lower temperature to precipitate into a hydrogel (Nagarsekarn et al. 2003). A greater state of ionization, induced by pH and ionic strength considerations, also increases hydrophilicity therefore thermo-responsiveness requires an appreciation of these factors also. Amino acids, such as glutamic acid, with ionisable R-groups are more influenced by changes in pH and ionic strength.

Thermo-responsive hydrogelation takes advantage of the ability of water to solubilize hydrophobic moieties at reduced temperatures. Increasing temperature decreases the solubility of the hydrophobic species resulting in phase separation of these groups creating hydrogels due to formation of physically cross-linked networks (Badiger et al. 1998). Peptide folding is also sensitive to temperature changes. A transition from α-helix to β-sheet secondary structure was utilized by Zhang and colleagues to induce temperature responsive gelation in EAK-12 (AEAEAEAEAKAK) and DAR16-IV (ADADADADARARARAR) peptides. β-sheet formation and gelation predominantly occur at 25ºC for EAK-12 with α-helix conformation at 85ºC. DAR16-IV was proven to transform to an α-helix nature at 75ºC. An increase in temperature to trigger an α-helix to β-sheet conformational change was also used by Kammerer to trigger gelation in short coiled-coil peptides (Kammerer and Steinmetz 2006). There is increased interest in developing biomolecular hydrogels that mimic human tissue. The ability to tailor hydrogelation to a specific temperature may be of benefit for tissue regeneration and wound healing applications. The temperature of external human skin varies depending on a range of factors including health status and location but is estimated to be between 31 and 40°C (Benedict et al. 1919). A formulation that was liquid at room temperature would allow ease of application to the highly varied shape of wound cavities. Rapid gelation at skin temperature would enable a biomolecular hydrogel to fill this cavity, potentially promoting healing and preventing infection. Such a product would be highly valued by emergency services and within conflict zones.

Enzyme-Responsive Hydrogels

Self-assembly in biological systems is tightly controlled by spatially confined molecular mechanisms, often catalysed by enzymes, to form cellular architecture such as microtubules, actin filaments, DNA, vesicles and micelles (Rasale and Das 2015). Enzymes are viable molecules for instructing localized assembly of biomolecules, resulting in hydrogel formation. They are particularly promising as hydrogelation can be triggered within a cell by utilizing an activating enzyme that is not found extracellularly. Enzymatic responsive systems involving hydrolysis of a peptide (amide) bond (Guilbaud et al. 2013), phosphate ester (Yang et al. 2006) or methyl ester (Hirst et al. 2010) are more commonly utilized for research purposes. Enzyme instructed self-assembly can be achieved by either catalysing the addition or removal of a group to/from a molecule to form a self-assembling motif (Yang et al. 2004; Toledano et al. 2006). Enzymatic synthesis of a self-assembling molecule has been proven for peptides whereby an amide linkage forms from the reversible condensation/hydrolysis reaction of an amine and carboxylic acid under conditions that govern thermodynamic control. The laws of thermodynamics ensure that condensation will be favoured over peptide hydrolysis only for molecules that self-assemble to form stable structures (e.g., hydrogels) and have sufficient free energy to overcome