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Analysis of the Contraction of Fibroblast-Collagen Gels and the Traction Force of Individual Cells by a Novel Elementary Structural Model *

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Abstract— **Based on the experimental data of the contraction ratio of fibroblast-collagen gels with different initial collagen concentrations and cell numbers, we analyzed the traction force exerted by individual cells through a novel elementary structural model. We postulate that the mechanical mechanism of the gel contraction is mainly because that populated cells apply traction force to some of the surrounding collagen fibrils with such proper length potential to be pulled straight so as to be able to sustain the traction force; this traction induce the cells moving closely to each other and consequently compact the fibrillar network; the bending force of the fibrils in turn resists the movement. By employing fiber packing theory for random fibrillar networks and network alteration theory, the bending force of collagen fibrils was deduced. The traction force exerted by individual fibroblasts in the gels was balanced by the bending force and the resistance from interstitial fluid since inertial force can be neglected. The maximum traction force per cell under free floating condition is in the range of 0.27-9.02 nN depending on the initial collagen concentration and populated cell number. The most important outcome of this study is that the traction force of individual cells dynamically varies under different gel conditions, whereas the adhesion force between cell and individual fibrils is relatively converging and stable.**

I. Introduction

Collagen gels will contract as contractile cells populated within, a process regarded as the in vitro analog of wound healing and harnessed to engineer various tissue equivalents [1]. Hence, to understand the contraction process is vital for studying the related physiological and pathological processes and for the applications in tissue engineering [2]. Due to the active and dynamic cellular processes and cell-cell, cell-collagen interactions, the behind mechanism of the phenomenon is yet to be fully understand. Although it is commonly accepted that the essence of the phenomenon is that contractile force generated by the cytoskeletal dynamics transmits at focal adhesion via integrins to the collagen fibrils; the mechanical details and the role of cellular processes still remain unanswered. Meanwhile, the contractile force exerted by individual cells in collagen gels is also an intriguing issue not only because it is closely related to some physiological and pathological behaviors but also because the recent researches show that such mechanical stimulus is a critical cue for the differentiation of stem cells [3]. A multitude of studies on this issue gives rise to a wide range, 0.1-450 nN, for the contractile force exerted by individual cells [4], showing that this quantity is active and sensitive to the extracellular environment. The objective of this study is to shed some light on the behind mechanical mechanism of collagen gel contraction and to elucidate the traction behavior of the populated individual cells.

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II. Materials and Methods

A. Casting and Culturing of Fibroblast-Collagen Gels

Dermal fibroblasts explanted from Wistar rat embryos and subcultured to 5 passages were used in the experiment. Fibroblast–collagen–DMEM mixtures, each in 1.0 ml volume containing 1.0 mg or 1.5 mg type I collagen (extracted from rat tail) and 0.3, 0.9, or 1.5 million cells, respectively, were poured into circular casting molds (diameter=22 mm). The mixtures were allowed to form gels in a 5%-CO_2, 37°C incubator for 1 h. After the gelation culture medium (DMEM supplemented with 1% penicillin– streptomycin and 10% fetal bovine serum) was added to float the gels, the contraction was thus under free floating condition. The culture continued for 7 days and medium change was on the 2nd and 4th day. Gel area was obtained by image processing of gel pictures using Adobe Photoshop CS4 and the contraction was delineated by the ratio of the gel area to the initial area versus culture duration. A surface-marked cover glass was placed on the gels when gel thickness was measured, the distance between the lower surface of the cover glass and the bottom of the culture dish was measured by means of an inverted light microscope.

B. Elementary Structural Model

Gel contraction starts because the traction force exerted by the populated cells makes the system lose mechanical equilibrium. Through the observation by means of scanning electron microscopy (SEM), we found that the collagen fibrils in the gel with fibroblasts (Fig. 1a) are much more curved than those in the gel without cells (Fig. 1b), an evidence to show that the fibrils are bended during the contraction. However, bended fibrils will not sustain the traction force; there must be some fibrils in such proper length potential to be pulled

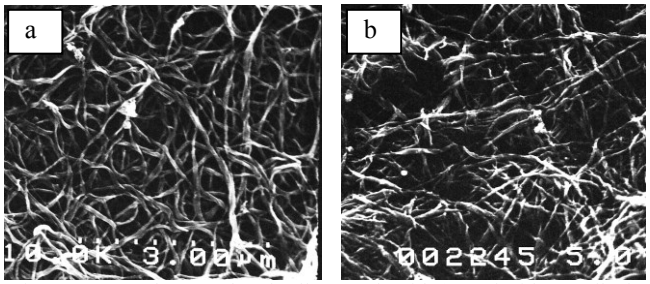

Figure 1. SEM photographs of collagen gels with (a) and without cells (b).

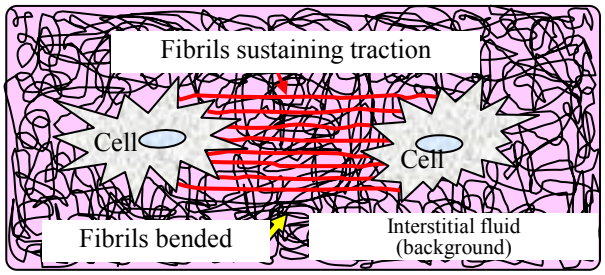

Figure 2. Illustration of the elementary structural model of gel contraction.

straight between two neighbor cells to sustain the force and induce the cells to move closely. Basing on this postulate, we develop an elementary structural model as shown in Fig. 2 to analyze the traction force exerted by individual cells. The critical points of the modeling are (1) the whole gel is divided into elementary units, each of which consists of one central cell and the surrounding collagen fibril network; (2) there exist some fibrils connecting two neighboring cells to be pulled straight so as to sustain the traction force; (3) the traction induce the two cells moving closely so that most other fibrils amid the units are bended to consequently resist the movement; (4) because of the ongoing contact and cross-linking among the fibrils, bending occurs on the fibril fragments sectioned by two succeeding intersections on the same fibril; (5) the elementary units are filled with interstitial fluid (culture medium), which also resists the movement.

The computation of bending resistance needs to estimate the length of the bending fragment. The estimation is based on contact point statistics of fiber packing theory developed by S Toll [5]. The theory deals with realistic random fibrillar networks and gives out a formula to estimate the average length of fiber fragment $<\lambda>$

$$<\lambda>=\frac{\pi d}{8\phi f}, \tag{1}$$

where ϕ is the fibril volume fraction; f is a scalar invariant of fiber orientation distribution, for 3D random orientation $f=\pi/4$; and d is the fiber diameter.

Bending force R_B on each fragment can be deduced by the definition

$$R_B=\frac{\partial E}{\partial \varepsilon}, \tag{2}$$

where ε is the fibril bending deformation and E is the bending energy which can be calculated by the following curve integration [6]

$$E=\frac{1}{2}k_B TA\int\kappa^2 ds, \tag{3}$$

where k_B is Boltzmann constant; T absolute temperature; A the persistence length of the collagen fibrils; and κ is the bending curvature of the fragment. The problem is how to calculate the curvature κ. Here we employ affine condition for the network deformation and furthermore, adopt network alteration concept, which regards the kinematics of filamentous network as a serial of newly formed structure different from the original one [7]. The version of network alteration in this study is reformed as that the movement of the cells and the attached fibril network is stepwise; at each step, there are new network state parameters ϕ, the fibril volume fraction, and $<\lambda>$, the average length of bending fragment. As the movement continues, ϕ increases and $<\lambda>$ decreases as indicated by (1). The bending at each step is thus not much large (gently curved as shown in Fig. 1a) and can be approximated by a sine curve according to [8]. Under this assumption, the curve integration in (3) becomes

$$\int\kappa^2 ds=\int_0^{\ell(<\lambda>)}\frac{\pi^4\alpha^2/\ell^4\sin^2(\pi x/\ell)}{(1+\pi^2\alpha^2/\ell^2\cos^2(\pi x/\ell))^{5/2}}dx, \tag{4}$$

where ℓ is the chord of the sine curve equal to the half of the period and α is the amplitude of the sine curve, thus $\alpha=\alpha(<\lambda>,\ell)$. The relationship between ℓ and $<\lambda>$ is determined by the affine condition.

The interstitial fluid resistance is calculated by

$$R_L=\frac{1}{2}\rho V^2 SC_D \tag{5}$$

where ρ is the fluid density; V the movement velocity; S the area projected at movement direction; and C_D is the resistance coefficient, which can be calculated by Ossen formula for the cell and by Lamb formula for the fibrils [9].

The cell movement is governed by the Second Law. However, because the cell mass and the acceleration are so small that the acceleration term can be neglected, the motion equation can be simplified as

$$F_T-R_B-R_L=0, \tag{6}$$

where F_T is the traction force exerted by individual cells.

To evaluate the adhesion force between individual cells and individual adhered fibrils, we introduce the following parameter

$$\tau=F_T\cdot\frac{4<\lambda>^2}{\pi c^2}, \tag{7}$$

where c is the cell diameter. It is the traction force F_T divided by the putative number of fibrils connecting to the cell surface projected at the mid-plane of two elementary units, and thus an estimate of the adhesion force to individual fibrils.

In practical computation, distance between two neighboring cells and the velocity of cell movement at each step were firstly calculated from experimental data, then ϕ, $<\lambda>$, R_B, R_L and finally the traction force F_T were computed by the above equations. Values of main parameters are listed in Table I.

TABLE I. PARAMETERS AND THEIR VALUES USED IN THE MODEL

Step interval (h)	c (μm)	d (μm)	A (μm)	T (K)	η (Pa s)
1.0	12.0	0.05	120.0	310	0.001

III. RESULTS

Time course of the area ratio of the contracted collagen gels (Fig. 3) shows the familiar facts that increasing cell number will increase gel contraction rate, whereas increasing initial collagen concentration will decrease it. We found the following empirical formula can fit the experimental data with good agreement.

$$z' = \frac{a + bt}{a + t}, \tag{8}$$

z' regresses experimental data, z, the area ratio; a and b are constant for each kind of gels; and t the culture duration in hour. The solid lines in Fig. 3 are the regression curves by (8). Table 2 gives the values of a and b. It can be seen that large cell number results in small a and b while large initial collagen concentration increases a and b. b is actually the contraction limit of the area ratio as t approaches to unlimitedness.

Fig. 4 shows the change of the collagen density in the gels having 0.9 million fibroblasts but different initial collagen concentration. Against intuitive expectation, after a few hours' contraction, gels with less initial collagen concentration have achieved larger collagen density than the gels with greater initial concentration. This phenomenon was also observed in the gels with 0.3 and 1.5 million cells. The features of the traction force of individual cells (Fig. 5) are summarized as follows: (1) there exists maximum of the traction force of individual cells, the time of the maximum positively related with the cell number if the initial collagen concentration is held constant; (2) the traction force increases as cell number increases; (3) cells generate larger traction force in the gels with less initial collagen concentration than those in the gels with greater initial concentration; (4) the maximum of the traction force falls into the range of 0.27-9.02 nN. Contrast to the "hill" profile of the traction force, parameter τ, the estimate of the adhesion for individual collagen fibrils, has large values at the early period and decreases later on (Fig. 6). The important outcome here is that except for the early contraction period, to ca. 10 hours from the beginning, the adhesion force for individual collagen fibrils is quite converged and stable under different gel conditions, particularly comparing with the wide range of the traction force of individual cells.

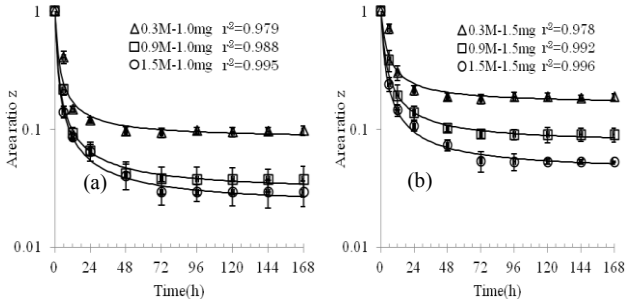

Figure 3. Change of area ratio of 1.0 mg gels (a) and 1.5 mg gels (b). Each data point is the mean±SD (n=3). Solid lines are the regression curves by (8), and $r^2=1-\Sigma((z_i-z')/z_i)^2/n$ is the coefficient of data determination.

TABLE II. VALUES OF a AND b IN (8) FOR DIFFERENT GELS

Collagen	1.0 mg		1.5 mg	
Cells ($\times 10^6$)	a	b	a	b
0.3	1.27	0.083	2.56	0.163
0.9	0.91	0.029	1.78	0.075
1.5	0.83	0.022	1.38	0.043

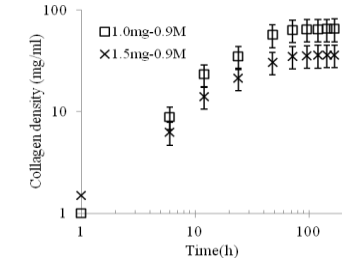

Figure 4. The change of the collagen density in the gels with different initial collagen concentration. Each data point is the mean±SD (n=3).

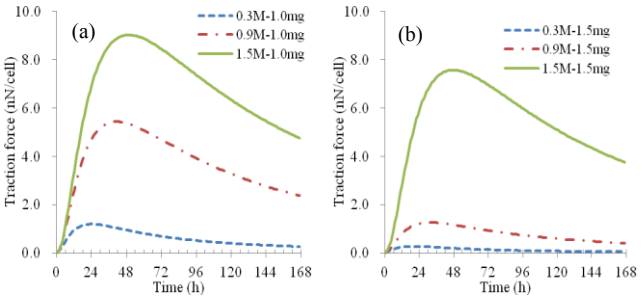

Figure 5. Traction forces of individual cells in gels with 1.0 mg/ml initial collagen concentration (a) and 1.5 mg/ml initial collagen concentration(b).

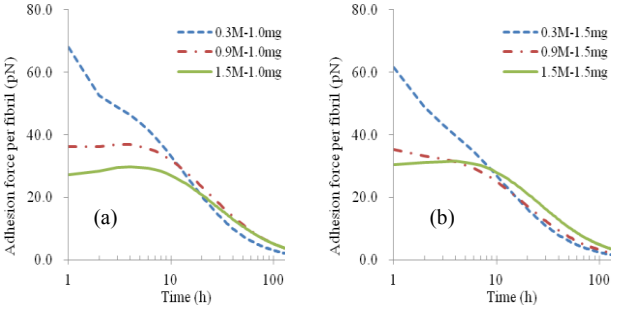

Figure 6. Estimate of the adhesion force for individual collagen fibrils in gels with 1.0 mg/ml initial collagen concentration (a) and 1.5 mg/ml initial collagen concentration(b).

IV. DISCUSSION

A novel model for the contraction of cell-populated collagen gels is developed in this study. We verified the validity of the model by the following evaluations: (1) the nature of collagen fibril bending is of elastic, the bending energy will finally diminish due to the ongoing cross-linking among fibrils and viscous dissipation from interstitial fluid. We have observed a ~3.0% expansion within 20 min in the diameter of 0.9 million-1.0 mg, 6 hour-culture gels after necrosis of populated cells was induced through adding 0.1 ml NaOH or 0.3 ml CH_3COOH to the culture medium, which shows the release of the elastic bending of the collagen fibrils primarily held by cell traction. (2) The macroscopic mechanical behavior of the collagen gels under external compression is Maxwell fluid-like, and the elastic element is mainly attributed to the elasticity of the collagen fibril network. We have measured the elasticity of the 0.9 million-1.0 mg collagen gels under external compression at low strain (<10%) and compared it with the theoretical value of the elasticity of the fibril network predicted by the model (Fig. 7). The model can fairly predict the elasticity of the collagen gels (the deviation between theory and experiment may be due to the effect of populated cells). (3) The adhesion force applying to one collagen fibril is

in the range of 1.0 to 70.0 pN (Fig. 6). The persistence length of collagen fibrils is estimated ca. 120 μm on the SEM photographs. Thus the characteristic force of entropic fluctuation of the collagen fibrils is $k_BT/A \approx 3.5 \times 10^{-5}$ pN, less than one 10000th of the adhesion force between cell and fibril. Therefore, the fibrils sustaining such force are quite fairly regarded as straight ones. (4) Similar time profiles of traction force of individual fibroblasts were reported [10, 11].

The modeling shows distinct roles of collagen fibrils as gel contraction. Some fibrils in the proper length potential to be pulled straight will sustain the traction force exerted by cells and induce the cells to move closely, while most of the other fibrils are bended due to the movement of the cells and thus in turn resist the movement. Cells extend dendrites during the early period of the culture, which makes them encounter more fibrils being able to sustain the traction force. Therefore, the traction force increases in the early period. The decrease of the traction force in the later period is partially because the number of fibrils to sustain the traction force no longer increases since dendritic extension has reached the limits, and partially because that the adhesion force to each fibril decreases, a phenomenon figured out by this study.

The more fibrils adhere to cells, the more fibrils can sustain the traction force; therefore, traction force of individual cells is basically related with the fibril density. Gels with less initial collagen concentration soon contract to larger density than that of gels with larger initial concentration (Fig. 4). Thus, traction force of individual cells in the gels with less initial collagen concentration is greater than that with larger initial collagen concentration. As for the effect of cell number, more cells means the cell-cell distance becomes closer, and then there are more fibrils can sustain the traction force because the length of the fibrils actually follows a probability distribution. In addition, more cells will enhance the cell-cell interaction, which will promote the contraction as reported by [12], and thereby increase the traction force.

The most interesting outcome of this study is that although traction force of individual cells in collagen gels is quite a dynamic quantity even under the free floating condition, the adhesion force between cell and individual fibrils is quite converging and stable except for the early contraction period. Time around 10-20 hour is a period when the adhesion of individual fibrils converges and decreases rapidly (Fig. 6), it may infer that gels with different conditions have achieve some similar characteristic state and start a new contraction phase during this period. We suppose these might be the formation of the network of cellular dendrite in this period as we have observed (data not shown here) and the beginning of cell migration (since large isotropic adhesion force to fibrils is not favorable to migration).

The advantage of the modeling is that it uses quite a few parameters (Table I), each of which is for the fundamental physical properties and can be easily measured. We realize that the calculated forces are the averages over the cell and fibril populations because (1) and (7) are both about the means of statistical variates. However, to discuss cellular behaviors at tissue-level these indices are much more important and

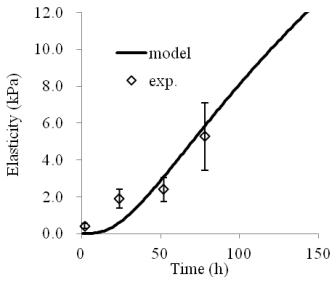

Figure 7. Elasticity of gels (0.9 million-1.0 mg) at different culture time measured by experiment and predicted by the model. Each experimental data point is the mean±SD (n=3).

representative to conceal cell-to-cell variation. The random network prerequisite assumed in (1) may limit the direct application of the modeling to other cases rather than free floating contraction. Particularly, fibril alignment is evident in the case of constraint contraction [2, 13], which requires new formula to estimate the length of bending fragment. Another limitation is that we did not include the influence of the change of cell size in (7). This influence may decrease the value of τ although the overall features of the parameter would not change. We are trying to re-evaluate f in (1) to incorporate the effect of fibrillar alignment and to measure the change of cell surface during gel contraction to address these limitations.

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