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Biphasic scaffolds for repair of deep osteochondral defects in a sheep model

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ABSTRACT

Background: To oppose the disadvantages of autologous osteochondral transplantation in the treatment of deep osteochondral defects such as donor site morbidity, size limitation, and insufficient chondral integration, we developed two biphasic scaffolds of either hydroxylapatite/collagen (scaffold A) or allogenous sterilized bone/collagen (scaffold B) and tested their integration in a sheep model.

Methods: We collected chondral biopsies from 12 sheep for the isolation of chondroblasts and cultured them for 4 wk. We created defects at the femoral condyle and implanted either scaffold A or B with chondrocytes or cell free. After 6 wk, animals were euthanized, we explanted the condyles, and evaluated them using histological, immunohistochemical, molecular biological, and histomorphometrical methods.

Results: Specimens with scaffold A showed severe lowering of the surface, and the defect size was larger than for scaffold B. We found more immune-competent cells around scaffold A. Chondrocytes were scarcely detected on the surface of both scaffolds. Histomorphometry of the interface between scaffold and recipient showed no significant difference regarding tissue of chondral, osseous, fibrous or implant origin or tartrate-resistant acid phosphatase–positive cells. Real-time reverse transcriptase–polymerase chain reaction analysis revealed significant up-regulation for collagen II and SOX-9 messenger ribonucleic acid expression on the surface of scaffold B compared with scaffold A.

Conclusions: Scaffold B proved to be stable and sufficiently integrated in the short term compared with scaffold A. More extensive evaluations with scaffold B appear to be expedient.

1. Introduction

The increasing incidence and impact of osteoarthritis as a result of cartilage injuries underlines its political and social significance. Because the self-healing competence of cartilage tissue is limited, several surgical options have been developed for repair. To date, there is no consensus on which treatment is the most effective. Nonoperative treatment is based mainly on medication such as nonsteroidal anti-inflammatory drugs or chondroprotective drugs and physiotherapy. Although this kind of treatment can reduce symptoms, none can actually heal preexisting cartilage damage [1]. The International Cartilage Society (ICRS) recommends surgical treatment of a cartilage defect >2 cm^2 and of grade III/IV according to the

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Outerbridge classification. Simple lavage and debridement are not considered to be effective in the longer term [2]. Bone marrow stimulation is a widely used method; in particular, the microfracture technique (MFX), which was introduced in the early 1990s has become popular. Early and midterm outcomes are reported to be positive, especially in younger patients [3–5], but decline in the longer term [6]. With osteochondral autologous transplantation (OAT), good results can be achieved in 71%–90% of patients, depending on the site of the defect [7–12]. Disadvantages of the procedure are limited areas for harvest and reports of ongoing pain at the donor site in up to 12% of patients [13].

Brittberg *et al* [14] introduced the technique of autologous chondrocyte implantation (ACI) in 1994; even after 10 years, 69% of patients showed improved function [15]. Compared with MFX or OAT, most studies reveal advantages for ACI [10–12,16–19]; thus, some reviewers recommend ACI as the method of choice [20]. Nevertheless, a Cochrane review did not give clear evidence for ACI as the best option [21].

For >1-cm-deep osteochondral defects, MFX and ACI alone are not a real option, and the limitations of OAT have to be considered as well. Although only 9% of all patients with cartilage lesions have deeper osteochondral defects [22], their treatment remains a challenge. One option is the so-called sandwich technique, with layers of autologous spongiosa, matrices, and chondrocytes [23,24], but it would be more desirable to have a complete scaffold that can be implanted. This model should be osteoconductive as well as chondroconductive, and also degradable.

To meet these demands, we developed two different biphasic scaffolds. After successful *in vitro* studies on cell toxicity, cell stability, and proliferation (unpublished), we tested them *in vivo* in the sheep model.

2. Methods

2.1. Scaffolds

Scaffold A consisted of an 8- to 10-mm-deep layer of hydroxylapatite (HA) and collagen in a ratio of 1:3. We produced HA in the form of nanoparticles by mixing phosphoric acid and calcium oxide slowly into a suspension of collagen. We then filled this mineralized collagen suspension in forms, freeze-dried them, vacuum-dried them, and sterilized them with ethylene oxide. The 2-mm upper layer consisted of porcine collagen I/III. We combined the two layers by dripping 20 μL collagen onto the plane surface of the cylinder five times, each time followed by a drying period.

Scaffold B consisted of allogenous bone derived from sheep coxae, and also an upper layer of porcine collagen I/III. The combination of the two layers followed the same protocol as for scaffold A. We then lyophilized the cylinders and sterilized them using gamma rays.

2.2. Study design and operations

In this study, we tested early ingrowth and reactions of the different scaffolds: scaffold A alone, scaffold A together with autologous chondrocytes, and scaffold B with autologous chondrocytes.

We performed animal surgery according to the legal requirements for animal protection and approved by the government of the county (presidial government of Darmstadt).

We took biopsies for chondrocyte cultivation from the left knee of 12 female Merino sheep. The sheep were all 2 y old, with an average body weight of 41.8 ± 3.6 kg. We cultivated the chondrocytes for 4 wk and implanted six sheep with scaffold A, either with ($n = 6$) or without ($n = 6$) chondrocytes, and six sheep with scaffold B with chondrocytes.

We performed all surgical procedures under general anesthesia and strict sterile conditions. A single injection of intravenous cephalosporin was given as antibiotic prophylaxis. The right knee joint was approached by a medial parapatellar incision and the weightbearing area of the femoral condyles was exposed. We created the osteochondral defects using a specially manufactured hollow drill with a diameter of 9.4 mm and depth of 1.1 cm. We then inserted the sterilized scaffolds press-fit into the defect. In six sheep, we implanted scaffold A into a defect on the medial and another one on the lateral condyle. We then dripped the autologous chondrocytes onto the superficial layer of the scaffold at the medial condyle; the scaffold at the lateral condyle was left without cells. In the other six sheep with scaffold B, only a defect on the medial condyle was set, and cells were dripped on all of the scaffolds. After a waiting time to allow the cells to adhere, we closed the wound in layers. Postoperative analgesia was provided by fentanyl transdermal patches and buprenorphine injections. We allowed all sheep to bear weight as soon as possible and put them back into a flock of sheep for the next 6 wk.

After 6 wk, we euthanized all 12 sheep with pentobarbital, explanted the right distal femur, and immediately sawed it into 2- to 3-mm-thick slices using a special saw (Exact, Norderstedt, Germany).

2.3. Macroscopic evaluation

Before we cut the condyles in layers, we evaluated the whole defect macroscopically according to criteria described by the ICRS [25] (Table 1).

2.4. Histological, enzyme, and immunohistochemical evaluation

We processed sections of each specimen for histological evaluation. We enzyme-histochemically detected tartrate-resistant acid phosphatase (TRAP)-positive cells, which indicate osteoclasts and macrophages. Collagen I and II and the glycoprotein “cluster of differentiation 68” (CD68) were detected immunohistochemically. We fixed at least one slice of each specimen in 4% phosphate-buffered paraformaldehyde (Merck, Darmstadt, Germany), decalcified with 10% ethylenediaminetetra acetic acid (pH 8.0; Sigma, Taufkirchen, Germany) in 3.5 mol/L Tris buffer (pH 7.4; Sigma) for 21 d, dehydrated it with graded concentrations of ethanol, saturated it in xylol, and embedded it in paraffin. We cut sections 3–5 μm thick with a rotation microtome (Leica, Bensheim, Germany), deparaffinized them, routinely stained them with toluidine blue and hematoxylin-eosin (HE), and