INFLUENCE OF CHITOSAN NONWOVEN FABRIC MODIFICATION ON ITS PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

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Introduction

The surface modification of the non-woven fabric, which is an architecturally close substrate for the external cellular matrix (ECM), is an attractive way to obtain scaffolding that stimulates the regenerative processes of the damaged tissue. Studies on scaffolds functionalization focus on the attachment of proteins or peptides activating adhesion processes [1]. Unfortunately, proteins tend to randomly curl during adsorption, making binding domains inaccessible to their cell membrane counterparts. In addition, since native ECM proteins may have several binding motifs, there is some difficulty in predicting the cellular response when used. Therefore, only protein fragments - short peptides containing specific motifs recognized by receptors - are used [2]. Such a solution guarantees greater stability of the system in relation to heat treatment, sterilization, pH changes or in vivo environment [3]. In the literature, examples of peptides derived from fibronectin (e.g. RGD, REDV, KQAGDV), collagen (e.g. DGEA, GFOGER), laminin (LRE, PDGSR, IKVAV, YIGSR) or elastin (e.g. VAPG) can be found [2,3]. The most commonly used of these is the arginine-glicyine-aspartate (RGD) sequence, which occurs in most ECM matrix proteins and acts as an adhesive [2].

Modification of nonwovens, which additionally have the character of partially cross-linked hydrogel, is challenge. Proceedings of chemical modification may change the attractive microstructure and physical methods of depositing - the results are insufficient for the needs (low concentration of peptides on the fiber surface) [4]. Additionally, the processing of the finished material significantly influences its physical properties (fibre morphology). The electrospraying method proposed in the paper seems to be a technique bringing significant advantages in comparison to the classical dip coating. Peptides being an extinguished part of biologically active collagen were applied by means of an electrodischarge device. Modified nonwovens were compared to the behavior of control materials (free of peptides).

Materials and Methods

Collagen-like peptides - K1 and K2 - were synthesized in the Institute of Organic Chemistry of the Technical University of Łódź. Modification of fibre surface was carried out by dip coating and lyophilization (method 1) and electrospraying and freeze drying (method 2). In Method 1, the first step was to immerse the CS nonwoven fabric in 0.5% peptide solution (PBS/24h/12°C), the next step was freeze drying of fibres with peptide (-50°C/0.03Torr/24h). In Method 2, the first step was to natrsy the nonwoven fabric (CS) with pepethide dissolved in water (conditions: 0.25-0.1%/15kV/15min) followed by 24-hour lyophilization (-50°C/0.03Torr). The obtained nonwovens were examined for morphology: NOVA NANO SEM 200 observing the changes on their surface and the distribution of the fiber size. Tests of absorbability (%N) of nonwovens before and after modification were carried out. The presence of biomolecules in fibrous scaffolds were confirmed by FTIR. The biological test: biocompatibility of the fibrous scaffolds (cytotoxicity, viability, adhesion to the scaffold) in contact with the fibroblastes were tested.

Results and Discussion

Physical modification of nonwovens with peptide solutions leads to a change in the morphology of the substrate, in the case of method 1 the visible peptide grid significantly reduces the copper distances, in the case of method 2 the peptides are adsorbed to the fiber surface. This is confirmed by spectroscopic studies where characteristic bands typical for peptides (I and II order amides) can be found. Thus, the average fiber thickness increases and the fiber becomes rough. It seems that visible changes may have a real effect on cell response, especially in terms of cell adsorption and adhesion.

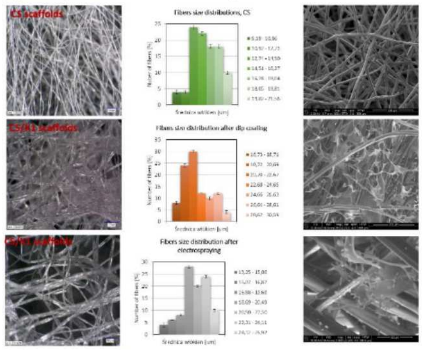

FIG. 1. Microstrucuture of chitozan non-woven fabric (CS/K1) after modyfication method 1 and method 2 with fibers size distribution.

Conclusions

Both dip coating and electrospraying are methods of fibrous substrate modification. In the case of the method 1, the substrate gains an additional microstructure made up of peptides. In the case of method 2, peptides adsorb to the fibres, affecting their surface development and physicochemical changes of the fibres. Thus, the substrates gain biomimetic character conducive to cell proliferation.

Acknowledgments

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