Dose Dependent Effect of Surface-Associated Arg-Gly-Asp on the Osteogenic Differentiation of Mesenchymal Stem Cells under Flow Perfusion

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Statement of Purpose: Arg-Gly-Asp (RGD) peptides incorporated into biomaterials have been shown to up-regulate mesenchymal stem cell (MSC) osteogenic differentiation. However, these effects have been assessed under static culture conditions, while it has been reported that flow perfusion also has an enhancing effect on MSC osteogenesis. The combination of MSC chemical stimulation, via RGD biomaterial modification, with mechanical stimulation, represented by flow perfusion, could serve as a window for novel and more efficient tissue engineering technologies. In this study, we evaluate the effect of different extents of RGD surface modification of poly(L-lactic acid) (PLLA) foams on the osteogenic differentiation of MSCs under conditions of flow perfusion at different flow rates.

Methods: PLLA porous foams were made by a salt leaching technique. Leached scaffolds were soaked in an acetone-water mixture, followed by incubation in a solution of PolyK in DMSO for 12 h. RGDC peptides were linked to the PolyK by disulfide bonding via previous incorporation of N-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP). RGDC levels were varied by changing the PolyK incubation concentrations, as previously reported[1]. Scaffolds were placed in a flow perfusion bioreactor and seeded with rat MSCs using an oscillatory flow technique developed in our laboratory[2]. After seeding, the constructs were cultured statically (in well-plates) or in the bioreactor, using osteogenic medium (a-MEM, 10% FBS, 1% antibiotics, 10^{-8}M dexamethasone, 0.05 mM ascorbic acid, and 10mM β-glycerophosphate). Flow rates in the perfusion bioreactor were 0.1 and 1.0 ml/min. Samples were taken at days 4, 8, and 16, and analyzed for cell proliferation, alkaline phosphatase activity (ALP), calcium deposition. Histology was performed to determine extra-cellular matrix morphology and distribution.

Results: All scaffolds supported cell growth. Cell proliferation was enhanced by the presence of RGD at all culture conditions (data not shown). ALP activity presented a peak after eight days under most culture conditions. RGD-modified scaffolds presented higher ALP activity and calcium levels than the controls. Under static culture, the ALP activity was statistically equal for all modification levels, reaching values as high as (4.26±0.55) pmol/hr/cell. Higher levels of ALP were observed in the middle level of modification ($1x10^{-4}$ mg polyK/ml) at 0.1 ml/min, while cells cultured at the lowest modification level presented the highest ALP activity at 1.0 ml/min. Under static conditions, there was no difference on calcium deposition between the different modification levels, reaching values as high as (195±30mg). At a flow rate of 0.1 ml/min, scaffolds modified at polyK incubation concentration of $1x10^{-4}$ mg/ml yielded the highest calcium levels at all time points, with a maximum of (473±55mg). At 1.0 ml/min, the highest calcium deposition was encountered at the lowest RGDC level, with a maximum of (786±120mg). Histology showed a large amount of homogeneously distributed ECM under dynamic cultures. Statically cultured scaffolds showed high degrees of degradation and little deposited ECM, which was distributed on the outer edges of the scaffold.

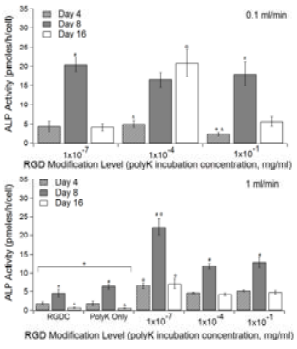

Figure 1. ALP Activity

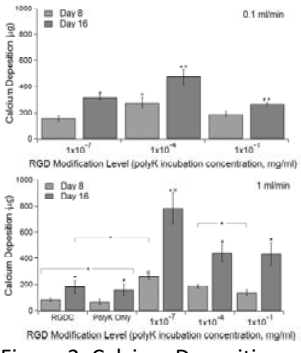

Figure 2. Calcium Deposition

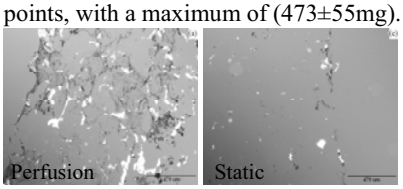

Figure 3. Histological Evaluation

Conclusions: RGDC surface modification combined with flow perfusion synergistically improved MSC osteogenesis with respect to their individual influences. There is a critical RGD surface concentration that yields greater degrees of osteogenesis and is different for every culture condition. It has been found that RGD may have an inhibitory effect on MSC osteogenic differentitation via $\alpha_v\beta_3$ receptor activation[3]. Additionally, flow perfusion enhances cell-matrix interactions. It is possible that at higher flow rates, cell-matrix interactions are further strengthened, increasing the inhibitory effect of the $\alpha_v\beta_3$ receptor activation. The osteoblastic differentiation would then be down-regulated when compared to the lower flow rates. To the best of our knowledge, this is the first detailed study on the combination of surface modification and mechanical stimulation to guide MSC osteogenesis. The synergism of combining chemical and mechanical stimulation of MSC may result in effective tissue engineering therapies.

References

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[3]Cheng SL. J Bone Miner Res. 2001;16(2):277-88