Durotaxis Directed Cell Migration for Enhanced Vascular Stent Endothelialization

By Edwin Shen

Abstract

Vascular stents often cause adverse physiological responses as a result of inadequate endothelial coverage. The new generation of vascular stent designs includes substratums with nanopatterned topographies that enhance cell functionality for rapid endothelialization. Quick endothelialization can be achieved by promoting cell migration rate and guiding cell migration direction, factors accomplishable by durotaxis, stiffness directed cell motility. However, the stiffness gradient required to induce durotaxis has not yet been implemented in materials viable for vascular stents. **Our objective is to improve upon existing stent designs through durotaxis by studying endothelial cell migration on biodegradable chitosan modified with nanopatterned topography and gradated stiffness.** We will fabricate three nanostructured substrates with 400 nm pitch rectangular grooves and ridges possessing different stiffness gradients of 1 Pa/um, 10 Pa/um, and 100 Pa/um and seed them with human umbilical vein and human aortic endothelial cells. We expect our nanostructured substrates will outperform our only topographically modified (no stiffness gradient) control and flat surface control. The proposed study will not only offer a potential solution for improved endothelialization but also offer a proof of concept for integration of durotaxis to vascular stent designs..

Lay Abstract

Coronary heart disease, caused by fat buildup in blood vessels, is estimated to kill one American every minute. Severe cases of this ailment are treated with vascular stents, which are hollow cylindrical implants that expand inside vessels to push aside the fatty buildup. However,

these stents injure the vessel upon expansion, often causing thrombosis, a life threatening blood clot inside the vessel. A solution to prevent stent thrombosis involves accelerating natural healing by shaping the surface of the stent to direct cell movement from healthy tissue to injured tissue. Cells have been found to preferentially travel from softer materials to stiffer materials, therefore, this stiffness directed cell migration can be applied to stents by modifying various sections of the stent's stiffness. We propose to integrate this stiffness gradient on top of previously devised microscopic structures that also control cell migration. This new substrate can potentially be used in vascular stent designs that accelerate natural healing and prevent life threatening stent thrombosis.

2. Specific Aims

We hypothesize that endothelial cells seeded onto anisotropically nanopatterned chitosan gels with gradated stiffness will display a significant increase in migration rate and better guided unidirectional movement compared to only nanopatterned chitosan and flat chitosan. We plan to test this hypothesis with the following specific aims:

2.1 Specific Aim 1: Fabricate and characterize chitosan substrates with gradated stiffnesses and anisotropically nanopatterned surfaces.

The surface topography of the polymer will be molded into rectangular grooves and ridges of 400 nm pitch (pitch width = groove width + ridge width) via a silicon master shaped with plasma etching. The stiffness gradient of chitosan will be modified by altering crosslink densities through photopolymerization. Three different stiffness gradients will be produced in approximate elastic modulus changes of 1 Pa/um, 10 Pa/um, and 100 Pa/um. For controls, nanopatterned chitosan and flat chitosan, both without stiffness gradation, will be produced. The elastic modulus of the chitosan and the surface topography of the master silicon platform will be confirmed through atomic force microscopy (AFM).

2.2 Specific Aim 2: Seed then analyze migration of human umbilical vein and human aortic endothelial cells on chitosan substrates.

Human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) will be separately seeded onto the five chitosan substrates for 5 days. The migration rates and migrational directions of individual cells will be determined by software assisted time-lapse confocal microscopy. The data between different substrates will be analyzed by two-tailed, independent t-tests.

3. Introduction

Coronary heart disease (CHD) is the leading cause of death worldwide, estimated at 7.2 million deaths each year [1]. In the US alone, more than one million people are afflicted by the disease. In 2007, one of every six deaths in the US was from CHD, and it is estimated that one American will die from a coronary event every minute [2].

CHD is characterized by stenosis, the narrowing of arteries, caused by atherosclerosis, the buildup of fatty deposits in the endothelial layer of the vessel. The narrowed artery restricts blood from entering the heart and, over time, can weaken heart muscles, leading to heart failure and arrhythmias [3].

A common treatment for severe cases of CHD, angioplasty, is the insertion of a stent, an expandable cylinder, to physically push back the plaque buildup and widen the artery (reviewed by Wilson et al. [4]). The first generation of stents, bare metal stents, have only mechanical functionality and are still widely used today. However, they frequently encounter problems of restenosis, the recurrence of stenosis after treatment [5]. The introduction of biodegradable, drug-eluting stents that release anti-inflammatory agents have since reduced instances of restenosis [5], but present other problems of delayed healing, hyperplasia (excessive tissue growth) and thrombosis (the obstruction of vessels from blood clots) [6 - 8]. Stent thrombosis, the most serious of these complications can cause deaths in over 40% of its incidences [9].

The most powerful predictor and cause of these symptoms is poor endothelial coverage,

or endothelialization, of the injured area [10]. A possible solution for promoting endothelialization is to cover the stent with antibodies that bind to endothelial progenitor cells [11]. Another solution, which we will explore, is to pattern the stent surface to provide a topography for which endothelial cells (ECs) from healthy tissue may be guided to adhere to and migrate towards injured tissue [5].

This substratum directed functionality is known as "contact guidance" and, for ECs alone, have been explored in many studies [12 - 20]. These studies have shown that nanostructured topographies can promote EC adhesion as compared to the flat surfaces of current stent designs [12, 16 - 19]. These topographies can also promote EC migration, and if anisotropic, can control directionality of migration [13 - 15, 19, 20].

Cell migration can also be guided via substrate stiffness gradients, an event known as durotaxis. First discovered by Lo et al. in 2000, it was found that fibroblasts migrated away from polymer sheets of lower elastic modulus onto sheets of the stiffer same material [21]. Furthermore, the cells increased migrational speed when transitioning to the stiffer portion of the substrate. Since then, several studies have confirmed that other cell types, including vascular smooth muscle cells, mesenchymal stem cells, epithelial cells, and aortic endothelial cells have responded similarly to gradated surface stiffnesses [22 - 25].

The migration enhancing properties of durotaxis can be a powerful promoter of speedy endothelialization. However, studies have yet to apply durotaxis to improving vascular stents. We propose to use durotaxis, along with surface topography previously proven to control and promote cell motility, to enhance EC migration and endothelialization for vascular stents. We will study, in vitro, the response of ECs seeded onto a biodegradable, nanopatterned substrate with gradated stiffness. If successful, this application could provide a potential solution to the inadequate endothelialization, and the many related complications, of vascular stent implants.

Application of this novel material may save the lives of many out of the millions that will undergo angioplasty.

4. Research Design and Methods

Unless specified otherwise, materials will be purchased from Sigma Aldrich.

4.1 Specific Aim 1: Fabricate and characterize chitosan substrates with gradated stiffnesses and anisotropically nanopatterned surfaces.

This purpose of this aim is to apply nanopatterning and stiffness graduation fabrication techniques to a chitosan substrate. The finished product will enhance EC migration rates and tightly control EC movement direction.

Note: we will not be integrating our material into a stent, we will only shape a flat substrate that represents a section of the stent.

4.1.1 Aim 1 Experimental Procedures

To shape chitosan with rectangular ridges and grooves of 400 nm pitch and 100 nm depth, we proposed molding the chitosan substrate with a silicon master created from plasma etching. The silicon substrate, prior to etching, will be washed in acetone and isopropanol in an ultrasonic cleaner then rinsed by distilled water and dried in a vacuum oven. Megaposit's SPR-955 (photoresist) will be coated onto the material with a sputter coater prior to etching. To remove the photoresist, the silicon master will be washed again in an ultrasonic cleaner after etching.

A photoreactive chitosan substance will be prepared according to Yeo et al.'s procedures [26]. Chitosan (93% w/w 140,000 - 220,000 MW) will be dissolved in distilled water. If the

chitosan is contaminated with macroscopic shell bits (a common occurrence), the solution will be purified via microcentrifuge. Three separate solutions of N,N,N,N-Tetramethylenediamine in of distilled water, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in distilled water, and 4-azidobenzoic acid in dimethyl sulfoxide will be added to the chitosan. The solution will be left to react overnight and the chitosan will be purified through an Amicon Ultra (MWCO 10 kDa) centrifuge filter the next day. The solution will be poured onto the silicon master and set to cure at 65 Celsius, forming a photoreactive, nanopatterned chitosan substrate. We will ensure thickness of at least 1 um as thin films are known to interfere with AFM stiffness characterization [27]. The resulting nanopatterned substrate is depicted in Figure 1.

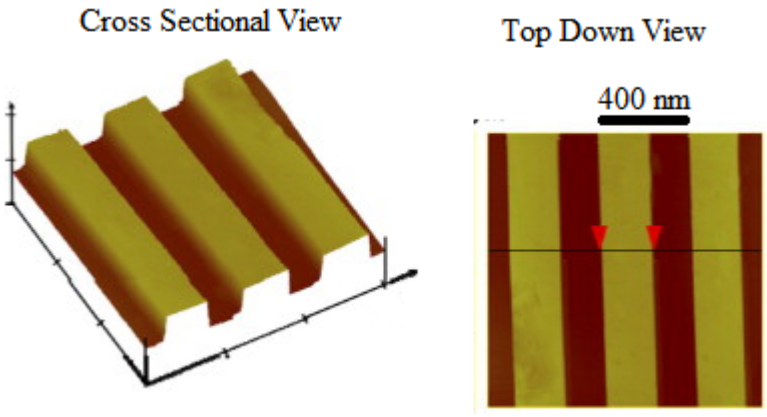

Figure 1. *The nanopatterned chitosan will have rectangular grooves and ridges of 400 nm pitch. Images were modified after taken from Lu et al.'s publication [12].*

UV photopolymerization will be used to generate a stiffness gradient according to procedures published by Sunyer et al. [28]. A Black Ray UV lamp (radiation range 315–400 nm, peak at 365 nm) will shine upon the masked chitosan solution, and as the mask moves away, varying the exposure of the chitosan, a gradient of crosslink densities will form. As higher crosslink density results in higher elastic moduli, a gradated crosslink density will result in

gradated stiffness. The movement of the mask will remain constant and controllable by a programmable linear motion stage designed by Johnson et al. [29]. The speed of the mask will need to be fine-tuned to create our expected stiffness gradients. This process is depicted in Figure 2.

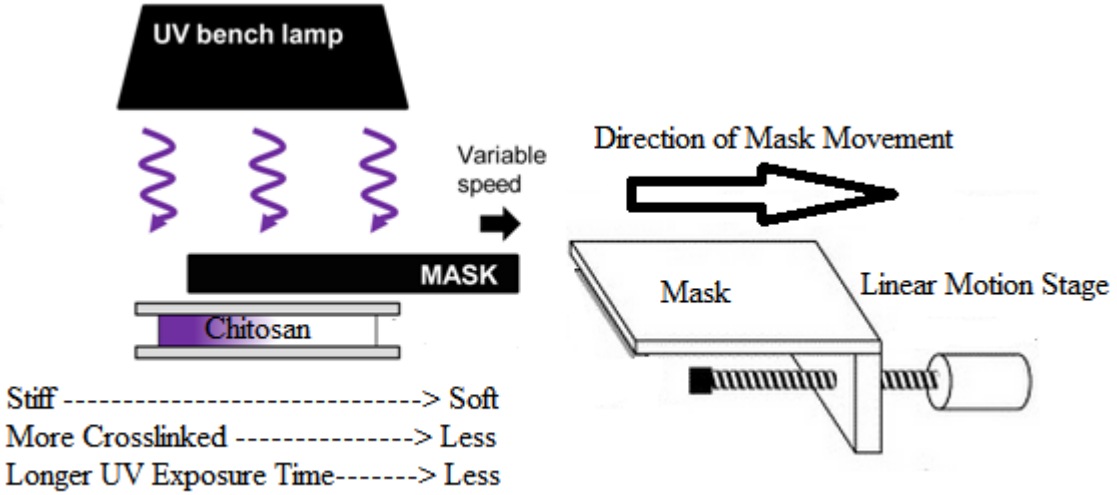

Figure 2. *Graduating the stiffness of the chitosan substrate will be accomplished through variable exposure photopolymerization. Images were modified after taken from Sunyer et. al and Johnson et al.'s publications [28 , 29].*

Fluorescent fibronectin (green fluorescent, HiLyte 488, Cytoskeleton Inc. Denver, CO) will be thinly coated over the chitosan for improved cellular adhesion. The fluorescence will be for characterization purposes later.

The final products will consist of five substrates. There will be four nanopatterned substrates, three of which will possess elastic modulus gradients of approximately 1 Pa/um, 10 Pa/um, and 100 Pa/um. One nanopatterned substrate will possess constant elastic modulus as a control. An additional substrate will be flat with a constant elastic modulus as a control.

To ensure that the substrate topographies possess properties as expected, the

stiffnesses and surface topographies of the substrates require characterization. For each substrate, AFM will detail the stiffness gradient, calculated with a Hertz model on Igor Pro [27], in 100 um steps across the length of the material. If the substrate does not exhibit elastic behavior, as one group encountered [23], the elastic modulus gradient can be estimated by fitting a linear model to the stiffness measurements. The pitch and depth parameters of the silicon master will also be measured with AFM. To ensure that fibronectin was evenly applied to all surfaces, we will measure for consistent fluorescent density across each substrate coated with fluorescent fibronectin.

4.2 Specific Aim 2: Seed then analyze migration of human umbilical vein and human aortic endothelial cells on chitosan substrates.

The purpose of this aim is to experimentally confirm the efficacy of the nanofabricated chitosan by measuring, in vitro, the migration velocities and trajectories of HUVECs and HAECs on the substrate.

4.2.1 Aim 2 Experimental Procedures

The HUVECs and HAECs (PromoCell, Germany) will be seeded on the softer end of the chitosan substrate and are expected to migrate to the stiffer end. The cells will be cultured in endothelial basal medium (PromoCell, Germany) and a pre-prepared endothelial cell growth kit, the EGM-2 BulletKit (Lonza, USA). The HUVECs and HAECs will be incubated at 37 Celsius and 5% CO2.

As tracking individual cells is tedious labor, the process will mainly be automated. The position of individual cells will be tracked with automated time-lapse confocal microscopy using a setup of a Zeiss custom incubated stage, a Marzhauser IM-EK32 motorized microscope stage, and a Zeiss Ludl MAC 6000 XY Stage Controller with Axiovision software. Microscope images, cell migration trajectories (defined as angle relative to the axis parallel to the direction of

stiffness gradation), and rates of migration will be processed and calculated by Igor Pro with an algorithm developed by McKee et al. [14]. As cell to cell interactions are known to interfere with contact guidance [13, 14, 21], cells that will come in contact with other cells will be excluded from the data. Cells that proliferate will be excluded as well. If the culture is too dense to measure individual cells, this specific aim will be repeated with fewer cells seeded.

Two-tailed, independent t-tests will test for statistically significant differences between the migration rates and directions of HUVECs and HAECs on the five different substrates. As cell migration follows a random walk model, we will also perform Kernel density estimations with MATLAB. This probability distribution will be more informative than the means and standard deviations of velocities and trajectories reported by many other articles.

4.3 Data Management

Data collected will be in the form of AFM outputs of the substrate stiffness, elastic modulus values calculated from those AFM outputs, AFM outputs of the substrate topography, data pertaining to the movement speeds of the UV mask and the resultant stiffness gradients, fluorescence density photographs of fibronectin, numerical values of those densities, time-lapse confocal microscopy images of ECs, migration velocities and trajectories of individual cells calculated from those images, Kernel density estimation results of those calculations, and statistical analysis results of those migration velocities and trajectories.

All data will be preserved in an external hard drive. The data will also be converted to .jpg files and spreadsheets before uploading to our lab website for peer scrutiny. The use of our data in other publications or products will be allowed only with our consent.

4.4 Timeline

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Aim 1	x	x	x	x	x	x	
Aim 2							x

Specific Aim 1: Fabricate and characterize chitosan substrates with gradated stiffnesses and anisotropically nanopatterned surfaces.

Specific Aim 2: Seed then analyze migration of human umbilical vein and human aortic endothelial cells on chitosan substrates.

This study is estimated to last at most 7 months.

Aim 1 involves a multi-stepped nanofabrication process. First, chitosan must be patterned with a plasma etched silicon master. This process is well established and should be a quick, straightforward process. Afterwards, the stiffness of the nanopatterned chitosan will be gradated through UV photopolymerization. The method we proposed was only published once and has never been applied to chitosan or any biodegradable polymer. Moreover, the movement speed of the UV mask must be fine-tuned through trial and error for us to produce a stiffness gradient of our desired ranges. The generous allocation of six months is primarily precaution for learning this relatively unknown section of procedure.

Aim 2 involves the setup of an automated time-lapse confocal microscope. This procedure is well established and should not encounter delay. The seeding and measurements span only ten days (5 days each for both cell types) and the data processing is a simple procedure. Why an entire month was allocated to Aim 2 is because cell cultures have tendencies for unexpected contamination or death. We also consider the slim possibility of having to repeat the seeding process in the case our culture is too dense to be suitable for migration calculations.

5. Rationale

This section will explain the decisions that formulated specific design elements of the procedure.

5.1 Rationale for Experiment Conceptualization and Significance

We hypothesize that endothelial cells seeded onto anisotropically nanopatterned chitosan gels with gradated stiffness will display a significant increase in migration rate and better guided unidirectional movement compared to only nanopatterned chitosan and flat chitosan.

Durotaxis, stiffness dependant contact guidance, has been proven to greatly improve cellular migration velocity and control migrational direction in many different types of cells [21 - 25]. Improving and controlling endothelial cell migration, via contact guidance, for improvement of vascular stents is an ongoing topic of study. These studies have proven nanostructured surface topographies to be highly effective [12 - 20], yet no designs have incorporated durotaxis, which requires a material of gradated stiffness. The reason for this gap is likely the difficulty of most nanofabrication techniques required to create a substrate of gradated stiffness. Moreover, no biodegradable polymers, the material of choice in the most advanced stents, have been modified to possess a gradated stiffness.

However, we devised a cost effective, stiffness graduating procedure that can be applied to chitosan, a biodegradable polymer known for its use in medical devices. Furthermore, by graduating the stiffness of a material that already possesses the topography of previous stent designs, we can create a substrate that combines the migration improving qualities of both durotaxis and nanopatterning. These qualities enhance endothelialization speed which mitigates the side effects of stent implants. As this design is the first of its kind, this study will also serve as a proof of concept for integration of durotaxis into vascular stents. Also, the flexibility of this

method can be adapted for integration of graduated stiffnesses to any surface topography and can potentially improve the migration enhancing properties of many stent designs. Although the scope of this experiment is only to test the material prior to integration into a stent, the ultimate goal is as depicted in Figure 3.

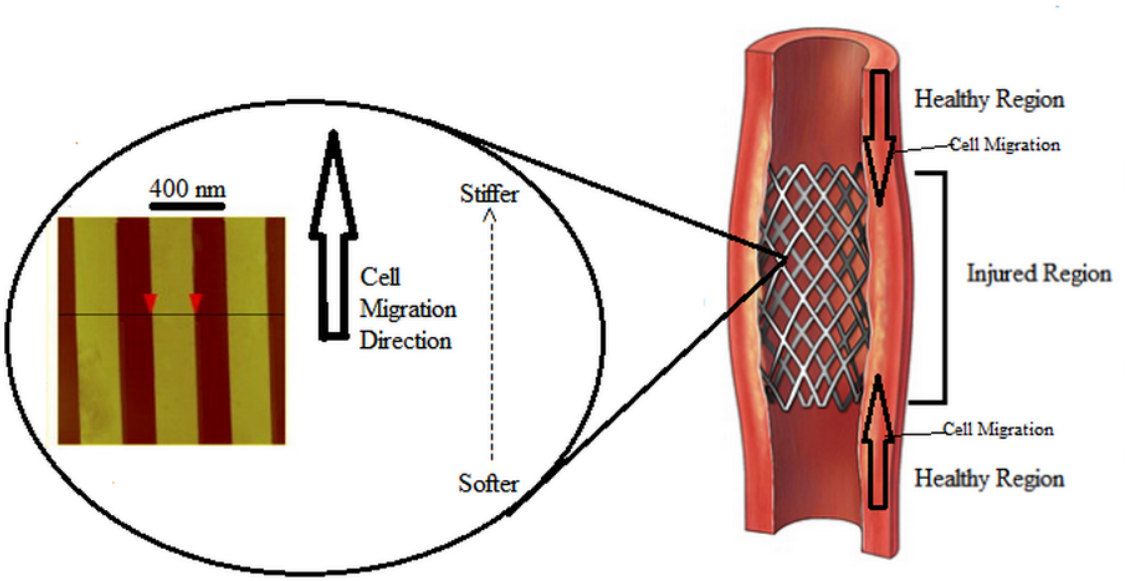

Figure 3. *The nanopatterned chitosan stent with a gradated stiffness is expected to better facilitate the migration of endothelial cells to vessel regions injured by angioplasty and mitigate adverse side effects. Images were modified after taken from Lu et al.'s publication and drbcshah.com [12].*

We expect the cells on the flat substrate to exhibit random walk with normal migrational speeds. We expect the cells on the nanopattern only substrate to exhibit bidirectional migrational direction parallel to the direction of the grooves and increased migrational speed. We expect the cells on the nanopatterned substrate with gradated stiffness to migration unidirectionally in the direction of the stiffness gradient from soft to stiffer material. In addition, the cells on this substrate is expected to have the highest migrational speeds. Between the three substrates with stiffness gradients, the substrates with the higher stiffness gradients will better enhance migrational velocity. The expected results are depicted in Figure 4.

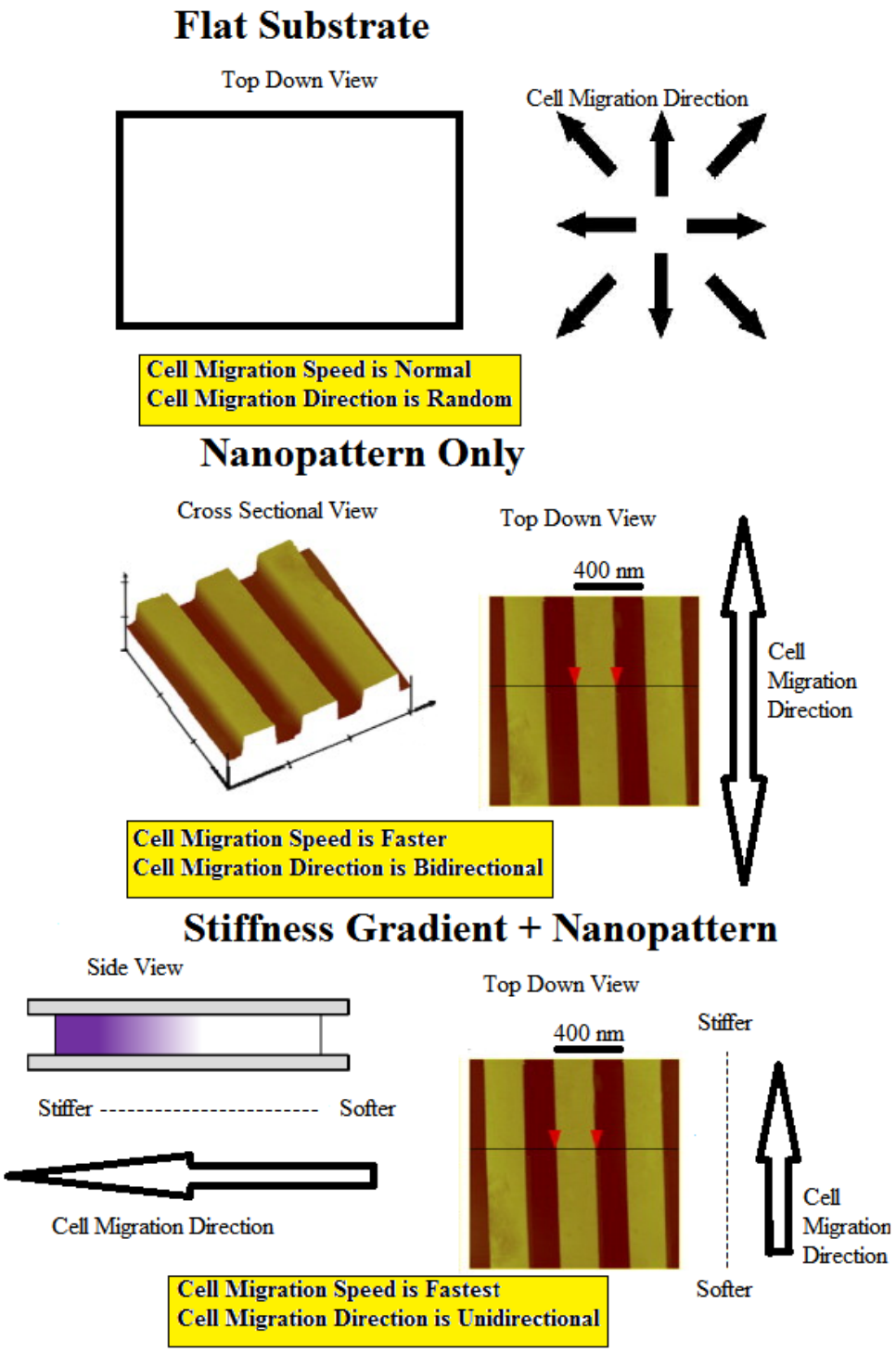

Figure 4. *The expected results of the different substrates. Images were modified after taken from Lu et al. and Sunyer et al.'s publications [12, 28]*

5.2 Rationale for Parameters of Substrates

400 nm pitch of rectangular grooves and ridges with 100 nm depth is selected as the feature scale and geometry based on previous success at promoting EC migration and controlling EC movement direction in prior studies of contact guidance [13, 15, 20].

The 1 Pa/um substrate is necessary in the case where graduating stiffness causes significant inconsistencies in the mechanical properties and biodegradability of the stent (one end of the stent is weaker than the other due to stiffness gradient). A gradient as low as 1 Pa/um would yield extremely mild inconsistencies. This mild stiffness gradient would still be sufficient to promote cell motility as demonstrated on mesenchymal stem cells [22].

The 100 Pa/um gradient is necessary in the case where graduating stiffness does not cause significant inconsistencies in the stent. The steep gradient of 100 Pa/um has been shown to be more effective, by a factor of six, in promoting cell motility than 1 Pa/um gradients as demonstrated on mesenchymal stem cells [22]. Even more impressive, cells on the 100 Pa/um gradient were not observed to reverse direction. Therefore the 100 Pa/um gradient, or even steeper gradients, may be most optimal for enhancing migration rates.

The 10 Pa/um substrate is selected as an intermediate range for comparison between the two extremes of 1 and 100 Pa/um.

The two controls are a substrate with nanopatterned surfaces without stiffness gradients and a flat substrate without stiffness gradient. These two controls serve as a means of comparison to gauge the efficacy of durotaxis in promoting cell migration as compared to designs without durotaxis and flat designs such as those found in modern stents.

5.3 Rationale for Nanofabrication Technique Choices: Plasma Etching and UV Photopolymerization

We propose to create a nanopatterned chitosan surface of the nanometer scale using a

plasma etched silicon master due to the prior success of such nanofabrication technique for similar studies of contact guidance [13, 15, 30].

Techniques to create gradated stiffness on polymers are much less established. Ways to create different stiffness on the same continuous piece of polymer range from simply placing multiple droplets of different density liquefied polymers under a glass coverslip [21] to light initiated polymerization through a gray-level photomask [31]. While simple, these methods are limited in precision and range. Other techniques that overcome these shortcomings include soft lithography for fabrication of microposts [32] and combinations of microfluidic generators and photolithography [33]. However, these techniques are expensive and difficult to implement.

The aforementioned techniques were only tried with acrylamide and, for the purpose of application to stents, requires adaptation to a biodegradable polymer. We propose to adapt chitosan to a cost effective and easy to implement, stiffness graduating, UV polymerization technique published by Suyer et al. [28]. The results of this technique on polyacrylamide were reported as precise and tightly controllable. Furthermore, the equipment required are commonly found in many laboratories.

5.4 Rationale for Material Choice: Chitosan

An ideal material for vascular stents would be a biocompatible material with reasonable mechanical strength and biodegradability. For the purposes of this study, the stiffness of the material should be easily graduatable.

We considered poly lactic acid (PLA) as PLA is the preferred polymer of choice for most biodegradable vascular stents because of its ideal mechanical properties and biodegradability. However, PLA requires a costly technique of irradiation to induce crosslinking [34]. Another polymer, polyacrylamide, was also considered for being well established for creating stiffness gradients [21, 28, 31, 33, 34]. However, polyacrylamide creates extreme inflammatory responses

and is not biodegradable as an implant material [35].

We propose chitosan because chitosan is adaptable to a simple, tested technique, published by Sunyer et al., that graduates stiffness [28]. This experiment also requires the polymer to possess a decent range of elastic moduli. Although photopolymerized chitosan has yet to be characterized, chemically crosslinked chitosan has been shown to have a range of elastic moduli from 1 GPa (normal chitosan) to 4 GPa (highly crosslinked chitosan) [36]. Chitosan has also been proven an effective implant material (section 3.3 of Chen et al.'s review [37]), popular for its compatibility with drug eluting stents and its natural adhesiveness that allows stents to remain in position [26]. Chemically and UV crosslinked chitosan has been tested to be biodegradable and caused no thrombosis with in vivo animal trials, however, the material caused inflammatory responses [26, 38]. We are also aware of the unlikely case where UV crosslinked intermediates may produce undesirable effects in living tissue, although animal tests indicated no such issues [26].

5.5 Rationale for Cell Choices: Human Vein Umbilical Endothelial Cells and Human Aortic Endothelial Cells

Endothelial cells from different sections of the cardiovascular system are not homogeneous [39]. In fact, different types of ECs have been shown to respond differently to the same substratum [13, 19]. Therefore, we must ensure that our stent design is applicable to multiple EC types.

Angioplasty is mainly performed in the coronary artery and less seldom performed in other arteries and veins [40, 41]. Therefore, we selected two cell types representative of the two vessel types. Also, these cells types have been used in similar previous studies, therefore our results can be more comparable [13, 14, 19].

6. Budget

This section will list all required equipment, their manufacturer, their purpose, and their cost along with staff salary.

Equipment, Material, or Staff	**Purpose**	**Manufacturer**	**Cost**
Principal Investigator	N/A	N/A	$35,000
2 Graduate Students	N/A	N/A	$35,000
Plasma Etcher	nanopatterning of silicon master	N/A	Provided by University
Ultrasonic Cleaner	cleaning silicon master in plasma etching process	N/A	Provided by University
Sputter Coater	applying photoresist in plasma etching process	N/A	Provided by University
Microcentrifuge	purifying chitosan	N/A	Provided by University
Vacuum Oven	drying and baking silicon master	N/A	Provided by University
Atomic Force Microscope	characterizing substrate stiffness and topography	N/A	Provided by University
Inverted Microscope (with confocal and fluorescence attachments)	to image cells and fibronectin	N/A	Provided by University

CO_2 Incubator	to maintain cells	N/A	Provided by University
MATLAB	statistical analysis and Kernel density estimation	Mathworks	Provided by University
Silicon Wafer	master mold for substrate patterning	Sigma-Aldrich	$107
Chitosan	substrate material	Sigma-Aldrich (93% w/w 140,000 - 220,000 MW)	$148
N,N,N,N-Tetramethylenediamine	photoreactive chitosan synthesis	CarboSynth (Berkshire, UK)	$53
1-Ethyl-3-(3-Dimethylaminopropyl)-Carbodiimide	photoreactive chitosan synthesis	Fisher Scientific (EDAC)	$470
4-Azidobenzoic Acid	photoreactive chitosan synthesis	Sigma Aldrich	$436
Dimethyl Sulfoxide	photoreactive chitosan synthesis	Sigma Aldrich	$36
YM-10	purify chitosan	Sigma Aldrich (NMWCO 10 kDa)	$363
Acetone	to wash silicon master	Sigma-Aldrich	$82
Isopropanol	to wash silicon master	Sigma-Aldrich	$62

SPR 955 Photoresist	plasma etching	Megaposit	$100*
UV Lamp	photopolymerization of chitosan	Black Ray (radiation range 315–400 nm, peak at 365 nm)	$633
UV Mask(Industrial Lead)	photopolymerization of chitosan	Amazon	$10
Programmable Linear Motion Stage	photopolymerization of chitosan	University Machine Shop	$2,000*
Fluorescent Fibronectin	to enhance adhesion on substrate, fluorescence is for characterization of fibronectin evenness	Cytoskeleton Inc. (Green fluorescent, HiLyte 488) (Denver, CO)	$785
Igor Pro Software	AFM stiffness and cell migration calculations	Wave Metrics	$435
HUVECs	in vivo tests of substrate efficacy	PromoCell (Germany)	$389
HAECs	in vivo tests of substrate efficacy	PromoCell (Germany)	$1,071
Endothelial Basal Medium	to culture cells	PromoCell (Germany)	$119
EGM-2 BulletKit	to culture cells	Lonza (USA)	$129

Axiovision Software	microscope imaging	Zeiss (Germany)	Free
Cell Migration Algorithm	to calculate cell migration rate and trajectory	McKee et al.	Free
Custom Incubated Stage	to maintain cell culture while imaging	Zeiss (Germany)	$8,000*
Marzhauser IM-EK32 Motorized Microscope Stage	for automated cell imaging	Marzhauser (Germany)	$3,000*
Ludl MAC 6000 XY Stage Controller	for automated cell imaging	Zeiss (Germany)	$1,000*

*Estimated pricing

Total Cost	**$89,428**

7. Public Health Impact Statement:

The current cure for severe cases of coronary heart disease, vascular stent implants, often result in side effects such as delayed healing, hyperplasia, and thrombosis; ailments mainly caused by poor endothelialization of the afflicted area. The death rate from stent thrombosis alone is over 40% per incidence. Therefore, it is crucial that future stent designs accelerate endothelialization, a feat accomplishable by altering the vascular stent surface topography. This study will provide a novel stent design to enhance endothelialization and curb incidences of related stent side effects.

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