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Fabrication of electrospun mucoadhesive membranes for therapeutic applications in oral medicine

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ABSTRACT

Oral mucosal lesions are related to several etiologies including trauma, infection, and immunologic and neoplastic diseases. Their prevalence varies greatly depending on ethnicity, gender and exposure to risk factors. Currently, most oral mucosal lesions are treated with creams, mouthwashes or gels containing suitable drugs. However, topical medications may be relatively ineffective as they are removed rapidly from oral surfaces, limiting drug contact times. Systemic medications might be more effective, but are associated with unacceptable off-target side effects. The aim of this study was to produce novel polymeric mucoadhesive membranes for therapeutic applications on the oral mucosa using electrospinning. Polyvinylpyrrolidone (PVP) and Eudragit RS100® (RS100) were used for the fabrication of membranes, whilst dextran (Dex) or poly(ethylene oxide) (PEO) particles were incorporated to enhance their mucoadhesive properties. An electrospun poly(caprolactone) (PCL) backing layer was added to create a dual-layer system. Solution properties were studied using rheometry, and membranes were characterized using differential thermal analysis and scanning electron microscopy. Solubility, surface hydrophobicity and adhesion properties were also investigated. Solution viscosity varied depending on composition and concentration, affecting fiber production. The addition of RS100 to PVP resulted in reduced membrane porosity and solubility, and increased surface hydrophobicity and *in vitro* adhesion times. Dex and PEO particles were located on the surface of the fibers. A PCL backing layer was successfully produced, with enhanced attachment between layers achieved through thermal treatment. PVP homopolymer membranes did not adhere to plastic or porcine mucosa, whereas PVP/RS100 membranes with and without PEO or Dex were tightly adherent. In conclusion, PVP and RS100 may be combined to tailor membrane properties. Furthermore, electrospinning facilitated the production of membranes consisting of

mucoadhesive-fabricated fibers displaying increased surface area and long-lasting adhesive properties. These novel compositions exhibit great potential for the fabrication of mucoadhesive patches for therapeutic applications in oral medicine.

1. INTRODUCTION

Lesions of the oral cavity are surprisingly common, and can range from ulcers, wounds and abrasions to lesions produced by conditions such as recurrent aphthous stomatitis (RAS, often termed aphthous ulcers) or oral lichen planus (OLP). RAS and OLP are debilitating lesions with unclear etiology and susceptible to reoccurrence that often form severe lesions at various sites within the oral cavity[1,2]. It is estimated that OLP affects 1-2% of the general population[3], whereas RAS affects up to 25% of the population at some point in their lives[4]. Current therapeutic treatments of these conditions involve the use of immune-modulating steroids that suppress the inflammatory response and reduce symptoms. A crucial problem of these treatments is the efficient delivery of an appropriate amount of drug to the affected area to elicit a therapeutic effect. There is currently no steroid containing creams, ointments or pastes specifically formulated, tested and approved for use in the oral cavity. Consequently, clinicians often have no choice but to use steroid products designed for the skin and whose use is not approved for the mouth. This means that their use in the oral cavity is '*off-label*' or 'unlicensed' and that their indications, recommended doses, contraindications and precautions for use can only be estimated from data obtained from use on the skin. Additionally, the adhesion of most creams, ointments and pastes to saliva coated oral mucosal surfaces is extremely poor, and the movements of the mouth and tongue accelerate their removal from the application site, making drug contact times short and frequently ineffective. This is particularly the case for mouthwashes. Systemic steroids are generally more effective but tend to rapidly induce unacceptable and sometimes serious systemic side effects, and consequently are administered sparingly. All these factors result in irregular dosage and/or undesired off-target toxicity, substantially reducing treatment effectiveness. These issues greatly emphasize the clinical need

for developing systems capable of delivering controlled amounts of drug locally to the lesion site to reduce off-target toxicity, maximize the drug dose applied to the lesion, and minimize dose variability. To achieve this, these systems should be capable of remaining adherent to the delivery site for prolonged periods of time so that the therapeutic effect may be enhanced.

Several approaches for the development of mucoadhesive drug delivery systems intended for the treatment of oral mucosal conditions have been investigated, including particulates[5,6], tablets[7,8] and films[9,10]. In recent years, the manufacturing technique of electrospinning has attracted increasing attention for this purpose[11–13]. Electrospinning is a versatile manufacturing method that uses high voltage electrical currents to produce membranes composed of polymeric fibers. Usual fiber diameters range from several hundred nanometers to a few micrometers, resulting in structures with large surface areas. This, in combination with the high loading capacity and encapsulation efficiency of electrospun fibers, makes electrospinning an ideal technique for drug delivery applications[14–16]. Additionally, electrospinning allows for the combination of a wide range of polymers, solvents and other substances, providing further structural and/or biological functionalities in order to tailor the properties of the membranes to the intended application[17–19]. Many of these characteristics cannot be easily achieved using conventional manufacturing techniques, suggesting that electrospinning is a promising candidate for the fabrication of bioadhesive patches for applications on the oral mucosa.

The aim of this study was to determine whether it was possible to fabricate innovative membranes with properties optimized for adhesion to oral mucosa using electrospinning. The resulting structures might work as protective patches once applied on mucosal lesions and, with further development, might also be used for the targeted release of therapeutic drugs. For this, polyvinylpyrrolidone and Eudragit® RS100 were investigated as fiber-forming polymers, and

particles of dextran or poly(ethylene oxide) were added to enhance the mucoadhesive properties of the final structure. The selection of widely available polymers already Food and Drug Administration (FDA) approved for clinical use minimizes toxicological concerns, increases confidence in biocompatibility, and facilitates translation for clinical use.

2. EXPERIMENTAL SECTION

2.1 Materials

Polyvinylpyrrolidone (PVP) (mw 2,000 kDa) and Eudragit RS100® (RS100) were kindly donated by BASF (UK) and Evonik Industries AG (Germany), respectively. Dextrans (Dex) (mw 2,000 kDa) were purchased from Pharmacosmos (Denmark). Poly(ethylene oxide) (PEO) (mw 2,000 kDa) and poly(caprolactone) (PCL) (mw 80 kDa) were purchased from Sigma Aldrich (UK). Ethanol 99.8+% (EtOH), dichloromethane (DCM) and dimethylformamide (DMF) were purchased from Fisher Scientific (UK).

2.2 Electrospinning system

Electrospun membranes were fabricated using a system composed of a KDS200 syringe pump (KdScientific, USA) and an Alpha IV Brandenburg power source (Brandenburg, UK). Plastic syringes (1 mL volume; Becton Dickinson, UK) were used to drive the solutions into 15 gauge blunt metallic needles (Intertronics, UK).

2.3 Preparation of polymeric solutions and fabrication of membranes

The composition of the polymeric solutions and the electrospinning conditions used for the fabrication of the bioadhesive membranes are shown in Table 1. The required amounts of PVP

and RS100 were weighed and added to 97% v/v EtOH prepared in distilled water, and mixed at room temperature using a magnetic stirrer until the polymers dissolved completely. Dex and PEO powders were then added to the solutions and, because Dex and PEO are insoluble in EtOH, the resulting suspensions were stirred until the particles were uniformly distributed (approximately 30 minutes).

2.4 Conductivity of polymeric solutions

Electrical conductivity of the polymeric solutions was measured using a Mettler Toledo FG3 conductivity meter (Mettler Toledo, Switzerland) applying a conductivity standard of 1413 μS cm^{-1}.

2.5 Rheology of polymeric solutions

The viscosity of the polymeric solutions was measured using a MCR 301 rheometer (Anton Paar, Austria) with a cone-plate measuring system CP50-1 (50 mm diameter and 1° cone) at a constant temperature (25°C ± 0.2 °C) and a sample volume of approximately 1 mL. The shear rates in the shear sweep tests ranged from 1 s^{-1} to 100 s^{-1}. When analyzing solutions containing PEO particles, a parallel-plate measuring system PP-50 with a 0.5 mm gap was used as the particles did not fit under the CP50-1 plate. A solvent trap was used on all experiments to prevent solvent evaporation from the solution.

2.6 Fabrication of hydrophobic backing layer

The hydrophobic backing layer was prepared by electrospinning a PCL solution on top of the bioadhesive layer. PCL was added to a blend of DCM and DMF (90:10 vol% DCM:DMF) in

order to prepare a solution of concentration 10 wt%, and stirred at room temperature using a magnetic stirrer until the polymer dissolved completely. To enhance the attachment between the bioadhesive and backing layers, a thermal treatment was applied by clamping the membranes between glass slides and heating at 70°C for 15 minutes in a dry oven. This temperature was selected based on the melting temperature of PCL (59°C - 64°C)[20], which is significantly lower than the melting temperatures of PVP (≥130°C)[21] and Eudragit RS100 (≥265°C)[22].

2.7 Scanning electron microscopy

Electrospun membranes were imaged using a Philips XL20 scanning electron microscope (SEM). Samples were sputter coated with gold and imaged using an emission current of 15 kV. All images were processed using GNU Image Manipulation Program (GIMP, http://www.gimp.org) and Fiji[23] software tools. Average fiber diameters were calculated using Fiji software by measuring >400 measurements for each composition.

2.8 Differential thermal analysis

Differential thermal analyses (DTA) of electrospun membranes were performed using a Perkin Elmer Thermogravimetric/Differential Thermal Analyzer. Samples (9-10 mg) were loaded into platinum crucibles and heated from room temperature to 700°C at a rate of 10°C/min under a nitrogen atmosphere. Recorded data was processed using Pyris (Perkin-Elmer, USA) and MjoGraph (Ochiai Laboratory, Yokohama National University, Japan) software tools.

2.9 Membrane solubility and surface hydrophilicity

The potential of electrospun membranes to dissolve after exposure to water was studied by placing samples (n = 3) on distilled water (0.1 mL) spread evenly on a glass slide for 30 s. Afterwards, the samples were removed if possible and imaged using SEM. The level of surface hydrophilicity was determined using the sessile drop technique. Images of distilled water droplets on the different surfaces were taken using a DCC1545M high resolution camera (Thorlabs, US) with MLH-10X lens (Computar, US), and analysed using ImageJ software and Drop Snake plug-in [24]. The contact angles were calculated using six different measurements at randomly selected locations.

2.10 *In vitro* adhesion study of electrospun membranes

The adhesive properties of electrospun membranes made of (i) 10 wt% PVP (PVP), (ii) 10 wt% PVP and 10 wt% RS100 (PVP/RS100), (iii) 10 wt% PVP, 10 wt% RS100 and 10 wt% PEO (PVP/RS100 + PEO), and (iv) 10 wt% PVP, 10 wt% RS100 and 10 wt% Dex (PVP/RS100 + Dex) were investigated *in vitro*. All compositions were studied before and after being subjected to thermal treatment to enhance backing layer attachment. Samples (1 x 1 cm; n = 4) were prepared from each membrane composition and applied to tissue culture plastic (i.e. petri dishes). For this, drops of distilled water (200 μl) were initially placed on the plastic surface using a micropipette, and the electrospun samples were applied on the drops by pressing uniformly with the index finger on the hydrophobic backing layer for 5 s. Afterwards, 20 mL of distilled water were added to the dishes, which were continuously shaken on an orbital shaker tray (45 rpm) for a total time of 240 minutes. The dishes were examined every 15 minutes to determine the time at which the backing layer detached from the surface.

2.11 *Ex-vivo mucoadhesion study using porcine oral mucosa*

Buccal mucosa was excised from bisected porcine heads immediately after slaughter and a full-thickness buccal mucosal graft (0.6 mm) taken using a Braithwaite grafting knife (Swann Morton, Sheffield, UK). Excised buccal mucosa tissue was stored in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 0.625 μg/ml amphoceterin B and used within 24 hours. The mucoadhesion (residence) time of patches was determined by measuring the time taken for more than 50% of the patch to detach from the mucosa. *Ex vivo* porcine mucosa was dried and attached firmly to a 100 mm petri dish using cyanoacrylate glue. The epithelial surface of the mucosa was moistened using artificial saliva (BioXtra) and patches applied to the mucosa using gentle fingertip pressure for 5 seconds. Once attached, the mucosa was submerged in artificial saliva and the petri dish shaken on an orbital shaker at 40 rpm. Data was obtained from 3 separate electrospun patches and buccal mucosa from 4 different pigs.

For histological analysis, patches were adhered to porcine buccal mucosa as previously described and then snap frozen in optimum cutting temperature (OCT) gel using a plastic mould (Surgipath, Leica Biosystems). Eight-micron thick sections were mounted onto Superfrost™ Plus slides (VWR, Pennsylvania, US) and air-dried for one hour. H&E staining required a modified protocol to minimise the effects of solvents on patch integrity. Slides were stained with filtered 0.1% Mayers haematoxylin (VWR, Pennsylvania, US) for 10 minutes, rinsed in cool, running ddH_2O for 5 minutes and dipped in 0.5% eosin (VWR, Pennsylvania, US) 10 times. Slides were dipped in ddH_2O 10 times (or until the eosin stopped streaking), dried for 1 minute and coverslips mounted using DPX (Sigma, Poole, UK).

2.12 Statistical analysis

Statistical analyses were performed using GraphPad Prism v6.00 software tool (GraphPad Software, La Jolla, CA, USA) using two-tailed Student T-test, and one-way or two-way ANOVA with post-hoc Tukey tests. In all cases, p values <0.05 were considered statistically significant.

3. RESULTS

3.1 Physical properties of polymeric solutions: electrical conductivity and viscosity

The outcomes of the electrospinning technique are strongly influenced by a series of processing parameters, many of which are closely related to the properties of the polymeric solutions employed[25]. The electrical conductivity and viscosity of the solutions used in this study were measured to determine how the various components affect these properties, and to correlate them with the results obtained from other analyses. Regarding the electrical conductivity of the solutions (Figure 1A), increasing the concentration of PVP resulted in significant increases in the potential of the solutions to carry surface charges. One-way ANOVA reported statistically significant differences between groups ($p<0.0001$), and post-hoc Tukey tests reported statistically significant differences between 5 wt% and 10 wt% PVP ($p = 0.032$). However, there were no significant differences between 10 wt% and 15 wt% PVP ($p = 0.178$). The addition of RS100 further increased the conductivity, but the presence of Dex or PEO particles resulted in significant reductions, which were not correlated to the concentration of particles added as no statistically significant differences between solutions containing similar amounts of dextran and PEO particles was observed (10 wt% PEO vs 10 wt% Dex, $p > 0.9999$; and 20 wt% PEO vs 20 wt% Dex, $p = 0.2857$). Rheometry showed that increasing the concentration of PVP from 0 wt% to 15 wt% resulted in an exponential increase in viscosity at the shear rate of 2 s^{-1} (Figure 1B). The addition of RS100 to 10 wt% PVP also resulted in greater solution viscosity at the same shear rate (Figure 1C) that was significantly different between 10 wt% RS100 ($p = 0.009$) and 15 wt% RS100 ($p = 0.0065$). The effect of the addition of Dex or PEO particles on solution viscosity (Figure 1D) was studied using the full range of shear rates (i.e. 1 s^{-1} to 100 s^{-1}), showing that doubling the quantity of particles added to the solutions (from 10 wt% to 20 wt%)

increased the viscosity measured at the shear rate of 2 s^{-1} noticeably up to 4-fold for solutions containing Dex, and 11-fold for solutions containing PEO. Additionally, the viscosity of solutions containing PEO particles was greater than the viscosity of solutions containing Dex particles at all shear rates.

3.2 Fabrication and characterization of PVP electrospun membranes

Electrospun membranes could be produced with no or little presence of defects using solutions of 5 wt% to 20 wt% PVP. Solutions with a concentration of 2.5 wt% PVP were not viscous enough to facilitate the formation of a continuous electrospinning jet, resulting in electrospraying. Although solutions of concentration 25 wt% PVP could be processed, the increased viscosity resulted in the appearance of marked amounts of electrospinning defects such as sputtering (Data now shown). SEM micrographs of membranes made with solutions 5 wt% to 15 wt% PVP (Figure 2A-C) showed that the samples were composed of fibers exhibiting regular diameters and no apparent defects. Fibers in materials made with solutions of 20 wt% PVP (Figure 2D) exhibited a flattened morphology and appeared to be more densely packed. Fiber diameters increased as the concentration of PVP increased (Figure 2E). Diameter variability also increased in response to the increased concentration of PVP (Table 2). Based on these findings, it was decided that 10 wt% PVP would be taken forward for subsequent studies.

3.3 Fabrication and characterization of 10 wt% PVP and RS100 electrospun membranes

To investigate decreasing the solubility of the electrospun membranes, increasing concentrations of RS100 were added to 10 wt% PVP solutions. The addition of more than 2.5 wt% of RS100 resulted in a noticeable change in fibre morphology, with the material displaying a flat or ribbon

shape (Figure 3C-H). Addition of RS100 above 2.5 wt% also resulted in an apparent increase in the compactness of fibre packing in the membranes, potentially reducing material porosity and flexibility, as well as an apparent increase in the variability of fibre diameter. During handling of the materials, we found that electrospun membranes exhibited increased fragility and rigidity as the concentration of RS100 increased in the composition. DTA analysis of electrospun materials fabricated made using solutions of PVP showed an endothermic peak at 444°C (Figure 3I), while those made using RS100 showed an endothermic peak at 404°C (Figure 3J). DTA patterns of electrospun materials made using PVP+RS100 showed an area of the graph formed by the overlap of two endothermic peaks at temperatures similar to those observed individually for RS100 and PVP, respectively (Figure 3K). The sudden reductions of μV of these peaks were due to polymer decomposition, as they matched the temperature range at which the samples dramatically lost most of their mass.

3.4 Fabrication of PVP and RS100 electrospun membranes containing bioadhesive particles

Dex or PEO particles were incorporated into the polymeric solutions to enhance the bioadhesive properties of the electrospun membranes. Solutions containing up to 30 wt% of Dex particles and up to 20 wt% of PEO particles could be successfully processed. A further increase of 10 wt% in the proportion of both substances (i.e. 40 wt% for Dex and 30 wt% for PEO) markedly affected the formation of electrospun fibers, resulting in the apparition of manufacturing defects. Greater amounts of bioadhesive particles resulted in high solution viscosity, with the consequence that the electrospinning process was unattainable. SEM micrographs of the membranes revealed that the Dex and PEO particles were located on the surface of the fibers and not embedded within the

polymeric matrix (Figure 4A-D). The particles of Dex, roughly spherical in shape, were observed either forming aggregates (Figure 4A) or dispersed individually (Figure 4B). PEO particles, possessing generally a larger diameter and more irregular morphology compared to Dex particles, appeared to be covered with a thin film of polymer (Figure 4C-D).

3.5 Fabrication of the mucoadhesive membrane-backing layer

A hydrophobic backing layer was created on top of the mucoadhesive layer to direct any potential drug release towards the mucosa and not into the oral cavity, and to act as protection against movement and friction by teeth and the tongue or from contact with food (e.g. if patches are to be worn whilst eating). Dual layer membranes composed of a mucoadhesive layer and a hydrophobic backing layer (10 wt% PCL) were successfully fabricated. SEM micrographs (Figure 5A-B) showed that, initially, there was no attachment between the layers after fabrication. Application of a thermal treatment (70°C for 15 minutes) induced morphological changes in the backing layer, causing it to acquire a smoother surface and to enhance the attachment with the bioadhesive layer (Figure 5D). No changes were observed in the morphology of the bioadhesive layer that retained its fibrous structure (Figure 5C-D).

3.6 Characterization of electrospun membranes solubility and hydrophobicity

The addition of RS100 to 10 wt% PVP resulted in the production of electrospun membranes with markedly reduced solubility compared to membranes made with PVP only. Samples made with 10 wt% PVP and 0 wt% or 2.5 wt% RS100 rapidly absorbed distilled water, transforming into a gel-like substance with no structural integrity. When 5% RS100 or greater concentrations were used, the resulting electrospun materials exhibited decreased solubility and, consequently,

enhanced structural integrity for a greater length of time. SEM images showed that the thickness of the region in the membrane affected by the uptake of water was smaller as the concentration of RS100 increased from 5 wt% to 20 wt% (Figure 6A-C). Contact angle measurements showed that the addition of RS100 to 10 wt% PVP increased the hydrophobicity of the electrospun membranes significantly (One-way ANOVA, $F(5, 30) = 678.3$, $p < 0.0001$), increasing the contact angle from 0° for 10 wt% PVP to 127° ± 4° for 10 wt% PVP with 10 wt% RS100. Surface hydrophobicity was significantly ($p < 0.0001$) decreased with the addition of Dex or PEO particles, reducing the contact angles to 108° ± 2° and 101° ± 7°, respectively. In addition, the surface of the thermally treated PCL backing layer was significantly ($p < 0.0001$) more hydrophilic (73° ± 2°) than the surface of untreated PCL (121° ± 6°). No statistically significant differences between the adhesive layer compositions containing Dex or PEO ($p = 0.1075$) were found (Figure 6D).

3.7 *In vitro* and *ex vivo* study of membrane adhesion

An *in vitro* adhesion assay demonstrated that membranes composed of 10 wt% PVP detached from the plastic surface immediately upon addition of distilled water. However, membranes made of PVP/RS100 and PVP/RS100 + PEO remained adhered to the surface for at least 240 minutes. Thermally treated PVP/RS100 + Dex remained attached for an average of 221 minutes, whilst untreated PVP/RS100 + Dex remained adhered for 225 minutes. There were no statistically significant differences between all compositions containing RS100 ($p = 0.1938$; two-way ANOVA), or between samples that had been thermally treated compared those that had not been treated ($p = 0.6600$; two-way ANOVA) (Figure 7A). Interestingly, for untreated PVP/RS100 + Dex membranes that detached, a substantial amount of the mucoadhesive layer

remained attached to the plastic after the backing layer separated, while this was not observed for thermally treated samples. Similar data was observed for thermally treated membranes in an *ex vivo* porcine mucosal adhesion assay. As with plastic, PVP membranes detached from the mucosa within minutes of the start of the assay. Membranes composed of PVP/RS100 alone or in the presence of Dex were tightly adherent with adhesion times averaging 360 minutes. In contrast, membranes composed of PVP/RS100+PEO were significantly ($p<0.05$) less adherent than PVP/RS100 but not when the membranes contained Dex, with adhesion times averaging approximately 200 minutes (Figure 7B). Histological sectioning of porcine tissue showed that the adhesive layer forms intimate contacts with the surface of the epithelium but at no point did we observe penetration into the mucosa, and there was no structural damage to the epithelium. The backing PCL layer remained intact and was associated with the adhesive layer all along the membrane (Figure 7C).

4. Discussion

In this study, we report the successful development and fabrication of novel polymeric membranes with mucoadhesive properties through electrospinning, intended for use as oral mucosal patches and ultimately for targeted drug delivery. The choice of electrospinning allowed for the fabrication of fibrous materials exhibiting large surface areas due to the small fiber diameter, which may enhance water absorption by the patch and thus facilitate drug release from the membrane into the mucosa. Additionally, electrospinning has shown great potential for drug delivery applications due to the production of materials with high loading capacity and encapsulation efficiency[14–16]. The main polymers selected for this purpose, PVP and Eudragit® RS100 have been frequently employed in the pharmaceutical industry for the development of drug delivery compositions, thus minimizing potential risks associated with material toxicity and immune reactions[26].

PVP proved to be a very versatile polymer to produce electrospun fibers, as relatively small increments (i.e. 5 wt%) in the concentration of polymeric solutions resulted in significant changes in fiber diameter. Solutions of 5 wt% PVP produced fibers with mean diameters of 305 nm, within the range of what is frequently considered in the literature as nanofibers.[27] As expected for electrospun materials, increasing the concentration of PVP resulted in greater fiber diameters.[25] However, this also resulted in greater diameter variability, which may be attributed to the increased viscosity of the solutions and, consequently, the greater difficulty of processing. Electrospinning outcomes can be strongly affected by changes in solution parameters, including polymer concentration (directly influencing solution viscosity) and electrical conductivity. Usually, fiber diameter is expected to increase proportionally to the concentration of the solution,

producing fibers with smooth surfaces. However, if a certain concentration threshold is surpassed, changes in fiber morphology will likely occur, leading to the appearance of defects. In this study, fibers obtained with solutions of 20 wt% PVP presented a flattened morphology that differed distinctly from the morphology of fibers produced with solutions of lower concentration. Rheometry studies showed that the viscosity of PVP solutions increased exponentially as the polymer concentration grew linearly, an effect that may explain the difficulties experienced. Regardless, these findings suggested that PVP processed using electrospinning offered great potential to tailor the architecture of membranes with the aim of enhancing the mucoadhesive properties and, potentially, the drug release properties of the system.

Electrospun PVP membranes were observed to quickly absorb water and change their physical appearance to a gel-like consistency, losing all structural integrity. This rapid dissolution may be inconvenient if localized drug delivery is to be performed over a prolonged period. Eudragit® RS100, a copolymer of ethyl acrylate, methyl methacrylate and trimethylammonioethyl methacrylate chloride, was thus combined with PVP to reduce the solubility of the membranes. The addition of increasing amounts of RS100 to the solutions while PVP was maintained constant at 10 wt% resulted in the successful production of electrospun membranes with reduced solubility in water. These membranes changed morphologically after exposure to water for a fixed length of time, but this effect was limited, especially for membranes with 10 wt% RS100 or more. In those cases, the face of the membranes not directly exposed to water retained the original fibrous structure. This effect may be due to the greater hydrophobic properties of materials containing RS100, which is insoluble at physiological pH and can swell in water.[28] This was confirmed using the sessile drop technique, which showed that electrospun membranes

made of 10 wt% PVP and 10 wt% RS100 exhibited greater surface hydrophobicity than membranes without RS100. SEM analysis showed that the dimensions of electrospun fibers made with PVP and RS100 appeared to increase as their morphology changed from cylindrical to ribbon-like. As with PVP-only fibers, this was an expected outcome due to the increase in solution viscosity after the addition of RS100. However, no significant increase in viscosity was observed when the concentration of RS100 increased from 0 wt% to 5 wt%, suggesting that there may be other underlying causes for this effect. DTA analyses suggested that PVP and RS100 might not blend well and that different phases may form in the electrospun fibers, each decomposing at a temperature similar to that observed in membranes made of the individual polymers. Indeed, solutions of PVP and RS100 had a completely transparent appearance individually but adopted an opaque form when mixed prior to electrospinning, further implying polymeric phase separation. Nevertheless, this change did not have a significant impact on manufacturing of the electrospun membranes, and it is plausible that the existence of two distinct phases might be advantageous in comparison to fibers made using a uniform blend of both polymers by exposing the hydrophilic PVP, facilitating water absorption. However, further work would be required to verify this potential benefit.

Dex and PEO are substances with proven bioadhesive properties.[29] Both were successfully incorporated into the electrospun materials through addition of the particulate material to the polymeric solutions. SEM showed that the bioadhesive particles were located on the surface of the fibers rather than being embedded inside the polymeric fibers. This is different to what has been reported previously for electrospun composite materials made of biocompatible polymers and additional particulate materials, such as bioactive glass.[19] Dex particles appeared in clusters

or individually, while PEO particles, of considerable larger size, were observed individually and possibly covered by a thin layer of polymer. The agglomeration of Dex particles in clusters and the larger PEO particles may have an effect in the electrospinning process as they may disrupt the electrospinning jet or increase solution viscosity. This effect was observed for solutions containing PEO, displaying values for the viscosity several times larger than that of solutions containing similar amounts of Dex. The reduction of surface hydrophobicity observed in membranes containing Dex or PEO particles may be due to the highly hygroscopic properties of both substances.

Regarding the potential effect of electrical conductivity on the processing of the polymeric solutions, it was observed that in most cases the conductivity increased significantly due to the addition of the polymers, especially RS100, most likely due to the charged Cl-N group within its molecular structure. In general, it is expected that greater electrical conductivity will result in the production of thinner electrospun fibers, as the electrical field effects a greater pull on the electrospinning jet due to the greater number of charges in the solution.[25] However, it is possible that in this particular case the effect of solution viscosity on fiber size was much more predominant, since the diameters were always observed to increase. Additionally, although the addition of particulate Dex or PEO resulted in significant reductions in electrical conductivity, the membranes produced with those solutions did not appear to be distinctly different to those produced with solutions without Dex or PEO. Therefore, these findings seem to suggest that electrical conductivity may be a minor influence on fiber diameter and morphology in this system. However, it is also possible that the greatly increased conductivity caused by RS100 may

facilitate the electrospinning process and the production of fibers with minimized presence of defects.

A dual layer system comprised of the mucoadhesive layer and a backing layer made of PCL was successfully produced. The sessile drop technique confirmed that the surface of the backing layer was hydrophobic, a desired feature for the development of drug delivery systems maximizing drug dosage towards the mucosa while preventing unwanted release into the oral cavity. However, as the electrospun PCL layer is porous, it is likely to result in the eventual diffusion of the drug through the membrane after it has been thoroughly wetted. To solve this problem, we heated the dual-layer patches to a temperature above the melting point of PCL. This thermal treatment caused the PCL layer to melt, resulting in a backing layer surface that was smoother, more uniform, and with increased hydrophilicity than non-treated membranes. Taken together, these data and the SEM analyses suggested that thermally treated PCL layers displayed reduced porosity than their non-thermally treated counterparts, although additional experiments are required to verify this. Additionally, the thermal treatment appeared to increase the level of bonding between the adhesive and backing layers, probably due to the creation of multiple attachment points between the PVP and RS100 fibers and molten PCL. Indeed, in the *in vitro* adhesion experiments we found that the backing layer became detached from the adhesive layer over time in the membranes that were not thermally treated, leaving the mucoadhesive layer attached to the plastic surface. In contrast, thermally treated membranes detached as a single unit with the adhesive and backing layers firmly attached to one another, suggesting that thermal treatment creates a tight association between the two layers. Although this method requires

further optimization, the thermal treatment appears to be an interesting approach to produce dual layer patches due to its simplicity and ease of use.

The studies of *in vitro* and *ex vivo* adhesion showed that the electrospun membranes were highly adhesive, except those that were made of PVP only. The addition of RS100 to PVP was therefore required to formulate a polymeric composition with increased adhesive properties, and this is likely due to the reduced solubility properties of these compositions. In the case of PVP only membranes, it is possible that water was absorbed quickly and the membrane transformed into a gel-like layer that did not retain any structural integrity. Interestingly, for plastic adherence, no significant differences were observed between the adhesion times of membranes that were thermally treated and those that were not, suggesting that the enhanced attachment between layers did not have a major effect or that the agitation used in the *in vitro* assay was not sufficient to test the protective capacity of the backing layer. The presence of bioadhesive substances (PEO and Dex) may be crucial to attain the most appropriate mucoadhesive properties of the membranes.

Membranes containing PEO or Dex were equally as adherent to plastic; however, the mucosal adhesion data suggest that membranes containing Dex are more tightly adherent to the mucosal epithelium than those made with PEO. This is particularly important and indicates that membranes can be potentially tailored to function; slow release drugs may be incorporated within Dex-containing membranes that will adhere to the mucosa for prolonged periods, whereas PEO membranes, that are not as adherent, may be the choice for drugs that may need to be released quickly. Moreover, the presence of a backing layer provides protection whilst enabling directed drug delivery to the mucosa and not into the oral cavity. These features ensure a

significant improvement over current treatment modalities such as creams and ointments. The development of drug-loaded mucoadhesive patches has the potential to radically alter current treatment for oral mucosal lesions.

5. Conclusions

Here we report the successful fabrication of novel mucoadhesive electrospun membranes with great potential for use in therapeutic applications in oral medicine, including the future treatment of oral mucosal lesions through localized drug delivery. PVP and Eudragit® RS100 were shown to be useful as fiber forming polymers. A range of PVP concentrations (5 wt% to 20 wt% PVP) were electrospun, resulting in the production of fibers with a smooth surface and increasing diameter in relation to the polymer concentration. The addition of RS100 to 10 wt% PVP significantly reduced membrane solubility, a property that could further enhance the potential of these compositions for long term application to oral mucosa. The incorporation of Dex and PEO particles onto the surface of the fibers by incorporation into the electrospinning process was successful over a relatively wide range of concentrations. It was ultimately possible to manufacture a complex dual-layer system, comprised of a mucoadhesive layer and a hydrophobic backing layer fabricated using electrospun PCL. *In vitro* and *ex vivo* studies demonstrated that membranes made with 10 wt% PVP, 10 wt% RS100 and 10 wt% PEO or Dex exhibited prolonged adhesion times. In conclusion, mucoadhesive patches with tailored adhesive properties may be manufactured using electrospinning for clinical applications in oral medicine.

FIGURES

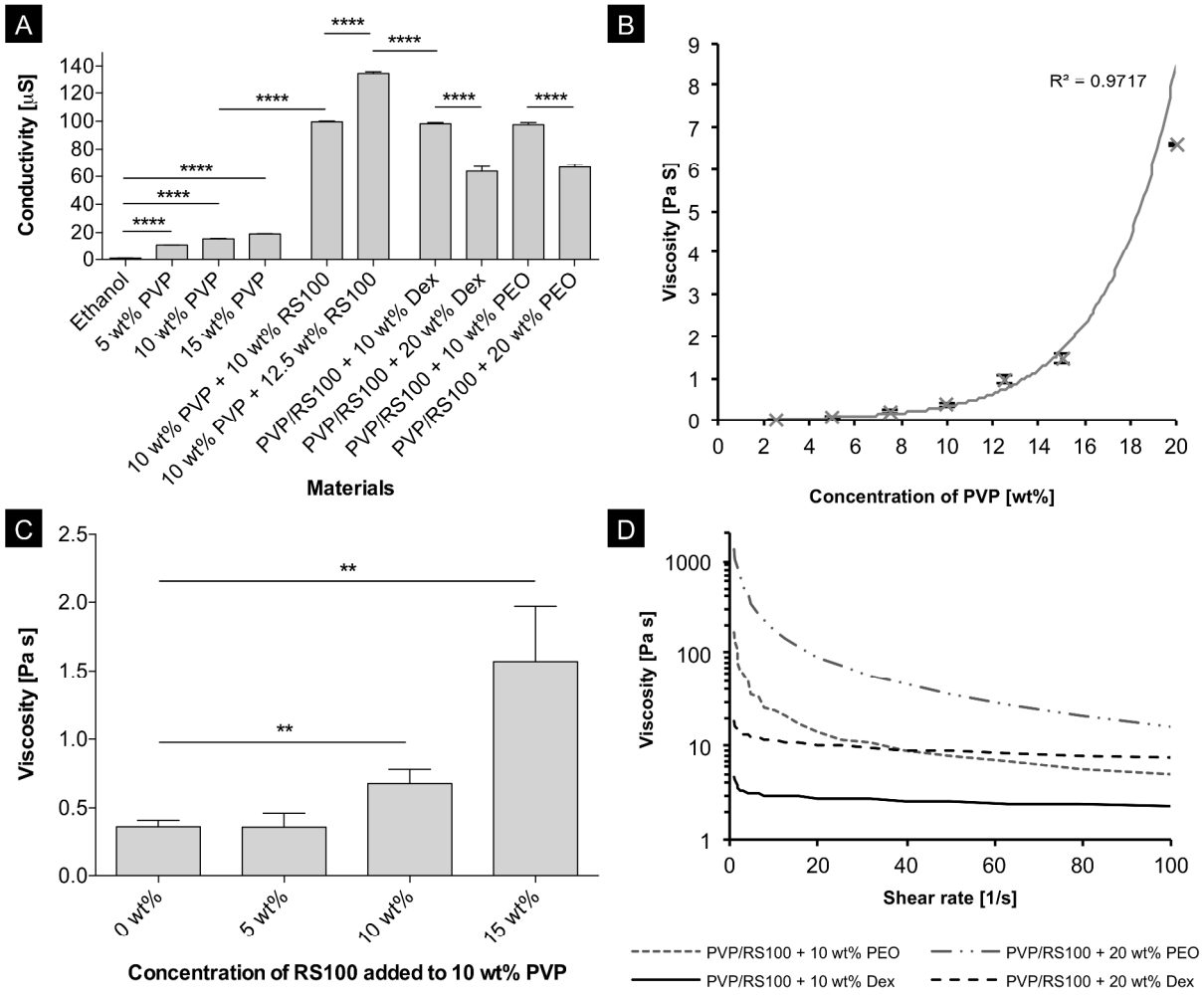

Figure 1. (A) Electrical conductivity measurements of ethanol and polymeric solutions of polyvinylpyrrolidone (PVP), Eudragit RS100 (RS100) and particles of dextran (Dex) and poly(ethylene oxide) (PEO). (B) Viscosity of solutions of increasing concentration of PVP measured at a shear rate of 2 s^{-1}. (C) Viscosity of solutions of 10 wt% PVP and increasing concentration of RS100 measured at a shear rate of 2 s^{-1}. (D) Viscosity of solutions of 10 wt% PVP and 12.5 wt% RS100 with 10 wt% and 20 wt% particles of Dex and PEO, measured at a range of shear rates from 0.1 s^{-1} to 100 s^{-1}. Data shown for (A-C) are mean ± SD. **, p = 0.01 to 0.001; ****, p = <0.0001.

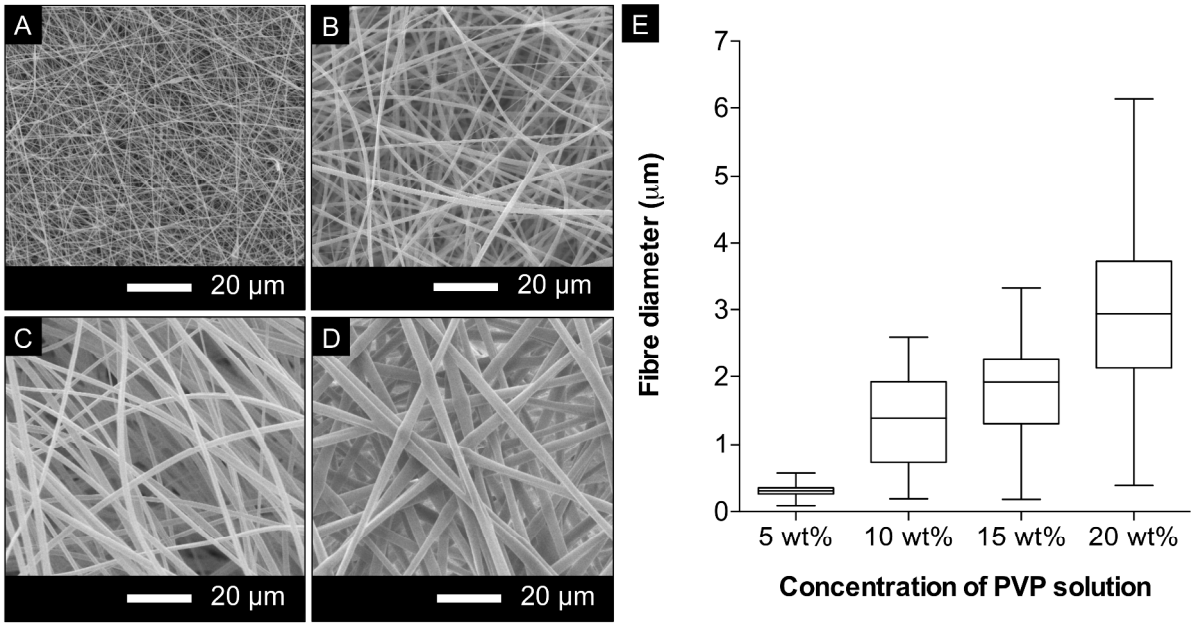

Figure 2. (A-D) Scanning electron microscopy micrographs and (E) fiber diameters of electrospun membranes fabricated using polymeric solutions of 5, 10, 15, and 20 wt% PVP. Scale bar = 20 µm.

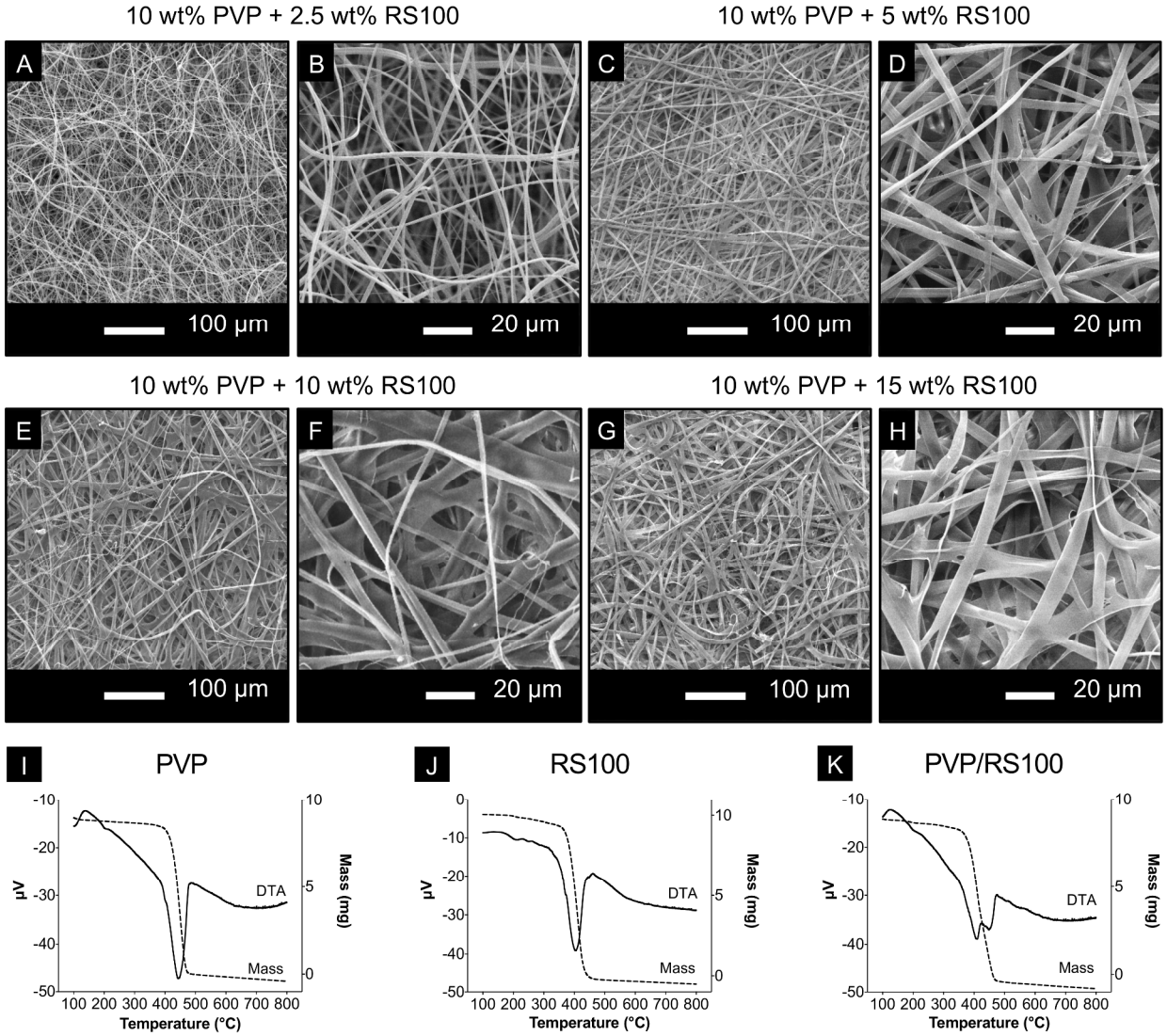

Figure 3. Scanning electron microscopy micrographs of electrospun membranes produced using polymeric solutions of 10 wt% PVP 2.5 with (A-B) wt% Eudragit RS100® (RS100), (C-D) 5 wt% RS100, (E-F) 10 wt% RS100 and (G-H) 15 wt% RS100. Scale bars = 20 and 100 µm. Differential thermal analysis of (I) PVP, (J) RS100 and (K) PVP/RS100 using a Thermogravimetric/Differential Thermal Analyzer following heating to 800°C at a rate of 10°C/min under a nitrogen atmosphere.

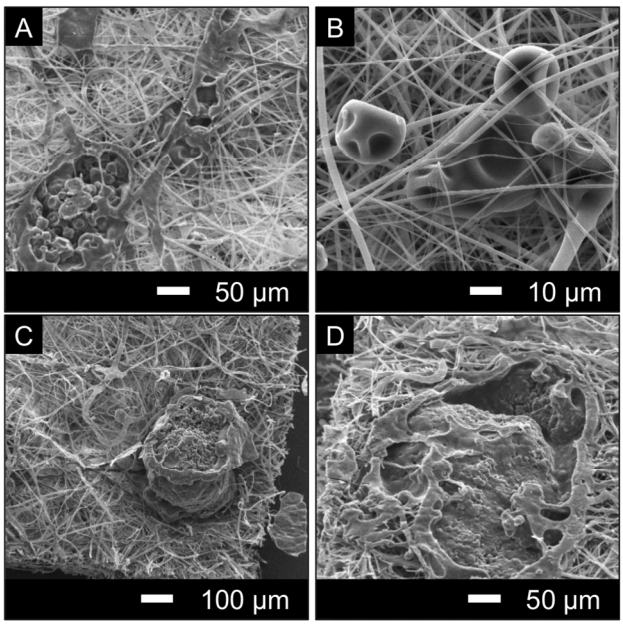

Figure 4. Scanning electron microscopy micrographs of electrospun membranes produced using polymeric solutions of 10 wt% PVP and 10 wt% RS100 containing particles of (A-B) dextran and (C-D) poly(ethylene oxide). Scale bars = 10, 50 and 100 µm.

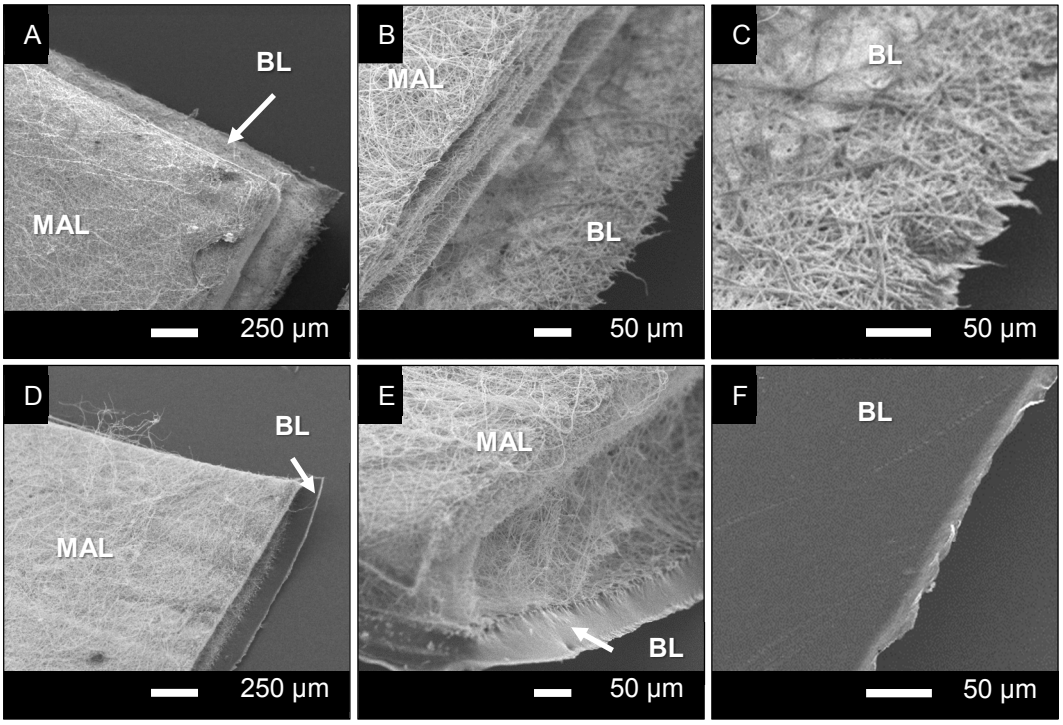

Figure 5. Scanning electron microscopy micrographs of dual-layer system composed of a mucoadhesive layer (MAL) made of 10 wt% PVP and a backing layer (BL) made of electrospun 10 wt% polycaprolactone. (A-C) Dual-layer system before thermal treatment and (D-F) after thermal treatment. (Scale bars = 50 and 250 µm.

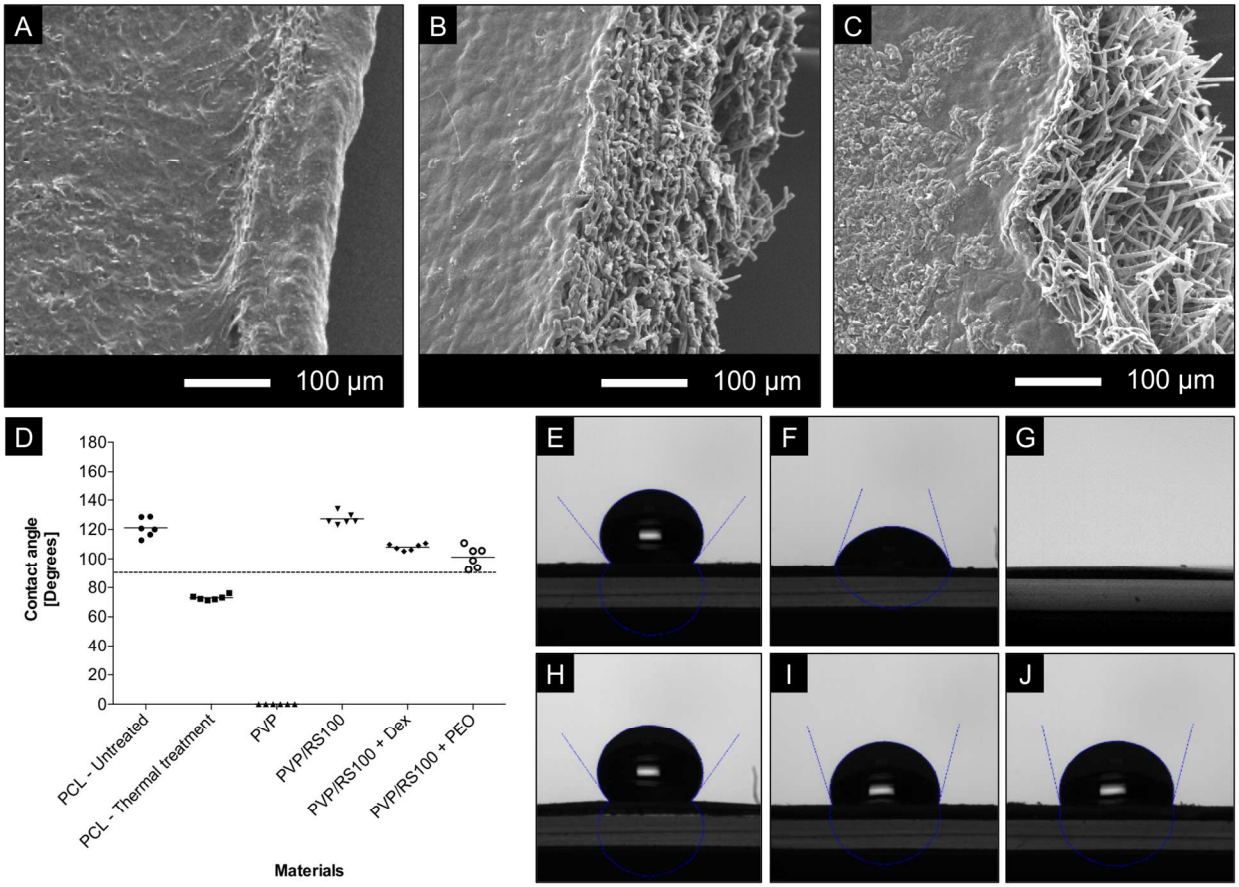

Figure 6. Scanning electron microscopy micrographs of electrospun 10 wt% PVP and Eudragit RS100 (RS100) with concentrations of (A) 5 wt% (B) 10 wt%, and (C) 15 wt% after exposure to distilled water for 30 s. (D) Contact angles obtained using the sessile drop technique for the different compositions and representative images of water drops on the surface of (E) untreated 10 wt% PCL, (F) thermally treated 10 wt% PCL, (G) 10 wt% PVP, (H) 10 wt% PVP and 10 wt% RS100, (I) 10 wt% PVP, 10 wt% RS100 and 10 wt% Dex particles, and (J) 10 wt% PVP, 10 wt% RS100 and 10 wt% PEO particles. Scale bars = 100 µm.

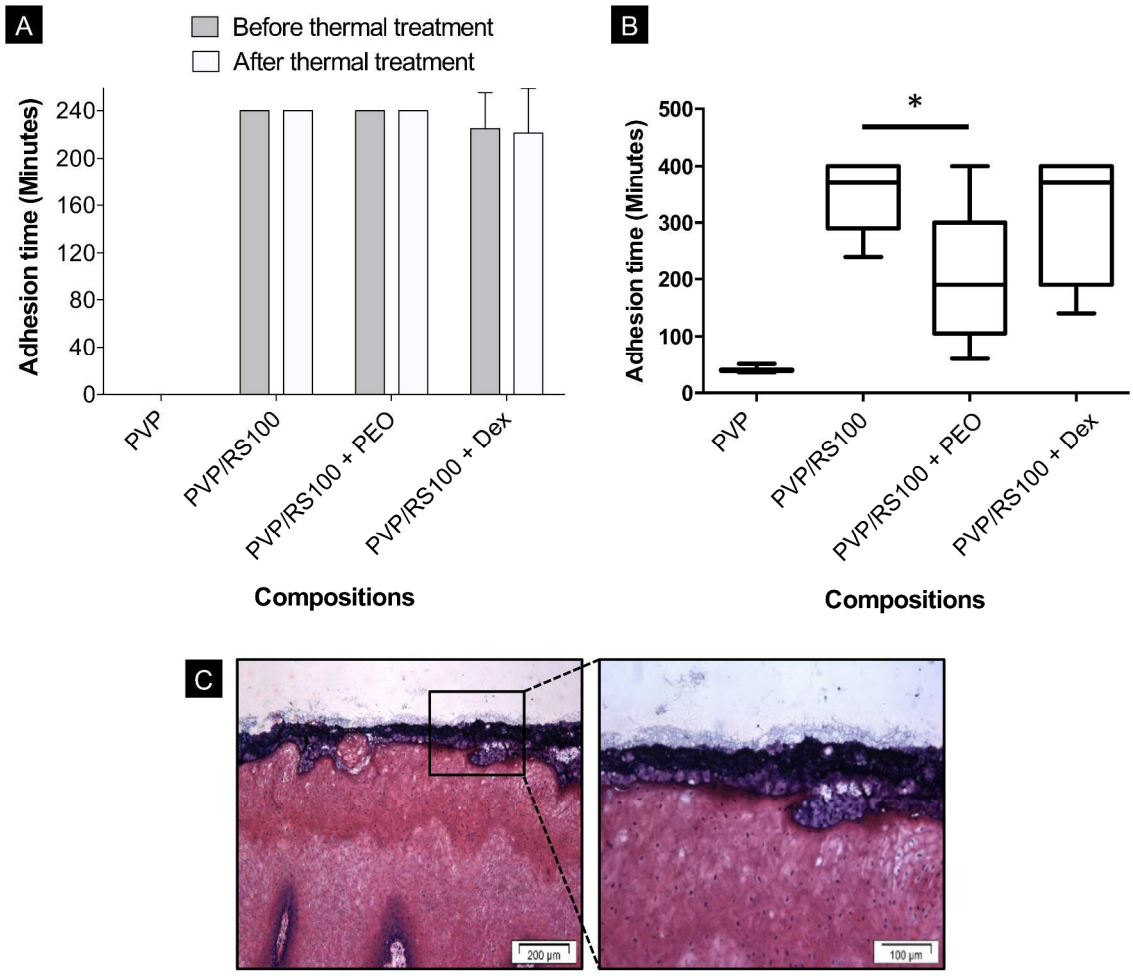

Figure 7. Adhesion times (minutes) of electrospun membranes made using polymeric solutions of 10 wt% PVP (PVP); 10 wt% PVP and 10 wt% RS100 (PVP/RS100); and 10 wt% PVP, 10 wt% RS100 and 10 wt% PEO (PVP/RS100 + PEO) or Dex (PVP/RS100 + Dex) to tissue culture plastic (A) or porcine buccal mucosa (B). For adherence to plastic all samples were tested before and after application of thermal treatment; adhesion to porcine mucosal was performed with thermally treated membranes only. The study was terminated after 240 minutes (for plastic) and 400 minutes (for porcine mucosa) respectively. Data shown are mean +/- SD of four independent experiments, * $p<0.05$ ANONA with Tukey's multiple comparison test. (C) Histological image of PVP/RS100+PEO attached to porcine buccal mucosa showing the intimate contact made between the adhesive layer and the oral mucosal epithelium.

TABLES

Table 1. Composition of polymeric solutions and electrospinning conditions used in the fabrication of the membranes.

Components				*Electrospinning conditions*		
PVP (Wt%)	RS100 (Wt%)	Dex (Wt%)	PEO (Wt%)	Voltage (kV)	Flow rate (mL/h)	Distance (cm)
2.5 – 25	0	0	0	15	1 – 5	19 – 23
10	2.5 – 15	0	0	15 – 17	2.5	19
10	10	5 – 50	0	17	2.5	19
10	10	0	5 – 50	17	2.5	19

Table 2. Descriptive statistics of the study of diameters (μm) of electrospun fibers fabricated using solutions of PVP.

PVP solution concentration	*Minimum*	*25% Percentile*	*Median*	*Mean*	*75% Percentile*	*Maximum*
5 wt%	0.088	0.2625	0.304	0.305	0.35	0.57
10 wt%	0.19	0.728	1.393	1.347	1.924	2.593
15 wt%	0.18	1.312	1.917	1.766	2.25	3.319
20 wt%	0.388	2.126	2.939	2.918	3.734	6.131

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Author Contributions

Martin E. Santocildes-Romero, Jens Hansen, Paul V. Hatton, Helen E. Colley, and Craig Murdoch conceived and designed the research. Martin E. Santocildes-Romero, Lucie Hadley, Katharina H. Clitherow performed the experiments, analyzed the data, conducted statistical analysis and interpreted the results. The manuscript was written and figures prepared by Martin E. Santocildes-Romero and further edited by Craig Murdoch, Helen E. Colley, Martin H. Thornhill, Paul V. Hatton, Lucie Hadley, and Jens Hansen. Jens Hansen contributed essential reagents. Jens Hansen, Martin H. Thornhill, Paul V. Hatton, Craig Murdoch and Helen E. Colley contributed essential expert knowledge. All authors are aware of the content and have read and edited the manuscript.

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