DEVELOPMENT AND CHARACTERIZATION OF CHITOSAN AND CHONDROITIN SULFATE BASED COMPOSITE SCAFFOLD FOR DRUG RELEASE

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ABSTRACT

The current study deals with the development and characterization of polymer composite/porous scaffold formed by varying the proportion of chitosan and chondroitin sulfate. It was studied that chitosan and chondroitin sulfate were crosslink to form a biomaterial to support control drug release. Composites were highly hemocompatible in nature. Polymer composites were characterized by yield percentage and fourier transform infrared analysis. The microstructures of the composite scaffolds analyzed through scanning electron microscopy. Ofloxacin a model drug was incorporated with composite solution and release kinetics was studied under *in vitro* conditions. Ofloxacin loaded scaffolds were effective against *Bacillus subtilis* and *Escherichia coli*. Based on the preliminary results, it was concluded that the scaffolds being used for the evaluation of the drug release in pathological state of cartilage degeneration. Further polymer composite/porous scaffold being shows good potential to be used with cell for *in vivo* study.

KEY WORDS: Polymer; Composite; Scaffold; Cartilage

INTRODUCTION

Tissue Engineering is an emerging field with the aim to repair, replace injured or damaged tissues. The research regulated in different type of organs including skin, cartilage, blood vessels, bone, muscle, nerves, liver, kidney etc. (Baran, Kiani et al. 2014). The mechanism of tissue engineering is to formed tissue *in vitro* by seeding cells into a biomaterial matrix after that implant the regenerated tissue into the body. Chitosan and chondroitin sulfate (CS) ironically cross linked to form a composite and its scaffold for the purpose of drug release. Composite shows various tissue engineering and biomedical applications. The freeze dried composites being used for the evaluation of the ofloxacin (OX) drug release in pathological state of cartilage inflammation. 9-Fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid is the systematic name of OX. The drug OX is treated to be a broad spectrum antibiotic effective against common gram-positive and gram-negative strains. It was studied that OX go through in to the tissue in the higher concentration and further analyzed through for soft tissue diseases (Wellinger, Gality et al. 2012; Ramineni, Eluri et al. 2015).

Chitosan is a natural cationic linear polysaccharide having β-(1-4)-linked D-

glucosamine and N-acetyl-D-glucosamine (Peluso, Petillo et al. 1994). Chitosan is extracted from crustacean shells showing biocompatible and biodegradable in nature and the most abundant natural polymer (Al Sagheer, Al-Sughayer et al. 2009). Chitosan is soluble in the presence of dilute acidic solution due the presence of amino group on the other hand insoluble in water is the major drawback in many application. Chitosan structure is quite related to glycosaminoglycans (GAG) often used as suitable candidate for the connective tissue engineering (Pillai, Paul et al. 2009; Pradas and Vicent 2014). Chitosan is generally semi crystalline polymer showing high degree of biocompatibility. Chitosan composites mainly assist the attachment, morphology and proliferation of different kind of cells like chondrocytes, hepatocytes, dermal fibroblasts and adrenal chromaffin cells (Thein-Han and Kitiyanant 2007). For the treatment of osteochondral defects chitosan plays an important role also the composites are mainly used for cartilage regeneration (Sharma, Gautam et al. 2011). Chitosan is a modified natural homo polymer having biocompatible, biodegradable, safe and nontoxic features. Chitosan based nanoparticles and micro particles were developed for the protein delivery and also used for the pulmonary and nasal delivery of peptides and proteins (Kushwaha, Rai et al. 2010). Chitosan are also facilitates as a matrix for controlled drug release in humans, animals along with non-steroidal agrochemicals in agriculture and used as mucoadhesive microcapsules (Patil, Patil et al. 2009; Rajan and Raj 2013). CS is a sulfated glycosaminoglycan mainly composed of N-acetylgalactosamine and glucuronic acids which are chains of alternating sugars. Bovine cornea or chemical treatment is the main source to get CS. The main role of CS in cartilage regeneration includes reduce in catabolic activity of chondrocytes and also exerts anti-inflammatory activity (Muzzarelli, Greco et al. 2012).

In the current study Chitosan and CS based composites and freeze dried were developed. Freeze dried scaffolds were prepared for the morphological analysis, drug release and antimicrobial study. The composites were thoroughly characterized using yield percentage and molecular properties.

MATERIAL AND METHODS

Chitosan, CS, sodium acetate and OX were obtained from Himedia, Mumbai, India. Ethanol was obtained from Honyon International Inc., Hong Yang Chemical Corporation, China. Acetic acid and sodium carbonate were obtained from Merck Ltd., Mumbai. Double distilled water (DDW) was used all over the study.

Preparation of polymer composites/scaffolds

2 % (w/v) polymeric solution were prepared by dissolving 2 g of chitosan and CS separately in 98 ml of 2% (v/v) acetic acid and acetate buffer (pH-5.0) in double distilled water and kept stirring at 500 rpm (40°C) for 24 h at room temperature. Chitosan and CS solution were mixed in the proportion of (100:0, 70:30, 50:50, 30:70, 0:100) on volume basis. Precipitates were separated by centrifugation for 5 min at 10000 rpm. Freeze dried chitosan and CS mixture were used for the SEM, drug release and antimicrobial study. The different proportion of the mixtures were kept in -20°C for the duration of 24 h, after that the whole mixture with different proportion were kept in a lyophilizer at -50°C for 48 h to get freeze dried product. The OX loaded polymer composite were prepared by the addition 2% (w/w) of the drug to the composite solution and further freeze dried. The polymer composites are highly hemocompatible in nature as per the previous reported literature (Ng, Hutmacher et al. 2001).

Morphological studies

Scanning electron microscopy (SEM) was done (QUANTA 200 F) for the freeze dried scaffolds. Gold sputter coating was done before the SEM analysis.

Molecular properties

The interactions between the component chitosan and CS in the polymer composites and the raw materials were studied in the FTIR (ATR spectroscopy) (Thermo Nicolet FTIR). Prior to the analysis the composite were dried in the hot air oven at 40ºC for 48hrs and crushed in to powder form. The adsorption spectra were measured at a scanning wave number range from 4000 cm^{-1} to 500 cm^{-1}.

***In vitro* drug release**

Freeze dried drug loaded samples were used for the drug release. *In vitro* drug release carried out in the USP 24 type-2 dissolution apparatus in 900ml of phosphate buffer (pH 7.4) at the paddle rotation of 100±5 rpm and dissolution media temperature was maintained to 37 ± 3°C. The experiment carried for a time interval of 10 h. Five ml of samples were taken at a time interval of 30 min for 10 h and replaced with fresh media. After 10 h the replaced media were examined spectrophotometrically (UV-1800 Spectrophotometer, Shimadzu) at 290nm.

Antimicrobial studies

Antimicrobial efficiency test were performed for the drug loaded samples against gram positive bacterium *Bacillus subtilis* and gram negative bacterium *Escherichia coli*. In the nutrient agar medium 100µl of the bacterial suspension having 10^6 CFU/ml were spread over it. The polymeric scaffolds treated to be negative control on the other hand 2% (w/w) drug loaded treated to be the control. The plates were then incubated for 24 h at 37°C. Zone of inhibition (ZOI) was measured after 24 h using the help of a ruler.

RESULT AND DISCUSSION

Yield percentage (polymer composites)

Polymer composites were prepared by varying the ratio of chitosan and chondroitin sulfate. There was a uniform mixing in the polymer samples. The yield percentage can be found out by using the formula yield percentage (%) = $W_0/W_t \times 100$, where W_0 is the weight of the polymer composite obtained and W_t is the total weight of the polymer taken(Kaur and Kaur 2013). The various compositions of the polymer composites have been tabulated in table 1.

Morphological studies

Freeze dried composites were analyzed for the SEM analysis shown in the Figure 1. The composition F1 shows the planer matrix. The composition F2, F3, and F4 shows homogeneous structure with porous network structure. Porous structure also formed in composition F5 in the freeze dried CS composite scaffold. All the composition shows appropriate morphology due to their heterogeneous structure along with well-organized porous structure.

Molecular properties

The ATR spectra of polymer composites formed by different proportion of chitosan and CS showed characteristic band shown in the Figure 2. It is evident that F1 shows peak at 1559 cm^{-1} and 1330 cm^{-1} this may be attributed to the vibration of -NH3+ and C-H groups (Anicuta, Dobre et al. 2010). In the formulation F2, F3 and F4 shows the broad peak in the range 1400-1600 cm-1 this may be attributed due to the presence of –CONH2, -COO- and –OSO3- groups. F5 shows broad peak around

1200 cm-1 this may be due to the presence of H and SO3H groups.

In vitro drug release

The in vitro drug release profiles of ofloxacin from freeze dried composite have been shown in the Figure 3. The rate of drug release depends upon the proportion of chitosan and chondroitin sulfate. Equal proportion of chitosan and chondroitin sulfate shows higher percentage of drug release (Murata, Miyamoto, & Kawashima, 1996; Oprea, Nistor, Profire, Popa, Lupusoru, & Vasile, 2013). The drug release take place very slowly in the composition F1 this may be attributed to higher water uptake rate. The release of drug from the freeze dried composite follows higuchian kinetics (Dash, Murthy, Nath, & Chowdhury, 2010; Amrutkar, & Gattani, 2009). Thus it can be concluded that there the drug release is diffusion controlled. Antimicrobial efficiency of the ofloxacin loaded composites was found out.

Table 1: Composition of polymer composites.

Sample No.	Chitosan:CS (Proportion)	Ofloxacin %(w/w)	Yield Percentage
F1	100:0	-	37.34±2.4
F2	70:30	-	41.63±3.7
F3	50:50	-	42.59±2.3
F4	30:70	-	39.43±4.4
F5	0:100	-	44.41±3.5
F1D	100:0	2	-
F2D	70:30	2	-
F3D	50:50	2	-
F4D	30:70	2	-
F5D	0:100	2	-

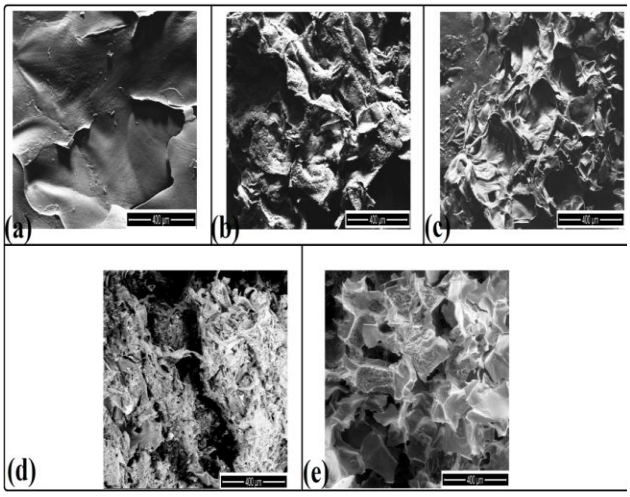

Figure 1: Scanning electron microscopy (SEM) of freeze dried composites (a) F1 (b) F2 (c) F3 (d) F4 and (e) F5.

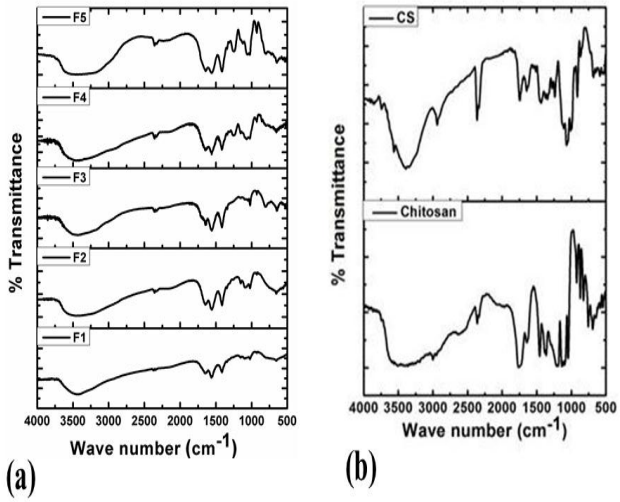

Figure 2: FTIR spectra of polymer composites and raw materials.

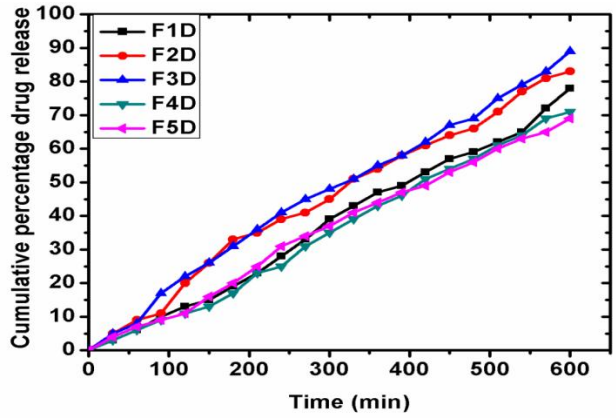

Figure 3: In vitro drug release profiles of freeze dried composites.

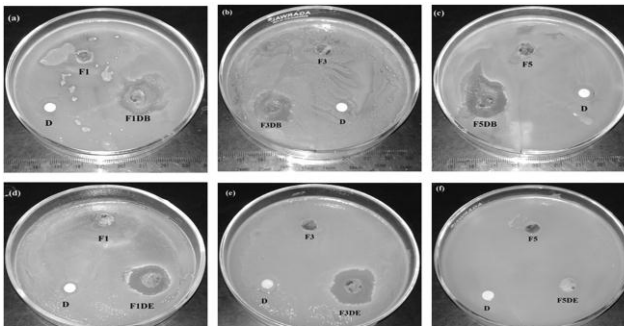

Figure 4: Antimicrobial activity of freeze dried scaffolds against *B. subtilis* (a) F1, (b) F2, (c) F3 and *E. coli (d) F1, (e) F2 and (f) F3*.

Antimicrobial studies

The antimicrobial efficiency of the OX loaded scaffolds were analyzed (Figure 4). The composition F3 shows the higher zone of inhibition compared to the other samples due to the higher rate of drug release in the composition F3. The drug loaded scaffolds shows good antimicrobial propertiesSynthetic procedure

CONCLUSION

This study which has involved chitosan and CS as unique combination composites to be used for drug release has given new dimension to scaffolds being used as drug carriers in tissue engineering. The composites scaffolds loaded with drugs can be used as a potential support with necessary anti-infective nature for tissue constructs. The morphological studies and chitosan and CS crosslink's exhibit good distribution matrix which can further be used to support chondrogenesis in cartilage regeneration. The drug loaded matrix supported good rate of drug release in pathological state of cartilage degeneration. The results have been duly supported by SEM, FTIR and compatibility.

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