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STRAIN RATE DEPENDENCE OF CELL MEMBRANE RESERVOIR IS KEY TO IMPACT-INDUCED CHONDROCYTE DEATH

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SUMMARY

Impact loading of articular cartilage causes extensive chondrocyte death. Cell membranes have a limited elastic range of 3-4% strain, but are protected from direct stretch during physiological loading by their membrane reservoir, an intricate pattern of membrane folds. Using a Finite Element (FE) model, we suggested previously that access to the membrane reservoir was strain rate dependent and that during impact loading the accessible membrane reservoir is drastically decreased, thus, strain applied to chondrocytes is directly transferred to cell membranes which fail when strains exceed 3-4%. However, experimental support for this proposal was lacking. In this investigation, we measured the accessible membrane reservoir size for different membrane strain rates using membrane tethering techniques with atomic force microscopy (AFM). Force spectroscopy was conducted on isolated chondrocytes (n=87) using AFM. A micron-sized cantilever was used to extract membrane tethers from cell surfaces at constant pulling rates. Membrane tethers could be identified as force plateaus in the resulting force-displacement curves. Six pulling rates were tested (1, 5, 10, 20, 40, 80µm/s). The size of the membrane reservoir, represented by the membrane tether surface areas, decreased exponentially with increasing pulling rates. The current results support our theoretical findings, which suggested that chondrocytes exposed to impact loading die because of membrane ruptures caused by high tensile membrane strain rates.

INTRODUCTION

Osteoarthritis (OA) is a debilitating disease of synovial joints involving erosion of articular cartilage. Chondrocytes are the only cell type in cartilage: they have a slow turnover rate. Therefore, cell death decreases the number of cells and is typically associated with a gradual cartilage degradation due to a failure of the remaining cells to maintain normal tissue metabolism [1]. Previous experimental results suggest that compressive loads applied to cartilage at impact rates causes excessive chondrocyte deaths in the superficial zone [2, 3], while similar load magnitudes applied at physiological rates produce no harm to cells. During tissue compression, cells become flat [4] and cell membranes experience local tensile strains [5]. Although cell membranes have a small elastic range (3-4%) [6], they are thought to be protected from direct tensile strains by the 'membrane reservoir', a pattern of intricate membrane folds [7], which is unfolded when cell membranes are stretched. In our previous theoretical work, we predicted that impact loading resulted in cell death through excessive tensile membrane strain rates [5]. We proposed that access to the membrane reservoir was strain rate dependent and that during impact loading the accessible membrane reservoir was drastically decreased, thus, strain applied to chondrocytes was directly transferred to the membrane, resulting in membrane failure when strains exceeded 3-4% [5]. However, there is no experimental evidence supporting this theoretical finding. The size of membrane reservoirs can be quantified using "membrane tethering" techniques [7]. This study was aimed at measuring the membrane reservoir size for different strain rates using a membrane tethering technique with AFM. We hypothesized that the accessible membrane reservoir decreases with increasing strain rates.

METHODS

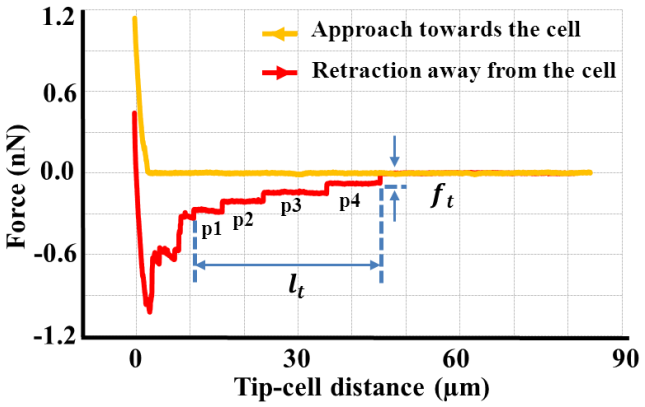

Figure 1: Force-distance curve obtained from force spectroscopy on a cell. The cantilever approaches the cell and deforms it with an initial force of 1.2 nN (orange curve, approach from right to left). After maintaining contact with the cell in a constant position for 20s, the cantilever is retracted from the cell (red curve, retraction from left to right) at a constant speed while pulling on membrane tether(s) (identified by the force plateaus). In this example, four tethers were extracted (marked as p1- p4). The variables measured were tether length (l_t) and force step (f_t)

Primary articular chondrocytes were seeded on a round-shaped coverglass and force spectroscopy was conducted on single cells (n=87) using AFM. A silicon nitride cantilever (BL-RC150VB, Asylum Research, Canada) with a nominal stiffness of 30 mN/m was used to approach cells at a speed of 2µm/s until the cantilever tip made contact and deformed

the cells with a force of 1nN. The cantilever tip was then kept stationary for 20s, after which the cantilever was retracted from the cell surface at a constant speed, extracting membrane tethers. Membrane tethers can be identified as force plateaus in the force-displacement curves (Figure 1). Six retraction speeds (1µm/s, n=62; 5µm/s, n=71; 10µm/s, n=36; 20µm/s, n=50; 40µm/s, n=47; 80µm/s, n=10) were used. Membrane tethering was performed at different locations on a cell surface and retraction speeds were randomized to avoid possible ordering artifacts. Force plateaus with a slope of $\leq$ 1 pN/µm were considered membrane tethers (Figure 1). Tether length was measured as the length of the force plateau(s). Assuming tethers to be cylindrical and that the tether radius, r_t, is related to the tether force, f_t [8] by:

$$r_t = 2\pi\kappa / f_t \qquad (1)$$

where κ is the membrane bending stiffness (= 0.2 pNµm) [9], the tether surface area could be calculated as $2\pi r_t l_t$.

The size of the accessible membrane reservoir is represented by the surface area of the last attached tether, measured from the first to the last force plateau. Each force step following a force plateau represents the detachment of a tether from the AFM tip. Tether force (force needed to hold a specific tether) is measured from force steps following a force plateau and is used to calculate the radii of the tether right before detachment (Equation 1). All data were expressed as means ± 1 standard error of the mean (SEM). Means were compared between pulling speeds using generalized estimating equations (α=0.05).

RESULTS AND DISCUSSION

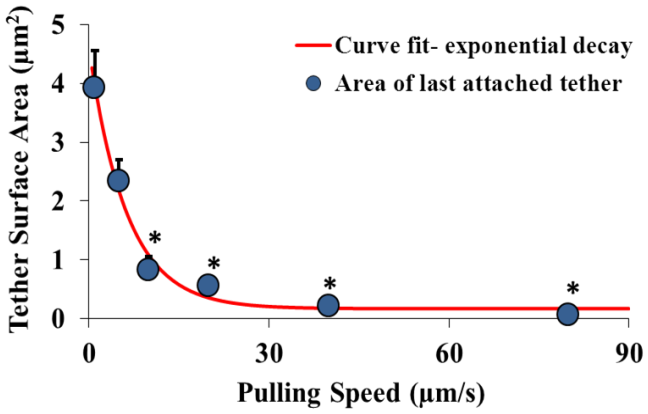

Figure 2: The tether surface area (representing membrane reservoir size) measured experimentally (blue circles) decreases exponentially with pulling speeds. The relationship is fitted well by an exponential decay law (red curve). The symbol '*' indicates significant differences in tether area compared to the area extracted at 1µm/s and 5µm/s.

The formation of membrane tethers indicates the existence of membrane reservoirs [7, 10, 11] because the force required for extension of a tether remains constant. The phospholipids extracted into the membrane tethers come from the cell membrane reservoir [7]. Since chondrocyte membranes experience tensile strains when cells are compressed [5], membrane tethering represents a way to quantify the size of the membrane reservoir as tensile loads are applied at different rates. We found that the size of membrane reservoirs decreases with increasing pulling rates (Figure 2). The relationship between membrane reservoir size and pulling rates was approximated well by an exponential decay law (red curve) using the formula:
surface area=4.44exp[-(pulling speed)/6.29] + 0.17 (Figure 2). This result agrees with our previous theoretical work on the causes of chondrocyte death during impact loading of articular cartilage, where we found that tensile strains not harmful under physiological loading conditions become harmful at impact loading rates because the accessible membrane reservoir is decreased to virtually zero at impact loading rates [5].

The tether force represents primarily the cell-cytoskeleton adhesions, and secondarily the membrane tension [12]. In agreement with previous studies [13], we found that the tether forces increased non-linearly with increasing pulling rates and that the relationship was well approximated by a weak power law (red curve): tether force=29.37(pulling speed)$^{0.34}$ (Figure 3). This result suggests that the retrieval of the membrane reservoir exhibits shear-thinning behavior and represents a viscoelastic process.

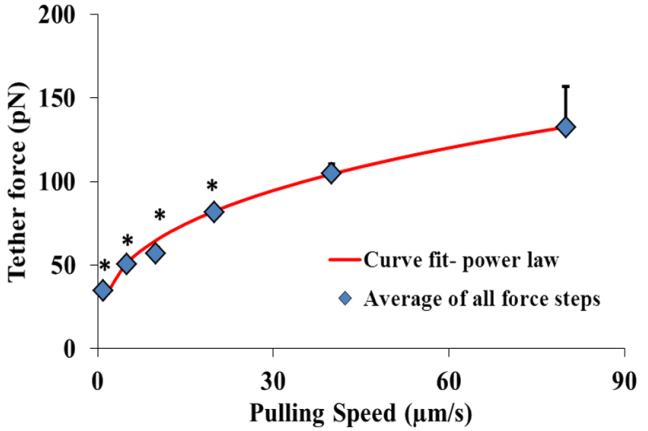

Figure 3: Tether forces measured experimentally (blue diamonds) depend non-linearly on the pulling speed. The relationship is fitted well by a power law (red curve), implying a shear-thinning behavior of the membrane tethering process. The symbol '*' indicates a significant difference in tether force compared to the tether force/radius obtained at 40µm/s.

CONCLUSIONS

We found that the size of the membrane reservoir accessible for strain buffering decreases exponentially with increasing strain rates, thus providing less protection to stretch of cell membranes. The increase in tether force with increasing strain rates implies that the recruitment of membrane reservoirs is viscoelastic. Therefore, our theoretical findings, which suggested that chondrocytes exposed to impact loading die because of membrane rupture due to excessive tensile membrane strain rates [5] is supported by our experimental findings.

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