Structuration and improvement of the resistance to culture medium of a modified photocrosslinkable glycosaminoglycan

Mª Carmen Márquez-Posadas[1], J. Ramiro[1], S. Merino[1], J. Becher[2], R. Wyrwa[2], M. Schnabelrauch[2], S. Rapoport[3], B. Castro[3]

[1] TEKNIKER-IK4, Micro and Nano Manufacture Unit. Avda. Otaola, 20. 20600 Eibar, Spain.
[2] INNOVENT e.V., Biomaterials Department. Prüssingstraβe 27 B. D-07745 Jena, Germany.
[3] HISTOCELL. Parque Tecnológico de Bizkaia 800, 2º. 48160 Derio, Spain.
smerino@tekniker.es

Abstract

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to the development of biological substitutes that restore, maintain, or improve tissue function. In tissue engineering approaches, an exogeneous three-dimensional polymer matrix is critical to the success of the approach. The material should perform as a vehicle to transport the cells and bioactive molecules to the desired sites in the body, as a synthetic extracellular matrix (ECM) to regulate the function of cells, or as a template to guide the growth of new tissues. For this purpose, a variety of biodegradable synthetic or naturally derived polymers, such as polysaccharides, have been employed to date [1]. The materials utilized in tissue engineering can be processed into various physical forms, including hydrogels. Hydrogels are hydrophilic cross-linked materials that are insoluble, but that can absorb large quantities of water and are capable of swelling in aqueous solutions. Hydrogels based on polysaccharides have many advantageous features: they are mainly composed of water, they have a structural similarity to the normal ECMs of tissues and often exhibit good biocompatibility and low toxicity. In particular, photopolymerized hydrogels are very promising materials for tissue engineering due to their high water content and tissue-like elastic properties.

Progress in both micro and nanofabrication has powered the field of tissue engineering in many aspects. One of these aspects is the study the responses to micro and nanopatterned topographical and biochemical cues in terms of cell morphology and functionality. It is well known that the architecture of the extracellular environment influences cell behavior with respect to morphology, cytoskeletal structure and biofunctionality [2]. Thus, substrates with different architectures can serve as platforms to study the effects of cell-substrate interaction on cell adhesion, orientation, migration and differentiation. As pointed before, one of the main features of hydrogels is that they must be insoluble in water, and in the case of cell differentiation and proliferation studies, they must resist immersion in a cell culture medium. In this study, the main objective was to find a method to improve the stability to a cell culture medium of microstructured hydrogels based on a modified photocrosslinkable glycosaminoglycan (GAG), methacrylated hyaluronan (m-HA, with high molecular weight and DS_{MA} = 0.61), at the same time as the structuration quality is maintained.

Microstructuring of hydrogels was carried out combining soft-lithography and photo-crosslinking over silicon substrates treated with 3-(trimethoxysilyl) propyl methacrylate, in order to have a good adhesion of the hydrogel. The PDMS stamps used for microstructuration had grooves of 2, 25, 50 and 100 μm width and 230 nm depth. Two different photoinitiation systems were used, one based on Irgacure 369 for curing under UV light and other based on eosin-Y for curing under visible light.

Two different approaches were used to increase the stability of the structured hydrogel to a cell culture medium. The first approach involved the addition of a crosslinking agent, triethyleneglycol dimethacrylate, in order to increase the crosslinking density, and thus, the rigidity of the hydrogel. The second approach involved blending (75:25 ratio) the modified GAG of interest with another modified polysaccharide, methacrylated dextran (m-Dx), whose hydrogels are known to swell less and to have higher rigidity. Both approaches showed a good microstructuration quality, as can be seen in Figure 1.

After structuration, hydrogels were sterilized using UV light for 10min. Then, hydrogels were immersed in culture medium, containing phenol red, L-Glutamine fetal bovine serum (10%), physiological levels of salt and water and they were kept in an incubator for 24 hours at 37°C in 5% CO2. Afterwards, the hydrogels were characterized in immersion using a microscope, and whereas the hydrogels composed exclusively of m-HA had lost microstructuration (only a layer of the material remained on the substrate), the microstructuration remained visible both in the m-HA with the additional crosslinking agent and, less clearly, probably due to a higher swelling degree, in the m-HA blended with m-Dx, as can be seen in Figure 2.

References

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Figures

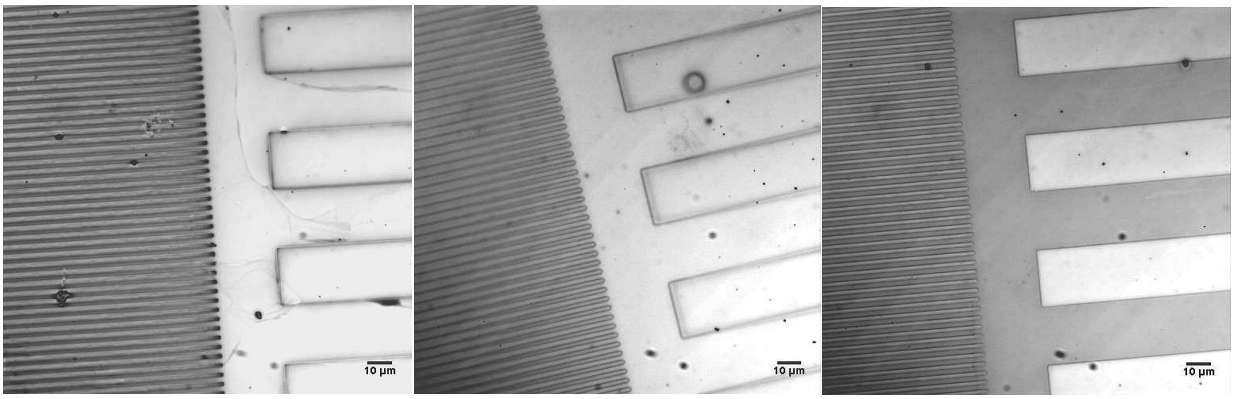

Figure 1. Microstructured m-HA cured with UV light (left), m-HA with additional crosslinker cured with visible light (center) and m-HA blended with m-Dx cured with UV light (right)

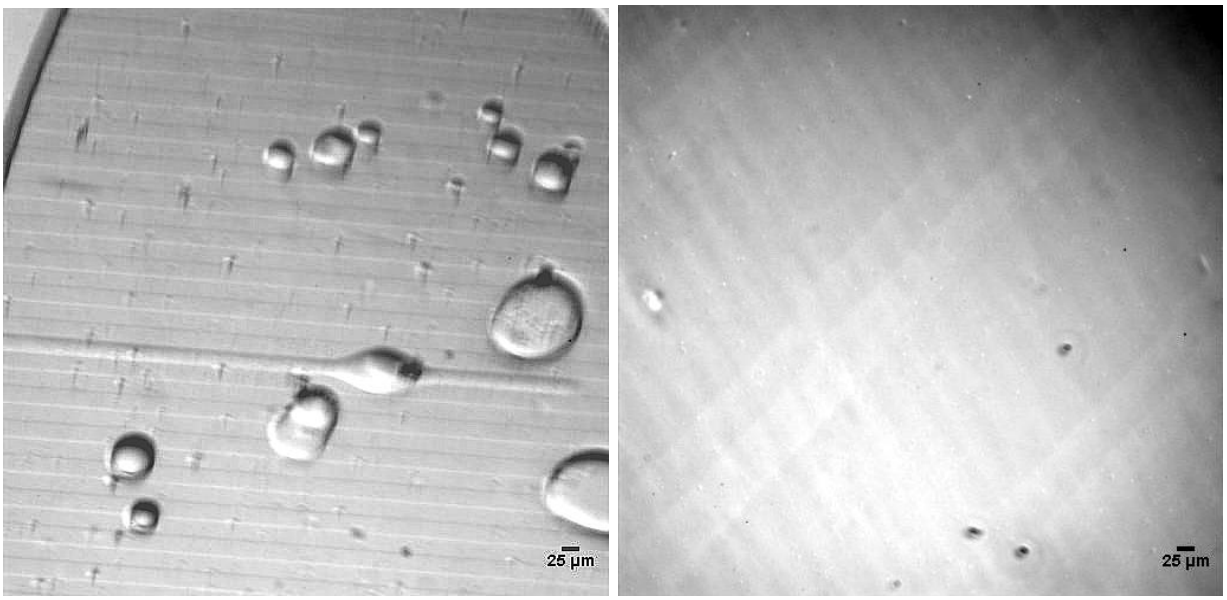

Figure 2. Photographs after 24 h immersion in cell culture medium. Left, microstructured m-HA with additional crosslinker; right, microstructured m-HA blended with m-Dx