Regeneration of a goat femoral head using a tissue-specific, biphasic scaffold fabricated with CAD/CAM technology

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ABSTRACT

Tissue engineering is considered as a promising approach for the regeneration of biological joint theoretically and thus provides a potential treatment option for advanced osteoarthritis. However, no significant progresses so far have been made in regenerating biological joint. In this study, a biphasic scaffold, which was consisted of polyactic acid-coated polyglycolic acid (PGA/PLA) scaffold and poly-

caprolactone/hydroxyapatite (PCL/HA) scaffold, was designed and used for regeneration of goat femoral head. The content of PLA and HA was optimized to a proper ratio, thus the scaffolds could achieve appropriate stiffness which was more conducive to articular cartilage and bone regeneration respectively. Furthermore, computer-aided design and manufacturing (CAD/CAM) technology was employed to fabricate the biphasic scaffolds into the desired shape and structure. The biphasic scaffolds with fine cell biocompatibility matched perfectly. Chondrocytes and bone marrow stromal cells (BMSCs) were seeded into the scaffolds for cartilage and bone regeneration respectively. After 10 weeks of implantation in nude mice subcutaneously, the cell-scaffold constructs successfully regenerated goat femoral heads. The regenerated femoral heads presented a precise appearance in shape and size similar to that of native goat femoral heads with a smooth, continuous, avascular, and homogenous cartilage layer on the surface and stiff bone-like tissue in the microchannels of PCL/HA scaffold. Additionally, histological examination of the regenerated cartilage and bone showed typical histological structures and biophysical properties similar to that of native ones with specific matrix deposition and a well-integrated osteochondral interface. The strategy established in the study provides a promising approach for regenerating a biological joint which could be used to reconstruct the impaired joint.

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1. Introduction

Osteoarthritis, which manifests as structural breakdown of cartilage and subchondral bone, is a chronic disability that affects approximately half of individuals over 65 years old worldwide [1,2]. Arthroplasty is the main treatment option for advanced osteoarthritis. Unfortunately, current arthroplasty based on titanium alloy, stainless steel, ceramic, and ultra-high molecular weight polyethylene (UHMWPE) is not a biological reconstruction. Wear particles will be generated inevitably during use, which lead to aseptic loosening frequently [3–7]. No matter how to improve the materials and processing technology, the presence of mechanical wear particles still could not be completely avoided. In addition, the match between the implant and native joint is another important factor that influences the longevity of the implant, because standard prosthetics could not match perfectly with every joint of individuals [8,9]. Clearly, there is an urgent need to develop a biological technology for regeneration and reconstruction of joint tissue, additionally, the regenerated biological
joint should match with native one well. Tissue engineering is such an emerging biological technology, which may regenerate an articular graft with biological structure and function similar to normal joint tissue (including cartilage, subchondral bone, and osteochondral interface) theoretically, and thus hopefully becomes a promising method instead of the current artificial arthroplasty [10].

A few studies have preliminarily reported the feasibility of regenerating tissue-engineered biological joints [11–16]. Although these studies were successful in some aspects, they did not obtain satisfactory results in homogeneity of cartilage regeneration and interface integration between regenerated cartilage and subchondral bone. Therefore, no practical breakthrough so far has been achieved in regenerating biological joints with homogeneity, continuous cartilage layer and well-integrated subchondral bone simultaneously. The main difficulty to regenerate such a biological joint lies in the proper tissue-specific scaffold design. The joint consists of both cartilage and subchondral bone, and thus the scaffold should be designed respectively suitable for cartilage and bone regeneration in different parts, since different tissues have different requirements in the scaffold design.

Polylactic acid (PLA) coated polyglycolic acid (PGA) scaffold has proved to be ideal scaffolds for cartilage regeneration [17,18]. However, how to fabricate the PLA/PGA scaffold to achieve a proper stiffness and accurate shape favorable for articular cartilage formation and its integration with underlying bone scaffold is one of important issues that need to be explored. Polycaprolactone/hydroxyapatite (PCL/HA) is a biodegradable, avirulent, biologically active, and osteo-conductive material. Moreover, HA is the major component of the mineral phase in bone. Therefore, PCL/HA were reported as a proper scaffold for bone regeneration [16]. Likewise, how to accurately control the stiffness and shape of PCL/HA scaffold to direct bone regeneration with a specific shape and size is also an important issue.

To address above issues, in this study, we designed a tissue-specific, biphasic scaffold, in which PLA/PGA scaffold was designed for cartilage regeneration while PCL/HA scaffold for bone regeneration. It has been reported that bone marrow stromal cells (BMSCs) respond to scaffold elasticity by differentiating into lineages corresponding to the stiffness of the native environment [19]. Therefore, as an important modification, the PLA content in the PLA/PGA scaffold was optimized to a ratio that provided appropriate stiffness suitable for cartilage regeneration. Likewise, the HA content in the PCL/HA scaffold was also optimized to a ratio that provided appropriate matrix stiffness favorable for bone regeneration. Furthermore, the technology of computer-aided design and manufacturing (CAD/CAM) was employed to fabricate the biphasic scaffolds into the desired shape and structure. Finally, chondrocytes and BMSCs were seeded into the PGA/PLA and PCL/HA scaffolds respectively, and the feasibility of regenerating goat femoral heads was explored by subcutaneously implanting the grafts in athymic nude mice.

2. Materials and methods

2.1. General experimental design

All animal experiments were approved and conducted according to the guidelines outlined by the Shanghai Jiao Tong University School of Medicine on animal use. All animal subjects received humane care in compliance with the “Guide for Care of Laboratory Animals” as detailed by the National Ministry of Science. In total, twenty 8-week-old athymic nude mice (Experimental Animal Center of Chinese Academy of Sciences, Shanghai, China) were used in this research. Mice were randomly assigned to either the experimental group (Exp, n = 10), in which mice were implanted with the cell–scaffold constructs, or the control group (Ctrl, n = 10), in which mice were implanted with cell–free scaffolds. Proximal femoral condyles from ten goats aged 10 months were used as the normal control group (NC, n = 10). The general experimental protocol is shown in Fig. 1.

2.2. Analysis of complex scaffold characteristics

2.2.1. Analysis of PGA/PLA scaffold characteristics with different PLA contents

PGA/PLA scaffolds were prepared according to our previously established method [20,21]. Briefly, 25 mg of unwoven PGA fibers (provided by the National Tissue Engineering Center of China) with varying PLA content were compressed into a cylinder that was 12 mm in diameter and 1.2 mm in thickness. The scaffold mechanical properties were analyzed as previously described methods [22]. The cell seeding efficiency of scaffolds was calculated based on previous methods [23]. Microstructure and biocompatibility of the scaffold was evaluated by scanning electron microscope (SEM; S-2150 Hitachi Ltd, Japan).

2.2.2. Analysis of PCL/HA scaffold characteristics with different HA contents

PCL/HA scaffolds with different HA contents were fabricated according to previously established methods [24]. Compression tests were performed to evaluate the mechanical properties of the scaffolds [25]. Water uptake ratios were measured, as previously described methods to evaluate PCL/HA scaffold wettability [25]. Microstructure and biocompatibility of the scaffold was evaluated by scanning electron microscope (SEM; S-2150 Hitachi Ltd, Japan).

2.3. Isolation and culture of cells

Both chondrocytes and BMSCs were obtained from 10-month-old goats. Cartilage samples were harvested from the femoral compartment of the knee joint. Chondrocytes were isolated, cultured, and expanded according to the reported methods [26]. Chondrocytes in passage two were harvested for regeneration of articular cartilage. BMSCs were isolated from the marrow of tibial condyle, cultured, and expanded as previously described methods [27,28]. BMSCs in passage two were harvested for the construction of subchondral bone.

2.4. PGA/PLA scaffold preparation for cartilage regeneration of the femoral condyle

Firstly, CAD/CAM technology was employed to fabricate the mold of PGA/PLA scaffold. The surface morphology of a goat femoral head with and without articular cartilage was obtained by laser scanning at 8 μm resolutions (Konica Minolta, Sakai, Osaka, Japan). The data were further processed to generate articular cartilage three-dimensional (3D) data. CAD software was used to design the mold and then input into a CAM system (Spectrum 510, Z Corporation) to fabricate a resin model by 3D printing. Then, PGA/PLA scaffolds were prepared according to previously established methods [20,21]. Finally, the PGA/PLA scaffolds with 10% PLA were molded into the corresponding anatomic shape of the articular surface with a thickness of about 1.2 mm.

2.5. PCL/HA scaffold preparation for bone regeneration of the femoral condyle

The 3D data of the surface morphology of femoral head without articular cartilage were reconstructed and designed through CAD software for the fabrication of osteochondral component scaffold. Two key points were involved in the scaffold design: (1) An extra intramedullary stem was added for surgical fixation; (2) Modularizing interconnecting microchannels were designed throughout the scaffold to promote cell distribution, nutrient transportation, blood vessel ingrowth, and thus enhanced bone regeneration. 40 wt% HA powder (Sigma, St Louis, MO, USA) was mixed with 60 wt% PCL pellets (Solvay, Brussels, Belgium; average Mw ~ 40,000, polydispersity index of 1.2) in a slurry at 120 °C (Fortus, Stratasys, Eden Prairie, MN, USA). PCL/HA scaffolds were fabricated by fused deposition modeling (FDM) that was controlled by a CAM software [24,25].

2.6. Preparation of cell–scaffold constructs

Chondrocytes in passage two were seeded into the PGA/PLA scaffolds at a density of 5.0 × 10^7 cells/ml. The cell suspension was dropped into the scaffolds and incubated for 5 h to allow sufficient cell adhesion [20,21]. A subset of PGA/PLA scaffolds were seeded with chondrocytes and cultured in vitro for 2–3 weeks in chondrogenic medium supplemented with 10 ng/ml transforming growth factor β1 (TGF-β1, InterGen, Burlington, MA), 40 ng/ml of dexamethasone (Sigma–Aldrich, St. Louis, MO, USA), 100 ng/ml insulin-like growth factor 1 (IGF-1; Sigma–Aldrich), 1% insulin-transferrin-selenium (ITS; Sigma–Aldrich), and so forth.

Similarly, BMSCs in passage two were seeded into the microchannels of PCL/HA scaffolds at a density of 2.5 × 10^6 cells/ml. The cell suspension was dropped into the microchannels and incubated for 4–5 h to allow sufficient cell adhesion. A subset of PCL/HA scaffolds were seeded with BMSCs in scaffold microchannels and cultured in vitro for 2–3 weeks in osteogenic medium supplemented with 10 nm β-glycerophosphate disodium (Sigma–Aldrich), 0.1 μM dexamethasone (Sigma–Aldrich), and 50 μM ascorbic acid 2-phosphate (Gibco, Grand Island, NY, USA).

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2.7. In vivo implantation of femoral head constructs

Together, the upper cartilaginous component and the lower osseous component formed the osteochondral construct. Following approval of the Institutional Animal Care and Use Committee at Shanghai Jiao Tong University School of Medicine, athymic nude mice were anesthetized and the constructs were implanted into the dorsum subcutaneously. No complications or adverse reactions were observed after surgery. At 10 weeks post-implantation, the animal subjects were sacrificed under anesthesia, and the specimens were harvested and fixed in 10% phosphate-buffered formalin.

2.8. Gross observations

At 10 weeks post-implantation, the constructs were harvested and grossly examined. The constructs were then dissected sagittally to observe the interface between the regenerated cartilage and subchondral bone. Surface smoothness, size, shape, and vascularization were grossly evaluated.

2.9. Histological analysis

Some of the harvested tissue and scaffold samples were decalcified in 0.5N ethylene diamine tetraacetic acid (EDTA) solution, then embedded in paraffin with standard histological procedures and sectioned at 8 \( \mu \)m thickness, the others were directly embedded in poly(methyl-methacrylate) and sectioned sagittally at 30 \( \mu \)m thickness. The former sections were stained with haematoxylin and eosin (HE), safranin O-fast green (SO/FG), toluidine blue (TB), Goldner’s trichrome (GT), while the latter sections were stained with von Kossa (VK) and TB.

An International Cartilage Repair Society (ICRS) visual histological assessment scale was performed to quantitatively evaluate cartilage regeneration according to previously reported methods [29]. Cell-free scaffolds were implanted likewise as control group (Ctrl, \( n = 10 \)). No complications or adverse reactions were observed after surgery. At 10 weeks post-implantation, the animal subjects were sacrificed under anesthesia, and the specimens were harvested and fixed in 10% phosphate-buffered formalin.

2.10. Immunohistochemical analysis

Immunohistochemical staining was performed to further identify expression of tissue-specific proteins in regenerated tissue according to previously established methods [32]. Type II collagen (COL II) (monoclonal antibody ab34712, 1:100, Abcam, Cambridge, MA, USA) was immunolocalized to identify expression of chondrogenic protein. Type-I collagen (COL I) (monoclonal antibody 5D8, 1:200, Enzo, Dural, NSW, Australia), Osteopontin (OPN) (monoclonal antibody N/A, 1:200, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and osteocalcin (OCN) (monoclonal antibody OC4-30, 1:400, Abcam, Cambridge, MA, USA) were immunolocalized to identify expression of osteogenic proteins.

2.11. Biomechanical analysis

All the tissue samples were trimmed into a cylinder shape with a diameter of 5 mm and compressive tests were performed using a testing system (Instron, Grove City, PA, USA) according to previously established methods [33]. Stress–strain curve, compression modulus, and compressive strength were recorded and analyzed, respectively.

2.12. Statistical analysis

All statistical analyses were performed with SPSS software (version 13.0). A \( p \)-value less than 0.05 was considered as statistically significant. Upon confirmation of a normal data distribution, quantitative data for the experimental, normal control, and control groups were evaluated with one-way ANOVA and post-hoc least significant difference tests. For the data with unknown distributions, non-parametric Wilcoxon signed-rank tests were performed.
3. Results

3.1. Influence of PLA and HA contents on the scaffold properties

By incorporating PLA coating, PGA fibers could be processed into porous scaffolds with specific geometry and stiffness (Fig. 2A–B). The compressive modulus of PGA/PLA scaffolds increased with the increase of PLA content from 0% to 30% (Fig. 3A), while cell seeding efficiency decreased (Fig. 3B). The compressive modulus of PGA/PLA scaffold containing 10% PLA (5.10 ± 1.00 MPa) reached the stiffness range of native articular cartilage (Fig. 3G) which has been reported to be favorable for chondrogenic differentiation of BMSCs [19]) with good biocompatibility (Fig. 2C) and a relatively higher cell seeding efficiency (over 85%) (Fig. 3B). Therefore, the scaffold containing 10% PLA was used for the next cartilage scaffold preparation.

PCL/HA could be fabricated into a designed 3D shape with a regular porous structure and good biocompatibility (Fig. 2D–F). The mechanical properties of PCL/HA scaffold, such as compressive stress curve, compressive modulus, and compressive strength, gradually increased with the increase of HA content (Fig. 3C–E). Furthermore, the water uptake ratio of the scaffold was also improved with the increase of HA content (Fig. 3F). The compressive modulus of PCL/HA scaffold containing 40% HA (57.90 ± 5.70 MPa) reached the stiffness range of native spongy bone (Fig. 3C) which has been reported to be favorable for osteogenic differentiation of BMSCs [19]) with good biocompatibility (Fig. 2F) and high water uptake ratio (Fig. 3F). Therefore, the PCL/HA scaffold containing 40% HA was used for the next bone scaffold preparation.

3.2. Construction of a complex scaffold with the shape of femoral head

The complex scaffold consisted of an upper PGA/PLA scaffold (Fig. 4F) and a lower PCL/HA scaffold (Fig. 4D, E). These two part scaffolds showed perfect match at both size and shape and could be combined into an intact biphasic scaffold with a precise shape and size of goat proximal femoral condyle (Fig. 4G).

The PCL/HA scaffold was fabricated according to the 3D data achieved from goat proximal femoral condyle (Fig. 4A–C) (not containing cartilage layer) to form a 3D hemispherical scaffold with the dimension of 18 × 17 × 15 mm³ (length × width × height), which was equal to the anatomic size of goat proximal femoral condyle (not containing cartilage layer). To promote cell distribution and nutrient transportation towards the inner of scaffold, the PCL/HA scaffold was designed to contain regular 3D interconnecting microchannels (200–400 μm in pore size) with a porosity of 54.6 ± 1.2% (Fig. 4H).

PGA/PLA scaffold was compressed into the shape of the articular surface with a thickness of about 1.2 mm, which was similar to the thickness of the goat femoral head articular cartilage (Fig. 4F). For preventing loss of chondrocytes from the scaffold, the PGA/PLA was compressed into a relatively compact porous scaffold with irregular pores (Fig. 4I).

3.3. Regeneration of goat femoral head

Both the PGA/PLA scaffold and the PCL/HA scaffold showed satisfactory cell biocompatibility with abundant matrix production (Fig. 5A–B). After cell seeding, both the scaffolds retained the original shape and size and could be integrated into an intact biphasic cell–scaffold construct with a precise shape of goat proximal femoral condyle (Fig. 5C).

After 10 weeks of in vivo implantation, the constructs basically retained the original size and shape (about 20 × 19 × 16 mm³ in length × width × height) with a smooth, continuous, avascular cartilage-like surface (Fig. 5D–E). At the cross section, a continuous, homogenous cartilage layer with a thickness about 1.5 mm was observed on the surface of engineered femoral head and the white, stiff bone-like tissue filled with the microchannels of PCL/HA scaffold (Fig. 5F). Particularly, it was worth notice that the cartilage layer and the subchondral bone part tightly integrated with each other with no observable seam at the interface (Fig. 5F). However, neither visible cartilage layer nor bone-like tissues was observed in the samples of control group (without cells), in which only sparse fibrous tissue and residual PCL/HA were observed (Supplemental Fig. 1A–B).

3.4. Evaluation of cartilage formation in regenerated femoral head

Continuous and homogeneous cartilage formation was the most important issue of regenerating biological joint. Histologically, the
The surface layer of regenerated femoral heads showed continuous, homogeneous cartilage tissue with typical characteristic of mature articular cartilage, in which abundant lacunae structures and positive staining of cartilage-specific matrices (such as glycosaminoglycan, collagen type II) was observed (Fig. 6). A few residual PGA fibers were also observed among the cartilage matrices (Fig. 6). No visible blood vessels were found in the whole cartilage layer (Fig. 6).

In control group, only sparse fibrous tissue with negative cartilage matrix staining was observed in the surface of the specimens (Supplemental Fig. 1C–F).

Histological quantitative analysis showed that the average of ICRS visual histological assessment scale in Exp group was a little lower than that in NC group while the average number of chondrocytes per total tissue area in Exp group was a litter higher than that in NC group, but both of them showed no significant differences between Exp and NC groups (n = 10, p > 0.05) (Fig. 7A–B). Mechanical analysis also showed no significant differences between Exp and NC groups, although both compressive modulus and compressive strength in Exp group were a little lower than those in NC group (n = 10, p > 0.05) (Fig. 7C–D). In control group, all of above indexes could not be analyzed normally due to lack of cartilage-like tissue in the surface of specimens (Fig. 7).

3.5. Evaluation of interface between cartilage and subchondral bone in regenerated femoral head

The interface integration between cartilage and subchondral bone was another key issue of regenerating biological joint. As shown in Fig. 8, the regenerated cartilage and subchondral bone showed a satisfactory integration with a typical osteochondral interface, in which cartilage tissue, immature calcified tissue, transitional trabecular bone as well as typical populations of hypertrophic chondrocytes could be observed at the same area, indicating a typical osteochondral interface. Particularly, it was noticed that the above osteochondral structure only was observed in the interface area and that neither ossified tissue in cartilage layer nor cartilage tissue in subchondral bone area was observed, indicating a tissue-specific regeneration in different microenvironments.

3.6. Evaluation of bone formation in regenerated femoral head

Bone regeneration provided a basic support for regenerating the whole biological joint. The hard tissue sections distinctly showed that partially degraded PLC/HA scaffolds were observed and calcified trabecular bone filled with the microchannels of the scaffolds (Fig. 9A–B). In paraffin sections, typical trabecular bone structure...

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was observed in microchannels of the PLC/HA scaffolds (the blank area was caused by the dissolution of PLC/HA scaffold during the treatment of specimens) (Fig. 9C–F). The surface of new formed bone trabecula was lined by cuboidal osteoblast-like cells and abundant osteocytes were found in the regenerated bone trabecula (Fig. 9C–F). Blood vessels were observed in both trabecular bone area and mesenchymal tissue area (Fig. 9C–F). Furthermore, osteogenic specific proteins, such as OPN, collagen type I, and OCN, were detected in the regenerated bone trabecula (Fig. 9G–I). In control group, no bone tissue was found after 10 weeks of implantation (Supplemental Fig. 1C–F).

Histological quantitative analysis demonstrated that BV/TV (bone volume/tissue volume), BS/BV (bone surface/bone volume), osteoblast surface/T.Ar (osteoblast surface/tissue area), and blood vessel number/T.Ar (blood vessel number/tissue area) showed no significant differences between Exp and NC groups (n = 10, p > 0.05), indicating fine bone formation and vascularization in Exp group (Fig. 10A–D). BV/TV, BS/BV, and Osteoblast surface/T.Ar were not assessed in Ctrl group because no bone formation was observed. A significant difference was observed between Exp group and Ctrl group in Blood vessel number/T.Ar (n = 10, *p < 0.01). Mechanical analysis revealed that both compressive modulus and compressive strength in Exp group reached over 90% of normal level with no significant differences between Exp and NC groups (n = 10, p > 0.05) (Fig. 10E–F). The compressive modulus and compressive strength in Ctrl group were significant lower compared to those in Exp and NC groups, although they still retained a certain level due to the presence of residual PCL/HA scaffolds (Fig. 10E–F).

4. Discussion

Tissue-engineering of biological joints is considered as a promising approach for biological treatment of advanced osteoarthritis [10]. However, no significant advances have been achieved in regenerating a tissue-engineered biological joint with a homogeneous, continuous cartilage layer and well-integrated subchondral bone structure [1,11–16]. In this study, the CAD/CAM technology was employed to fabricate tissue-specific, biphasic scaffolds which were used to regenerate goat femoral heads with precise shape and size in athymic nude mice successfully. The regenerated femoral heads showed a continuous, homogeneous, avascular cartilage layer on the surface and typical bone tissue in the subchondral bone part. Moreover, the regenerated cartilage and subchondral bone were well integrated with each other and presented the typical osteochondral transitional structure in the interface regions. The present study provides a preliminary exploration toward the
successful regeneration of biological joint for clinical applications in the future.

The proper design of scaffold is critical for successful regeneration of biological joint with well-integrated cartilage and subchondral bone. Briefly, the ideal scaffold for regeneration of biological joint mainly includes the aspects as follows [11–13]: (1) Biphasic component which is suitable for regeneration of cartilage and bone respectively; (2) Biocompatibility and biodegradable materials with suitable mechanical properties; (3) A precise contour appearance with a perfect integration of the cartilaginous and osseous component which is similar to the native joint; (4) A porous scaffold component with an interconnecting micropore structure for cell distribution and nutrient transportation. All these factors should be considered in the scaffold design.

Cartilage is the most important component of joint and regeneration of articular cartilage is a great challenge in regenerating a biological joint. Scaffolds play an important role in directing cartilage regeneration with a desired shape. The PGA/PLA scaffold has been known as one of the most successful scaffolds for cartilage regeneration because of their desirable mechanical properties, biocompatibility, and ability to be shaped. Since Cao et al. engineered cartilage with the shape of human auricle in a nude mouse model [34], further study has focused on the regeneration of cartilage tissue by molding PGA/PLA in complex shapes [21]. In the present study, the contour of regenerated articular cartilage was accurately controlled and retained by the PGA/PLA scaffold, which was precisely molded by means of CAD/CAM technology. The regenerated cartilage showed a homogeneous and continuous

![Image](https://example.com/image.png)
Histological assessment and biomechanical analysis of the regenerated cartilage. ICRS visual histological assessment scale (A) and chondrocyte number per total tissue area (chondrocyte number/T.Ar) (B) show no significant differences between the experimental group (Exp) and the normal control group (NC) (n = 10, p > 0.05). Similarly, no significant differences are observed in compressive modulus (C) and compressive strength (D) between Exp group and NC group (n = 10, p > 0.05). In control group (Ctrl), all of above indexes can not be analyzed normally due to lack of cartilage tissue in the surface of specimens (*).

Fig. 8. Histological examination of the interface between regenerated cartilage and subchondral bone. The regenerated cartilage presents well-integration with the regenerated subchondral bone and no obvious seam is observed at the interface (A–D). A typical transitional structure area is observed between regenerated cartilage and subchondral bone (C, D), in which cartilage tissue, hypertrophic chondrocyte populations (green arrows), incompletely calcified tissue, and transitional trabecular bone are observed simultaneously. Scale bar = 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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cartilage-like appearance with a regular hemispherical shape and size, which matched well with native articular cartilage in the shape and size. Most importantly, the regenerated cartilage presented typical cartilage characteristics with apparent lacuna structures, abundant cartilage-specific matrix deposition, and sufficient biophysical properties similar to that of native cartilage. All these results indicated that the PGA/PLA scaffold mentioned in the study was suitable for regeneration of homogeneous and continuous articular cartilage.

The integration between cartilage and subchondral bone in the interface is another great challenge in regenerating a biological joint. In the present study, the regenerated cartilage and subchondral bone integrated with each other well and typical populations of chondrocytes were observed in the osteochondral interface regions. Additionally, hypertrophic chondrocytes, calcified cartilage matrix, and transitional trabecular bone could also be observed in the regions. The reason of satisfactory integration in the interface was considered mainly as the following aspects: (1) The precise well-matched PGA/PLA and PCL/HA scaffold appearance; (2) The relatively rough surface and the micropores of PCL/HA scaffold provided a favorable topographical surface for chondrocyte attachment, meanwhile extracellular matrix (ECM) and regenerated cartilage could be anchored in the micropores; (3) Moreover, a few chondrocytes migrated into or on the surface of osseous component scaffold may be induced into hypertrophy calcified chondrocytes in the osteogenic microenvironment [35]; (4) Meanwhile, a few undifferentiated BMSCs that migrated from PCL/HA scaffold to PGA/PLA scaffold may be induced towards chondrocyte lineages when exposed to decreased matrix stiffness and chondrogenic microenvironment produced by chondrocytes [19]; Accordingly, the regenerated cartilage and subchondral bone could be able to integrate with each other well.

Bone regeneration is an important issue in regenerating a biological joint. The following aspects might be conducive to bone regeneration. Firstly, BMSCs have the capacity to be induced into several mesenchymal lineage pathways resulting in the formation of definitive tissues such as bone and cartilage [36]. Secondly, HA has osteo-inductive capability in the presence of BMSCs [37]. Thirdly, another big part is that BMSCs responded to scaffold elasticity by differentiating into lineages that corresponded to the stiffness of the native environment [19,38–40]. The stiffness of the PCL/HA scaffolds was in the range that of normal spongy bone and therefore presented a favorable osteogenic environment and enhanced the regenerative capacity of bone. Finally, the design of interconnecting microchannels with a diameter of 200–400 μm in PCL/HA scaffold was conducive to cell distribution, nutrient transportation, and ingrowth of blood vessels, which was apparently helpful for improving bone regeneration [41,42]. In addition, BMSCs

Fig. 9. Histological examination of the regenerated subchondral bone. In hard tissue sections, trabecular structure (A; toluidine blue staining, TB) and mineral deposition (B; von Kossa staining, VK) are observed among the PCL/HA scaffold, which has partially degraded (red arrows). In paraffin sections, trabecular structures, typical osteoblasts (blue arrows), osteocytes (yellow arrows), and vascularization (black arrows) are observed in the microchannels of PCL/HA scaffold (C, D, E, F). Positive immunohistochemical localization of osteopontin (G, OPN), type-I collagen (H, COL I) and osteocalcin (I, OCN) are further observed in the osseous region. Scale bar = 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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were also reported to have the ability to enhance angiogenesis by up-regulating VEGF synthesis \cite{43,44} and thus promoted bone formation further.

Besides suitable for regeneration of a biological joint subcutaneously ectopically, the present scaffold might also be suitable for reconstruction of a biological joint in situ. Firstly, the scaffold could be designed to match native joint perfectly. Secondly, the articular microenvironment is more favorable for cartilage and bone regeneration by providing proper growth factors and mechanical stimulation \cite{17,35}. Thirdly, due to the modularized interconnecting microchannels, the nutritional supplements will be more sufficient for tissue regeneration \cite{41,45}. Finally, the PCL/HA scaffold has a relatively slow degraded rate, which basically guarantees the maintenance of shape and initial mechanical properties \cite{46}. So an important extension of this study is regenerating a femoral head ectopically or in situ in vivo for biological joint replacement in animal models based on the present strategy, and the study is now ongoing. Up to now, certain progress has made. We hope that the regeneration of biological joint for patients in vivo will come true in the near future.

5. Conclusions

The present study established a feasible strategy for regeneration of a biological joint based on the tissue-specific, biphasic scaffold fabricated by CAD/CAM technology. The regenerated goat femoral heads presented a precise appearance compared to native ones with smooth, continuous, avascular, and homogeneous articular cartilage and well-integrated subchondral bone. Moreover, the regenerated cartilage and subchondral bone showed the similar histological structures and biophysical properties compared to native ones, meanwhile well-integrated osteochondral transitional regions in the interface were observed simultaneously.
Author disclosure statement
The authors declare no conflict of interest.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jbiomaterials.2013.05.038.

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