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Novel meloxicam releasing electrospun polymer/ceramic reinforced biodegradable membranes for periodontal regeneration applications

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ABSTRACT

Periodontal disease is associated with the destruction of periodontal tissues, along with other disorders/problems including inflammation of tissues and severe pain. This paper reports the synthesis of meloxicam (MX) immobilized biodegradable chitosan (CS)/poly(vinyl alcohol) (PVA)/hydroxyapatite (HA) based electrospun (e-spun) fibers and films. Electrospinning was employed to produce drug loaded fibrous mats, whereas films were generated by solvent casting method. *In-vitro* drug release from materials containing varying concentrations of MX revealed that the scaffolds containing higher amount of drug showed comparatively faster release. During initial first few hours fast release was noted from membranes and films; however after around 5 h sustained release was achieved. The hydrogels showed good swelling property, which is highly desired for soft tissue engineered implants. To investigate the biocompatibility of our synthesized materials, VERO cells (epithelial cells) were selected and cell culture results showed that these all materials were non-cytotoxic and also these cells were very well proliferated on these synthesized scaffolds. These properties along with the anti-inflammatory potential of our fabricated materials suggest their effective utilization in periodontital treatments.

1. Introduction

Periodontitis is an inflammatory disease which damages periodontal ligament, gingival, alveolar bone, cementum, and triggers bone resorption and finally results complete tooth lost [1]. Chronic periodontitis has been accepted as one of the most leading and widely spreading dental disease [2]. In 2012, the centers for disease control and prevention (CDC) reported that in US 47.2% of adults aged 30 years or older had some form of periodontal disease this number increases to 70% in over sixty years [3]. Fig. 1 shows the difference between the normal tooth/gum and periodontitis.

The on-site generation and release of prostaglandins (PGs) [4] and arachidonic acid metabolites [5] are mainly responsible for periodontal destruction [6]. Non-steroidal anti-inflammatory drugs (NSAIDs) have delivered promising results in periodontal treatment due to their ability to decrease the inflammatory destruction [7–9]. NSAIDs suppress the formation of eicosanoids by blocking the cyclooxygenase (COX) enzyme systems which converts arachidonic acid to the individual metabolites [10]. These metabolites are responsible for periodontal destruction [11]. The COX-1 and COX-2 are two forms of the COX enzyme. Generally, the COX-2 NSAIDs deliver better clinical benefits than the classical non selective NSAIDs [12]. In this research, MX was chosen to obtain it's controlled and local release. Meloxicam (MX) belongs selective COX-2 inhibitor NSAIDs and is also known as a potent inhibitor of severe exudation and protects cartilage and bone loss [12]. In addition, MX is identified as an effective drug in preventing alveolar bone loss in animal models [13]. Due to the problems associated with the difficult locations of the periodontal pockets and also the local production of inflammatory mediators in these periodontal pockets. It suggests the intra-pocket NSAIDs delivery systems the most logical and effective solution. Additionally, intra-pocket delivery systems require overall less amount of the active agent required for treatment and a controlled drug delivery device holds a number of benefits such as better patient compliance and effective drug release onto the damaged site [14,15].

The synthetic guided tissue regeneration (GTR) membranes have attracted great attention due to their successful use in biomedical applications. GTR membranes are generally composed of polymers, which behave as barrier materials. They prevent epithelial down growth and allow alveolar bone cells and periodontal ligament to grow and repair the defects [16]. In the last 2 decades the potential of chitosan as a biomaterial for tissue engineering and regenerative medicine has been extensively investigated [17–20]. Chitosan is easily obtained by simple

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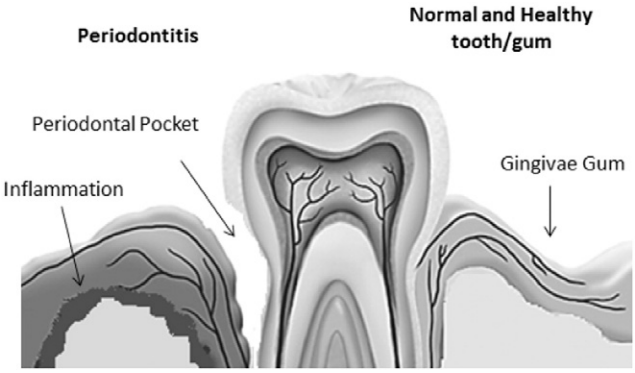

Fig. 1. A comparative representation of normal and healthy tooth/gum and periodontitis.

deacetylation reaction under basic conditions from abundantly available natural polymer chitin. It has bacteriostatic properties; inhibits the growth of both gram negative and gram-positive bacteria [21]. The application of chitosan gel in patients with chronic periodontitis has showed the reduction of gingival inflammation markers, due to its antimicrobial properties [22].

Calcium phosphates (CaP) are bone cells carriers and are also osteoinductive (capable of osteogenesis) materials having ability to bind endogenous bone morphogenetic proteins in circulation Therefore, CaP are attractive and extensively used biomaterials for hard tissues engineering applications [16]. One of the most widely used CaP for bone graft applications is hydroxyapatite (HA) [23]. HA holds chemical composition similar to natural bone mineral [23] and therefore it interacts directly to bone when it is implanted [24].

Recently, an effective composite for bone repairs was prepared from HA nanocrystals with incorporated chitosan [25,26]. In another study, Tang et al., reported the impact of HA in the thermo sensitive hydrogels containing chitosan/poly(vinyl alcohol) and HA, in this study HA facilitated sustained release of tested protein [27]. PVA is a non-toxic polymer with good flexible and foldable films making properties, all these properties of PVA make it suitable agent for the preparation of flexible membranes for soft tissue engineering applications [28,29].

Numerous techniques have been used for the fabrication of porous cell-supporting membranes. Particularly, electrospun (e-spun) fibers have attracted great attention in biomedical applications due to their unique morphology and composition which mimics extracellular matrix [30]. 3D fibrous sheets support cell attachment, migration, proliferation and differentiation greater as compared to traditional 2D scaffolds [30]. E-spun fibers have been widely exploited in tissue engineering, wound healing and drug delivery applications [31,32]. The uses of bioceramic materials alone or as composites with biopolymers have revolutionized the field of regenerative medicine [33].

In literature, the authors could not find any study in which MX loaded CS/HA/PVA e-spun nanofibrous membranes/films were prepared. This study reports the development of MX encapsulated biodegradable chitosan (CS)/poly (vinyl alcohol) (PVA)/hydroxyapatite (HA) based electrospun fibers and films with potential anti-inflammatory properties to treat periodontitis. We believe the immobilized MX drug would play an important role to inhibit periodontal inflammation and its local release would make this system more effective.

2. Materials and methods

2.1. Materials

Chitosan (CS) (Mw: 146,105.g/mol., degree of deacetylation (DD): 83%, intrinsic viscosity: 30.78 ml/g), poly(vinyl alcohol) (PVA) (Mw: 72,000, degree of hydrolysis 98%) was purchased from Merck (Germany) and hydroxyapatite (HA) CAPTAL® 'S' grade was purchased from Plasma Biotal Limited (UK). Sodium hydroxide (Sigma Aldrich) hydrochloric acid (Merck, Germany) and glacial acetic acid (BDH Laboratory Supplies UK) were of analytical grade reagents and were used without further purification. Meloxicam (MX) was provided by Empire Pharmaceuticals (Pvt.) Ltd, Lahore, Pakistan. Chitosan was prepared by following the procedure published elsewhere [34–36] with some modifications [37].

2.2. Preparation of Meloxicam-loaded CS/HA/PVA fibrous membranes and films

Four different formulations (with different MX concentration were prepared by adding 0.03%, 0.06%, 0.09% and 0.12%MX (Table 1). HA (1% w/w) particles were dispersed in glacial acetic acid (85%, 12 ml) to form a suspension and were magnetically stirred to decrease agglomeration. To this suspension chitosan (2.5% w/w) was added while stirring. Then PVA (3.7% w/w) which was separately dissolved in water at 80 °C was added to the solution while stirring. The mixture was allowed to cool at room temperature and further stirred for 18 h to attain homogeneity. This homogenous solution was divided into four equal parts and to each part 0.03%, 0.06%, 0.09% and 0.12%MX was added respectively (Table 1) and were stirred overnight at room temperature. The mixtures were further homogenized by using an ultrasonicator (Wise Clean, WUC-DO6H) for 30 min. The solution having MX (0.12%) was divided into two equal parts to prepare e-spun fibers and films, whereas the remaining 0.03%, 0.06%, 0.09% MX containing solution were used to prepare e-spun membranes.

The solutions were e-spun at a voltage of 18KV to create micro/nanofibers with a needle having 24 gauge inner diameter and a 0.3 mL/h flow rate of solution using a syringe pump. The distance between needle tip and charged aluminum collector was 8 cm. The e-spun fiber mats were removed from the collector. Then half portion of the MX loaded e-spun fibers membranes were heat treated at 80 °C (below the glass transition temperature of PVA *i.e.* 85 °C) and these were called heat treated and the remaining parts which were not heat treated were called control. For the preparation of films, the homogenized solution was poured into petri dishes and was kept in a vacuum oven for 2 h to remove air bubbles then dried at 37 °C for 24 h to form a thin film (control). Half of this film was then heat treated (heat treated).

The overall protocol for the preparation of fibrous membranes and films and chemical structures of the chitosan, HA, PVA and MX are shown in Fig. 2.

2.3. Characterization of heat treated and control fibrous mats and films

The morphology of the e-spun fibers and films was investigated by Scanning Electron Microscope (JEOL JSM-6480 LA). An accelerating voltage of 10 kV was applied and samples were sputter coated. SEM images were used for calculating the average fiber diameter (was average of 100 fibers) by using image-processing software (Image J).

The degree of swelling of all the synthesized materials was measured by adding the pre-weighted scaffolds into phosphate buffer solution (PBS) at 37 °C for 24 h. The below given formula was followed to determine the degree of swelling:

$$\text{Degree of swelling } (\%) = [(M - Md)/Md] \times 100.$$

M is the weight of sample after submersion in the buffer solution for 80 min; M*d* is the weight of the sample in its dry state.

Fourier Transform Infrared Spectroscopy (Thermo Nicolet 6700P, USA) was used to evaluate the chemical structural properties of the prepared materials. The spectra were recorded at room temperature using photo acoustic mode with carbon background and helium purging, with resolution of 8 cm^{-1}, 256 scan numbers and range of 4000–400 cm^{-1}.