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Synthesis and characterisation of gelatin/zeolite porous scaffold

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ABSTRACT

Exploring the possibility of using zeolites in tissue engineering scaffolds is a potential approach in regenerative medicine because of their biocompatibility and cation exchange ability. A novel method to synthesize formaldehyde crosslinked gelatin/zeolite scaffolds by lyophilisation technique is reported in this paper. AFM images of gelatin solutions obtained before and after the addition of formaldehyde, revealed the coil to helix transformation of gelatin after crosslinking. The pore size of gelatin control scaffold was in the range of 50–750 μm while it was greatly reduced to 10–350 μm after incorporation of 0.5% zeolites in gelatin matrix, G(0.5%). Micro-CT analysis showed that porosity of G(0.5%) was 81% and the pores were well interconnected. The elemental analysis and crystallinity studies confirmed the presence of zeolites in G(0.5%). Interestingly, contact angle was found to increase from 88.6° to 108° with the increase in concentration of zeolites in gelatin. G(0.5%) showed the highest glass transition temperature (~37 °C) as well as dynamic compression modulus (~737 kPa). Swelling and degradation of scaffolds were tuned by adjusting concentration of zeolites in the composite scaffolds. All these results suggest that they can be further investigated for their application in tissue engineering.

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1. Introduction

Tissue engineering scaffolds are porous structures fabricated from synthetic and naturally derived biodegradable polymers which serve as transitory artificial extracellular matrix (ECM) that promotes cell attachment and 3-dimensional (3D) tissue formation [1]. There are various techniques to fabricate 3D porous scaffolds namely, fiber bonding [2], lyophilisation [3], supercritical fluid technology [4], compression moulding and salt leaching [5], gas foaming [6], rapid prototyping [7] and electrospinning [8]. Lyophilisation or freeze drying is a dehydration technique in which liquid samples are frozen below its glass transition temperature (Tg) or melting point and frozen solvents are removed by sublimation process, thereby obtaining porous, interconnected structures [9]. Compared to other techniques, the distinct benefits of lyophilisation are that no toxic organic solvents are being used and the low temperature helps to maintain the activity of biomacromolecules and pharmaceutical products for long period. Unlike normal drying process, this technique involves low surface tension which can maintain the pore structure. Hence, scaffolds with wealth of pore morphologies and

Abbreviations: CAF, copper activated faujasites; G(0%), gelatin without CAF; G(0.25%), gelatin with 0.25% CAF; G(0.5%), gelatin with 0.5% CAF; G(2.5%), gelatin with 2.5% CAF; G(5%), gelatin with 5% CAF; 3D, 3 dimensional; ECM, extra cellular matrix; Tg, glass transition temperature; V_d, defect volume; V_s, volume of scaffold material; W_i, initial weight of sample before immersing in water; W_t, final weight of sample after water uptake; W_0, initial weight of scaffold before degradation studies; W_1, final weight of scaffold after degradation studies; mV, milli volt; μm, micrometer; SD, standard deviation; PBS, phosphate buffered saline.

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nanostructures can be synthesised by adjusting various parameters in freeze drying [10].

Natural or synthetic biopolymers are used for fabricating lyophilised scaffolds. Natural polymers have several advantages over synthetic polymers as they could be easily recognised by surrounding biological microenvironment and are metabolically processed through established pathways. On the other hand, synthetic polymers may generate toxicity and chronic inflammation due to lack of cell recognition signals [11]. Among the various natural polymers, gelatin is an ECM protein with extensive pharmaceutical and medical applications. It is obtained by the partial hydrolysis of collagen, which is extracted from bone, skin, tendon and cartilage [12]. It gels and melts below normal body temperature. Gelatin polypeptide chains exist as flexible, unfolded coils at elevated temperatures and undergo coil to helix transformation at lower temperatures [13]. It is pro-angiogenic [14], non-immunogenic [15], biocompatible and biodegradable [16]. Literature reports a wide application range for gelatin based scaffolds in bone tissue engineering [17], gene transfection [18], wound dressing [19], drug delivery [20], corneal endothelial cell therapy [21], and innumerable other fields. Due to the different functional groups in gelatin like —NH_2, —SH, —COOH, double bonds, gelatin can be modified with bio-molecules and even nanoparticles [22]. Zhang et al. has utilised this property to synthesis microspheres of gelatin and zeolite for controlled drug delivery [23]. Our attempt was to innovate a method by which we could incorporate inorganic carriers like zeolites in 3D porous scaffolds that allow high cell seeding density for dermal tissue growth and diffusion of nutrients and waste products from the cells.

Zeolites are microporous molecular sieves made up of crystalline aluminosilicates. They are biocompatible and are used as safe oral magnetic resonance imaging (MRI) contrast agents [24], drug carriers [23], skin whitening agents [25], anticancer agents [26], reduce TiO_2 induced reactive oxygen species in fibroblast cell lines [27] and enhance oxygen delivery to cells under hypoxia [28]. In the present study, copper activated faujasite (CAF), a mineral group in the zeolite family, was used. According to the International Zeolite Association, faujasites (Framework type code FAU) have cubo-octahedral sodalite cages, connected by hexagonal prisms. Copper ions balance the negative charge of faujasite lattice. These have excellent ion exchange capacity and lattice stability. Very few works are reported on the application of CAF for tissue engineering applications.

Owing to the merits and versatility of freeze drying, its application was exploited to synthesize a novel 3D porous scaffold based on gelatin/CAF composite. The structural, mechanical, thermal, water uptake and *in vitro* degradation studies of the prepared composite scaffolds were investigated in this paper. The antibacterial activity, cytotoxicity and *in vivo* studies are discussed in detail in forthcoming paper.

2. Experimental

2.1. Materials

Gelatin powder (type B) was purchased from Merck Chemicals, India. Glycerol (purity – 99%) and formaldehyde (36.5–38%) solutions were supplied by Sigma Aldrich (France). CAF was a kind gift from IRMA (France). Phosphate buffered saline (PBS) powder was purchased from Sigma Aldrich. All chemicals used for synthesis were of analytical grade and were not further purified.

2.2. Preparation of porous gelatin/CAF scaffold

A 2% (w/v) gelatin solution was prepared by dissolving gelatin powder in distilled water by magnetic stirring at 60 °C. To this solution, 5% (v/v) glycerol was added as plasticiser and again stirred. Then, 0.5% (w/v) of CAF was dispersed in distilled water and sonicated for half an hour, before being added to gelatin solution by syringe addition. After complete interdispersion of the solutions, the temperature was decreased to 37 °C. 0.38% (v/v) formaldehyde was added to this solution under thorough and continuous mixing to crosslink gelatin containing CAF. The solution was then poured in petri plates, pre-frozen at −20 °C in deep freezer, followed by lyophilisation (Christ Alpha 1–2 LD Plus Freeze Dryer) at −50 °C to prepare porous gelatin/CAF composite scaffolds. By keeping the concentration of gelatin and formaldehyde constant, composite scaffolds with 0.25%, 0.5%, 2.5% and 5% (w/w) of CAF namely, G(0.25%), G(0.5%), G(2.5%), G(5%), were prepared along with a control scaffold, G(0%), without any CAF.

2.3. Characterisation

The hydrodynamic diameter and distribution of CAF particles were determined by a light scattering technique using Malvern Zeta sizer Nano ZS. Surface charge and stability of CAF suspension were also analysed.

A commercial multimode Nanoscope IIIa atomic force microscope from Veeco (Santa Barbara, CA) equipped with typical Silicon tips (LTESP, Veeco) was used to analyse the morphology of gelatin coils before and after the addition of crosslinker. 5 μL of sample was dispersed in 100 ml of Millipore water. The solution was filtered and drop deposited on freshly cleaved mica and dried at 25 °C in vacuum oven. Tapping mode was used to record the images. The spring constant was 0.57 N/m and imaging was done at a scan speed of 3.5 Hz. Obtained images were analysed.

Micro-CT experiments were performed at the technical resource centre, Morlaix (France) using monochromatic beam of X-rays. The system allowed scanning objects with voxel sizes of 4 μm, using its 240 kV/320 W directional V(TOMEX) 240D X-ray tube and precision manipulator. The sample with thickness of 2.5 mm was rotated step by step through a full 360° rotation at increments of less than 1° per step and a series of 2-dimensional (2D) X-ray images were acquired, which were then reconstructed to obtain 3D images. The reconstruction process involved several steps like recording of projections, logarithm, ramp filtering and back projection. The object was rotated such that it was always inside cone X-ray. In case of manipulator, the maximum size of object diameter was 500 mm and length was 600 mm. The maximum distance between sample and detector was 1500 mm. The high resolution detector, Type XRD 1640 had output type directional angle at 60°. During reconstruction of 3D images, the first 25 back

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