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Asymmetry in traction forces produced by migrating preadipocytes is bounded to 33%

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ABSTRACT

Wound healing by gap closure is accomplished by the migratory cells within tissues. In fat tissue, the preadipocytes, stem cells committed to the adipose (fat) lineage, typically migrate into the wound area, and then differentiate into mature adipocytes to facilitate tissue repair and regeneration. While cell migration has previously been characterized, typically for fibroblasts, little is known about the dynamic, mechanical interactions of migrating cells with their microenvironment; cells crawl on a two-dimensional (2D) substrate by attaching and applying forces that allow them to extend leading edges and retract their rear. Moreover, preadipocytes, the highly migratory precursors of fat cells, have not been studied from this aspect. Here, we have evaluated the migration of preadipocytes, through their speed and directionality as well as the magnitude of the lateral forces applied during their migration on a 2D gel with Young's modulus of 2.44 kPa. We have found that the preadipocytes migrate non-directionally in the absence of chemoattractant, at an average rate of 0.27 μm/min, similar to fibroblasts. The preadipocytes exhibited a wide range of total traction forces (100–800 nN), and migrated along the long axis of their elongated morphology. Interestingly, we have observed an asymmetry in the location of force application between the lead and rear of the cells that was bounded in magnitude, where cells applied only up to 33% more force on either side; cell sides were defined relative to the minor axis of a bounding ellipse. These quantitative mechanobiological aspects of natural preadipocyte migration may shed light on the wound healing processes occurring in adipose tissue.

1. Introduction

Wound healing is one of the most challenging medical problems and is responsible for a significant fraction of costs of medical treatments. There are two general types of wounds, trauma wounds due, for example, to surgery or acute injury, and chronic wounds, such as diabetic or pressure ulcers. Pressure ulcers, for example, have a prevalence of 5.7–12.8% of patients in hospitals and 6.2–12.8% in surgical intensive care units, and their incidence is as high as 25–33% in community settings [1,2]; each serious case is estimated to cost $10k. Beyond the substantial costs associated with wound treatment, wounds reduce the quality of life o patients and can often be life threatening. Thus, there is an urgent need for development of cost-effective and efficient treatment approaches.

A central stage in wound healing is cell migration into the wound-gap in the tissue, and often also differentiation of migratory stem cells, which replace damaged cells to repair the tissue [3]. Consequently, the migration of highly motile fibroblasts and stem cells has been studied extensively. Migration can be triggered by gap (wound) formation, addition of a chemoattractant, or in the case of highly migratory cells, such as fibroblasts, can occur naturally, as part of physiological processes [4]. The cells' migration cycle includes various complex processes that rely on mechanical interactions between the cell and the surrounding matrix, such as structural polarization of the adhered cell, extension of a protrusion at the leading edge, and retraction of the cell rear [5-9]. These types of mechanical processes require cells to apply forces between cells and on the surrounding matrix or substrate [10]. The stiffness of the substrate and variations in it can affect cell migration [11]. Cell migration occurs either collectively, in groups or monolayers [12], as in embryonic development [13], or as single cells. Single cells may, for example extravasate through a blood vessel or cross tissue layers to reach a target location where they may differentiate and form tissues and organs [14,15].

Stem cells and fibroblasts typically constitute the population of cells that migrate during gap closure of wound healing. In fat tissue, adipose-derived stem cells migrate and affect the migration of neighboring fibroblasts during wound healing [3,16,17]. Stem-cells can remain highly migratory even after commitment to a

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specific lineage, but prior to differentiation. Thus, preadipocytes, the fibroblast-like cells derived from stem cells and committed to the adipocyte lineage, are highly migratory. The preadipocytes migratory nature allows them to be recruited and displaced by the body to increase fat storage capacity or to regenerate damaged tissue; their mobility is lost following differentiation into mature adipocytes. While the importance of preadipocyte migration is clear, little is known about their dynamic, mechanical interactions with during migration, and specifically, the forces applied by preadipocyte while crawling on a two-dimensional (2D) substrate have not been evaluated.

Here, we evaluate the dynamics and forces associated with migration of single, undifferentiated preadipocytes on a soft, elastic polyacrylamide gel (Young's modulus of 2.44 kPa). We concurrently evaluate the directionality of the preadipocyte motion, the migration rate, and the traction forces applied by the migrating preadipocytes. We show that natural, unstimulated (i.e. with no chemoattractants) preadipocytes migrate non-directionally on the 2D substrate, along the cell's long axis. The preadipocytes applied a wide range of total traction forces (100–800 nN), likely depending on the captured stage of migration. To highlight possible stages of migration, we have evaluated the asymmetry in the location of applied force, relative to the cells' minor axis; the cell axes and sides (i.e. left or right) were defined relative to the minor axis of a bounding ellipse. We observe that while the forces vary widely, the asymmetry of the force application is bounded, where cells apply only up to 33% more force on either of their sides.

2. Materials and methods

Cell culture. We cultured mouse embryonic 3T3-L1 preadipocytes (American Type Culture Collection, ATCC no. CL-173) in growth medium consisting of high-glucose (4.5 mg/ml) Dulbecco's modified Eagle's medium (DMEM, Gibco-Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum, 1% each of L-glutamine and sodium pyruvate (all from Biological Industries, Kibutz Beit Haemek, Israel), and 0.1% penicillin-streptomycin (Sigma-Aldrich, St Louis, MO). Cells were cultured and maintained in a humidified incubator at 37 °C, 5% CO_2 and were used at passages 10-20 from ATCC stock [18].

Polyacrylamide gel preparation. Polyacrylamide gels (PAM) were prepared as described previously [19-21]. Briefly, we used a glass cover slip, 30 mm diameter, #5 thickness (Menzel, Germany) as the basis for the gel. The glass was first coated with hydroxyl groups using 0.1 M NaOH, and then activated using 3-aminopropyltrimethoxysilan (both from Sigma-Aldrich, St Louis, MO), and then 2 μm diameter green florescent beads (Molecular Probes, Invitrogen Life Technologies, Carlsbad, CA) were glued to the glass by overnight drying; the fixed beads' position is used to remove small drifts occurring with time. After 24 h the glasses were activated with glutaraldehyde rinsed and dried for at least 2 h.

To produce gels with Young's modulus of 2.44 ± 0.04 kPa we mixed 34 μl of 40% vol. acrylamide and 3.8 μl of 2% vol. BIS acrylamide (both from Bio-Rad, Israel) in 203 μl of distilled water. To the monomer solution we added fluorescent, carboxylate-coated polystyrene particles, 200 nm in diameter (Molecular Probes, Invitrogen Life Technologies, Carlsbad, CA). Gelation was induced with 1:200 vol. ammonium persulfate (APS) as initiator and 1:500 vol. of tetramathylethylenediamine (TEMED) as a catalyst (both from Sigma-Aldrich, St Louis, MO). To reduce the polymerization rate, gels were prepared on ice and solutions were kept at 4 °C. A volume of 25 μl of the mixed solution was applied on the glass cover slip and locally confined (Gene Frame, 25 μl, 10 × 10 mm^2, ABgene Thermo-Scientific, Waltham, MA). Polymerization (gelation) was performed at 2 °C while centrifuging for 30 min at 300 g while the gels were turned upside down, so as to localize the 200 nm fluorescent beads to the gel surface [20,22]. After polymerization, the gels were rinsed with HEPES at pH 8.5 (Sigma-Aldrich, St Louis, MO) and stored in phosphate buffered saline. To facilitate adherence of living cells to the PAM gels, the gel surface was activated with Sulfo-SANPAH (Pierce, Thermo Scientific, Waltham, MA) and coated with collagen type 1 (Rat tail, Sigma-Aldrich, St Louis, MO). Glass cover slips with gels were placed in a custom made 6-well plate and stored at 4 °C until use.

Gel stiffness determination. The Young's modulus of the gels was obtained using a TA Instruments AR-G2 rheometer (New Castle, Delaware). The gels were prepared, at the 2.44 ± 0.04 kPa formulation, directly on the rheometer plate and their shear modulus was measured using a 2 cm diameter, stainless steel, parallel plate fixture. Time sweep experiments were run, with oscillatory strain of 0.5% and angular frequency of 3.14 rad/s, during and following gelation to determine the elements of the shear modulus: the storage modulus G' and the loss modulus G'' moduli. The storage modulus was 1-2 orders of magnitude larger than the loss modulus, indicating the elasticity of the PAM gel. Thus, the Young's modulus, E, was obtained using the following relation: $E = 2|G'|(1 + \nu)$, where $\nu = 0.49$ is the polyacrylamide gel Poisson's ratio [23].

Cell seeding and image acquisition. The preadipocytes in culture media were seeded on gels (30,000–40,000 cells/2 ml) and were maintained in a humidified incubator at 37 °C, 5% CO_2 for 24 h to facilitate cell adhesion. The amount of cells was chosen to enable single-cell imaging in each field of view. Thus, mechanical interactions are not expected to influence neighboring cells that are several cell-widths apart [24]. Cells and gels were imaged with an Olympus IX81 inverted, epifluorescence microscope, using a 60×/0.7NA differential interference contrast (DIC, Nomarsky Optics) air-immersion, long working-distance objective lens. Images were taken using an XR Mega-10AWCL camera (Stanford Photonics Inc., Palo Alto, CA), at a final magnification of 107.8 nm/pixel. To monitor the migration of preadipocytes, single cells at random locations on the gel were imaged at 20 min intervals, where each cell was observed for at least 3–4 time points, and a total of 45 separate images of single cells were obtained. At each time-point and each field of view, three images were acquired: DIC image of the cells on the gel, fluorescence image of the 200-nm diameter particles embedded at the gel surface, and a fluorescence image of the 2 μm diameter particles glued to the glass cover slip; measurements were carried out in the same location in each field of view throughout the experiment. Cells were imaged up to 90 min, until they migrated out of the field of view. Following imaging, the cells were removed using trypsin (EDTA 0.02%, Solution C, Biological Industries, Kibutz Beit Haemek, Israel), and images of the undeformed-gel were obtained in each field of view.

Traction force microscopy data analysis. The traction forces exerted by the cells were evaluated using modules in MATLAB 2012; codes were kindly provided by Ramswami Krishnan, Harvard University [25]. In short, we measure the displacement of beads in the gels, which are deformed by cell applied forces. The traction stresses applied by the cells to the gel are then determined using the Boussinesq solution that directly correlates the bead displacements (indicative of gel deformation) to the inducing traction stress through a convolution integral [26].

The bead displacements were obtained by cross-correlating images of the fluorescent beads at the gel surface at different time points, with the undeformed gels following cell removal. That provided the beads displacement maps, due to cell-gel interactions. The displacement maps were obtained using a sliding window of 32 × 32 pixels with a shift of 16 pixels. Displacement maps were transformed to Fourier space, and using an inverse Green's function, the traction stresses were obtained by simple tensor multiplication; the Green's function includes the gel Young's modulus