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ADVANCES IN EXPERIMENTAL APPROACHES FOR INVESTIGATING CELL AGGREGATE MECHANICS†

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ABSTRACT Cells tend to form hierarchy structures in native tissues. Formation of cell aggregates *in vitro* such as cancer spheroids and embryonic bodies provides a unique means to study the mechanical properties and biological behaviors/functions of their counterparts *in vivo*. In this paper, we review state-of-the-art experimental approaches to assess the mechanical properties and mechanically-induced responses of cell aggregates *in vitro*. These approaches are classified into five categories according to loading modality, including micropipette aspiration, centrifugation, compression loading, substrate distention, and fluid shear loading. We discussed the advantages and disadvantages of each approach, and the potential biomedical applications. Understanding of the mechanical behavior of cell aggregates provides insights to physical interactions between cells and integrity of biological functions, which may enable mechanical intervention for diseases such as atheromatosis and cancer.

KEY WORDS cell aggregates, loading method, biomechanics, biomedical applications

I. INTRODUCTION

Most cells exist in a format of cell aggregates *in vivo*, which are defined as cell clusters that aggregate and/or interact with each other, including loose cell clusters and compact spherical clusters (i.e., spheroids)[1]. Cell aggregates play an important role in many cellular processes, such as tissue functionality[2], embryonic development[3] and cancer metastasis[4]. Under physiological conditions, cells tend to form some basic functional units (e.g., lobules in the liver, nephrons in kidney, islets in pancreas), which are integrated into tissue structures. For example, liver is made up by many hepatic lobules, which are small histological and functional units of liver tissue. These lobules are essentially cell aggregates that are comprised of hepatocytes. *In vitro* studies show that hepatocyte aggregates rather than single cells exhibit hepatic specific function[5]. Other cell aggregate types include lacuna in bone or cartilage, and alveoli in lung. In addition, cells form aggregates during embryonic development (i.e., embryonic body

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(EB)), which control the developmental direction when interacting with extracellular matrix (ECM) *in vivo* [6] and *in vitro*[7]. Further, cell aggregates promote cancer metastasis, resulting in the development of drug resistance[8], tumor growth[9] and invasion[10] to adjacent and remote tissues. Thus, insight to the features of cell aggregates (e.g., structure and mechanics) is of importance to understand various biological behaviors and to explore the mechanisms to restrain disease progression.

Studies have shown that the mechanical properties of cell aggregates and their interaction with microenvironment have significant effects on functionality and biological behaviors. For instance, stem cell differentiation strongly depends on the relative elasticity or stiffness of microenvironment. As reported mesenchymal stem cells (MSCs) promote differentiation towards neurons, muscle lineages, or osteoblasts with increasing stiffness of culture substrate[11]. EB growth and differentiation in three-dimensional (3D) structures were inhibited when the elastic modulus of scaffold increased from 16 to 34 Pa[12]. On the other hand, the mechanical properties of tissue significantly affect cancer progression[13]. Changes in cancer ECM mechanics activate integrins through Erk and Rho signaling, which can enhance the stiffness of ECM[14]. Besides, engineered microscale cellular units can be used to mimic native functional units[15], and they can be assembled into larger constructs *in vitro*[16, 17], where mechanical forces also play an important role in this process.

With advances in micro- and nano-technologies, various methods have been developed to explore the mechanical and biological behaviors of cell aggregates. In this paper, we present advances in the mechanical testing methods of cell aggregates, including micropipette aspiration technique, centrifugal force method, compression loading method, substrate distention method, and methods based on fluid shear stress. We also discussed the advantage and disadvantages associated with each method.

II. EXPERIMENTAL APPROACHES FOR STUDYING CELL AGGREGATE MECHANICS

2.1. Micropipette Aspiration

The micropipette aspiration technique is one of the common methods for studying the mechanical properties (e.g., deformation and adhesion) of single cells and cell aggregates. The deformation of single cells or aggregates is induced by a negative pressure, which is applied using a microfluidic device containing two hydraulic pressure control systems (Fig.1)[18]. Briefly, cell aggregates are placed a cell chamber and aspirated using a micropipette, which is filled with medium and connected to a hydrostatic system. The hydrostatic system consists of a reference reservoir and a variable reservoir, where the liquid level of the reference reservoir is adjusted to be the same as that in the cell chamber. The micropipette, precisely controlled by a micromanipulator, is used to move cell aggregates. Suction pressure is then applied to cell aggregates, which equals to the pressure difference between the variable and reference reservoirs. The resultant morphology change of cell aggregates is recorded using an inverted microscope. The microscopic images are used to analyze the deformation of cell aggregates, which may be compared to numerical simulation (generally finite element modeling) to obtain mechanical properties of cell aggregates (e.g., Young's modulus)[19].

Compared with earlier models, more accurate rheological interpretation of micropipette aspiration experiments has been carried out, enabling prediction of subcellular stress and deformation in response to physical forces[20, 21]. The micropipette aspiration technique has also been improved in precision using micropipettes of different shapes, specifications and functional properties[22]. The micropipette aspiration method can also be modified to study mechanical interactions between adjacent cells via double micropipette aspiration[23]. The micropipette aspiration method offers several advantages, such as high precision[24], easy standardization and good reproducibility (Table 1). However, there are also several limitations associated with this technique: i) both the inner diameter and focusing error of the micropipette need optical correction; ii) the micropipette taper needs measuring and compensating.

2.2. Centrifugation

The centrifugal force method is generally used to characterize the adhesion force between cells and basement (Fig.2) . A case in point is that adhesion force between HT1080 cells and Tat-immobilized 96 well plate is quantified by applying a buoyant force. First, HT1080 cells are placed in a medium

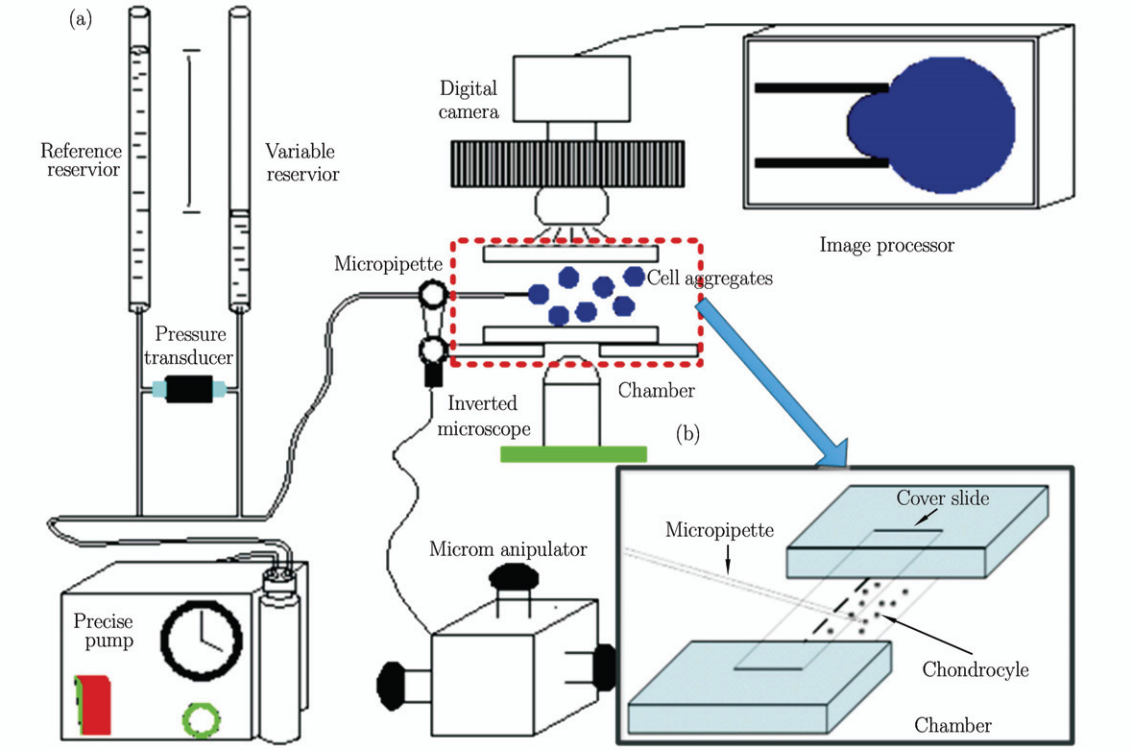


Fig. 1. Micropipette aspiration technique. (a) Schematic of the setup for micropipette aspiration technique; (b) Micropipette aspiration system. Negative pressure is induced using a microfluidic device containing two hydraulic pressure control systems: one is used to precisely control microscopic movement of a micropipette, and the other to control the negative pressure produced by the micropipette to adsorb cell aggregates.

Table 1. **Experimental approaches to studying cell mechanics of cell aggregates**

	Mechanical parameters	Precision	Reproducibility	References
Micropipette aspiration	Viscosity coefficient, elastic modulus	High	Good	[19–21]
Centrifugation	Viscosity coefficient	Low	Poor	[25,26]
Compression	Elastic modulus	Low	Good	[29,31]
Substrate distention	Viscosity coefficient, elastic modulus	Low	Good	[33,34,37,43]
Fluid shear	Viscosity coefficient, elastic modulus	Low	Good	[49–51,60]

Note: Spherical shape of cell aggregates was used in the reported literature.

that has a density higher than the cells. As such, cells are subject to cell adhesion, gravity and buoyant forces[25]. Upon applying increasing centrifugal force, weak and strong cell adhesion between cells and the plate can be distinguished according to their resistance. Via close monitoring of the whole process, the morphology of cell aggregates can be recorded and used to characterize the mechanics. This method has also been used to measure the surface tension of cell aggregates[26]. The centrifugal force method is simple and accurate to measure the cell aggregates mechanical properties, and it can be extended to a wide variety of cell-matrix and cell-cell interaction studies[27]. However, cell aggregates suspended in a matrix cannot reflect real cell-living environment *in vivo*, since cell aggregates are not attached to the matrix. Further, the effects of centrifugal force on cell aggregates are yet unclear.

2.3. Compression Loading

The compression loading method is used to apply pressure, either negative or positive, on cell aggregates, mimicking the conditions under which cells reside *in vivo*. Two modalities have been used to apply a compression force on cells: indirect loading method and direct loading method, depending on where the pressure is applied (Fig.3).

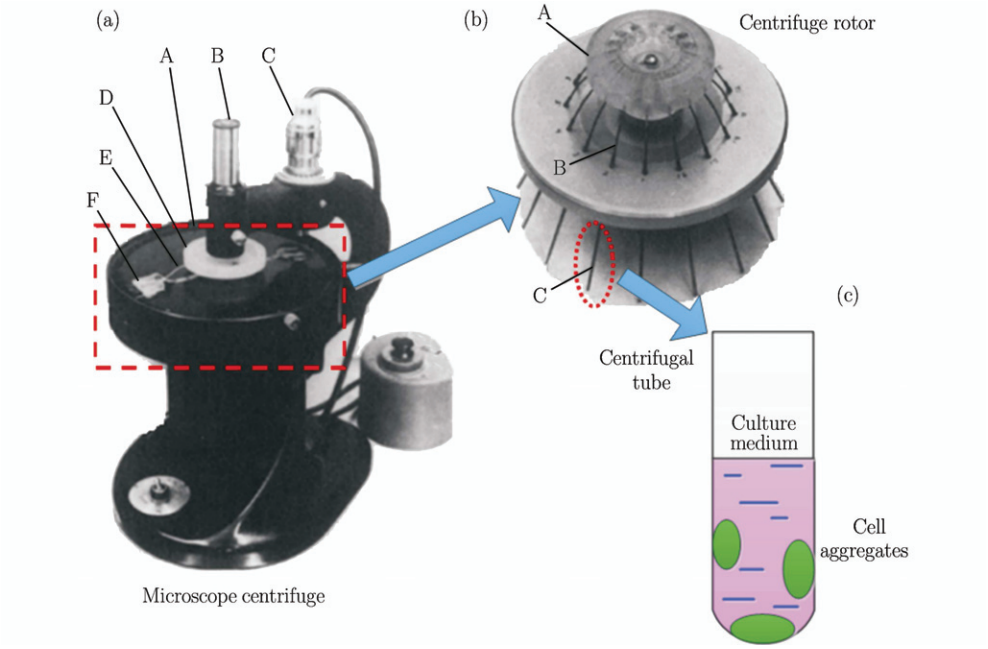


Fig. 2. Centrifugation based method[27]. (a) Microscope-centrifuge with apparatus permits direct observation of cell aggregates during centrifuge experiment. A, rotor; B, ocular; C, light source; D, distributor; E, connecting tube; F, specimen chamber; (b) Centrifuge rotor: A, distributor with central vertical well into which fixative can be injected during centrifugation; B, a connecting tube extending to a cannula from a horizontal well in the central chamber wall; C, cannula descending through the rotor lid will exit into a centrifuge tube containing aggregates to be fixed; (c) Centrifuge tube containing cell aggregates.

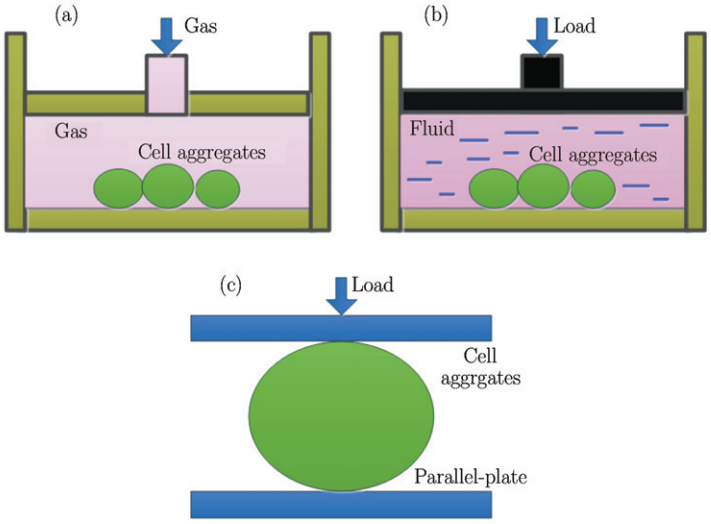


Fig. 3. Compressive loading methods. Indirect compressive loading by (a) gas pressure and (b) hydrostatic pressure. (c) Direct compressive loading using parallel plates.

2.3.1. Indirect loading

In the indirect loading method, pressure is applied via gas[28] (Fig.3(a)) or liquid[29] (Fig.3(b)). So far, three approaches have been used to generate a negative or positive pressure on cultured cells. First, a vacuum pump is used to generate a negative pressure in a cell culture chamber. Second, gas is injected into an airtight cell culture chamber to apply a positive pressure[30]. In these two approaches, cell aggregates are placed at the bottom of the cell culture chamber, and a ventilation hole is present at the top of the chamber. Through this ventilation hole, a vacuum environment can be obtained with a pump

generating a negative-pressure in the cell culture chamber. The negative-pressure induces tensile stress on the cell aggregates. Alternatively, nitrogen gas (without affecting the activity of cell aggregates) is pumped into the chamber through the ventilation hole to introduce a positive pressure, leaving cell aggregates compressed. Third, a hydrostatic pressure is applied on cultured cells by pumping liquid instead of gas through a moveable piston at the top of the chamber. The effect of hydrostatic pressure on the viability of bovine aortic endothelial cells has been studied by adjusting the height of the moveable piston. Indirect loading can generate uniform mechanical forces on cultured cells. However, since cells are cultured in an airtight cell culture chamber, media need to be changed regularly to maintain proliferation and normal metabolism of cells for an extended period of time.

2.3.2. Direct loading

The direct loading method applies direct mechanical pressure on cell aggregates placed between two parallel glass slides (Fig.3(c)). Briefly, cell aggregates are placed on a glass slide that serves as a lower compression plate. The glass slide is located at the bottom of a medium chamber, and can be moved vertically using an electronic micromanipulator. The upper compression slide is connected with another glass slide through an inbox wire to a copper-beryllium cantilever. The deflection of the cantilever is measured with a noncontact eddy current position measurement apparatus. The setup can be mounted in a thermally insulated chamber to maintain desirable temperature using a temperature controller. The deformation profile of cell aggregates is recorded using an inverted microscope and a digital camera, and the obtained images or videos are analyzed (e.g., using Matlab or other calculation software). This method ahs been used to study the effects of static as well as dynamic mechanical compression on chondrocytes[31] and the ability of chondrocytes to synthesize and assemble cartilage-like ECM[32]. Compared to indirect loading, it is simpler to control the pressure on cell aggregates over a wide range to create a deformation profile. However, the mechanical loading on the cells (e.g., stress and strain) is heterogeneous for spherical cell aggregates.

2.4. Substrate Distention

In this method, tensile strain is applied to cell aggregates (generally thin layered cell cluster) using an artificial flexible substrate. Briefly, cell aggregates are firstly seeded on a flexible silicone substrate, which is prepared by polymerizing a silicone fluid in a desired frame. The stiffness of the substrate can be varied by altering the crosslinking time and initial viscosity of the silicone fluid. Then, the substrate is coated with ECM proteins to promote cell adhesion and attachment. Once a stretching force is applied, cell aggregates attached firmly to the substrate are subjected to stretching deformation. Subsequently, wrinkling patterns appear on the surface of cell aggregates, which can be visualized under a microscope. Images of the wrinkling patterns can be taken using a digital camera and analyzed using an image processing system. Two shapes of the tensile substrate have been used, including a rectangle (Fig.4) and a circle (Fig.5).

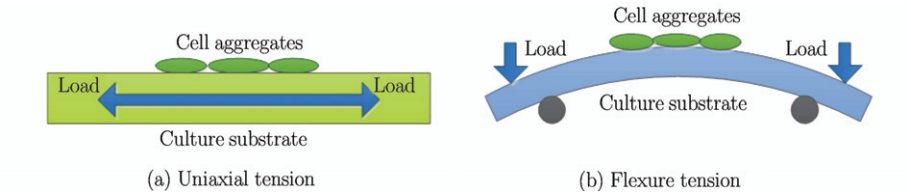


Fig. 4. Methods of rectangular substrate tension.

The rectangular tensile substrate can be employed to generate uniaxial tension[33,34] (Fig.4(a)) or flexure tension[35,36] (Fig.4(b)). Cells cultured on a rectangular elastin substrate are subject to cyclic stretching at different frequencies and magnitudes. Via controlling the period frequency and stretching magnitude, the rectangular substrate and the attached cell aggregates undergo a deformation. This method has been used to assess the effects of shear stress, pressure, and mechanical strain on vascular cell structure and function[37].

For the circular tensile substrate method, cells are cultured on a circular silicone rubber mold, and a tensile strain can be applied to the attached cells by stretching the mold using fluid displacement

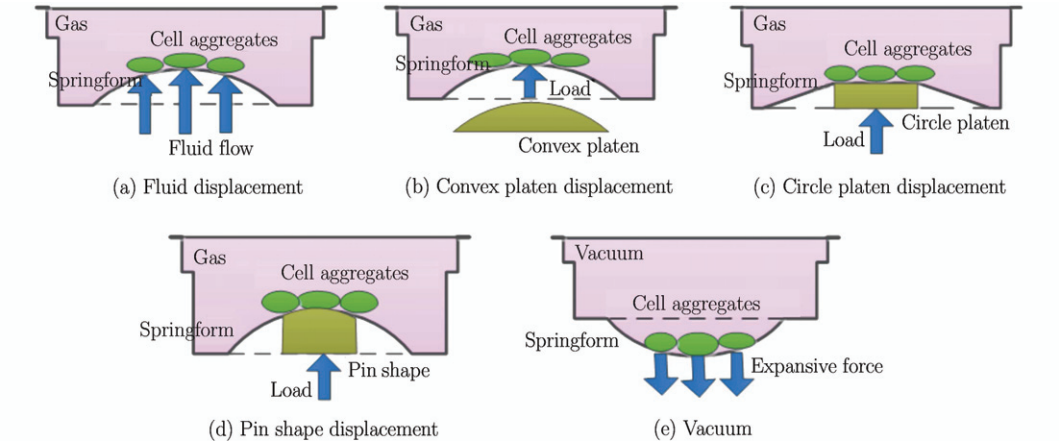


Fig. 5. Methods of circular substrate tension.

(Fig.5(a)), convex platen displacement (Fig.5(b)), circle platen displacement (Fig.5(c)), pin shape displacement (Fig.5(d)), or vacuum method (Fig.5(e)). The detailed parameters that have been studied using these methods are summarized in Table 2. In the method of fluid displacement (Fig.5(a)), gas or liquid is introduced to the bottom of the substrate with a certain flow rate. The induced flow pressure leads to the deformation of the elastic substrate, and meanwhile stretches the cells attached to the substrate[38,39]. In this method, fixation of the substrate is more convenient and reliable. It has been used to study the generation of intracellular signals of cells under deformation[40].

Table 2. **Substrate distention on a circular tensile substrate**

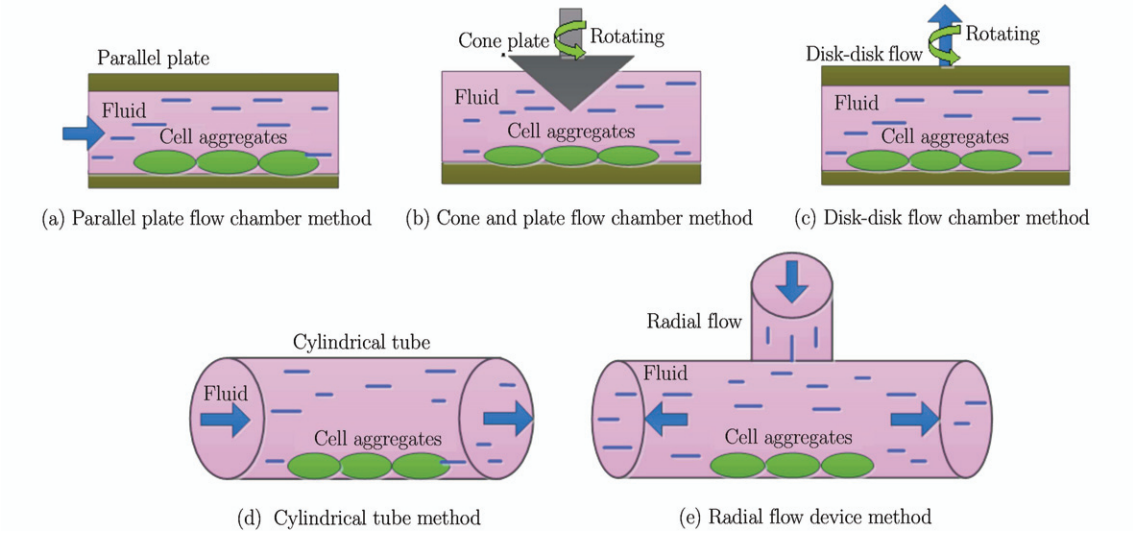
Methods	Driving force	Characteristics	References
Fluid displacement	Constant flow to displace cell attachment	Flow loading easily controlled	[38,39]
Convex platen displacement	Stretch force induced using an eccentric motor	Achieving different magnitude and frequency load	[41–43]
Circle platen displacement	Stretch force induced by friction	Friction induce the cell strain	[44,45]
Pin shape displacement	Stretch force induced by a pin shape compression bar	Uniform uniaxial strain	[46]
Vacuum method	Vacuum stress	Achieving a big strain rate	[47,48]

With the convex platen displacement method (Fig.5(b)), cell aggregates firmly attached to the elastic substrate are stretched by applying continuous or intermittent stretch force to the elastic substrate using an eccentric motor[41,42]. The stretch of elastic substrate is altered with different degrees of curvature of the convex platen governed by the frequency. This method has been used to study the mechanical properties of bone cells[43]. In the circle platen displacement method (Fig.5(c)), a stretch force is applied onto the substrate circle platen controlled by a motor[44,45] and, as a consequence, the cells attached to the substrate are subject to deformation. In the pin shape displacement approach (Fig.5(d)), a pin shape compression bar is used to deform the substrate and the attached cells. A uniform uniaxial strain can be produced on the whole substrate and the cells are under the same strain field independent of position. With this method, Vandenburgh[46] studied the mechanical characteristics of muscle cells and observed real-time changes in cell membrane, cytoskeleton, nucleus and fluorescence-marked calcium. Deformation of cell aggregates can also be induced by using a vacuum stress device (Fig.5(e))[47,48].

Regardless of the approaches for introducing substrate distention, the magnitude and frequency of the stretching load can be easily controlled. With an uniaxial stretch force applied to the substrate, the surface of the substrate remains flat, ensuring uniform straining of cells. Also, a homogeneous uniaxial strain-field can be created on the substrate, allowing all the cells to undergo the same extent of deformation independent of their position. In this method, however, cells may not adhere to the substrate tightly, which may lead to cell sliding and hence affect the measurement accuracy.

2.5. Fluid Shear Stress Loading

The fluid shear stress method is used to mimic shear stress applied on cells *in vivo* so as to study cell morphology and cell adhesion (Fig.6). So far, four types of chamber have been developed: (1) parallel-plate flow chamber to apply laminar flow; (2) cone and plate flow chamber to generate laminar or turbulent flows; (3) disk-disk flow chamber to generate laminar or turbulent flows; and (4) cylindrical tube device to generate radial flow. The parallel plate flow chamber (Fig.6(a)) can provide constant shear stress by the hydrostatic pressure of elevation difference, or transient shear stress using a micropump[49–51]. Since the upper and lower plates of the influx tube and the efflux tube have pressure differences, cells are subject to uniform or pulse shear stress, depending on the speed of fluid flow and/or the length/width of the chamber. The parallel plate flow chamber has been used to study the effects of shear stress on cell functions[52–54].



(a) Parallel plate flow chamber method (b) Cone and plate flow chamber method (c) Disk-disk flow chamber method

(d) Cylindrical tube method (e) Radial flow device method

Fig. 6. Methods based on fluid shear stress.

A cone and plate flow chamber (Fig.6(b)) has been used to generate a vertical uniform shear stress from the cone chamber to the plate. In this setup, the cone-plate and the plate are rotating to the opposite direction with an equivalent speed. Cells cultured on the low plate are relatively static to the ground and they are subject to a wide range of shear stress by altering the flow rate of liquid from the top chamber and the rotating speed[55, 56]. The cone and plate flow chamber can be improved by introducing a disk-disk flow chamber (Fig.6(c))[57], where the upper cone-plate is changed to a plane plate. Using a disk-disk flow chamber, the effects of haemodynamic forces on vascular endothelial cells, and their role in localizing atherosclerotic lesions within specific regions of the vasculature were studied[58] . A cylindrical tube (Fig.6(d))[59] and a radial flow device (Fig.6(e))[60] are also introduced to provide shear stress on cells. With the cylindrical tube device, a radial flow is obtained by flowing a fluid from the horizontal side. In contrast, a fluid is poured into the radial flow device from the top.

The fluid shear stress method can control precisely the shear stress within a biological relevant range. In addition, this method can offer a uniform shear stress, minimizing the variations in mechanical measurements. However, it should be noted that factors including flow rate, viscosity of the fluid, as well as the channel width and height need to be considered for a desired shear stress. In addition,

the resultant biological effects such as cell adhesion, deformation and growth by shear stress cannot be simply distinguished from the hydrostatic pressure present in the experimental process. Another confounding factor is downstream cells may also be affected by paracrine signals released by upstream cells as seen in a cone and plate flow chamber[61].

III. CONCLUSIONS

Accumulating evidence indicates cell mechanics plays an important role in understanding the nature of cell aggregates, tissues and organs, which can benefit mechanical intervention for disease progression and artificial organ transplantation. In this review, we summarized the advances in developing experimental approaches for measuring the mechanical properties of cell aggregates, including the micropipette aspiration, centrifugation, compression loading, substrate distention, and fluid shear stress loading. We pointed out the advantages and disadvantages and exemplified the use of these stress loading approaches. However, the goal of translating cell mechanics to clinical medication requires further development of experimental approaches to mimic the situations in *vivo*. In addition, advanced sensing technologies based on optics, acoustics and electronics are needed to achieve high precision, resolution and fidelity results.

References

[1] Guevorkian,K., Colbert,M.J., Durth,M., Dufour,S. and Brochard-Wyart,F., Aspiration of biological viscoelastic drops. *Physical Review Letters*, 2010, 104(21): 1-4.

[2] Hughes,L.C., Archer,C.W. and Gwynn,I.A., The ultrastructure of mouse articular cartilage: collagen orientation and implications for tissue functionality. *European Cells and Materials*, 2005, 9: 68-84.

[3] Ulitsky,I., Sive,H., Jan,C.H., Shkumatava,A. and Bartel,D.P., Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Elsevier Science*, 2011, 147(7): 1537-1550.

[4] Smid,M., Wang,Y., Zhang,Y.X., Klijn,J.G.M., Siewerts,A.M., Atkins,D., Martens,J.W.M. and Foekens,J.A., Genes associated with breast cancer metastatic to bone. *Journal of Clinical Oncology*, 2006, 24(15): 2261-2267.

[5] Powers,M.J., Janigian,D.M., Wack,K.E., Baker,C.S., Stolz,D.B. and Griffitch,L.G., Functional behavior of primary rat liver cells in a three-dimensional perfused microarray bioreactor. *Tissue Engineering*, 2004, 8(3): 499-513.

[6] Krieg,M., Arboleda-Estudillo,Y. and Puech,P.H., Tensile forces govern germ-layer organization in zebrafish. *Nature Cell Biology*, 2008, 10(4): 429-436.

[7] Xu,F., Sridharan,B. and Wang,S.Q., Cell printing for controlled-size embryoid body formation. *Biomicrofluidics,* 2011: p. doi: 10.1063/1.3580752.

[8] Meads,M.B., Gatenby,R.A. and Dalton,D.S., Environment-mediated drug resistance: a major contributor to minimal residual disease. *Nature Reviews Cancer,* 2009, 9(9): 665-674.

[9] Chen,L., Park,S.M., Tumanov,A.V., Hau,A., Sawada,K., Feig,C., Turner,J.R., Fu,Y.X., Romero,I.L., Lengyel,E. and Peter,M.E., CD95 promotes tumour growth. *Nature*, 2010, 465: 492-426.

[10] Liotta,L.A. and Kohn,E.C., The microenvironment of the tumour-host interface. *Nature*, 2001, 411(6835): 375-379.

[11] Engler,A.J., Sen,S., Sweeney,H.L. and Discher,D.E., Matrix elasticity directs stem cell lineage specification. *Cell*, 2006, 126(4): 677-689.

[12] Battista,S., Guarnieri,D., Borselli,C., Zeppetelli,S., Borzacchiello,A., Mayol,L., Gerbasio,D., Keene,D.R., Ambrosio,L. and Netti,P.A., The effect of matrix composition of 3D constructs on embryonic stem cell differentiation. *Biomaterials*, 2005, 26(31): 6194-6207.

[13] Butcher,D.T., Alliston,T. and Weaver,V.M., A tense situation: forcing tumour progression. *Nature Reviews Cancer*, 2009, 9(2): 108-122.

[14] Huang,S. and Ingber,D.E., Cell tension, matrix mechanics, and cancer development. *Cancer Cell*, 2005, 8(3): 175-176.

[15] Watanabe,S., Sato,S., Ohtsuka, K. and Takenaka, S., Electrochemical DNA analysis with a supramolecular assembly of naphthalene diimide, ferrocene, and beta-cyclodextrin. *Analytical Chemistry*, 2011, 83(19):7290-7296.

[16] Xu,F., Wu,C.M., Rengarajan,V., Finley,T.D., Keles,H.O., Sung,Y., Li,B., Gurkan,U.A. and Demirci,U., Three-dimensional magnetic assembly of microscale hydrogels. *Advanced Materials*, 2011, 23(37): 4254-4260.

[17] Xu,F., Finley,T.D., Turkaydin,M., Sung,Y., Gurkan,U.A., Yavuz,A.S., Guldiken,R. and Demirci,U., The assembly of cell-encapsulating microscale hydrogels using acoustic waves. *Biomaterials*, 2011, 32(31): 7847-7855.

[18] Hochmuth,R.M., Micropipette aspiration of living cells. *Journal of Biomechanics*, 2000, 33(1): 15-22.
[19] Pandey,R., Gupta,R.K., Shahid,M., Maiti,B., Misra,A. and Pandey,D.S., Synthesis and characterization of electroactive ferrocene derivatives: ferrocenylimidazoquinazoline as a multichannel chemosensor selectively for Hg2+ and Pb2+ ions in an aqueous environment. *Inorganic Chemistry*, 2012, 51(1):298-311.
[20] Zhou,E.H., Xu,F., Quek, S.T. and Lim,C.T., A power-law rheology based finite element model for single cell deformation. *Biomechanics Modeling Mechanobiology*, DOI: 10.1007/s10237-012-0374-y, 2012.
[21] Guevorkiana,K., D.Gonzalez-Rodriguez,D., Carlierb,C., Dufour,S. and Brochard-Wyart,F., Mechanosensitive shivering of model tissues under controlled aspiration. *PNAS*, 2011, 108(33): 13387-13392.
[22] Guevorkian,K.C., M.J. and Durth,M.L., Aspiration of biological viscoelastic drops. *Physical Review Letters*, 2010, 104: 1-4.
[23] Daniel,S.J. and N.Chao,L., Noncontact measurement of the local mechanical properties of living cells using pressure applied via a pipette. *Biophysical Journal*, 2008, 95(6): 3017-3027.
[24] Forgacs,G.K. and Kosztin,I., Cellular aggregates under pressure. *American Physical Society*, 2010, 3(43): 3017-3027.
[25] Marmottanta,P.M. and Audrenb,B., The role of fluctuations and stress on the effective viscosity of cell aggregates. *PNAS*, 2009, 106(41): 17271-17275.
[26] Kalantarian,A., Ninomiya,H., Saad,S.M., David,R., Winklbauer,R. and Neumann,A.W., Axisymmetric drop shape analysis for estimating the surface tension of cell aggregates by centrifugation. *Biophysical Journal*, 2009, 96(4): 1606-1616.
[27] Evseenko,D.D. and Zhu,G., Identification of the Critical Extracellular Matrix Proteins that Promote Human Embryonic Stem Cell Assembly. *Stem Cells and Development,* 2009, 18(6): 919-928.
[28] Liu,C.Z. and Wang,Y., Effects of cyclic hydraulic pressure on osteocytes. *International Bone and Mineral Society*, 2010, 46(5): 1449-1456.
[29] Elder,B.D., Hydrostatic pressure in articular cartilage tissue engineering: from chondrocytes to tissue regeneration. *Tissue Engineering*, 2009, 15(1): 43-53.
[30] Liu,L.L. and Liao,D., A microfluidic device for continuous cancer cell culture and passage with hydrodynamic forces. *Lab on a Chip*, 2010, 10(14): 1807-1813.
[31] Shahin,K.D., Tissue engineering of cartilage using a mechanobioreactor exerting simultaneous mechanical shear and compression to simulate the rolling action of articular joints. *Biotechnology and Bioengineering*, 2012, 109(4): 1060-1073.
[32] Thorpe,S.D.B. and Vinardell,T., The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-β3 induced chondrogenic differentiation. *Annals of Biomedical Engineering*, 2010, 38(9): 2896-2909.
[33] Dunkers,J.C. and Pakstis,L., Solutions for determining equibiaxial substrate strain for dynamic cell culture. *Journal of Biomechanics*, 2010, 43(13): 2613-2617.
[34] Yu,H.Y.R. and Sandham,A., Mechanical tensile stress effects on the expression of bone sialoprotein in bovine cementoblasts. *The Angle Orthodontist*, 2009, 79(2): 346-352.
[35] Durst,C.A. and Mansfield,E.G., Flexural characterization of cell encapsulated PEGDA hydrogels with applications for tissue engineered heart valves. *Acta Biomaterialia*, 2011, 7(6): 346-352.
[36] Poapongsakorn,P., Time-dependent deformation of closed-cell PVC foam. *Journal of Cellular Plastics*, 2011, 47(4): 323-336.
[37] Ferdousa,Z.J. and Nerem,R.M., Differences in valvular and vascularcell responses to strain in osteogenic media. *Biomaterials*, 2011, 32(11): 2885-2893.
[38] Connolly,S.C.S. and Fairbank,N.J., Chronic oscillatory strain induces MLCK associated rapid recovery from acute stretch in airway smooth muscle cells. *Journal of Applied Physiology*, 2011, 111(4): 955-963.
[39] Bartalena,G.G. and Sharma,R.I., A novel method for assessing adherent single-cell stiffness in tension: design and testing of a substrate-based live cell functional imaging device. *Biomedical Microdevices*, 2011, 13(2): 291-301.
[40] Muravyov,A.V.T. and Maimistova,A.A., Extra- and intracellular signaling pathways under red blood cell aggregation and deformability changes. *Clinical Hemorheology and Microcirculation*, 2009, 43(3): 223-232.
[41] Huang,L.M. and Helmke,B.P., A stretching device for high-resolution live-cell imaging. *Annals of Biomedical Engineering*, 2010, 38(5): 1728-1740.
[42] Cheng,T.P. and Dunkers,J., Solutions for determining equibiaxial substrate strain for dynamic cell culture. *Journal of Biomechanics*, 2010, 43(13): 2613-2615.
[43] Andrew,A. and Pitsillides,S.C., Using cell and organ culture models to analyze responses of bone cells to mechanical stimulation. *Bone Research Protocols*, 2012, 816(7): 593-619.
[44] Billiar,K.L., The mechanical environment of cells in collagen gel models global and local effects in three-dimensional biological hydrogels. *Tissue Engineering and Biomaterials*, 2011, 4: 593-619.

[45] Sander,E.A. and Swickrath,M.J., Out of many, one: modeling schemes for biopolymer and biofibril networks. *Chemistry and Physics Science*, 2010, 9: 557-602.
[46] Vandenburghc,H. and Yunga,Y.C., Cellular strain assessment tool (CSAT): precision-controlled cyclic uniaxial tensile loading. *Journal of Biomechanics*, 2009, 42(2): 178-182.
[47] Shin,H.S. and Sim,S.J., Shear stress effect on transfection of neurons cultured in microfluidic devices. *Journal of Nanoscience and Nanotechnology*, 2009, 9: 1-6.
[48] Schwartz,G. and Uhricha,C., Efficient and long-term stable organic vacuum deposited tandem solar cells. *Proceedings of SPIE Digital Library*, 2009, 7416: 1-11.
[49] Yamamoto,K., Shear stress mechanotransduction via endogenous ATP release in vascular endothelial cells. *IFMBE Proceedings*, 2012, 37(1): 777-780.
[50] Yamamoto,J.A., Shear-stress-sensing and response mechanisms in vascular endothelial cells. *Interface Oral Health Science*, 2010, 1: 3-9.
[51] Obi,S. and Shimizu,N., Fluid shear stress induces arterial differentiation of endothelial progenitor cells. *Journal of Applied Physiology*, 2009, 106(1): 203-211.
[52] Brown,A. and Brian,J., Modeling of shear stress experienced by endothelial cells cultured on microstructured polymer substrates in a parallel plate flow chamber. *Biotechnology and Bioengineering*, 2011, 108(5): 1148-1158.
[53] Condorelli,L. and Arrigoni,C., Effect of fluid shear stress on tubular kidney epithelial cell structure. *IFMBE Proceedings*, 2010, 25(10): 50-52.
[54] Huesa,C. and Richard,M., Parallel-plate fluid flow systems for bone cell stimulation. *Journal of Biomechanics*, 2010, 43(6): 1182-1189.
[55] Wang,X.Q. and Fujiwara,K., Thioredoxin interacting protein promotes endothelial cell inflammation in response to disturbed flow by increasing leukocyte adhesion and repressing kruppel-like factor 2. *Circulation Research*, 2012, 110(4): 560-568.
[56] Dreyer,L. and Autschbach,R., An advanced cone-and-plate reactor for the in vitro-application of shear stress on adherent cells. *Clinical Hemorheology and Microcirculation*, 2011, 49(1): 391-397.
[57] Obi,S. and Masumura,T., Shear stress induces arterial differentiation of bone marrow-derived endothelial progenitor cells. *Micro-NanoMechatronics and Human Science*, 2009, 9: 650-655.
[58] Chiu,J.J. and Chien,S., Vascular endothelial responses to altered shear stress: Pathologic implications for atherosclerosis. *Annals of Medicine*, 2009, 41(1): 19-28.
[59] Geitmann,A., How to shape a cylinder: pollen tube as a model system for the generation of complex cellular geometry. *Sexual Plant Reproduction*, 2010, 23(1): 63-71.
[60] Tilton,N.M. and Serre,E., Pressure-driven radial flow in a Taylor–Couette cell. *Journal of Fluid Mechanics*, 2010, 660: 527-537.
[61] Conway,D.E. and Eskin,S.G., Endothelial cell responses to atheroprone flow are driven by two separate flow components: low time-average shear stress and fluid flow reversal. *Heart and Circulatory Physiology*, 2010, 298(2): 367-374.