Resilin, a structural protein found in insects, is another example of engineered polypeptide gaining increasing attention in biosynthetic scaffold for 3D cell culture (Li et al. 2013; Su et al. 2014). Resilin, as suggested by its name, has excellent resilience and energy storage capabilities, which allow it to recover from high-strain cyclic loading with no hysteresis. Polypeptide constructs containing resilin consensus sequence (e.g., GGRPSDSYGAPGGGN) have been produced from Drosophila CG15920 gene via recombinant DNA techniques (Su et al. 2014). These resilin-like polypeptides (RLPs) have been used to fabricate 3D hydrogels by means of temperature-induced transition or chemical crosslinking. Similar to the incorporation of cell-responsive motifs in ELPs, the biological functionality of RLPs can be improved through incorporating cell adhesive or protease sensitive sequences in the peptide backbone. Other functional peptide sequences, such as RGD, have also been designed into RLPs for improving differentiation of human mesenchymal stem cells (hMSCs) (Renner et al. 2012; McGann et al. 2016a; McGann et al. 2016b).

Small molecular weight hydrogelators

Supramolecular assembly describes the association of molecules by means of hydrogen bonding, metal chelation, van der Waals forces, hydrophobic interaction, π-π stacking, or other non-covalent and directional bindings (Nagarkar and Schneider 2008). This attractive class of macromolecular chemistry has found numerous applications in non-covalent surface modification, affinity-based drug delivery, imaging and diagnostic applications, and more recently hydrogel synthesis and fabrication (Liebmann et al. 2007; Das et al. 2009; Shao and Parquette 2010). Two types of supramolecular hydrogels are suitable for 3D cell culture: namely hydrogels fabricated from small molecular weight 'hydrogelator' and those prepared from macromolecular host-guest interactions (see section below). In addition to the gelation mechanisms, these two classes of supramolecular hydrogels also differ in cell loading method. In small molecular hydrogelator (e.g., aromatic Fmoc-linked or alkyl group oligopeptides), hydrogels are formed due to π-π stacking of the hydrophobic aromatic Fmoc groups or hydrophobic interactions between the alkyl chains (Fleming and Ulijn 2014). Due to the hydrophobicity of the constituent molecules, the precursor solutions are often prepared using solvents (e.g., dimethyl sulfoxide, DMSO). Upon dilution using aqueous buffer, the hydrophobic interactions between Fmoc or alkyl groups yield nanoscale molecular assembly that can further entangle to form hydrogels with higher order fibrillar structures and interconnected pores. Due to the use of organic solvents during gelation, cells are loaded to the Fmoc-peptide hydrogels post-gelation. Cell adhesive peptides can be readily incorporated in the form of Fmoc-linked peptide (e.g., Fmoc-RGD) (Zhou et al. 2009; Zhou et al. 2014; Zanuy et al. 2016). When Fmoc-peptide hydrogels are utilized for 3D cell culture, care must be taken in terms of residual solvents. Another concern is that the assembly of these small molecular weight gelators often leads to kinetically trapped aggregates. Nonetheless, this form of supramolecular hydrogel can be controlled to produce nanoscale fibrillar structure, which resembles fibrillar structure found in native ECM.