A variety of biomaterial-based 3D cell culture platforms have been developed to circumvent the disadvantages associated with animal-derived 3D matrices (Tibbitt and Anseth 2009; DeForest and Anseth 2012; Liu et al. 2015). For example, polymeric scaffolds can be fabricated to have porous structures for 'housing' cells and permitting their proliferation, migration, and other cellular activities (Elbert 2011). Solid and porous scaffolds can be fabricated prior to cell seeding, thus allowing a variety of materials to be used. These scaffolds are typically fabricated to have larger pore sizes, usually on the order of tens of micrometer or sub-millimeter to facilitate uniform cell seeding. Scaffold with a larger pore size is ideal for cell seeding, but the trade-off is weaker mechanical properties. It also cannot truly recapitulate a 3D extracellular microenvironment where cells are often in close contact with their surrounding matrix. Additionally, creating a porous scaffold with interconnected pores is necessary but challenging. An easier route to creating interconnecting space in a polymeric scaffold is to use fibrous materials (Stephens-Altus et al. 2011). Fibrous scaffolds are ideal for many tissue engineering applications because they mimic the fibrous architectures of many native tissues, especially those rich in fibrous collagens (Wade et al. 2015).

Another attractive class of biomaterial suitable for 3D cell culture is semi-synthetic biomimetic hydrogels (DeForest and Anseth 2012; Lin et al. 2015). These crosslinked and water-imbibing polymeric network are increasingly used for modeling diseases *in vitro* and for regenerative medicine applications. Hydrogels are particularly suitable for investigating the influence of extracellular milieu on cell fate because the components of a biomimetic hydrogel can be precisely engineered. The mesh size of hydrogels is on the order of tens of micrometers, a scale much smaller than the size of a cell but larger than most of the growth factors, cytokines, chemokines. Hence, the accessibility of encapsulated cells to these small molecular weight proteins are not significantly hindered (Lin and Metters 2006). The high water content and good permeability of a hydrogel allow facile nutrient-waste exchange, while the crosslinked polymeric network gives rise to tunable elasticity and easy tethering of bioactive motifs for supporting cell survival and function in 3D (Lin and Anseth 2009). In the past few decades, the field of biomaterials science and engineering has witnessed drastic improvement in hydrogel-based 3D cell culture, which is the focus of this chapter.

## Design Criteria in Preparing Hydrogels for 3D Cell Culture

For all biomimetic hydrogels intended for cell culture application, the most important design criterion is that the components (e.g., macromers, crosslinkers, initiators, catalysts, functional motifs, etc.) used to prepare cell-laden hydrogels must be non-cytotoxic. Furthermore, the crosslinking conditions must be mild for maintaining viability and phenotype of the cells. Ideally, these hydrogels should present functional moieties found in the native ECM for sustaining long-term cell function or for guiding cell fate processes. These preferential properties include, but not limited to, permitting facile transport of biomolecules (e.g., sugars, lipids, polysaccharides, proteins, and cellular metabolites), matching matrix mechanics to native tissues, sequestration and liberation of bioactive growth factors, as well as providing cell-matrix interactions through ligand-receptor binding and protease-medicated matrix cleavage (Drury and