Zinc in Composite Scaffolds Promotes Cell Growth and Mineralized Matrix Production

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Statement of Purpose: Approximately 5-10% of all bone fractures result in non-union [1]. Current surgical strategies involving stabilizing the fracture with rods and screws or stimulating fracture healing with bone grafts or graft substitutes have limited success due to the lack of biological activity and integration. As an alternative, bone tissue engineering strategies have been investigated to promote bone regeneration by mimicking the extracellular matrix. Zinc is a known insulin mimetic and has been shown in soluble form to influence osteogenesis as well as bone formation in animal models and stimulation of collagen production in osteoblast cells [2,3]. However, treatment strategies utilizing zinc in tissue engineering scaffolds have been limited. We hypothesize that zinc containing composite scaffolds will promote osteogenesis. In this study, zinc is incorporated into polycaprolactone (PCL) fibers containing ceramic particles of hydroxyapatite (HA) and beta-tricalcium phosphate (TCP). Zinc release and mesenchymal stem cells (MSC) growth and osteogenesis are evaluated.

Methods: Scaffolds were fabricated by electrospinning PCL with 30wt% ceramic particles consisting of 20:80 HA:TCP and zinc at either 0, 0.1, 0.025, 0.05, 0.075, or 0.1 wt%. To evaluate the effect of the ceramic, scaffolds containing no ceramic particles was also examined for zinc release. Scaffolds were immersed in PBS (pH 7.4) and incubated at 37°C for up to 28 days. Zinc concentrations were determined with inductively coupled plasma mass spectrometry (ICP-MS). Human MSCs at passage 4 were seeded at $3x10^4$ cells per 7 mm diameter scaffold and cultured for up to 28 days. The scaffolds were cultured in either growth media (GM; DMEM, 10% fetal bovine serum, and 1% antibiotic-antimycotic), osteoinductive media (OM; growth media supplemented with 100 nM dexamethasone, 0.05 mM ascorbic acid, and 10 mM β-glycerophosphate) or chondrogenic media (CCM; high glucose DMEM, 4mM Glutamax, 10 nM dexamethasone, 170nM ascorbic acid, 35uM proline, 1mM sodium pyruvate, 1% antibiotic-antimycotic, and 0.1% ITS+Premix). Scaffolds cultured in CCM were switched to OM on day 14 until day 28 (CCM/OM) to mimic osteochondrogenic differentiation. Cell growth was measured by PicoGreen ® dsDNA assay (Invitrogen). Collagen production was determined by the hydroxyproline kit (Sigma-Aldrich). Matrix mineralization was quantified using the QuantiChrom Calcium Assay kit (Bio-Assay Systems). Osteocalcin was determined by ELISA kit (Invitrogen). Cell morphology and collagen type I production were assessed by immunostaining. Data analysis was performed using factorial ANOVA or one way ANOVA and post hoc Tukey HSD at $p<0.05$ significance level (SPSS-24).

Results: The PCL and PCL with ceramic have different release profiles. Approximately 25% of the total zinc was released from the PCL scaffold containing 0.1wt% zinc while 1.5% of total zinc was released from the PCL containing ceramic scaffold (figure 1 a). Cell growth was supported by the scaffolds in all three media types where 0.05 wt% zinc containing scaffolds was significantly higher than control (0 wt.% zinc) in GM (figure 1b). In OM, all zinc containing groups had significantly higher cell numbers than the control by day 28 (figure 1c). Collagen production increased for zinc containing groups in all media conditions, where 0.01 and 0.1 wt% zinc groups were higher than the control in GM and 0.05 and 0.075% zinc groups were higher than the control in OM (figure 2a and 2d). Mineralization for the 0.05 wt% zinc scaffold group was statistically higher than the control in OM (figures 2e). Although mineralization for the other groups increased, they were not statistically different from the control (figure 2b). Osteocalcin production was significantly higher for 0.025 and 0.05 wt% zinc containing groups in GM as compared to the control (figure 2c). Though osteocalcin production was higher in OM, they were not statistically different from the control (figure 2f).

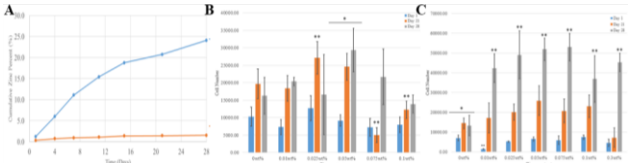

Figure 1: A. Zinc release from scaffolds containing ceramic (orange) or without ceramic (blue) over 28 days. Cell numbers over time in (B) GM and (C) OM. *$p<0.05$ as compared with other scaffold. ** $p<0.05$ as compared to control for time point

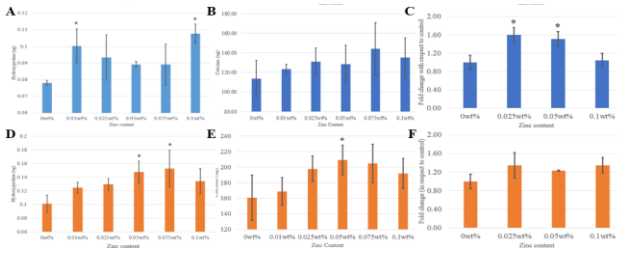

Figure 2: Collagen production (hydroxyproline), mineralization (calcium), and osteocalcin for GM (a-c) and OM (D-F), respectively. *$p<0.05$ compared to control

Conclusions: This study demonstrated the influence of zinc in composite scaffolds in promoting cell growth and osteogenesis. The ceramic in the scaffolds limited the release of zinc potentially due to the displacement of calcium with zinc in the ceramics; however, zinc still influenced cell behavior. The zinc containing scaffolds improved cell growth, collagen production and matrix mineralization. Future studies will investigate the effect of the zinc containing scaffold on MSC osteogenesis examining gene expression and zinc transporters.

References: [1] (Leng Y et al., Mater Des. 2019;183,108151). [2] (Wey A et al., J Orthop Res., 2014;6:834-41). [3] (Wang T et al., J Trace Elem Med Bio. 2007;21:84-91).