FIBRONECTIN POLYMERIZATION ENHANCES THE TENSILE MECHANICAL PROPERTIES OF CELL-IMBEDDED COLLAGEN GELS

Candace D. Gildner (1), Denise C. Hocking (1, 2)

(1) Department of Biomedical Engineering
University of Rochester
Rochester, NY

(2) Department of Pharmacology and Physiology
University of Rochester
Rochester, NY

ABSTRACT

The polymerization of fibronectin (FN) into the extracellular matrix (ECM) increases actin organization and regulates the composition of the ECM. Previous studies have shown that both the ECM and the actin cytoskeleton contribute to the mechanical properties of collagen-based tissues. In the present study, we performed uniaxial tension tests to determine the contribution of FN polymerization to the tensile mechanical properties of collagen-based tissue constructs. Our data indicate that FN polymerization specifically induced a 5-fold increase in the tensile ultimate strength and a 13-fold increase in the toughness of collagen biogels. In contrast, FN polymerization did not affect the tensile moduli of these biogels. The actin cytoskeleton was required to initiate FN-induced changes in the mechanical properties of collagen biogels. However, once FN-induced contraction occurred, the actin cytoskelecton did not contribute to the mechanical properties. These data indicate that the mechanical properties of collagen biogels can be enhanced by the cell-mediated polymerization of a FN matrix.

INTRODUCTION

Tissue development depends on dynamic interactions between cells and their ECM. The ECM is a fluid-filled network composed of collagens, proteoglycans, and glycoproteins [1]. Transmembrane integrin receptors mechanically couple the ECM to the actin cytoskeleton [1]. Both the ECM and the actin cytoskeleton contribute to the mechanical properties of tissues [2]. In turn, the mechanical properties of load-bearing tissues, such as blood vessels and ligaments, influence their functionality [3]. A current shortage of natural replacements for load-bearing tissue has created a demand for artificial tissues that can withstand in vivo mechanical forces.

The contributions that individual components of the ECM make to the mechanical properties of tissues are not known. FN is a glycoprotein that exists in a soluble form that circulates in the plasma and in an insoluble form that localizes to the ECM [1]. Soluble FN is incorporated into the ECM through a highly regulated cell-mediated process, termed FN matrix polymerization [1]. In its ECM form, FN increases cell contractility [4], influences the structure of the actin cytoskeleton [4], and regulates the deposition of ECM proteins [5]. Collagen-based tissue constructs have the potential to serve as replacements for load-bearing tissue. The purpose of this study was to determine the effect of FN matrix polymerization on the tensile mechanical properties of cell-imbedded collagen gels. In addition, the role of the actin cytoskeleton in the initiation of FN-induced changes in the mechanical properties and its contribution to the mechanical properties of contracted cell-imbedded collagen gels were assessed.

MATERIALS AND METHODS

Mouse embryonic FN-null cells [5] were imbedded in type I collagen gels (0.8 mg/ml) as previously described [4]. FN (10 - 80 nM), laminin (40 nM), fibrinogen (40 nM), or an equal volume of PBS were then added to the collagen/cell solution. To form rings (I.D. 25.4 mm, O.D. 35 mm), the neutralized collagen solution was polymerized at 22°C around a central mandrel. At 1 h, the mandrel was removed, serum-free media was added, and dishes were scored along their inner edge to insure that gels were free floating. After a 20 h incubation at 37°C/8% CO_2, gels were removed from their dishes and looped around BSA-coated nylon grips attached to an MTS Q-test 5 machine. A uniaxial tension test was then performed at a constant speed of 1 mm/min (~2-3% initial strain). The load was recorded using a 5 N load cell (MTS). During testing, gels were hydrated with DMEM/Hepes. The initial cross-sectional area and length of each gel were determined from front-view digital images and were used to calculate the engineering stress and strain. The ultimate strength was defined as the maximum stress that a gel withstood prior to failure. The toughness was calculated using the trapezoid rule. The instantaneous modulus was linearly dependent on the stress [6], and was obtained by fitting stress-strain data to the non-linear solution of the differential equation [6].

Figure 1: Collagen biogel on the mechanical testing apparatus. Bar = 10 mm.

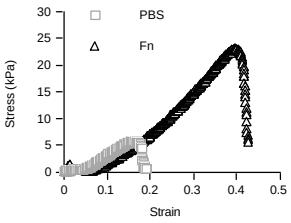

Figure 2: Stress-strain relationship for biogels incubated in the absence or presence of FN (40 nM)

RESULTS

A representative stress-strain relationship of FN-treated biogels is shown in Fig. 2. The addition of FN to cell-imbedded collagen gels induced a significant dose-dependent increase in their ultimate strength and toughness. Biogels treated with 80 nM FN had an ultimate strength of 25.7 kPa and a toughness of 4.64 kJ/m^3. The moduli of FN-treated biogels were not significantly different from controls. The addition of laminin or fibrinogen did not increase the ultimate strength or toughness of collagen biogels. Moreover, the inhibition of FN polymerization with the FN-specific monoclonal antibody 9D2 [7] prevented the FN-induced increase in the mechanical properties of biogels (Fig. 3). These data indicate that FN polymerization into the ECM specifically increases the mechanical properties of collagen biogels.

To determine the role of the actin cytoskeleton in the initiation of FN-induced increases in the mechanical properties, biogels were treated with both FN (40 nM) and 2 μM cytochalasin D (CD), an inhibitor of actin polymerization, prior to contraction. These biogels did not demonstrate FN-induced increases in their mechanical properties (Fig. 4). In contrast, when contracted biogels were treated with 10 μM CD for 2 h prior to mechanical testing, their ultimate strength and toughness were comparable to FN-treated biogels (Fig 4). These data indicate that the actin cytoskeleton is required to initiate FN-induced changes in the mechanical properties of biogels. However, once FN-induced contraction has occurred, the actin cytoskeleton does not contribute to the mechanical properties of these biogels.

DISCUSSION

Our data indicate that the polymerization of FN into the ECM increases the ultimate strength and toughness of collagen biogels. Previous studies have shown that FN polymerization increases cell-generated tension and actin organization [4]. Furthermore, studies have correlated biogel contraction with increases in the stiffness of the actin cytoskeleton [8]. Together, these data suggest that FN-induced changes in the actin cytoskeleton may contribute to increases in the mechanical properties of biogels. However, contracted biogels lacking a functional actin cytoskeleton demonstrate a FN-induced increase in their mechanical properties. These data suggest that, once organized, the FN matrix, and not the actin cytoskeleton, is a primary contributor to the mechanical properties of contracted collagen biogels. Previous studies have shown that FN polymerization regulates the deposition and maintenance of collagen I into the ECM [5]. Other studies suggest that collagen fibril alignment influences the mechanical properties of collagen gels [9]. As such, the FN matrix may influence the mechanical properties of biogels by regulating collagen deposition and organization.

CONCLUSIONS

In summary, the cell-mediated polymerization of an FN matrix

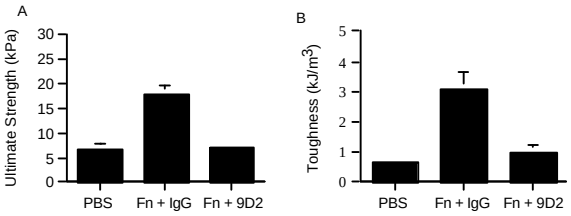

Figure 3: Effect of FN matrix polymerization on the (A) ultimate strength and (B) toughness of biogels.

stimulates an FN-specific dose-dependent increase in the ultimate strength and toughness of cell-imbedded collagen gels. The actin cytoskeleton is required to initiate FN-mediated changes in the mechanical properties of biogels. However, once FN-induced contraction occurs, the actin cytoskeleton does not contribute to the mechanical properties of collagen biogels.

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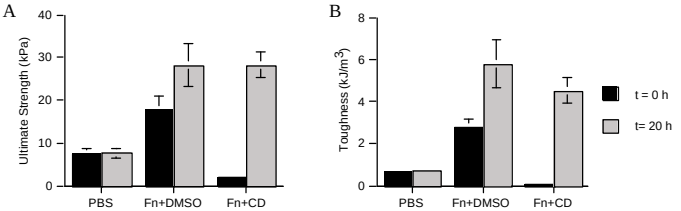

Figure 4: Effect of actin cytoskeleton on the (A) ultimate strength and (B) toughness; t = time CD added