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Influence of select extracellular matrix proteins on mesenchymal stem cell osteogenic commitment in three-dimensional contexts

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ABSTRACT

Growth factors have been shown to be powerful mediators of mesenchymal stem cell (MSC) osteogenic differentiation. However, their use in tissue engineered scaffolds not only can be costly but also can induce undesired responses in surrounding tissues. Thus, the ability to specifically promote MSC osteogenic differentiation in the absence of exogenous growth factors via the manipulation of scaffold material properties would be beneficial. The current work examines the influence of select extracellular matrix (ECM) proteins on MSC osteogenesis toward the goal of developing scaffolds with intrinsically osteoinductive properties. Fibrinogen (FG), fibronectin (FN) and laminin-1 (LN) were chosen for evaluation due to their known roles in bone morphogenesis or bone fracture healing. These proteins were conjugated into poly(ethylene glycol) diacrylate (PEGDA) hydrogels and their effects on encapsulated 10T½ MSCs were evaluated. Specifically, following 1 week of culture, mid-term markers of various MSC lineages were examined in order to assess the strength and specificity of the observed osteogenic responses. PEG–LN gels demonstrated increased levels of the osteogenic transcription factor osterix relative to day 0 levels. In addition, PEG–FG and PEG–LN gels were associated with increased deposition of bone ECM protein osteocalcin relative to PEG–FN gels and day 0. Importantly, the osteogenic response associated with FG and LN appeared to be specific in that markers for chondrocytic, smooth muscle cell and adipocytic lineages were not similarly elevated relative to day 0 in these gels. To gain insight into the integrin dynamics underlying the observed differentiation results, initial integrin adhesion and temporal alterations in cell integrin profiles were evaluated. The associated results suggest that α_2, α_v and α_6 integrin subunits may play key roles in integrin-mediated osteogenesis.

1. Introduction

Mesenchymal stem cells (MSCs) are being increasingly recognized as a viable cell source for bone regeneration applications due to their ability to be expanded in vitro and to differentiate into a number of cell lineages. MSC differentiation is known to be influenced by a range of environmental stimuli, among the most potent of which are growth factors. However, the use of exogenous growth factors in tissue engineering scaffolds not only can be costly but also can induce undesired responses in surrounding tissues. Thus, MSC-based bone regeneration strategies would benefit from the identification of scaffold material properties which intrinsically promote osteoblast lineage progression in the absence of exogenous growth factors.

A number of two-dimensional (2-D) studies have demonstrated MSC osteogenic differentiation to be tightly regulated by cellular interactions with the surrounding extracellular matrix (ECM) [1–13]. However, comparatively little is known regarding the effects of various ECM components in regulating MSC osteogenesis in three-dimensional (3-D) scaffold environments [14–16]. This is significant since recent studies suggest that effects observed in two dimensions may not be indicative of the effects of the same scaffold variables in more biomimetic 3-D culture systems [17–19]. Therefore, the current work focuses on elucidating the influence of select ECM constituents on MSC osteogenic differentiation in 3-D contexts.

Towards this goal, we incorporated specific ECM molecules into hydrogel scaffolds designed to have moduli within the "osteogenic" range identified in the 3-D human and mouse MSC studies of Huebsch et al. [20]. In selecting molecules for examination, we chose to focus on several ECM proteins associated with bone morphogenesis (fibronectin [21] and laminin-1 [22,23]) and/or bone fracture

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