International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 6; July-September, 2015, pp. 364-367

http://www.krishisanskriti.org/ijbab.html

Characterization of Fibrous Scaffold using Quantitative Nano-Mechanical Mapping Mode of Atomic Force Microscope

Mohammad A. Hussain[1], Adnan Memic[2], Nazeeh S. Alothmani[3], Rabah W. Al-dhaheri[4], Faten Al-hazmi[5], Hani A. Alhadrami[6] and Ali Khademhosseini[7]

[1,2,3,4,5,6]KAU, Jeddah, KSA
[7]Harvard-MIT, USA
E-mail: [1]mhussain2@kau.edu.sa, [2]amemic@kau.edu.sa, [3]nothmany@kau.edu.sa, [4]raldhaheri@kau.edu.sa, [5]fialhazmi@kau.edu.sa, [6]hanialhadrami@kau.edu.sa, [7]khademh@gmail.com,

Abstract—*Electrospun polymeric fibers with nano and submicron diameters amalgamated with inorganic nanoparticles, in particular, those constructed with uniform reinforcements can be used as scaffolds in tissue engineering. Importantly it warrants a characterization of their mechanical properties particularly at the nanoscale. The PeakForce Quantitative NanoMechanics (PF-QNM) AFM mode allows to probe nanomechanical properties of the material namely, DMT modulus, adhesion, dissipation and deformation, at the same time along with topographical imaging. In this paper we present results of PF-QNM characterization of three kinds of electrospun nanofibers made from a 1:1 blend of two polymers: PCL and PMMA with further addition of inorganic nanoparticles (Ag and ZnO) at three ratios thereby adding another degree of nanocomposition to the resulting nanocompoite nanofiber scaffold. The results showed the inorganic nanoparticles Ag and ZnO, with different shapes and sizes, appearing at the surface of the nanofibers. Heterogeneous Ag and ZnO nanoparticle distribution was observed on the nano fiber mesh. The Ag and ZnO contents were different at different locations along the nanofibers lengths based on their ratios in three different types of nanofiber mesh. The different ratios of Ag:ZnO inorganic nanoparticles affected the DMT Modulus as well as hydrophilic nature of the three kinds of nanofiber surfaces. Increasing ZnO amount increased both the hardness and water contact angle of the nanofiber mesh. For the same increase in the (Ag:ZnO) ratio, ZnO doubled the %increase in hydrophobicity as compared to Ag. From Ag:ZnO (30:30) to (100:30) increased the water contact angle by 8% while altering the ratio (30:100) increased it by 16%. The average DMT modulus was 0.62 ± 0.26 MPa for control mesh, 2.22 ± 0.61 MPa for Ag:ZnO (30:30), 5.62 ± 1.39 MPa for Ag:ZnO (100:30), and 63.87 ± 81.82 MPa for Ag:ZnO (30:100) mesh.*

1. INTRODUCTION

The last decade has seen an increasing trend in designing scaffolds for tissue engineering from biodegradable polymeric fibers with nano and submicron diameters specifically produced by electrospinning method. Partly successes in using such scaffolds are attributable to structural similarity to the extracellular matrix (ECM), convenient porosity for cellular proliferation, high surface area to volume ratio, reduced immunogenicity and biodegradability among others [1]. After implantation of such scaffolds, in biomechanical environment of the body, however the fibers in the scaffolds are subjected to stresses and strains in physiological conditions, thereby standing a chance to permanent deformation or even failure to scaffold structure. Therefore, there is a pressing need to characterize their mechanical properties, especially at the nanoscale as well as to assess resulting surface properties such as wettability.

1.1 Nano-mechanical properties.

Atomic force microscopy (AFM) in the family of scanning probe microscopy has emerged as a promising tool with a host of powerful techniques that have been employed in imaging of the components of biomaterials scaffolds and probing selected mechanical properties under physiological conditions [2-4]. The PeakForce Quantitative NanoMechanics (PF-QNM) is relatively a new addition to the host of techniques allowing to measure nano-mechanical properties of the materials including reduced Young`s modulus, adhesion, dissipation, and deformation concurrently with topographical height imaging [5].

1.2 Wettability.

Wettability pertains to the interaction between solid and fluid phases at the interface. Strictly the contact angle of the fluid with the solid phase at which the liquid–vapor interface meets the solid–liquid interface, explains the wettability. This in turn is determined by a force balance between adhesive and cohesive forces. An increasing tendency of a drop to spread out over a flat, decreases the contact angle, thereby providing an inverse measure of wettability. A contact angle less than 90° (low contact angle) usually indicates that wetting of the surface is very favorable, and that the surface is hydrophilic while a contact angles greater than 90° (high contact angle) commonly means that wetting of the surface is unfavorable and that the surface is hydrophobic.

2. EXPERIMENTAL PROCEDURE

In the present work two polymers namely Polycaprolactone (PCL) and poly methyl methacrylate (PMMA) were blended in 1:1 ratio to make base nano-composite fibers, produced by electrospinning from solution at applied voltage of 12 kV. Thereafter this was transformed into three different kinds of nanofiber meshes through sputtering of Ag and ZnO in three different ratios viz., Ag:ZnO in a) 30:30, b) 30:100 and c) 100:30. The sputtering was done using RF Sputtering System following an optimized protocol at the nanotechnology central facility of KAU. We used a Bruker Dimension Icon AFM (Bruker Corporation, CA, USA) with ScanAsyst™ to determine modulus of the fibers in the scaffold as well to image simultaneously the surface topographical information as height sensor mages. The recording of all images were performed in the PeakForce QNM (Quantitative NanoMechanics) imaging mode using typical silicon tips (TESPA, spring constant 20-80 N/m, Veeco, Santa Barbara, CA, USA). All imaging were conducted in air under ambient conditions. The information available in AFM force-distance curves are fed to PeakForce QNM mode, and the maximum force by the tip exerted to the sample is constant as shown in Fig. 1.

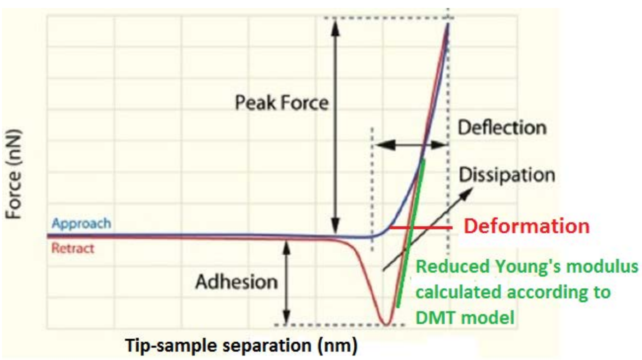

Fig. 1: Schematic of force/distance (FD) curve illustrating the obtainable information. By analyzing FD curve the nano scale mechanical properties can be calculated. Automatic analysis of curve using NanoScope Analysis Software generate maps of mechanical properties distribution concurrently with topographical imaging. (Adapted from [6]).

The corresponding deflection of the cantilever at this maximum force is used to generate topological mapping. The slope of the retraction curve near zero separation yields the stiffness/modulus, and the minima in the retraction curve gives the adhesion pull-off force [7]. The Derjaguin-Muller-Toporov (DMT) model were used to measure the reduced Young`s modulus of the fiber sputtered with inorganic nanoparticles [5] including other mechanical properties of adhesion, deformation and dissipation with high spatial resolution (data not shown). The images were analyzed using the offline AFM software (NanoScope Analysis, version 1.5).

A typical output of the DMT modulus analysis is presented in Fig. 2. DMT modulus Rms values were

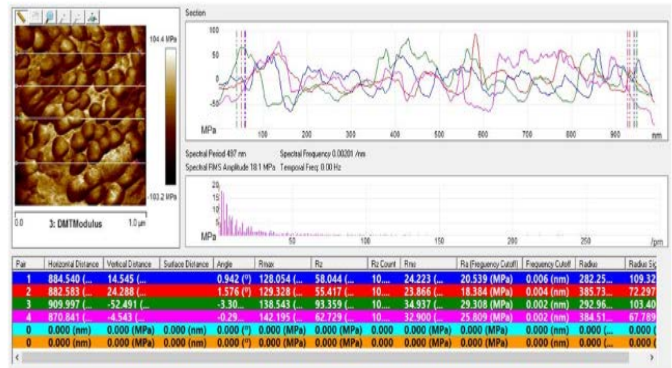

Fig. 2: A typical DMT modulus calculation output of the NanoScope Analysis software. Each DMT modulus map was analyzed at least using three cross sections across the modulus map.

calculated across each line cross section of the DMT modulus map as shown in Fig. 2. At least three such cross sectional values of DMT modulus were averaged across each mapping done on the fibers.

The water contact angle was measured using Attension Optical Tensiometer Theta 200 (Biolin Scientific, Stockholm, Sweden) following a standardized protocol in the biomaterials and tissue engineering laboratory, ECE department at KAU.

3. RESULTS AND DISCUSSION

Fig. 3 shows the micrographs of PCL:PMMA electrospun nanofiber meshes sputtered with three combinations of Ag:ZnO obtained by AFM operating in PF-QNM mode. The high magnification DMT Modulus image (f) of Ag:ZnO combination of 30:100 sputtering show predominantly Ag nanoparticles (relatively smaller sizes) while mage (h) of Ag:ZnO combination of 100:30 sputtering show predominantly ZnO nanoparticles (relatively larger sizes). The later combination shown in (h) of Fig. 3 corresponds to higher values of Young modulus as compared to 100:30 combination of Ag:ZnO as shown in (f) of Fig. 3.

The corresponding height sensor images (larger size) shown in the first column do not have distinct information regarding Ag and ZnO nanoparticle anchored on the fiber surfaces as a result of the sputtering method.

In general DMT modulus values were increased significantly as compared to control base fiber mesh. This fact can be of course explained by inorganic nanoparticle addition to the composite fiber mesh. Comparing between different combinations of ZnO and Ag nanoparticles, mean values of DMT modulus were much higher for ZnO:Ag (100:30) as compared to ZnO:Ag (30:100) combinations (Table 1). This can be attributed to hard nature of ZnO by virtue of which copper is transformed to a harder brass by addition of zinc.

Adhesion force between AFM probe and sample is yet another important quantity that can be calculated from PF-QNM. As cells need to adhere properly to the biomaterial to regenerate new tissue, adhesion remains of utter importance for utilization of electrospun fibers as scaffolds for cells seeding. Our results demonstrated that sputtering of inorganic ZnO and Ag nanoparticles in 30:30, 30:100 and 100:30 combinations caused decreasing adhesion (data not shown) and increasing measured DMT modulus value (Table 1).

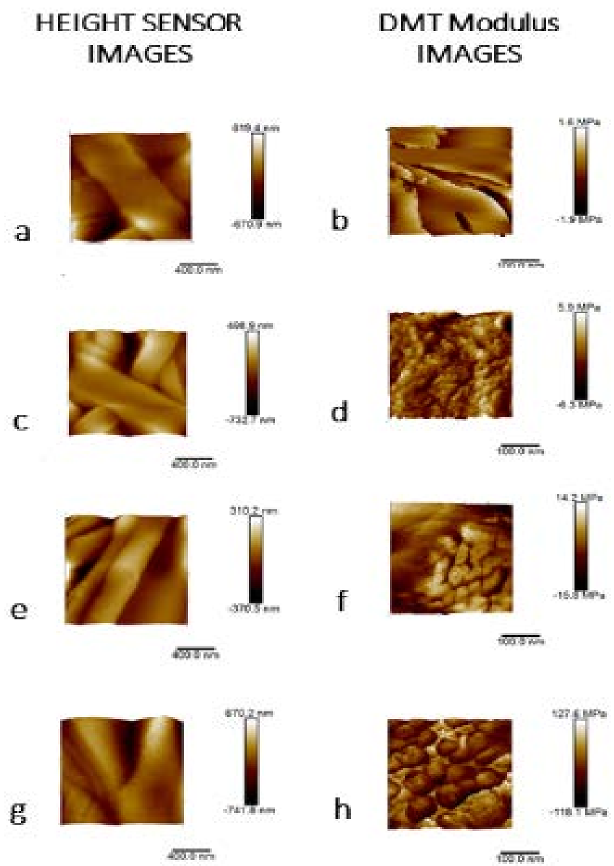

Fig. 3: AFM height sensor images (column-1) and DMT modulus images (column-2). Height sensor images are low magnification images showing no distinct appearance of inorganic nanoparticles sputtered on fiber surfaces. High magnification DMT modulus images of the fiber surface in column-2 clearly shows and identifies Ag and ZnO nanoparticles in micrograph (f) and (h) respectively (Scale bars: column-1 is 400 nm and column-2 is 100 nm).

The water contact angle values doubled (16%) for Ag:ZnO combination of 30:100 as compared to 100:30 combination of Ag:ZnO which increased by 8% from the value exhibited by 30:30 combination of Ag:ZnO. The average values of water contact angles of three different combinations of Ag:ZnO sputtering are shown in the Table 2.

Table 1: Mean DMT Modulus (MPa) of fibers sputtered with three different ZnO:Ag combinations.

Control mesh	ZnO:Ag mesh (30:30)	ZnO:Ag mesh (30:100)	ZnO:Ag mesh (100:30)
0.62 ± 0.26	2.22 ± 0.61	5.62 ± 1.39	63.87 ± 81.82

Table 2. Mean water contact angle on meshes of three different ZnO:Ag combinations.

Control mesh	ZnO:Ag mesh (30:30)	ZnO:Ag mesh (30:100)	ZnO:Ag mesh (100:30)
132 ± 2.0	135 ± 5.1	146 ± 1.4	157 ± 0.58

4. CONCLUSIONS

A concurrent topographical imaging with corresponding nanomechanical probing of biomaterial samples using the PF-QNM is undoubtedly a very promising technique. With the advent of this technique we may claim that the nanomechanical mapping thus achieved, is successful for identifying the composition in biomaterial nano-composite blends. In conclusion, we have obtained the nanomechanical mapping on a polymer blend of PCL/PMMA sputtered with inorganic nanoparticles. With this technique, we can not only measure the Young' s modulus of the PCL/PMMA blend but also map the morphology of the sputtered inorganic components based on Young's modulus of the constituting polymers. For comparison the fibers may be examined by a scanning electron microscope as well as XPS techniques.

5. ACKNOWLEDGEMENTS

This project was supported by the National Science, Technology and Innovation Plan (NSTIP) strategic technologies program in the Kingdom of Saudi Arabia—Project No. 11NAN1544-03. The authors thank the Science and Technology Unit, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, for providing technical support.

REFERENCES

[1] Kijeńska, E., Prabhakaran, M. P., Swieszkowski, W., Kurzydlowski, K. J., Ramakrishna, S., "Electrospun bio-composite P(LLA-CL)/collagen I/collagen III scaffolds for nerve tissue engineering", *Journal of Biomedical Materials Research: Part B – Applied Biomaterials* 100, 2012, pp. 1093-1102.

[2] Wozniak, M.J., Chen, G., Kawazoe, N., and Tateishi, T., "Monitoring of mechanical properties of serially passaged bovine articular chondrocytes by atomic force microscopy", *Micron*, 40, 2009, pp. 870-875.

[3] Wozniak, M. J., Kawazoe, N., Tateishi, T., Chen, G., "Change of the Mechanical Properties of Chondrocytes during Expansion Culture", *Soft Matter*, 6, 2010, pp. 2462-2469.

[4] Roguska, A., Hiromoto, S., Wozniak, M. J., Pisarek, M., Yamamoto, A., "Collagen immobilization on 316L stainless steel surface by applying electrochemical treatment: surface characterization and in-vitro study", *Applied Surface Science*, 257, 2011, pp. 5037–5045.

[5] Chlanda, A., Rebis, J., Kijenska, E., Wozniak, M. J., Rozniatowski, K., Swieszkowski, W., Kurzydlowski, K.J., "Quantitative imaging of electrospun fibers by PeakForce Quantitative NanoMechanics atomic force microscopy using etched scanning probes", *Micron*, 72, 2015, pp.1-7. doi: 10.1016/j.micron.2015.01.005. Epub 2015 Feb 9

[6] Heu, C, Berquand, A, Elie-Caille, C, Nicod, L, "Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells", *J Struct Biol.*,178, 1, 2012, pp.1-7. doi: 10.1016/j.jsb.2012.02.007. Epub 2012 Feb 17.

[7] Cranston, E.D., Eita, M., Johansson, E., Netrval, J., Salajková, M., Arwin, H., Wågberg, L., "Determination of Young's modulus for nanofibrillated cellulose multilayer thin films using buckling mechanics", *Biomacromolecules*, 12, 2011, pp. 961–969.