EMULSION ELECTROSPINNING - METHOD TO INTRODUCE PROTEINS FOR BIOMEDICAL APPLICATIONS

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Introduction

The possibility of manufacturing of nanofibrous scaffolds mimicking the microstructure of the extracellular matrix (ECM) is an advantage of electrospinning techniques used in tissue engineering and regenerative medicine. Modification of the electrospinning conditions as well as the possibility to modify the solution used to produce nanofibers give new opportunities to prepare scaffolds with desired behaviour in the organism. One of the modifications used to release biologically active compounds or drugs is emulsion electrospinning. The advantage of this solution is the control over the release kinetics of the active ingredient or biomolecule and the high-efficiency encapsulation [1]. Moreover, encapsulation of proteins and pharmaceuticals in electrospun fibers is one of the strategies to overcome the barriers associated with maintaining the stability and effectiveness of the active ingredient during the formulation process [2].

In our study, we focused on the possibility of loading protein into nanofibers using emulsion electrospinning, which creates core-shell nanofibers from an emulsion consisting of an organic phase (polymer) with surfactant and aqueous phase. To prevent negative interaction of the model protein (albumin, alanine) suspended in the aqueous phase with the polymer organic solvent (PCL) during the electrospinning process, commercial surfactants used in cellular research (Tween 80, Triton X100) were used. The next step was to develop electrospinning conditions to obtain fibers enriched in encapsulated proteins. Specifically, we investigated the solution and emulsion process parameters for electrospinning to achieve high protein loading into nanofibers, preservation of protein bioactivity prolonged formulation stability, and controlled protein release for future biomedical applications.

Materials and Methods

Both oil and water phases for emulsion were prepared separately. 15% (w/v) Polycaprolactone (PCL, Mw = 80kDa, Sigma-Aldrich, Germany) and surfactant (Tween80, Sigma-Aldrich) at different concentrations (0,1%, 0,5% , 1% v/v) were dissolved in dichloromethane (DCM, Chemland SA) and dimethylformamide (DMF, Chemland SA). Alanine (L-alanina, Sigma-Aldrich, Germany), as well as Bovine Serum Albumin (Mw = 64kDa, Sigma-Aldrich, Germany), were dissolved in a distilled water to prepare a water phase. Then, both alanine and albumin solutions were dropwise added to the polymer solution and stirred in an ice bath. The microstructure of nanofibers was observed with a scanning electron microscope (NOVA NANO SEM 200). To determine the physicochemical properties of a scaffold, contact wet angle and surface free energy were measured with a goniometer (DSA 25 Kruss). The presence of the surfactants was checked by ATR mode in FTIR study (BioRad Tensil 60). The release kinetics of peptides in the material was assessed by turbidimetry (2100AN IS Laboratory Turbidimeter). Cytocompatibility was assessed by seeding fibroblasts on a fibrous scaffold after 7 days of contact with the fibrous scaffold.

Results and Discussion

The results of the study confirm that the surfactant addition strongly affects the physicochemical properties of the fiber surface; the hydrophobicity decreases and the fibers from superhydrophobic materials become hydrophilic. As shown in FIG. 1 the contact angle of pure fibrous PCL decreases from 130° to about ≈20° for PCL fibers obtained from PCL/Triton, PCL/Tween mixture. The observed effect is independent of the surfactant concentration. This is proved by the hydroxyl bands in the range 3500 cm^{-1} and 1190 cm^{-1} typical for the structure of both surfactants. On the other hand, a change in the ratio of the 1190:1170 cm^{-1} bands is observed in materials where triton/albumin and triton/alanine emulsions were introduced into the fibers during the electrospinning process. Protein macromolecules encapsulated in micelles do not yield bands characteristic of the proteins used, it is cover by surfactants layer. Surfactant addition does not affect the diameter of PCL fibers, whose distribution ranged from 280-1800nm for pure PCL and remained unimodal with a maximum at 1000-1500 nm for PCL/Tween80. In contrast, the addition of emulsion to the spinning solution with PCL resulted in a significant increase in the fiber size, whose distribution remained unimodal but the range widened to 3000 nm (for PCL/Tween80/albumin). The observed changes are a consequence of the larger micelle sizes present in the emulsion. None of the tested scaffolds showed a cytotoxic effect.

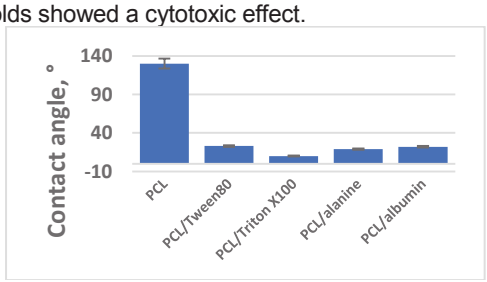

FIG. 1. Contact angle of the samples.

Conclusions

The emulsion electrospinning process allows obtaining fibres modified by micelles with biological compounds. The addition of the surfactants as well as surfactants with encapsulated protein increase hydrophilicity and diameter of polymer fibres. Moreover, the presence of an external layer of a polymer in a shell part provides more controllable kinetics of biomolecules release. Neither surfactants nor micelles with surfactants and proteins did not induct cytotoxicity of fibrous scaffolds.

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References

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