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Review

Design of cell–matrix interactions in hyaluronic acid hydrogel scaffolds ☆

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ABSTRACT

The design of hyaluronic acid (HA)-based hydrogel scaffolds to elicit highly controlled and tunable cell response and behavior is a major field of interest in developing tissue engineering and regenerative medicine applications. This review will begin with an overview of the biological context of HA, which is needed to better understand how to engineer cell–matrix interactions in the scaffolds via the incorporation of different types of signals in order to direct and control cell behavior. Specifically, recent methods of incorporating various bioactive, mechanical and spatial signals are reviewed, as well as novel HA modifications and crosslinking schemes with a focus on specificity.

1. Introduction

Hyaluronic acid (HA), a naturally occurring glycosaminoglycan (GAG) and one of the primary components of the extracellular matrix (ECM), is increasingly being utilized in biomedical applications due to both its ability to serve as a blank slate and its biological activity. In particular, it is commonly used to form hydrogel scaffolds for tissue engineering [1], which may in turn be employed for localized drug [2] and DNA delivery purposes [3]. This review aims to give a brief overview of the biological functions of HA, knowledge that is needed to fully realize the potential HA offers for tissue engineering applications, before describing recent and novel ways in which HA scaffolds have been engineered to deliver various classes of signals to influence and ultimately command greater control over cell–matrix interactions and cell behavior (Fig. 1).

HA is a linear polysaccharide that consists of two alternating units, B-1,4-D-glucuronic acid and B-1,3-*N*-acetyl-D-glucosamine (Fig. 2). This unmodified polymer has a molecular mass between 10^3 and 10^4 kDa, and can reach a length of 25 μm when fully extended. Its negative charge attracts positive ions and results in an osmotic balance that brings in water. This allows HA to exist as a highly hydrated molecule and paves the way for a myriad of uses in the body.

After synthesis in the inner face of the plasma membrane of fibroblasts, HA is translocated to the pericellular space and the interstitial matrix [4]. This characteristic makes HA distinct from other GAGs, which are typically synthesized in the Golgi and contain a protein core. Three transmembrane glycosyltransferase isoenzymes, HAS1–3, regulate the synthesis process. Hormones and growth factors, among other bioactive molecules, can also help regulate the production of HA.

HA was first characterized in the vitreous of the eye [5]. A few years later, it was successfully isolated from umbilical cord tissue where its main saccharide components, glucoronic acid and glucosamine, were found [6]. A thorough analysis of HA throughout the body of the rat can be applied to approximate the distribution of the polysaccharide in other mammals [7]. For instance, the 40–60 mg that exists in a 250 g rat can be extrapolated to ~11–17 g in a 70 kg human. A little over half (~56%) of the HA found was located in the skin, with another 27% found in the skeleton and supporting tissues, while approximately 8–9% was found in muscle and the internal organs. Among the organs, the highest amount of HA was present in connective tissue, the lung, the kidney and the brain, with low amounts present in the liver and blood serum. Two reviews in particular provide a more thorough explanation of HA distribution throughout the organs in different mammals [8,9].

2. Biological function

One property of HA that can potentially be exploited is the dependence of its biological function on its molecular weight. While HA typically exists as a high molecular weight polymer over 10^6 daltons, it can be cleaved by the enzyme hyaluronidase (HAse) to obtain molecules of much lower molecular weights [10]. In fact, high and low molecular weight HA exhibit opposing effects on cell

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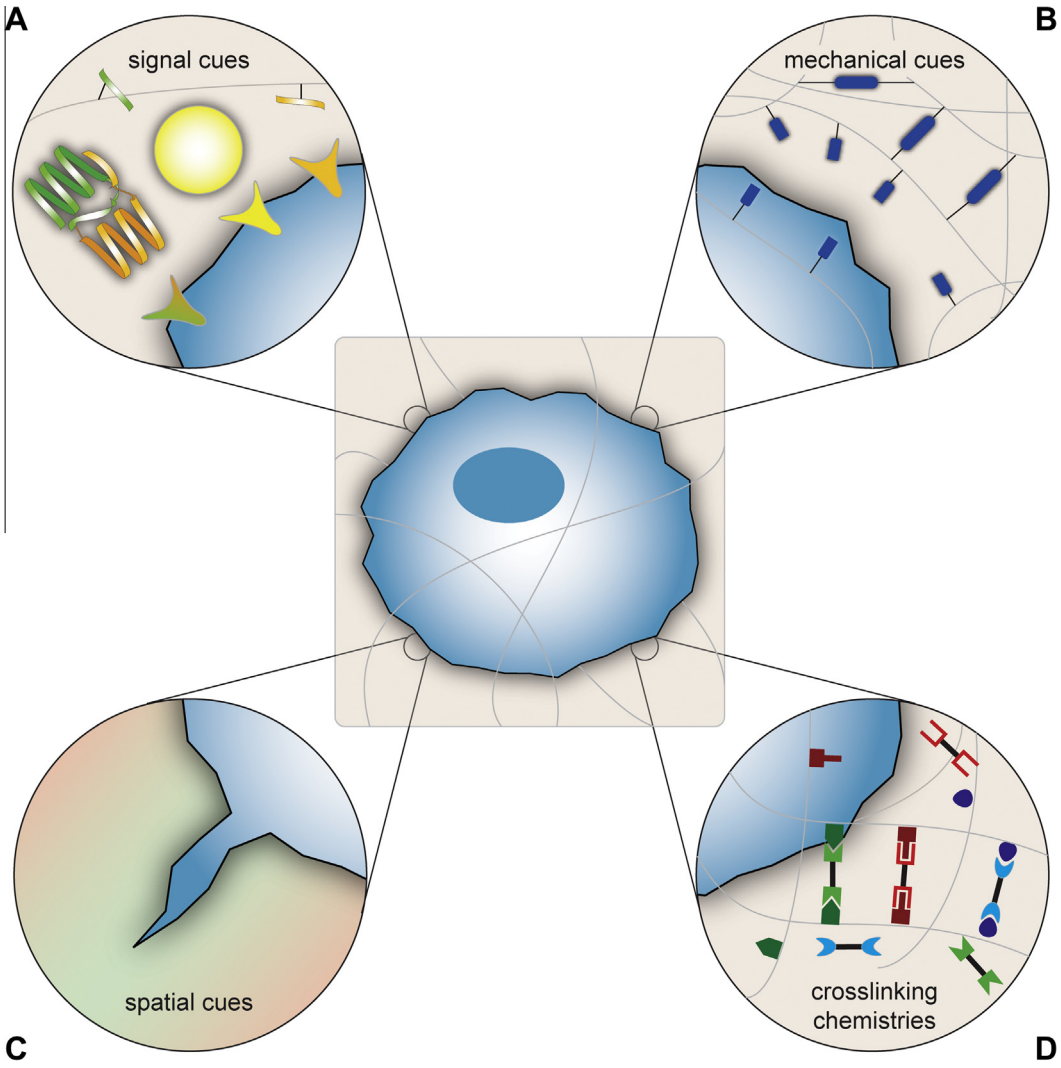

Fig. 1. Hyaluronic acid scaffolds can be engineered in several ways to control encapsulated cell behavior. (A) Bioactive signals can be incorporated into the scaffold in suspension or covalently bound to the polymer. (B) Tuning the mechanical properties by utilizing degradable crosslinkers and/or degree of crosslinking can regulate cell mechanosensing. (C) Spatial cues such as patterned bioactive signals, porosity control and topographical patterns can direct cell migration. (D) New "click chemistries" have been employed to provide new ways to covalently crosslink the hydrogel network.

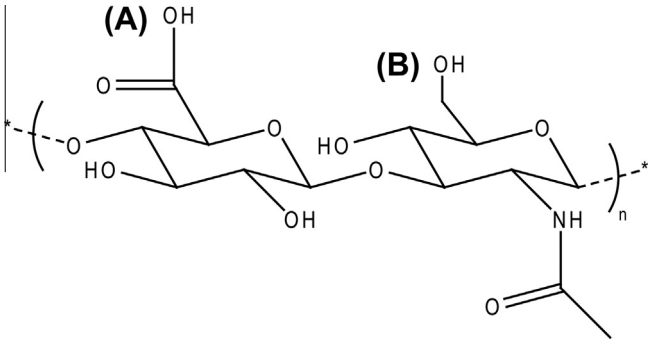

Fig. 2. Structure of hyaluronic acid. Chemical modifications are commonly performed at (a) and (b).

behavior. In addition to inhibiting cell proliferation, high molecular weight HA is also anti-angiogenic [11]. In fetal development of rat follicles, HA acts as a high molecular weight barrier that blocks endothelial cell migration and subsequent angiogenesis. However, cleaving the polymer into shorter fragments with hyaluronidase enables endothelial cells to migrate and initiate angiogenesis [12]. These high molecular weight polymers are also anti-inflammatory and immunosuppressive [13,14]. This feature may be particularly important as the amniotic fluid of fetuses has high concentrations of high molecular weight HA. In contrast, low molecular weight HA ($<3.5 \times 10^4$ Da) has been implicated as pro-inflammatory and pro-angiogenic [15]. In particular, oligomers of the 6–20 kDa range activate antigen-presenting cells, such as dendritic cells, while slightly larger HA (2×10^4–4.5×10^5 Da) stimulates inflammatory cytokines [16]. In addition, fragments consisting of 4–25 disaccharide units are able to promote blood flow to injury sites and subsequent angiogenesis [11,15,17].

On the molecular level, HA primarily interacts with the cell-surface receptor cluster determinant 44 (CD44) and the receptor for hyaluronate-mediated motility (RHAMM). CD44 plays a key role in tissue organization by mediating ECM remodeling, cell–cell interactions and cell–matrix interactions [18–19]. A normal wound healing model in CD44-knockout mice exhibited decreased cell migration, motility and ECM turnover [20]. Interactions between HA and RHAMM can result in a variety of downstream signaling pathways that affect protein kinases such as focal adhesion kinases, MAP kinases, phosphatidylinositol kinases, and tyrosine ki-