Resveratrol Nanoparticle-incorporated Scaffolds to Modulate Inflammation and Promote Osteogenesis

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Statement of Purpose: Tissue engineering applies principles of biology to engineering fundamentals in order to generate functional tissue replacements for diseased or damaged organs (1). This is accomplished by utilizing a specific combination of cells, biomaterials, and signaling cues. Many tissue engineering approaches are successful in vitro, however fail when translated to in vivo applications due to the immune response from the host. The complicated interplay of cells and cytokines at the interface of an implanted biomaterial that governs success versus failure of a scaffold must be deconstructed in order to properly design an approach to harness inflammation and promote wound healing. Resveratrol is a polyphenol found in the skin of grapes and has demonstrated anti-inflammatory (2) and osteogenic properties (3). To this end, we propose a resveratrol nanoparticle-incorporated scaffold to i) direct macrophage phenotype from M1 pro-inflammatory to M2 wound healing and ii) drive the osteogenic differentiation of bone marrow mesenchymal stem cells (hMSCs) to facilitate graft assimilation and tissue regeneration.

Methods: Modulation of Inflammation: To analyze the ability of resveratrol to inhibit inflammatory cytokine production of macrophages, we placed M1 macrophages in basal THP-1 medium supplemented with either 0, 1, 10, or 25 μM resveratrol, and gene expression was analyzed by qRT-PCR after 6 and 24 hours. Osteogenic Potential: The effect of resveratrol on the osteogenic differentiation of hMSCs was examined by culturing undifferentiated hMSCs in hMSC basal medium (BM), basal medium supplemented with 12.5 μM resveratrol (BMr), osteogenic medium (OM), and osteogenic medium containing 12.5 μM resveratrol (OMr). Osteogenic differentiation was based on quantitative calcium and alkaline phosphatase (ALP), calcium and ALP staining, and qRT-PCR gene expression of Runx2 and Osteocalcin (OCN). Co-Culture: To assess the interplay between macrophage phenotype and hMSC differentiation towards osteogenic lineage, we cultured hMSCs with monocytes, M0 macrophages, and M1 macrophages in OM at a cell number ratio of 1:10. Osteogenic differentiation was determined by calcium and ALP quantification, and qRT-PCR gene expression of OCN. 3D Study: To combine the above two-dimensional (2D) experiments into a three-dimensional (3D) scaffold design, we created PLGA resveratrol nanoparticles by using a nanoprecipitation technique, and sintered them to PLGA microsphere scaffolds. M1 macrophages and hMSCs were seeded on to the scaffolds, cultured for 21 days, and analyzed for osteogenic markers (calcium, ALP, Runx2, and OCN).

Results: The 2D culture studies demonstrated that resveratrol has the ability to reduce inflammation as well as differentiate hMSCs towards bone tissue. Modulation of Inflammation: For all concentrations of resveratrol added to M1 macrophage culture medium, VEGF expression increased. The level of inflammatory cytokine TNF-α was reduced in all conditions, with the greatest net decrease in the 10 μM concentration. IL-6 expression was reduced in all groups, and resveratrol increased the initial expression of IL-10 as well as prolonged cytokine release after 24 hours in dosages of 10 and 25 μM. Osteogenic Potential: hMSCs in OMr demonstrated the highest calcium levels. Cells cultured in OM expressed the second highest levels of calcium on days 14 and 21, followed by BMr. Day 7 hMSCs cultured in BMr exhibited calcium levels only slightly lower than OMr, indicating the ability of resveratrol to stimulate the early onset of differentiation. ALP expression in hMSCs was the highest in OMr, followed by cells cultured in OM, BMr, and lastly BM. Furthermore, qRT-PCR gene expression of Runx2 and OCN indicated the greatest osteogenic differentiation of hMSCs cultured in OMr at days 14 and 21 (Figure 1). Interestingly, hMSCs in BMr demonstrated the highest OCN levels at day 7, which further exhibits the ability of resveratrol to stimulate the early onset of osteogenic differentiation without osteogenic growth factors. Co-Culture: The highest amount of calcium was detected in M1/hMSC group followed by M0/hMSC, monocytes/hMSCs, and hMSCs. ALP expression was the greatest for the M1/ hMSC co-culture, followed by the monocytes/hMSCs and M0/ hMSCs groups for days 14 and 21. Furthermore OCN levels were the highest in the M1/ hMSC culture group for days 7, 14, and 21. 3D Study: Resveratrol nanoparticle-incorporated scaffolds provided the highest levels of Calcium, ALP, and OCN for all time points as compared to the controls.

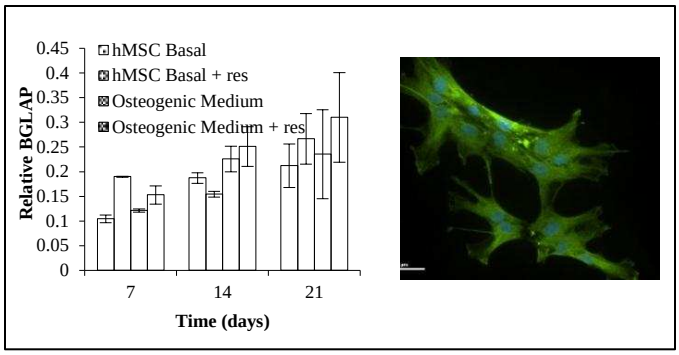

Figure 1. Osteocalcin Expression

Conclusions: Together, these studies demonstrate the potential of utilizing resveratrol to harness the inflammatory response towards enhancing osteogenic differentiation of hMSCs. We were able to control macrophage behavior and switch cells from a pro-inflammatory M1 phenotype to wound healing M2 macrophages by using resveratrol. Additionally, we demonstrated the osteogenic potential of resveratrol in differentiating hMSCs, and showed how macrophages co-cultured with hMSCs enhance stem cell differentiation towards bone tissue. Furthermore, PLGA-resveratrol nanoparticles can be designed to release specific concentrations of resveratrol within bone tissue grafts so that inflammation is controlled and host hMSCs will be recruited to differentiate into bone tissue. This research provides proof of concept for a method of translating in vitro bone tissue engineering successes into relevant clinical applications.

References:

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3. Shakibaei M. PloS one. 2012; 7: e35712.