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Hybrid scaffold bearing polymer-siloxane Schiff base linkage for bone tissue engineering

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ABSTRACT

Scaffolds that can provide the requisite biological cues for the fast regeneration of bone are highly relevant to the advances in tissue engineering and regenerative medicine. In the present article, we report the fabrication of a chitosan–gelatin–siloxane scaffold bearing interpolymer-siloxane Schiff base linkage, through a single-step dialdehyde cross-linking and freeze-drying method using 3-aminopropyltriethoxysilane as the siloxane precursor. Swelling of the scaffolds in phosphate buffered saline indicates enhancement with increase in siloxane concentration, whereas compressive moduli of the wet scaffolds reveal inverse dependence, owing to the presence of siloxane, rich in silanol groups. It is suggested that through the strategy of dialdehyde cross-linking, a limiting siloxane loading of 20 wt.% into a chitosan -gelatin matrix should be considered ideal for bone tissue engineering, because the scaffold made with 30 wt.% siloxane loading degrades by 48 wt.%, in 21 days. The hybrid scaffolds bearing Schiff base linkage between the polymer and siloxane, unlike the stable linkages in earlier reports, are expected to give a faster release of siloxanes and enhancement in osteogenesis. This is verified by the in vitro evaluation of the hybrid scaffolds using rabbit adipose mesenchymal stem cells, which revealed osteogenic cell-clusters on a polymer-siloxane scaffold, enhanced alkaline phosphatase activity and the expression of bone-specific genes, whereas the control scaffold without siloxane supported more of cell-proliferation than differentiation. A siloxane concentration dependent enhancement in osteogenic differentiation is also observed.

1. Introduction

Silicon is an element which has been shown to promote osteogenic differentiation, alkaline phosphatase activity and gene expression [1–4]. Silicon plays an important role in calcification during bone formation and increased concentrations of silicon have been found in connective tissues and bone [5,6]. Epidemiological studies have proven that silica intake rate has a positive effect on bone mineral density and bone strength at the hip site of men and pre-menopausal women [7,8]. Consequently, ceramic and polymer-inorganic hybrid scaffolds containing silicon have been extensively explored for bone tissue engineering applications [9–12]. In the perspective of a homogeneous regeneration of bone, nano-hybrids with uniformly distributed silicon are favored as scaffolds for bone tissue engineering [13,14].

Siloxane, an oxidized form of silicon can be incorporated into polymer matrices through the sol–gel method for achieving a desired hybrid having uniformly distributed silicon content. The most commonly used precursor for the covalent incorporation of siloxane into the polymer matrix is glycidoxypropyltriethoxysilane, through a secondary amine linkage stable towards hydrolysis [10,11,15,16]. Other methods reported for the covalent incorporation of siloxane include ester linkage generated on the limited number of carboxylic groups on gelatin, and also through urethane and urea linkages [17,18]. The stability of urea and urethane linkages towards hydrolysis and the limited possibility of formation of ester linkages on gelatin can reduce the ready availability of siloxane to the cells for enhanced osteogenesis. It has been reported that the release of inorganic ions like calcium and silicon into the culture medium and their internalization can enhance osteogenesis significantly [5,19]. These inorganic ions can also serve as nucleating agents for the mineralization of osteoids, helping in the acceleration of bone nodule formation and maturation, which is a key factor for the success of bone tissue engineering [5,20,21].

Schiff base or an imine, which is produced through the reaction of an aldehyde or ketone with an amine, has been used for various bio-medical applications, including cross-linking of polymers for bone tissue engineering [22–24]. However, no report is available hitherto, which explored the cross-linking chemistry using a dialdehyde to covalently incorporate a siloxane into a polymer matrix for fabricating a hybrid scaffold for bone tissue engineering. It is expected that the incorporation of siloxane and simultaneous cross-linking of polymers through the Schiff base linkage, which is reactive towards hydrolysis can yield a scaffold that can provide enhanced osteogenesis [25]. In the present study,

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covalent incorporation of amine-containing siloxane through Schiff base formation into gelatin, a partial derivative of collagen; and chitosan, an amine-bearing polysaccharide is attempted. Though supports cell adhesion and proliferation, chitosan's major drawback is its high brittleness and low mechanical strength and a combination of gelatin and chitosan was opted to compensate for the brittleness of chitosan [26,27]. Covalent incorporation of siloxane in to chitosan-gelatin matrices through Schiff base formation has not been reported yet. Structural and in vitro evaluation of the scaffold for bone tissue engineering applications using rabbit adipose mesenchymal stem cells (AD-MSC) is presented in the article.

2. Experimental section

2.1. Materials

Gelatin (acid extracted from bovine skin with bloom number equal to 225, Mw ~ 50,000), chitosan (with a degree of deacetylation of ≥75%) and 3-aminopropyltriethoxysilane (AS) were purchased from Sigma (USA). Glutaraldehyde solution (25% w/w, aqueous), all the inorganic salts for simulated body fluid and hydrochloric acid were of analytical grade and purchased from Merck Specialties Pvt. Ltd., India. Dulbecco's Modified Eagles Medium, High Glucose (DMEM-HG), fetal bovine serum (FBS), and penicillin-streptomycin were procured from Gibco (USA).

2.2. Methods

2.2.1. Fabrication of the scaffolds

The hybrid scaffolds were prepared in two steps, cross-linking and freeze drying. The combination of gelatin and chitosan was opted to compensate for the brittleness of chitosan [25,26]. A chitosan:gelatin weight ratio of 1:4 was chosen to provide sufficient mechanical strength for the scaffold. Representative siloxane concentrations of 10 and 20% with respect to the total weight of the polymers were opted and the scaffolds obtained were denoted as CG10 and CG20, respectively. The maximum siloxane concentration was limited to 20 wt.% because a siloxane concentration of 30 wt.% (CG30) resulted in extensive degradation and shrinkage of the scaffold up on cross-linking with glutaraldehyde, leading to the loss of dimensional stability. Briefly, to a 1.2 wt.% solution of chitosan in 0.1 N HCl, gelatin (4.8 wt.%) was added so that the total polymer content will be 6 wt.%, and the solution was heated at 40 °C for 1 h to ensure complete dissolution of gelatin. 3-aminopropyltriethoxysilane was hydrolysed by aging in 0.1 N HCl for 48 h to obtain the hydrolysed siloxane solution. To the polymer solution, the hydrolysed siloxane solution was added and stirred using a mechanical stirrer for 30 min. The viscous solution thus obtained was further cross-linked by mixing with glutaraldehyde at a polymer:glutaraldehyde weight ratio of 14:1, and the highly viscous solution thus obtained was immediately transferred in to cylindrical moulds and allowed to incubate at 4 °C for 6 hours, to obtain a gel. Subsequently, the cross-linked hybrid gel was frozen in cylindrical molds by being held at −80 °C for 5 h and then lyophilized by holding for 48 h to obtain the hybrid scaffold. The scaffold was washed twice with neutralizing buffer followed by washing four times with distilled water to remove the adsorbed acid and unreacted siloxane (each washing using a shaker took an average of 2 h). Scaffolds of the required dimension for various studies were cut from the bulk scaffold using a scalpel. Chitosan–gelatin scaffold without siloxane, was used as the control scaffold (CG). In order to confirm the incorporation of siloxane using glutaraldehyde cross-linking, the hydrolysed siloxane solution was precipitated by cross-linking with glutaraldehyde and the powder obtained (SIL) was vacuum dried at 80 °C. Fabrication of the scaffolds is schematically shown in Fig. 1. The hybrid scaffold CG30 showed a weight loss of 48 wt.% in 21 days, and was not opted for the in vitro evaluation (Fig. S1, Supplementary Information).

2.2.2. Structural evaluation of the scaffolds

The scaffolds were characterized using X-ray diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FT-IR), thermogravimetry (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), energy dispersive spectra (EDS), for its structure and morphology. XRD data for 2θ between 5° and 25° were collected on a Philips X'pert Pro X-ray diffractometer equipped with a graphite monochromator and X'celerator detector. FT-IR measurements were made on a Perkin-Elmer Spectrum one spectrophotometer in the range of 4000–400 cm^{-1} using KBr pellets containing 2 wt.% samples. TGA was performed on a TGA-50 (Shimadzu) thermogravimetric analyzer employing a heating rate of 10 °C/min from 30 to 775 °C under a nitrogen flow of

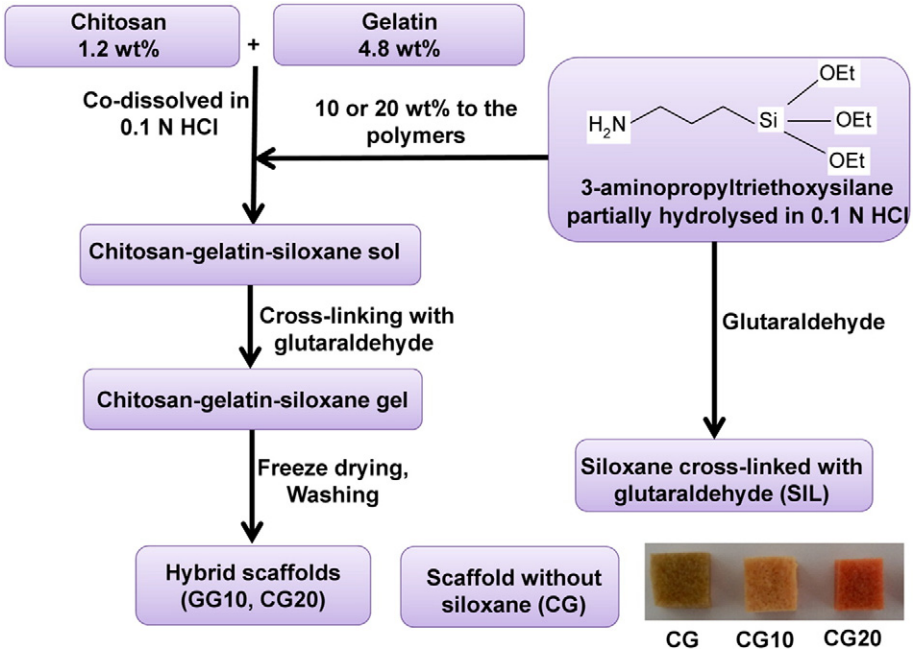

Fig. 1. Scheme showing the steps involved in the fabrication of the hybrid scaffolds CG10 and CG20. Scaffold without siloxane (CG) was fabricated as the control and SIL was precipitated for confirming the incorporation of siloxane in to the polymer matrix.