Abstract

The present study describes the synthesis of macroporous polymeric composite matrix using natural polymers like alginate and agarose by the process of cryotropic polymerization. The synthesized matrix was examined for their thermal stability and mechanical property at different temperature. The results indicated thermal tolerance of the composite matrix. However with increasing temperature, elastic behavior of matrix shows increase in tensile property but decrease in elastic modulus. The results suggest that there is potential in the use of naturally derived polymers for the synthesis of porous biomaterials with thermo-elastic properties for various biomedical applications.

Introduction

One of the most exciting areas of advanced materials science research is in the applications of biomaterials to healthcare and especially to reconstructive surgery. At a time when technological research should contribute to improvements in the quality of life, it is obvious that the developments of advanced materials for application either inside or outside the body in order to treat disease and injury have a high priority. The variety of natural chemical structures together with the precise control of their molecular architecture and morphology, rationalize the numerous uses of biopolymers (natural polymers) as biomaterials in high technological and biological applications. Because of inherited properties, biopolymers are of interest as a bio-mimetic component for tissue engineering application but there are also some concerns like loss of bioactivity of the biopolymer during its extensive processing prior to application. In addition, they have also been widely used in drug and protein delivery system, cell culture, medical and biological sensors, water absorbent pads, hygiene products, breast implants, wound dressing and for enzyme and cell immobilization. Porous polymeric matrices are attractive class of substrates for various biotechnological applications. Among other properties, the thermal stability and mechanical properties are important parameters to understand the biomaterial behavior in a biological (in situ) environment.

We aimed to synthesize a composite porous polymeric matrix, which could retain 3D porous structure even after drying, show improved thermal stability and elastic behavior for biomedical application. We have used different ratios of polymers and optimized the matrix properties. The thermo-elastic stability was accessed by thermo-gravimetric and mechanical analysis. The use of cryotropic polymerization technology to produce customized porous matrix with controlled internal micro-architecture is also addressed.

Experimental

Synthesis of agarose-alginate (Ag-Al) cryogel monolith

Ag-Al cryogel was synthesized using N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide
hydrochloride (EDC) with N-hydroxysuccinimide (NHS) for chemical crosslinking. Low viscosity alginate solution (3.75%) was prepared in a plastic tube (50 mL) using deionized water as a solvent. On the other hand, agarose (Low EEO; gelling temperature 38-40 °C) (6%) was dissolved in deionized water by placing the agarose containing tube in the boiling waterbath until the solution became transparent. Further, 4 mL of stock solution of alginate (3.75%) was added to 5 mL of hot agarose solution (6%) and then cooled at room temperature. When the temperature of the heterogeneous solution reached to 45 °C, 500 μL of 0.35 M freshly prepared EDC solution was added which was followed by adding 500 μL of 0.2 M NHS solution. The reaction mixture of Ag-Al was transferred into 5 mL plastic syringe and immediately freezed at -12 °C for 16 h in liquid cryobath (Grant, Cambridgeshire, UK). After completion of incubation, gels were thawed in deionized water and dried at room temperature till further use.

Characterization of porous agarose-alginate matrix

The structural morphology of synthesized Ag-Al cryogel was analyzed by scanning electron microscopy (SEM). Thermo-gravimetric (TGA) analysis of Ag-Al cryogel was performed using calorimeter under nitrogen atmosphere over the temperature range of 20 to 500 °C at increment of 10 °C/min. Cylindrical cryogel samples of height 5 mm and diameter 13 mm were saturated with 0.1 M phosphate buffer saline (PBS) and then used for conducting the tensile tests. Uniaxial elongation was performed to the cryogel samples with 10 kN load cell under displacement control at the rate of 1 mm/min. The slope of the graph with stress (kPa) on Y-axis and strain (%) on X-axis was measured at 10% strain to calculate elastic modulus and tensile strength.

Results and discussion

Ag-Al cryogels were synthesized by optimizing the concentration of agarose in co-polymer. The synthesis is based on cryotropic gelation of agarose and alginate chains at subzero temperature in the presence of crosslinker EDC and NHS. Synthesized Ag-Al cryogel was white in color, soft, spongy and retained 3D porous architecture (Fig. 1a). Upon complete drying, cryogel was shrunk approximately 1 to 2 mm in diameter from its original size (13 mm diameter). Interestingly, Ag-Al gel shows shape memory property i.e. returns to its original shape and size while soaking in aqueous medium.

![Fig 1. Physical form of AA monolith gels (a) and its scanning electron microscopic image showing homogenous macropores distribution (b). (SEM scale bar: 50 μm).](image-url)
will have better heat transfer ability and maintain the temperature compared to bigger sample. So, during the cryogelation process, variations in the temperature besides other parameters can change the structural homogeneity of a cryogel.

The thermal stability of alginate gel and Ag-Al gel was accessed by thermo-gravimetric analysis (TGA). The graph of percentage weight loss v/s time showed onset of decomposition approximately at 60 °C, which is due to the dehydration of gel in both the cases. The second decomposition was observed at 200 °C in both the matrices, but the degree of abjection was higher in case of plain alginate with 50% weight loss at 250 °C, which is a typical behaviour of alginate. While in the composite Ag-Al gel, a linear decomposition was observed, which began at 200 °C and 50% decomposition was noticed at 340 °C. This might be due to the higher intermolecular/interfibrillar crosslinking of polymer chains of agarose and alginate resulting in increased thermal stability.

Unconfined compression tests showed significant elasticity of Ag-Al cryogels and maintained their physical integrity even after compressing them up to 80% of their original length. The elastic modulus was observed in the range of 36-39 kPa. The aggregate tensile modulus showed good elasticity of the Ag-Al cryogels. At different temperatures i.e. 27 °C and 37 °C, the tensile modulus was 320 ± 63 kPa and 250 ± 94 kPa, respectively. These results suggest that temperature affects the elastic behavior of the matrix.

Conclusion

Nevertheless, the physico-mechanical studies compliment the macroporosity of these polymeric biomaterials, which is an important parameter in tissue engineering, bioreactors and bio-separation applications. This particular polymer combination has also been synthesized by authors in other type of formats like disks, sheets and beads⁴. Agarose itself has physical crosslinking property and can make a porous three-dimensional scaffold. However, the presence of alginate in the composite increases the softness of the cryogel as well as the spongy nature. On the other hand, alginate presence is also beneficial to cultivate different cell types for tissue engineering applications and immobilization purposes. Alginate has also been used for capturing of heavy metals and bacteria from the waste water⁵. Our group is using these cryogels with suitable chemistry, porosity as well as thermal and mechanical stability, modulated as per the application. Our purpose is to provide a novel approach to design such materials which can be cost effective and highly efficient for cell and enzyme immobilization, bio-processing, separation technologies as well as in regenerative medicine and tissue engineering applications.

References