Electrospun nanofibers for Sustained Delivery of Cetylpyridinium Chloride for Buccal Topical Application

MHA. Zanin[*], VA. Santos[**] , AM. Oliveira[*], NNP Cerize[*] and MA. Borsatti[**]

[*] Institute for Technological Research of the State of São Paulo – IPT, Laboratory of Chemical Processes and Particle Technology - Bionanomanufacturing, São Paulo- SP, Av. Prof. Almeida Prado 532, Cidade Universitária, CEP 05508-901, Brazil, mhzanin@ipt.br, ncerize@ipt.br, amarim@ipt.br
[**] University of São Paulo, São Paulo, Brazil
Department of Stomatology, School of Dentistry, Av. Professor Lineu Prestes, 2227
Cidade Universitária 05508-000, São Paulo, Brazil, valalves@usp.br, maborsat@usp.br

ABSTRACT

Electrospun polymeric nanofibers were developed using polyvinylpyrrolidone (PVP-Kollidon® 90F), ammonio methacrylate copolymer type B (PMMA-Eudragit® RS100) and cetylpyridinium chloride (CPC), 10:10:1 (wt/wt%) for buccal topical application. The morphology of mats with and without the CPC was analyzed and also the the thermal behaviors and the physicochemistry characteristics of the nanofiber mats. The SEM images revealed that all of the fibers were smooth and the average diameter of fibers was from 200 nm to 500 nm. The thermal analysis showed that the incorporation of cetylpyridinium chloride in the nanofiber through electrospinning process changed the cristallinity of cetylpyridinium chloride and/or conferred to this drug a new amorphous state. The *in vitro* release of CPC from the fiber in PBS buffer (pH 7.4) showed a sustained release over the experimental time of 24 h and the drug did not permeated all the skin achieving the medium receptor.

Keywords: Eletrospinning, nanofiber, cetylpyridinium chloride, Drug Delivery System

1 INTRODUCTION

Cetylpyridinium chloride is a drug known for its antifungal activity *in vitro* and *in vivo* against *Candida* albicans [1]. The Candida albicans is a comensal organism that can be isolated from the gastrointestinal tract, oral and vaginal mucosa of up to 80% of healthy individuals. Normally it doesn´t represent a problem, however it can cause symptomatic infections of mucosal membranes[2].

Currently, there are a variety of topical and systemic antifungal agents available for treating oral candidiasis. The topical antifungal agents most commonly used are nystatin and miconazole (MCZ), which have undesirable side effects and the risk of developing fungal resistance. The main adverse effects observed with nystatin are unpleasant taste, nausea and motion sickness. Miconazole may cause drug interactions, even in the case of topical use. Mouthwashes containing antiseptics, such as chlorhexidine, cetylpyridinium chloride, triclosan and others, have been studied as alternatives for traditional antifungal agents. It has been shown that CPC has proven antifungal activity in vitro and in vivo, with advantages over triclosan, and chlorhexidine[3]. A CPC delivery-controlled system, transported in polymer nanofibers (PVP/PMMA) was developed as an altenative to protect the drug and improve the drug bioavailability in contact with the oral mucosa.

2 MATERIAL AND METHOD

2.1 Materials

Cetylpyridinium chloride (Sigma-Aldricht Corp., Saint Louis, USA); Polyvinylpyrrolidone (PVP-Kollidon® 90F, BASF, Ludwigshafen, Germany); Ammonio methacrylate copolymer type B (PMMA, Eudragit® RS100, Evonik, Germany); Acetone and Ethyl alcohol (Synth).

2.2 Production of CPC nanofiber by electrospinning process

A 10.0% (wt/wt) PVP:PMMA (1:1) blend was solubilized in acetone and ethyl alcohol solution (50:50 v/v) and the CPC was added at 5% (wt/wt). The mixed solution was pumped (infusion pump PHD2000 – Harvard Apparatus) at a constant rate of 1 mL/h, forming a bead of solution at the tip of syringe. A high voltage (ES30P-10W Gamma High Voltage Research, Florida, USA) of 18 kV was applied between the nozzle needle, a negative potential, and the grounded collector. As the jet breaks up into fibers from the Taylor cone, the liquid was evaporated and gave rise to dry fibers which were subsequently spun on the wrapped collector with aluminum foil. The operation occurred until that a multilayer fiber mat was obtained. The distance between the tip of syringe and the collector was of 6 cm. The velocity of rotating collector used was 30 rpm. The Figure 1 shows the set up of the electrospinning

equipment in lab scale where the CPC nanofiber was developed.

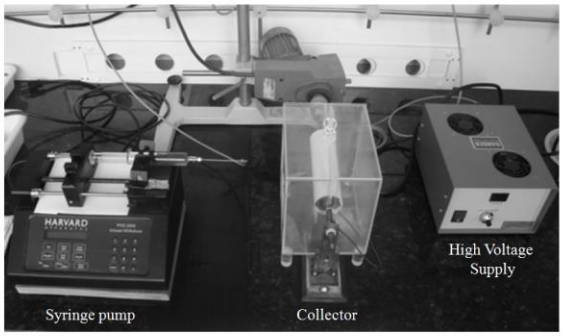

Figure 1: Picture of the Lab-built device employed in all of the electrospinning experirments.

2.3 Morphology analysis

The Scanning electron microscopy (SEM) was done using a Scanning Electron Microscopy of High Resolution - FEG - SEM (FEI Quanta 3D).

2.4 Thermal analysis

The techniques used was Differential Scanning Calorimetry (DSC). The DSC curves were obtained with a Mettler Toledo 822e 202 System. Aliquots of about 5 mg of each sample were placed in an aluminum pan without pin. Convencional DSC measurements were performed by heating the sample from 25 ^{0}C to 700 ^{0}C at a rate of 5^{0}C/min under a nitrogen flow of 50 mL/min.

2.5 FT-IR analyses

FT-IR spectrum was recorded on NICOLET 6700, Thermo Scientific FT-IR spectrometer. The samples were scanned from 600 to 4000cm^{-1}.

2.6 Permeation studies using esophageal mucosa as membran model

In vitro permeation study was done in Franz cell diffusion using a pig esophageal mucosa as model for buccal tissue [4] and phosphate-buffered-saline (PBS) (pH 7.4) as receptor medium.

Briefly, the Franz cells with a diffusion area of 1.77 cm2 were used. The connective side of tissue (Pig Esophageal Mucosa) was spread over the PVP/PMMA nanofiber and the ensemble was mounted in the diffusion cell. It was used a frozen tissue that was first placed in PBS pH 7.4 for 15 min before assembly on the diffusion cells. The donor and receptor compartments were filled with the same buffer and the cells were allowed to stabilize for 30 min in a water bath at 37^{0}C.

The receiver compartment volume was 6.0 mL and 1 mL samples were collected sequentially and replaced by fresh buffer. The CPC concentrations were determined by UV-vis spectrophotometty. The diffusion experiments were conducted for 24 h with a agitation velocity of 300 rpm.

2.7 Quantification of Cetylpyridinium chloride of the nanofiber

The amount of CPC pure and the remaining in the PVP/ PMMA nanofiber mats was determined using UV-vis spectrophotometer, GBC10 MODEL, Cintra, based on the literatura[5].

3 RESULTS

The Figure 2 illustrates a picture of the PVP/PMMA blend nanofiber developed and the Figure 3 presents the SEM micrographs of nanofiber with incorporated CPC. The PVP/PMMA blend nanofibers with CPC presented a average diameter from 200 to 500 nm and showed a round-shaped and smooth morphology.

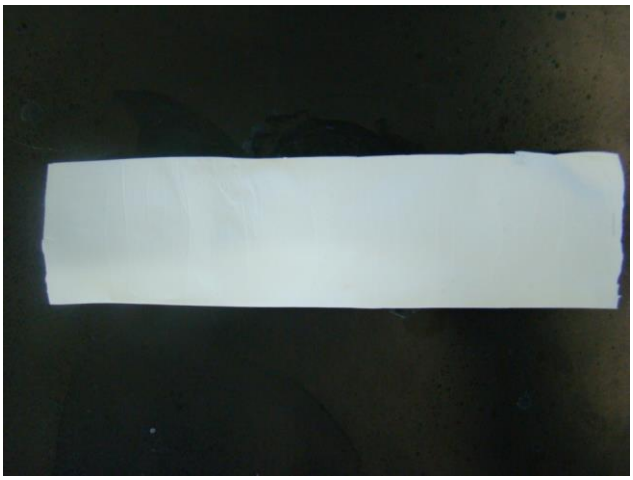

Figure 2: Picture of an electrospun PVP/PMMA fiber mat with CPC (5% wt/wt).

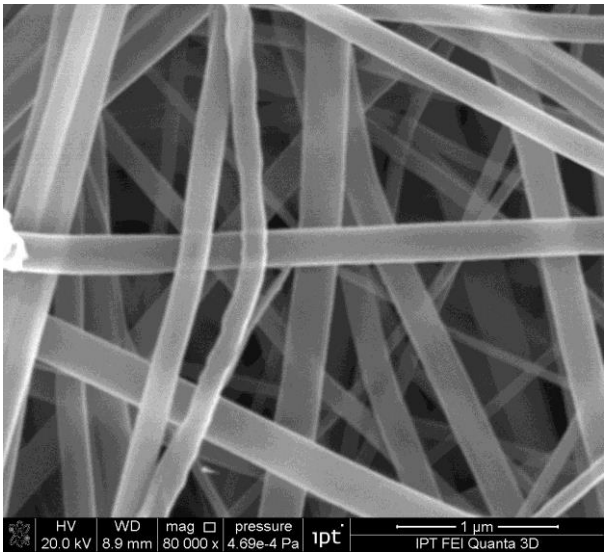

Figure 3: SEM photograph of an electrospun PVP/PMMA fiber mat with CPC (5% wt/wt). 80000x

The DSC profiles showed a single endothermic peak at 85^0C [6] for the CPC profile that was not detected in the nanofiber with CPC. These results suggest that the incorporation of CPC in the electrospinning process change the cristallinity of CPC and/or confers to this drug a new amorphous state (Figure 4).

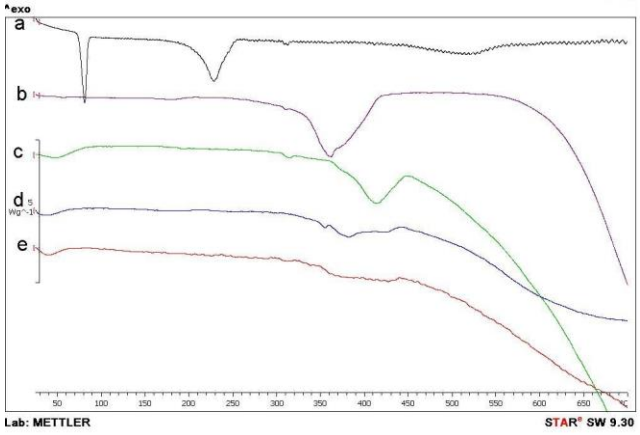

Figure 4: (a) CPC; (b) PMMA Polymer; (c) PVP Polymer;(d) PVP/PMMA nanofiber without CPC and (e) PVP/PMMA nanofiber with CPC (5% wt/wt) .

The FT-IR and DSC were techniques used in order to know the interactions among the polymers and the CPC.

IR spectra of the nanofibers without CPC presented a similar spectra to the nanofiber with CPC (Figure 5).

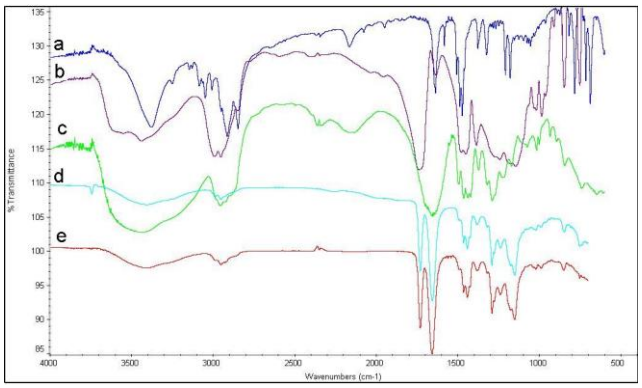

Figure 5: (a) CPC; (b) PMMA Polymer; (c) PVP Polymer;(d) PVP/PMMA nanofiber without CPC and (e) PVP/PMMA nanofiber with CPC (5% wt/wt) .

The in vitro release of CPC from the fiber in PBS buffer (pH 7.4) showed a sustained release over the experimental time of 24 h (Figure 6) and the CPC did not achieve the medium receptor, according to the permeation study.

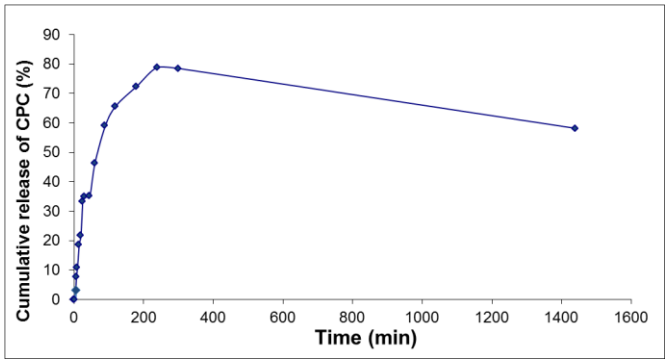

Figure 6: Release profile of CPC from nanofiber comprising PVP/PMMA with CPC (5% wt/wt).

4 CONCLUSIONS

An alternative to buccal delivery of drug, such as cetylpyridinium chloride (5% wt/wt), was developed through the electrospinning technology using the polymers polyvinylpyrrolidone (PVP) and ammonio methacrylate copolymer (PMMA). The nanofiber presented an average diameter varying from 200 nm to 500 nm. The electrospinning process seems to change the cristallinity of the CPC after its nanofiber incoporation.

The *in vitro* release of CPC from the fiber in PBS buffer (pH 7.4) showed a sustained release over the experimental time of 24 h and the drug did not permeated all the skin achieving the medium receptor. This system open a new possiblity to control the CPC release at buccal mucosa. In addition to this, this new system can improve the drug bioavailability in contact with the oral mucosa.

ACKNOWLEDGMENTS

The authors acknowledge the financial support by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to the infrastructure to do development of the nanofiber and for the Institute for Technological Research of the State of São Paulo – IPT.

REFERENCES

[1] Giuliana G, Pizzo G, Milici ME, Musotto GC, Giangreco R. In vitro antifungal properties of mouthrinses containing antimicrobial agents. J Periodontol. 68(8):729-33, 1997.

[2] Kim J, Sudbery P. Candida albicans, a major fungal pathogen. J Microbiol., vol. 49, 171-177, 2011.

[3] Santos, VA., VIEIRA, P V A., Oliveira, AM., Zanin, MHA., Borsatti, MA. Antifungal effect of electrospun nanofibers containing cetylpyridinium chloride against Candida albicans. Brazilian Oral research, v 28, 1-6, 2014.

[4] Consuelo, ID; Pizzolato, GP; Falson, F; Guy, RH; Jacques, Y. Evaluation of Pig Esophageal Mucosa as a Permeability Barrier Model for Buccal. Tissue journal of pharmaceutical sciences, vol. 94, 2777-2788, 2005.

[5] Benamor M., Aguersif, N., Draa, MT. Spectrophotometric determination of cetylpyridinium chloride in pharmaceutical products. Journal of Pharmaceutical and Biomedical Analysis. Vol.26, 151-154, 2001

[6] Krajisnik ,D.; Dakovic, A, Milojevic, M; Malenovic, M; Kragovic, M; Bogdanovic, DB; Dondur, V, Milic, J. Properties of diclofenac sodium sorption onto natural zeolite modified with cetylpyridinium chloride. Colloids and surfaces B: Biointerfaces, vol.83, 165-172, 2011.