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Engineering preliminaries to obtain reproducible mixtures of atelocollagen and polysaccharides

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ABSTRACT

The critical stage in producing blends of biomacromolecules consists in the mixing of component solutions to generate homogenous diluted colloidal systems. Simple experimental investigations allow the establishment of the design rules of recipes and the procedures for preparing homogenous and compositionally reproducible mixtures. Starting from purified solutions of atelocollagen, hyaluronan and native gellan, *having as low as possible inorganic salts content*, initial binary and ternary mixtures can be prepared up to a total dry matter content of 0.150 g/dL, in no co-precipitating conditions. Two pH manipulation ways are feasible for homogenous mixing: (i) unbuffered prior correction at pH 5.5, and (ii) "rigid" buffering at pH 9.0, using organic species. Atelocollagen including co-precipitates can be obtained in the presence of one or both polysaccharides, preferably in pH domains far from the isoelectric point of scleroprotein. A critical behavior has been observed in mixtures containing gellan, due to its macromolecular dissimilarities compared with atelocollagen. In optimal binary mixtures, the coordinates of threshold points on the phase diagrams are 0.028% w/w atelocollagen/0.025% w/w hyaluronan, and 0.022% w/w atelocollagen/0.020% w/w gellan. Uni- or bi-phasic ternary systems having equilibrated ratios of co-precipitated components can be prepared starting from initial mixtures containing up to 0.032 g/dL atelocollagen, associated with, for example, 0.040 g/dL hyaluronan and 0.008 g/dL gellan, following the first pH manipulation way.

1. Introduction

Reproducibility, seen as the batch-to-batch and piece-to-piece variability of the values of products properties and characteristics, represents one of the main issues in engineering. In the particular case of biomaterials and scaffolds, ensuring reproducible reactivity and internal morphology holds a major place on the practitioners' agenda. Synthetic biomaterials provide the ability to rigorously design and ex-vivo "construct" substrata having reproducible physical–chemical and structural properties [1], but their similarity with natural extracellular matrix (ECM) is still humble comparing to biological-origin materials and tissue-derived structures or macromolecules [2], and do not support the attempts to biomimic tissue environments. This is why biomacromolecule bearing cell recognition domains are preferred [3], fibrillar collagen being one of them [4]. An important drawback of biomacromolecules (especially of proteins, polysaccharides and their conjugates) consists in their limited reciprocal compatibility in aqueous colloidal mixtures, above a certain concentration. Either segregative or associative separation occurs, in an uncontrolled manner, due to thermodynamic incompatibility, or to electrostatic driven coacervation, respectively [5]. Hence, the reproducibility of the compositional characteristics of biomacromolecule mixtures remains poor, and the predictability of those characteristics must be investigated, prior to any studies on their colloidal dispersions, blends or composites.

The present paper aims to describe a set of simple techniques, able to provide the feasible weight ratios of biomacromolecules that can be mixed, together with the mixing parameters, in order to ensure an intimate and reproducible blending, before any further processing of the blends. The pragmatic target is to obtain long term stable fluid blends of a quasi-native scleroprotein, the atelocollagen (*aK*), together with two types of polysaccharide: a mammalian tissue-derived one, the hyaluronan sodium salt (*NaHyal*), and a bacterial biosynthesized one, the native gellan (*Gellan*). Such blends are susceptible to generation of cyto-friendly substrata that benefit of the peculiarities of individual components: the GFOGER cell-recognition domain of atelocollagen [4,6], the support for cell proliferation and migration given by hyaluronan [7], and the ability to reconstitute extended three-dimensional networks, even at low temperatures, provided by the high acyl gellan [8]. Starting from ternary blends of the above mentioned biomacromolecules, various porous forms of dry and controlled rehydrated scaffolds can be obtained, such as cryo- and vitri-gels, which can be post-processed to generate spatial shapes. If covalent cross-linking bridges are induced between the blended macromolecules, matrices with limited swellability can be also

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