Aggrecan (AGG) is a large, aggregating proteoglycan present throughout the body, but predominantly found in articular cartilage. The principle features of AGG, its hyaluronan (HA) binding domain and its abundance of covalently attached glycosaminoglycans (GAGs), make it an essential component of the functional ability of articular cartilage. Current tissue engineering constructs have attempted to stimulate AGG production, but have been unable to produce adequate amounts of mature AGG, and hence have suffered a mismatch in mechanical properties. To address these deficiencies, an AGG mimic was synthesized to match AGG functional properties and provide greater control within tissue engineering constructs. Chondroitin sulfate was functionalized with HA-specific binding peptides to replicate both the GAG presence and HA-binding ability of AGG, respectively. Upon characterization and testing, the mimic was able to effectively bind to HA, increase the compressive strength of cartilage extracellular matrix-based constructs, and protect the other extracellular matrix (ECM) components from degradation, replicating the important functions of AGG. In particular, the mimic produced a 78% increase in compressive strength of the ECM-based constructs, and was able to significantly reduce the degradation of both HA and collagen. The initial characterization of the newly synthesized AGG mimic demonstrates its potential in tissue engineering constructs, and provides an essential basis for more explorative studies of the AGG mimic’s abilities as an AGG substitute and beyond.

Synthesis and characterization of an aggrecan mimic
Jonathan C. Bernhard, Alyssa Panitch*

Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA

1. Introduction

Aggrecan (AGG) is a large, aggregating proteoglycan found throughout the body, but predominantly found within cartilage extracellular matrix (ECM) [1,2]. The protein backbone of AGG is approximately 220 kDa in size, and can be defined in three main segments [1,3]. The first segment contains the binding domain of the proteoglycan to hyaluronic acid (HA), a long, unbranched glycosaminoglycan [1,4]. A link protein also binds within the first segment of AGG and to the HA, stabilizing the AGG–HA binding [5]. The second segment of AGG contains the glycosaminoglycan (GAG) binding region [1,5]. On average, 100 chondroitin–6-sulfate (CS), 30 keratan sulfate (KS) and N/O-linked oligosaccharides bind within this region [6,7]. With the appropriate attachment of GAGs, the proteoglycan is approximately 3 MDa [2]. The final segment is lectin-like in nature, and is believed to play a role in GAG attachment [2,7,8]. The aggregating property of AGG is due to the binding of the first segment to HA [9–12]. On average, 100 AGG bind to the HA chain, creating a structure that resembles a bottlebrush [13,14]. HA in general is slightly negatively charged, and with the sulfate and carboxyl negative charges of the AGG GAGs, the AGG–HA aggregates contain a high negative charge density [3,15]. This high concentration of negative charges provides AGG with its unique functional properties within the body [16]. AGG is predominantly found within cartilage, where it is the most prevalent proteoglycan, constituting 5–15% of the cartilage ECM dry weight [17–19]. The presence of AGG helps provide cartilage with its unique resiliency and compressive strength [6,20]. The presence of the high concentration of negative charges of AGG attracts mobile cations, resulting in an osmotic pressure within the cartilage ECM [7,21]. When a compressive force is applied to the cartilage, the osmotic pressure created by the negative charge density hinders flow of incompressible water, increasing the compressive strength of the cartilage [9,22–24]. Therefore, the specific composition of AGG is responsible for the functional properties of cartilage, protecting the underlying bone from compression and friction-based injury.

Osteoarthritis (OA) is a disease that degrades the ECM components of cartilage, AGG in particular, and exposes the underlying bones to injury. If left untreated, the cartilage can be completely destroyed, leaving the patient immobile and in extreme pain [6,25]. A preliminary event in OA involves the degradation of the AGG by upregulated ADAMTS 4 and 5 [26–28]. Besides removing the compressive properties associated with AGG, studies have shown the possibility that AGG protects the remaining ECM components from degradation [29–31]. The studies presume that once