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Modified gum arabic cross-linked gelatin scaffold for biomedical applications

P.R. Sarika [a], Kuriakose Cinthya [b], A. Jayakrishnan [c], P.R. Anilkumar [b,*], Nirmala Rachel James [a,**]

[a] Department of Chemistry, Indian Institute of Space Science and Technology, Valiamala, Thiruvananthapuram, Kerala 695 547, India
[b] Tissue Culture Laboratory, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Thiruvananthapuram, Kerala 695 012, India
[c] Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600 036, India

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ABSTRACT

The present work deals with development of modified gum arabic cross-linked gelatin scaffold for cell culture. A new biocompatible scaffold was developed by cross-linking gelatin (Gel) with gum arabic, a polysaccharide. Gum arabic was subjected to periodate oxidation to obtain gum arabic aldehyde (GAA). GAA was reacted with gelatin under appropriate pH to prepare the cross-linked hydrogel. Cross-linking occurred due to Schiff's base reaction between aldehyde groups of oxidized gum arabic and amino groups of gelatin. The scaffold prepared from the hydrogel was characterized by swelling properties, degree of cross-linking, in vitro degradation and scanning electron microscopy (SEM). Cytocompatibility evaluation using L-929 and HepG2 cells confirmed non-cytotoxic and non-adherent nature of the scaffold. These properties are essential for generating multicellular spheroids and hence the scaffold is proposed to be a suitable candidate for spheroid cell culture.

1. Introduction

Collagen is a major structural protein present in human body and gelatin is derived by partial denaturation of collagen. Gelatin is used extensively in biomedical applications due to its non antigenicity, non immunogenicity, biodegradability and biocompatibility [1]. Gelatin has been extensively used in the form of coacervatives, hydrogels, micro and nanoparticles for drug delivery and tissue engineering applications [2–5]. Recently, gelatin-carrageenan hydrogel was investigated for controlled delivery of quercetin (Q,3,5,7,3′,4′-pentahydroxyflavone), a member of the flavonoid family [6]. Gelatin has many integrin binding sites for cell adhesion and differentiation which makes it suitable to be used in tissue engineering applications [7,8]. Detailed studies on calorimetric properties and dynamic behavior of the water at the interface of gelatin–glycosaminoglycans blends have been reported [9,10]. (See Scheme 1.)

Cross-linking agents such as glutaraldehyde [11], hexamethylenediisocyanate, carbodiimide [12] and acyl azides [7] are being employed for cross-linking of gelatin to improve the water resistant ability [13], thermal stability and mechanical properties. These materials, if released in to the body due to degradation or remaining unreacted in the structures, are reported to cause toxicity [14,15]. Hence, there have been numerous attempts to prepare gelatin based scaffolds by avoiding toxic cross-linking agents. Gelatin hydrogels, stabilized by partially oxidized polysaccharides such as dextran, alginic acid, chondroitin sulphate, and carboxymethyl cellulose have been reported in literature [16–20]. These hydogels were developed to be used as wound dressings, tissue adhesives and scaffolds for tissue engineering. This was found to be an excellent stabilization method also for protein molecules such as collagen and chitosan and enabled replacement of toxic cross-linking agents. Balakrishnan et al. utilized this route to prepare injectable hydrogels based on gelatin and oxidized alginate [21]. Rapid cross-linking between amino groups of gelatin and aldehyde groups of oxidized alginate led to hydrogel formation in presence of borax in an aqueous medium. Recent articles by Boanini et al. and Yang et al. also examine the biocompatibility of alginate cross-linked gelatin and collagen matrixes [22,23].

Scaffolds play an important role in tissue engineering [24–27]. Biopolymer based scaffolds are versatile because of their non toxicity and high functionality [28]. In this study, we explored the possibility of utilizing a natural gum, namely gum arabic as cross-linker for scaffold based on gelatin. Gum arabic (GA) is a biocompatible, nontoxic, water soluble, natural gum obtained from acacia tree. It is a branched, slightly acidic complex polysaccharide containing arabinose, rhamnose, galactose and glucoronic acid residues with a backbone consisting of 1,3 linked β-D-galactopyranosyl units. Side chains are composed of two to five 1,3 linked β-D-galactopyranosyl units, joined to the main chain by 1,6 linkages [29]. It is an inexpensive polysaccharide which is being extensively used as stabilizing, emulsifying and thickening agent in the food industry. Gum

* Corresponding author. Tel.: +91 471 2520 271.
** Corresponding author. Tel.: +91 471 2568 538; fax: +91 471 2568 541.
E-mail addresses: anilkumarpr@sctimst.ac.in (P.R. Anilkumar), nirmala@iist.ac.in (N.R. James).

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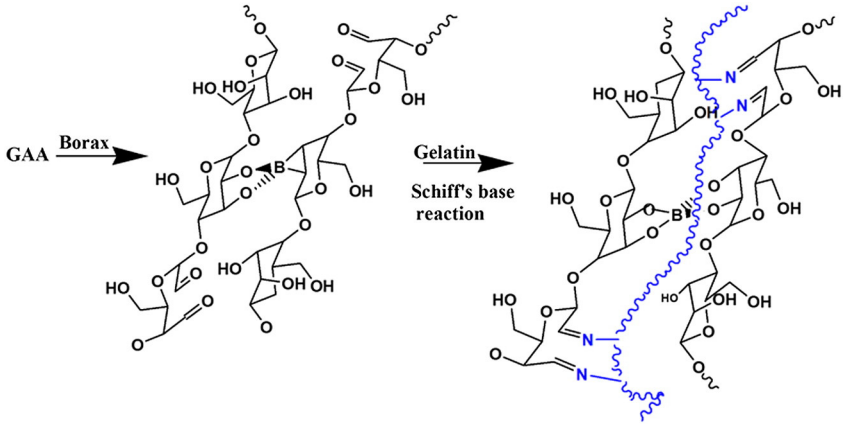

Scheme 1. Gum arabic aldehyde cross linked with gelatin in the presence of borax.

arabic–drug conjugates were investigated for controlled drug delivery applications [30,31]. Gum arabic based microparticles and nanoparticles have already been reported in the literature [32–37]. Gum arabic was also used as a surface modification agent and a non toxic phytochemical construct for metal nanoparticles [36,38,39]. Reis et al. modified gum arabic with glycidyl metharylate to prepare pH responsive hydrogels for magnetic nano particles [40].

Though GA has been proposed as a potential biomaterial, its use in scaffolds for tissue engineering has not been explored yet. In this study, the possibility of using modified gum arabic as stabilizer for gelatin has been explored. Gum arabic was modified by periodate oxidation and characterized. Gelatin was cross-linked with oxidized gum arabic leading to the development of scaffold based on gelatin and gum arabic.

The scaffold was developed, characterized and evaluated for its cytocompatibility and cell adhesion. The studies proved that the scaffold is noncytotoxic and exhibits non-adherent characteristics. Hence the scaffold can function as a substrate for spheroid cell culture which requires a non-adherent surface, where cells adhere to each other to attain tissue like architecture with less adherence to the substrate [41,42].

2. Materials and methods

2.1. Materials

Gum arabic (from acacia tree) of approximate molecular weight 250 kDa, gelatin (Type A), sodium metaperiodate, sodium tetra borate (borax), trinitrobenzenesulfonic acid (TNBS), minimum essential medium (MEM), neutral red, propidium iodide (PI), fluoresceine diacetate (FDA) and glutaraldehyde were purchased form Sigma Aldrich, Saint Louis, USA. Sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, silver nitrate, hydroxyl amine hydrochloride, sodium hydrogen carbonate, sodium hydroxide and isopropanol were obtained from Merck (Mumbai, India). Trypsin EDTA (0.25%, 0.02%) was procured from Invitrogen, USA. Dialysis tubing (6000–8000 MWCO) was procured from Spectrum Laboratories Inc., CA, USA. Double distilled water was employed in all synthesis experiments and Milli Q water (Millipore) for cell culture work.

2.2. Preparation of oxidized gum arabic

Gum arabic was oxidized to gum arabic aldehyde (GAA) using sodium metaperiodate [33,34]. Gum arabic, 10 g (0.058 mol) was dissolved in 80 mL of distilled water and required amount of sodium periodate was dissolved in 20 mL of water (for 10% oxidation,1.24 g, 0.0058 mol and for 20%, 2.48 g, 0.0116 mol).

Sodium periodate solution was added to gum arabic solution and the reaction mixture was stirred with a magnetic stirrer at 20 °C for 6 h in dark. Purification was done by dialysis using a dialysis tube of MWCO 6000–8000 for three days against distilled water. The absence of periodate in the dialysate was checked by adding 1 mL of 1% solution of silver nitrate to 1 mL of the dialysate. After the complete removal of periodate (no turbidity with silver nitrate), the dialysate was frozen and lyophilized. Oxidized gum arabic was obtained in high yield of 80–85%.

2.3. Viscosity studies

Intrinsic viscosities of gum arabic and gum arabic aldehyde were measured using Ostwald viscometer. Stock solutions of GA, 10% and 20% oxidized gum arabic (GAA-1 and GAA-2 respectively) were prepared in 0.1 M borax. From the stock solution, dilute solutions (1%, 2%, 3% and 4%, *w*/*v*) were prepared using borax. Borax solution (15 mL, 0.1 M) was introduced into the viscometer and its time of flow was measured (t_0). Time of flow (t) for all the solutions was determined as above and intrinsic viscosity was determined by õ-plotting the graph between η_{red} (reduced viscosity) and η_{inh} (inherent viscosity).

2.4. Determination of aldehyde content

Aldehyde content in oxidized gum arabic was determined by titrimetric method [16]. GAA-1 (0.1 g) and GAA-2 (0.1 g) were dissolved in 25 mL, 0.25 N aqueous solution of hydroxyl ammonium chloride. Liberated hydrochloric acid resulting from the reaction of aldehyde group in GAA with hydroxyl ammonium chloride was titrated against 0.1 N NaOH. Methyl orange (0.05% solution, *w*/*v*) was used as indicator. At the end point, red color solution was changed into yellow. The number of moles of NaOH consumed is equivalent to the number of moles of aldehyde present in the sample.

2.5. Preparation of gum arabic–gelatin hydrogel and scaffold (GGA)

Scaffolds were prepared from oxidized gum arabic and gelatin. This is based on the Schiff's base reaction between free amino groups of gelatin and aldehyde groups of gum arabic aldehyde (GAA). GAA-1 solution and GAA-2 solution were prepared in 0.1 M borax and 10% (*w*/*v*) solution of gelatin was prepared in distilled water. GAA-1 or GAA-2 solution (0.5 mL) was taken in a glass vial and to this 0.5 mL of gelatin solution was added and vortexed at 37 °C to obtain hydrogel. Gelation occurred very fast leading to the formation of a smooth clear hydrogel. The hydrogel thus obtained was frozen and lyophilized for 12 h. Samples were then rinsed with distilled water to remove borax