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Research Article

The Application of Polysaccharide Biocomposites to Repair Cartilage Defects

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Owing to own nature of articular cartilage, it almost has no self-healing ability once damaged. Despite lots of restore technologies having been raised in the past decades, no repair technology has smoothly substituted for damaged cartilage using regenerated cartilage tissue. The approach of tissue engineering opens a door to successfully repairing articular cartilage defects. For instance, grafting of isolated chondrocytes has huge clinical potential for restoration of cartilage tissue and cure of chondral injury. In this paper, SD rats are used as subjects in the experiments, and they are classified into three groups: natural repair (group A), hyaluronic acid repair (group B), and polysaccharide biocomposites repair (hyaluronic acid hydrogel containing chondrocytes, group C). Through the observation of effects of repairing articular cartilage defects, we concluded that cartilage repair effect of polysaccharide biocomposites was the best at every time point, and then the second best was hyaluronic acid repair; both of them were better than natural repair. Polysaccharide biocomposites have good biodegradability and high histocompatibility and promote chondrocytes survival, reproduction, and spliting. Moreover, polysaccharide biocomposites could not only provide the porous network structure but also carry chondrocytes. Consequently hyaluronic acid-based polysaccharide biocomposites are considered to be an ideal biological material for repairing articular cartilage.

1. Introduction

Articular cartilage plays a vital role in the function of joint action. Complete articular cartilage is the foundation of the normal function of the joint to exercise. Articular cartilage is a single tissue without supply of blood and lymphatic. As the cartilage cells divide very slowly, its ability to repair itself is low and it usually cannot be repaired. Therefore articular cartilage defects are a significant problem in orthopedic surgery. For the reason of primary osteoarthritis or from trauma, it will cause the felling of joint pain. Owing to lack of ability of self-repairing, cartilage injuries are kept for years and can result in further degeneration [1]. With the rapid development of tissue engineering, techniques to restore articular cartilage defects have also made tremendous progress [2], but these are still unsatisfactory for the effect of repairing articular cartilage defect. For decades, lots of researches have been conducted on articular cartilage, we had a better understanding of the biological repair process, and it has recently been shown that articular cartilage has a spontaneous repair reaction in the case of full-thickness cartilage defects [3]. However, the extent of this repair response is finite. By contrast, few useful elaborations with respect to repair processes in terms of partial-thickness lesions restricted the cartilage itself [4].

Despite the fact that lots of methods have been employed to repair cartilage damages, they work ineffectively, such as chondrectomy [5], drilling [6], cartilage scraping [7], arthroplasty [8], grafting of autogenic or allogenic chondrocytes [9, 10], and periosteum [11] as well as cartilage and bone flap being the most commonly applied [12]. Although the bone repair could be induced [13], it will remain to be a great challenge in terms of repairing large defects of articular cartilage [14].

Rebuilding cartilage makes it possible to repair cartilage defects with the rapid development of tissue engineering.

The primary method is to repair cartilage defects by the use of seeding cells and scaffolds [15]. Scaffolds are generally employed to restore cartilage defects mainly because of their three-dimensional surroundings needed for regeneration of cartilaginous tissues [16]. Both synthetic [17–20], natural [21–25] scaffolding materials and other composites containing fillers biomaterials [26] have been used for cell conveying in special cell regeneration.

Synthetic scaffolds are artificial option for biological repairing. In comparison with natural scaffolds, their advantage lies in the ability to stand weight-bearing forces by means of regulating their mechanical performance. Also, it has been reported for the successful use of different synthetic polymers in repairing the cartilage defects. However, synthetic materials have the following disadvantages: first, acidic byproducts will be created and accumulated in the use of scaffolds; second, they take on a poor biocompatibility; last, the underlying toxicity of byproducts in the course of these materials' degradation may give rise to an inflammatory reaction [27].

In all natural materials, chitosan [28, 29], collagen [30, 31], atelocollagen gel [32, 33], fibrin [34], alginate [35], and agarose [36] have been employed as effective scaffolds for cartilage repairing, and the repair effect of all above natural materials are relatively satisfactory natural materials. De Franceschi et al. demonstrated that implanting chondrocytes via the carrier of an atelocollagen gel could boost the repair of the articular cartilage in the knee [30]; especially when the scaffold exhibits nanostructure, the effect of repair will be more clear and effective [37].

For the existing form of polyanion, hyaluronic acid with linear high molecular mass polysaccharide is generally known as hyaluronan; also α-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine are component unit of hyaluronic acid. Generally speaking, the molecular weight of hyaluronic acid lies in the range of 103 to 107 [38]. Hyaluronic acid is the component not only existing inthe ECM of various connective tissues, but also interacting with binding proteins. Proteoglycans which function as lubricant safeguarding the surface of articular cartilage help in the control of water balance. Additionally, hyaluronic acid plays a role of selecting and protecting around the cell membrane; what is more, special cell receiverswhich control inflammation, cell behavior, angiogenesis, and healing processes could be easily identified by hyaluronic acid [37]. Umbilical cord, synovial fluid, rooster comb, and vitreous humor are major source for commercially available hyaluronic acid [39, 40]; however, hyaluronic acid could be also obtained by means of massive microbial fermentation, avoiding the danger of animal-derived pathogens [40], and sometimes natural materials like hyaluronic acid could be guided by other specific materials [41].

As a result of excellent biocompatibility and viscoelastic performances, hyaluronic acid has been broadly studied and employed in the biomedical area for cell encapsulation, carrier systems, and tissue engineering. It is just because of its nonimmunogenic performances, extensive use, and simple operating of chain size that hyaluronic acid is especially suitable for tissue engineering applications. What is more, by means of interaction with cell-surface receptors, it immediately imposes an influence on tissue organization, which advances the transfer of ECM remodeling and special cell. Hyaluronic acid is well known to interact with chondrocytes by means of every exterior receptor related to signaling pathway, which enable chondrocytes to keep their original phenotype. Additionally, hyaluronic acid could activate collagen II and aggrecan along with cell proliferation. What is more, by means of integrating alginate, chitosan, and fibrin gel matrices, hyaluronic acid possesses the ability to offer artificial ECM surroundings. So hyaluronic acid scaffold is a very promising biomaterial to repair articular cartilage defects. In this study, polysaccharide biocomposites (hyaluronic acid hydrogel containing chondrocytes) were used to repair full-thickness articular cartilage defects in rats. According to the evaluation of histological and biochemical criteria, the repair effect of restoration materials will provide a basis for its application.

2. Materials and Methods

2.1. Grouping. In this experiment, we selected 120 250 ± 20 g clean-grade SD rats (The Animal Experimental Center of Hebei Medical University) and classified them into three groups randomly: natural repair (group A), hyaluronic acid repair (group B), and polysaccharide biocomposites repair (hyaluronic acid hydrogel containing chondrocytes, group C). Each group has 36 rats. The remaining 12 rats are used to get chondrocytes.

2.2. Obtaining Chondrocytes. Cut rats' xiphoid cartilage under sterile conditions, and shear cartilage into pieces. Then, digest cartilage pieces by 0.25% trypsin (GIBCO Company, France) at 37°C for 3 minutes, drain the supernatant, and after that digest treated cartilage pieces by 0.25% trypsin (GIBCO Company, France) again at 37°C for 1 hour; then digest them by 0.2% collagenase II (Baiao Biotechnology Company) at 37°C for 2 hours. Get the supernatant and centrifuge at 800 rpm for 10 minutes for collecting chondrocytes. Via Toluidine blue staining, we made an identification of chondrocytes. By means of Trypan blue staining, the fact that the viability of acquired cells was greater than 90% was detected. Few medium was added into collected cells, and cells density was adjusted at 1×10^5/mL and mixed evenly by vortex shaker to reserve.

2.3. Preparation of Polysaccharide Biocomposites. The achieved chondrocytes were added into 1% hyaluronic acid hydrogel (The Experimental Center of The First Hospital of Hebei Medical University; pH: 6.8~7.8, osmotic pressure ratio: 1.0~1.2). The cell density was adjusted to 5×10^4/mL and mixed evenly by vortex shaker to reserve.

2.4. Surgical Methods

2.4.1. Preparation of Cartilage Damage Model. Animals were banned food and water 12 hours before surgery and injected with anesthetic (ketamine 1%, 10 mg/kg, diazepam 1 mg/kg)

in the upper left thigh. The experimental animals that had been anaesthetized were put on the sterile surgical drapes with both their legs' extension position fixed in a supine position. The surgical area in right leg was disinfected by 2% iodine, 75% alcohol. Skin was slit in longitudinal orientation from the 2 mm on the pole of patella to the 2 mm under the lower pole of patella on its right knee. Medial support belt and joint capsule were slit in level at 1 mm inside the inner edge of the patella. The patella was dislocated to the outside. Femoral trochlear appeared in flexion at 90°. In order to form full-thickness cartilage defects, use a hollow drill (with a diameter of 2.5 mm) to drill several 3 mm holes in the middle of trochlea humeri. After ful hemostasis, medial retinaculum, joint capsule, and the skin were sutured in turn. After surgery, 0.1 mL of hyaluronic acid hydrogel was injected into articular cavity of group B. Also 0.1 mL of polysaccharide biocomposites (hyaluronic acid hydrogel containing chondrocytes) was injected into articular cavity of group C; the rats of group A would be naturally repaired. All surgeries were performed by experimenter under the help of same assistants.

2.4.2. Postoperative Treatment. The operative incision was disinfected by 75% alcohol once a day and surgical suture was removed after 7 days.

2.5. The Experimental Details of Some Steps

2.5.1. HE Staining. Prepare the tissue slice and dewax in the xylene for 5–10 min; then remove slice into the mixture of xylene and absolute ethanol (1 : 1). Soak the slice in the 100%, 90%, and 85% ethyl alcohol in sequence each for 2–5 min; at last, after dealing with the distilled water, transfer it to dye liquor; stain the slice with hematoxylin dyeing for 5–15 min, wash redundant dye off the slide with water, and separate color using 0.5–1% hydrochloric acid alcohol for a moment; perform a microscopy control until the nucleus and chromatin are clear. Flush with running water for 15–30 minutes, and then the nuclei will turn blue. Then wash the slice with distilled water for a short time, and stain with 0.1–0.5% eosin dye for 1–5 min, dehydrate with 85%, 90%, and 100% ethyl alcohol in sequence each for 2-3 min. Treat sample with xylene twice for about 10 min totally. In the end, wipe the excess xylene around the section, and drop proper amount of neutral gum rapidly; then seal the slice with coverslip.

2.5.2. Masson Staining. Fix the tissue with neutral formaldehyde liquid, prepare paraffin section, and dewax to water routinely. Then, stain section with Masson compound staining liquid for about 5 min, and wash with 0.2% acetic acid aqueous solution for a short time. Treat section with 5% phosphotungstic acid for 5–10 min, and then embathe section with 0.2% acetic acid aqueous solution twice; then stain with aniline blue for 5 min and wash with 0.2% acetic acid aqueous solution for a moment. Finally, dehydrate section with absolute alcohol and treat using xylene; seal slice with neutral balsam.

2.5.3. Scanning Electron Microscope Observation. Select 4 rats randomly in each group 40 days after cartilage damage repair operation; cut open along original operative incision immediately after rabbits' execution. Obtain materials regularly according to electron microscope specimen. Cut and acquire a tissue block (with the size of $2.5 \times 2.5 \times 1.0\,mm^3$) in the local damage cartilage rapidly, fix the tissue block with 4% glutaraldehyde fixation fluid, observe the scanning electron microscope, and take pictures.

2.5.4. Immunohistochemistry. Fix the tissue, prepare paraffin section and dewax routinely. Conduct a digestion using enzyme and then carry out rehydration. Wash section using PBS (0.01 mol/L, pH: 7.2–7.4) for 5 min, dry with cold wind, and put into the wet box. Drop diluted fluorescent antibody at temperature of 37°C for 30–60 minutes. Wash sections with PBS twice and distilled water once, respectively, in sequence. Eventually, seal slices with 50% glycerol buffer, examine the section under fluorescence microscope, and then conduct control staining.

2.5.5. Measuring the Contents of Type II Collagen. In the experiment, the content of type II collagen was calculated via determination of content of hydroxyproline. Specifically, the average content of hydroxyproline in collagen is 13.4%, and the content of type II collagen accounts for 80% in articular cartilage collagen; as a consequence, as long as we make a determination of content of hydroxyproline in articular cartilage, we will figure out the content of type II collagen.

Determination of content of hydroxyproline: cut and acquire a tissue block (with the size of $2.5 \times 2.5 \times 1.0\,mm^3$) in the local damage cartilage, and the block weighs about 60–80 mg. Then dehydrate the tissue block with 1 mL anhydrous ethanol for 1 h after physiological saline flushing, discard the supernatant, and degrease the tissue block 2 times with 1.6 mL mixture of acetone and ethyl ether, each time of degreasing operation stays overnight. Take out and dry out the tissue block; then put it into oven (at temperature of 110°C) and dry to constant weight, weigh 5 mg cartilage samples accurately, and put into a test tube; then add 0.7 mL muriatic acids (at concentration of 6 mol/L) into tube and hydrolyze in the oven (at temperature of 105°C) for 24 h; adjust the solution to pH 6 with 6 mol/L sodium hydroxide. Then, adjust the volume to 5 mL accurately, centrifuge at the rate of 2500~3000 rpm for 10 min, and gain 0.6 mL supernatant for determining content of hydroxyproline.

2.6. Statistical Analysis. Data were analyzed with SAS software, and expressed as mean ± SD. The difference between the two groups was compared with Student's t-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

In the observation of electron microscope observation, specimen staining revealed that the bottom of restoration area in group A appeared to be a small amount of red granulation tissue whose surface was covered by pale, relatively

smooth, and transparent membrane after 10 days of operation (Figure 1(a)). The restoration area of groups B and C was filled with regenerated tissue which was white and soft and also had an uneven surface and clear boundary (Figures 1(b) and 1(c)). After 20 days of surgery, repair tissue of restoration area in group A was white and slightly hard and had uneven surface and clear boundary (Figure 1(d)); repair tissue of restoration area in groups B and C was milky white and tough and also had a flat surface and clear boundary (Figures 1(e) and 1(f)). After 40 days of surgery, the bottom of restoration area group A was filled with granulation tissue (Figure 1(g)). Restoration area in group B was filled with repair tissue which was yellowish white and had a relatively clear boundary, tough quality, and uneven surface (Figure 1(h)). The repair tissue of group C without clear boundary with normal cartilage (Figure 1(i)) was tough and translucent. After 10 days of surgery, the case of inflammatory cell (most were lymphocytes) infiltration was found in repair tissue. 20 days later, inflammatory cell infiltration went clearer. after 40 days of operation, lymphocytes cell infiltration reduced. For groups A and B, slight lymphocytes cell infiltration appeared in the repair tissue of both groups after 10 days of operation; lymphocytes cell infiltration got a little more after 20 days of operation, but obviously less than group C; lymphocytes cell infiltration reduced obviously after 40 days of operation. According to the specimen staining, group C (Figure 2(a)) performed best in the synthesis of collagen in repair area. The second best is group B (Figure 2(b)) and both group C and group B outperformed group A (Figure 2(c)).

By the observation of scanning electron microscopy, collagen fibers in parallel with articular surface arranged regularly as with group C (Figure 3(c)) in the restoration area, and the boundary of collagen fibers in the restoration area is not clear with normal cartilage after 40 days of operation. In case of group B (Figure 3(b)), the arrangement of collagen fibers was relatively regular; also fibers were relatively thinner and partially fractured or sunken. For group A (Figure 3(a)), collagen fibers in the restoration area were in disorder and thick; just like group B, collagen fibers in group A were partially fractured too. In the results of immunohistochemical detection, brown and yellow positive reaction product was found in every cell cytolymph in the restoration area. The amount of group C (Figure 4(a)) was the most and well dispersed; the second was group B (Figure 4(b)) and the reaction product dispersed uniformly, and just few positive reaction products were found in group A (Figure 4(c)).

In Table 1, We can obviously see that the content of type II collagen was the highest in group C in the tissue of repair area; then the second highest was group B; the last is group A after 10, 20, and 40 days of operation.

In a word, at every time point, through the evaluation of histology and biochemistry, cartilage repair effect of group C is the best; the second best is group B; both of them were better than group A.

4. Discussions

Articular cartilage is hyaline cartilage, whose tissue's metabolic activity is low, without blood supply and lymphatic drainage, and cartilage cells divide very slowly, so its ability to repair itself is low; usually it cannot be repaired [42–44]. Therefore, how to promote the repair of damaged cartilage is one of the key research points to scholars for a long time. With the development of tissue engineering technology, cartilage injury repair has made some progress [45–48]. Currently, cartilage tissue engineering research's main content focused on seed cells, the carrier material, and the interaction of seed cells and carrier [49]. In the process of tissue engineering materials application, there is no generally accepted ideal implant because of the compatibility of the seed cells; the carrier's adhesion and degradation should be considered [50–52]. Ideal carrier material whose extracellular matrix components are as close as to the nature chondrocytes should have good tissue compatibility and biodegradability [53]. Currently there are two categories: natural materials and synthetic materials. Synthetic materials include polylactic acid, polyglycolic acid copolymer, hydroxyapatite, and calcium phosphate [54, 55]. Owing to lack of binding sites which cells can recognize, so synthetic material has no biological activity and its degradation products may be toxic. Natural materials are mainly collagen, fibrin gel, hyaluronic acid, and so on. Its biocompatible and biodegradable properties are better than synthetic materials'. But its different resources make obviously different structure and performance [56–59].

Hyaluronic acid is a nonsulfated polysaccharide-based natural cartilage matrix, whose three-dimensional structure has high porosity and surface and space area are bigger, which is benefit for growth of cell adhesion, extracellular matrix deposition, the take-up of gases and nutrients, and metabolic product discharge, and offers a good interface of material-cell function. Hyaluronic acid can improve adhesion between cells and extracellular matrix burial and play an important role in cartilage nutrition, maintaining cartilage characteristics and joint lubrication [60–62]. Hyaluronic acid also can maintain normal growth of cartilage cells and promote the integration of transplanted chondrocytes and damaged cartilage [63–66]. Certainly, along with other natural materials, such as chitosan [67, 68] and collagen [69], the hyaluronic acid could be used as biomaterials scaffolds for repairing articular cartilage. The purity of hyaluronic acid is higher, the immunogenicity is lower, and the biocompatibility is better. Hyaluronic acid's degradation products can promote wound healing [70–73]. Hyaluronic acid as a treatment for osteoarthritis medication has been in clinical use for many years [74]. Its efficacy is satisfactory with fewer side effects. And its operation is simple as an injectable material. So in this study hyaluronic acid is selected as a carrier material. Experimental results indicate that the repair effect of group B is better than that of group A in terms of tissue science and biochemical evaluation, which fully proved that hyaluronic acid can promote cartilage repair.

In this experiment, inflammatory cells, mainly lymphocytes, are in infiltration in each group restoration area at early stage after surgery under light microscope and Group C is the most obvious. With time going, inflammatory cells' quantity becomes less. 10 days after the injection of repair materials, infiltration of inflammatory cells can be seen in restoration area for identification of phagocytic cells. Groups A and B have the same level of infiltration of inflammatory

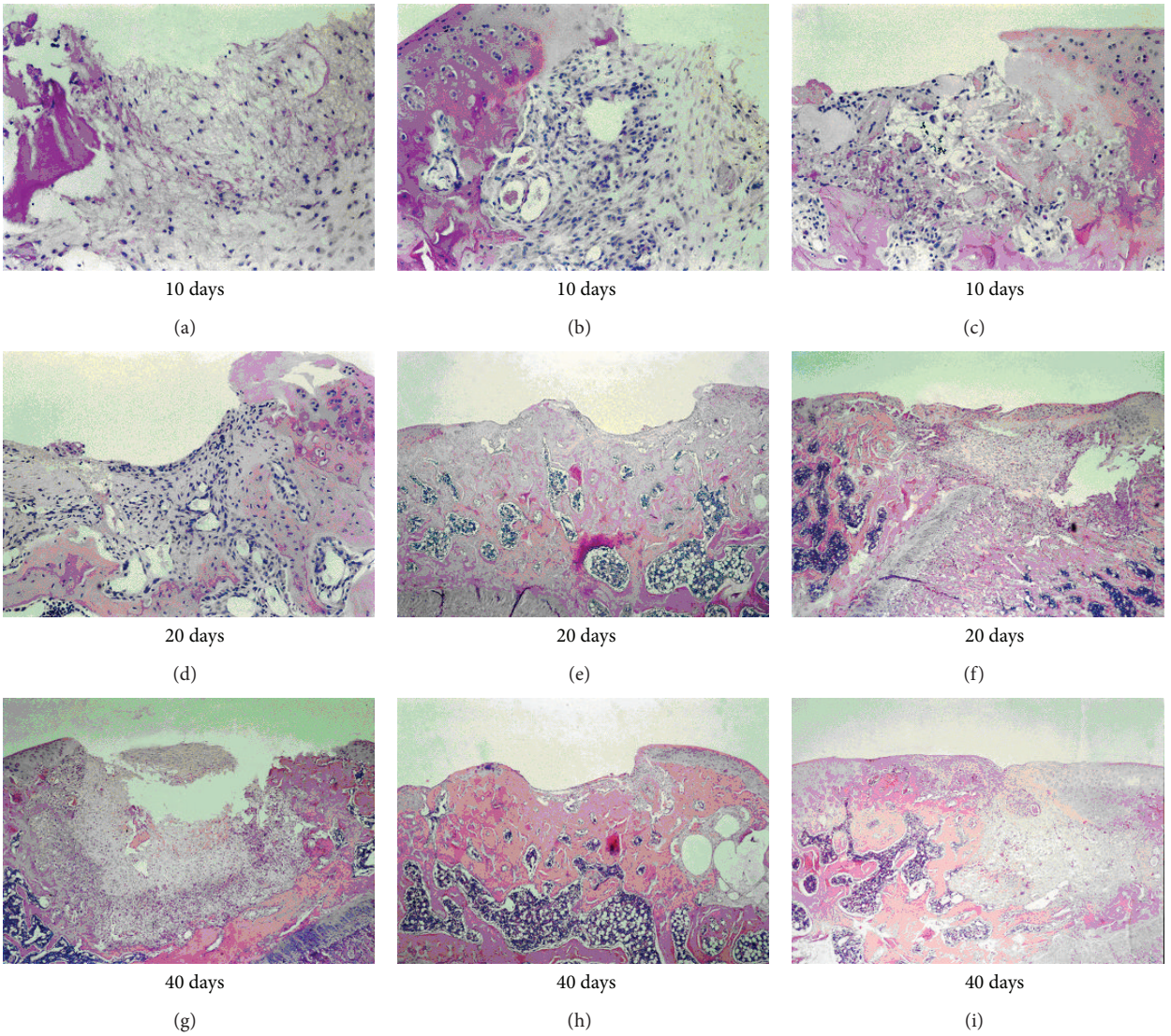


Figure 1: The results of specimen HE staining in restoration area; magnification times of all pictures in Figure 1 are 200. Pictures (a), (d), and (g) belong to group A; pictures (b), (e), and (h) belong to group B; pictures (c), (f), and (i) belong to group C.

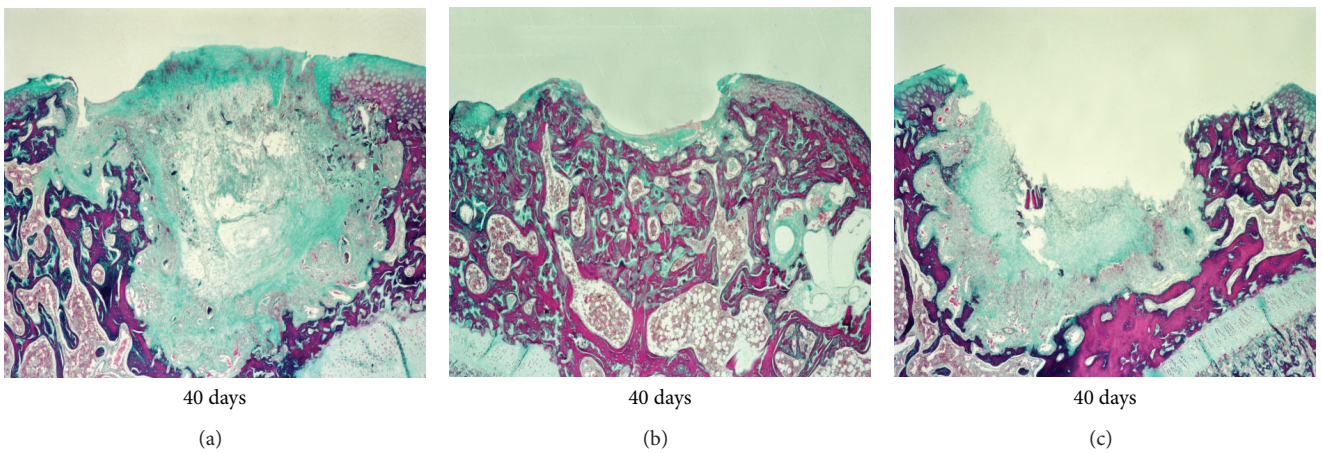
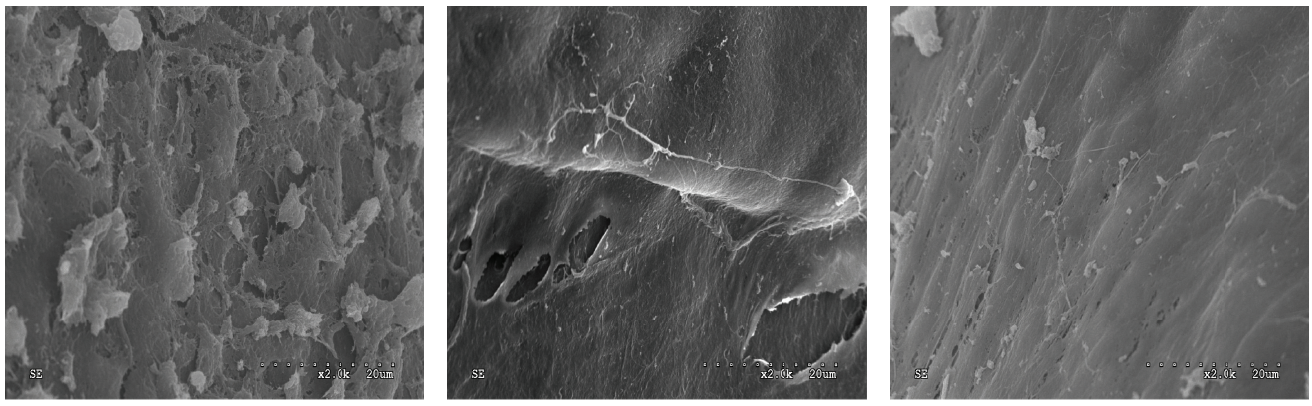
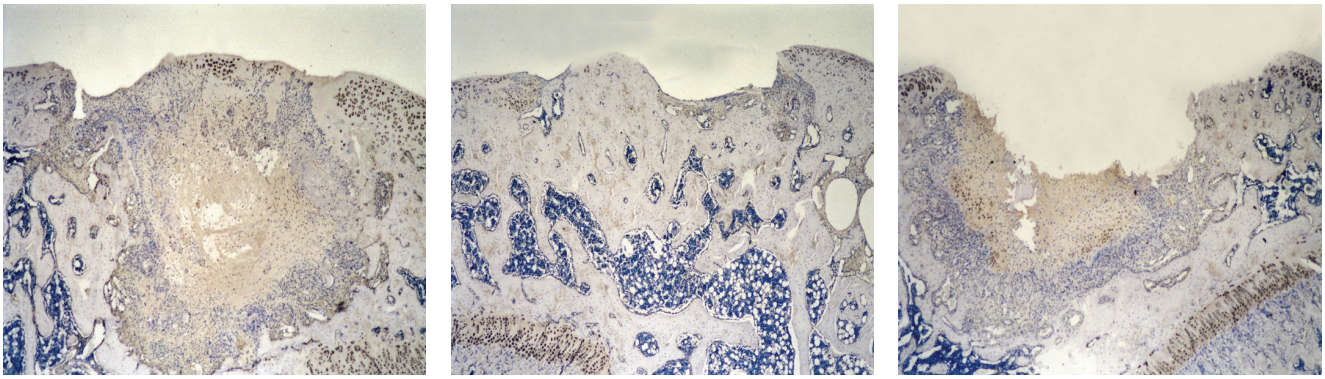


Figure 2: The results of specimen Masson staining in restoration area; magnification times of all pictures in Figure 2 are 200. Pictures (a), (b), and (c) belong to groups A, B, and C, respectively.



40 days (a) 40 days (b) 40 days (c)

Figure 3: The scanning electron microscopy images of collagen fibers in repair area; magnification times of all pictures in Figure 3 are 2000. Pictures (a), (b), and (c) belong to groups A, B, and C, respectively.



40 days (a) 40 days (b) 40 days (c)

Figure 4: The results of immunohistochemical detection in restoration area; magnification times of all pictures in Figure 4 are 200. Pictures (a), (b), and (c) belong to groups A, B, and C, respectively.

cells, which indicates that hyaluronic acid as a carrier material has good biocompatibility. The level of infiltration of inflammatory cells at each time point is the best, which indicates that resource of immune resource is major histocompatibility antigens on cartilage cell's surface. The result of immunohistochemistry shows that positive reaction product of Group A restoration area is the least, which indicates that its ability of proteoglycan synthesis was significantly reduced; positive reaction product of group C restoration area is the most in the all 3 groups, which indicated that hyaluronic porous network structure is conducive to the growth of cartilage cells and extracellular matrix is proteoglycan-rich both of which can slow down the degradation of type II collagen synthesis of chondrocytes. The sign of the maturity level of cartilage is collagen because the structural basis of cartilage is collagen. Masson staining indicates that a large number of collagen fibers can be seen in group C restoration area, which is more than groups A and B. As with hydroxyproline content, group C contained the most; then the second is group B; the last is group A. Three groups' difference is statistically significant. All can prove that this repair material can effectively promote cartilage repair. Through the experiment, compared to natural repair, the overall repair effect of hyaluronic acid performed better. This fact fully demonstrated that hyaluronic acid played a catalytic role for cartilage repair. When compared with hyaluronic acid, the repair effect of polysaccharide biocomposites (hyaluronic acid hydrogel containing chondrocytes) was better, which fully illustrated that the combined action of hyaluronic acid and chondrocytes was advantageous to the growth of cartilage cells and collagen and proteoglycan synthesis. Of course, its porous network structure also played a driving role in repairing cartilage defects. These polysaccharide biocomposites, which were obtained easily and prepared simply, are a relatively ideal biomaterial for cartilage repair and could be made into injection.

Because clinical cartilage damage is mostly closed, selection of repair material tends from solid to liquid and the method of transplantation tends from open graft with large injury to simple, minimally invasive intra-articular

Table 1: The content of type II collagen in the repair area at different time points (μg/mg).

Groups	10 days (μg/mg)	20 days (μg/mg)	40 days (μg/mg)
Natural repair	80.83 ± 4.91	81.03 ± 5.01	80.94 ± 5.35
Hyaluronic acid	90.35 ± 9.59*	88.39 ± 7.96**	89.17 ± 8.52**
Composites	99.78 ± 7.56*	105.55 ± 7.49*	101.97 ± 7.34*

*$P < 0.01$; **$P < 0.05$.

injection. In this experiment, polysaccharide biocomposites (hyaluronic acid containing chondrocytes) were applied in intra-articular injection to repair cartilage defects, whose result indicates that this method is feasible, satisfactory, and provides a theoretical basis for clinical application. However, due to the separation and culture of chondrocytes being directly related to repair effect, the isolation and culture techniques of chondrocytes are needed to further improve.

5. Conclusion

Transplantation of isolated chondrocytes which belongs to the category of tissue engineering has tremendous potential for treatment of cartilage injury and regeneration of articular cartilage tissue. As to transplantation of isolated chondrocytes and formation of cartilage tissue, the determining factor of success lays in particular cell-carrier materials. In terms of restoring articular cartilage, hyaluronic acid biomaterials outperformed natural repair obviously; of course, polysaccharide biocomposites performed best in effect of restoration. Polysaccharide biocomposites could offer the porous network structure which could promote chondrocytes proliferation, and it could also carry chondrocytes effectively. The porous network structure has high histocompatibility with the surrounding and good biodegradability, and it also favors chondrocytes survival, reproduction, and splitting. So the polysaccharide biocomposites is an appropriate specific cell-carrier material. Consequently hyaluronic acid-based polysaccharide hydrogels biocomposites are considered to be an ideal biological material for repairing articular cartilage.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Feng Zhao and Wei He contributed equally to this work.

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