Bioactive Modification of End-functionalized Multi-arm Poly(ethylene glycol) Affects Macroscopic Properties and Cellular Behavior

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End-functionalized multi-arm poly(ethylene glycol) (PEG) hydrogel synthesized by Michael-type addition is useful for many tissue engineering applications due to the tunable material properties that can be decoupled from the biological activity. The chemistry of bioactive modification of PEG hydrogels with integrin binding sequences, like RGD, competes with the crosslinking chemistry of protease sensitive peptides. As a result, PEG hydrogels modified with increasing concentrations of RGD have inferior physical properties compared to unmodified hydrogels. Here, we investigated the effects of the macromers' functionality and the chemistry of RGD modification on PEG's macroscopic network properties, such as swelling and stiffness. Modification of the hydrogels with increasing concentrations of RGD caused a greater extent of swelling in the 4-arm PEG (Fig.1). 8-arm PEG supported efficient network crosslinking up to 3.75 mM RGD modification, (Fig.1B) when the maximal load in 4-arm PEG gels was 1.25 mM of RGD (Fig.1A). Overall, PEG gels with greater functionality demonstrated superior mechanical properties and allowed greater bioactive modification resulting in a larger spectrum of favorable combinations of mechanical and biological properties.

To further investigate the combined effect of mechano-biological properties of the PEG hydrogel on cellular growth and behavior, we encapsulated human bone marrow stromal cells in PEG gels modified with 0.25 and 1.25mM RGD concentrations (Fig. 2). The stiffness of hydrogels in all tested conditions were kept the same. Spheroid formation and restricted cell growth were observed in 4 and 8-arm PEG gels with low RGD concentration. In high RGD conditions, only 8-arm PEG supported cell growth, providing an optimal mechanical and biological environment. These results suggest that PEG with a greater functionality allows more precise control over material properties, which directly regulate cellular behavior and function.

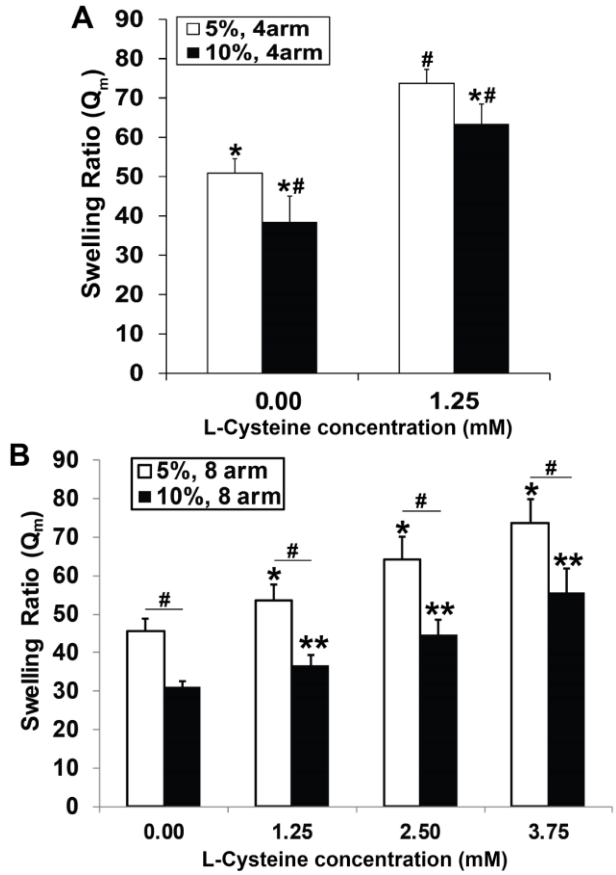

Figure 1 : The effect of the L-Cysteine concentration on swelling ratio (A) All data is reported as mean ± standard deviation (n = 30). *Significantly different from 5% 4-arm PEG with 1.25 mM L-Cysteine # Significantly different from 5% 4-arm PEG with 0 mM L-Cysteine (*,#: $p < 0.05$) **(B)** All data is reported as mean ± standard deviation (n = 30). *Significantly different from 10% 8arm PEG with lower L-Cysteine concentration **Significantly different from 5% 8arm PEG with lower L-Cysteine concentration (*,**,#: $p < 0.05$)

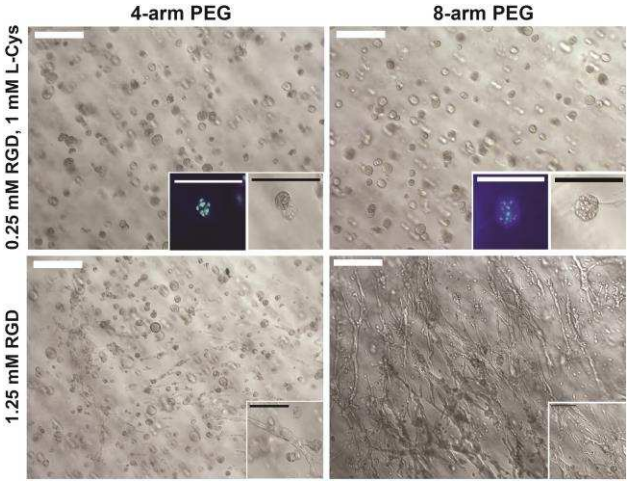

Figure 2 : The effect of the RGD concentration on human bone marrow stromal cells growth and the network formation at Day 12 Scale bar: White = 200 μm, Black/DAPI = 100 μm