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Original Article

Preliminary study of *in vitro* drug permeation using a pumpless microfluidic platform

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Abstract

A 3-layer microfluidic device is developed using an inexpensive and readily accessible printer with commercially available printing material, polylactic acid. In addition, to serve as a semi-permeable membrane in microfluidic chip, chitosan/polyvinyl alcohol (CS/PVA) membrane was prepared using an electrospinning process. Electrospun membrane was prepared from a mixed solution of 10% w/v of PVA and 2% w/v of CS. The obtained electrospun CS/PVA membrane showed water resistance with a swelling degree of 97-98%. Their morphology observed by field emission scanning electron microscopy revealed uniform and completely bead free structures at the CS/PVA volume ratio of 30/70, whereas at the volume ratio of 40/60 produced some bead structure. The electrospun membrane was assembled into 3D printing microfluidic chip to preliminary evaluate the drug permeability. By comparing between two nano-carrier systems for curcumin, the nano-carrier with smaller particle size showed faster drug permeation than another one with larger particle size. The developed 3D printing microdevices can be utilized gravity as the driving force, allowing for preliminary drug permeation screening with small amount of sample.

Keywords: chitosan, polyvinyl alcohol, membrane, microfluidic chip, electrospinning

1. Introduction

Recently, biomimetic microfluidic platforms are employed as a potential technique to improve predictive power of preclinical new drug delivery systems. Microfluidic technology is based on the science that deals with the fabrication of micro-devices where the flow behavior of small volumes of fluids in micro-channels and micro-chambers could be controlled (Ahn *et al*., 2018). Thus, a microfluidic device, which is comprised of both a vascular chamber and a tissue chamber separated by a porous membrane, would enable *in vitro* drug transport study and has been considered as an alternative approach to improve in vitro dissolution studies (Wang, Abaci, & Shuler, 2017).

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Microfluidic devices can be produced by a 3D printer. 3D printing technology is advanced considerably over the last decade in both availability and ease of use, accompanied by a substantial decrease in price. This provides the ability to create custom objects quickly and easily (Morgan *et al*., 2016). Polydimethylsiloxane (PDMS), a biocompatible thermo-curable elastomer, has been a material of choice in most microfluidic systems due to transparency, gas permeability, flexibility, and relatively easy to manufacture at small scales (Song *et al*., 2020; Wang & Burghardt, 2018). However, PDMS is notorious for highly solvent evaporation, absorption of hydrophobic compounds, and unstable surface treatment. These defects can lead to inconsistent and unpredictable biological outcomes (Wang & Burghardt, 2018). To alleviate these drawbacks, in our study, polylactic acid (PLA) a biocompatible thermoplastic material, has been used to produce environmentally microfluidic devices. PLA, one of most common materials used in 3D

printing, is considered as safe and has been widely applied to tissue engineering (Pranzo, Larizza, Filippini, & Percoco, 2018).

Several membrane-integrated microfluidic devices have recently been reported for several targeted tissue, such as blood brain barrier, gastrointestinal tract (Arık *et al*., 2018). The selection of materials for membrane should prioritize optical transparency and their capacity for cell adhesion under shear stress (Jiang, Li, Zheng, Li, & Huang, 2019). To achieve these properties, electrospun nanofibers prepared by electrospinning method offer many unique properties such as high surface area-to-volume, pore size within a nano-range, and high porosity with suitable for cell adhesion (Morgan *et al*., 2016). Electrospinning is considered as a fast-developing technique for improving membrane properties for *in vitro* drug permeation study. Biopolymers are also gaining importance as an alternative membrane material (Castro-Muñoz & González-Valdez, 2019). Chitosan (CS) is one type abundantly available polymer that has gained attention because its non-toxicity, biocompatible and biodegradable (Wang *et al*., 2020). It is a natural polycationic linear polysaccharide with primary amino groups and hydroxyl groups (Bakshi, Selvakumar, Kadirvelu, & Kumar, 2019). However, CS derived membranes are often brittle that limit its application (Jeencham, Sutheerawattananonda & Tiyaboonchai, 2019). On the other hand, polyvinyl alcohol (PVA), a biocompatible, non-toxic and water-soluble polymer, can reduce the crystallinity of chitosan via hydrogen bonding (Agrawal, & Pramanik, 2016).

In this study, a pumpless microfluidic model was fabricated from PLA using 3D printing. CS/PVA membranes were prepared by electrospinning method and their physicochemical properties were characterized including morphology and swelling degree. In addition, the performance of developed microfluidic chip was preliminary evaluated in term of drug permeation study. The developed electrospun CS/PVA membrane was equipped with a microfluidic chip to evaluate the curcumin permeation from two different nano-carrier systems.

2. Materials and Methods

2.1 Materials

Chitosan (from shrimp with >90% deacetylation, 890 kDa) was obtained from Marine Bio-Resources Co., Ltd (Samutsakhon, Thailand). Curcumin was purchased from Thai-China Flavors and Fragrances Industry Co., Ltd. (Bangkok, Thailand). Polyvinyl alcohol (PVA, 115 KDa), ethanol, methanol and commercial acetic acid were purchased from Loba Chemi (Raipur, India). Polylactic acid was purchased from Amazon (JAYO, Raipur, India)

2.2 Electrospinning preparation

CS/PVA membrane was prepared using electrospinning device (E-SPIN NANOTEC, super ES-2, Kanpur, Uttar Pradesh, India). Briefly, PVA was dissolved in distilled water and heated to 80 ^{o}C to obtain 10% (w/v) PVA solution. 2% (w/v) CS solution was prepared by dissolving CS in 2% (v/v) acetic acid. Then, CS and PVA solution were mixed at different volume ratio under continuously stirring and heated at 60°C. The spinning CS/PVA solution was loaded into a 10 ml syringe connected with a blunt needle, 0.7 mm internal diameter, and pumped through needle at a flow rate of 1.2 ml/h, while a high voltage of 23 kV was applied across the needle and ground collector. The ground collector covered with aluminum foil was used for the nanofiber deposition with a distance between needle tip to collector of 10 cm. Finally, the obtained electrospun CS/PVA mats ware soaked in ethanol overnight before dried in an oven at 80 °C for 30 min.

2.3 Physicochemical properties

Film thickness was measured at three random positions with a vernier micrometer (Yuzuki, Meena Bazar, Jama Masjid Delhi, India). All measurements were carried out at room temperature. Viscosity of the solution was measured by microviscometer (Lovis 2000ME, Anton Paar, Gurgaon, Haryana, India) at room temperature. Swelling properties of electrospun CS/PVA membrane was determined by immersion in phosphate buffer saline (PBS, pH 7.4) for 24 hrs. The wet weight (W_{wet}, swollen weight) and dry weight (W_{dry}, dried at 45 °C for 8 hrs) were measured. Then, the swelling index was calculated as shown in equation 1.

$$\text{Swelling index} = \frac{(W_{wet} - W_{dried}) \times 100}{W_{wet}} \quad (1)$$

Morphological observation was performed using a field emission scanning electron microscope (FESEM, Quanta 250 FEG, FEI Co., OR, USA). Samples were sputter-coated with gold to produce a conductive coating and to prevent charging.

2.4 Device design and fabrication

A triple layer microfluidic device is first designed using a commercial computer-aided design (CAD) drawing software (SolidWorks, 2016), which translated into STL format, a suitable file for 3D printer language. Using a 3D printer (TECH B, Bhilai, Chhattisgarh, India), the microfluidic device was fabricated by adding printing material, polylactic acid (PLA), to 3D model data layer by layer at a print speed of 50 mm/s and a nozzle temperature of 190 °C.

2.5 Preliminary *in vitro* permeability study

In vitro permeability was performed using prepared microfluidic device equipped with electrospun CS/PVA membrane. Two different nanocarrier systems of curcumin, C1 and C2, were used to perform permeability test. Both C1 and C2 were separately dissolved in phosphate buffer saline (PBS), pH 7.4, which equivalence to curcumin concentration of 0.5 mg/ml. The sample was put into the donor chamber at a constant rate of 2 µl/min and circulated between the two reservoirs by reversing the tilt direction periodically. At predetermined time intervals of 1, 2, 3 and 4 hrs, the sample was collected. The amount of curcumin transported across the membrane was determined using a microplate spectro fluorometer at 425 nm (Thermo Scientific Multiskan Sky

Microplate Spectrophotometer, Powai, Mumbai, India), with a curcumin standard curve ranging from 1-10 µg/ml.

3. Results and Discussion

3.1 Preparation of CS/PVA electrospinning

In the electrospinning process, a jet of melted polymer solution is discharged from an injection needle and electrically charged to create a loosely connected web of nanofibers. Electrospinning is influenced by the type of polymer, viscosity and electrical conductivity properties of the polymer solution (Xue, Wu, Dai, & Xia, 2019). Unfortunately, the solution of pure CS was failed to produce continuous and uniform nanofibers. CS, a cationic polyelectrolyte, is only dissolved in acidic medium, in which the NH_2 group of CS protonates into NH_3^+. Thus, in an electric field, large repulsive forces are developed between the NH_3^+ positively charged groups, which preventing continuous fiber formation and leading to a non-uniform beaded morphology (Koosha, & Mirzadeh, 2015). To solve this problem, a nonionic polymer PVA, was chosen as a co-polymer. Blending with PVA could help disrupt the self-association of CS chains by the formation of additional hydrogen bond between PVA and CS. This led to a reduction in the repulsive force between polycationic groups, thereby creating improved chain entanglement, and inducing the production of continuous and uniform nanofibers (Cheng, Gao, Wang, & Hu, 2015).

Results showed that PVA concentration and the volume ratio of CS and PVA are important parameters determining the electrospinnability of solutions. When preparing the membrane by varying the PVA concentration from 9 to 11% (w/v), while maintaining the CS/PVA volume ratio at 40/60 and the CS concentration at 2% (w/v). The blended solution with 9% PVA produced fibers with a lot of beads. Increasing the PVA concentration to 11% (w/v), a few electrospun fibers were obtained due to the higher solution viscosity. Thus, only the blended solution of 2% CS with 10% of PVA produced uniform nanofibers. Then, the effect of CS/PVA volume ratio was further elucidated. The blended solutions were prepared with 2% CS and 10% PVA, while varying the CS/PVA volume ratio at 50/50, 40/60 and 30/70. The electrospun membrane obtained with CS/PVA volume ratio of 50/50 showed more beads than the continuous fibers with visual observation. This could be a result from increased the NH_3^+ repulsion force on the CS backbone, which impeding the formation of polymer chain entanglement during jet stretching, whipping, and bending (Biranje, Madiwale, & Adivarekar, 2017). Consequently, this leads to droplet formation instead of nanofibers. On the contrary, the electrospun membrane prepared with lower amount of CS, at CS/PVA volume ratio of 40/60 and 30/70, showed smooth membrane with no beads with visual observation. The membrane thickness was approximately 100 µm.

Unfortunately, the obtained electrospun CS/PVA membrane could be dissolved in water, which is not applicable for *in vitro* permeation study. To improve the water resistance properties, physically crosslink was performed by soaking the mats in ethanol following by thermal treatment. Ethanol and thermal treatments could improve the water resistance of CS/PVA electrospun membrane by removal of residual water within the fibers and increase inter- and intramolecular hydrogen bonding (Çay, Miraftab, & Kumbasar, 2014). Consequently, these processes could increase the crystallinity of CS/PVA nanofibers, which resulting in the mats stable in water (Koosha *et al*., 2015).

3.2 Physicochemical characterization

3.2.1 Viscosity measurement

The viscosity of the solutions plays an important role in electrospinning process. A low-viscosity polymer solution implies less entanglement of polymer chains in the solution which may lead to poor electrospinning, resulting in the formation of beads or spindle-like structures (Kuo, Jhang, Lin, Hsien, & Hsieh, 2017). However, the solution with high viscosity of > 40,000 mPa.s was too thick to form nanofibers (Xue *et al*., 2019).

Before electrospinning, the viscosities of the solutions were measured. CS/PVA mixed solutions were optically clear, transparent and homogeneous, which denoted miscibility between the two polymers. The viscosity of CS/PVA solutions increased as the ratio of PVA increased. The viscosity of CS/PVA at the volume ratio of 40/60 and 30/70 are 521.72 and 641.85 mPa.s, respectively. Both formulations showed suitable viscosity which easily discharged from the needle tip to create fine fibers at the ground collector.

3.2.2 Swelling and weight loss studies

The electrospun CS/PVA membrane at the volume ratio of 40/60 and 30/70 showed swelling index of 98.20±0.07 and 97.21±0.35, respectively. The high-water uptake could be a result from hydrophilic functional groups CS and PVA, such as NH_2 and OH (Fathi *et al*., 2020). It is also notable that the swellable nanofibrous membrane suitable for supporting the attachment and proliferation of cells without any cytotoxic effect (Koosha *et al*., 2015).

3.2.3 Morphology characteristics

The field emission scanning electron microscope (FE-SEM) has been widely used in studying the morphology of the nanofiber network structure. FE-SEM micrographs of both CS/PVA electrospun membranes, at the ratio of 40/60 and 30/70, revealed uniform distribution of nanofibers, Figure 1. Nevertheless, completely bead free nanofiber structure was achieved at the blend ratio of 30/70, while some beads were observed at the blend ratio of 40/60.

3.2.4 Microfluidic chip design and fabrication

The designed triple layer microfluidic device made from PLA was successfully fabricated using a 3D printer. This printing technique is benefit for low cost, high speed and simplicity of the process (Ngo, Kashani, Imbalzano, Nguyen, & Hui, 2018). The microfluidic platform, with 320 µl of total volume, was composed of three layers; perfusion, middle and lid (Figure 2). The perfusion layer is composed of one reservoir connected with two chambers via two parallel microchannels of 100 x 200 µm (width x height). The reservoir has a total holding volume of 160 µl. The two

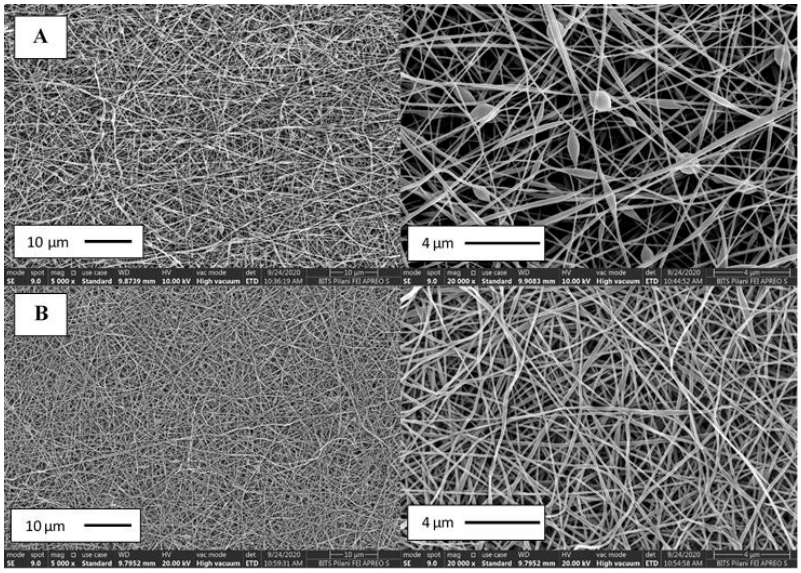

Figure 1. FE-SEM micrographs of electrospun CS/PVA blend fibers: (A) CS/PVA (40/60), (B) CS/PVA (30/70)

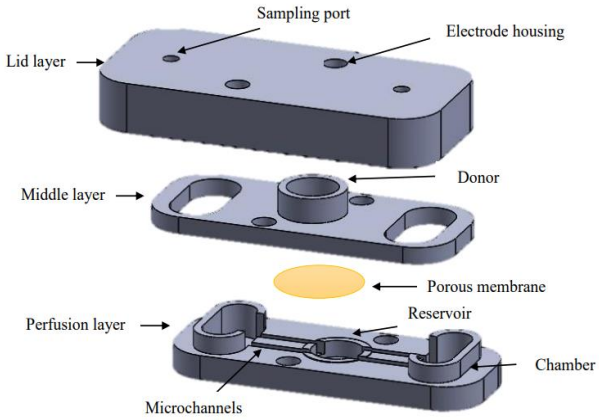

Figure 2. Schematic exploded view of the microfluidic platform. The device consists of three layers: (i) a bottom perfusion layer with 1 reservoir connected with two chambers via two parallel microchannels; (ii) a middle layer with one donor that covers the perfusion layer; and (iii) a top lid layer with sample ports and electrode housing that covers the middle and perfusion layers to minimize water evaporation. The membrane was assembled between the perfusion and the middle layers.

chambers have a 6 mm diameter. The middle layer, composed of a donor compartment, covers the perfusion layer. The lid layer, integrated with sampling ports, is designed to cover the middle layer to minimize water evaporation. All three layers have a groove for the electrodes to monitor the cell viability in case of cell culture is employed. During 3D printing process, PLA is heated at the nozzle to reach a semi-liquid state and then extruded onto the platform or over the top of the previously printed layers. The thermoplasticity of the polymer filament is an essential property for this method, which allows the filaments to fuse together during printing and then to solidify at room temperature after printing.

3.2.5 Permeability studies

We next evaluated the performance of microfluidic chip integrated with electrospun CS/PVA membrane to investigate the permeability of two curcumin formulations; C1 and C2. C1 is curcumin loaded polymer based nano-carriers with a mean size of 120 nm, while C2 is curcumin loaded surfactant based nano-carriers with a mean size of 26 nm. In addition, the curcumin powder was used as a control. The electrospun membrane was equipped between the middle layer and the perfusion layer. Fluids are delivered to the perfusion reservoir via gravity driving force, with no failure from air bubbles, which eliminating the need for external pumps and tubing. The fluid flow circulated between the two chambers via microchannels by a tilted rocking platform periodically. As expected, curcumin powder showed no curcumin permeation through the membrane due to its low water solubility (Pan-On, Rujivipat, Ounaroon, & Tiyaboonchai, 2018). On the contrary, C1 and C2 provided curcumin freely soluble in water with a droplet size of 120 and 26 nm, respectively. As a result, the C1 showed ~78% curcumin permeation within three hours, Figure 3. However, C2 showed ~96% curcumin permeation within two hours. There was statistically significant difference among these two formulations by one way analysis of variance (ANOVA analysis, $P < 0.05$). An explanation for this result could be attributed to the particle size, which effect to the rate of drug transport across the membrane (Beloqui, Rieux, & Préat, 2016). It has been reported that smaller particle size could enhance dissolution behavior by increasing both final solution concentration and the dissolution rate (Zheng *et al*., 2019). Therefore, the small particle size of C2 could facilitate faster curcumin diffusion through the membrane than the larger particle size of C1. The results suggested that this 3D printing microfluidic chip embedded with electrospun CS/PVA membrane model is a platform to allow robust for drug permeability studies with a low amount of sample and dissolution medium.

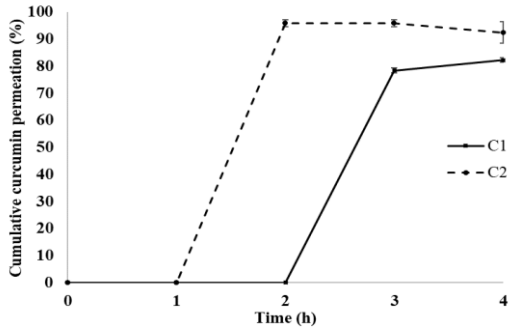

Figure 3. *In vitro* permeation study of curcumin nanocarrier using microfluidic device: (A) C1 and (B) C2. Error bars show standard deviation for n = 3.

4. Conclusions

In this work, the CS/PVA membrane with bead free nanofiber was successful fabricated with electrospinning technique. By post-treatment with ethanol and heat, the obtained electrospun CS/PVA membrane exhibited good physical stability in an aqueous solution with a swelling degree of 97-98%. The 3-layer microfluidic model was fabricated from a commercially available printing material, PLA, using a 3D printer. The 3-layer microfluidic model embedded with CS/PVA membrane is suitable for drug permeability studies. The pumpless design using gravity driven flow for recirculating luminal perfusion allows for robustness. In addition, this platform requires low amount of sample and medium. Therefore, this platform can be helpful for drug permeation screening which is benefit during drug development before performing animal study or human clinical trials.

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