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Quantitative imaging of electrospun fibers by PeakForce Quantitative NanoMechanics atomic force microscopy using etched scanning probes

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ABSTRACT

Electrospun polymeric submicron and nanofibers can be used as tissue engineering scaffolds in regenerative medicine. 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 their structure. Therefore, there is a growing necessity to characterize their mechanical properties, especially at the nanoscale.

Atomic force microscopy is a powerful tool for the visualization and probing of selected mechanical properties of materials in biomedical sciences. Image resolution of atomic force microscopy techniques depends on the equipment quality and shape of the scanning probe. The probe radius and aspect ratio has huge impact on the quality of measurement.

In the presented work the nanomechanical properties of four different polymer based electrospun fibers were tested using PeakForce Quantitative NanoMechanics atomic force microscopy, with standard and modified scanning probes. Standard, commercially available probes have been modified by etching using focused ion beam (FIB). Results have shown that modified probes can be used for mechanical properties mapping of biomaterial in the nanoscale, and generate nanomechanical information where conventional tips fail.

1. Introduction

In the last twenty years, a growing interest in new biomaterials for medical applications can be observed. Fibrous polymer scaffolds which can replace traditional metal implants become increasingly important in this field. In order to fulfill their task, fibrous biomaterials should be biocompatible and characterized by suitable mechanical properties.

Electrospinning technique offers the ability to control material properties, as well as structure, and mechanical functions of the scaffolds for tissue engineering applications (Baker et al., 2012). Due to their biodegradability, interfibrous pore size, high surface area to volume ratio, immunogenicity, structural similarity to the tissue extracellular matrix (ECM) (Elsabee et al., 2012; Bosworth et al., 2013; Khadka and Haynie, 2012; Raghavan et al., 2012; Sun et al., 2013; Kijeńska et al., 2012), electrospun fibers are good candidates for scaffolds. The mechanical properties of the material might have a strong influence on the biological function of the scaffold (Reilly and Engler, 2010). It is also worth to be noted that overall mechanical properties of any fibrous structure is based on three quantities (Carlisle et al., 2009): the architecture of the electrospun mesh, the properties of the single fibers and the junction between the fibers.

To study the mechanical properties of the electrospun fibers at the nanoscale atomic force microscopy (AFM) can be used. It is a powerful tool for the visualization, and probing of selected mechanical properties of materials in broadly defined life sciences (Wozniak et al., 2009, 2010).

This is mainly due to the ability of the technique for measuring force and distance at a high resolution and exploring surface in air and liquid with minimal sample preparation. It allows to examine topography, and mechanical properties of cells and tissues, as well as engineered biomaterials for tissue engineering. Testing mechanical properties of the electrospun fibers is crucial, and must be taken into account, because fibrous structures should be able to withstand the external forces acting on them after implantation (Chew

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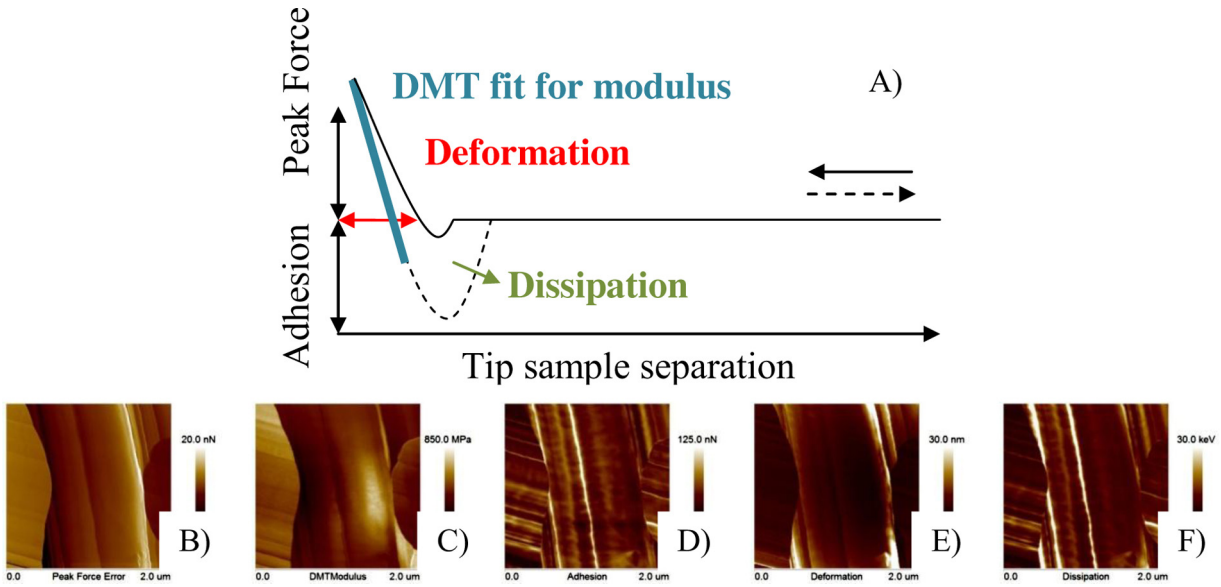

Fig. 1. Force curves (A), and corresponding information that can be obtained from them for PLCL fibers (2 μm × 2 μm scan area): peak force error data channel (B), DMT modulus data channel (C), adhesion data channel (D), deformation data channel (E), and dissipation data channel (F).

et al., 2006). In addition, different types of cells may need different mechanical properties of the scaffolds (Yang et al., 2008). One can find literature positions describing testing of mechanical properties of electrospun fibers using AFM, with nanoindentation (Zhang et al., 2011), stretching (Baker et al., 2012) and bending method (Croisier et al., 2012), but to the authors' knowledge it is the first time when new AFM technique – PeakForce Quantitative NanoMechanics (PFQNM) is used to characterize electrospun polymeric fibers.

PeakForce Quantitative NanoMechanics is a new atomic force microscopy imaging mode developed recently, which allows to measure some mechanical properties of the material, such as: reduced Young's modulus, adhesion, deformation and dissipation with high spatial resolution by probing at the nanoscale. Very few publications describing use of PFQNM have been printed, including stiffness mapping of amyloid fibrils (Adamcik et al., 2011), bitumen (Fischer et al., 2013), or polymers deformation testing (Liu et al., 2012).

Each series of nanomechanical measurements on a sample is equivalent to 256 × 256 force-separation curves, providing a map of mechanical properties with the same resolution as the topographic image (Fig. 1). This technique can be used with any standard AFM probes, but for quality as well as quantity results, proper calibration must be done. Both – appropriate tip, and suitable reference sample choice are crucial to obtain adequate modulus values. The Derjaguin-Muller-Toporov (DMT) model (Butt et al., 2005) implemented into the dedicated Bruker NanoScope Analysis software is used. If not, the modulus map will only represent qualitative instead of quantitative information (Pittenger et al., 2010).

To calculate the reduced Young's modulus, the retract curve (Fig. 1A) was fitted into the DMT model:

$$F - F_{adh} = \frac{4}{3}E^* \sqrt{R(d - d_0)^3}$$

where F is the force acting on the cantilever, F_{adh} the adhesion force between probe and the surface, R is the tip radius, $d - d_0$ the sample deformation, and E^* is the reduced Young's modulus. If the sample Poisson ratio is known, the Young's modulus can also be calculated.

Next factor that was taken into account for quantitative analysis was deformation of the sample material. Maximum deformation during the PFQNM examination can be described as the penetration of the probe into the sample. The deformation channel may include information from both plastic and elastic deformation components (Pittenger et al., 2010).

Image resolution for AFM techniques depends mostly on the scanning probe parameters. As probe radius decrease, image resolution increase, thus probe geometry is crucial for high resolution imaging. There are several ways of probes modification described in the literature: carbon nanotube attachment (Lee et al., 2008), PVD (Physical Vapour Deposition) (Chen et al., 2000), and T – CVD (Thermal Chemical Vapour Deposition) methods (Chattopadhyay et al., 2006) or ion sputtering (Bale and Palmer, 2002). According to our knowledge, there is no information regarding work that propose a use of modified AFM probes for PFQNM examination. In this paper the AFM tip probe FIB modification procedure for PeakForce QNM application is described. It has been conducted in order to compare images obtained by standard, and modified probes.

2. Materials and methods

2.1. Materials

Poly(L-lactide-co-caprolactone) (PLCL) with molecular weight of 150 000 was purchased from Evonik (Germany). Polycaprolactone (PCL) with a molecular weight of 80 000 g/mol, hydroxyapatite powder of grain size below 200 nm, and 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) used as a solvent in electrospinning, were purchased from Sigma–Aldrich [USA]. Collagen type 1 has been purchased from Koken (Japan).

2.2. Electrospun fibers fabrication

Solutions of 9 w/v P(LCL)/collagen/HAp, and 9 w/v PCL/HAp (both with 90:10 ratio) were prepared by dissolving the polymer and proteins/ceramics together in HFP and stirred overnight at room temperature. P(LLA-CL) solutions with concentrations of 9 w/v, and PCL solutions with concentrations of 9 w/v were prepared as a reference, using the same procedure. Each of prepared solutions were placed in a 3 ml plastic syringe attached with flat-ended steel needles with 27G inner diameter, and then the syringe were placed in the pump (KD Scientific) and dispensed at a rate of 0.8 ml/h. Fibers were fabricated using electrospinning at 12 kV of