitro* and in animal models. NELL-1 is a novel growth factor believed to specifically target cells committed to the osteochondral lineage. However, NELL-1 is a secreted protein and has a short half value period *in vivo*, which affects its bioactivity and longevity. Therefore, controlled delivery of NELL-1 may be a possible modality to regenerate osteochondral defects.

Recent study found that NELL-1-incorporated chitosan microparticles provide controlled delivery and maximize biological efficiency, and promote chondrocytes proliferation and deposition of cartilage-specific extracellular matrix materials *in vitro*[14]. Aghaloo *et al.*[22] reported another drug delivery system of NELL-1, in which Ad-NELL-1 gene was transferred into primary adult goat bone marrow stromal cells (BMSCs) and it was found that goat BMSCs were successfully transduced at a relatively high efficiency of approximately 70% and survived at least 4 weeks after *in vivo* intramuscular injection of nude mice. In the current study, we also used autogenous BMMSCs as a vehicle to carry NELL-1 gene, and transplanted into the osteochondral defect utilizing PLGA scaffolds. To our knowledge, this is the first report to evaluate Nell-1's therapeutic potential for the repair of large osteochondral defects using an *ex vivo* gene therapy technique. Better cartilage resurfacing and subchondral bone formation were observed in the NELL-modified BMMSCs/PLGA group when compared with the BMMSCs/PLGA group. We thought that this controlled release method can effectively extend the duration of bioactive NELL-1, and make a contribution to osteochondral regeneration.

The *in vivo* tracing experiment confirmed that these BMMSCs can survive for at least 6 weeks *in vivo*, verified by either X-gal staining. This finding is consistent with a previous study in knee joint[8]. Additionally, the transplanted BMMSCs were observed both in the regenerate cartilage and subchondral bone, suggesting that the transplanted cells, at least in part, directly participate in cartilage and bone regeneration. On the other hand, although the BMMSCs do provide a practical source of autogenous cells with chondrogenic

and osteogenic potential, it is quite possible that host cells are also involved or even dominate in the repair process. Local transplantation of BMMSCs was proven to recruit more circulating stem/progenitor cells to the region of injury and contributed to healing[23]. In the present study, the transplanted BMMSCs also served to recruit host stem cells in the subchondral bone or to stimulate replication of chondrocytes in the recipient cartilage. It is clear that the topical persistence of bioactive NELL-1 released from BMMSCs contributes to and enhances these effects. However, the time period was only extended to 24 weeks. It is possible that persistence of NELL-1 in the fully repaired defect may be detrimental beyond 24 weeks. This limitation should be resolved in further investigations.

In conclusion, NELL-1-modified BMMSCs/PLGA can successfully induce fibrocartilage and subchondral bone within the period of 24 weeks. These findings demonstrate that this new composite would lead to an improvement in the quality and quantity in the repair of large osteochondral defects.

Author contributions

Dr Hu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version to be published.

Study conception and design. Hu, Zhu.

Acquisition of data. Zhu, Zhang.

Analysis and interpretation of data. Hu, Zhu, Zhang, Man.

Manuscript preparation. Zhu, Zhang, Hu.

Statistical analysis. Zhu, Ma.

Role of the funding source

The authors state that the study sponsors have no involvement in the study design, collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Competing interest statement

All authors have no financial and personal relationships with other people or organizations that could potentially and inappropriately influence (bias) their work and conclusions.

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