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Study the effect of silk-fibroin/nanochitosan/hyaluronic acid on adhesion, proliferation and osteoblast differentiation of bone using MC3T3-E1 cell line for tissue engineering

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Nanochitosan/silk-fibroin/hyaluronic acid (NCS/SF/HA) of ternary blend was prepared and then nanochitosan was chemically crosslinked were geared up by the simple ionic cross linking method using TPP to get better bioavailability. Characterizations of the ternary blends were investigated by thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC), *in vitro* studies were carried out. *In vitro* cell culture study using MC3T3-E1 cells has shown an enhanced cell attachment, proliferation, differentiation, scaffold porosity and hydrophilicity, were further achieved by the incorporation of the ternary scaffolds NCS/SF/HA. The assay studied in the cell line MC3T3-E1 include LDH. In the near future, it is most likely that the NCS/SF/HA/scaffold based systems would help to reconcile the clinical and commercial demands in tissue engineering.

Keywords: Silk fibroin, nanochitosan, hyaluronic acid, biomaterial, cell culture, *in vitro* studies.

Introduction

Current approaches to treat large bone defects involve transplantation of autologous bone grafts. Tissue engineering is a promising alternative approach to injury repair that uses biomaterials to restore function of damaged or disfunctional tissues[1,2]. An important goal in bone tissue engineering is to devise methods that can enable bone repair in cases where the loss/damage is significantly high, using biomaterial scaffolds to deliver vital cells to the defective site[3]. Scaffolds for osteogenesis should mimic the morphology, structure of the bone and facilitate normal function[4]. Chitosan is a biopolymer of polyaminosaccharide, consisting of unbranched chains of β-(1,4)-2-acetoamido-2-deoxy-glucose. Chitosan possesses more number of chelating amino groups and can be chemically modified[5]. Nano-sized particle possess good performance due to high specific surface area, small size and quantum size effect that aims to synthesize chitosan nanoparticles by ionic gelation of chitosan and tripolyphosphate could make it exhibit higher capacity towards cell binding and proliferation[6]. Silk biomaterials have received increasing attention as promising scaffolds for tissue engineering, although their biological suitability remains to be established[7,8]. Hyaluronic acid (HA) is a linear polysaccharide and it is an important constituent of the extracellular matrix of many soft tissues in the body[9].

This study investigates the preparation and characterization made from the blend of nanochitosan (NC), silk fibroin (SF) and hyaluronic (HA) acid. The thermal property of the fabricated scaffold are examined by Thermo Gravimetric Analysis (TGA). The potential use of scaffold in bone tissue engineering applications is evaluated through a MC3T3-E1 cell culture test.

Materials and methods:

Preparation of chitosan nanoparticles:

1 g of chitosan was dissolved in 200 ml of 2.0% (v/v) acetic acid. 20 ml of 0.8 g sodium tripolyphosphate was dropped slowly with stirring. Chitosan nanoparticles as a suspension were collected and stored in deionised water. Supernatant was discarded and nanochitosan was air dried for further use and analysis.

Preparation of silk fibroin/hyaluronic acid:

Silk fibers of 3 mm length were cut and 0.5 g of it was dissolved in 100 ml of 10% LiCl in formic acid. This silk fibroin solution was then stirred well under magnetic stirrer for a period of 2 h. After this process, hyaluronic acid (HA) (80–

150 kDa, 0.5 mg) was dissolved in deionized water (20 mL) and this solution was stirred well for 2 h at room temperature to activate the HA carboxylic group. This prepared solution mixture was then freezed dried to –80°C for overnight and the scaffold was subjected to further studies.

Results and discussion

Thermogravimetric analysis:

TGA is a technique in which, its endothermic properties, exothermic properties, the temperature upto which the material does not loss weight and starts decomposing[10] were identified from TGA studies. TGA also helps in predicting the sequence of arrangement of repeating units and side groups in the polymers chains.

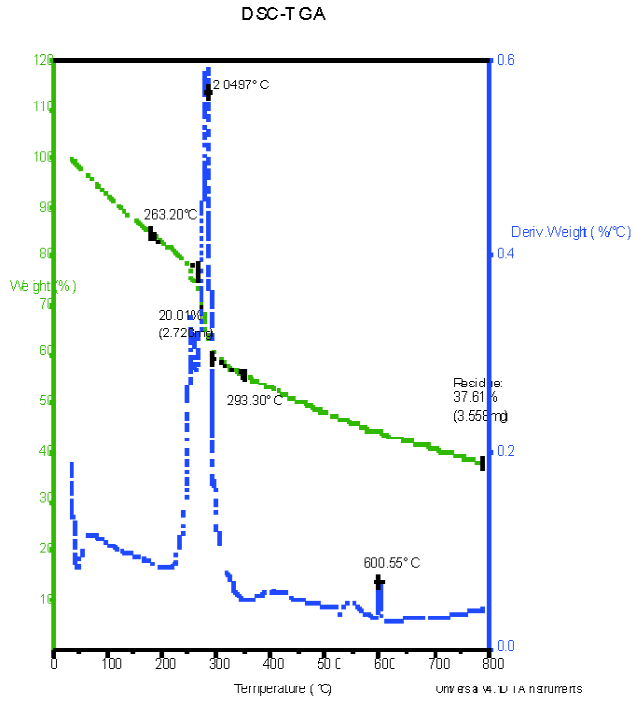

Fig. 1. TGA studies of 3D porous scaffold of ternary NCS/SF/HA blend.

The TGA thermogram details of nanochitosan/silk fibroin/hyaluronic acid ternary blend indicate that two stages of major weight losses. The initial weight loss, below 100°C, was mainly due to water evaporation and is followed by nearly constant weight from 150°C to 180°C[11]. The very low mass loss observed in the second stage range from 150°C to 180°C can be attributed to the loss of other low temperature volatile species[12]. The third major weight loss from 263.20°C to 293.30°C is associated with the breakdown of side chain groups of amino acid residues (cleavage of peptide bonds) present in silk fibroin material and the disassociation (degradation) of intermolecular side chains of hyaluronic acid and nanochitosan[13].

DSC thermal analysis:

DSC method is used to determine the miscibility of the molecules in a polymer and also properties of the solid state such as the glass transition temperature (T_g) of some synthetic polymers[14].

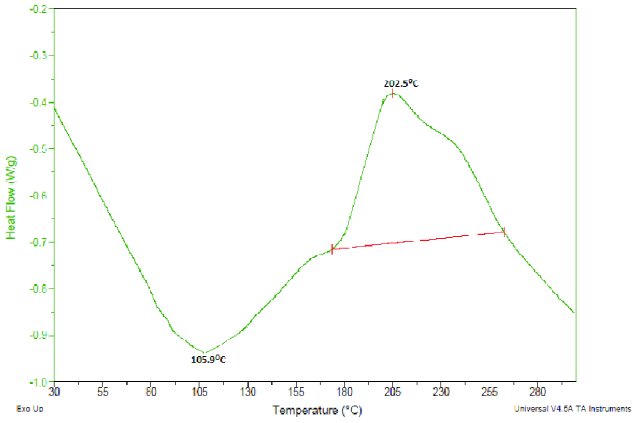

Fig. 2. DSC studies of 3D porous scaffold of ternary NCS/SF/HA blend.

DSC study of ternary scaffold NCS/SF/HA shows a broad endothermic peak at 105.9°C which is attributed to the elimination of absorbed water associated with the hydrophilic groups of the polymer and a sharp exothermic peak at 202.5°C is due to the decomposition of side chains in nanochitosan, amino acids and also the glycosidic units in hyaluronic acids[15]. The ternary scaffold shows a single glass transistion temperature at 175°C higher the T_g, the better will be the long term thermal stability of a material. From the observed result it was concluded that the ternary scaffold was found to be thermally more stable.

LDH (lactate dehydrogenase):

The lactate dehydrogenase is a cytosolic enzyme present in most eukaryotic cells, releases into culture medium upon cell death due to damage of plasma membrane and the increase of the LDH activity in culture supernatant is proportional to the number of lysed cells[16].

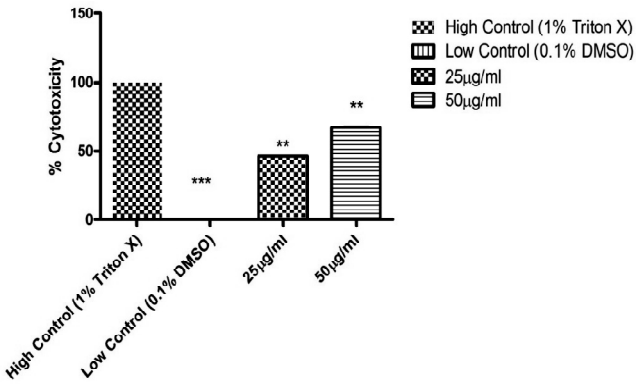

Fig. 3. Lactate dehydrogenase (LDH) activity in MC3T3-E1 cells culture assessed by 3D scaffold of ternary NCS/SF/HA blend.

Fig. 3. represents the lactate dehydrogenase (LDH) activity in MC3T3-E1 cells culture assessed by 3D scaffold of ternary NCS/SF/HA blend. *In vitro* cytocompatibility was evaluated by using MC3T3-E1 cells to confirm that the developed ternary blend were cytocompatible and nontoxic, and cells were found to be attached and well spread out on the scaffold by various concentration is due to chitosan is the only positively charged biopolymer and is able to interact with negatively charged scaffold matrix, without evident signs of cytotoxicity in the *in vitro* system[17]. Therefore, cytosolic enzymes can leak into extracellular fluids only when the cell membrane integrity is lost[18]. From the above obtained results it was evident that the ternary blended NCS/SF/HA scaffold shows less cytotoxicity when compared to the control and hence it was suggested that the NCS/SF/HA scaffold was found to be the most promising material for biomedical applications.

Conclusions

Biodegradable polymeric nanoparticle of chitosan, silk-fibroin and hyaluronic acid was incorporated with the highly potent 3D Scaffold are utilized to carry out tissue engineering applications by using MC3T3-E1 cell line. TGA and DSC study shows that the scaffold is highly thermally stable and suitable to carry out biomedical applications. The LDH assay study reveals that the scaffold is non-toxic and enhanced proliferations. The ternary scaffold prepared could be used to carry out further studies like clinical sutures, cartilages and wound healing in biomedical applications.

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