To obtain azide-modified HA (N_3-HA), azido-3,6,9-trioxaundecan-1-amine was grafted onto HA by EDC/NHS chemistry (Hu et al. 2011). To form a hydrogel, the $–N_3$ groups of N_3-HA were reacted with the acetylene groups of gelatin modified with propiolic acid, catalyzed by Cu(I), to form triazole rings. Chondrocytes seeded on the surface of the hydrogels were shown to remain viable and proliferate.

Alkyne-functionalized HA (obtained via EDC/NHS-mediated coupling of propargylamine to the HA carboxylic groups, Fig. 1B-iii) was crosslinked with linkers possessing two terminal azide functionalities (Piluso et al. 2011). Variation of the crosslinker density and crosslinker type (length and rigidity) created hydrogels with elastic moduli in the range of 0.5–4 kPa. Cytotoxicity assays did not show toxic effects on L929 cells.

Thermoreversible poly(N-isopropylacrylamide)-hyaluronan (PNIPAM-HA) hydrogels were synthesized through "click" chemistry and RAFT polymerization (Mortisen et al. 2010) using copper-catalyzed azide-alkyne cycloaddition of AL-HA with azido-terminated PNIPAM (N_3-PNIPAM). PNIPAM-HA hydrogels were shown to be cytocompatible to hTERT-BJ1 fibroblasts.

Enzyme-Mediated HA-Tyramine Hydrogels

As described previously, covalent crosslinking has been widely used to obtain HA hydrogels with precise control over crosslinking density. However, many of the crosslinking reagents used are toxic, precluding their use for cell encapsulation applications. Enzyme-mediated crosslinking reactions are finding increasing applications for developing hydrogels due to their mild conditions and enzyme specificity. Tyramine-modified HA (Fig. 1B-iv) has been used to induce covalent crosslinking (Loebel et al. 2015; Wang et al. 2014a; Lee et al. 2009) by horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2). HA derivatization with tyramine has been obtained through EDC/NHS chemistry (Lee et al. 2009; Lee et al. 2008), but the use of DMTMM was also recently reported (Loebel et al. 2015). The later method was shown to provide several advantages, compared with conventional method, such as accurate control of the degree of substituted (DS) tyramine (Tyr) on HA and consequently better control over the viscoelastic properties, *in vitro* swelling and enzymatic degradation of the crosslinked hydrogels. The mechanical strength of the HA-Tyr hydrogel was shown to be tuned solely by the H_2O_2 amount without affecting the gelation rate. Subcutaneous injections of HA-Tyr with H_2O_2 and HRP demonstrated that rapid gelation could prevent diffusion of the injected polymer solution and ensure localized gelation at the injection site (Lee et al. 2008). HA-tyramine hydrogels conjugated with the bioactive peptide epitope arginine-glycine-aspartic acid (RGD) promoted the adhesion, proliferation and migration of human umbilical vein endothelial cells (HUVECs), as well as capillary-like network formation and extension, in combination with co-culture of HUVECs and human fibroblasts (HFF1) *in vitro*, compared to an unmodified hydrogel. *In vivo* formation of vasculature in the cell-laden hydrogel constructs confirmed the anastomosis with the host vasculature (Wang et al. 2014a). By changing the polymer and H_2O_2 concentrations, Tyr-HA hydrogels with different mechanical properties (soft, medium and stiff) were obtained and tested for their ability to support the expansion of hESCs in 3D (Xu et al. 2015). Tyr-HA