Journal of Biomechanics 44 (2011) 2356–2361

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Determination of mechanical properties of soft tissue scaffolds by atomic force microscopy nanoindentation

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ARTICLE INFO

Article history:
Accepted 7 July 2011

Keywords:
AFM
Nanoindentation test
Soft scaffolds
Measurement of mechanical property of scaffolds
3D cultures

ABSTRACT

While the determination of mechanical properties of a hard scaffold is relatively straightforward, the mechanical testing of a soft tissue scaffold poses significant challenges due in part to its fragility. Here, we report a new approach for characterizing the stiffness and elastic modulus of a soft scaffold through atomic force microscopy (AFM) nanoindentation. Using collagen–chitosan hydrogel scaffolds as model soft tissue scaffolds, we demonstrated the feasibility of using AFM nanoindentation to determine a force curve of a soft tissue scaffold. A mathematical model was developed to ascertain the stiffness and elastic modulus of a scaffold from its force curve obtained under different conditions. The elastic modulus of a collagen–chitosan (80%/20%, v/v) scaffold is found to be 3.69 kPa. The scaffold becomes stiffer if it contains more chitosan. The elastic modulus of a scaffold composed of 70% collagen and 30% chitosan is about 11.6 kPa. Furthermore, the stiffness of the scaffold is found to be altered significantly by extracellular matrix deposited from cells that are grown inside the scaffold. The elastic modulus of collagen–chitosan scaffolds increased from 10.5 kPa on day 3 to 63.4 kPa on day 10 when human foreskin fibroblast cells grew inside the scaffolds. Data acquired from these measurements will offer new insights into understanding cell fate regulation induced by physiochemical cues of tissue scaffolds.

1. Introduction

Cells *in vivo* interact with various surrounding cells through cell–cell and cell-extracellular matrix (ECM) interaction in a three dimensional (3D) fashion. It has become clear recently that cell fate is regulated by not only soluble signaling molecules but also by physicochemical cues such as mechanical properties of an ECM. Thus, the determination of mechanical properties of an *in vitro* constructed ECM, i.e., a tissue scaffold, becomes more critical for controlling cell growth and differentiation in 3D environments. A variety of biomaterials have been developed and adopted for fabricating 3D scaffolds. Collagen and chitosan are two widely used natural biomaterials for 3D cultures (Chen et al., 2006; Ma et al., 2003; Yan et al., 2006; Zhang et al., 2006). Both materials can be readily cross-linked to form hydrogels for growing soft tissues (von Heimburg et al., 2001; Wang and Ye, 2009; Zustiak and Leach, 2010). Compared to scaffolds made from hard materials, cells grown inside soft scaffolds respond more significantly to scaffold's mechanical properties (Discher et al., 2009; Engler et al., 2006; Georges and Janmey, 2005; Levental et al., 2007). Besides, the elasticity of the soft scaffolds can be altered by cells through their secreted ECM (Mammoto et al., 2009). Studies suggest that cells can alter substrate's stiffness hundreds of micrometers away from their edges (Winer et al., 2009). The traction forces that cells apply to their matrix can also refashion the matrix stiffness of a hydrogel scaffold that exhibits strain-stiffening behaviors.

Unlike hard scaffolds, the mechanical properties of soft scaffolds are difficult to be characterized due to its fragility. They usually can only tolerate nN stress, making it difficult to measure. One solution is to determine their elastic modulus through indentation. Nanoindentation has been applied for characterizing many soft materials' mechanical properties (Doube et al., 2010; Isaksson et al., 2010). In nanoindentation test, small loads and a small tip can be used with an AFM (Barone et al., 2010). AFM can measure forces at the nN level (Chowdhury and Laugier, 2004; Clifford and Seah, 2006; Darling et al., 2007). For example, AFM nanoindentation has been applied to quantify quasi-static mechanical properties of newly synthesized cell-associated matrices of individual chondrocytes (Lee et al., 2010; Ng et al., 2007). Although AFM nanoindentation shows tremendous potentials for characterizing soft tissues, its application in determining mechanical properties of a hydrogel scaffold in liquid has not yet been explored. Here, we present a new approach of quantifying tensile strength of a soft tissue scaffold using AFM nanoindentation. A mathematical model was developed and used for determining the stiffness and elastic modulus of collagen–chitosan hydrogel scaffolds under various conditions.

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doi:10.1016/j.jbiomech.2011.07.010