Available online at www.sciencedirect.com
ScienceDirect
Advanced Drug Delivery Reviews 59 (2007) 1351 – 1365
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

# Hyaluronan-dependent pericellular matrix ☆

Stephen P. Evanko [a], Markku I. Tammi [b], Raija H. Tammi [b], Thomas N. Wight [a,*]

[a] *The Hope Heart Program, Benaroya Research Institute at Virginia Mason, 1201 9th Avenue, Seattle, WA 98101, USA*
[b] *Department of Anatomy, Kuopio University, FIN-70211 Kuopio, Finland*

Received 17 April 2007; accepted 1 August 2007
Available online 14 August 2007

**Abstract**

Hyaluronan is a multifunctional glycosaminoglycan that forms the structural basis of the pericellular matrix. Hyaluronan is extruded directly through the plasma membrane by one of three hyaluronan synthases and anchored to the cell surface by the synthase or cell surface receptors such as CD44 or RHAMM. Aggregating proteoglycans and other hyaluronan-binding proteins, contribute to the material and biological properties of the matrix and regulate cell and tissue function. The pericellular matrix plays multiple complex roles in cell adhesion/de-adhesion, and cell shape changes associated with proliferation and locomotion. Time-lapse studies show that pericellular matrix formation facilitates cell detachment and mitotic cell rounding. Hyaluronan crosslinking occurs through various proteins, such as tenascin, TSG-6, inter-alpha-trypsin inhibitor, pentraxin and TSP-1. This creates higher order levels of structured hyaluronan that may regulate inflammation and other biological processes. Microvillous or filopodial membrane protrusions are created by active hyaluronan synthesis, and form the scaffold of hyaluronan coats in certain cells. The importance of the pericellular matrix in cellular mechanotransduction and the response to mechanical strain are also discussed.


*Keywords:* Proteoglycan; Hyaluronan; Versican; Aggrecan; CD44; RHAMM; Mechanotransduction; Cell adhesion; Cell traction

**Contents**



## 1. Introduction

Hyaluronan, or hyaluronic acid, is a multifunctional glycosaminoglycan that forms the basis of the pericellular matrix. Hyaluronan is a linear polymer composed of repeating disaccharides of glucuronic acid and *N*-acetylglucosamine [-β(1,4)-

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Natural and Artificial Cellular Microenvironments for Soft Tissue Repair".

* Corresponding author. Tel. +1 206 341 1377; fax: +206 341 1370.
*E-mail address:* twight@benaroyaresearch.org (T.N. Wight).

0169-409X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2007.08.008

GlcUA-β(1,3)-GlcNAc-]$_n$, and is synthesized by 3 different but related hyaluronan synthases, HAS1, HAS2 and HAS3 [1,2]. These are enzymes with multiple transmembrane domains that synthesize hyaluronan at the inner surface of the plasma membrane. During synthesis, the growing polymer chain is extruded through the membrane into the pericellular space. This is in contrast to the mode of synthesis of other glycosaminoglycans, which are made and covalently linked to core proteins in the Golgi apparatus to make a proteoglycan, and secreted by normal exocytotic mechanisms. Hyaluronan chains can be anchored to the cell surface *via* the synthase enzyme or through binding to a cell surface receptor such as CD44 or RHAMM (receptor for hyaluronic acid mediated motility). Hyaluronan is cleaved by one of several hyaluronidases. There are six hyaluronidase genes in humans, encoding enzymes with different properties and different cell locations [3]. Under normal physiological conditions, hyaluronan ranges in relative molecular mass from $10^6$–$10^7$ (~2000–25,000 disaccharides) with polymer lengths of 2–25 μm (see review by Toole [4]). Hyaluronan is capable of an amazing variety of conformations when deposited on mica surfaces; from extended chains, to relaxed coils, to condensed rods, and pearl necklaces of helical coils, rods, hairpins, and toroids [5]. Hyaluronan can also self-associate to form fibers, networks, and stacks. When retained at the cell surface, hyaluronan can form a voluminous pericellular matrix or "coat", which has also been termed "glycocalyx". The hyaluronan-dependent coat has multiple important roles, from serving structural and mechanochemical functions, to the regulation of cell division and motility, as well as cancer progression and metastasis. This review will discuss various aspects of hyaluronan-dependent pericellular matrix structure and function.

## 2. Pericellular matrix structure

Several studies have investigated the structure and formation of the pericellular matrix. One of the most widely used techniques to view the hyaluronan-dependent pericellular matrix is the particle exclusion assay, which was first utilized nearly forty years ago [6]. In this assay, a suspension of particles, usually fixed erythrocytes, is allowed to settle and a clear zone surrounding the cell is made apparent by virtue of the exclusion of the red blood cells by the gel-like hyaluronan coat (Fig. 1). Treatment of cells with hyaluronan-specific *Streptomyces* hyaluronidase removes the pericellular coat, indicating that matrix integrity is hyaluronan-dependent.

Since the thickness of the hyaluronan coat often exceeds 20 μm, which roughly corresponds to the extended length of a single high molecular mass hyaluronan, it is obvious that there must be a way to stretch out the chains, rather than allow their random coil formation close to the cell surface. This notion is in line with the fact that the pericellular coat in many cells requires an aggregating proteoglycan, such as aggrecan or versican, in order to exclude erythrocytes in the particle assay [7]. The repulsion between the highly charged chondroitin sulfates in these proteoglycans apparently forces the perpendicular, extended state of cell surface hyaluronan, and results in the formation of a thick coat. The aggregating proteoglycans interact with

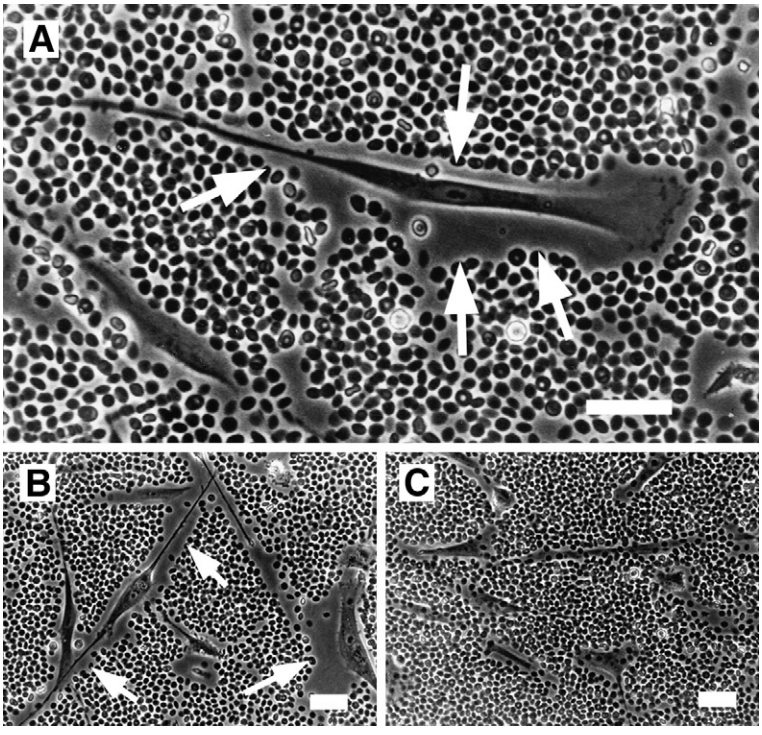


Fig. 1. Hyaluronan-dependent pericellular matrix in human smooth muscle cells visualized using the particle exclusion assay. The cell coat excludes the fixed erythrocytes and is seen as a clear zone surrounding the cell (arrows). A. A typical locomoting cell with a small amount of pericellular matrix at the lammellipodium in front and more abundant matrix along the cell flanks and trailing uropod. B, C. Pericellular matrices were visualized before, B, or after, C, digestion with *Streptomyces* hyaluronidase. Bars equal 50 μm. Panel A originally published in: S. Evanko, J. Angello, T. Wight, Formation of hyaluronan and versican rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol., 1999, 19(4):1004–1013. Used with permission.