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Carboxymethyl guar gum synthesis in homogeneous phase and macroporous 3D scaffolds design for tissue engineering

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Dimethyl amino pyridine (PubChem CID: 14284)
Monochloroacetic acid (PubChem CID: 300)
Lithium chloride (PubChem CID: 433294)
Ammonium bicarbonate (PubChem CID: 14013)
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ABSTRACT

Guar gum (GG) is a galactomannan obtained directly from the C*yamopsis tetragonoloba* seeds pericarb. The biopolymer hydrates hugely in three chain associated coil formations. Chaotropic Hofmeister ion like lithium interacts at the hydrogen bonding sites and render GG homogenization in polar solvents like dimethyl sulfoxide. This phenomenon was used for the first time for galactomannan derivatisations in homogeneous phase. Higher degree of substitution (DS) that was hereto unattainable in GG was achieved due to Hofmeister ion assisted assembly deformations. Furthermore, carboxymethyl guar gum (CMGG, DS = 1.10) blends well in poly-vinyl alcohol (PVA) at 2:1 mass ratio and enabled hydrophilic porous scaffold design for cell propagation. CMGG-PVA scaffolds porosity was 70–90% and the tensile strength was 6.32 MPa. CMGG-PVA scaffolds were useful as cell factories and in tissue engineering. New generation guar gum derivative scaffolds were non cytotoxic and permitted cell propagation in growth medium.

1. Introduction

Polysaccharides are convenient tools for tissue engineering scaffolds design (Ahadian et al., 2015; Balakrishnan & Banerjee, 2011). Polysaccharide hydrogels are risk free and elicit low or no immunogenic reactions. Covalently modified hyaluronates and biohybrid alginates were extensively investigated in tissue engineering areas (Collins & Birkinshaw, 2013). Polysaccharides in tissue engineering are however often constrained due to lack of structural robustness, specificity and soft matter characteristic. Alginates, experienced uncontrolled polymer degradation and depletion of cell proteins due to electrostatic repulsions. Tissue engineering cellulose nano-crystals are generally not available in consistent quality and cytotoxic reactions were widely reported.

Guar gum (GG) is a hydrated biopolymer obtained directly from the C*yamopsis tetragonoloba* seed pericarbs. GG is used regularly as functional food, in cosmetics, processed food like icecreams and drug delivery (Krishnaiah, Satyanarayana, Rama Prasad, & Rao, 1998). The biopolymer comprises of β 1–4 mannose chain interposed with α 1–6 galactose substituents in almost every second unit. GG hydrates hugely in three chain coil formations due to galactose units' preferential intra molecular hydrogen bonding. The mannose surfaced assembly of the biopolymer is one interesting platform in the protein interfaces (Ghosh, Abdullah, & Mukherjee, 2015). GG *per se* is a non-ionic polymer but it exceptionally binds with lectins and antibodies due to the presence of surface mannose moieties and glycobiology interactions (Pettolino et al., 2001). GG blends well with a range of polyalcohols, proteins and lipids. Protein interactions with partially substituted GG were intelligently applied in designing artificial tear to help circumvent dry eye diseases (Mafi, Pelton, Cui, & Ketelson, 2014). The unique biopolymer structural characteristic has incited us to explore in appropriate scaffolds design for tissue engineering applications.

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One challenge in case of GG is to attain high degree of surface functionality. Different techniques applied earlier for anionic and cationic derivatives synthesis have resulted in low DS products (Sharma, Kumar, & Soni, 2004). Tri-molecular tight coil structure in water is arguably a reason for that. Herein, we have used chaotropic Hofmeister ion guided functionalization techniques to obtain high DS carboxymethyl guar gum (CMGG) for the first time. The new generation biopolymer derivative can be readily cross-linked in near neutral pH due to higher number of functional groups. Furthermore, when cross-linked, GG and derivatives presented a bio-interactive mannose chain surface. GG is a very interesting material at the cell interfaces when some of the material characteristics are enhanced.

Macrofibrous scaffolds were designed from high DS CMGG and PVA blends following a facile gas foaming freeze drying technique. PVA is a biodegradable, nontoxic, inexpensive, semi crystalline polymer used often in biomedical devices and tissue engineering (Maheshwari, Kumar, Nagiah, & Uma, 2013). PVA blends well in cross-linked CMGG and 3D bioartificial networks were developed due to lamellar structuring. Cell adhesion, viability and proliferation within the network were unrestrictive and the scaffold provided a good candidate for tissue engineering applications.

2. Experimental section

2.1. Materials

Reagent grade dimethyl amino pyridine (DMAP) (99%) and lithium chloride (LiCl) were from Spectrochem (India). Monochloroacetic acid (MCA) was from CDH (India). Bio-reagent grade dimethyl sulfoxide (DMSO, 99.9%), boric acid (H_3BO_3) and ammonium bicarbonate (NH_4HCO_3) were from Sigma-Aldrich (St. Louis, MO, U.S.A). Guar gum powder (GG, CAS No. 9000-30-0) was received as a gift from Nuevo Polymers (India). Polyvinyl alcohol (PVA, M.W. 1.15 kDa) was from LobaChemie (India). Buffer reagents like sodium chloride (NaCl, SRL, India), potassium chloride (KCl, Himedia, India), disodium hydrogen phosphate (Na_2HPO_4, Qualigens, India) and potassium dihydrogen phosphate (KH_2PO_4, Himedia, India) were outsourced. High glucose Dulbecco's modified eagle medium (DMEM), heat inactivated fetal bovine serum (HI-FBS), antibiotic-antimycotic solution and trypsin–ethylenediaminetetraacetic acid (Trypsin-EDTA) were from Gibco (India). Triton-X 100 was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A). Staining agents like phalloidin were procured from Abcam (U.S.A) and 4′,6-diamidino-2-phenylindole (DAPI) from TCI (Japan).

2.2. Carboxymethyl guar gum synthesis

Prior to the synthesis of CMGG, native GG was washed by a facile solvent water washing technique (McCleary & Nurthen, 1983). Typically, 5 g of GG was soaked in 50% (v/v) aqueous isopropanol for 24 h and the filtered residue was thereafter transferred into 250 mL three necked round bottomed flask containing 50 mL of DMSO. The flask was placed over a magnetic stirrer at 100 rpm and allowed to swell for 12 h. LiCl (2 g) in 20 mL of DMSO was added into it and a homogeneous solution was soon developed. The flask was attached with nitrogen purging system, reflux condenser, mechanical stirrer, dropping funnel and placed over a mantle. The temperature was controlled at 30 ± 2 °C under nitrogen purging at 5 mL min^{-1}. DMAP (11.29 g), dissolved in 5 mL of DMSO, was added under stirring and the solution pH was checked at 8. MCA (8.7 g) in 8 mL of DMSO was then added dropwise under stirring. The reaction mixture was allowed to equilibrate for 3 h after complete addition. The solution was poured into a beaker containing 300 mL cold aqueous ethanol (50% v/v). The product CMGG was filtered, washed in water and soxlated for 72 h against ethanol until chloride test in the solvent was negative and the leachants checked free from solvents in UV spectrophotometer (EVO300 PC, Thermo Fisher, U.S.A). Different batch reactions were run using varying proportions of MCA to observe product DS variations.

2.3. Carboxymethyl guar gum-PVA scaffolds

Scaffolds from high DS CMGG and PVA were prepared by a gas foaming and freeze drying technique. Briefly, PVA powder (100 mg) was dissolved in 10 mL of deionized water under stirring for 30 min at 80 °C over a magnetic stirrer. After cooling, CMGG (200 mg) was added into the PVA solution and stirred for additional 30 min until a hydrogel was formed. Ammonium bicarbonate crystals (150–200 μm) was then added in 4:1 mass ratio, mixed well and the gel molded into small cylindrical shapes. The gel cylinders (2 cm × 4 cm) were placed in 5% (w/v) boric acid solutions (pH 6) for simultaneous foaming and cross-linking. Scaffold cylinders were further placed in a lyophilizer (Eyela FDU 1200, Japan) for 48 h, cut under a diamond cutter in 4 μm discs, washed subsequently in 70% (v/v) aqueous ethanol and sterile water. The scaffolds were stored in a −20 °C refrigerator (Bluestar CHF150C, India) for further studies. Different ratio of PVA to CMGG was used and the scaffold properties were recorded.

2.4. Carboxymethyl guar gum characterization

The DS in CMGG was determined in terms of free carboxylate groups present per unit mass of the biopolymer (Stojanović, Jeremić, Jovanović, & Lechner, 2005). Typically, 2 g of CMGG was weighed in exact half and dissolved in excess of 0.1 N NaOH, stirred for 20 min and the unreacted excess of NaOH was back titrated against 0.1 N HCl solution using methyl orange indicator. The remaining half portion was dried to constant weight at 110 ± 5 °C. DS was calculated as:

$$DS = 162w_A/(5900 - 58\,w_A) \quad (1)$$

$$\text{Where, } w_A = (C_{NaOH}V_{NaOH} - C_{HCl}V_{HCl})/m \quad (2)$$

C_{NaOH} and C_{HCl} were the molar concentration of NaOH and HCl solutions, w_A was the mass fraction of the substituent group, V_{NaOH} was the volume of NaOH taken and V_{HCl} was the volume of HCl consumed, m was the weight of polymer in g.

The viscosity of 1% (w/v) aqueous biopolymer solutions were recorded in Brookfield DV-II + Pro (Brookfield, U.S.A) at 25 °C. Infrared spectra in KBr pellets were recorded in FTIR spectrometer (Jasco 6300, Japan), over the range of 400–4000 cm^{-1}. Thermal properties were recorded in Pyris Diamond TG/DTA (Perkin Elmer, Singapore) instrument. Samples (~5 mg) were placed in platinum crucible and heated incrementally (10 °C min^{-1}) up to 400 °C under continuous N_2 purging. X-ray diffraction studies were carried out in PANalytical X.Pert Pro XRD (PW 3040/60, Netherland) with Cu anode and Kα radiation (λ = 1.54060 Å) at 40 kV and current 30 mA.

2.5. Scaffold characterization

In vitro scaffold swelling ratio was investigated in triplicate and the results averaged for comparative purposes. Each scaffold type was immersed in 25 mL PBS solution (0.05 M, pH 7.4) at 25 ± 2 °C. The scaffolds were removed, blotted and reweighed at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 22 and 24 h. The swelling extent was calculated as:

$$\text{Swelling ratio (\%)} = [(W_s - W_d)/W_d] \times 100 \quad (3)$$

Where, W_s and W_d were weights of the wet and dry matrix respectively.

Scaffold porosity (p) was measured by modified ethanol displacement method (Guan, Fujimoto, Sacks, & Wagner, 2005) and analysed as:

$$p = (v1 - v3)/(v2 - v3) \quad (4)$$

Where, $v1$ was the initial volume of ethanol in a cylinder, $v2$ was the volume after scaffold immersion and $v3$ was the volume after scaffold removal.