Mineralized Collagen Scaffold Pore Structure Enhances Immunomodulatory Potential of Mesenchymal Stem Cells

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Statement of Purpose: Craniomaxillofacial (CMF) defects are critical-sized missing bone from the skull, jaw, or neck. Due to their large-scale they require surgical intervention. Our laboratory has developed a class of mineralized collagen scaffolds designed to promote mesenchymal stem cell (MSC) osteogenic differentiation and subsequent CMF bone regeneration in the absence of exogenous growth factor supplements [1]. MSCs possess immunomodulatory functions that could serve to enhance macrophage recruitment and anti-inflammatory phenotypes [2]. Macrophages (MΦ) have prominent plasticity and can be polarized into inflammatory M1 or anti-inflammatory M2. In recent MSC-MΦ co-culture studies, MSCs led to the generation of M2 MΦ and the upregulation of interleukin 10 (IL-10) secretion. This effect resulted from soluble factor dependent signaling including the release of prostaglandin E2 (PGE_2) [3]. Finally, pro-inflammatory licensed MSCs displayed enhanced immunosuppressive functions. In this study we describe the capacity of MSCs to display an immunosuppressive behavior on mineralized collagen scaffolds with and without inflammatory stimulation and their influence on MΦ polarization.

Methods: Mineralized collagen scaffolds were fabricated by homogenizing and subsequently lyophilizing a slurry of type I bovine collagen, calcium hydroxide, phosphoric acid, and glycosaminoglycans (GAGs) into porous scaffolds. MSCs were cultured in basal media or primed with inflammatory cytokines 24 hours prior to seeding on scaffolds that had isotropic (Iso) or anisotropic (Ani) pores, or heparin (Hep) containing scaffolds for 21 days. MSC osteogenic and immunomodulatory responses were observed via protein secretion, gene expression, and metabolic activity. MSCs were directly and indirectly cultured with MΦ on anisotropic scaffolds, and MΦ polarization was observed via protein secretion, and gene expression over 14 days of culture. Statistical testing was performed using ANOVA with error reported as mean ± standard deviation.

Results: MSC osteoprogenitors exhibited increased metabolic activity in all groups with time. Primed MSCs on Ani scaffolds were significantly more metabolically active than those on Iso scaffolds, with significant differences observed by day 21. This shows that Ani scaffolds provide a favorable environment for MSCs to grow and differentiate regardless of treatment. Basal and primed MSCs produced similar quantities of osteoprotegerin (OPG) in early stages while basal MSCs on Hep scaffolds produced significantly more OPG than all other groups by day 21. OPG is known to inhibit osteoclastogenesis and promote osteogenesis. Primed MSCs in all scaffold groups secreted more IL-6 compared to basal MSCs with Ani scaffolds having the greatest production. Increased IL-6 production has been shown to upregulate IL-10 production from MΦs indicative of a M2 phenotype. Similar trends were observed with PGE_2 secretion as all primed groups displaying increased PGE_2 secretion compared to basal groups with the Ani scaffolds secreting the greatest amount. PGE_2 is known to influence the transition of M1 MΦ toward an M2 phenotype allowing for the resolution of the inflammatory response. Primed MSCs displayed a higher capacity to polarize MΦ toward an M2 phenotype agreeing with previous literature.

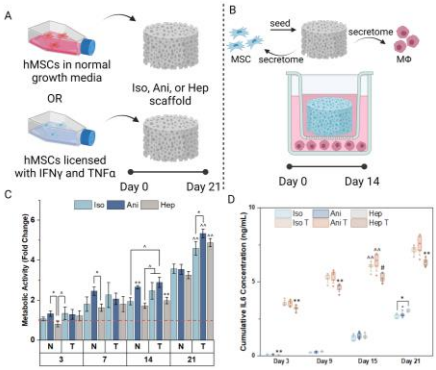

Figure 1: (A) Schematic of MSC and (B) of co-culture experimental set-up. (C) Metabolic activity profile. (D) IL-6 ELISA release profile. *,^, significance between indicated groups, and **, ^^ significance between all groups of same or other treatment respectively at ($p<0.05$).

Conclusions: In this study we looked at the influence of pore structure and GAG content on stem cell endogenous production of immunomodulatory factors when cultured in basal or primed media. We further observed the influence of basal or primed MSCs on MΦ polarization. Basal MSCs display increased OPG production on Hep scaffolds however express similar levels of immunomodulatory factors regardless of scaffold. Primed MSCs display heightened metabolic activity and production of immunomodulatory factors on Ani scaffolds suggesting the opportunity to alter scaffold pore structure to temporally modulate MΦ polarization in an inflammatory environment to improve osteogenic healing and remodeling. Future work will examine the crosstalk effects of MSCs, MΦ and endothelial cells to identify scaffold variants that induce an anti-inflammatory and pro-angiogenic phenotype.

References:

[1] D. W. Weisgerber et. al., Biomaterials science, 2015, [2] Wong S. et. al., Science Advances, 2020, [3] Németh K et. al., Nature medicine, 2009