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Synthetic hydrogel niches that promote hMSC viability

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Abstract

Photopolymerized poly(ethylene glycol) (PEG) hydrogels were used as a base platform for the encapsulation and culture of human mesenchymal stem cells (hMSCs). The base PEG formulation presents an environment completely devoid of cell–matrix interactions. As such, viability of hMSCs in unmodified PEG hydrogels is very low. This formulation was modified to contain pendant phosphate groups to facilitate the sequestering of osteopontin within the gel, as well as pendant cell-adhesive RGD peptide sequences, which are found in osteopontin and other cell adhesion proteins. The survivability of hMSCs was examined with culture time and as a function of the gel chemistry to examine the role of cell–matrix interactions in promoting long-term viability. In the absence of any adhesive ligands, hMSC viability drops to 15% after 1 week in culture. However, by incorporating the RGD sequence or pendant phosphate groups this low viability was rescued to 75% and 97%, respectively. It is believed that the phosphate groups promote mineralization of the hydrogel network, and this mineral phase sequesters cell-secreted osteopontin, resulting in enhanced cell–matrix interactions and improved cell viability.
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Keywords: Hydrogel; Human mesenchymal stem cells; Photoencapsulation; Osteopontin; Cell viability; Poly(ethylene glycol)

1. Introduction

Hydrogels are water swollen, but insoluble, polymer networks. The high water content and tissue-like elasticity lead to properties that are similar to many tissues, and as such, hydrogels are often explored as synthetic extracellular matrix analogs for the three-dimensional culture of cells. Hydrogels can be synthesized from purely synthetic components that create an environment that limits cellular interactions and only cell secreted extracellular matrix molecules can modify this niche. In contrast, chemical derivatization of hydrogels (e.g., with peptide epitopes or adhesion proteins) affords opportunities to control cell–matrix interactions in a highly controlled manner. In essence, these two situations allow the creation of cellular niches that simply permit cells to function or actively present cues that promote selected functions. To date, researchers have explored the development of hydrogels for the delivery of numerous primary cells, including chondrocytes (Bryant and Anseth, 2001, 2002; Bryant et al., 2004; Paige et al., 1995; Rice and Anseth, 2004; Sittinger et al., 2004), osteoblasts (Burdick and Anseth, 2002; Burdick et al., 2002; Sittinger et al., 2004), valvular interstitial cells (Masters et al., 2004), smooth muscle cells (Mann, 2003; Mann et al., 2001; Moffat and Marra, 2004; Ramamurthi and Vesely, 2005), fibroblasts (De Rosa et al., 2004; Shu et al., 2004; Zielinski and Aebischer, 1994), and mesenchymal stem cells (Lawson et al., 2004; Nuttelman et al., 2004; Schantz et al., 2003; Temenoff et al., 2004; Wang et al., 2003; Williams et al., 2003).

An emerging area of interest is the design of hydrogel carriers for therapeutic applications, such as the three-dimensional culture and expansion of stem cells, particularly mesenchymal stem cells (MSCs). For regenerative medicine applications, MSCs have many

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advantages including their ease of isolation (Ballas et al., 2002), high proliferative capacity (Banfi et al., 2002; Bruder et al., 1997a,b; Haynesworth et al., 1992a,b), and ability to differentiate into a variety of cell types that are capable of producing large amounts of tissue. Under the right environmental stimuli, MSCs are capable of differentiating into the cells of bone, cartilage, tendon, muscle, fat, and others (Bruder et al., 1997a,b; Caplan, 1991; Haynesworth et al., 1992a,b; Pittenger et al., 1999). Because of these properties, numerous groups are exploring the development of scaffolds for three-dimensional MSC culture. Interestingly, when MSCs are encapsulated in a permissive hydrogel environment (i.e., one that provides no cues, but simply permits cells to function), such as poly(ethylene glycol) (PEG), their viability drops off dramatically with culture time (Nuttelman et al., 2004). Specifically, less than 10% of human mesenchymal stem cells (hMSCs) survive after 4 weeks of culture in PEG hydrogels. This observation is in stark contrast to previous cell encapsulation studies with bovine chondrocytes (Bryant and Anseth, 2001), where cell viability remained high (>95%) after 6 weeks in culture, and neonatal rat calvarial osteoblasts (Burdick and Anseth, 2002), where cell viability was greater than 80% after 4 weeks in culture, in similar PEG hydrogels. Part of this difference may be related to the differences in anchorage dependence, species, and age of the various cells; however, in regards to hMSCs, interesting results have been reported with respect to expression of cell adhesive protein genes, especially osteopontin, when hMSCs are cultured in permissive PEG hydrogels.

Osteopontin is an especially important cell adhesion protein for hMSCs, regulating both adhesion of osteoprogenitor cells and the differentiation of stem cells to osteoblasts (Darimont et al., 2002; Ohgushi and Caplan, 1999; Yabe et al., 1997). Adhesion of osteoprogenitor cells to the bone surface during remodeling is one of the major turning points in their differentiation to mature osteoblasts; during this differentiation process, osteoprogenitor cells have been reported to make large amounts of bone tissue quickly (Yabe et al., 1997). When hMSCs were encapsulated in PEG hydrogels and cultured in basal media, osteopontin expression by these cells after 1 week was found to be nearly ten times higher than osteopontin expression by hMSCs cultured in monolayer culture (Nuttelman et al., 2004). In general, cells do not attach directly to highly hydrated synthetic gels, such as PEG, because of limited protein adsorption, so hMSCs encapsulated inside these gels are presented with a "blank" environment. We hypothesize that the encapsulated hMSCs are secreting elevated levels of osteopontin in an effort to modify their environment to enable cell attachment.

While cells can normally alter their environment by secreting adhesion proteins, there is little to no interaction of secreted adhesion proteins with the surrounding environment once the cells are encapsulated within a PEG gel and osteopontin can readily diffuse through the gel. As a result, there are few interactions between cell–surface integrins and the surrounding gel environment. Cell–matrix interactions can dramatically affect differentiation, survival, and tissue evolution; anchorage-dependent osteoprogenitor cells, such as hMSCs require a support matrix in order to survive (Ishaug-Riley et al., 1998). In the absence of cell–matrix interactions, anchorage-dependent cells undergo apoptosis, or more specifically, anoikis [i.e., apoptosis that occurs as a result of cell detachment from the extracellular matrix (Frish and Ruoslahti, 1997)]. Cell binding to the extracellular matrix through integrin receptors is thought to provide signals for suppression of apoptosis (Ruoslahti and Reed, 1994). Thus, when designing hydrogel niches to serve as synthetic extracellular matrix environments, preservation of matrix–integrin interactions is critical to promote long-term cell survival and function. In fact, nonspecific cell attachment to a substrate is not sufficient to prevent apoptosis; specific binding to integrins is required to prevent apoptosis. This specificity has been demonstrated using surface-immobilized antibodies against two types of non-integrin cell surface molecules, which did not prevent apoptosis, as opposed to surface-immobilized anti-integrin antibodies, which did lead to prevention of apoptosis (Meredith et al., 1993).

In this research, we aimed to better understand the role of the extracellular matrix in supporting hMSC viability and survivability by designing two specific types of hydrogel niches that incorporate functionalities designed to enhance specific matrix–integrin interactions. The first gels contain a pendant arginine–glycine–aspartic acid (RGD) peptide sequence that is found in numerous cell adhesion proteins, including osteopontin (Ruoslahti, 1996). The RGD sequence is known to bind to integrin receptors on cell surfaces, supporting cell adhesion and preventing apoptosis (Frish and Ruoslahti, 1997; Ruoslahti, 1996; Ruoslahti and Reed, 1994; Wadsworth et al., 2004). The second gels were designed to capture and sequester cell-secreted osteopontin. Osteopontin, like other sialoproteins, has an acidic domain that binds to the positively charged hydroxyapatite mineral matrix of bone. Cells attach to bone mainly through osteopontin that, through electrostatic interactions, has adsorbed to the mineralized matrix. Thus, negatively charged phosphate groups were incorporated into the PEG gel to indirectly promote cell adhesion by facilitating mineralization and subsequent adsorption of cell-secreted osteopontin. Through these two hydrogel formulations, we aimed to understand the role of matrix adhesion, as well as the relative ability of a peptide sequence versus an entire protein, to promote long-term survivability, and presumably prevent apoptosis, of encapsulated hMSCs.