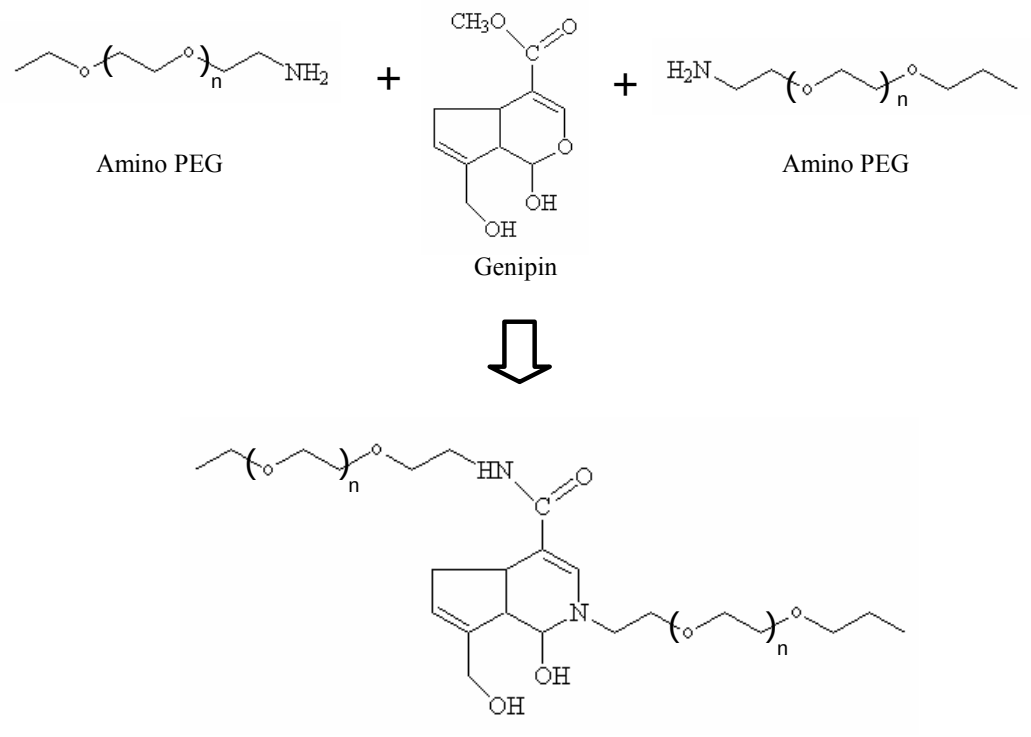
longer gelation time is required for hydrogel formation due to its relatively lower reactivity of the methacryl group. To further accelerate the gelation of the hydrogel for cell delivery procedure, an alternative system based upon the conjugate addition crosslinking between thiol-modified HA and PEGDA was developed, which satisfies most key requirements for injectable *in vivo* tissue engineering applications [115–116].

Figure 3. The reaction scheme of amino PEG-genipin hydrogel.



3.2.3. Genipin crosslinked hydrogels

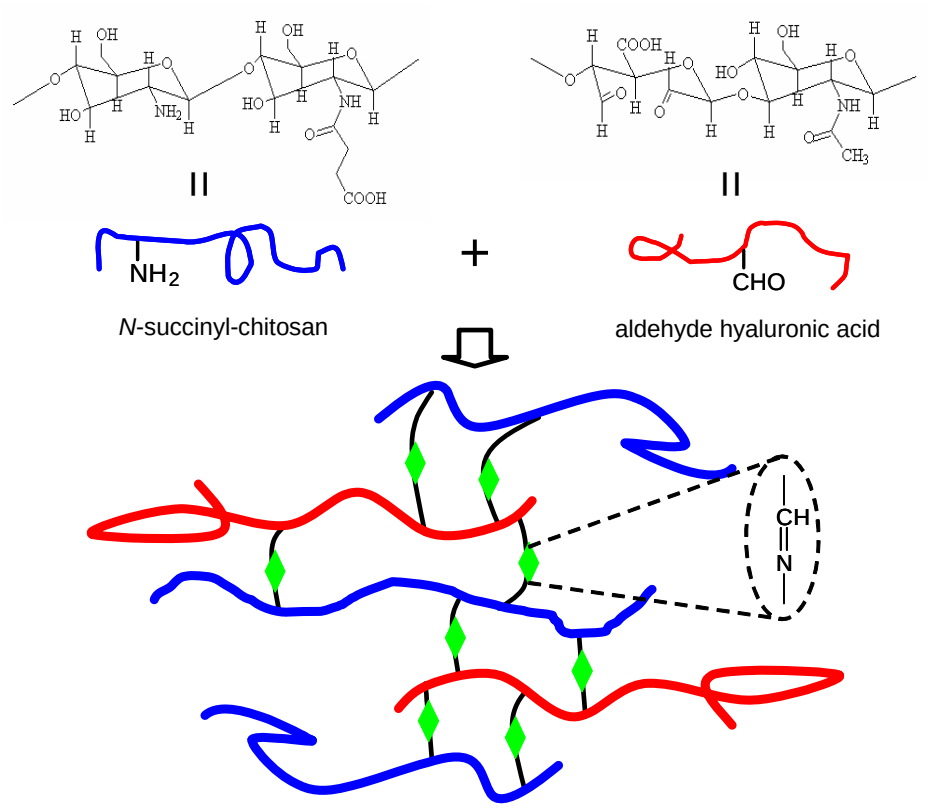
Genipin is a natural product extracted from the gardenia fruit, which overcomes the toxicity inherent in most commonly used synthetic cross-linkers [118–119]. Recent studies identified that genipin can be utilized to crosslink functional amine groups present in natural tissues and biomaterials with very minimal cytotoxic effects, compared to studies performed with glutaraldehyde, a commonly used crosslinker [119–123], resulting in materials with a deep blue color. The utilization of genipin (0.5~3.5 wt %) to crosslink natural biocompatible polymers, such as chitosan and gelatin, to form biodegradable hydrogels has the potential to produce novel scaffolds for various tissue engineering applications. Our laboratory has recently examined the synthesis of novel amino-terminated multi-arm PEG based hydrogels utilizing genipin as a crosslinking agent [124–127]. We examined 2 PEG structures: 4-arm PEG, a molecule with 4 PEG chains attached at a central point, and 8-arm PEG, a molecule with 8 PEG chains attached to a central molecule (Figure 3). The gelation time, swelling, water uptake and weight loss were dependent on the structure of the PEG hydrogel. Due to the molecular architecture, the 8-arm PEG hydrogel showed a much slower gelation reaction, more compact structure and lower water uptake than those of the 4-arm PEG hydrogel, as well as a better stability *in vitro* with a 35.2 mM genipin. Furthermore, human adipose derived stem cell study results indicated that both the 4-arm and 8-arm PEG hydrogels are able to support cell adhesion. This study

represents the potential opportunity to use genipin cross-linked, multi-arm PEG-genipin hydrogels, especially the 4-arm PEG-genipin, as an injectable scaffold in a variety of tissue engineering applications.

3.2.4. Schiff-base crosslinked hydrogels

More recently, we have developed a new injectable, *in situ* forming biocompatible and biodegradable hydrogel as cell carriers for tissue engineering applications [128]. The polysaccharide hydrogel derived from water-soluble chitosan and oxidized hyaluronic acid gel upon mixing, without employing any extraneous chemical crosslinking agents. The gelation is attributed to the Schiff-base reaction between amino groups of *N*-Succinyl-chitosan and aldehyde groups of oxidized hyaluronic acid (Figure 4). *N*-Succinyl-chitosan, a water soluble chitosan derivative, was synthesized *via* introduction of succinyl groups at the *N*-position of the glucosamine units of chitosan. Hyaluronic acid can be oxidized, and the carbon-carbon bonds of the cis-diol groups in molecular chain are cleaved and generate reactive aldehyde functions, which can develop chemical crosslinking action with amino functions *via* Schiff-base linkage. This polysaccharide hydrogel creates a biomimetic microenvironment with improved biocompatibility and biodegradation for tissue regeneration. Several other polysaccharides such as dextran, gum arabic and chondroitin sulfate can be partially oxidized and employed for Schiff-base linkage [129–134].

Figure 4. The scheme of *N*-succinyl-chitosan and aldehyde hyaluronic acid composite hydrogel *via* Schiff's base cross-linking reaction.



4. Applications of Injectable Hydrogels

4.1. Clinical Applications

The need for injectable, biodegradable hydrogels in biomedical applications is immense. One example is the utility of hydrogels in cartilage regeneration. The physical properties of the hydrogel can be designed to easily match those of articular cartilage in addition to matching mechanical properties of the scaffold with the native tissue. Further applications for hydrogels include soft tissue regeneration after tumor removal or trauma. A number of researchers have studied the combination of injectable hydrogels and biodegradable microspheres for controlled drug delivery in tissue engineering, including our own laboratory [135–139]. The following sections describe the pre-clinical and clinical studies of hydrogels for these applications.

4.2. Cartilage Repair

The need for tissue-engineered cartilage is immense and of great clinical significance. Traumatic and degenerative lesions of articular cartilage are leading causes of disability [140]. It is estimated that over 40 million Americans currently suffer from osteoarthritis [141]. Tissue engineering methods, including the use of injectable hydrogels, to improve cartilage repair and regeneration will therefore have high clinical impact. The advantage of injectable therapies for cartilage repair is that the implant is not only maintained within the defect, but also allows immediate weight-bearing due to the stiffness and strength that is achieved almost instantly. Additionally, a general advantage of injectable therapies is the utilization of minimally invasive surgery as compared to open surgery. As such, there have been numerous studies involving the use of injectable hydrogels for cartilage repair.

4.3. Soft Tissue Regeneration

Soft tissue reconstruction is a significant challenge in reconstructive surgery. There are several reasons for a lack of soft tissue, e.g., adipose tissue, such as congenital (e.g., in Parry-Romberg syndrome [142] or Poland syndrome [143], both of which can result in lipoatrophy), traumatic, or oncologic surgery. Due to a lack of better alternatives, transplantation of autologous adipose tissue has been used for soft tissue reconstruction for the past century. However, the clinical outcome of adipose tissue transplantation is unpredictable as there is variable graft resorption due to a lack of vascularization [144]. A desirable strategy to repair soft tissue is to induce adipogenesis *in situ*. One method to accomplish this is to utilize cells that can differentiate to form adipose tissue, and seed those cells into a scaffold, resulting in adipose tissue formation. Another strategy is to utilize injectable systems. As such, many injectable hydrogels based on both synthetic and natural biomaterials have been examined. For example, Hemmerich *et al*. reported the reconstruction of small defects using injectable hyaluronic acid-based gel which were mixed with undifferentiated adipose-derived stem cells (ASCs). Adequate adipose tissue formation was observed using ASCs and hyaluronic acid as the scaffold [145]. Hyaluronic acid, therefore, is applicable for generating adipose tissue in gels, displaying adipogenic as well as angiogenic properties [146].

Other injectable scaffold matrices include biodegradable, polymeric microspheres. For example, Yuksel *et al*. reported the release of insulin-like growth factor-1 (IGF-1) as well as insulin from PLGA microspheres enhanced *de novo* adipose tissue formation [147]. Their study demonstrated the potential of long-term local IGF-1 and insulin delivery to induce adipogenic differentiation to mature lipid-containing adipocytes from non-adipocyte cell pools (e.g., ASCs) that were administered directly to the deep muscular fascia of the rat abdominal wall.

In addition to PLGA microspheres, the use of extracellular matrix (ECM) particles for injectable systems for adipose tissue engineering has been studied [139,148]. We have previously reported the assessment of ASC attachment, proliferation, and differentiation on gelatinous microparticles, termed CultiSphers [139]. These results demonstrated the potential of using biodegradable particles as cell carriers for soft tissue repair.

5. Conclusions

Injectable scaffolds are promising substrates for tissue engineering with the advantage that drugs and cells can be readily integrated into the gelling matrix. Many efforts have been developed to improve injectable hydrogels and thus, support the development of more natural and functional tissues. The success of injectable tissue constructs is highly dependent on the design of the hydrogel scaffolds including physical, chemical and biological properties. An ideal injectable hydrogel would potentially mimic many roles of ECM found in tissues, resulting in the coexistence of both physical and chemical gels. Current biomaterials are unable to meet all the design parameters simultaneously (e.g., degradation, biocompatibility or mechanical properties). Furthermore, injectable hydrogel development will likely have a significant impact on the advancement of tissue engineering. An objective in future work is to design bioactive materials that would be readily injectable at or below room temperature, would form gels with relatively appropriate biodegradable properties under physiological conditions, and would support cell induction. Novel crosslinking methods should be developed, both to enhance the material biocompatibility as well as control the mechanical properties. In addition, cell induction ligands such as growth factors and genes can be incorporated into the injectable scaffolds such that specific signals could be delivered in an appropriate spatial and temporal manner.

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