Materials Science and Engineering C 68 (2016) 317–326

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

A novel smart injectable hydrogel prepared by microbial transglutaminase and human-like collagen: Its characterization and biocompatibility

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ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 23 April 2016
Accepted 23 May 2016
Available online 25 May 2016

Keywords:
Hydrogels
Microbial transglutaminase
Human-like collagen
Injectable
Characterization
Biocompatibility

ABSTRACT

Various tissue scaffold materials are increasingly used to repair skin defects by cross-linking because of the ability to fill and implant in any form via operation. However, crosslinker residues cannot be easily removed from scaffold materials prepared by chemical crosslinking methods, limiting their use for skin tissue engineering. Here, microbial transglutaminase (MTGase), a nontoxic crosslinker with high specific activity and reaction rate under mild conditions, was employed crosslinks in human-like collagen (HLC) to yield novel smart MTGase crosslinked with human-like collagen (MTGH) hydrogels, which are sensitive to temperature and/or enzymes. Various ratios of MTGase/HLC were performed, and their physicochemical properties were characterized, including the swelling ratio, the elastic modulus, the morphology and the porosity. The degradation behavior and mechanism of MTGase in concentration-dependent manner involved in formation hydrogels were identifying *in vitro*. The cell attachment *in vitro* and biocompatibility *in vivo* were also investigated. The results demonstrated that the use of different concentrations of MTGase to crosslink HLC produced products with different degradation times and biocompatibilities. The 50 U/g MTGase-prepared MTGH hydrogels had a higher density of crosslinks, which made them more resistant to degradation by collagenase I and collagenase II. However, 40 U/g MTGase-prepared MTGH hydrogels were more suitable for cell attachment. In addition, compared with the Collagen Implant I® (SUM) used in animal experiments, the 40 U/g MTGase-prepared MTGH hydrogels had a lower toxicity and better biocompatibility. Therefore, 40 U/g MTGase crosslinked with HLC should be used to prepare MTGH hydrogels for potential application as soft materials for skin tissue engineering.

1. Introduction

Skin is the largest organ in the human body. Due to its significant clinical need, convenient access, and thin construction, skin has been at the forefront of engineered tissues. Research into bioengineered skin over the past 30 years has yielded a number of commercial bovine spongiform encephalopathy (BSEs) [1]. However, none of these products meet the criteria for fully functional skin [2], and approximately half have been approved by the FDA for various indications. Thus, tissue engineering of artificial skin requires a biocompatible, non-immunogenic, degradable scaffold as well as cells that support tissue repair and function. Hydrogels have three-dimensional elastic structural networks that absorb and store large amounts of water and are an interesting topic in materials science or skin tissue engineering [3]. "Smart" hydrogels that are sensitive to external stimuli such as temperature, pH, electric fields, magnetic forces, light, and enzymes can actively react to environmental changes. Meanwhile, smart hydrogels, as homogeneous materials, could mimic scaffolds to restore skin tissue [4] or be used for skin tissue engineering [5] as a soft-tissue filling in defective tissue [6]. Conductive polymer hydrogels (such as polyaniline (PANI) or polypyrrole (PPy)) have been introduced into a widely studied thermally responsive hydrogel, poly (*N*-isopropylacrylamide) (PNIPAM), and have led to the development of conductive smart gels (PNIPAM/PANI) that are sensitive to temperature change [7]. They are also promising materials for use in drug delivery and adhesion [8]. However, its non-degradable or toxicity may be limited used in skin tissue restoration. So, exploit smart hydrogels for application in skin is necessary.

Hydrogels are biomacromolecules and degradable materials made from polysaccharide and protein raw materials such as collagen,

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http://dx.doi.org/10.1016/j.msec.2016.05.108
0928-4931/

chitosan, alginate, and hyaluronan, or other synthetic materials such as polyacrylamide, polylactic acid and poloxamer [9]. Collagen is a particularly promising biomaterial that is the major constituent of extracellular matrices (ECM) of interstitial tissues, including skin, bone, cartilage, tendons, and ligaments. As a result of its excellent biocompatibility and natural biodegradability, collagen has been widely used as a scaffold in skin and bone tissue engineering [10]. Commercially available collagen, usually obtained from buffalo and hide bones, has a weak mechanical performance and is potentially subject to rejection, which restricts its application as a biomaterial [11]. Human-like collagen (HLC) is highly expressed by recombinant *Escherichia coli* BL21 containing a partial cDNA clone obtained from human collagen mRNA [12,13]. Due to its water solubility, low immunogenicity, good biocompatibility and biodegradability, HLC has become a novel biomaterial used in studies of artificial bones [14] and vascular scaffolds [15]. Owing to a lack of mechanical strength and sensitivity to *in vivo* enzymes, the biomedical applications of collagen are seriously limited. Therefore, it is essential to stabilize the collagen configuration to augment its physical performance and to strengthen its resistance to enzyme attack. To achieve this goal, crosslinks are usually introduced by physical or chemical crosslinking methods. However, physical methods (dehydrothermal drying (DHT) or UV-irradiation *etc.*) create weak bonds that have a high risk of degradation, so such hydrogels are more sensitive to hydrolysis [16]. Chemical crosslinking with a variety of agents is currently the most commonly used crosslinking method to fabricate collagen with improved physical properties. The crosslinkers glutaraldehyde, formaldehyde and EDC have been extensively discussed [17–19]. These agents enhance the mineralization or stiffness of the tissue, but are often toxic compounds to have to be remove/extracted from the hydrogels before they can be applied biomedically [3]. Recently, the use of enzymes as crosslinkers has drawn more interest, mainly because of the mildness of enzymatic reactions under normal physiological conditions and their high efficiency, selectivity and nontoxicity [20,21].

Transglutaminases (TGases) are a group of enzymes (EC 2.3.2.13) that catalyze an acyl-transfer reaction between the γ-carboxamide group of a protein or peptide-bound glutamine and the ε-amino group of a lysine residue, forming a relatively protease-resistant intermolecular or intramolecular ε-(γ-glutamyl)lysine isopeptide bond [22]. Moreover, they can covalently attach the primary amine in a compound to the peptide bond in glutamine to modify the physical, chemical and biological properties of a protein [23]. Therefore, they have been used commercially and in research in many different processes [24]. As a biocatalyst, the extracellular enzyme microbial transglutaminase (MTGase) shows broad substrate specificity and is also active over a broad range of pH values and temperatures [25]. It has been used to catalyze macromolecular grafting and to crosslink proteins [24]. MTGase has also been used to crosslink collagen matrices, raising their denaturation temperatures [26] and to combine cell adhesion factors within a hydrogel, leading to enhanced cell proliferation [27]. However, the degradation and biocompatibility of microbial transglutaminase-crosslinked collagen matrices need to be improved and optimized. Because HLC has the characteristic features of water solubility, low immunogenicity, good biocompatibility and biodegradability, crosslinking human-like collagen with microbial transglutaminase could improve its biodegradation resistance and the *anti*-inflammation properties of the resulting hydrogel.

In this study, our main objective was to prepare smart injectable MTGH hydrogels using HLC crosslinking by MTGase. Microbial transglutaminase was used as the biocatalyst and crosslinker. Properties such as the physical/chemical features, the degradation rate, cell adhesion, cytotoxicity and histocompatibility of the resulting hydrogels were analyzed. Our study sheds light on the degradation and crosslinking MTGase interaction of human-like collagen regarding its role in smart filling *in vivo* as well as their possible applications for tissue engineering of skin.

2. Materials and methods

2.1. Materials

Human-like collagen (HLC) [12,13] was provided by our laboratory. Microbial transglutaminase was purchased from Shanghai yuanye Bio-Technology Co., Ltd. Rabbits as an animal model were obtained from Xi'an Jiaotong University, and all other solvents and reagents were of analytical grade.

2.2. Preparation of the MTGH hydrogel

HLC was dissolved in pH 6 PBS buffer to prepare a 10% (w/v) solution, and then a specific amount of MTGase (20, 30, 40, 50, or 60 U/g HLC) was added to the solution (Table 1). After immediate and complete mixing, the mixtures were incubated at 37 °C for 12 h, and then transferred to 4 °C for another 12 h to consolidate. Finally, the prepared gels were placed at 90 °C for 5 min to stop the enzymatic reaction.

2.3. Characterization of the MTGH hydrogels

2.3.1. Gelling time measurement

The gelling time of hydrogels was measured by the tube inversion method at constant temperature. A specific amount of MTGase was added to centrifuge tubes filled with prepared HLC solutions at different ratios of MTGase/HLC and mixed completely. Then, the tubes were put into a water bath at 37 °C until the gel solution stopped flowing. The time for this process to occur was taken as the gelling time.

2.3.2. Swelling measurement

The swelling ratios of the hydrogels were determined by the classical gravimetric method. Dried gels were put into PBS buffer (pH 7.4) at 37 °C. The gels were weighed at various times until the swelling equilibrium was reached. The time to reach the swelling equilibrium was used to calculate the swelling ratio (SW) according to following equation:

$$SW = (W_s - W_d)/W_s$$

where W_s and W_d represent the weights of the swollen HLC hydrogel and the dried HLC hydrogel, respectively. To study the effect of temperature on the swelling equilibrium of the hydrogels, dried gels were put into PBS (pH 7.4) and ultrapure water at 37 °C and 4 °C until the swelling equilibrium was attained.

2.3.3. Compression experiments

Compression experiments were performed with a gel strength instrument (Electronic Universal Testing Machine). First, the height of the compression plate was set to 10 mm and the ultima height was set to 6 mm. The disc of the instrument was circular with a diameter of 10 mm, and the compression speed was set to 2 mm/min. The gel was cut to a length of 10 mm and then put on the instrument disc. The test was stopped when the compression displacement reached 6 mm. The compression modulus and the relationship between the compression load and compression displacement were calculated. The

Table 1
Compositions of the MTGH hydrogels with different ratios of MTGase/HLC.

Hydrogels	The ratios of MTGase/HLC(U/g)	HLC (mL)[a]	MTGase(mg)
MTGH2	20	5	50
MTGH3	30	5	75
MTGH4	40	5	100
MTGH5	50	5	125
MTGH6	60	5	150

[a] HLC is 10 wt.% aqueous solution.