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Crosslinked hydrogels based on biological macromolecules with potential use in skin tissue engineering

Raluca Vulpe [a,c], Marcel Popa [a,f,*], Luc Picton [b,c,d], Vera Balan [e], Virginie Dulong [b,c,d], Maria Butnaru [e], Liliana Verestiuc [e]

[a] "Gheorghe Asachi" Technical University, Faculty of Chemical Engineering and Environmental Protection, Department of Natural and Synthetic Polymers, 73 Prof. dr. docent Dimitrie Mangeron Street, 700050 Iasi, Romania
[b] Normandie Université, France
[c] Université de Rouen, Laboratoire Polymères Biopolymères Surfaces, F-76821 Mont Saint Aignan, France
[d] CNRS UMR 6270 & FR3038, F-76821 Mont Saint Aignan, France
[e] "Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Medical Bioengineering, Department of Biological Sciences, 9-13 Kogalniceanu Street, 700454 Iasi, Romania
[f] Academy of Romanian Scientist, 54 Splaiul Indenpendentei Street, 050094 Bucuresti, Romania

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ABSTRACT

Zero-length crosslinked hydrogels have been synthesized by covalent linking of three natural polymers (collagen, hyaluronic acid and sericin), in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide. The hydrogels have been investigated by FT-IR spectroscopy, microcalorimetry, *in vitro* swelling, enzymatic degradation, and *in vitro* cell viability studies. The obtained crosslinked hydrogels showed a macroporous structure, high swelling degree and *in vitro* enzymatic resistance compared to uncrosslinked collagen. The *in vitro* cell viability studies performed on normal human dermal fibroblasts assessed the sericin proliferation properties indicating a potential use of the hydrogels based on collagen, hyaluronic acid and sericin in skin tissue engineering.

1. Introduction

Due to their ability to tailor or tune the mechanical surface and their regeneration features, protein based biomaterials, and especially hydrogels, became an important direction in the biomaterial area [1,2]. Among proteins, collagen (COL) provides a combination of strength and toughness required for specific tissue functions [1], meanwhile sericin (S), glue like protein from the silkworm cocoons of *Bombyx mori*, proved to be an important presence in the composition of collagen scaffolds, with stimulatory potential on cellular collagen production [3]. Sericin is a glycoprotein [4] that contains 18 amino acids, including essential amino acids [5] and a high content of hydrophilic amino acids, mainly represented by serine, glycine, histidine, glutamic acid, aspartic acid, threonine, and tyrosine [4,6]. It is important to mention that both amino acid composition and molecular weight distribution could be dependent on the extraction method used [7]. Due to its biological properties, sericin has been proposed as a versatile material especially for tissue engineering applications, as pointed out in several representative reviews [8–10].

Hyaluronic acid (HA) is a glycosaminoglycan with an important impact in the embryonic development, tissue organization and wound healing [11]. Thus, a combination of these three natural polymers can potentially lead to a hydrogel with excellent properties for tissue regeneration.

The formulation of the hydrogels could be a key factor for their biological performance, in terms of cell viability, proliferation or spreading. In this regard, several attempts to obtain tridimensional structures based on collagen and sericin have been proposed using crosslinkers like glutaraldehyde [12–14] or genipin [15,16]. However, the hydrogels obtained by these methods displayed a certain amount of cytotoxicity. Encouraging results have been reported by Dinescu et al. [17], who developed a 3D porous scaffold based on sericin and collagen, improved with prochondrogenic factors such

* Corresponding author at: "Gheorghe Asachi" Technical University, Faculty of Chemical Engineering and Environmental Protection, Department of Natural and Synthetic Polymers, 73 Prof. dr. docent Dimitrie Mangeron Street, 700050 Iasi, Romania.
E-mail address: marpopa@tuiasi.ro (M. Popa).

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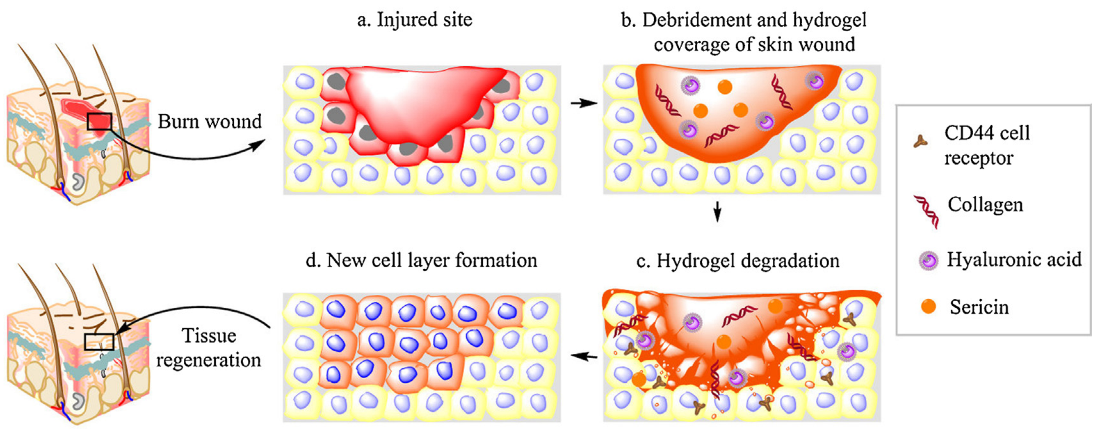

Fig. 1. Schematic representation of skin tissue regeneration steps.

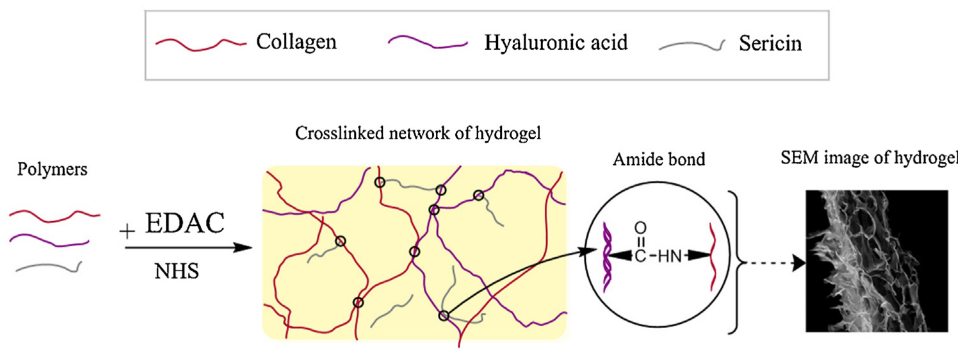

Fig. 2. Schematic representation of hydrogel preparation by carbodiimide crosslinking.

as chondroitin sulfate or hyaluronic acid intended for cartilage tissue engineering.

Collagen was first crosslinked with EDAC in 1995, by Damink et. al [18], which developed a new method for carboxylic groups activation. Likewise, collagen and hyaluronic acid were crosslinked *via* EDAC/NHS and the obtained biomaterials had different tissue engineering applications like: dermal reconstruction or adipose tissue engineering [19–21]. Due to its extraordinary physico-chemical and biological properties, such as gelling ability, moisture retention capacity and wound healing properties when used in creams [22,23], recently, sericin drew attention of the researchers.

In this context, this paper proposes the development of new hydrogels based on collagen, sericin and hyaluronic acid through the crosslinking of the natural polymers *via* carbodiimide chemistry, in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) and *N*-hydroxysuccinimide (NHS). This crosslinking method was preferred due to the biostability and mechanical properties of scaffolds provided by carbodiimides [23], EDAC crosslinking representing a way to manufacture stable hydrogels able to overcome the high degradability rate of native collagen [24]. EDAC is a carbodiimide, one of the zero-length crosslinkers, the smallest compounds that mediate the bonding of two molecules by forming a bond containing no additional atoms. The addition of NHS increases the stability of the intermediates formed and recent studies recommend the use of a co-solvent (*e.g.*, ethanol) to increase the solubility of NHS which is relatively water-insoluble [25].

The use of naturally derived materials has been considered vital in tissue engineering, especially in the development of artificial scaffolds for cell proliferation and differentiation, natural polymers providing an optimum micro medium for a better cell adhesion and tissue development. Due to their architectural context in which the extracellular matrix plays an important role in tissue regeneration, biomaterials represent the central components of many tissue engineering strategies [26].

Wound healing is a complex series of events that are interconnected and dependent on one another. Depending on the wound type, acute or chronic, different models of healing can be applied, with different methods of wound bed preparation. In Fig. 1 a schematic representation of skin tissue regeneration steps is depicted, when a hydrogel based on collagen, sericin and hyaluronic acid are used.

Briefly, the injured site of a burn wound must be first subjected to debridement, an important step for tissue regeneration. Debridement is defined as the removal of necrotic tissue and foreign material that prevents the wound from healing [27]. Afterwards, the hydrogel coverage of the skin wound will prevent water loss and through its biodegradation process, the released content of the hydrogel will stimulate the cell proliferation. CD44 is a broadly distributed cell surface protein that mediates the cell attachment to extracellular matrix and it is the principal cell surface receptor for hyaluronate [28]. Therefore hyaluronic acid plays an important role in tissue regeneration. On the other side, collagen is present in the natural extracellular matrix [26] and together with sericin and hyaluronic acid it can mimic some features of extracellular matrix and stimulate direct migration, growth and organization of cells during wound healing and tissue regeneration.

The aim of this study was to present the synthesis, characterization and preliminary biocompatibility evaluation of new porous hydrogel based on collagen, sericin and hyaluronic acid which combines the special abilities of collagen and hyaluronic acid with the remarkable properties of sericin in order to fully exploit their potential for further skin tissue engineering.