Structural and Mechanical Characterization of Biomaterials for Tissue Engineering: Fibrous Scaffold -Quantitative Nano-Mechanical Mapping by Atomic Force Microscopy

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Structural and Mechanical Characterization of Biomaterials for Tissue Engineering: Fibrous Scaffold - Quantitative Nano-Mechanical Mapping by Atomic Force Microscopy

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Abstract

Biodegradable polymeric fibers with nano and submicron diameters, produced by electrospinning can be used as scaffolds in tissue engineering. However is necessary to characterize their mechanical properties especially at the nanoscale. The PeakForce Quantitative NanoMechanics (PF-QNM) is recently developed AFM mode, which allows to probe mechanical properties of the material, such as: reduced Young`s modulus, deformation, adhesion, and dissipation, simultaneously with topographical imaging. In this paper we are presenting results of PF-QNM characterization of two kinds of electrospun fibers: PCL and PCL/HAp. The average calculated from DMT theory Young modulus was 3 ± 1 MPa for PCL mesh and 17 ± 3 MPa for PCL+HAp mesh.

Keywords: PeakForce Quantitative NanoMechanics (PF-QNM), Atomic force microscopy, Mechanical properties at nanoscale, Fibrous scaffold, Electrospinning.

1. Introduction

Biodegradable polymeric fibers, produced by electrospinning method, with nano and submicron diameters can be used as scaffolds in tissue engineering. It is possible due to their interfibrous pore size, high surface area to volume ratio, immunogenicity, biodegradability and structural similarity to the extracellular matrix (ECM) [1]. In physiological conditions fibers are subjected to stresses and strains from the surrounding biological environment. Such stresses can cause permanent deformation or even failure to scaffold structure. Therefore, there is a growing need to characterize their mechanical properties, especially at the nanoscale. Atomic force microscopy (AFM) has emerged as a powerful tool in the imaging of cells and biomaterials and probing selected mechanical properties under physiological conditions [2,3,4]. The PeakForce Quantitative NanoMechanics (PF-QNM) is recently developed AFM mode, which allows to measure mechanical properties of the material, such as: reduced Young`s modulus, deformation, adhesion, and dissipation, simultaneously with topographical imaging [5].

2. Experimental Procedure

In presented work two different kinds of fibers, produced by electrospinning from solution at applied voltage of 12 kV [1]. Polycaprolactone (PCL) and Polycaprolactone with 10 wt% of hydroxyapatite (PCL/HAp). The Bruker MultiMode AFM NanoScope 8 atomic force microscope and APP NANO - ACST probe were used to measure morphology, and related nanomechanical properties of electrospun fibrous meshes. The AFM microscope were eqipted with PeakForce Quantitative NanoMechanics (PF-QNM) module. The principle of PF-QNM bases on force-distance curve of interaction between AFM probe and sample [2,3], what is shown in **Figure 1**. The Derjaguin-Muller-Toporov (DMT) model were used to measure mechanical properties of the material: reduced Young`s modulus, adhesion, deformation and dissipation with high spatial resolution [5]. For comparizon the fibers were examined by a scanning electron microscope (Phenom Pro X, FEI SEM).

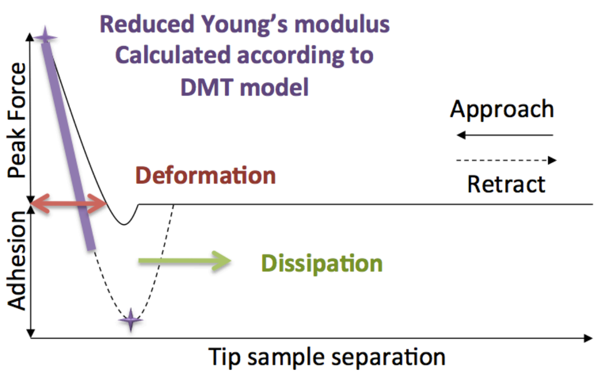

Fig.1 Schema of force-distance (FD) curve. The curve represents interaction during approaching of AFM probe to the sample surface, contact with sample and retracting away from surface. By analyzing FD curve the mechanical properties at nano scale can be measured. Automatic analysis of curve set allows to generate maps of mechanical properties distribution simultaneously with topographical imaging.

3. Results and Discussion

Figure 2 shows the micrographs of PCL and PCL/HAp electrospun fibers obtained by SEM and AFM (Height images) operating in PF-QNM mode. The DMT Modulus images show higher values of Young modulus (brighter color) for composite PCL/HAp material in comparison with pure PCL. The average values of selected nanomechanical properties are shown in the plot at the bottom of Fig. 2.

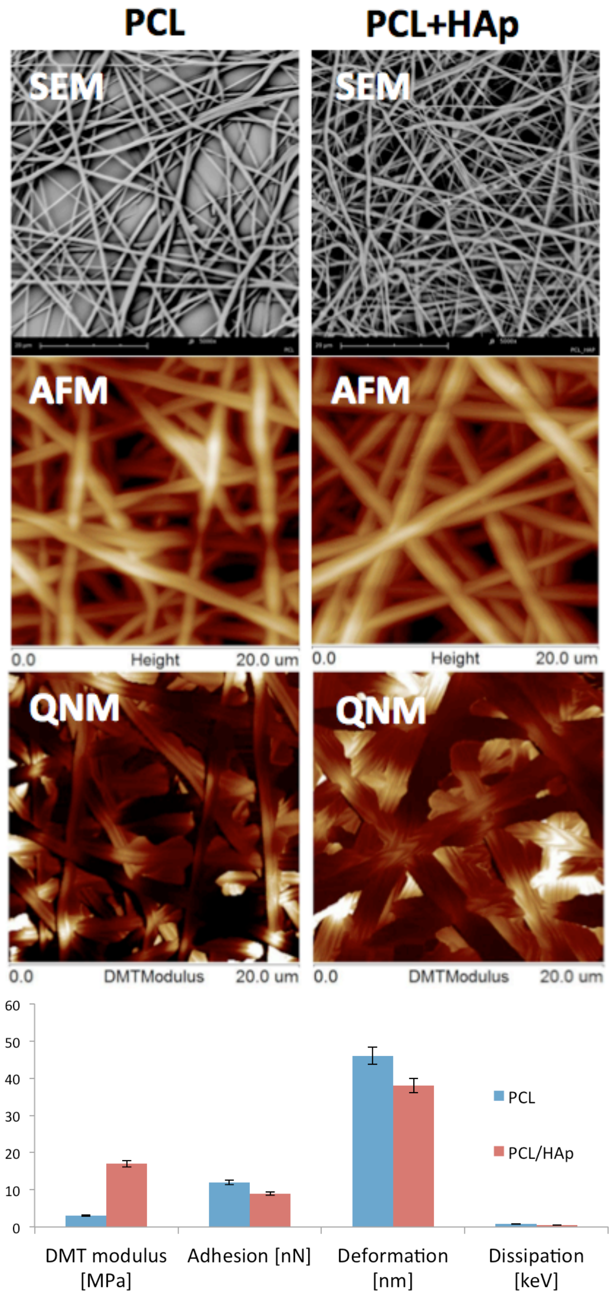

Fig.2. Comparison of results obtained on PCL and PCL/HAp composite. Imaging: SEM (scale bar 10μm), AFM, PF-**QNM** (DMT Young Modulus). Average results of nanomechanical properties calculated from PF-**QNM** images.

Mean values of Young modulus were equal: 17 ± 3 MPa and 3 ± 1 MPa for PCL/HAp and PCL respectively. This fact can be of course explained by ceramic addition to composite. It should be mentioned that these data were collected from polymeric meshes, so results are average for wholes constructs, and they are different than that for bulk material. However results obtained from single fibers (data not shown) confirmed these for meshes. Another important quantity, which can be calculated from PF-QNM is adhesion force between AFM probe and sample. Adhesion is extremely important for use of electrospun fibers as scaffolds for cells seeding. Cells need to adhere properly to the biomaterial to grow new tissue. It should be mentioned, that the adhesion force and other mechanical properties depends on the radius of AFM probe. In our previous work [5)], we sharpened AFM probes using focused ion beam (FIB) and demonstrated that decreasing probe radius caused decreasing adhesion and increasing measured DMT modulus value. High magnification mapping of nanomechanical properties on PCL/HAp sample allowed to distinguished in easy way bioceramic filler in polymeric matrix (data not shown).

4. Conclusions

The PF-QNM is very promising technique for probing nanomechanical properties of biomaterials simultaneously with topographical imaging.

Mechanical properties of nanomaterials can differ from the properties of materials at macroscale, and can depend on geometry of AFM probe.

Acknowledgements

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