Active Manipulation of ECM Stiffness and Its Effect on Endothelial Cell Migration during Angiogenesis

Peter C. Y. Chen, Sahan C.B. Herath, Dong-an Wang and Harry Asada

***Abstract*—We have developed an engineering approach for actively manipulating the local stiffness of the extracellular matrix (ECM) and studied its effect on the migration of endothelial cells (ECs). In this approach, magnetic beads are embedded in an ECM through bio-conjugation between the Streptavidin-coated beads and the collagen fibers in order to alter the local stiffness of the ECM with the application of an external magnetic field. Our experimental results show that the magnetic forces acting on the embedded beads influence the behaviour of ECs during angiogenesis. These results suggest the possibility of creating desired stiffness gradients in an ECM for manipulating cell behavior *in vitro*.**

***Index Terms*—Stiffness, Manipulation, Magnetic field, Force, Angiogenesis**

I. Introduction

CELL migration, proliferation and differentiation rely, to a great extent, on their microenvironment. Two groups of factors, namely the soluble cues and the mechanical cues, influence cell behaviour. Soluble cues include chemical growth factors such as the vascular endothelial growth factor (VEGF), metabolites and dissolved gases, while mechanical cues are concerned with composition, architecture and mechanical properties of the extracellular matrix (ECM), in addition to cell-cell interactions.

It has been shown that the stiffness of the extracellular microenvironment, an insoluble cue, influences many types of cell behaviour [3][4]. For endothelial cells (ECs) in particular, cell migration behaviour is significantly influenced by the stiffness ECM [6][7][8]. This motivates our exploration of engineering techniques for mechanical manipulation of the ECM components in order to produce the desired change in its stiffness. The reaction of the ECs to the induced change in stiffness in their microenvironment will provide useful information on the significance of this insoluble cue during angiogenesis. This paper describes our proposed approach for mechancially manipulating the microscale stiffness of the ECM, and its effect on endothelial cell migration.

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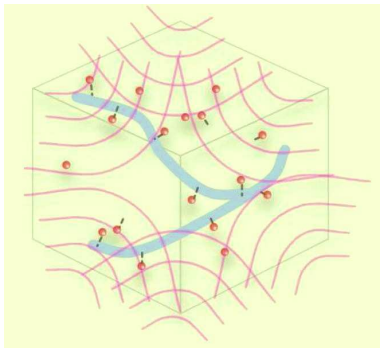
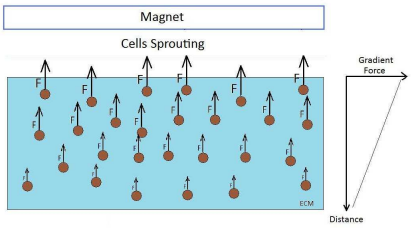
II. Material and Methods

We embedded magnetic beads in the ECM and delivered both the ECM and endothelial cells into a microfluidic device. The magnetic beads are embedded in the ECM via bio-conjugation between the beads and the ECM fibers as shown in Fig. 1a. Applying an external magnetic field on the ECM produces a force gradient (as illustrated in Fig. 1b) on the beads to resist the dislocation of the ECM fibers due to migration of ECs during migration. This increases the apparent stiffness in the vicinity of the ECs. With this approach, it is possible to observe the changes in sprouting behaviour of ECs when the apparent stiffness of the extracellular matrix changes.



(a) Bead Embedded ECM



(b) Force Gradient

Fig. 1: Schematic illustration of the proposed approach

A. Embedding beads in ECM

To embed beads in the ECM with bio-conjugation, magnetic beads were prepared with a coating that has a good affinity with the collagen fibers to form a strong attachment. We used streptavidin for the coating since it will affix to collagen-rich areas. 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 [12][13].

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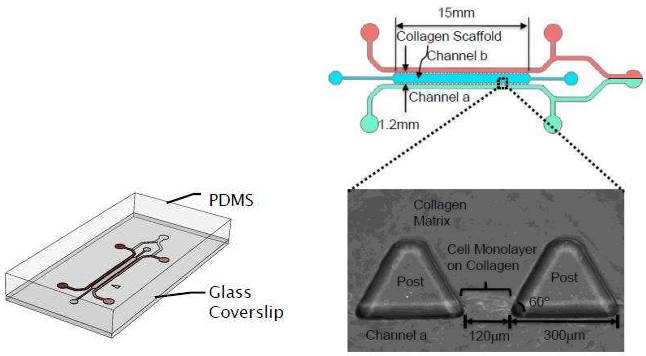
B. Preparation of samples

The collagen was prepared according to the recipes listed in Table I. All of the samples contain 2.5mg/ml of Rat Tail Collagen Type 1 (obtained from BD Biosciences, NJ, USA). The streptavidin-coated magnetic beads BM551 were procured from Bangs Laboratory, IN, USA. The collagen with a bead concentration of 0.075 mg/ml, was prepared according to the Table I.

TABLE I: Recipes for collagen preparation.

Collagen (4.62 mg/ml Stock Concentration)	0.216 ml (Diluted to 2.5 mg/ml)
Water PBS (10x) NAOH (0.5 N)	0.131 ml 0.040 ml 0.007 ml
Beads (5 mg/ml Stock Concentration)	0.006 ml (Diluted to 0.075 mg/ml)
Total	0.400 ml

The microfluidic devices (shown in Fig. 2) were fabricated according to the design by Farahat et al [14].

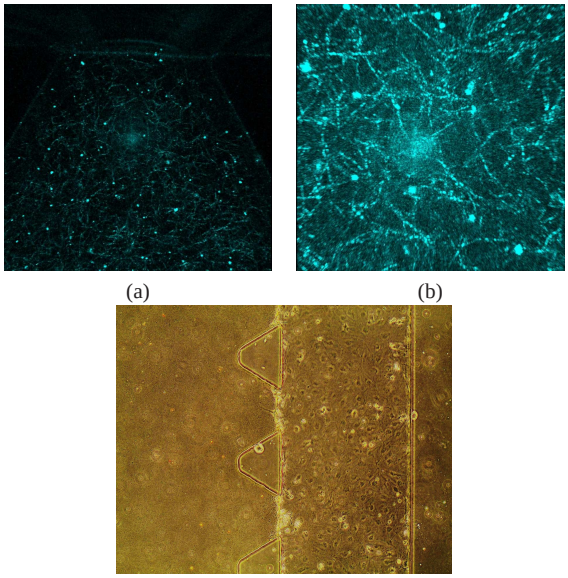


(a) (b)

Fig. 2: (a) The device is constructed from a PDMS layer made by standard soft lithographic techniques and bonded to a glass slide (b) The device consists of a central 3D collagen gel matrix (channel b) with a media channel on either side. The gel channel is bounded by 37 posts for the growth of endothelial cells. The endothelial cells were seeded in channel a.

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 pipetted into the central 3D collagen gel port (channel b) carefully so that no visible air cavities were formed. Fibrillogenesis was 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 gelation occurred throughout the entire collagen strip.

Once fibrillogenesis has occurred the magnetic beads and the collagen fibers were observed by reflectance microscopy. Fig. 3a and 3b show the reflectance microscopy image of beads while Fig. 3c shows the phase image of the cells and collagen with beads in channel b and channel a respectively. It can be observed from the reflectance images that after fibrillogenesis, the ECM consists of a network of magnetic beads (bright spots) and collagen fibers (bright lines).



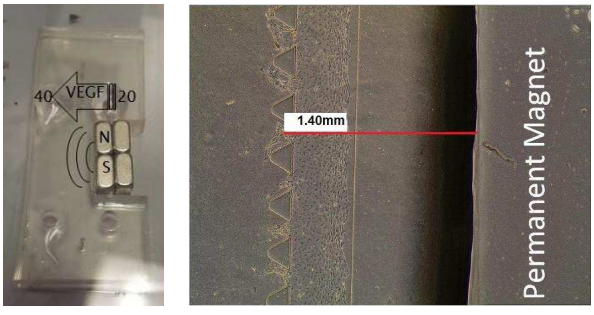
(a) (b)

(c)

Fig. 3: (a) (b) Reflectance image of collagen embdedded with magnetic beads. The bright lines indicate collagen fibers and the bright spots are magnetic beads (c) Phase image of gel in the middle channel and cells confluent in the side channel. The yellowish spots are the magnetic beads.

C. Device setup

A permanent magnet, made of an alloy of neodymium, iron and boron (NdFeB), capable of producing a magnetic field of 1.2 T at the surface was placed at the cell seeding side of the micrfluidic device. The magnet was located approximately 1.4 mm away from the EC sprouting region as shown in Fig. 4.



(a) (b)

Fig. 4: Experimental Setup

D. Magnetic force gradient

To compute the forces experienced by superparamagnetic particles, it is necessary to first compute the magnetic field, its magnetic field gradient and also obtain the magnetic moment of the beads m_{sat}. The force experienced by a single magnetic bead is given by the formula [16]

$$F = \nabla (m_{sat} \bullet B) \quad (1)$$

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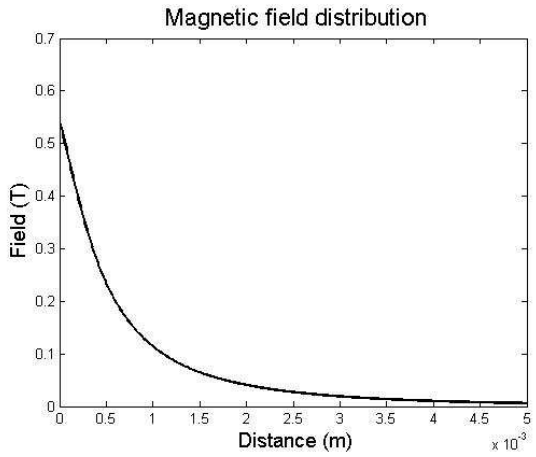
The magnetic field equation along the x-axis for a cuboid permanent magnet is given by [15]

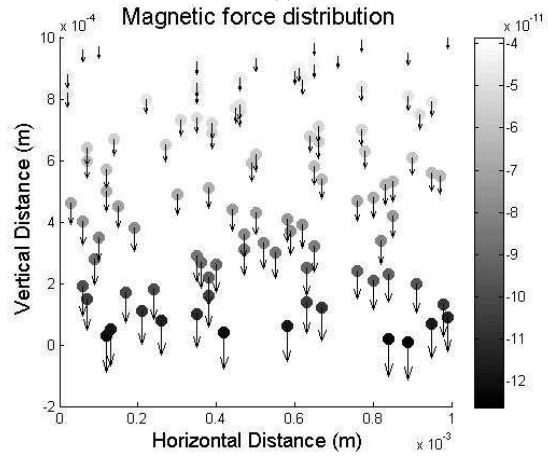
$$B_x(x) = \frac{B_r}{\pi}\left[tan^{-1}\left(\frac{ab}{2x\sqrt{4x^2+a^2+b^2}}\right) - tan^{-1}\left(\frac{ab}{2(c+x)\sqrt{4(c+x)^2+a^2+b^2}}\right)\right] \quad (2)$$

where a, b and c are the height, width and thickness of the permanent magent respectively.

The magnetic field distribution along the x axis for the permanent magnet that was used in this experiment is shown in Fig. 5a, with $a = b = c = 5$ mm and $B_r = 1.2$ T. It can be calculated that each of the superparamagnetic beads experiences a force of about 0.12 nN towards the permanent magnet at a distance of 1.4 mm. This force decreases as the distance between the magnetic beads and the magnet increases and it is shown in the simulated force gradient in Fig. 5b. The permanent magnet is attached to the channel with the cells (channel a) and a VEGF gradient of 20 40 is added as shown in the Fig. 4b.



(a)



(b)

Fig. 5: (a)Magnetic field generated, (b)Magnetic force on individual beads. The length of arrows and the grayscale intensities indicate the magnitude of force acting on each individual bead.

E. Experiment procedure

The medium was added once every 24 hours and the growth of the endothelial cells were observed under a microscope. Phase images were taken daily. To ensure repeatability, three independent experiments were performed. There were eight ECM samples (divided into two sets) in each experiment conducted under the conditions as summarized in Table II.

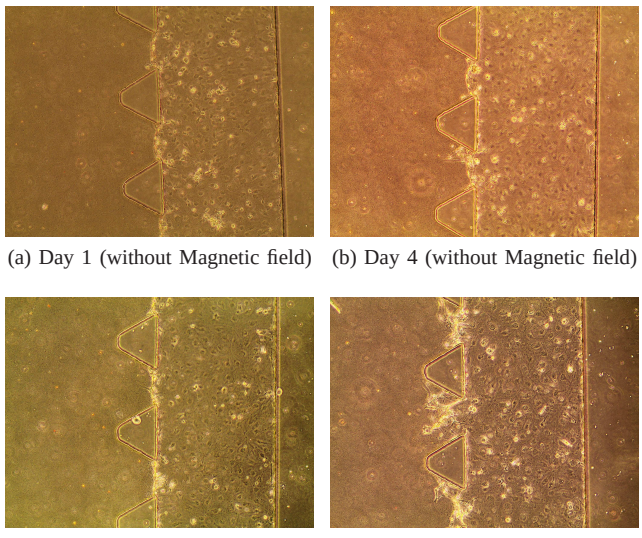
TABLE II: Tests

Set No.	No. of tests	Bead concentration	Magnetic field
1	4	0.075 mg/ml	off
2	4	0.075 mg/ml	on

III. Results and Discussion

Fig. 6 shows the phase images taken on Day 1 and Day 4 of the experiment. From Fig. 6b it can be observed that, although a very high VEGF gradient of 20 40 was added to the device, very little sprouting occured during the first four days. When considering ECs migration in this particular experiment, it has to be noted that the stiffness of Type 1 collagen embedded with magnetic beads is higher than that of pure collagen gel. This would also have an effect on EC sprouting, even without the external magnetic field applied.

It can be clearly observed that there was a higher level of angiogenic activity at Day 4 (Fig. 6d), for the case where the device was exposed to an external magnetic field. The amount of cell invasion into the collagen with gel has markedly increased in this case.



(a) Day 1 (without Magnetic field) (b) Day 4 (without Magnetic field)

(c) Day 1 (with Magnetic field) (d) Day 4 (with Magnetic field)

Fig. 6: Images of ECs migration on Day 1 and Day 4 in collagen gel with beads, without magnetic field (a and b) and with magnetic field (c and d).

Fig. 7 shows two sets of images of the microfluidic channel taken on Day 8. It can be seen that on Day 8 the ECs in the microfluidic device under the influence of the magnetic field had migrated further into the region of higher VEGF conentration, as compared to the ECs in the microfluidic device without the magnetic field.

The active signals 405 hoechst blue and 488 phaloidin green (produced from the confocal image in Fig. 7) indicate

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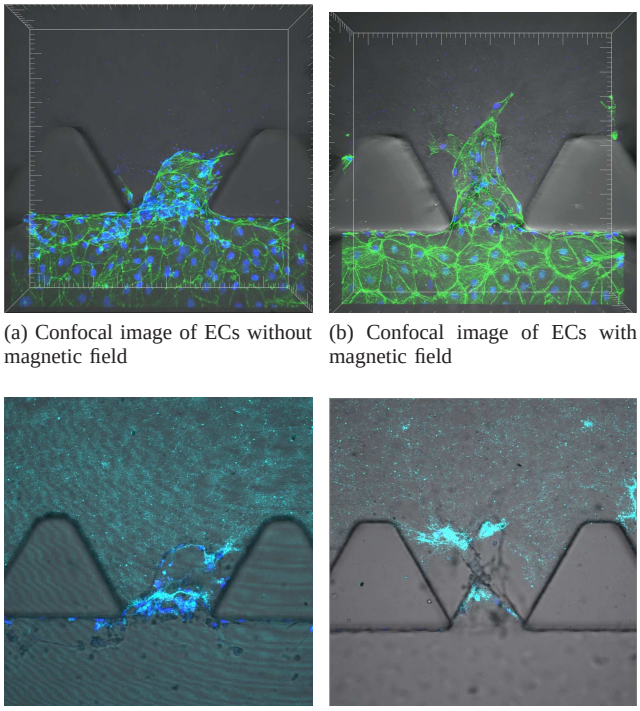

(a) Confocal image of ECs without magnetic field

(b) Confocal image of ECs with magnetic field

(c) Reflectance image of collagen gel without magnetic field

(d) Reflectance image of collagen gel with magnetic field

Fig. 7: Confocal images of ECs on day 8. The nucleus is stained with 405 hoechst blue and the actin filament is stained with 488 phaloidin green

no toxicities inflicted by the magnetic beads on the ECs during their proliferation stage. It can be concluded that the beads embedded in the ECM provided a platform similar to pure collagen for ECs to proliferate and migrate.

It can also be verified that the cell density (number of blue dots in Fig. 7a and Fig. 7b) along the monolayer was consistent between the two sets of samples. The green colour (from the stains for the actin filament) shows the cell structure of the ECs during angiogenesis. It can be observed from Fig. 7a and Fig. 7b that cell structure were similar in both cases. From the reflectance images shown in Fig. 7c and Fig. 7d it can be seen that the brighter areas, which indicate higher concentrations of collagen density, were locations of the tip cells [19]. Table III summarizes the key observations from these experiments.

TABLE III: Summary of ECs activity

Magnetic field	Beads	Activity during Day 1 to Day 4	Activity during Day 5 to Day 8
off	No beads	Average of 13 slots showed EC sprouting height of above 60 μm	Average of 16 slots showed EC sprouting height of above 120 μm
off	With beads (0.075 mg/ml)	Average of 14 slots showed EC sprouting height of above 60 μm	Average of 12 slots showed EC sprouting height of above 120 μm
on	With beads (0.075 mg/ml)	Average of **23** slots showed EC sprouting height of above 60 μm	Average of **26** slots showed EC sprouting height of above 120 μm

It can be seen that the application of the magnetic field on the beads significantly affected the activities of ECs. Embedding these particles in the ECM creates a crosslink zone as the beads bind onto the collagen fibers, resulting in an increase in the stiffness of the collagen. More significantly, when the magnetic field is present, a bead located approximately 1.4 mm away from the permanent magnet is estimated to exert a force of 0.12 nN. Collectively such forces in general oppose the cell traction forces (estimated to be up to 57 nN for an EC [20]) as ECs move away from the monolayer and into the collagen gel. This resistance can be considered as an increase in the apparent stiffness of the ECM in the vicinity of the migrating cells. Therefore the cells tend to migrate preferentially in such an environment, since ECs are known to increase motility and spreading in areas with higher stiffness [21].

IV. Conclusion

The results from this work have demonstrated the possibility of creating desired stiffness gradients in an ECM *in vitro* to influence cell behavior. Making this approach for active manipulation of ECM stiffness practicable requires further experimentation and analysis to clarify various issues. First, 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 the strength of the magnetic field, etc.

Second, although magnetic beads have been used in many applications (both *in vivo* and *in vitro*), their effect on the actual biological behaviour of cells are not yet fully understood. This is particularly true when an ensemble of beads are embedded in the ECM via bio-conjugation. It may be argued (based on available experimental results, e.g., [18][22][23]) that the bio-compatibility and toxicity of these magnetic beads with respect to its biological environment solely depend on the coating on the surface of the magnetic particles. Hence it is essential to verify that such an ensemble of beads do not directly interfere with the natural behaviour of cells.

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