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# Immobilization of gelatin on electrospun fibers: a comparative analysis of aminolysis-based procedure and physisorption for three aliphatic polyesters

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**Introduction**

Immobilization of adhesive proteins, such as gelatin, collagen, fibronectin on the scaffold surface has become a widely reported method that can improve the interaction between scaffold and cells. It is achieving through various methods originated from chemical binding or physical interaction between biomolecule and polymer [1,2]. In this study, we modified the surface of three types of electrospun fibres using chemical immobilization of gelatin basing on aminolysis and glutaraldehyde cross-linking or physisorption of gelatin and evaluated the change of materials properties as well as L929 cells response.

**Experimental Methods**

Poly(caprolactone) (PCL), poly(L-lactide-co-caprolactone) 70:30 (PLCL) and poly(L-lactide) (PLLA) fibers were obtained by electrospinning. The aminolysis reaction was carried out by immersing nonwovens in ethylenediamine solution in isopropanol under various treatment conditions to test the susceptibility of fibers to the reaction. Two sets of conditions were chosen to evaluate immobilization for samples with lower and higher concentrations of free NH2 groups. Then, gelatin was chemically immobilized on the surface of given samples using glutaraldehyde as a cross-linking agent. Samples with physisorbed gelatin were prepared by simple immersion in gelatin solution. Ninhydrin test and BCA test were used to measure the density of amine groups and gelatin on the surface, respectively. Change of morphology, average molecular weight and crystallinity were determined using scanning electron microscopy, gel permeation chromatography and wide-angle X-ray scattering technique, respectively. The wettability of modified samples was measured by a goniometer. Mechanical properties were determined using uniaxial tension testing. The stability of the coating originated from chemical binding or physical interaction was verified via incubation in phosphate-buffered saline (PBS) from 1 to 90 days. L929 cells were cultured on modified samples to investigate the biological response to modified samples after 3 and 5 days.

**Results and Discussion**

It was shown that aminolysis-based immobilization could provide a higher concentration of gelatin on the fiber surface in the case of all investigated polyesters (Fig.1.). However, gelatin concentration was relatively high in the case of physisorption, especially when comparing to samples aminolyzed under mild reaction conditions. However, the incubation test showed that the chemically immobilized gelatin layer is more stable than physisorbed one. After 90 days of immersion, more than 60% of the initial concentration of protein was detected for a physically modified PCL sample while the values were higher than 90% and 80% for aminolyzed samples with lower and higher concentration of free NH2 groups, respectively (Fig.2.). Despite a similar concentration of free NH2 groups among all polyesters, the concentration of gelatin was significantly higher in the case of PCL fibers. The cause of this phenomenon could be the difference in the accessibility of groups on the surface, the difference in fiber diameter or roughness, which could influence the amount of immobilized gelatin for each polymer. For chosen conditions, there was no difference in fibers morphology between chemically and physically modified samples, however, it is known that aminolysis could cause fracturing of fibers [3]. Gelatin immobilization contributed to the change of the fibres properties from hydrophobic to completely hydrophilic after each studied modification. Additionally, the time of water drop absorption