International Journal of Biological Macromolecules 70 (2014) 10–19

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Tailoring the properties of gelatin films for drug delivery applications: Influence of the chemical cross-linking method

P. Coimbra*, M.H. Gil, M. Figueiredo

CIEPQPF, Chemical Engineering Department, University of Coimbra, Polo II, 3030-290 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form 5 June 2014
Accepted 10 June 2014
Available online 24 June 2014

Keywords:
Gelatin films
Chemical cross-linking
Carbodiimide
Methacrylic anhydride

ABSTRACT

Two types of chemically cross-linked gelatin films were prepared and characterized. The first type of films was cross-linked with 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (EDC) under heterogeneous conditions and are named Gel-E. In the second type of films, gelatin was previously functionalized with methacrylamide side groups by the reaction with methacrylic anhydride and for that is named Gel-MA. The modified gelatin was subsequently cross-linked by a photoinitiated radical polymerization.

These films were characterized relatively to their degree of cross-linking, buffer uptake capacity, resistance to hydrolytic and proteolytic degradation, and mechanical and thermal properties. Results show that the employed cross-linking method, together with the degree cross-linking, dictate the final properties of the films. Gel-E films have significant lower buffer uptake capacities and higher resistance to collagenase digestion when compared to Gel-MA films. Additionally, Gel-E films exhibit higher values of stress at break and lower strains at break. Moreover, the films properties could be modified by varying the extent of the chemical cross-linking, which in turn could be controlled by varying the concentration of EDC, for the first type of films (Gel-E), or by using gelatins with different degrees of functionalization, in the case of the second type of films (Gel-MA).

1. Introduction

Gelatin is a water-soluble proteinaceous material obtained from the denaturation and partial hydrolysis of collagen [1,2]. Generally, gelatin is obtained by an acidic or alkaline treatment of collagen from animal byproducts such as cattle bones and pork skins. Due to the variety of collagen sources and production processes, a large diversity of gelatins, with variable chemical compositions and physical properties, can be produced [2].

As known, gelatins can be dissolved in water and form low viscosity solutions at temperature above ca. 35 °C, but undergo a sol–gel transition when cooled, forming physical gels. The mechanism of this thermoreversible gelation has been extensively investigated [3–6]. The gelation involves a conformational transition of part of the gelatin chains from a random coil state to collagen-like triple-helix structures.

Gelatin has been used for a long time in the food and pharmaceutical industry in a large number of applications [2]. Besides being biodegradable and biocompatible, gelatin is nonimmunogenetic and has hemostatic properties [1]. Due to these favorable properties, gelatin is also used in several biomedical applications, such as a hemostatic sponges for surgical purposes [7], wound dressing materials [8,9], and sealants for vascular prostheses [10]. Further, gelatin has already been investigated as a material for the construction of scaffolds for tissue engineering [11–16], vehicles for the controlled delivery of bioactive macromolecules, such as peptides, proteins, and oligo- and polynucleotides [17–19], and as carrier for transplanted fragile tissues, like retinal sheets [20].

Since gelatin gels formed by simple thermal induced gelation readily dissolve at physiological temperatures, the gelatin based materials used in many of the described biomedical applications are usually submitted to an irreversible cross-linking treatment, in order to enhance their thermal and mechanical stability, as well as to retard their rate of degradation *in vivo* [17]. Permanent cross-link is achieved by the introduction of intermolecular covalent bonds between gelatin chains. This can be accomplished either by physical methods like, for example, the treatment with heat or irradiation [21,22], or by chemical methods, by the reaction of gelatin with various types of chemical agents. Dialdehydes, like formaldehyde

* Corresponding author at: CIEPQPF, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Polo II, 3030-790, Coimbra, Portugal.
Tel.: +351 239798700; fax: +351 239798703.
E-mail address: patricia@eq.uc.pt (P. Coimbra).

http://dx.doi.org/10.1016/j.ijbiomac.2014.06.021
0141-8130/

or glutaraldehyde, are recognized as very efficient cross-linking agents of gelatin and collagen [21,23–25]. Unfortunately, their use has been frequently associated with problems such as cytoxicity and calcification [26–28], which prompt the investigation of more biocompatible cross-linkers, like the water soluble 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) [7,10,18–20,29,30]. Other synthetic chemical agents already investigated include various diisocyanates and diepoxides [24,31]. Alternatively, a variety of non-toxic natural occurring molecules, like genipin [32–34] and phenolic compounds such as tannic acid [35] and nordihydroguaiaretic acid [36], have been used. Gelatin can also be cross-linked by the use of naturally occurring enzymes, like transglutaminase [11,25,37,38]. In this case, the enzymes work by catalyzing reactions that create covalent bonds between gelatin chains.

Other approach involves the pre-functionalization of gelatin with pendant methacrylate side groups, or other photosensitive moieties, and the subsequent chemical cross-linking by light irradiation in presence of an appropriate photoinitiator. This method is of particular interest for biomedical applications, since it permits the 3D encapsulation of viable cells simultaneously to gel formation [14,15], or even *in situ* gel formation [39].

Independently of the method chosen to prepare chemical cross-linked gelatins, the phenomenon of physical gelation is always present, and will happen, in a greater or lesser extent, as soon as the temperature is sufficiently low. Thus, the final network structure of chemical cross-linked gelatins and, consequently, their properties, will be determined essentially by the way both of these processes take place, i.e., if they occur at the same time or one after the other. This particular aspect has been investigated by some authors [38,40–42], that used rheological and optical rotation measurements to follow the formation of gelatin networks under different thermal protocols, in which physical cross-linking and chemical cross-linking occurred simultaneously or sequentially. These studies demonstrated that the viscoelastic properties of the formed networks are highly dependent upon the sequence of formation and on the relative amount of triple helices and covalent bonds formed.

In the present work, we prepared and characterized two types of chemical cross-linked gelatin films, with several degrees of cross-linking. The first type of films, designated hereinafter as Gel-E, were cross-linked with EDC whereas in the second type of films, Gel-MA, gelatin was first functionalized with methacrylamide side groups and subsequently cross-linked by a photo-initiated radical polymerization of the added moieties.

The objective of this work is to gain insight on how different chemical cross-linking methods, thermal protocols, and cross-linking levels can be used to tailor the properties of gelatin films, such as the buffer uptake capacity, enzymatic degradation, and mechanical and thermal properties. We hope that the findings of this work will serve as basis for the achievement of our ultimate goal, which is the development of an implantable drug release gelatin film. More specifically, we intend to develop a gelatin film that can release, after being implanted in a body site subject to a surgical tumor resection, a chemotherapeutic agent, in order to reduce the probability of tumor recurrence.

2. Materials and methods

2.1. Materials

Gelatin type A (from porcine skin, 300 bloom), collagenase (from *Clostridium histolyticum*, EC 3.4.24.3, 245 CDU/mg), 2,4,6-trinitrobenzene sulfonic acid (TNBS) solution (5% w/v), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), and methacrylic anhydride (MAA), were purchase from Sigma–Aldrich. *N*-hydroxysuccinimide (NHS) was obtained from Acros Organics and the photoinitiator 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propan-1-one (Irgacure®2959) was a kind gift from Ciba Speciality Chemicals. All the other chemicals were of reagent grade and were used as received without further purification.

2.2. Preparation of chemical cross-linked Gel-E films

These films were produced following the procedure reported by Kuijpers et al. [18,29]. A gelatin solution of 8% (w/v) was prepared by dissolving gelatin in deionized water at 50 °C. After being sonicated, in order to remove air bubbles, 20 ml solution were poured into polystyrene petri dishes (diameter = 8.5 cm), and left to air-dry at room temperature (around 25 °C). The formed films, with a thickness of 156 ± 10 μm, were then cut into squares of 1 cm width. After being dried in vacuum for 1 day, samples were cross-linked with variable amounts of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC). For each reaction, 25 gelatin squares, with a total weight of approximately 625 mg (average weight of 25 mg each), were immersed in 25 ml of 2-morpholinoethanesulfonic acid (MES) buffer (pH 5.5, 0.1 M) and cross-linked with the addition of EDC and *N*-hydroxysuccinimide (NHS). The amount of EDC was calculated in order that the molar ratio of EDC to the carboxylic acid groups of gelatin varied from 0.25 to 4.0, assuming that there are 0.080 mol of carboxyl groups per 100 g of gelatin type A [18,29]. The molar ratio of NHS to EDC was kept constant at 0.2. The reaction was allowed to proceed for 5 h at 4 °C and then it was quenched by submerging the samples in a solution of 0.1 M disodium hydrogenphosphate and 2 M sodium chloride (pH ≈ 8.7) for 20 min. Finally, samples were repeatedly washed with deionized water and dried in a vacuum oven at ambient temperature.

2.3. Preparation of photo-cross-linked Gel-MA films

2.3.1. Synthesis of Gel-MA

Gelatin methacrylamide (Gel-MA) was prepared by the reaction of methacrylic anhydride (MMA) with the primary amino groups of gelatin, according to the procedure originally reported by Van den Bulcke et al. [43]. In order to prepare Gel-MA with various degrees of substitution, the amount of MAA used was varied between 0.13 ml and 8.4 ml, in order that achieve molar ratios of MMA to free amino groups of 0.25, 0.5, 1.0, 2.0, 4.0 and 16.0. In the calculations it was assumed that there are 0.035 mol of free amino groups per 100 g of gelatin [43]. For the reaction, 10 g of gelatin were dissolved in 100 ml of phosphate buffer saline (PBS, pH 7.4) at 50 °C. MMA was then added under vigorously magnetic stirring conditions and left to react for 1 h at 50 °C. The reaction was stopped and the mixture was poured in to dialysis bags and dialyzed for 4 days against distilled water at room temperature. The reaction products were then frozen with liquid nitrogen and freeze-dried during 3 days.

2.3.2. Photo-cross-linking of Gel-MA films

For the preparation of the films, 1.6 g of freeze-dried Gel-MA was dissolved in 20 ml distilled water at 50 °C. The water soluble photoinitiator Irgacure®2959 was added to the solution in the amount of 0.15% relatively to the Gel-MA weight. The warm solution was poured into polystyrene petri dishes (diameter = 8.5 cm), left to cool during 5–10 min, and then irradiated with a long-wave ultraviolet (LWUV) light (10 mW/cm^2) for 10 min. The formed films were then left to dry at room temperature. After complete drying, films with a thickness of 190 ± 15 μm were formed.