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CELLULAR STIFFNESS MEASUREMENT FOR 3D BIOLOGICAL PRINTING

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ABSTRACT: During 3D biological printing, cells can sense their environment and change their own properties accordingly. In order to understand how cells modulate their stiffness with respect to their environmental stiffness, micropipette aspiration method was used to measure the aspiration lengths of porcine mesenchymal stem cells (pMSCs), which were cultured on polydimethylsiloxane (PDMS) substrates with different stiffness for different time periods, under certain pressure. After the measurements, both elastic and viscoelastic models were used to analyze the elasticity of the cell. Clear relationship between PDMS stiffness and cell stiffness could not be obtained with elastic model. However, from viscoelastic model, it gives that cells cultured on softest PDMS had the largest elastic modulus while on stiffest PDMS had lowest elastic modulus.

INTRODUCTION

There have been many studies on the mechanical properties of single cell as it is able to provide much useful information for the benefits of the medical industry (Bao & Suresh 2003; Khani et al. 2013; Shojaei-Baghini et al. 2013) as well as 3D cell printing process (Mironov et al. 2003; Fedorovich et al. 2011; Othon et al. 2008). One of the studies included the investigation of the mechanism and development of a disease by the stiffness of the cell (Shojaei-Baghini et al. 2013) as a healthy cell will have a different stiffness from the unhealthy one. Also, by altering the stiffness of cell would enable the regeneration of tissues and can serve to help increase the success rate of organ printing which can be used to replace failed organs (Mironov et al. 2003).

Many techniques have been developed to study single cell mechanics, such as atom force microscopy method (Radmacher et al. 1996) and optical tweezers method (Dai & Sheetz 1995), among which micropipette aspiration is a quite accurate and broad used one. In micropipette aspiration, mechanic properties of cells can be obtained through model fitting of aspiration lengths with time (Lim et al. 2006). Previous studies have shown that the stiffness of cells can be changed by changing the stiffness of the substrates (Tee et al. 2011; Vichare et al. 2014; Discher et al. 2005). However, the precise relationship between the substrate stiffness and cultured cell stiffness is still not very well understood. The goal of this project is to find out the stiffness of the cell in different substrate stiffness and how the period of time the cell is cultured on the substrate will affect the stiffness of the cell. For this study, porcine Mesenchymal Stem Cells (pMSCs) were used for investigating the mechanical properties and polydimethylsiloxane (PDMS) was used as the substrate. Micropipette aspiration method was applied to measure the cell properties. And a total of 7 culturing days of the cells on the substrate were studied.

MATERIALS AND METHODS

Preparing of Substrate

PDMS substrates with three different stiffness (base to cross linking agent ration of 5:1, 10:1, and 20:1) were mixed in petri dishes respectively. They will be named as PDMS 5:1, PDMS 10:1 and PDMS 20:1 here after. Mixture with lesser amount of cross linking agent is softer. Hence, PDMS 20:1 substrate was the softest and PDMS 5:1 was the hardest. After well mixing, they were placed in a vacuum to vacuum out any gases present in the mixtures. When there were no more bubbles present, which generally took about 20 to 30 minutes, the samples were placed in an oven at temperature of 70-80 °C for curing. The samples were then ready for cell culture.

Cell Culture

Porcine Mesenchymal Stem Cells (pMSCs) were cultured on the PDMS prepared. Dulbecco's Modified Eagle Medium (DMEM) was used as the medium for culturing. It took six steps to prepare the cultured cells ready for MA measurement: (1) rinse the culture flask with Phosphate Buffer Solution (PBS); (2) add 1ml of enzyme (Tryple E) into the flask, shake the flask gently, and leave it at 37°C for about 2 minutes for it to react with the surface; (3) pour the enzyme away and add 3ml of DMEM into a tube; (4) centrifuge the tube for 5 minutes to precipitate the cell; (5) pour away the DMEM and leave only the precipitate; (6) add in 5ml of fresh DMEM to the precipitate and mix them well. In this report, the properties of pMSCs were studied after culturing on PDMS for 1 day, 5 days, and 7 days.

Micropipette Aspiration

Micropipettes with inner diameters of 0.2-0.8 times of the cell diameter were fabricated using a puller (P-97 Flaming/Brown Micropipette Puller from Sutter Instruments) from borosilicate glass capillary tubes with 1mm outer diameter and 0.5mm inner diameter (Sutter Instruments). These pipettes were used to apply negative pressure on the cell, which could lead to the aspiration of cell into the micropipette. All the experiments were done under a microscope (Axiovert 40 MAT, Carl Zeiss) and images were taken with a high speed camera (AxioCam, Carl Zeiss) to measure the aspiration length. Except aspiration length, the negative pressure applied and the time taken for the deformation were also obtained and recorded. This information was then used to analyze cell mechanical properties with appropriate models.

Theoretical Modeling

pMSC as reported earlier is a solid-like material with viscoelastic properties (Tan et al. 2008; Pan et al. 2005). Hence, solid models were used. There are two kinds of models can be applied to pMSCs, elastic model and viscoelastic model. The elastic model is a simplified version of the viscoelastic model with which time factor is neglected. Usually, it is insufficient to describe the mechanical properties using only the elasticity model; viscoelastic model would be more accurate as it takes into consideration of loading history and loading rate which is important for cell study (Lim et al. 2006). Here, both models were used for the analysis.

For elastic model, the elasticity of the cell can be calculated using the following equations (Shojaei-Baghini et al. 2013):

$$E = \Phi \left(\frac{3R_p}{2\pi} \right) \left(\frac{\Delta p}{L_a} \right) \quad (1)$$

Where E is young's modulus, Φ is a dimensionless constant, taken to be 2.0-2.1 when the ratio of pipette wall thickness to radius is 20%-100%, R_p is pipette radius, L_a is equilibrium length, ΔP is aspiration pressure.

For viscoelastic model, the viscoelasticity of cell can be obtained from fitting the data into the following formula (Khani et al. 2013):

$$L(t) = \frac{\Phi R_p \Delta P}{\pi k_1} \left[1 - \frac{k_2}{k_1 + k_2} e^{-t/\tau} \right] \quad (2)$$

Where k_1, k_2 are elastic constants, and τ is time constant. Fitting results of k_1, k_2 and τ can be used to calculate the instantaneous (E_0) and equilibrium (E_{equi}) elasticity and the viscosity (μ) respectively (Pan et al. 2005) as following:

$$E_0 = \frac{1}{2}(k_1 + k_2) \quad (3)$$

$$E_{equi} = \frac{1}{2}k_1 \quad (4)$$

$$\mu = \frac{\tau k_1 k_2}{(k_1 + k_2)} \quad (5)$$

RESULTS AND DISCUSSION

Elastic Model

Results of the aspirated pMSCs are shown in Figure 1. Three cells of each condition (different PDMS stiffness and number of days cultured) were aspirated. For the analysis of elasticity using the linear elastic model, Eq. (1) mentioned earlier was analyzed in this section.

First, for cell on PDMS 5:1, it is found that the elastic modulus of the cell on day 1 was the smallest, having 34 ± 14 Pa. Later, on day 5, the elastic modulus increased to 223 ± 189 Pa and it remained about the same on day 7. This also implies that the cell treated with PDMS of ratio 5:1 got stiffer as the day passed. While for cell treated on PDMS of ratio 10:1, the stiffness of the cell got stiffer on day 5 but became less stiff on day 7. It is found that on day 1, the elastic modulus of the cell was 114 ± 58 Pa and on day 5 it increased to 198 ± 139 Pa. however, on day 7, the cell became softer, having elastic modulus of 88 ± 41 Pa, which was the softest among the three days. The elastic modulus of cell cultured on PDMS 20:1 showed a similar trend as of the cell cultured on PDMS 10:1. From day 1 to day 5, the elastic modulus increased from 39 ± 16 Pa to 174 ± 140 Pa. however, from day 5 to day 7, the elastic modulus decreased to 90 ± 54 Pa.

Figure1. (a) Average trend of stiffness VS PDMS stiffness for different day. (b) Average trend of stiffness VS time for different PDMS stiffness

Viscoelastic Model

Typical viscoelastic behavior of the solid-like cell should be one that the aspirated length of the cell can reach to equilibrium after certain time period; further aspiration will not increase the length of the aspiration as shown in Figure 2.

Figure 2. Typical aspiration length VS time of a viscoelastic cell

The viscoelastic model, Eq. (2) mentioned above, was used to fit experimental data and the elastic constant, k_1 and k_2 , and time constant τ were obtained. Instantaneous and equilibrium elasticity as well as the viscosity were calculated using Eq. (3) to Eq. (4).

Figure 3. (a) Trend of the instantaneous elastic modulus and (b) equilibrium elastic modulus VS time for different PDMS stiffness

It can be seen from Figure 3 that both the instantaneous and equilibrium elastic modulus show an increasing trend in general as time passing by, which could be due to the time period of cell culture. The longer the cell is attached on the PDMS, the stiffer the cell is.

Figure 4 shows the cell elastic modulus change with different PDMS stiffness. From the figure, it can be seen that cell on PDMS 20:1 had higher elastic modulus hence stiffest; while cell on PDMS 5:1 had a relatively lower elastic modulus hence softest. Based on these observations, conclusion can be made that softer substrate resulted in stiffening of pMSCs, which is quite different from the previous studies where stiffer cells were found on stiffer substrate (Tee et al. 2011). Opposite results might be due to the facts that different cells and different substrates were used in their studies.

Figure 4. (a) Trend of the instantaneous elastic modulus and (b) the equilibrium elastic modulus VS PDMS stiffness for different times

CONCLUSION

The stiffness of polydimethylsiloxane (PDMS) does affect the elasticity of porcine Mesenchymal Stem Cells (pMSCs). From the elastic model, no clear trend can be observed on how the substrate stiffness changes the elasticity of the cells. However, from the viscoelastic model, it shows that day 7 will yield the stiffest cell, and softer substrate will result in stiffer pMSC and vice versa.

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REFERENCES

- Bao, G. & Suresh, S. (2003). Cell and molecular mechanics of biological materials. *Nature materials*, 2(11), pp.715–25.
- Dai, J. & Sheetz, M.P. (1995). Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers. *Biophysical journal*, 68(3), pp.988–96.
- Discher, D.E., Janmey, P. & Wang, Y.-L. (2005). Tissue cells feel and respond to the stiffness of their substrate. *Science (New York, N.Y.)*, 310(5751), pp.1139–43.
- Fedorovich, N.E. et al. (2011). Organ printing: the future of bone regeneration? *Trends in biotechnology*, 29(12), pp.601–6.
- Khani, M.-M. et al. (2013). Evaluation of Mechanical Properties of Human Mesenchymal Stem Cells During Differentiation to Smooth Muscle Cells. *Annals of biomedical engineering*.
- Lim, C.T., Zhou, E.H. & Quek, S.T., 2006. Mechanical models for living cells—a review. *Journal of biomechanics*, 39(2), pp.195–216.
- Mironov, V. et al. (2003). Organ printing: computer-aided jet-based 3D tissue engineering. *Trends in biotechnology*, 21(4), pp.157–61.
- Othon, C.M. et al. (2008). Single-cell printing to form three-dimensional lines of olfactory ensheathing cells. *Biomedical materials (Bristol, England)*, 3(3), p.034101.
- Pan, W. et al. (2005). Viscoelastic properties of human mesenchymal stem cells. *Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference*, 5, pp.4854–7.
- Radmacher, M. et al. (1996). Measuring the viscoelastic properties of human platelets with the atomic force microscope. *Biophysical Journal*, 70(1), pp.556–567.

- Shojaei-Baghini, E., Zheng, Y. & Sun, Y. (2013). Automated micropipette aspiration of single cells. *Annals of biomedical engineering*, 41(6), pp.1208–16.
- Tan, S.C.W. et al., 2008. Viscoelastic behaviour of human mesenchymal stem cells. *BMC cell biology*, 9, p.40.
- Tee, S.-Y. et al. (2011). Cell shape and substrate rigidity both regulate cell stiffness. *Biophysical journal*, 100(5), pp.L25–7.
- Vichare, S., Sen, S. & Inamdar, M.M. (2014). Cellular mechanoadaptation to substrate mechanical properties: contributions of substrate stiffness and thickness to cell stiffness measurements using AFM. *Soft matter*, 10(8), pp.1174–81.