Relative Influence of Collagen Concentration versus Substrate Modulus on MSC Fate Decisions

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Statement of Purpose

The ability to rationally design culture environments to guide stem cell differentiation for specific organ applications would represent a significant advance in regenerative medicine. However, rational selection of culture environments to evoke desired stem cell differentiation requires a deeper understanding of stem cell responses to specific stimuli than currently exists. A myriad of environmental signals are known to influence stem cell differentiation, including scaffold modulus as well as the concentration and identity of bioactivity presented to the cells. Although a range of studies have explored the separate impact of these two microenvironmental variables on stem cell behavior, few studies have evaluated the relative influence of these factors. This is significant, because, if one environmental signal proves dominant, then more attention can be focused on appropriately tuning this variable so as to elicit desired differentiation.

The present study was designed to evaluate the influence of scaffold modulus relative to that of collagen type I concentration (bioactivity) on mesenchymal stem cell (MSC) differentiation. To isolate the influence of modulus from that of bioactivity, we employed hydrogels prepared from diacrylate-derivatized poly(ethylene glycol) (PEGDA). PEGDA has several properties which make it desirable for studies focusing on elucidating the relative impact of specific stimuli on cell behavior, including its biological "blank slate" character and its tunable mechanical properties. In the current work, three separate moduli and two distinct collagen type I concentrations were probed. The modulus and bioactivity ranges examined were selected to be similar (~ factor of 5-6 difference between the "high" versus "low" set points) to enable consistent evaluation and comparison of stimuli effects.

Methods

Macromer Synthesis. Photo-sensitive acrylate groups were introduced to the terminal ends of linear 8 kDa and 20 kDa PEG according to known methods. Acrylate-derivatized collagen type I (Ac-Col I) was synthesized by reaction of rat tail-derived collagen type I with excess ACRL-PEG-NHS at pH 8.5. Construct Preparation and Maintenance. At each collagen concentration (0.04 and 0.2 mg/g), three distinct scaffold moduli (80, 300, 550 kPa) were investigated by tuning the molecular weight and concentration of PEG. MSCs encapsulated within the hydrogels were cultured for 21 days, with time points collected every 7 days. Endpoint Analyses. Differentiation was assessed by competitive ELISA for cell markers specific to various mesenchymal lineages.

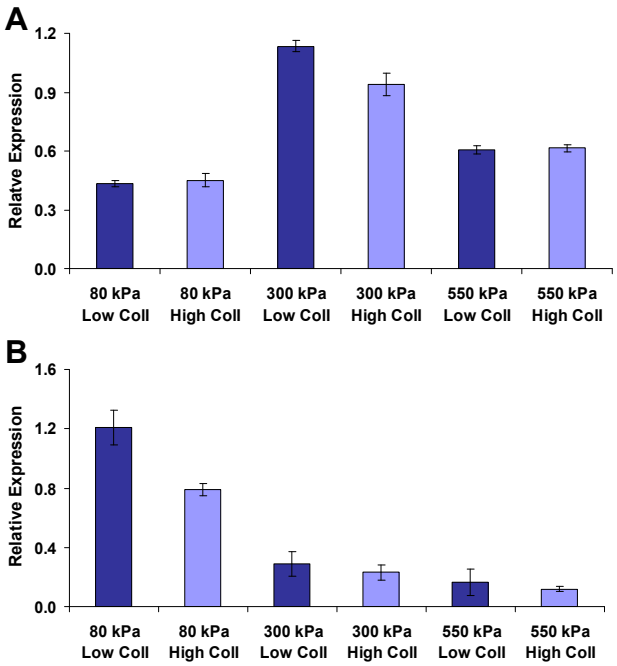

Fig. 1. (A) Relative expression of osteocalcin among formulations. (B) Relative expression of AFABP among formulations. Results associated with "low" collagen (0.04 mg/g) and "high" collagen (0.2 mg/g) gels are denoted in dark blue and light blue, respectively.

Results/Discussion

ELISA analyses were conducted for osteocalcin, adipocyte-fatty acid binding protein (AFABP), SM22α, and collagen type II. Each of these molecules served as mid/late-term markers of differentiation toward osteoblast-, adipocyte-, smooth muscle-, or chondocyte-like cells. Osteocalcin expression was heightened in the ~300 kPa constructs relative to the 550 kPa and the 80 kPa scaffolds (**Figure 1A**), whereas AFABP levels were higher in the 80 kPa hydrogels relative to the 300 kPa and the 550 kPa formulations (**Figure 1B**). In contrast, collagen concentration appeared to have limited influence on cell differentiation relative to modulus. Similar results were observed for collagen type II and SM22α (data not shown).

Conclusions

Over the time course of the study, modulus appeared to dominate the observed MSC fate decisions, suggesting that modulus gradients may be more powerful in guiding MSC differentiation than collagen gradients, at least over the bioactivity-modulus range examined.

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