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Property peculiarities of the atelocollagen–hyaluronan conjugates crosslinked with a short chain di-oxirane compound

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ABSTRACT

Minimal amounts of a short-chain bifunctional crosslinker of about 1.3 nm length, the 1,4-butanediol-diglycidyl ether (BDDGE), were used to generate atelocollagen–hyaluronan conjugates in hydrogel state. Two a priori constraints were considered in recipe/procedure developing: (i) working in nondenaturing conditions, and (ii) ensuring a low cytotoxicity of the final product. Both atelocollagen (aK) and hyaluronan (NaHyal) were accurately purified to reduce their molecular-weight dispersity, in order to ensure the reproducibility of hydrogels characteristics. 1:5 aK:NaHyal weight ratios and 1:2.5 to 1:5 α-NH_2:BDDGE molar ratios were found to be the most favorable recipe prescriptions that allow the obtaining of rheo-mechanically stable hydrogels, able to be manipulated during cell culturing protocols. Experiments revealed two unexpected effects due to the crosslinking reactions mediated by a short-chain molecule: (i) the occurrence of two thresholds in the rheological behavior of the hydrogels, related with the amount of added crosslinker, and (ii) a quasi-denaturation side-effect induced over the protein component by large or in excess amounts of crosslinker.

1. Introduction

Although tempting in terms of availability, reproducibility, and ability to be chemically designed, synthetic polymers are still not exclusively used in scaffolding realm. Actually, biomacromolecules are increasingly preferred to generate extracellular matrix (ECM) substitutes [1], especially because they are native carriers of biochemical cues and recognition domains, which guide the cell life and evolution.

The main issue in tissue engineering is to gradually mimic the chemo-, morpho- and bio-peculiarities of the extracellular matrix. To provide high levels of chemo-mimicry, it is desirable to produce scaffolds by using organotypic native biomacromolecules, and, if any, as low as possible quantities of synthetic polymers, or alien natural biopolymers. The "deconstruction and rebuilding approach" in biomacromolecule-based scaffold manufacturing faces the need of inducing crosslinking bridges, as the only way to reduce the solubility of the components, and to consolidate the structural morphology of the new edifice. When different types of biomacromolecules are used, and if covalent bridges are generated in a quasiregular manner, from the chemical point of view, the resulted scaffold represents a conjugate [2]. If biomacromolecule species interact in a "scrambled self-assembling" way, exclusively by physical-type bridges, the resulted product represents an aggregate, and if the bridges that irregularly connect their "scrambled" chains are of covalent type and have a non-zero length, a network results [3]. Unless special precautions are taken, a non-reproducible mixture of conjugate–aggregate–network type is obtained when covalent crosslinking is induced in biomacromolecule blends. In order to ensure the tissue engineering applicability of the mentioned crosslinking products, a prevailing conjugate nature must be provided to the crosslinked final product (usually of hydrogel type, but also in the form of cryo- and vitri-gel).

Biomacromolecule conjugation provides complex structures having combined properties of the components, offering a broad versatility in ECM chemo-mimicking attempts. Scleroprotein and glycans are the preferred candidates in producing applicable ECM substitutes of conjugate-type [4], especially if the crosslinkers are short-chain bifunctional molecules. Two biomacromolecular precursors, atelocollagen (aK) and hyaluronan (NaHyal), both in quasi-native states, possess some peculiarities that make them ideal candidates for ECM chemo-mimicking. Atelocollagen bears sterically exposed GFOGER cell-recognition domains [5,6], and it is available in large enough quantities. Hyaluronan is ubiquitous in animal connective tissues, is significantly involved in water homeostasis at ECM level [7], and acts as both structural and signaling molecule [8].

Transglutaminase-induced zero length crosslinking bridges [9] are of interest in producing protein-based scaffolds because they do not

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