In vitro Modulation of Macrophage Behavior by Ceramic-based Scaffolds

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Statement of Purpose: The inflammatory response plays a central role in bone repair and regeneration. Macrophages, the primary cells of the immune system, are major contributors to this process. Though originally believed to serve as only destructive, proinflammatory phagocytes, it is now recognized that macrophages display high plasticity and exist on a spectrum of phenotypes. We have shown that M1 (classically activated) macrophages are required at early stages of healing to initiate angiogenesis, while M2 (alternatively activated) macrophages are needed at later stages to facilitate blood vessel anastomosis and tissue maturation [1]. Recently, Roohani and Zreiqat engineered novel ceramic-based scaffolds Zirconium ($Ca_3ZrSi_2O_9$) referred to as Baghdadite and Strontium, Zinc ($Sr\text{-}Ca_2ZnSi_2O_7$) and Gahnite ($ZnAl_2O_4$) referred to as Sr-HT Gahnite, and demonstrated *in vivo* their unique ability to regenerate large bone defects under load [2-3]. We hypothesize that a potential mechanism behind their success is that they promote the proper M1-to-M2 sequence of macrophage activation needed for healing, and that this behavior is related to their unique microstructure. The goal of this research is to elucidate the interactions between these scaffolds and cells of the inflammatory response to aid in designing biomaterials that can facilitate tissue repair and regeneration. In this work, the time-dependent effects of macrophages exposed to Baghdadite and Sr-HT Gahnite are investigated and compared to a tricalcium phosphate and hydroxyapatite clinical control (TCP/HA), manufactured to the same specifications.

Methods: Primary human monocytes were isolated from blood and differentiated into macrophages as described previously [1]. Unactivated macrophages were seeded directly onto steam-sterilized, pre-equilibrated scaffolds at a density of 1×10^6 cells/cm^3 and allowed to attach at 37°C and 5% CO_2. After 1 h, additional culture media was added and the samples were incubated for 6 days, with a media change on day 3. Unactivated macrophages (M0) exposed to culture media alone served as a control. At 36 h and 6 days after cell seeding, the scaffolds were transferred into TRIzol for RNA extraction. Gene expression for 10 markers of macrophage phenotype was conducted as described previously[1]. Data shown (Fig. 1A-E) represent the mean fold change over M0 ± SEM (n=4). These data were subsequently converted into a combinatorial score (Fig. 1F) indicative of the M1-M2 character of the macrophages resulting from scaffold interactions. This score is calculated as the ratio of expression of multiple M1 markers over M2 markers [4], so that higher scores represent increased proinflammatory (M1-like) behavior with respect to M2 behavior. Statistical analysis was performed in GraphPad Prism 6.0 using a two-way ANOVA. Dunnett's or Sidak's multiple comparisons tests were performed with TCP/HA scaffolds as the control; significance is indicated for $p<0.05$.

Results: Gene expression analysis (Fig. 1 A-E) revealed upregulation by Baghdadite scaffolds of M1 (*TNFa, CCR7*) and M2a (*TIMP3*) markers on day 1.5, and M2c marker *CD163* on day 6. Sr-HT Ghanite scaffolds induced upregulation of M2a markers (*MDC, TIMP3*) on day 1.5, and suppression of M1 marker *IL-1β* by day 6, relative to the TCP/HA control (Fig. 1a-e). These findings suggest that Baghdadite and Sr-HT Ghanite scaffolds induce a mixed M1/M2 phenotype. Intriguingly, Baghdadite scaffolds promote an M1-to-M2 transition that is consistent with the natural sequence of macrophage activation during normal healing, in support of our hypothesis. Combinatorial M1/M2 scoring (Fig. 1F) revealed a temporal increase in the proinflammatory behavior of macrophages exposed to TCP/HA, which was inhibited by Baghdadite and Sr-HT Ghanite scaffolds.

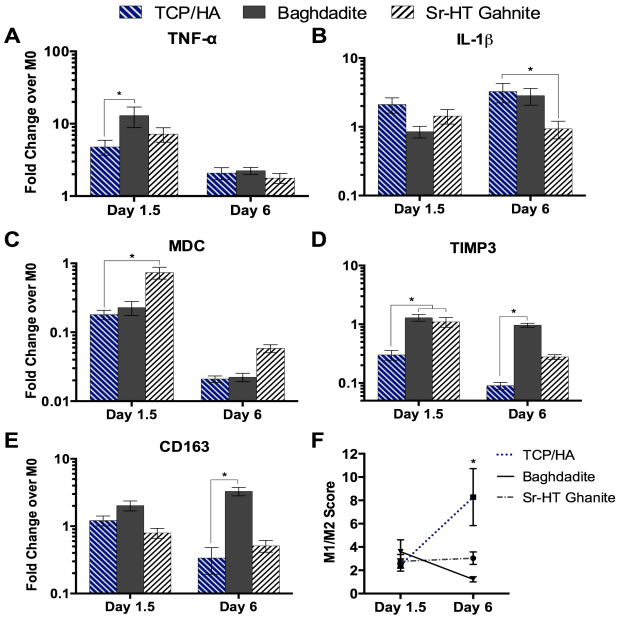

Figure 1. Representative gene expression data for M1 (A-B), M2a (C-D) and M2c (E) markers. (F) Combinatorial M1/M2 score resulting from macrophage-scaffold interactions; *p<0.05.

Conclusions: Overall, these results suggest that Baghdadite and Sr-HT Ghanite scaffolds have the potential to modulate macrophage behavior and maximize bone repair by recapitulating the proper M1-to-M2 transition observed in normal healing. Future work is needed to confirm these findings at the level of protein secretion, and to identify the difference among these scaffolds that leads to changes in macrophage behavior. Understanding these contributions will enable us to design improved biomaterials that can facilitate tissue repair and regeneration.

References: [1] Spiller KL *et al*. Biomaterials. 2014; 35:4477-88. [2] Roohani-Esfahani SI *et al*. Acta Biomaterialia. 2012; 8:4162-72. [3] Roohani-Esfahani SI *et al*. Acta Biomaterialia. 2013; 9:7014-24. [4] Nassiri S *et al.* manuscript in review. **Acknowledgements:** The authors gratefully acknowledge the Australian NHMRC and ARC, and NSF-CNIC support of this work (award 1425737).