Repair of Rat Calvaria Defect with Injectable Strontium (Sr^{2+})-Doped Polymeric Brushite Ceramics

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Statement of Purpose: Calcium phosphate cements (CPCs) are mechanically weak and have poor anti-washout capability. We developed a polymeric brushite ceramic (P-DCPD) that is mechanically strong and capable of sustained drug release. Our previous in vitro study showed a sustained release of Sr^{2+} from P-DCPD and the released Sr^{2+} significantly enhanced the growth and differentiation of osteoblastic cells. The aim of this study is to examine the bone healing effects of Sr^{2+}-doped P-DCPD using a rat calvaria defect model.

Methods: P-DCPD without or with 10% Sr^{2+} (Sr-PDCPD) were prepared. Thirty adult male Fischer 344 rats were divided into three groups: (1) Control (without P-DCPD); (2) with P-DCPD implantation, and (3) with Sr-P-DCPD implantation (n=10 each). Cements were injected into 8-mm calvarial critical-sized defects. Calvariae were harvested at 12 weeks post-surgery. Bone repair efficacy was evaluated by micro computed tomography (μCT) and bone histometric analysis. In addition, the new bone formation at the bone-cement interface was evaluated using sequential tetracycline/calcein labeling two weeks and three days before sacrifice, respectively.

Results: Handling: Both P-DCPD and Sr-P-DCPD were injectable, moldable to the defect, and stable in vivo for up to 12 weeks because of their excellent cohesion behavior.
Histomorphometry of hard tissue section: P-DCPD significantly enhanced new bone formation. New bone formation covering the entire bone defect area was found in both ceramic groups. There was a close interaction between degrading ceramic fragments (particles) with surrounding newly formed bones (Fig.1).
In vivo fluorescence labeling: As compared to control (<1%), much stronger fluorescent signals were observed in P-DCPD group (85%± 2.5) and in Sr-P-DCPD group (60%± 2.5). The fluorescent signals were distributed within the entire defect area evenly and surrounded by degrading ceramic residues (Fig. 2).
H&E stained paraffin sections: No host inflammatory response to implanted ceramics was found. There were more ceramic fragments left in the Sr-P-DCPD group than that of P-DCPD group.
μCT analysis: The implanted ceramics markedly increased the density that hindered the accurate evaluation. The doping of Sr^{2+} significantly increased the density (reduction of the porosity) of P-DCPD. The new bone formation (BV/TV) at different thresholds (220-420) indicated that it was difficult to define an appropriate threshold setting that can be used to quantify the dynamic interaction between new bone formation and ceramic degradation because of the much higher density of implanted cements.

Conclusion: P-DCPD ceramic was biocompatible, degradable and enhanced new bone formation. However, the Sr^{2+} doping did not provide additional advantages of bone healing efficacy as proposed. The performance of Sr-P-DCPD in vivo disagrees with what we observed in vitro. The possible reasons were, at least in part, due to the unoptimized loading dose and slower degradation, increased material density and the reduction of material porosity. Therefore, Sr-P-DCPD does not represent an adequate substrate for new bone ingrowth instead of proven osteogenic activity of embedded Sr in pre-osteoblastic cells proven in vitro. In conclusion, injectable P-DCPD represents a new bone graft substitute that can be used for the treatment of bone defects. Further investigation should be conducted using the P-DCPD ceramic doped with different Sr^{2+} concentrations in large animal long bone defect model.

References:

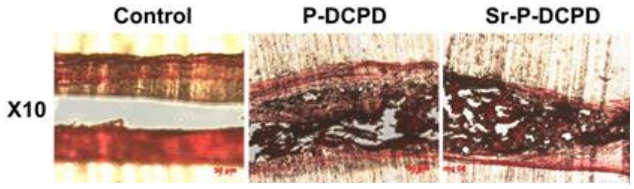

Fig. 1. Hard tissue section analysis of Masson-Goldner stained sections shows new bone formation

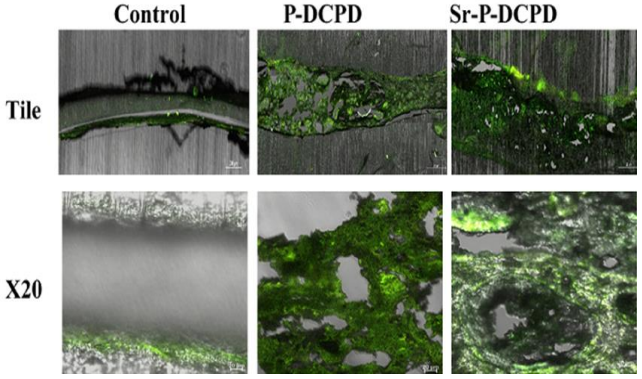

Fig. 2. Confocal images of hard tissue sections with fluorescent signals of tetracycline/calcein double staining. (A) Tile reconstruction of the entire defect area, (B) Magnification X 200 showing the local details

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