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Biocompatibility and resorption of a brushite calcium phosphate cement

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Abstract

A hydraulic calcium phosphate cement with β-tricalcium phosphate (TCP) granules embedded in a matrix of dicalcium phosphate dihydrate (DCPD) was implanted in experimentally created defects in sheep. One type of defect consisted of a drill hole in the medial femoral condyle. The other, partial metaphyseal defect was located in the proximal aspect of the tibia plateau and was stabilized using a 3.5 mm T-plate. The bone samples of 2 animals each per group were harvested after 2, 4, 6 and 8 weeks. Samples were evaluated for cement resorption and signs of immediate reaction, such as inflammation, caused by the cement setting in situ. Differences regarding these aspects were assessed for both types of defects using macroscopical, radiological, histological and histomorphometrical evaluations. In both defects the brushite matrix was resorbed faster than the β-TCP granules. The resorption front was followed directly by a front of new bone formation, in which residual β-TCP granules were embedded. Cement resorption occurred through (i) extracellular liquid dissolution with cement disintegration and particle formation, and (ii) phagocytosis of the cement particles through macrophages. Signs of inflammation or immunologic response leading to delayed new bone formation were not noticed at any time. Cement degradation and new bone formation occurred slightly faster in the femur defects.

Keywords: Biocompatibility; Resorption; Calcium phosphate; Cement; In vivo; Brushite

1. Introduction

The use of bone substitution is indicated in trauma and orthopaedic surgery [1], where autologous bone graft is still the "gold standard". Although high in biological valence [1–4], disadvantages as donor site morbidity and limited availability [4–7] restrict its use and have led to the search of appropriate alternatives at an early stage.

Calcium-phosphate compounds (CaP) as bone substitute materials already have a firm place in clinical applications [3], with tricalcium phosphate (TCP) and hydroxyapatite (HA) accounted as those most frequently investigated. Both compounds are considered biocompatible, bioactive in the sense of osteoconduction and bioresorbable, though it should be noted that HA degrades considerably slower [8]. Mostly, they are used in the form of blocks or granules. The incorporation of blocks may be limited due to defect geometry leading to incomplete bone–implant contact and thus, to insufficient osteointegration [9]. Granules risk migrating from the original defect and thus, exhibit lower mechanical properties [6,10–13].

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LeGeros [14] as well as Brown and Chow [15,16] were the first to present liquid, injectable calcium phosphate cements (CPC), a new family of bone substitute materials. The common property of this class of biomaterials, which is also responsible for the term "hydraulic" cement, is that after mixing of one or several calcium phosphate powders and an aqueous phase they turn into a pasty, mouldable compound that sets to a firm mass at room or body temperature [17–19].

Two types of cements are distinguished depending on the end product of the setting reaction: HA (HA-CPC) and brushite (dicalcium phosphate dihydrate) cements (DCPD-CPC), of which the latter raised great interest in recent times [20–23]. DCPD-CPC are resorbed faster compared to HA-cements [8] due to the fact that brushite is a metastable compound when used under physiological conditions [24]. The transformation of brushite into apatite after implantation, resulting in an increase of the resorption time [18,25], should be prevented by adding a magnesium salt of low solubility [26].

As in calcium phosphate ceramics [6,27], the same ways of decomposition are presumed for CPCs [12], although the cell type involved in the breakdown varies according to the type of cement. Fast resorbed CPCs are decomposed by macrophages and giant cells, slowly resorbed CPC (months–years) by osteoclast-type cells [10]. Type and extent of cement degradation depends on numerous factors, such as chemical and physicochemical material properties (composition, crystallinity, porosity, density, form, size), the animal model (species-specific characteristics) as well as on the implantation site [18,28–30].

The DCPD-CPC (chronOS^{TM} Inject, RMS Foundation, Bettlach, Switzerland and Synthes Biomaterials, Switzerland) investigated here has already shown to exhibit a significantly higher rate of cement resorption and new bone formation without any signs of inflammatory or immunologic response at 2, 4 and 6 months compared to a commercially available HA-CPC [31].

The early phase reactions of the organism and surrounding tissue caused by an implanted material, particularly during setting of the cement, is important in testing the biocompatibility [32]. Thereafter, the decomposition of a resorbable substance starts immediately after its implantation. Dissolution of a chemical compound as well as the cell-mediated resorption may reach a particularly high level in this early phase.

The objective of this study was to describe the surrounding bone tissue's cellular reactions after implantation, the mechanism of cement decomposition, taking two different defect locations and defect characteristics into consideration. The study was based on the hypothesis that the DCPD-CPC degrades by a combination of dissolution as well as by cellular decomposition and then is substituted by new bone, while HA-CPC degrades more slowly and mainly through cellular resorption mechanisms in the sense of creeping substitution [33,34]. It was also assumed that the DCPD-CPC used here showed a good biocompatibility and caused no inflammatory response also in the early phase.

2. Material and methods

2.1. DCPD-CPC

The cement powder and the 0.5% sodium hyaluronate solution [31] were mixed using a metal bowl and a spatula for 1 min before being filled into a 10 ml syringe. After 3 min (from the beginning of the mixing procedure), the cement was injected into the prepared defects. The cement set via the reaction: β-$Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \bullet H_2O + 7H_2O \rightarrow 4CaHPO_4 \bullet 2H_2O$. After setting, the cement was biphasic, i.e. consisted of large granules of β-TCP (30 wt%; 0.3 mm in diameter) embedded in a matrix containing fine DCPD crystals (55 wt%) and remnants of β-TCP powder (15 wt%, disregarding the water, pyrophosphate and magnesium-salt content).

2.2. Animal model

Eight adult, female, Swiss alpine sheep (4 years old) with a mean body weight of 68 kg (56–76 kg) were used for this study. Observation periods were 2, 4, 6 and 8 weeks with 2 animals each. The animal experiments were conducted according to the Swiss regulations of Animal Welfare and permission granted by the Ethical committee (application # 176/2000). Animals were accommodated to the new environment and clinically examined approximately 3 weeks before surgery. Food was withdrawn 24 h before induction of anaesthesia, while drinking was permitted ad libitum.

Medetomidine (5 μg/kg i.m., Domitor^{TM}, Orion Animal Health, Finland) served as premedication before induction of anaesthesia with diazepam (0.1 mg/kg, Valium^{TM}, Roche Pharama, Switzerland) and ketamin (2 mg/kg, Narketan^{TM} 10, Chassot GmbH, Germany). Anaesthesia was maintained by isoflurane–oxygen inhalation (FORENE^{TM}, Abbot AG, Switzerland). As peri- and postoperative infection, pain and inflammation prophylaxis, penicillin (Hoechst AG, Germany), gentamycin (Streuli & Co AG, Switzerland) and carprofen (Rimadyl^{TM}, Pfizer Inc., NY, USA) were administered at 12 h intervals for 3 days. Animals were placed in lateral recumbency and were operated alternately on the left or right posterior limb.

The medial femoral condyle and the medial side of the tibial metaphysis were exposed through a skin incision of approximately 13 cm in length over the medial femoral condyle and beyond the proximal section of