ELECTROSPUN KERATIN/POLY(BUTYLENE SUCCINATE) MATS FOR WOUND HEALING AND DRUG DELIVERY APPLICATIONS

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EXTENDED ABSTRACT

Regenerated wool keratin is a promising natural material for wound dressing. In the present study, keratin extracted from wool was blended with poly(butylene succinate) in 1,1,1,3,3,3-hexafluoro-2-propanol (blending ratio 50/50) and electrospun into nanofibrous mats. Compared to the pure poly(butylene succinate) nanofibres, the keratin based ones showed lower diameters, lower mechanical properties but an increased biodegradability, a delayed diclofenac release and increased bioactivity.

Keywords: wound dressing, drug delivery, keratin, electrospinning

1. INTRODUCTION

In response to the growing global health problem of acute and chronic wounds induced by various diseases (diabetes mellitus, cancer, psoriasis, etc.), an ambitious challenge in the biomedical field concerns the design of wound dressings with peculiar functions that fully respond to the different needs of patients.

Wound dressings made of electrospun non-woven nanofibers are considered effective scaffolds for skin tissue regeneration, since their high surface to volume ratio and their microporous structure, which mimics the extracellular matrix (ECM), promote cell adhesion and proliferation.

The electrospinning of naturally derived polymers, such as fibroin or collagen have been massively investigated, due to their biocompatibility, bioresorbability and biological activity.

In this framework, keratin appears to be an ideal candidate, since, like collagen, it possesses the amino acid sequences responsible for cell adhesion (Arg-Gly-Asp and Leu-Asp-Val)[1].

Moreover, keratin can be easily obtained from low cost renewable sources, such as by-products from wool textile industry, raw wool not fitting for spinning deriving from dairy industry and feather, horns and nails from butchery.

However, because of the low molecular weight and the brittle properties of keratin, the transformation of this protein into nanofibers by electrospinning is a great challenge. Blending keratin with a proper polymer is a widely used strategy to address this drawback.

Poly(butylene succinate) (PBS) is an aliphatic polyester, recently approved by the Food and Drug Administration, which is emerging thanks to its thermal stability, high melting temperature and, most importantly, its biocompatibility and biodegradability.

In the present work, keratin was blended for the first time, with PBS, using the 1,1,1,3,3,3-hexafluoro-2-propanol (HIFP) as common solvent.

After that, the pure PBS and keratin/PBS blend solutions were loaded with diclofenac sodium salt and electrospun into nanofibers. The electrospun mats were characterized from

morphological and mechanical point of view and in terms of biodegradability in enzymatic conditions, cell viability and drug release.

2. EXPERIMENTAL

2.1 Preparation of pure PBS and keratin/PBS electrospun membranes

Keratin was extracted from wool by sulphitolysis reaction according to a published method[2]. After that, keratin powder was dissolved with PBS in HIFP, at a blending ratio of 50/50 and at a total polymers concentration of 15% wt. The mixture (4mL) was electrospun using a voltage of 20 kV, a tip-to target distance of 15 cm and a flow rate of 0.03 mL/min. A solution of pure PBS dissolved in HFIP at a concentration of 15% wt was electrospun using the same process conditions.

2.2 Preparation of diclofenac loaded pure PBS and keratin/PBS electrospun membranes

The diclofenac loaded mats were prepared as described before, adding diclofenac sodium salt (23% *vs* polymers) in the solutions, before the electrospinning.

2.3 Characterization of keratin/PBS electrospun membranes

The prepared nanofibers were analyzed by the scanning electron microscope (SEM), using a Zeiss EVO LS 10 LaB6 with an acceleration voltage of 5 kV and a working distance of 5 mm. Samples were gold-sputtered for 1 min before the analysis. The fiber diameters were analyzed by means of freely distributed software GIMP 2.8 (GNU Image Manipulation Program). For each samples, 150 measures were taken from different images.

Mechanical tests were conducted using an Instron 4465 dynamometer with 10 KN load cell. Rectangular strips with the dimensions of 5x50 mm and thickness of about 100μm were used for the measurements. The breaking stress, the elongation at break and elastic module were reported as mean value ± standard deviation of the tests carried out on different 6 tests.

Enzymatic degradation experiments were conducted under physiological conditions (pH 7.4 and 37°C) and in the presence of trypsine (500 μg/mL). The samples (about 10 mg) were incubated in tryspine (2.5 μg / mg of sample). Periodically the samples were removed from the solution, washed with distilled water and acetone and left to dry until reaching a constant weight. The samples were then weighted to measure the weight loss. These measurements were carried out in triplicate.

Mouse embryonic fibroblast (NIH-3T3/GFP) cells were cultured under standard conditions in the DMEM medium, supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 0.1 mM MEM Non-Essential Amino Acids (NEAA), 100 U mL^{-1} penicillin and 100 U mL^{-1} streptomycin in a humidified incubator set at 37 °C with 5% CO_2. Cells were seeded on diclofenac-free electrospun membranes in 24-well plates at a density of 10^5 cells per cm^2.

Diclofenac release from the prepared mats was evaluated by inserting the samples in a dialysis membrane of cellulose acetate (cut off 12–14 kDa) and immersed in phosphate buffer. The system was maintained under shaking at 37 °C and aliquots of 100 μL were withdrawn at appropriate time intervals, while an equal amount of fresh phosphate buffer was added (sink conditions). The amount of diclofenac released in the medium from the drug loaded mats was evaluated by UV–Vis absorption band at 276 nm, using an appropriated calibration curve. Drug-free mats were used as control.

3. RESULTS AND DISCUSSION

3.1 Keratin/PBS nanofibers

In figure 1, the nanofibers of pure PBS and keratin/PBS 50/50 are shown. Both samples displayed homogenous and defects free nanofibers. Compared to pure PBS nanofibers, those containing keratin posses a narrower diameter distribution shifted towards lower diameters.

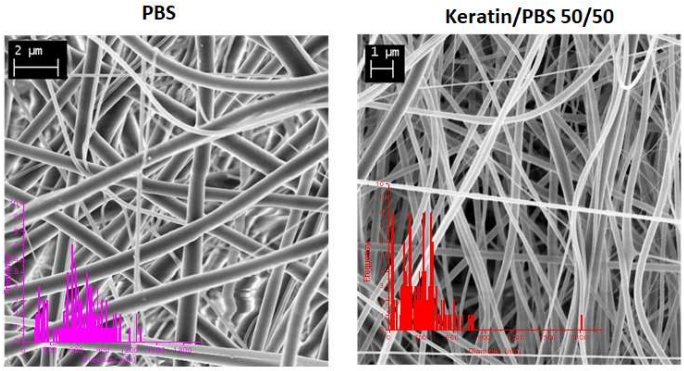

Figure 1. PBS and keratin/PBS 50/50 nanofibers and related diameter distributions

The mechanical tests revealed that the two samples showed comparable elastic modulus and tensile stress values, confirming that it was possible to obtain a keratin-based wound dressing easier to handle. Instead, the elongation at break of keratin/PBS scaffold is slightly lower than that of pure PBS and this is due to the presence of keratin which reduces the plastic behavior of the sample.

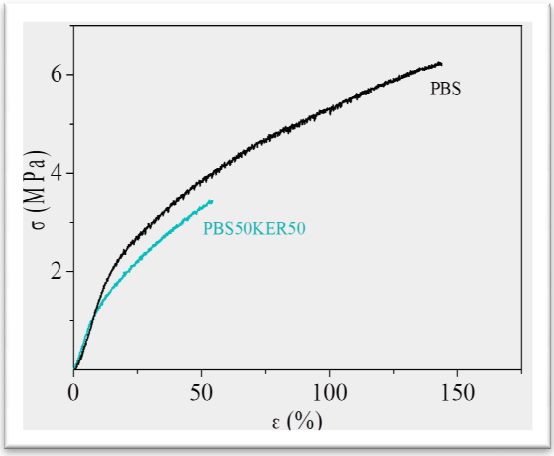

Figure 2. Stress-strain curves of the PBS and keratin/PBS 50/50 nanofibers

3.2 Biodegradability

In figure 3, the weight loss of the samples evaluated in physiological conditions and in the presence of trypsine are reported. As can be seen, while the biodegradation of the PBS scaffold reached only the 10% after 17 days, that of the sample containing keratin reached the 45% after 9 days. This behavior is attributable to the degradation of keratin present inside the sample, which degrades completely within 9 days.

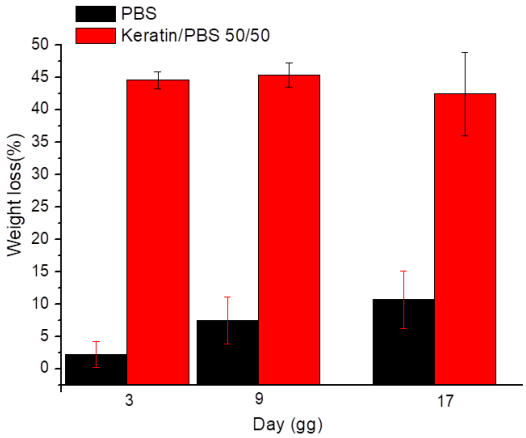

Figure 3. Weight loss of the samples evaluated under physiological conditions (pH 7.4 and 37°C) and in the presence of trypsine (2.5µg per mg of the sample).

3.3 Cells growth and drug release

The diclofenac release profiles from the electrospun PBS and keratin/PBS 50/50 nanofibers are shown in figure 4a. About 30% of diclofenac was released from both the electrospun mats after 1h. Instead, the amount of drug release from keratin based mat, after 3h, is lower than that of pure PBS mat. This indicates that the presence of keratin delayed the drug release process, probably because of the stronger chemical interactions between the protein and diclofenac.

In order to evaluate the bio-activity of the electrospun mats, both samples were undergone to the cell viability test using fibrobalst cells (NIH-3T3). As shown in figure 4b, the cells proliferate on both mats, indicating that both of them are biocompatible. However, the cells adhesion was higher in the presence of keratin, evidencing that the protein increased the bioactivity of the sample.

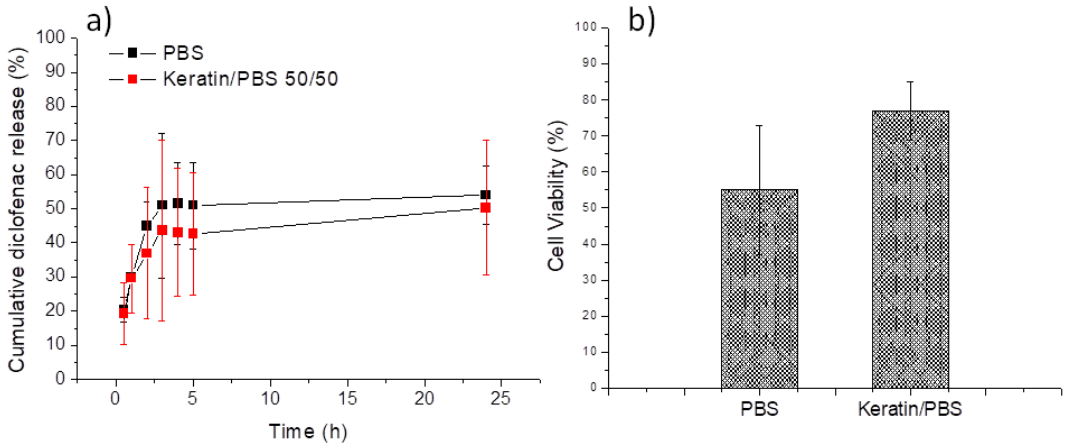

Figure 4. (a) diclofenac release profiles and (b) cell viability of NIH-3T3

3. CONCLUSION

In this work, keratin/PBS 50/50 nanofibers without drug, as well as loaded with diclofenac sodium salts were successfully electrospun from mixture of the two polymers dissolved in HFIP. The PBS has been proved to be an excellent blending polymer that improved the

processing of the protein, as well as the mechanical properties of the keratin-based electrospun membrane.
Biological in vitro cell culture demonstrated that keratin stimulated a more significant cell viability than pure PBS. Moreover, the presence of keratin delayed the diclofenac release.
The results of drug (diclofenac) release and cell culture assays suggest that the keratin/PBS nanofibers are promising biomaterials for wound healing and drug delivery.
In conclusion, the properties of the final keratin-based electrospun membrane have proved to be extremely interesting in view of possible applications in the field of tissue engineering anddrug delivery, despite the results obtained are preliminary and represent a starting point.

3. REFERENCES

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