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Effect of temporal onsets of mechanical loading on bone formation inside a tissue engineering scaffold combined with cell therapy

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ABSTRACT

Several approaches to combine bone substitutes with biomolecules, cells or mechanical loading have been explored as an alternative to the limitation and risk-related bone auto- and allo-grafts. In particular, human bone progenitor cells seeded in porous poly(L-lactic acid)/tricalcium phosphate scaffolds have shown promising results. Furthermore, the application of mechanical loading has long been known to be a key player in the regulation of bone architecture and mechanical properties. Several in vivo studies have pointed out the importance of its temporal offset. When an early mechanical loading was applied a few days after scaffold implantation, it was ineffective on bone formation, whereas a delayed mechanical loading of several weeks was beneficial for bone tissue regeneration. No information is reported to date on the effectiveness of applying a mechanical loading in vivo on cell-seeded scaffold with respect to bone formation in a bone site. In our study, we were interested in human bone progenitor cells due to their low immunogenicity, sensitivity to mechanical loading and capacity to differentiate into osteogenic human bone progenitor cells. The latest capacity allowed us to test two different bone cell fates originating from the same cell type. Therefore, the general aim of this study was to assess the outcome on bone formation when human bone progenitor cells or pre-differentiated osteogenic human bone progenitor cells are combined with early and delayed mechanical loading inside bone tissue engineering scaffolds. Scaffolds without cells, named cell-free scaffold, were used as control. Surprisingly, we found that (1) the optimal solution for bone formation is the combination of cell-free scaffolds and delayed mechanical loading and that (2) the timing of the mechanical application is crucial and dependent on the cell type inside the implanted scaffolds.

1. Introduction

Cells such as mesenchymal stem cells (MSCs), human bone progenitor cells (hBPCs), and bone marrow-derived MSCs, have shown their potential for bone tissue engineering (BTE) in several in vitro studies, by producing mineralized extra cellular matrix under osteogenic conditions (Krattinger et al., 2011; Krebsbach et al., 1999; Montjovent et al., 2004; Owen et al., 1987; Phinney et al., 1999; Pittenger et al., 1999; Wu et al., 2015). In different experimental and in vivo implantation conditions, those cells have demonstrated their capacity to induce bone formation when implanted with BTE scaffolds. In citing a few studies, Serafini et al. have highlighted the ability of bone marrow-derived MSCs to form bone marrow and hematopoietic niches when implanted in heterotopic sites (Serafini et al., 2014), whereas other studies have shown an increase in bone formation when implanted in bone sites (Corre et al., 2015; Dupont et al., 2010; Jäger et al., 2007; Liu et al., 2013; Montjovent et al., 2008; Srouji and Livne, 2005; Xu et al., 2010; Yasko et al., 1992).

In parallel, it has long been known that mechanical loading plays an important role in the regulation of bone architecture and properties (Carter et al., 1989; Huiskes et al., 2000). Capitalizing on this phenomenon for applications, several studies demonstrated in vivo that the temporal onset of mechanical loading on bone formation in scaffolds was crucial (Boerckel et al., 2012; Roshan-Ghias et al., 2010, 2011). The application of early mechanical loading, applied a few days post-implantation, was seen to be ineffective or moderate compared to delayed mechanical loading, applied several weeks post-implantation.

Therefore, in the present work, based on longitudinal microCT images and histological analysis we investigated the effect of the combination between mechanical loading and cell therapy in the

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outcome of a tissue engineering scaffold implanted in a rat model. We were able to score the effects of the different bone tissue engineering treatments in scaffold bone formation under these experimental conditions.

2. Materials and methods

2.1. Cell culture and scaffold seeding

The interest in using hBPCs arose from their low immunogenicity (Montjovent et al., 2009) and capacity for osteogenic differentiation into mature osteoblasts (Montjovent et al., 2004), referred in the present work to as osteogenic hBPCs (hOBPCs). This differentiation capacity allows us to test in vivo two different bone cell fates originally derived from the same type of cell.

The culture conditions and scaffold seeding were performed as thoroughly reported elsewhere (Hausherr et al., 2017). In short, hBPCs were harvested from fetal bone tissue of 15 weeks gestational age following a voluntary interruption of pregnancy (Biobank, CHUV, Switzerland, Protocols 51/10). hBPCs were first expanded in standard culture medium composed of DMEM basal culture medium high glucose (Invitrogen, USA), supplemented with 10% (v/v) FBS (Thermo Fisher Scientific, USA) and 1% (v/v) L-Glutamine (200 mM, Invitrogen, USA). We showed in a previous study that under these culture conditions, hBPCs kept their osteoblastic phenotype (Hausherr et al., 2017). At passage 4, the cells were seeded into scaffolds composed of poly(L-lactic acid) (PLA, Boehringher Ingelheim, Germany) and 5% β-tricalcium phosphate (5% β-TCP, Fluka, Sigma-Aldrich, ST Louis, USA) (Mathieu et al., 2006). Before seeding hBPCs at a concentration of 0.5×10^6 cells per PLA/5% β-TCP scaffold using a pressure-driven technique, the scaffolds were sterilized by ethylene oxide, perfused with 0.9% NaCl solutions (B. Braun, Germany) and sonicated to avoid micro-air bubbles inside the scaffolds. Three different scaffold conditions were prepared: cell-free scaffolds (CF), scaffolds seeded with hBPCs (CS) and scaffolds seeded with hOBPCs (OCS). For CS scaffolds, hBPCs were cultured in standard culture medium and were seeded three days before implantation. In the case of OCS scaffolds, hBPCs were seeded two weeks before implantation. To induce osteogenesis, the medium of OCS scaffolds was changed three times a week with osteogenic differentiation medium, composed of α-MEM (Gibco, USA), 10% (v/v) FBS (Thermo FisherScientific, USA), 1% (v/v) L-Glutamine (200 mM, Invitrogen, USA), 1% (v/v) Vitamin C (5 mg/mL, Sigma-Aldrich, USA), 1% (v/v) β-glycerophosphate (500 mM, Sigma-Aldrich, USA) and 1% (v/v) dexamethasone (1 mM, Sigma-Aldrich, USA). Just before implantation, the scaffolds were washed three times with sterile 0.9% NaCl solution (B. Braun).

2.2. Animal study design

Tissue engineering PLA/5% β-TCP scaffolds were implanted in a pre-drilled hole in both femoral condyles of female rats. The bone trauma site in the femoral condyle was situated under the growth plate, therefore corresponding to a metaphysis location. The study included 5 experimental groups with 5 to 6 animals assigned to each group. Each experimental group corresponded to one scaffold condition and two loading cases. For scaffold conditions, we implanted either CF, CS or OCS scaffolds bilaterally, while for the mechanical loading, we defined three cases: early, delayed and no external mechanical loadings. In each experimental group, one leg of each rat was subjected to either an external early or an external delayed mechanical loading while the other leg received no specific external mechanical loading. In the early mechanical loading case, the application of the mechanical loading started 2 days post-implantation, while in the delayed mechanical loading case the mechanical loading started 14 days post-implantation. A longitudinal in vivo micro-computed tomography (microCT) imaging follow-up was performed to evaluate the bone formation inside the different scaffold conditions and loading cases.

2.3. Animal model and surgical procedure

The animal model and surgical procedure were used as described elsewhere (Hausherr et al., 2017; Kettenberger et al., 2014). Briefly, female Wistar rats (280–300 g, licence N° 2631.0, EXPANIM, SCAV, Epalinges, Switzerland, provided by Janvier Labs, Saint-Berthevin, France) were anesthetized with Isoflurane (Piramal Entreprise Ltd., Bombay, India) and their legs shaved. Before the surgery, they were injected subcutaneously with Buprenorphine (0.03 mg/kg/day, Temgesic®, Reckitt Benckiser AG, Wallisellen, Switzerland) as analgesic and their eyes were covered with tears fluid (Viscotears®, Alcon, Forth Worth) to avoid eye drying. Prior to scaffold implantation, one leg was put in a flexed position to fix and stabilize the knee. After skin incision and muscle fascia splitting, a hole measuring 3 mm in diameter and 3 mm in depth was drilled in the lateral side of the femoral condyle using a motorized dentist's drill (DEC 100, Nobelcare, Sweden). Bone and blood remaining in the hole were rinsed with 0.9% NaCl solution (B. Braun) and removed with a surgical aspiration, followed by scaffold implantation. The scaffold (CF, CS or OCS) was press-fitted inside the drilled hole before muscles and skin were closed. The same surgical intervention was done on the contralateral femur of each animal. As post-operative care, the rats were injected with Buprenorphine (0.03 mg/kg/day, every 8 h for 48 h, Temgesic®) and paracetamol (Dafalgan 500 mg effervescent tablet, UPSA Bristol-Myer Squibb SA, Barr, Switzerland) was added to the drinking water for one week. The rats were euthanatized with an intracardiac Pentobarbital (< 200 mg/kg, Esconarkon, Streuli Pharma SA, Uznach) injection 12 weeks after scaffold implantation.

2.4. In vivo mechanical loading

After the surgery, either external early or delayed mechanical loading protocols were applied depending on the experimental group. In both cases, one leg of each rat received an external controlled mechanical loading for 5 min (10 N at 4 Hz, every two days over a period of 9 days) using a machine design based on previous studies (De Souza et al., 2005; Fritton et al., 2005; Stadelmann et al., 2009). The contralateral leg of the rat was used as a control (no loading during the 5 min sessions). The loading parameters were based on studies described elsewhere (Roshan-Ghias et al., 2010, 2011). For both loading cases, the rats were kept under anaesthesia during the loading sessions and were free to move between the sessions.

2.5. In vivo microCT imaging and data analysis

A longitudinal in vivo microCT imagings of both femurs at 6 time points (2, 4, 6, 8, 10 and 12 weeks after scaffold implantation) was performed using a SkyScan 1076 scanner (Bruker microCT, Kontich, Belgium), except for the OCS scaffold experimental group at week 6 due to a source breakdown of the microCT. Each leg was scanned separately and introduced in a plastic tube to stretch and fix the leg during scanning. The scanning parameters were the same for all scans (pixel size: 18 μm, filter: 0.5 mm aluminium, voltage: 80 kV, current: 120 μA, exposure time: 360 ms, rotation step: 0.5°). The chosen scan frequency had no impact on the structural bone parameters as described elsewhere (Brouwers et al., 2007).

The 2D reconstruction (ring artefact: 4, beam hardening: 20%, no smoothing) was done using NRecon software (Brukuer microCT), followed by the selection of the volume of interest (VOI) on 3D reconstructed datasets of each leg on Amira® (FEI Visualization Sciences Group, Burlington, USA). As the scaffold was not visible in microCT images because of its low absorption values, the VOI was selected as a cylinder with the same dimension than the scaffold (3 mm diameter and 3 mm high). In the present study, we were interested to compare the