In-Vitro Release and Bioactivity of BMP-2 Released from TCP/HA Granules Coated with Collagen and Heparin

Hannink, G; Geutjes, P J; Van Kuppevelt, T H; Buma, P
Orthopaedic Research Lab, Department of Orthopaedics, Radboud University Nijmegen Medical Centre, Netherlands

Introduction
Many systems have been designed for the delivery of bone morphogenetic proteins (BMPs). Although these delivery systems enhanced bone repair, there are still problems in controlling release rate, often resulting in a high initial burst release. Therefore, it would be ideal to develop a system for the sustained delivery of BMPs over an extended time period. The attachment of heparin to biomaterials may result in an appropriate matrix for the binding, and controlled sustained release of BMPs. Binding to heparin stabilizes these growth factors and protects them from proteolytic degradation. The half-life of BMPs in culture media is prolonged 20-fold [1]. In order to create a carrier based delivery system with a localized sustained release, heparin was covalently attached to type I collagen-coated tricalciumphosphate/hydroxyapatite (TCP/HA) bone substitute and subsequently loaded with BMP-2. Although the osteogenic capacity of BMP-2 has been tested extensively, little is known about the effect of prolonged growth factor retention on the stability (bioactivity) of BMPs. Therefore, we investigated the release kinetics and bioactivity of BMP-2 from TCP/HA granules coated with collagen and heparin.

Methods
Three carriers were compared, TCP/HA granules (BoneSave, Stryker Orthopaedics, Limerick, Ireland), TCP/HA granules coated with crosslinked collagen (TCP/HA-Col) and TCP/HA coated with crosslinked collagen and heparin (TCP/HA-Col-Hep). rhBMP-2 (R&D Systems Europe, UK) was loaded onto the granules by soaking in 100 µl 1.0 µg/ml BMP-2 in PBS (pH 7.4) for 2 hours, followed by washing 3 times in PBS. Preparation and characterization of the granules has been described in detail previously [2].

The release kinetics of the BMP-2 from the TCP/HA, TCP/HA-Col and TCP/HA-Col-Hep granules were determined by immersing the granules (50 mg) in 100µl PBS (pH 7.4) with 0.02% (w/v) sodium azide. The release from the granules was determined by immersing the granules in microcentrifuge tubes containing 1.5 ml PBS and 0.05% (w/v) sodium azide. At various time points, the supernatant was withdrawn and the tubes were replenished with buffer. The amounts of BMP-2 in the supernatant were determined using SDS-PAGE, staining with 0.2% AgNO3, 0.2% NH4NO3, 5% Na2CO3 and 1% tungsten acid [3].

In vitro biological activity of the released BMP-2 was determined in a human mesenchymal stem cell (hMSC Poietics, Cambrex BioScience, Belgium) culture model over a period of 35 days (n=4). hMSCs are known to respond to BMP-2 with an increase in alkaline phosphatase (ALP) activity. Before the start of the experiment, the cells were expanded, pooled to create a homogeneous mixture, and cryopreserved in multiple aliquots. For each time point, an aliquot was thawed, expanded and plated at 30,000 cell/cm² in a 24 wells plate. One day post plating, the medium was changed and transwells containing the implants were inserted into each well. The culture media were changed at 3.5 days. At days 3.5, 7, 14, 21, 28, and 35, the transwells were transferred to new cell culture plates and the previous plates were assayed for ALP. The ALP activities were normalized to protein contents of the cell lysate as measured by DNA (picogreen assay). Empty transwells were used as negative controls and known BMP-2 concentrations in the culture media (0, 12.5, 25, 50, 75, 100 ng/ml) were used as positive controls. At each time point ALP activity was normalized to the negative control.

Results
TCP/HA granules showed a burst release of BMP-2 within the first 4 h. After an initial burst, the TCP/HA-Col granules showed a more gradual release of BMP-2. In contrast, BMP-2 release from the TCP/HA-Col-Hep granules was sustained up to 21 days (Fig. 1). From the cell culture model, data is available up to day 14. The BMP-2 released from the TCP/HA-Col-Hep showed to be bioactive up to day 14 (Fig. 2). At the first two time points, however no increase in bioactivity over basal level was found. At these time points little BMP-2 was released from the granules (Fig. 1). In contrast, BMP-2 released from the TCP/HA and TCP/HA-Col appeared to be bioactive only at the first two time points. At day 14, no difference over the negative control could be observed. Results from both SDS-PAGE (release) and cell culture seem to be consistent.

Discussion
This new system has several advantages for BMP-2 delivery. The TCP/HA-Col-Hep granules exhibited a sustained release of BMP-2, while BMP-2 was released more rapidly from the TCP/HA and TCP/HA-Col granules. In the cell culture model, the released BMP-2 appeared to be bioactive. TCP/HA and TCP/HA-Col showed BMP-2 bioactivity only for a short period of time. This is probably due to the rapid release from the granules. After 7 days no more BMP is being released from these granules. Preliminary results indicate that the BMP-2 present on the TCP/HA-Col-Hep will retain its bioactivity for a longer period. However, no conclusions can be drawn until data from the additional time points has been collected. This delivery system could probably also be applied to deliver dual or multiple growth factors that have affinities for heparin such as VEGF, BMPs and PDGF, which could synergistically enhance angiogenesis and osteogenesis. This new local sustained delivery system may provide a powerful modality for bone regeneration.

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References
2. Hannink, G et al. Proc ORS 2009; 34, 1347