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ACTIVE MANIPULATION OF ECM STIFFNESS

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INTRODUCTION

The mechanical properties of the microstructures surrounding cells influence the behavior of cells in differentiation, proliferation, and apoptosis, etc. The stiffness of the extra- cellular microenvironment has been shown to be one such mechanical property [1][2]. Studies reported in the literature concerning the stiffness of the extracellular microenvironment mainly sought to understand the scientific principles and mechanisms underlying its effect on cell-environment interaction [3]. This paper describes an approach that achieves such manipulation, and reports experimental results that demonstrate the effectiveness of this proposed approach.

MATERIAL AND METHODS

Superparamagnetic particles are embedded in the extracellular matrix (ECM) and manipulated with an external magnetic field to achieve active manipulation of the stiffness of the extracellular microenvironment.

Bead-ECM bio-conjugation

To have the beads bound onto the collagen fibers, a specific coating is necessary. This coating should have a good affinity with the collagen fibers such that it forms a strong attachment between the magnetic beads and the collagen fibers. We used beads coated with streptavidin since streptavidin will affix to areas dense with collagen. Streptavidin contains an Arg-Tyr- Asp (RYD) amino acid sequence that mimics the Arg-Gly-Asp (RGD) receptor domain of fibronectin. This form of bio-conjugation involves four different types, namely, hydrophobic, van der Waals, hydrogen bonding network, and covalent bonds. The complementary shapes, charges, polarity, and hydrophobicity of the streptavidin and the collagen fibers permit multiple weak interactions which in combination produce a tight binding [7][8].

ECM sample preparation

The collagen was prepared according to the recipes listed in Table I. Both samples (containing 2.0mg/ml of Rat Tail Collagen, Type 1) were obtained from BD Biosciences. The streptavidin-coated magnetic beads BM551 were procured from Bangs Laboratory. Two types of samples were prepared: one with magnetic beads and one without. For samples with magnetic beads, a concentration of 0.5mg/ml of the beads is added. The collagen (or the collagen with beads mixture) was thoroughly vortexed for two minutes until a homogeneous solution was formed and all the components of the mixture were spread throughout the entire volume. The mixture was then pipette into the mold carefully so that no air cavities would be formed. Fibrillogenesis was usually done in an external incubator at 37˚C and 5% CO_2. To achieve self- assembly of collagen molecules into fibers and binding of beads to the collagen fibers, the samples were placed in an incubator for at least 22 hours to ensure that gellation occurred throughout the entire collagen strip.

Evaluation of ECM stiffness

The micro-force tester has a load cell rated at 10N with a 0.001N resolution. Stretch tests were carried out on different sets of ECM samples at the fixed rate of 10 mm/min. While performing the stretch tests, a certain portion of these samples was exposed to a static magnetic field produced by a permanent magnet. This permanent magnet is made of an alloy of neodymium, iron and boron (NdFeB) and it is capable of producing a magnetic field of 2T. Two magnets form an aligned configuration such that the ECM sample is located between the magnets with a gap of 1.5 *cm* between one side of the ECM and a magnet, allowing the magnetic field to pass though the magnetic beads embedded in the ECM.

RESULTS

Six sets of tests (involving four ECM samples in each set) were conducted under the conditions as summarized in Table I.

TABLE I
TESTS

Set No.	No. of tests	Bead concentration	Magnetic field
1	4	0	Off
2	4	0	On
3	4	0.1 mg/ml	Off
4	4	0.1 mg/ml	On
5	4	0.5 mg/ml	Off
6	4	0.5 mg/ml	On

Stiffness of pure ECM

The slopes of the average of Set 1 and Set 2 differ by $5x10^{-4}$ in magnitude. To demonstrate that this difference in stiffness was due to sample variations and that the presence of the magnetic field did not affect the stiffness of pure ECM samples, the eight sets of data are randomly divided into two groups by picking two curves each from Set 1 and Set 2. The slopes of these two randomly divided sets differ by $5x10^{-4}$ in magnitude.

Change in ECM stiffness due to 0.5 mg/ml concentration of embedded beads

To see the effect of the embedded beads on the stiffness of the overall ECM, the linear approximations of the force-displacement relationships of ECM samples with and without beads and under the condition that the magnetic field was absent. The plots show that embedding 0.5 mg/ml of beads in the pure ECM slightly increases its stiffness. The difference (in magnitude) in the slopes of the linear approximations is $1\ x10^{-3}$.

Change in ECM stiffness due to magnetic manipulation of 0.5mg/ml concentration of embedded beads

There is a clear separation of the two set of curves from set 5 and 6 in the elastic region before sample rupture occurs. The set of four curves exhibits a higher slope (as compared to the other four curves) in the force-displacement relationship that corresponds to Set 6. Figure 1 shows the linear approximations of the force-displacement relationships from Set 5 and Set 6.

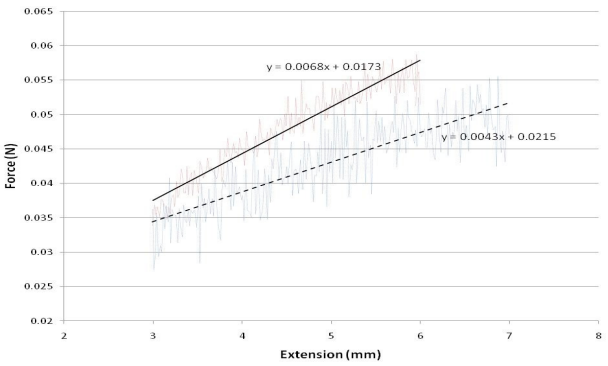

Fig. 1. Force-displacement relationships from Set 5 (dash line) and Set 6 (solid line).

Change in ECM stiffness due to 0.1 mg/ml concentration of embedded beads

To see the effect of the embedded beads on the stiffness of the overall ECM the stretch results are obtained from carrying out experiments according to set 3 and 4. The plots show that embedding 0.1 mg/ml of beads in the pure ECM reduces the difference in stiffness between pure collagen and magnetic bead embedded collagen. The difference (in magnitude) in the slopes of the linear approximations is $9\ x10^{-3}$.

CONCLUSION

The experimental results reported in this paper demonstrate that, by embedding magnetic beads in a ECM through bio- conjugation between the Streptavidin-coated beads and the collagen fibers, the stiffness of the ECM can be actively manipulated by the application of an external magnetic field. Such active manipulation can produce significant change in the stiffness of the ECM. The effect of a number of variables on the stiffness of the bead-embedded ECM remains to be explored; these variables include bead concentration, size and placement of the magnets and their distance to the ECM sample, and strength of the magnetic field, etc. The experiments described in this paper only concern manipulation of the ECM along a single axis. To study how ECM stiffness affects cell behavior in a realistic *in vitro* environment requires the ability to manipulate the stiffness gradient in the ECM in 3D. We are currently pursuing our research in this direction.

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