

Force Measurement Study of Engineered Collagen-Chitosan Scaffold Using Atomic Force Microscopy

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Abstract—The structure and properties of scaffold are important in cell-based tissue engineering, especially the mechanical property. Here, we quantify the dynamic oscillatory mechanical behavior of two kinds of porous collagen/chitosan scaffolds. The Young's Modulus were measured in PBS using Atomic Force Microscopy (AFM)-based nano-indentation in response to an imposed oscillatory deformation as a function of force, which can be converted to Young's Modulus. Collagen/chitosan scaffolds with different ratio (8:2 and 7:3, V/V), which already showed good properties for cell culture, were tested. The Young's Modulus of collagen/chitosan scaffold with ratio 7:3 is bigger than that of 8:2, which is consistent with our expectation. Force curves were obtained first from indentation, and then Young's Modulus was determined using a proper Hertz contact mathematical model. Meanwhile, the mechanical properties of mice pancreas and heart were obtained as controls. The results indicated that AFM-based nano-indentation is a good method for the mechanical property testing of porous scaffold.

Keywords—component; Collagen/Chitosan; Scaffold; Tissue Engineering; AFM; Nano-indentation

I. INTRODUCTION

Cell-based tissue engineering holds great potential for therapies involving regeneration and/or replacement of damaged tissue. Such approaches typically involve seeding cells within scaffolds and subjecting them to stimulatory biochemical and/or mechanical factors in vitro or in vivo to promote the development of engineered tissue. The ultimate goal and challenge is to develop a graft with structural, biochemical and biomechanical properties similar enough to healthy tissue so that upon maturation in vivo it can restore physiological function. To achieve this objective, it is important to know the mechanical property of engineered tissue.

Different tissue has different mechanical property, since the mechanical property is important for different tissue construction, it's necessary to test the mechanical property of scaffold before and after seeding cells. Because the stiffness of engineered tissue cannot be inferred simply from the

concentrations of biopolymers or synthetic polymers used to prepare porous scaffold, hydrogel or other flexible substrates, but needs to be tested directly, since the elasticity is often very strongly dependent on the precise concentration of active crosslinkers, filament length, network architecture, and other factors that vary from one experiment to another. Collagen and chitosan are two natural biomaterials, both of them can be used as scaffold of different engineered tissue construction [1-4], but it seemed it's hard to test the mechanical properties of porous scaffolds, especially the scaffold with cells in the medium.

AFM is an important tool for the investigation of biological processes on the nanometer scale. It can not only be used for imaging the topography of surfaces but also for measuring forces on the molecular level, so it was used as a nano-indenter measuring elastic properties. Recently, Laurel Ng and BoBae Lee reported atomic force microscope (AFM) -based indentation of the newly synthesized cell-associated matrices of individual chondrocytes to quantify their quasi-static mechanical properties [5-6]. This approach provides an advantage over macroscopic testing of the entire tissue-engineered construct, which is complicated by the combined behavior of the cells and scaffold, a significant effect at early times [7].

In this paper, we propose an AFM-based method with which nano-indentation on these porous scaffolds is able to be implemented and their corresponding force curves are obtained. Eventually, the Young's Modulus of the scaffold can be calculated and determined after applying the force curve to a proper Hertz contact mathematical model.

II. EXPERIMENTAL SETUP

A. Fabrication of Porous Scaffolds

Collagen/chitosan porous scaffolds were made according to the previous method [8]. Briefly, 0.5% (w/v) type I collagen solution and 2% chitosan solution were prepared in 0.1M acetic acid. The mixtures were then frozen at -80°C for 2h and then lyophilized for 24h in a freeze dryer (LABCONCO Co., Kansas, MO, USA). The scaffolds were then cross-linked by carbodiimide (EDC)/ methyl ethanesulfonate (MES)/ N-hydroxyl succinimide (NHS). Cross-linked porous scaffolds were freeze-dried again and sterilized with UV. According to the properties of scaffolds we made before, we chose two cross-linked scaffolds 8:2 and 7:3 (collagen:chitosan, v/v) to do

the mechanical strength testing. Both of the scaffolds have suitable pore size, porosity, water content and maybe suitable for the growth of fibroblast and embryonic stem cell.

B. Atomic Force Microscopy

We tested the mechanical strength of scaffold using AFM. Heart and pancreas tissue from mouse were used as controls. Agilent 5500 ILM was used to carry out the experiments. A rotated monolithic silicon probe with a spring constant of 0.2 N/m was used, in both AC imaging mode and the indentation. This probe uses an “on scan angle” symmetric triangle tip to provide a more symmetric representation of features over 200nm and its resonance frequency is 13kHz in air, which will vary accordingly in liquid. The tip radius is about 10nm and its half cone angle is 25°. The indentation was done in liquid environment and Fig. 1 shows the experimental setup.

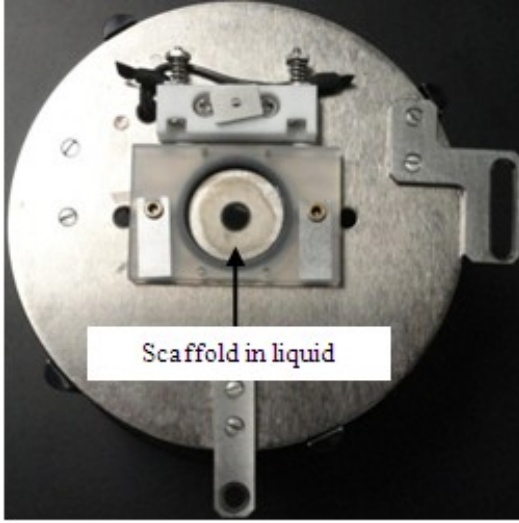


Fig. 1. Liquid cell setup for the indentation of scaffold.

C. Nano-Indentation

The mechanical properties of linear, isotropic, elastic materials considered here may be completely described by two intrinsic parameters, the Young's Modulus E (Pa) and the Poisson's Ratio ν (dimensionless). From AFM-based nano-indentation, we are able to obtain the force (nN) versus indentation distance (nm), which is the pre-condition to calculate the Young's modulus of the sample. Force curves were collected by monitoring the cantilever deflection while ramping the piezo scanner in z direction (vertical), with the x , y scanning disabled, resulting in a plot of force versus sample position. Figure 2 illustrates the schematic of indentation. As we can see, Δz is the piezo-actuator translation under user's control, Δd is the deflection of the cantilever and δ is the indentation distance on the sample. The relationship of these three parameters can be described as in Eq. (1). Furthermore, according to Newton's third law, the magnitude of the force acting on the sample is equal to the force exerting on the cantilever, which is able to be calculated as in Eq. (2). Thus, the force curve can be obtained.

$$\Delta z = \Delta d + \delta \quad (1)$$

$$F = k_c \cdot \Delta d \quad (2)$$

Before plotting the force curve, a calibration is necessary since the AFM software provides us with the amplitude of the cantilever deflection volte. The sensitivity (nm/V) is needed in order to obtain the deflection of the cantilever while indenting the sample. For the calibration, we indented a mica surface with the same tip. The mica surface is hard enough to work as a rigid substrate, which means there will not be a deformation of the surface and the translation distance is equal to the deflection of the cantilever (imagine there is no sample in Fig. 2). Hereby, the sensitivity is able to be determined with this method and it is 89.0nm/V.

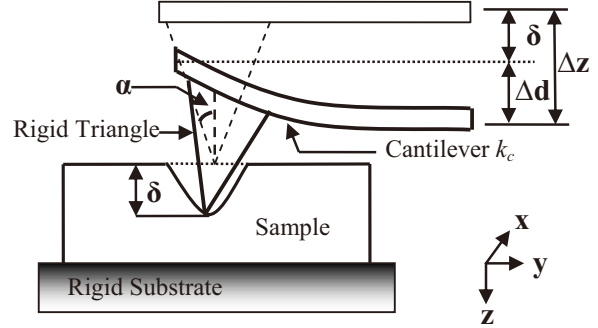


Fig. 2. Schematic of AFM-based nano-indentation: indenting a thin biological sample with a triangle AFM tip.

D. Calculation of Young's Modulus

The slope of a force curve describes the elastic properties of a sample in a qualitative way. On an infinitely stiff sample, the deflection Δd of the cantilever is identical to the movement of the piezo in Δz direction. In the case of a soft sample, the cantilever tip will indent the sample. This indentation distance δ leads to a smaller deflection Δd , resulting in a flatter force curve with a smaller slope. Also the loading force can be determined as in (2) according to Hooke's law.

The elastic deformation of two spherical surfaces touching under load was calculated theoretically in 1882 by H. Hertz. Sneddon extended the calculation to other geometries, like a cone pushing onto a flat sample as used here. We will still call this model a Hertz model, to distinguish it from others, including other effects such as adhesion or plastic deformation. The Hertz model gives the following relation between the indentation δ and the loading force F :

$$F = \left(\frac{2}{\pi}\right) \left[\frac{E}{(1-\nu^2)}\right] \delta^2 \tan(\alpha) \quad (3)$$

where, F is the loading force on the sample, E is the Young's Modulus, ν is the Poisson's Ratio (assumed to be 0.5 [9-11]), δ is the indentation distance, and α is the half-opening angle of the AFM tip ($\alpha = 25^\circ$). Therefore, E can be determined once the force curve is obtained.

III. CURRENT RESULTS

A. AFM Scan

Before the indentation process, the surface information of the scaffold was obtained under liquid environment using AC imaging mode. Scaffold samples were immersed in PBS to simulate the same surroundings as it is inside human body. The topography image obtained during scanning is depicted in Fig. 3 for the engineered Collagen-Chitosan scaffold. The scan is suspended right after it starts and no valuable information about the sample surface was obtained. The reason for this is that the position of the AFM tip varies fiercely than it does for a normal scan because of the liquid environment scanning which affects the stability of the scanning motion. Furthermore, this happens because of the softness of the engineered Collagen-Chitosan scaffold. Additionally, the approach is always hard to complete as the scaffold sample is so soft that it is very unstable while sitting on the sample plate in PBS. A small ladder-like surface would have the tip stuck as the scan range of the AFM scanner is in microns. Despite this particular setback, the nano indentation of the engineered Collagen-Chitosan scaffold will not be affected.

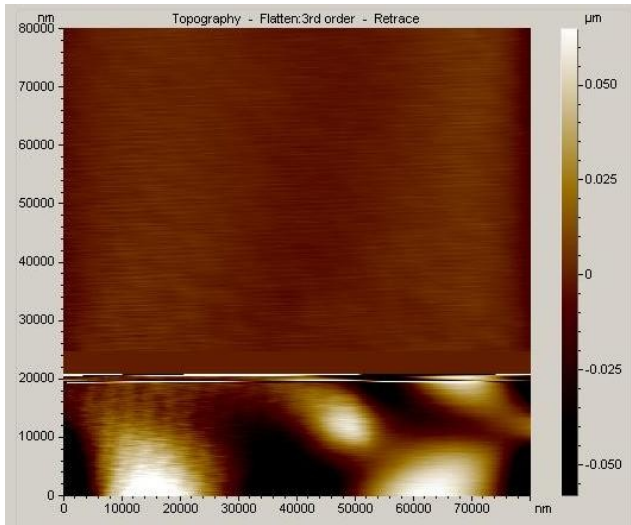


Fig. 3. A topography scan of the scaffold in PBS.

B. Indentation and Force Curve

Indentation tests performed on two kind of scaffold in PBS, three different locations for each scaffold, mice pancreas and heart tissue were used as controls. Nonlinear increase in force with indentation depth was observed, by linear fitting, the slope rate of a curve may reflect the gross stiffness of a sample. From Fig. 4, we can see that the gross stiffness of collagen/chitosan scaffold with volume ratio of 7:3 (scaf2) is bigger than the scaffold with ratio of 8:2 (scaf1), and this result is consistent with our macroscopic testing. Also we can see that the stiffness of heart tissue is much bigger than pancreas as shown in Fig. 5.

C. Calculation of Young's Modulus

The Young's Modulus value (E) was obtained from six force curve measurements, which revealed the average modulus of the sample. The E value of scaffold2 was 11.6kPa,

which is much higher than the E value of scaffold1 (3.69kPa). The E value of tissue samples sacrificed from 3 mice, and six force curves were used to calculate the E value. The same with force curve, the E value of heart tissue (47.4kPa) is much higher than pancreas (8.25kPa), which means the heart tissue is much stiffer than pancreas. Figure 6 and 7 illustrate the calculation results of E for the two kinds of scaffolds, mice pancreas and heart. The results are presented by the mean values of 6 measurements of each scaffold or tissue and the standard deviations are obtained as well.

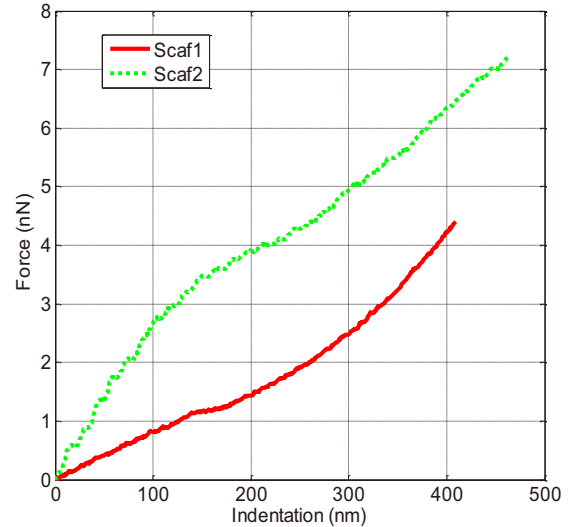


Fig. 4. Force curve comparison between scaffold 1 and 2: from linear curve fitting, the slopes are 0.00974 and 0.01368 respectively.

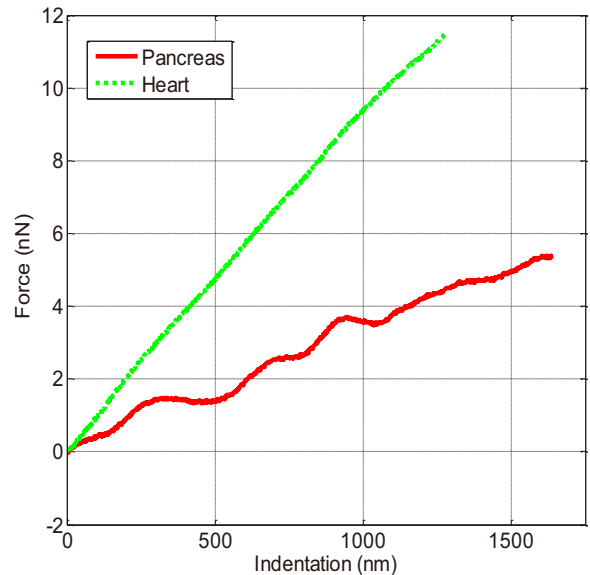


Fig. 5. Force curve comparison between pancreas and heart: from linear curve fitting, the slopes are 0.00329 and 0.00908 respectively.

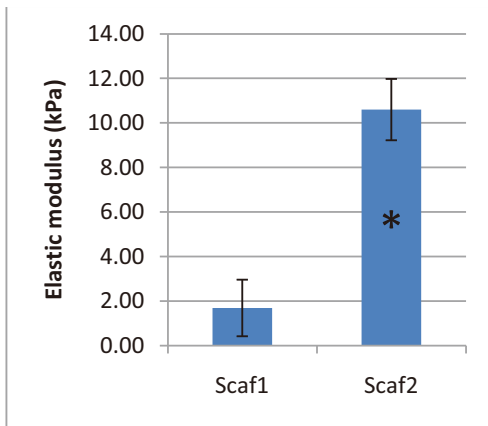


Fig. 6. Average Young's Modulus E of two scaffolds determined by AFM-based nano-indentation. Standard deviation is included.

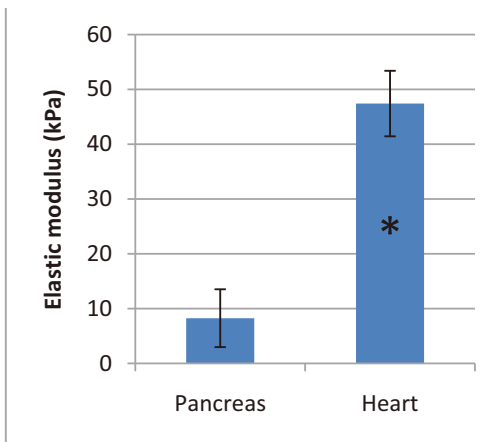


Fig. 7. Average Young's Modulus E of mice pancreas and heart tissue determined by AFM-based nano-indentation. Standard deviation is included.

IV. CONCLUSION

Based on the current results stated in this paper, we may conclude that AFM-based nano-indentation is capable to study the mechanical property of the collagen-chitosan porous scaffolds. Actually, it is impossible for almost every existing tool to measure the elastic property of these extremely thin, light, soft and fragile porous samples in a liquid environment except for AFM. The consistence of the current measurement results with the results of our expectation encourages us. By

comparing the mechanical properties of the scaffolds with the ones of the normal tissue, we also can determine which kind of scaffold is more suitable for some special tissue construction. In the future, the effect of cell-incubation on the mechanical property of the scaffolds will be studied. Furthermore, AFM-based nano-indentation is also capable to characterize the mechanical property of different samples, such as cells and air bubbles. Once the mechanical property of a sample is determined, various applications are able to be realized in many areas including engineering, biology and medicine.

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