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The mechanisms of fibroblast-mediated compaction of collagen gels and the mechanical niche around individual fibroblasts

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ABSTRACT

Fibroblast-mediated compaction of collagen gels attracts extensive attention in studies of wound healing, cellular fate processes, and regenerative medicine. However, the underlying mechanism and the cellular mechanical niche still remain obscure. This study examines the mechanical behaviour of collagen fibrils during the process of compaction from an alternative perspective on the primary mechanical interaction, providing a new viewpoint on the behaviour of populated fibroblasts. We classify the collagen fibrils into three types – bent, stretched, and adherent – and deduce the respective equations governing the mechanical behaviour of each type; in particular, from a putative principle based on the stationary state of the instantaneous Hamiltonian of the mechanotransduction system, we originally quantify the stretching force exerted on each stretched fibrils. Via careful verification of a structural elementary model based on this classification, we demonstrate a clear physical picture of the compaction process, quantitatively elucidate the panorama of the micro mechanical niche and reveal an intrinsic biphasic relationship between cellular traction force and matrix elasticity. Our results also infer the underlying mechanism of tensional homoeostasis and stress shielding of fibroblasts. With this study, and sequel investigations on the putative principle proposed herein, we anticipate a refocus of the research on cellular mechanobiology, in vitro and in vivo.

1. Introduction

Fibroblast-mediated compaction of collagen gels attracts widespread attention in studies related to wound healing [1,2], cellular fate processes [3–6], and regenerative medicine [7–11]. Remarkably, however, more than three decades after this phenomenon was first described in detail by E Bell et al. [12], the underlying mechanism and the micro mechanical environment around individual cells still remain obscure.

Fibroblasts in newly formed collagen gels are characterised by their striking ability to protrude pseudopodial extensions – as long as several tens of μm within a few hours. These pseudopodia possess a microtubule core and actin-rich tips [13]; their extension has been demonstrated to be integrin mediated [14], and Rho-family kinases are involved in the regulation of the actin-myosin dynamics of the cytoskeleton [5,15]; and, they ultimately constitute a network via intercellular contacts [16,17]. In this process, collagen fibrils adjacent to the pseudopodia (or in the front of the tips thereof) become aligned with the long axis of the pseudopodia, which induces a local compaction of the network [18,19]. Morphological investigations have generally suggested that this compaction is caused by cells exerting a traction force on the attaching collagen fibrils, which in turn results in the accumulation of fibrils around the cells. However, recent studies have challenged this opinion [20].

At present, the anisotropic biphasic theory (ABT) [21] is the only sophisticated theory on this subject. This approach regards the

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collagen gel as a continuum biphasic material wherein the cells are simply a component of the network phase, which is phenomenologically modelled as a Maxwell-like fluid. The factor that drives gel compaction in the governing equations of ABT is a multiplicative term comprising cell-generated stress, cell concentration, and cell orientation. In other words, ABT explains collagen gel compaction as an integrated effect involving cell traction, cell migration and proliferation, and cellular and fibrillar reorientation. The deeply interactive nature of the various biological and physical elements of the gel compaction process deters the application of a simple cause-and-effect description, and it would be appropriate to approach the problem with due presumption of complexity [22–26].

The micro mechanical environment around individual cells undoubtedly plays a vital role in gel compaction, initially drawing attention due to its close relation to the physiological and pathological behaviours of wound healing [27,28]. And recently, the importance of this issue was raised in studies of the stem cell's niche [29–32]. The force exerted by individual fibroblasts in collagen gels has been measured in the range of 0.1–450 nN by means of direct measurement in gel slabs [33–36] or extrapolation through the deformation of the substrate [37–40]. Although these data show that the cellular contractile force is variable and sensitive to the extracellular environment and culture conditions, the overall micro mechanical environment has not been fully elucidated, quantitatively.

Therefore, despite the multitude of studies based on this model tissue, there is still a lack of clear answers to the following important questions. What is the physical process by which cells exert traction force on the surrounding fibrils while compacting the entire construct? While the effects of cellular random migration and fibrillar orientation are spatially limited to the immediate vicinity of the populated cells, the majority of the macro-compaction of the gels is attributable to those fibrils distal to the cells. This begs the question: In what state are those distal fibrils and what roles do they play in the process? A specific value of traction force results from the interplay between cells and the surrounding collagen fibrils. The mystery herein is the underlying regulatory mechanism by which a cell acquires so particular a value from within the broad range of its force-generating capacity.

These questions motivate us to try to disentangle the convolution of cause-and-effect in the hope of uncovering the initiating agent of collagen gel compaction, and to expose the micro mechanical niche around individual fibroblasts, by means of mechanical modelling. We particularly focus on the analysis of the collagen network because this, as the passive partner to the active populated cells in the compaction, is much more stable and easily identified. Although the fibril network itself is also a complex structure composed of random cross links and entangled fibrils, it is more readily elucidated than the populated living cells whose behaviour it exactly reflects, as the cell-fibrillar network provide the only primary mechanical action-counteraction coupling within the system [41]. Our approach is based on the statistics of fibrillar networks [42] and is inspired by network alteration theory [43]. We introduce the classification of fibrils into three different types according to their roles during the compaction process and deduce the respective governing equations that exactly describe the mechanical behaviour of each type. In particular, a putative principle based on the stationary state of the instantaneous Hamiltonian of the mechanotransduction system is employed in the deduction. We justify our theory by applying it to two representative fibroblast-collagen gel compactions: free-floating and quasi-uniaxial constraint. In the course of this justification, intriguing findings about the micro mechanical environment around individual fibroblasts in these two systems are obtained. Finally, we discuss the implications of our findings in this model tissue with regard to its predicated equivalence to the relevant cellular biology and connective tissue physiology.

2. Experimental materials and methods

2.1. Gel formation and compaction measurement

Two types of compactions, free floating (FF) and quasi-uniaxial constraint (UC) (Fig. 1), were experimentally investigated in this study. Modelling was evidenced and verified by the experimental data from these gels, and in turn revealing the (micro) mechanical niche around individual fibroblasts within the gels. Fabrication of the two types of fibroblast-collagen gels was as described in our previous studies [9,10]. Briefly, type I collagen (extracted from rat tail) and dermal fibroblasts (explanted from Wistar rat embryos and subcultured to 5 passages) were mixed with DMEM solutions and poured into two types of casting moulds (Fig. 1A,B). Each gel had a 1.0 ml initial volume; cell number and collagen mass are listed in Table 1. For UC gels, two T-shaped stainless steel wires were initially placed, one anchored to the well edge and the other connected to a vision-based micro force sensor (described below in *Measurement of the constraint force in UC gels*), for measuring the constraint force during gel compaction. The mixtures were allowed to gelate in a 5%-CO_2, 37°C incubator for 40 min. After gelation, the FF gels were displaced from the dish bottom by adding culture medium, resulting in a free-floating culture. The ends of the UC gels wrapped spontaneously around the T-shaped anchors. After culture medium was added to float the gels, compaction along the anchor direction was essentially constrained to the region between the two anchors, while compaction could occur freely in the orthogonal directions. As a result, the gels compacted to a string-like shape. The gels were cultured in 6 ml of DMEM supplemented with 1% penicillin–streptomycin and 10% foetal bovine serum (FBS) for one week.

Gel size was obtained by image processing of gel pictures using Adobe Photoshop CS4, and compaction was delineated as the ratio of the gel area (for the FF gels) or of the gel width (for the UC gel) to the corresponding initial values. A surface-marked cover glass was placed on the gels when gel thickness was measured, and 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.

2.2. Measurement of the elasticity of FF gels under external compression

Gel was immersed in phosphate buffer saline (PBS) and settled on the stage of an analytical balance. A controlled press moved downward to compress the gel at constant speed. After the compression strain reached 5%, the compression was halted and stress relaxation occurred. The compression/relaxation profile was regressed via a model composed of two parallel Maxwell fluid models (details in Supplementary information S1). The elasticity was regarded as owing mainly to the collagen fibrillar network, and was used to verify the theory of fibrillar bending (see 3. Modelling Theory).

2.3. Measurement of the constraint force in UC gels

We developed twenty vision-based micro force sensors and individually calibrated them. The principle of these sensors is that transverse force can be investigated by measuring the deflection of glass fibre cantilever through microscope (details in Supplementary information S2). Each sensor was settled onto one UC gel mould (as shown in Fig. 1B) and the constraint force during the gel compaction can be measured.

2.4. Observation of cellular morphology in gels

Following the addition of 0.3 ml acetic acid (99.7%, Sigma) to the culture dishes of 1 mg collagen-0.9 million fibroblasts gels (with a confirmed decrease in pH to 3.20) and incubation in a 37 °C incubator for 30 min, the collagen gel was dissolved. This allowed the morphology of the embedded fibroblasts to be clearly observed under a biological inverted microscope. Some of the cells that had formed a pseudopodial network within the gel could be separated by gentle pipetting. Pictures were taken of cells with pseudopodial processes, and measurements were made using Adobe Photoshop CS4; these included the diameter of the cellular soma, and the diameter and length of the pseudopodial processes. From these data, cellular surface area could be estimated.

2.5. Observation of collagen fibril morphology by scanning electron microscopy (SEM)

SEM samples for FF gel, UC gel, and cell-free gel were prepared by using standard protocol for biological tissues.

2.6. Observation of the expansion of the gel immediately after cellular necrosis

This experiment was to demonstrate that the nature of the fibrillar bending as gel compaction is of elastic. Cell necrosis was induced by adding 0.1 ml of 1 N sodium hydroxyl to the FF gel dishes containing 0.9 million cells in 1.0 mg collagen at 8 h culture. Then, gel diameter was measured by means of an inverted microscope.