EXTENDED TIMOSHENKO BEAM FORMULA FOR CELLULAR CONTRACTION FORCE CALCULATION

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ABSTRACT

The inherent viscoelastic properties and low aspect ratio of micro-fabricated PDMS micropillars compromise the accuracy of these devices to measure the cellular contraction forces. In our previous work, these issues have been studied by finite element analysis. However, a more in-depth analytical formula is still lacking. In this paper, an extended viscoelastic Timoshenko beam formula was developed and validated by micro-beam bending tests. In addition a case study of the contraction force of cardiac myocytes was performed, and the formula showed excellent accuracy comparing to the traditional elastic Euler beam formula.

KEYWORDS: Polydimethylsiloxane, Timoshenko Beam, Viscoelastic, Aspect Ratio, Cellular Force, Cardiac Myocytes.

INTRODUCTION

Polydimethylsiloxane (PDMS) based micropillars have been used as bio-transducers for measuring the cellular forces [1,2]. The accuracy of these sensitive devices depends on appropriate modeling to convert the micropillar deformations into the corresponding reaction forces. The traditional approach to calculating the reaction force is based on the Euler beam theory with the consideration of a linear elastic slender beam for the micropillar. However, the low aspect ratio in geometry of PDMS micropillars does not satisfy the slender beam requirement. In addition, the inherently time-dependent behavior in PDMS has to be considered for accurate force conversion. Although these issues have been discussed using finite element analysis in previous paper [3], we herein introduced a more in-depth analytical formula, namely the extended viscoelastic Timoshenko beam formula. The formula was validated by the micro-beam bending test, and a case study of the contraction force of cardiac myocytes was also performed.

THEORY

The viscoelastic property of PDMS was represented by the Young's relaxation modulus $E(t)$, which was obtained by a stress relaxation test on the PDMS film and expressed in a general Maxwell model

$$E(t)=0.7467 + 0.0713e^{-t} + 0.0257\, e^{-0.1t} + 0.0315\, e^{-0.01t} \quad (1)$$

The force-displacement conversion formula was based on the Timoshenko beam formula [4]. The low aspect ratio of PDMS micropillars was considered by a shear coefficient α, and the Young's relaxation modulus was also integrated into the formula. The reaction force P can be expressed as

$$P(t) = \frac{3IAv_0}{L[AL^2 + 6\alpha I(1+\nu)]}[E_\infty t + \sum_{i=1}^{N} \frac{E_i}{\lambda_i}(1 - e^{-\lambda_i t})] \quad (2)$$

where v_0 is the loading rate, I is the moment of inertia, A is the cross section area, L is the length, and v is the Poisson's ratio. The detailed derivations can be found in our recent publication [5].

EXPERIMENTAL

The PDMS micropillars were fabricated by soft lithography, replicating from a patterned SU-8 mode. The PDMS were prepared by mixing the prepolymer Sylgard 184 (Dow Corning) with the curing agent at a volume ratio of 10:1, and cured at 65 °C for 90 min. Upon peeling off from the mold, PDMS micropillars were formed (SEM micrographs are shown in Figure Figure 1 (a)).

The micro-beam bending test was conducted on a TI 900 Triboindenter (Hysitron). The micropillars were mounted horizontally on a nanoindenter holder and indented vertically by the wedge indenter tip (Figure 1(b)). The initial indenter tip position along the longitudinal axis was set to 160 μm (measured from the bottom substrate, which was also the fixed end of the micro-beam under bending) by monitoring the optical micrograph of the nanoindenter. The maximum tip displacement of 2.5 μm attained with three loading rates, namely 250, 500 and 1000 nm/s, was used in the tests.

The microchip containing PDMS micropillars was pre-coated with 15mM laminin to enhance cellular adhesion prior to plating the cells. The laminin solution was then removed and the cells were plated on the PDMS substrate for 2 h to allow cell attachment. After that the chip was gently rinsed with fresh culture media to remove the unattached floating cells. The cells were incubated in 5% CO_2 at 37.2ºC, and the culture media was changed every 72 h. Cell contractility was stimulated with Isoproperno perfusion and images were captured using a Cool SNAP EZ CCD Camera. The locations of the centroids, or centers of each pillar, were recorded on a frame-by-frame basis and used to determine the displacement of each pillar over time.

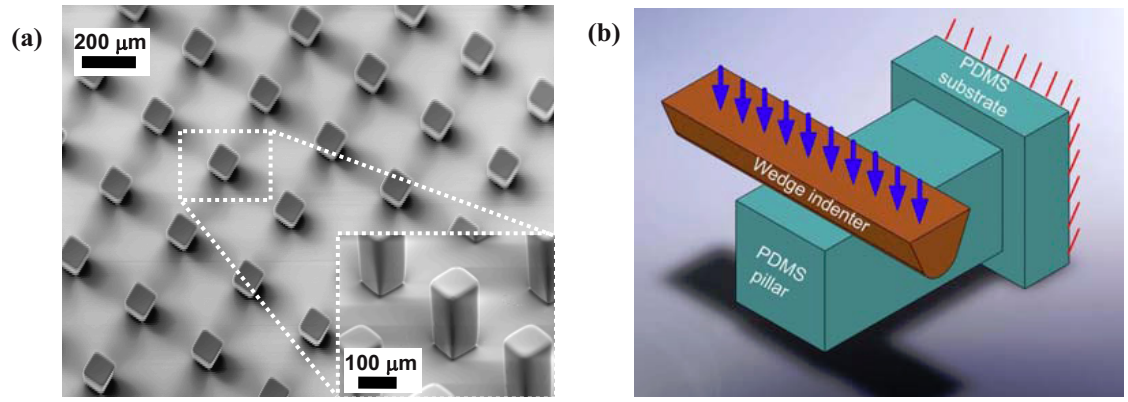

Figure 1: *(a) The SEM micrographs of PDMS micropillars with a length of 200 μm and a square cross section of 100 x 100 μm^2. (b) Schematic diagram of the PDMS micropillar indented by a wedge indenter tip (The PDMS substrate was fixed to the sample holder and a uniform vertical load was applied by the indenter).*

RESULTS AND DISCUSSION

The viscoelastic Timoshenko beam formula was validated by the micro-beam bending tests. The accuracy of the micro-beam bending model depends primarily on two aspects: the geometry (aspect ratio of a micropillar) and the material constitutive law (elastic or viscoelastic). To evaluate the accuracy of our viscoelastic Timoshenko beam model, three more models were constructed based on the combinations of these two aspects. For the first aspect, the Euler and Timoshenko beam models were compared at a fixed loading rate. For the second aspect, the elastic and viscoelastic models were compared at different loading rates.

As shown in Figure 2(a), both the two Euler beam formulas overestimate the forces by ~30 %, while the other two Timoshenko formulas agree well with the experimental data. In addition, the elastic and viscoelastic Timoshenko beam formulas were compared with the experimental data at three different loading rates. As shown in Figure 2(b), the elastic formula tends to underestimate the forces, while the viscoelastic formula essentially agrees with the experimental data, and it becomes more accurate at higher loading rates.

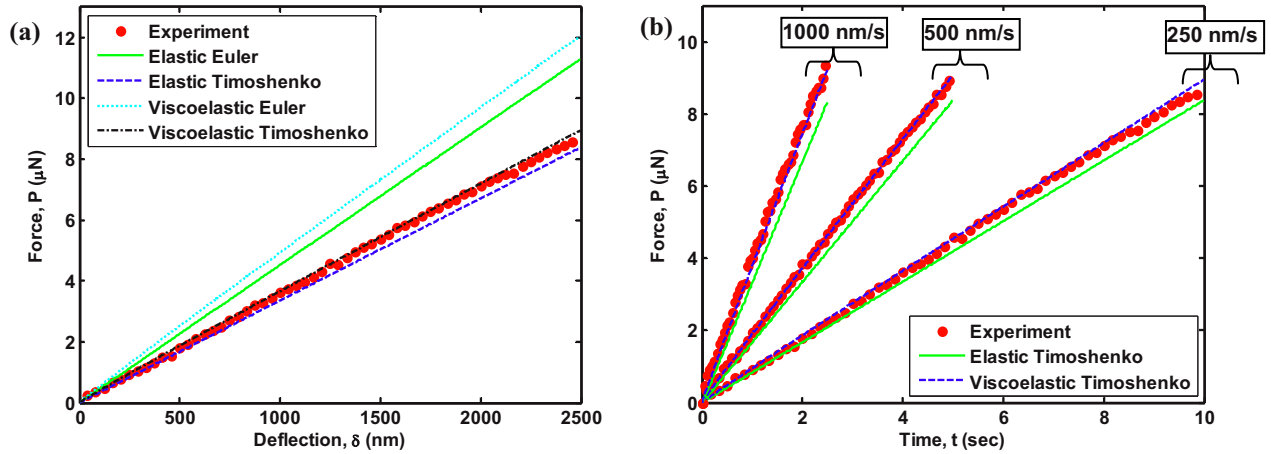

Figure 2: *(a) The reaction forces of PDMS micropillars from the experimental data, elastic Euler, elastic Timoshenko, viscoelastic Euler and viscoelastic Timoshenko beam formulas at a loading rate of 250 nm/s. (b) The reaction forces of PDMS micropillars from the experimental data, elastic Timoshenko and viscoelastic Timoshenko beam formulas at three loading rates.*

In the case study of cardiac myocytes, the micropillar displacements induced by the cardiac myocytes contraction show regularly cyclic behavior (Figure 3(a)). The average maximum displacement was 0.036 μm, and the contraction frequency was 10.8 Hz. The contraction force was calculated by our formula and expressed in stress. The maximum contraction stress from our formula is 172.6 Pa, while that from the elastic Euler beam formula is 162.8 Pa. Although the difference is relative small (5.6 %) due to the specific micropillar dimension and cellular contraction rate, a parameter study (Figure 3(b)) shows that the difference between these two formulas can vary for a large range (-12 ~ 30 %), which depends on the micropillar dimension and loading rate.

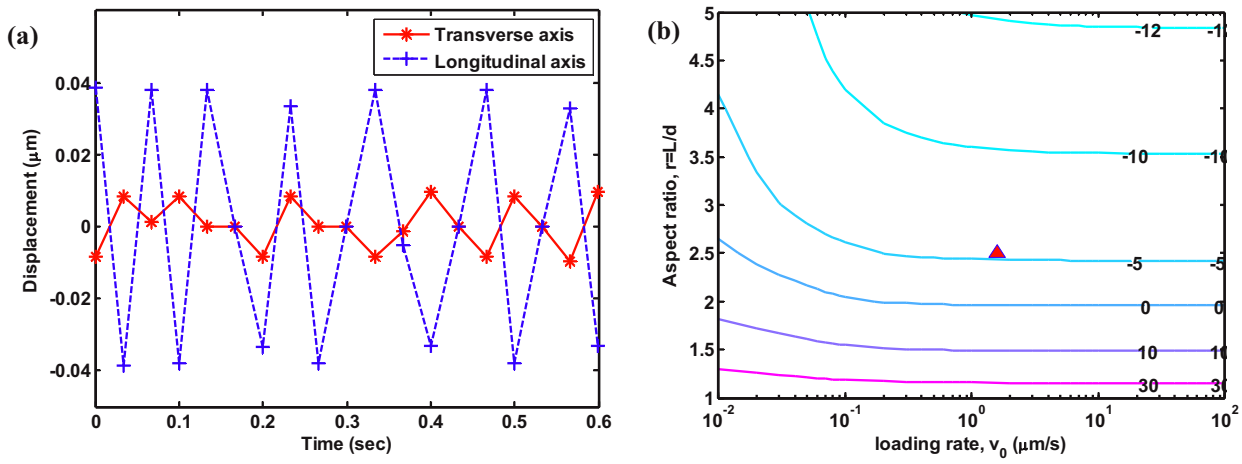

Figure 3: *(a) The micropillar displacements by the cardiac myocyte contraction along the longitudinal axis (contraction axis) and transverse axis. (The micropillar was 5 μm tall with a circular cross section of 2 μm diameter.) (b) The difference between force predictions from elastic Euler beam and viscoelastic Timoshenko beam formulas at various loading rate v_0 and micropillar aspect ratio r (length L divided by diameter d). The difference for the case in this work is plotted by a red triangle.*

CONCLUSION

To the best of our knowledge, this work is the first to develop an analytical formula for viscoelastic bending behavior on polymer micropillars for the force measurement, with the consideration of the loading rate dependent modulus and the shear deformation due to the low aspect ratio. The technique allows biologists to measure cellular forces with much improved accuracy. It can also be used as a general guideline for the design of other cantilever-based soft polymer bio-transducers at micro- or nano-scales.

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