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Adhesion, phenotypic expression, and biosynthetic capacity of corneal keratocytes on surfaces coated with hyaluronic acid of different molecular weights

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ABSTRACT

In ophthalmology, hyaluronic acid (HA) is an important extracellular matrix (ECM) component and is appropriate for use in generating a microenvironment for cell cultivation. The aim of this work was to evaluate the rabbit corneal keratocyte (RCK) growth in response to HA coatings under serum-free conditions. After modification with HA of varying molecular weights (MWs: 35–1500 kDa), the surfaces were characterized by atomic force microscopy and contact angle measurements, and were used for cell culture studies. Our data indicated that the substrates coated with higher negatively charged HA become rougher and are more hydrophilic, resulting in the decrease of cell adhesion and cell–matrix interaction. This early cellular event was likely responsible for the determination of keratocyte configuration. Additionally, for the growth of RCKs on dry HA coatings with surface roughness of 1.1–1.7 nm, a strong cell–cell interaction was observed, which may facilitate the formation of multicellular spheroid aggregates and maintenance of mitotically quiescent state. At each culture time point from 1 to 5 days, a better biosynthetic capacity associated with a higher prevalence of elevated ECM production was found for the cells in a spherical configuration. Irrespective of polysaccharide MW of surface coatings, the RCKs presented good viability without hypoxia-induced death. As compared with a monolayer of adherent keratocytes on tissue culture polystyrene plates and low MW HA-modified samples, the cell spheroids (76–110 μm in diameter) showed significantly higher expressions of keratocan and lumican and lower expressions of biglycan, similar to those of keratocytes in vivo. Moreover, the expression levels of corneal crystallin aldehyde dehydrogenase (7–9-fold increase) and nestin (10–16-fold increase) were greater in larger-sized spheroids, indicating higher ability to maintain cellular transparency and self-renewal potential. It is concluded that the cultured RCKs on surfaces coated with HA of different MWs can sense ECM cues, and the multicellular spheroids may potentially be used for corneal stromal tissue engineering applications.

1. Introduction

Tissue engineering represents a novel technology for the creation of functional living replacements by cultivating cells on biomaterial scaffolds in combination with signaling cues [1]. As an extracellular matrix (ECM) molecule during tissue repair, biomaterial can provide a template for cell growth and organization. Over the past few years, the biomaterial coatings have extensively been investigated in the field of corneal regenerative medicine. After surface modification with collagen, the poly(2-hydroxyethyl methacrylate) membranes become favorable for corneal epithelial migration and stratification [2]. Moreover, we, as others, have shown that the development of poly(*N*-isopropylacrylamide)-based artificial ECM is beneficial to establish a smart cell culture platform [3,4]. By means of temperature-induced cell adhesion and detachment, the bioengineered corneal epithelial and endothelial sheets are successfully fabricated to improve the therapeutic efficacy of cell grafts. These findings suggest the crucial roles of biomaterial coatings in the modulation of cell behavior and regeneration of tissue function.

It is known that the cell–material interaction is a complex multi-step process consisting of early events, such as cell adhesion and spreading, and late events, such as cell proliferation, differentiation, and functioning [5]. Several surface properties of culture substratum, including hydrophilicity [4] and nanotopography [6], are important to determine these cellular responses, given that the cells can recognize ECM molecules via cell surface receptors. In

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