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RESEARCH ARTICLE

Chitosan/PVA nanofiber for the application in implantable drug delivery systems

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ABSTRACT

In this study, we prepared methotrexate (MTX) loaded chitosan/polyvinyl alcohol (PVA) nanofibers using electrospinning method. The prepared nanofiber mats were soaked into the glutaraldehyde solution for crosslinking. The ratio of chitosan/PVA was 1:9 and 1:7, and the crosslinking time was 24 hrs and 36 hrs. In vitro release study was performed on four formulations of nanofibers. Determination of contact angle, SEM, and MTT assay on U-87 MG cell line and MCF-7 cell line were carried out by those formulations of nanofibers. SEM images showed that the average fiber diameter was 221 nm with a range of 94–410 nm and also water contact angle was 24.3°. In vitro release profile of nanofibers with the ratio of chitosan/PVA of 1:9 and crosslinking time of 36 hrs was more acceptable compared to other ones. After 24 hrs, Chitosan/PVA nanofibers mat containing MTX had 18% and 20% cytotoxicity on U87MG and MCF7 cell lines, respectively. In conclusion, MTX loaded chitosan/PVA nanofibers would be an appropriate therapeutics implant for cancer therapy; however, more studies are also needed.

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INTRODUCTION

Implants have been used in medical practices for over six decades[1]. Traditionally, these medical implants were used to provide physical and functional restoration or compensation. Common examples of traditional medical implants are applied in dental[2], orthopedics[3], tissue implants[4], stents[5], and pacemakers[6]. Technological advances have ushered new types of multifunctional implantable medical devices capable of delivering xenobiotic therapeutic compounds such as hormones and drugs during recent years [7, 8]. These new devices are expected to complement existing treatments for complicated diseases such as autoimmune diseases, neurodegenerative ones, and cancers.

With regards to cancers, it has been observed that over 80% of cancer cases require invasive medical procedures such as surgical removal of the tumor[9]. This surgical removal of tissue leaves a cavity behind, which can potentially impact the surrounding tissues structural and functional integrity. As a result, medical implants can restore both physical and functional integrity and can be envisioned as a panacea to tissue restoration after surgery. Biocompatible tissue implants have been prepared by a variety of techniques such as electrospinning[10], phase separation [11] and also 3-D printing[12] methods. It has been observed that transplanting a functional scaffold in the brain cavity left after surgery resulted in a six-fold reduction in the cavity volume[13]. Scientists have

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further investigated the potential of "therapeutic" scaffolds, which are scaffolds loaded with anti-cancer agents. Implantation of therapeutics-loaded scaffold in the tumor bed immediately after removal of the tumor via surgery can significantly increase drug localization, potentially improving drug efficacy and decreasing other organs toxicity[14-16]. 3D printed drug delivery implanted loaded with multiple agents exhibited minimal toxicity to normal tissues with sustained drug release in breast cancer treatment[14].

There are several studies with well characterized physiochemical properties of chitosan [17] and polyvinylalcohol (PVA) [18, 19] in the development of an implantable drug delivery system. Chitin, the source of chitosan, is the second most abundant natural polymer; thus, it is a cheap material. Chitosan is a non-toxic, biodegradable polysaccharide with natural wound healing and antibacterial properties[20, 21]. Unfortunately, similar to most natural polymers, the weak mechanical properties of chitosan decrease its biological applications. In order to improve the mechanical properties of chitosan, a synthetic polymer, PVA was added to the chitosan to make a chitosan/PVA blend. PVA is hydrophilic polymer which is generally regarded as a safe and biocompatible polymer[22]. Chitosan/PVA scaffold loaded with therapeutics can be prepared using electrospinning technique. Aside from its simplicity, by electrospinning method coaxial tubes can be prepared, which are desirable for incorporating other agents such as biomolecules and therapeutics[23]. In previous works, preparation of MTX loaded NPs, MTX/curcumin co-loaded nanoparticles and Paclitaxel/MTX co-loaded PLGA nanoparticles was investigated [24-26]. In this work, cancer inhibitory agent, MTX was loaded in nanofiber mats and both its physicochemical and anti-cancer properties were studied.

MATERIALS AND METHOD

Materials

PVA (Mw 70000 Da, fully hydrolyzed) and chitosan were bought from Sigma Aldrich. MTX was purchased from Xian Xinlu Biotechnology company. U-87 MG cells and MCF-7 cell lines were purchased from Pasteur institute of Iran. Acetic acid and glutaraldehyde were from Dr. Mojallali Co. Cell culture media, Fetal Bovine Serum (FBS) and Trypsin were bought from Bio Idea Co.

Preparation of chitosan/PVA solution

Chitosan/PVA solutions with the weight ratio of 1:9 and 1:7 were prepared in acetic acid solution. The chitosan/PVA mixtures and the acetic acid solution in double distilled water (DDW) had constant concentrations of 10% w/v and 0.1% w/v, respectively. The solutions were stirred at room temperature until the components were completely dissolved into a homogeneous mixture. Afterwards, drug-loaded nanofibers were then prepared by adding 5 mg of MTX and further stirring the solution for 3 hrs. The chitosan/PVA solutions with or without drugs were drawn into a 5 ml syringe with an 18-G blunt metal needle as the spinneret. The distance between the needle and the rotating collector was adjusted to 17 cm, and the solution feeding rate was set to 1 ml/h. The covered collector with aluminum foil was set with a rotating speed of 20 rpm, voltage of 15 kV was applied between the rotating collector and the needle tip. The MTX loaded chitosan/PVA nanofibers were then crosslinked with glutaraldehyde for 28 hrs and 36 hrs and dried at room temperature.

Characterization of Chitosan/PVA properties

Drug Loading Efficiency

The efficiency of the nanofibers to entrap MTX was investigated by first weighing 50 mg of fiber mat from all formulations and dissolving it in 15 ml of acetic acid (1% v/v). The amount of free drug in the supernatant was then measured indirectly using UV-Vis spectrophotometer (Cecil CE 7250, England) at the 300 nm wavelength for MTX. The drug loading efficiency was then calculated using the equation below.

$$\text{Drug loading efficiency (DL\%)} = \frac{\textit{Initial amount of drugs} - \textit{amount of drugs in the supernatant}}{\textit{Initial amount of drugs} + \textit{weight of chitosan / PVA}} \times 100$$

Drug release

MTX loaded chitosan/PVA electrospun mats were incubated in 25 ml PBS with pH 7.4 at 37 °C. Due to the MTX tendency to attach to the surface of the nanofibers, within the first hour, four samples were collected to fully characterize the burst release. Afterward, the samples were taken every four hours. The amount of MTX released is proportional to the absorbance of the sample at 300 nm using UV-Vis at spectrophotometer (Cecil CE7250, England).

Physical properties of nanofibers

Afterward, we selected the best formulation for further studies, including morphology, mean diameter, and contact angle. The morphology of the nanofibers was analyzed by SEM after gold coating. The apparent water contact angle (WCA) of the fiber mat was measured with a DSA10 contact angle goniometer (Krüss, Hamburg, Germany). Water droplets (3 µl) were placed carefully on the surface of the nanofibers mat and the contact angles representing the wettability of the material were determined using the low bond axisymmetric drop shape analysis (LBADSA) method.

In vitro cytotoxicity assay

The cells, U87MG and MCF7, were cultured in Dulbecco's modified Eagle medium, containing 10% FBS and 1% penicillin/streptomycin. The dried nanofibrous samples were cut and weighed on a Taishi, JF2004 scale. The samples (2.0 ± 0.08 mg) were placed in 24-well plates, followed by the addition of U87MG cells and MCF-7 cells in DMEM medium supplemented with 10 % FBS. Each well was seeded at a cell density of 300000 cells of either U87MG or MCF7. Afterward, the plates were placed in a humidified incubator containing 5 % CO_2 at 37 °C for 24 hrs. Cells grown in a culture medium without drugs and nanofibers mat were used as a negative control. After 24 hrs the culture media were aspirated and an equivalent amount of MTT solution was added to the samples, and the plate was left for 3 hrs. Afterward, the MTT solution was removed carefully, and DMSO was added to the wells to dissolve the formazan crystals. Both test and control samples were transferred to individual wells of 96-well plate and read at 570 nm using a microplate reader (BioTek ELx808).

RESULTS AND DISCUSSION

SEM and wettability study

Sample B2 with achitosan/PVA ratio1:9 and 36 hrs crosslinking time was selected for SEM, wettability, and cell viability assay investigations. SEM results of the electrospun chitosan/PVA fiber mat revealed an average fiber diameter about 221 nm with a range of 94–410 nm (Fig. 1). It can be readily seen that the mat was fairly porous and beadless with a dense network due to the thickness of the nanofibers.

Deacetylation of the insoluble chitin results in the formation of chitosan which possesses more amino groups. It is well known that the degree of

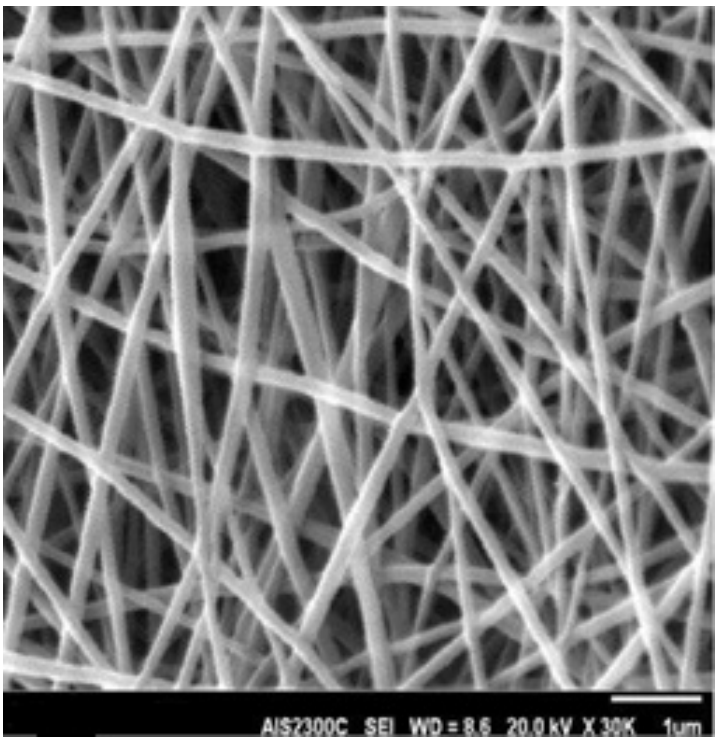

Fig.1. SEM image of chitosan/PVA nanofibers

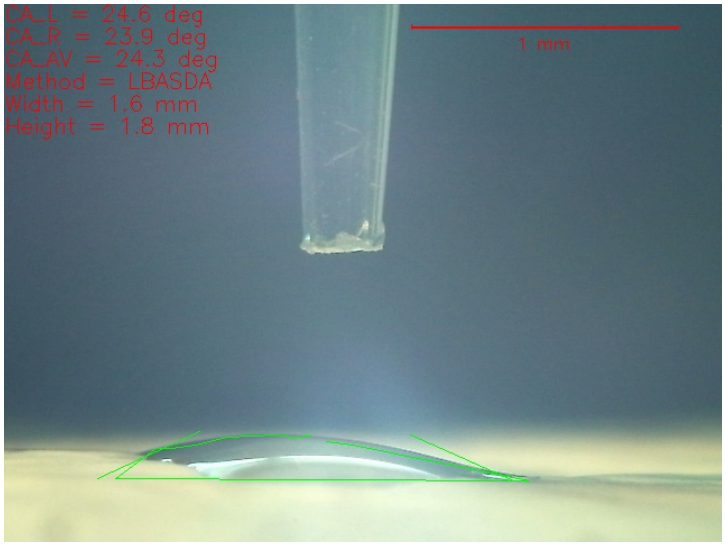

Fig. 2. Water contact angle measurement of chitosan/PVA nanofiber

deacetylation is proportional to the solubility of chitosan due to an increase in the number of amino groups which can interact with water[27]. PVA is also a well-known hydrophilic synthetic polymer. In a study, the contact angle of different PVA film structures was obtained from 6°-65°[28]. Moreover, in another study, different samples of water-soluble chitosan/PVA nanofiber mats had various contact angles from 14°-64°[29]. We also obtained a water contact angle of chitosan/PVA nanofiber mats of 24.3° (Fig. 2).

Drug loading efficiency and drug release

The amount of drug loaded in chitosan/polyvinyl alcohol (PVA) nanofiber mats was determined by UV-Vis at spectrophotometer. All four formulations had high loading efficiencies of 9%. This loading efficiency of MTX was observed in chitosan nanoparticles, and PVA stabilized nanoparticles [30].

MTX release profile was investigated for a period of 480 min at a pH of 7.4 in PBS. As shown in Fig. 2, in A1 sample (chitosan/PVA ratio of 1:7 and crosslinking time of 28 hrs), the drug was released for about 1 h, and in A2 and B1 samples, the drug was released within 4 hrs, whereas drug was released about 6 h for B2 sample. In our earlier study, we reported that loading MTX in nanoparticles depends on the adsorption of MTX on the surface of the nanoparticles [31]. Cui *et al.* reported that PVA/CS nanofibers crosslinked with glutaraldehyde could effectively decrease the drug release rate and the burst effect of drug from the composite [32].

In addition, the release period of the drug enhanced as crosslinking period increased from 28 to 36 hrs. This is in agreement with Laha *et al* results [33].

In vitro cytotoxicity assay

The ability to live cells to reduce tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan is proportional to the number of metabolically active cells. After exposing cells chitosan/PVA nanofibers mat without MTX, cell viability was 99.2% and 99.0% for U87MG and MCF7 after 24 hrs, respectively. MCF7 breast cancer cells were slightly more sensitive to MTX compared to U87MG cells. After 24 hrs, chitosan/PVA nanofibers mat containing 10% MTX had cytotoxicity of 18% and 20% in U87MG and MCF7, respectively.

CONCLUSION

In this study MTX-loaded chitosan/PVA nanofibers mat was prepared using the electrospinning method for drug delivery systems. The average diameter of nanofibers was about 221 nm with a water contact angle of 24.3°. Loading MTX in nanofibers was about 9%, and its release from nanofibers of B2 samples (chitosan/PVA ratio of 1:9 and crosslinking time of 36 hrs) extended to 8 hrs. Moreover, the prepared nanofiber mats was able to kill 18% and 20% of U87MG and MCF7

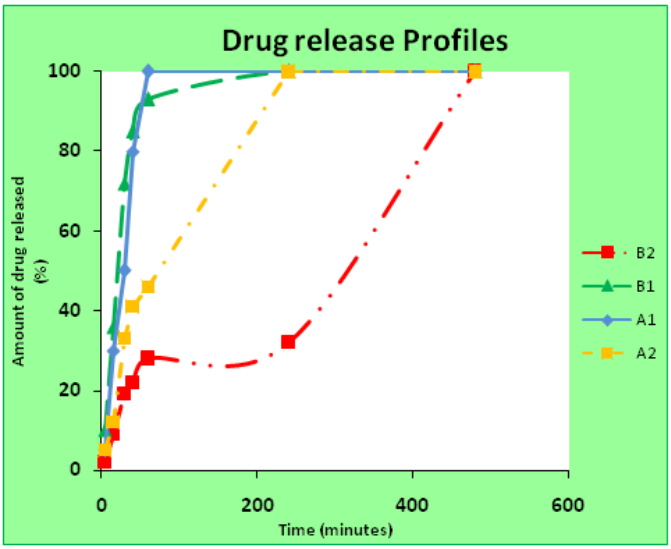

Fig. 3. MTX release profile of chitosan/PVA nanofibers.

Table 1. Physiochemical properties of chitosan/PVA nanofibers containing 9% MTX.

Formulation	Chitosan/PVA ratio	Crosslinking time (hrs)	Fiber diameter (nm)	Contact angle (deg)
A1	1:7	28	-	-
A2	1:7	36	-	-
B1	1:9	28	-	-
B2	1:9	36	221	24.3

cancer cells during in vitro assays. It is proposed that the prepared nanofiber mats may be useful for the implantable drug delivery system in cavity tissue reconstruction. However, more studies are required.

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