Synthetic Hydrogel Materials Suitable for 3D Cell Culture

Synthetic polymers for crosslinking of cell-laden hydrogels

Many hydrophilic synthetic polymers and co-polymers are utilized as backbone of cell-laden hydrogels for 3D cell culture. Synthetic hydrogels can be prepared from small molecular weight monomers (e.g., acrylamide, N-isopropylacrylamide, N-vinylpyrrolidone, hydroxyethyl methacrylate, etc.) or crosslinked from high molecular weight macromers (e.g., poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), etc.) or their derivatives (e.g., PEG-diacrylate, PEG-vinylsulfone, PEG-norbornene, thiolated-PVA, tyramine-PVA, etc.). When polymerized from small molecular weight monomers, extensive washes post-gelation are required to remove residual unreactive monomers and initiators that might be harmful for the cells. *In situ* cell encapsulation is less likely to be compatible with hydrogels polymerized from small molecular weight monomers, although these gels can be made with highly controllable properties, such as crosslinking density, degradability, and even porosity (when porogens are used). On the other hand, hydrogels prepared from polymerizable hydrophilic macromers often are more compatible for *in situ* cell encapsulation, given that all components used for hydrogel crosslinking are cytocompatible. Regardless of the route of hydrogel preparation, synthetic polymers do not contain bioactive motifs critical for cell survival, proliferation, and morphogenesis. Therefore, major research efforts in preparing synthetic hydrogels are to conjugate or co-polymerize biomimetic ligands for improving cell-material interactions (DeForest and Anseth 2012).

Radical-mediated chain- and mixed-mode polymerizations

The most commonly used method to prepared hydrogels for 3D cell culture is through radical-mediated chain-growth polymerization of functionalized macromers (e.g., acrylated or methacrylated PEGs). Immobilization of pendant ligand is easily achieved through co-polymerization of acrylated or methacrylated peptides/proteins in the crosslinked polymer network. Hubbell and colleagues pioneered the field by using N-hydroxysuccinimidyl (NHS)-activated esters (with or without a PEG spacer) to immobilize acrylated integrin-binding peptides to the otherwise inert PEGDA hydrogels (West and Hubbell 1995; Hern and Hubbell 1998; West and Hubbell 1999). This approach has become a popular method of functionalizing PEG hydrogels with bioactive motifs. Even though this approach is simple, it may not be ideal for some applications due to the fact that the pendant peptides are linked to hydrophobic poly(acrylate) or poly(methacrylate) kinetic chains, resulting in decreased accessibility of the peptides/proteins to the cells (Lin and Anseth 2009). Furthermore, the immobilization efficiencies (i.e., percentage of immobilized peptides/proteins) of (meth)acrylated pendant peptides are relatively low (~60%) (Elbert and Hubbell 2001; Hao and Lin 2014), which means that the non-crosslinked peptides may bind to cell surface receptor and act as soluble agonist or antagonist.

Acrylated PEGs are a unique class of macromer in that they are crosslinkable through radical-mediated chain-growth or mixed-mode polymerization (Salinas and Anseth 2008; Hao and Lin 2014; Hao et al. 2014; Lin et al. 2015). The latter describes