Online Data Supplement

Lung Self-Assembly is Modulated by Tissue Surface Tensions

Margaret A. Schwarz [1], Haihua Zheng [2], Susan Legan[1] and Ramsey A. Foty[2]

[1] University of Texas Southwestern Medical Center at Dallas, Dallas, TX and [2]Robert Wood Johnson Medical School - University of Medicine and Dentistry of New Jersey, New Brunswick, NJ

Measurement of tissue cohesion by TST:

The tissue surface tensiometer. The apparatus is shown schematically in Fig. S1. The compression cell is composed of two chambers. The outer chamber (OC) is connected to a 37°C circulating water pump, and serves to regulate the temperature of the inner chamber (IC). The chambers are constructed of milled Delrin and contain quartz windows for visualization of the aggregate. The lower assembly (LA) screws into the base of the inner chamber and is used to 1) position the aggregate in the inner chamber; 2) seal the bottom of the inner chamber; 3) elevate the aggregate to initiate compression; and (4) control the distance between the parallel plates and hence the compression of the aggregate. The central core (CC) of the assembly is adjustable. The tip of the central core (the pedestal) is composed of smooth Teflon and acts as the lower compression plate (LCP). The upper compression plate (UCP) is a Teflon cylinder 15mm long that hangs from the balance arm (B) by a flame-straightened nickel-chromium wire (NCW). During the course of an experiment, the cell aggregate (A) is positioned on the lower plate and raised until it contacts the upper plate. The upper plate is connected to the balance arm (B). Compression of the aggregate causes displacement of the balance arm. The balance is a Cahn/Ventron model 2000 recording electrobalance, which operates on the null balance principle. The fulcrum of the balance arm has an armature within a permanent magnetic field. When the balance is operating, it continuously modulates the current passing through the electromagnetic assembly, which in turn maintains the balance arm in the horizontal position. When an object is suspended from the balance arm, the voltage, which the balance applies to keep the arm in the horizontal position, is proportional to the object's weigh. This voltage measures the external compressive force applied to the aggregate. The aggregate's shape is monitored by visual observation through a 25 x Nikon SMZ10A stereoscope coupled to a computer equipped with a frame grabber. In order to minimize adhesion of cell aggregates to the compression plates, both the lower and upper compression plates are coated with poly (2-hydroxyethylmethacrylate) {poly(HEMA)}, a polymeric material to which cells do not adhere (1).

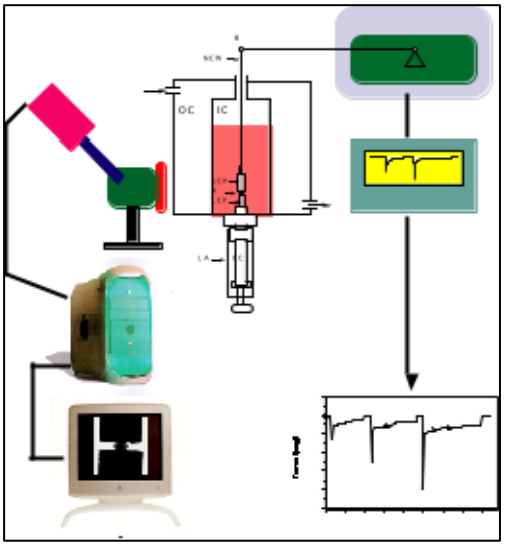


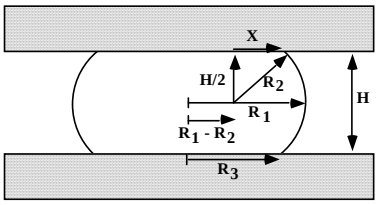
Fig. S1. The tissue surface tensiometer.

Aggregate compression. The inner chamber is filled with pre-warmed CO_2-independent tissue culture medium (Gibco/BRL, NY). Aggregates ranging in size from about 200-300 μ are positioned on the lower compression plate. The upper compression plate (UCP) is positioned above the aggregate and allowed to settle, establishing a pre-compression apparent UCP weight baseline. The LCP is raised until the aggregate is compressed against the UCP. Adjusting the height of the inner core of the lower apparatus controls different degrees of compression. Compression is monitored by observation through a dissecting microscope equipped with a CCD video camera. Aggregate images are captured, digitized, and analyzed using ImageJ software. Apparent UCP weight change is continuously recorded on a strip chart recorder, achievement of shape equilibrium being denoted by the leveling-off of the Cahn balance's voltage output. Each aggregate is subjected to 2 different degrees of compression, the second greater than the first.

Calculation of aggregate cohesivity. At shape equilibrium, the cohesivity of an aggregate of cells compressed between parallel plates to which it does not adhere can be obtained from the Young-Laplace equation (Eqn. S1, Fig. S2), where σ is cohesivity, F is the force acting to compress the aggregate, πr^2_3 is the area of the surface of the aggregate upon which force F is exerted, and R_2 and R_3 are, respectively, the radius of the equator of the compressed aggregate and the radius of an arc defining its surface profile normal to the compressing plates and extending between them. Measuring the compressive force and geometry at force and shape equilibrium and applying these measurements to the Young-Laplace equation generates numerical values of *apparent* tissue surface tension. Upon reaching equilibrium and calculation of σ_1, aggregates will be decompressed and allowed to approach a second equilibrium σ_2 will be calculated as described above.

Eqn. S1 $$\sigma = \frac{F}{\pi R_3^2}\left(\frac{1}{R_1}+\frac{1}{R_2}\right)^{-1}$$

Fig. S2



Confirmation of aggregate liquidity. The two likely material states to be considered as they apply to tissue aggregates are liquidity and elasticity. The calculated surface tension of a liquid aggregate, when subjected to two different compressions will remain constant. In such aggregates the ratio of σ_2/σ_1 will be equal to 1 and will be less than the ratio of the force applied at each successive compression (F_2/F_1). In contrast, the calculated surface tension of an elastic aggregate will obey Hooke's law and increase proportionately to the applied force. For elastic aggregates the ratio of σ_2/σ_1 will not be equal to 1 but will instead approach the ratio of F_2/F_1. The surface tension of liquid aggregates will also be independent of aggregate size (2, 3). Only measurements in which surface tension is independent of the applied force and size are used to calculate average σ for each cell line.

Validation of TST measurements. In order to confirm the validity of our TST measurements, the tissue surface tensiometer was calibrated by compressing an air bubble in culture medium and comparing the calculated surface tension with that obtained by the de Noüy ring method. Surface tension measured by TST and by the de Noüy technique were essentially identical and within 5% of the published value (2).

References Cited

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3. Foty RA, Pfleger CM, Forgacs G, Steinberg MS. Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development* 1996;122(5):1611-1620.