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Tracking mechanical and morphological dynamics of regenerating Hydra tissue fragments using a two fingered micro-robotic hand

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Regeneration of a tissue fragment of freshwater polyp Hydra is accompanied by significant morphological fluctuations, suggesting the generation of active forces. In this study, we utilized a two fingered micro-robotic hand to gain insights into the mechanics of regenerating tissues. Taking advantage of a high force sensitivity ($\sim$1 nN) of our micro-hand, we non-invasively acquired the bulk elastic modulus of tissues by keeping the strain levels low ($\varepsilon < 0.15$). Moreover, by keeping the strain at a constant level, we monitored the stress relaxation of the Hydra tissue and determined both viscous modulus and elastic modulus simultaneously, following a simple Maxwell model. We further investigated the correlation between the frequency of force fluctuation and that of morphological fluctuation by monitoring one "tweezed" tissue and the other "intact" tissue at the same time. The obtained results clearly indicated that the magnitude and periodicity of the changes in force and shape are directly correlated, confirming that our two fingered micro-hand can precisely quantify the mechanics of soft, dynamic tissue during the regeneration and development in a non-invasive manner. © *2016 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4943402]

The freshwater polyp Hydra is a paradigm for an unlimited regenerative capacity.[1] The regeneration from dissociated cell aggregates[2] (re-aggregates) or spherical pieces of cut tissues (regenerates) is an extreme case, demonstrating that complete structures (whole organisms) can be achieved from a broad range of initial conditions. Recently, it has been shown that Wnt signaling dictates the molecular patterning, which is currently understood within the framework of reaction-diffusion mechanisms.[3–6] However, if one focuses on the morphological dynamics during the initial phase of regeneration, the formation of the major body axis from a spherical re-aggregate or regenerate is accompanied with extremely dynamic fluctuations of both size and shape, indicating an active force generation. A healthy epithelial bi-layer creates high osmotic pressure inside the Hydra regenerate, which acts as a "hydro-skeleton" against the epitheliomuscular cells. The water flow into spherical Hydra regenerates leads to an inflation of the regenerate and results in a rupture of the epithelial layer, which is in contrast to matured Hydras that can release the excess water through the mouth opening.[2,3] In recent studies, correlations between mechano-genetic and mechano-chemical coupling have been suggested as possible factors involved in Hydra regeneration.[7,8] Therefore, the quantification of the mechanical aspect, i.e., the viscoelasticity of osmotically stabilized tissues and active forces simultaneously, is an essential step that has not been addressed enough so far. In this study, we focus on the mechanics of freshly cut Hydra regenerates (Fig. 1(a)) during the earlier stage of regeneration, $t = 3$–5 h. We utilized a two fingered micro-hand[9] with plate-shaped end-effectors in order to precisely quantify (a) the viscous and elastic modulus as well as (b) the amplitude and frequency of forces actively generated by regenerates (Figs. 1(b) and 1(c)).[10] The two fingered micro-hand consists of two series of parallel mechanism modules. Each module has three piezoelectric devices that are used as actuators to independently drive two glass needles. Each needle has a 3 degrees of freedom motion, thus enabling dexterous grasping motion of micro objects like chopsticks.[11] The hand has a fine force sensor on the end of one needle whose force measuring capability includes $\Delta F_{\mathrm{lim}} \sim 10^{-9}$ N resolution and 1.5×10^{-8} N RMS noise level. In a previous account, the micro-hand device was utilized to monitor the Meyer hardness of human lung epithelial cells according to the increase in the level of viral proteins.[12] The advantage of the micro-hand, compared to conventional two-plates methods,[13,14] is that the contact area A_c between the tissue and a single micro-hand tip ($(11 \pm 2) \times 10^3 \mu\mathrm{m}^2$) is almost an order of magnitude smaller than the cross sectional area of a tissue ($(88 \pm 1) \times 10^3 \mu\mathrm{m}^2$), and thus this can be assumed to be constant. This enables precise and robust measurement of the viscoelastic properties of the object of interest, regardless of morphological changes or in the presence of active fluctuations.

In the first step, a regenerate was compressed with a micro-hand by linearly decreasing the separation distance D within 15 s (Fig. 2(a), red), reaching the maximum force level

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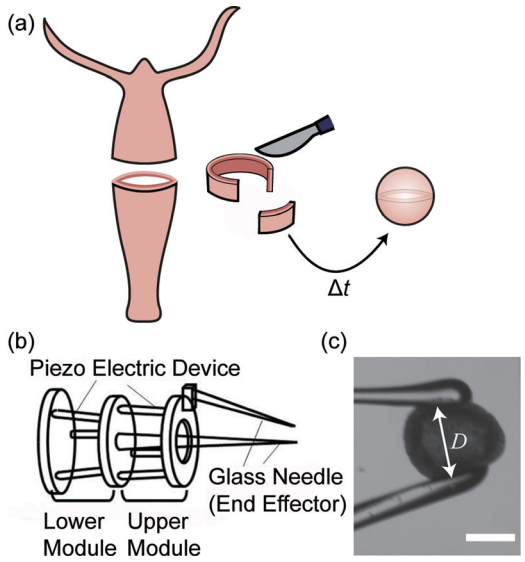

FIG. 1. (a) Preparation of Hydra tissue samples (regenerates). A ring-like piece of tissue removed from the upper body column (so called "gastric region") was cut into 4 pieces. Each of the cut Hydra fragments rounds up to minimize the line tension and forms spherical balls consisting of two cell layers (called "Hydra regenerates"). (b) Schematic illustration of the two fingered micro-hand used in this study. (c) A Hydra regenerate was clamped by precisely controlling the separation distance D, while the force generated by the clamped regenerate could be monitored within the precision of $\Delta F \sim 10^{-9}$ N. Scale bar corresponds to 200 μm.

F_{max} (Fig. 2(a), blue line). This yields the relationship between the maximum force F_{max} and the displacement ΔD. Then, the force relaxation over time was recorded while keeping the separation distance D constant (Fig. 2(a), red line). If the elastic response of a regenerate is linear, a Hookian spring constant k can be obtained from $k = dF_{max}/d\Delta D$. Here, we test different F_{max} levels to ensure that the system is responding in a linear fashion, which cannot be guaranteed with conventional methods.[13,14] By normalizing F_{max} with contact area A_c and ΔD with initial diameter of the regenerate D_0, one can gain a relationship between stress ($\sigma = F_{max}/A_c$) and strain ($\varepsilon = \Delta D/D_0$). In this study, only compression periods in which the slipping of the regenerate was negligible were used for analysis. The data presented in Fig. 2(b) were obtained from 11 measurements of three regenerates, suggesting that the elastic response of Hydra regenerates is linear within the strain level $\varepsilon = 0.03$–0.15. The bulk elastic modulus obtained from the linear fit $E = \sigma/\varepsilon \sim 185 \pm 13\,\mathrm{N\,m^{-2}}$ corresponds to a spring constant of $k = (8.1 \pm 0.7) \times 10^{-3}\,\mathrm{N\,m^{-1}}$, showing reasonable agreement with the values from previous accounts on other tissues squeezed between two plates.[13] However, it should be noted that the high force sensitivity of our micro-hand (approx. $\Delta F_{lim} \sim 10^{-9}$ N with RMS noise of 1.5×10^{-8} N) enables one to minimize potential artifacts caused by large, often invasive sample-probe contacts seen in previous studies.

In the next step, we analyzed the relaxation of force $F(t)$ at a constant separation distance D (Fig. 3), which can readily be translated into the stress relaxation over time $\sigma(t)$ at a constant strain ε_{const}. An exponential fit of the experimental data points (Fig. 3, red solid line) yields a characteristic time constant $\tau = 19 \pm 8$ s, which agrees with the longer time scale for other tissues measured by a two-plates method $\tau \sim 20$ s.[13]

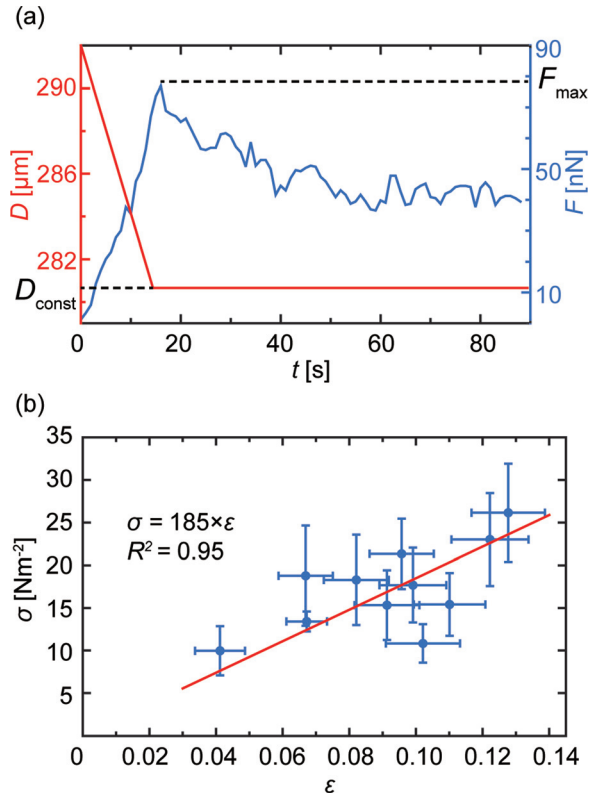

FIG. 2. (a) A representation of the micro-robotics measurements. Once a regenerate is clamped, the separation distance D was decreased to the target level in 15 s and kept constant (red). In parallel, the response force F was recorded over time (blue), yielding the maximum force response F_{max} and the force relaxation at a constant D. (b) The stress-strain ($\sigma - \varepsilon$) relationship of Hydra regenerates obtained from the experimental F-D relationships ($n = 11$). The linear regression yields a Young's modulus $E \sim 185 \pm 13\,\mathrm{N\,m^{-2}}$ and a spring constant of $k = (8.1 \pm 0.7) \times 10^{-3}\,\mathrm{N\,m^{-1}}$.

Such a single exponential force relaxation is well reproduced by the simplest serial connection of a spring and a dashpot, called the Maxwell's model (Fig. 3, inset). By adopting this simple model, one could gain the Stokes frictional coefficient $\gamma = k\tau = 0.17 \pm 0.08\,\mathrm{N\,m^{-1}}$ s and the viscous modulus $\eta = E\tau = (3.6 \pm 1.5) \times 10^{3}\,\mathrm{N\,m^{-2}}$ s simultaneously.

As one can see in Fig. 3, many of the force relaxation curves can be identified as a combination of an exponential decay and an oscillatory fluctuation. In fact, Hydra regenerates

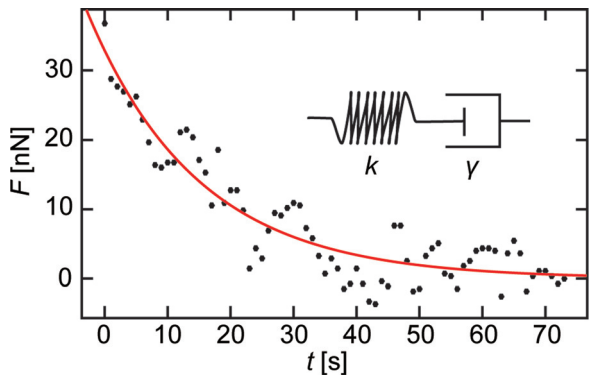

FIG. 3. A typical force relaxation curve from the micro-robotics measurement, which can be fitted with a single exponential function (red solid line). Employing a simple Maxwell model (inset), the Stokes frictional coefficient $\gamma = k\langle\tau\rangle = 0.17 \pm 0.08\,\mathrm{N\,m^{-1}}$ s and the viscous modulus $\eta = E\langle\tau\rangle = (3.6 \pm 1.5) \times 10^{3}\,\mathrm{N\,m^{-2}}$ s can be calculated.

exhibited extremely dynamic morphological dynamics.[10] Such morphological changes are vital for the Hydra during regeneration. They are caused by active forces and are correlated with the fluctuation of gene expression.[7] It is therefore essential to quantify the viscoelasticity and active forces of tissues simultaneously. For this purpose, we prepared two regenerates from the same animal at the same time (Fig. 4). We then monitored the force fluctuation by repeating (i) the clamping for 90–350 s at $F \sim 10^{-7}$ N and (ii) the releasing for 20–100 s for one regenerate (Fig. 4(a), left) over a time period of 94 min. By placing another (intact) regenerate side-by-side (Fig. 4(a), right), the periodicity of force fluctuation (Fig. 4(b)) was directly compared to that of shape fluctuation of an intact regenerate (Fig. 4(c)). None of the samples burst during the measurement. Fig. 4(b) represents a typical force fluctuation curve of one regenerate recorded over time, which can be characterized by a maximum-to-minimum difference of $\Delta F = (5 \pm 2) \times 10^{-8}$ N and a periodicity of $\Delta t = 45 \pm 5$ s. It should be noted that the decay in ΔF can be attributed to the drift of the baseline according to the viscoelastic relaxation as presented in Fig. 3. Fig. 4(c) represents the size fluctuation of an intact Hydra regenerate residing next to the "tweezed" Hydra plotted as a function of time. As shown in the figure, the typical size fluctuation can be characterized by the oscillatory change in the mean radius $\Delta r = (1.8 \pm 0.4)$ μm and the periodicity of size fluctuation $\Delta t = 59 \pm 12$ s. As the experimentally obtained Δr coincides with the strain of $\varepsilon \sim 0.013 \pm 0.003$,[10] a linear regression of the force-distance relationship predicts the active force that deforms the intact tissue to be approximately $F \sim (3 \pm 1) \times 10^{-8}$ N, which is in good agreement with the maximum-to-minimum difference obtained in Fig. 4(b), $\Delta F = (5 \pm 2) \times 10^{-8}$ N. Finally, good agreement between the periodicity of oscillatory force and that of size fluctuation implies that our two fingered micro-hand enables one to precisely measure the active forces generated by Hydra tissues without interfering with their native morphological dynamics.

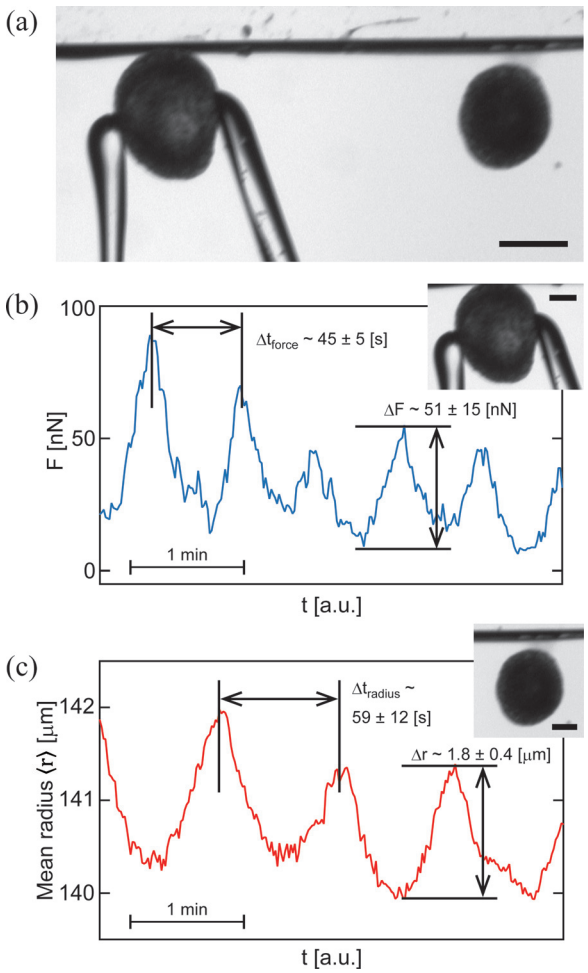

FIG. 4. (a) Parallel experiments of a "tweezed" Hydra regenerate (left) and an "intact" Hydra regenerate (right) enable one to determine conclusively whether the force readout from a micro-hand is correlated with the morphological dynamics.[10] (b) A typical force response of a Hydra regenerate "tweezed" at the constant separation distance D, exhibiting periodically oscillating forces, characterized by a maximum-to-minimum difference of $\Delta F = (5 \pm 2) \times 10^{-8}$ N and a periodicity of $\Delta t = 45 \pm 5$ s. (c) A typical morphological fluctuation of the mean radius of an "intact" Hydra regenerate recorded over time. The characteristic periodicity of force and size fluctuation shows good agreement, confirming that our micro-hand device can precisely record the forces from regenerating tissues that are merely in the order of several tens of nN. Scale bars correspond to 200 μm (for (a)) and 100 μm (for ((b) and (c))). (Multimedia view) [URL: http://dx.doi.org/10.1063/1.4943402.1]

To conclude, our findings demonstrated that the two fingered micro-robotic hand with a high force sensitivity ($\Delta F_{lim} \sim 10^{-9}$ N) allows for: (a) precise measurements of the bulk elastic modulus of large (diameter > 100 μm) soft tissues by the fine-adjustment of both stress and strain in a linear regime, (b) simultaneous determinations of not only elastic modulus but also viscous modulus of Hydra tissue from the stress-relaxation curve, and (c) parallel tracking of periodic oscillation of active force of a "tweezed" and an "intact" tissue to guarantee the non-invasiveness of the assay developed in this study. The combination of a highly sensitive micro-hand and tissues undergoing dynamic motions opens a large potential towards quantitative tissue mechanics, where collective dynamic motion of cells orchestrates to realize distinct morphology and functions.

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