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Augmentation of bone defect healing using a new biocomposite scaffold: An in vivo study in sheep

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ABSTRACT

Previous studies support resorbable biocomposites made of poly(L-lactic acid) (PLA) and β-tricalcium phosphate (TCP) produced by supercritical gas foaming as a suitable scaffold for tissue engineering. The present study was undertaken to demonstrate the biocompatibility and osteoconductive properties of such a scaffold in a large animal cancellous bone model. The biocomposite (PLA/TCP) was compared with a currently used β-TCP bone substitute (ChronOS™, Dr. Robert Mathys Foundation), representing a positive control, and empty defects, representing a negative control. Ten defects were created in sheep cancellous bone, three in the distal femur and two in the proximal tibia of each hind limb, with diameters of 5 mm and depths of 15 mm. New bone in-growth (osteoconductivity) and biocompatibility were evaluated using microcomputed tomography and histology at 2, 4 and 12 months after surgery. The in vivo study was validated by the positive control (good bone formation with ChronOS™) and the negative control (no healing with the empty defect). A major finding of this study was incorporation of the biocomposite in bone after 12 months. Bone in-growth was observed in the biocomposite scaffold, including its central part. Despite initial fibrous tissue formation observed at 2 and 4 months, but not at 12 months, this initial fibrous tissue does not preclude long-term application of the biocomposite, as demonstrated by its osteointegration after 12 months, as well as the absence of chronic or long-term inflammation at this time point.

1. Introduction

Not all bone losses are similar and correspondingly not every clinical situation needs the same treatment [1]. In particular, when load bearing situations are targeted and the quality of the bone is not optimal for an autograft the surgeon may be tempted to use an artificial scaffold to fill the bone defect. This situation often arises during revision of total knee replacement, where significant bone loss can be found either under the tibial tray or in the femoral part of the knee implant [2]. Unfortunately, in this case the use of calcium phosphate cement is not suitable for filling the bone defects, as this material is too brittle to sustain the shocks that will inevitably arise during normal daily activity [3].

An alternative to a ceramic scaffold could be to use porous bioresorbable polymers such as poly α-hydroxyacids [4,5]. However, despite allowing deformation of the scaffold, which is necessary for application in total knee revision, these polymer scaffolds do not offer good enough mechanical properties to resist significant loadings. To overcome this mechanical drawback ceramic–polymer composites have gained increasing interest [6–8]. In particular, a biocomposite of controlled porosity combining β-tricalcium phosphate (β-TCP) and poly(L-lactic acid) (PLA) was obtained by supercritical gas foaming [9]. This biocomposite displayed a cellular microstructure and elastic properties close to human trabecular bone [10] and was shown in a numerical study to be able to sustain the load if used as a tibial spacer to fill the bone defect during revision of total knee replacement [11]. In vivo studies in small animals have demonstrated the biocompatibility and osteoconductivity of the biocomposite developed [12]. The results in small animals such as rodents have to be considered with caution if we want to extrapolate them to clinical applications and need, at least, to be confirmed by studies in large animals. The sheep is often chosen as an experimental animal because its bone structure and bone remodelling rate are very similar to those of humans [13].

The aim of this study was to demonstrate the biocompatibility and osteoconductive properties of the new PLA/β-TCP biocompos-

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ite in a large animal model over a period of 2, 4 and 12 months and to determine new bone formation, implant degradation and inflammation compared with an untreated (empty) control. A positive control, made of porous β-TCP, already used clinically as a bone substitute (ChronOS™), was also included in the study.

2. Materials and methods

2.1. Scaffold

Manufacture of the new PLA/β-TCP biocomposite and characterization of its morphology and material properties have been described previously [10]. Briefly, the biocomposite is obtained by a two steps process, namely melt extrusion and supercritical CO_2 foaming. PLA granules (Boehringer Ingelheim) and β-TCP powder (Dr. Robert Mathys Foundation) are first dried to avoid polymer degradation. Both raw materials, in a 95/5 wt.% PLA/β-TCP ratio, are mixed and melt extruded in a micro-extrudor (Micro 5 DSM). The composite rods obtained are then cut, dried again, put into a mould and placed in a high pressure and temperature chamber (Autoclave France). The pressure is increased to 225 bar at which pressure PLA melted at 195 °C. Porosity was then created by CO_2 release and control of the depressurisation and cooling rates.

The positive control used in this study, ChronOS™ (Dr. Robert Mathys Foundation), is an established bone substitute. ChronOS™ consists of pure β-TCP and was obtained using the calcium phosphate emulsion method [14]. Briefly, a hydrophobic liquid is mixed with calcium phosphate paste and an emulsifier. After mechanical stirring an oil/cement paste emulsion is prepared. Once the cement has set and after oil washing porous ceramic blocks are then obtained. Finally, sintering at 1250 °C leads to porous β-TCP blocks.

Both types of material, biocomposite and ChronOS™, were then machined to obtain 15 mm long by 5 mm diameter cylinders. All scaffolds were sterilized by γ-irradiation before implantation.

2.2. Animal study

The animal model considered in this study has been described by Zeiter et al. [15]. All animal experiments were performed with the approval of the Veterinary Authority of the Canton Graubünden (no. 16/2006).

Briefly, 13 female Swiss Alpine sheep, with a mean age of 3 years and a mean weight of 50 kg, were used. Two custom made jigs, one with three holes and one with two holes, were used to position the defects in cancellous bone in the femur and tibia, respectively, in a standardized way. Three defects with a diameter of 5.1 mm were drilled in the distal part of both femora and two in the proximal part of both tibiae (bilateral model), cleaned with saline solution to remove osseous drilling particles and implanted with a scaffold or left empty. In each femur there was a defect filled with the biocomposite (Fig. 1), a defect filled with ChronOS™ as positive control and one defect left empty as a negative control. In the left tibia one defect was filled with the biocomposite and the other with ChronOS™. In the right tibia one defect was filled with the biocomposite and one left empty as a negative control. The site of the scaffolds within each bone was evenly distributed between animals to avoid a location bias. Small screws were also inserted in order to correctly position the removal jig at explantation.

Animals were killeded euthanized after 2, 4 and 12 months (4 animals per time point). After positioning of the custom made removal jig with the aid of the positioning screws, implants were harvested using a coring device (trephine attachment, Synthes) with an outer diameter of 12.5 mm. The resulting core consisted of the implant/defect in the centre with approximately 3 mm of surrounding cancellous bone. Samples were analysed by micro-CT and histology.

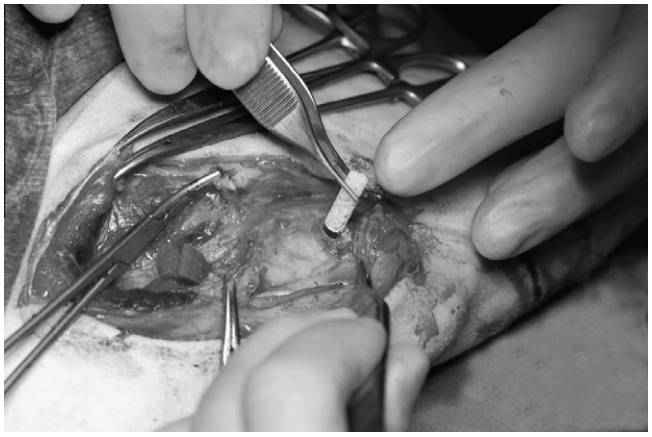

Fig. 1. Medial view of the distal femur. The defects were flushed with lactate Ringer's solution prior to inserting the implants in a drilled defect of 5.1 mm diameter and 15 mm depth.

2.3. Blood analysis

Blood was taken from each animal before surgery, 1 week and 2 weeks after surgery as well as before being killed euthanized. The total number of white blood cells and the differential blood count were determined.

2.4. Micro-CT

The harvested cores were fixed in 70% ethanol for 3 days prior to micro-CT analysis (μCT 40, Scanco Medical). The long axis of the bone samples was aligned orthogonally to the axis of the X-ray beam and a 5 mm region of interest in the middle of the sample was measured, representing the created defect. In that region bone volume to total volume (BV:TV) was evaluated by thresholding. Additionally three-dimensional (3D) reconstructions of the region of interest were created.

A measurement protocol was created to define the scanning parameters, including a source voltage of 70 kV and intensity 114 μA. The scans were performed using high resolution settings. Each scan yielded an image data set of 400 slices. The resulting two-dimensional images had an element size of 18 μm in all three spatial dimensions. A 3D Gaussian filter with $\sigma = 0.8$ and a support of one voxel was used to partially suppress noise.

2.5. Histology

Samples were dehydrated in an ascending series of alcohol (50–100%) and defatted with xylene. After infiltration of the samples with liquid LR-white in an evacuated container at 4 °C for 14 days they were polymerized with LR-white into ready to cut sample blocks. A circular saw (Leitz 1600 Saw microtome, Leica) was used to cut 200 μm thick cross-sections in the middle of the harvested core.

Macroradiographs (Faxitron X-Ray System, Hewlett Packard) were taken from each cross-section before further preparation of the samples. The sections were then ground and polished down to 80–100 μm thick sections (Exact Micro Grinding System, Exakt Apparatebau). Their surface was etched with 1% formic acid (Fluka) for 30 s, rinsed, stained with 1% Toluidine blue (Fluka) for 20 min and blot dried after washing in deionized water. Further, as a sec-

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