ABSTRACT

Nanofibrus polycaprolactone scaffolds embedded with silver nanoparticles by electrospray deposition (esd) technique

This thesis deals with the development of Polycaprolactone (PCL) nanofibrous scaffolds, with Electrospray Deposition Method, within the field of Regenerative Medicine and specifically to enhance the mechanical behavior of natural polymeric scaffolds for cartilage regeneration.

Polycaprolactone is a biodegradable polymer composite, which belongs to the class of aliphatic polyesters, is easily crystallized, tough and flexible, with slow biodegradation. Moreover, the mechanical properties of this polymer can be configured and designed according to the desired characteristics, making it a promising candidate synthetic polymer for tissue repair and regeneration, mostly in orthopedic applications. Silver nanoparticles were selected for enrichment of nanofibrous scaffolds because of their antimicrobial properties (prevention of bacterial biofilm formation on the surface of medical implants). Also they increase the surface roughness, and consequently increase the hydrophobicity , and strongly influence cell adhesion, depending on the size, morphology, shape, charge, reactivity, functionality and creating aggregates or not.

The ultimate objective was to create a biomimetic nanofibrous environment which simulates the extracellular matrix (ECM) of the articular cartilage and therefore promote the adhesion and proliferation of chondrocytes and essentially creating new cartilage tissue. The structural characteristics of the scaffolds in tissue engineering, affect cellular behavior and must have specific construction requirements to support cell attachment, proliferation and differentiation. The scaffold essentially acts as a temporary synthetic extracellular matrix (ECM) that interacts with the cells to form a new tissue.

The electrostatic deposition method (Electrospinning), is the most common method for the construction of nanofibrous scaffolds. The Electrospray Deposition, the method used in this work, based on the same principle of operation with the electrostatic deposition method. The electrospun polymer nanofibers have many

excellent properties, including small diameters, the large specific surface, a high degree of structural perfection and superior mechanical properties. Additionally, the nonwoven polymeric structures offer a unique ability to control pore sizes between the fibers.

In this work, polycaprolactone (Poly-caprolactone, PCL) was used, at various concentrations (20 %, 25 % and 30 %) in various solvents (90 % acetic acid and chloroform / methanol in the ratio (3:1)) to find the optimum one for the particular polymer. The optimization of growth conditions of scaffolding, proved to essentially concern the polymer concentration, the type of solvent (surface tension, volatility) , the distance of needle - collector, the applied voltage and the flow rate. The development time of the scaffolds was for one hour, for the results to be comparable.

The structural/ morphological/ topographical characterization of scaffolds, made by Scanning Electron Microscopy (SEM) and Scanning Atomic Force Microscopy (AFM) and the evaluation of wettability by measuring the Contact Angle, CA. The mechanical strength was studied by the method of Nanoindentation. The polycaprolactone scaffolds showed hydrophobic behavior, increasing with the addition of silver nanoparticles . The size and identification of silver nanoparticles was done by X-ray diffraction, which revealed their existence. The scaffolds were studied also on the biological response in cell lines. Specifically, the cell line L929s (immortalized fibroblast cell line isolated from fibrosarcoma (adipose tissue) cells) was cultured on the surface of scaffolds for two intervals (24hrs , 3 and 7 days), and became a quantitative estimation of the cells grown with the application of MTT protocol. To complete the cellular study, images were taken by Scanning Electron Microscopy (SEM). Some samples were selected as optimal scaffolds, which was cultured with human chondrocytes, with the same procedure as with the fibroblast cells. The latter study cell filled again with images using Scanning Electron Microscopy (SEM). In the groups of samples, the largest cell viability showed the 30% PCL in chloroform / methanol to the minimum distance needle - collector (D200 = 5cm). Scaffolds with silver nanoparticles showed less viability. Polymeric nanofibrous scaffolds show high biocompatibility, as consisting of components that are recognized by the cells. That is, it seems that the main mechanism of cell adhesion

involves direct interactions fiber - cells, due to roughness and increased the area available (ratio surface / volume).

Concluded that the chemical composition, roughness, wetting and surface topography are determining factors for the biocompatibility of a polymeric material, as they determine the mechanism of cell adhesion and influence cell growth, proliferation and migration.