Covalently Crosslinked HA Hydrogels

Covalent crosslinking is necessary to impart stability and improve functions of HA hydrogels. HA can be directly crosslinked without any chemical modifications via hydroxyl groups using bisepoxide or divinyl sulfone (DVS) (Table 1). However, carboxyl groups have been selected as the preferred site for derivatization due to their superior reactivity and hydrogels have been obtained via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) chemistry, biscarbodiimide and multifunctional hydrazides under acidic conditions (Xu et al. 2012). Some of these methods require toxic reagents and harsh conditions that are not suitable for cell and protein encapsulation.

Schiff base reaction

Schiff base reaction can be formed between an amine group and aldehyde group without additional chemical crosslinking reagents. HA can be oxidized with sodium periodate ($NaIO_4$) to produce aldehyde functionalities (Burdick and Prestwich 2011). Combinations of oxidized HA with adipic acid dihydrazide (ADH) hydrogels can be readily obtained by Schiff base reaction forming a hydrazine linkage (Su et al. 2010). Cell viability and cytotoxicity assays using nucleus pulposus (NP) cells showed good biocompatibility of these hydrogels, as well as an ability to promote gene expression of aggrecan and type II collagen, which are the major ECM components of NP cells. Computational finite element studies of the mechanical behaviour of ADH-HA hydrogels showed that the stiffness of the hydrogels were not affected by the presence of encapsulated adipocytes, indicating the application of these hydrogels for the delivery of preadipocytes in tissue reconstruction (Shoham et al. 2013).

Despite being a simple method to form hydrogels, the use of periodate oxidation to obtain aldehyde-modified HA causes degradation of the polymer chain. Thus, more chemo-selective chemistries for the synthesis of HA hydrogels that occur under physiological conditions without generating any toxic by-products have been explored.

Michael-type addition

The Michael addition reaction results in the nucleophilic addition of a nucleophile or a carbonation (e.g., amines and thiols) to an α,β-unsaturated carbonyl compound (Yang et al. 2014). This reaction has high selectivity, enabling efficient coupling under physiological conditions, without producing side products or toxic compounds. For example, the Michael addition reaction can occur between thiol and vinyl sulfone (VS) or aminoethyl methacrylate (Table 1). Cells and growth factors can be encapsulated by simply mixing them with the polymer precursor solutions.

Thiolated HA (DTPH-HA, Fig. 1B-i) can be readily crosslinked in contact with air via the formation of disulphide bond. To better control crosslinking kinetics and density, several crosslinkers have also been used, such as polyethylene glycol diacrylate (PEGDA), PEG divinyl sulfone (PEGDVS), PEG tetra-thiols ($PEG\text{-}SH_4$) (Table 1). SH-HA has been used to form covalently crosslinked hydrogels which have been utilized to control the release of growth factors (Pike et al. 2006; Burdick