Calcium Phosphate Cements Loaded With Proton Pump Inhibitors as Novel Bone Substitute Biomaterials

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Statement of Purpose: Calcium phosphate cements (CPCs) are known bone substitute candidates and may also serve as drug delivery systems for active agents (1).
The *in vivo* degradation of the CPCs is mainly cell-mediated (2), where osteoclasts create and acidic environment by secreting protons through vacuolar proton pumps (3). Proton pump inhibitor (PPI) drugs are agents that bind to these pumps inhibiting the development of the acidic resorption environment (4).
We wished to address the following questions: Can we control the in vivo degradation of CPCs by loading them with PPIs? And: Is the PPI-loaded CPC degradation delayed when compared to undoped cements?

Methods: The solid phase of the CPCs consisted of an equimolar mixture of Dicalcium Phosphate (DCPA) and Tetracalcium Phosphate (TTCP). Either Bafilomycin (BAF), Omeprazole (OMP) or Pantoprazole (PANT) were incorporated into the cement matrix. The liquid component consisted of a 0.2M phosphate solution. Dimethylsulphoxide (DMSO) solutions of BAF and OMP as well as a phosphate solution of PANT were prepared. For each 25mg of powder, 125µL of liquid was employed (P/L ratio 2:1). Solid and liquid components were mixed in order to obtain set cements, which were then crushed into particles (90-355µm). The CPCs were either left undoped (Controls) or were doped with BAF (1% w/w), OMP (5% w/w) or PANT (5% w/w). The final particulate material was then γ-sterilized. Control and Test materials were placed into bilateral 2.3mm diameter distal femoral defects in 200-250g male Wistar rats. Implants were left for either 1 or 3 weeks (n=3). Following the allotted time, animals were euthanized by cervical dislocation after exposure to CO_2. Femora were harvested and fixed in 10% buffered formaldehyde. Samples were then trimmed and scanned for MicroCT quantitative analysis of the volume of particles and bone formation. For the MicroCT quantitative analysis, the volumes of the remnant particles and bone activity were measured and compared to the volume of an averaged defect volume ($12mm^3$). The software Microview GE (London, ON, Canada) was used to calculate the above-mentioned volumes. Statistical analysis was performed using the software R (The R Foundation for Statistics Computing Version 2.3.0). Differences among groups were compared using ANOVA, followed by post hoc Tukey HSD. P-values lower than 0.05 were considered significant.

Results/Discussion: The MicroCT analyses showed bone formation in all samples. Moreover, directly bone-particulate contact was observed at 1 and 3 weeks. At 3 weeks postoperative, the volume of particles loaded with PANT had a statistically significant higher volume when compared to particles doped with BAF.

Small amounts of BAF and OMP particles were found after the first week. These diminished volumes are possibly due to the particulate leaching from the bone defect.
Bone formation was greater than the volume of the defects for all samples. After 3 weeks of implantation, the volume of reparative bone formation of the BAF-CPCs was statistically higher than those of the control and OMP-CPCs samples. On the other hand, the volume of bone formation of the PANT-CPCs was statistically higher than that of BAF-CPCs.
At 3 weeks, all PPI groups presented, proportionally, a delayed degradation rate when compared to the Control. From the first to the third week, the respective particle and bone resorption rates for the tested samples were as follows: Control (82.2% and 65.7%); BAF-loaded CPC (30.8% and 53.0%); PANT-loaded CPC (29.0% and 28.3%); OMP-loaded CPC (17.7% and 61.6 %).

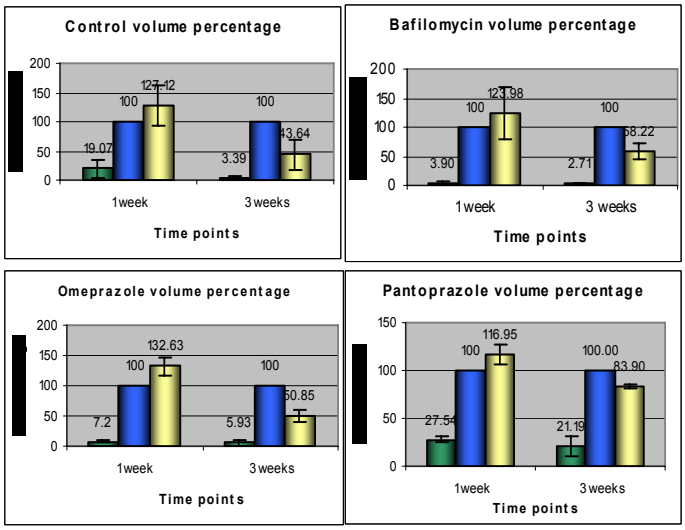

Figure 1.Particle and Bone volumetric percentage measurement for Control, BAF-CPC, OMP-CPC, and PANT-CPC. Green column- Particle volume ; Blue column - Defect volume ; Yellow column- Bone volume.

Conclusions: PPI-doped CPC particles were resorbed at a delayed rate when compared to the undoped controls. All samples presented a greater bone activity volume than that of the defect volume in the first week. However, a greater amount of bone was found surrounding the PPI samples at 3 weeks.

References:

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