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Influence of Hydrogel Substrate on Cell Growth and Virus Infection

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Abstract of the Thesis

Influence of hydrogel substrates on cell proliferating and virus infection

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The interaction of cells with their extracellular matrix is of great importance when cells adapt to their environment. The purpose of this thesis is to design substrates with controllable properties and to study cellular interaction on these substrates. Firstly, enzymatically cross-linked gelatin hydrogels with different elastic modulus were prepared. Then, we studied the condition of cell growth and virus infection on these hydrogels as a followed-up.

In the first part of the study, we made enzymatically cross-linked gelatin hydrogels with five different elastic modulus. As a parameter of stiffness, elastic modulus varies from 2.4 KPa to 7.5 KPa based on which hydrogels are graded from soft to hard.

In the second part, we studied the growth of rabbit kidney cells cultured on hydrogels of different stiffness. Growth curves of the cells were made to study the abilities of soft and hard hydrogels to support cell proliferation. Result shows that cell proliferation rate differs when using hydrogel substrates of different stiffness as substrates.

In the third part of this thesis, we infected rabbit kidney cells with pseudorabies virus for a period of time . And confocal fluorescence microscopy was used to investigate the influence of hydrogels with different elastic modulus on infectivity of the virus.

Dedicated to

My dearest parents

for

Their love and support

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Special thanks to all my good friends in Stony Brook University who helped me adapt to the new environment quickly and made these two years so special. You are all my friends for life.

This thesis would not be complete without mentioning the most important persons in my life, my parents. No words can express how much I love them. I am really grateful to all the love they gave and all the sacrifices they made while raising me up and to support my studying in the United State. I am really fortunate to be a son of yours, Mom and Dad.

Chapter 1

Introduction

1.1 Background

With the rapid pace of development in medical science, diseased or damaged human organs or tissues are able to be repaired or replaced nowadays. Due to the shortage of organs, there is always a stupendous demand of prosthetic or biological materials. Therefore, as tissue engineering (TE) emerged in the middle of 1980s, it has continuously been a multidisciplinary field full of attention and excitement. It has been rapidly developed to meet the demand of tissues and organs [1–4]. The aim of TE is to provide growing and functioning substitutes which can replace or regenerate damaged tissues [4–6]. The key factors of tissue engineering are cells, scaffolds and growth-stimulating signals which is also called the TE triad [4, 7]. Development of hydrogels applied in tissue reconstruction keeps in a rapid pace in recent two decades. In 1993, S. Woerly discussed the possibility for hydrogels being used in neural tissue reconstruction [8]. Y. L. Cao et al. successfully created a autologous tissue-engineered cartilage in the shape of a human nipple on an

immunocompetent porcine animal model in the year of 1998 [9]. C. Halberstadt et al, in 2004, made hydrogel injections into the subcutaneous space of a sheep to study the hydrogel in reconstructive applications [10]. In the year 2011, polyacrylamide hydrogel injection has been successfully applied to help HIV-infected patients suffering from severe facial lipoatrophy with facial reconstruction [11]. As an efficient scaffold, such as the one used in human facial reconstruction mentioned above, it should have similarities to the natural extracellular matrix (ECM) both physically and chemically to support cell proliferation, and even more, function controlling [12–14]. Hydrogels, due to their compositional and mechanical similarities to the ECM which leads to high biocompatibilities as seen in successful use in the peritoneum [15, 16] and other sites in vivo, have long received attention as great scaffolds for tissue engineering as well as drug delivery vehicles [17, 18]. The unique physical properties, that hydrogels have porous structures which can be easily controlled by managing the density of cross-links and the the ability to swell in aqueous environment, draw people's attention to hydrogels in drug delivery applications [15]. The fact that hydrogels can absorb from 10% to as much as thousands of times of their weight in aqueous media, becomes one of the reasons for cells to be able to proliferate, migrate and differentiate on hydrogels [19]. And the performance of hydrogel structures benefit from being similar to the natural ECM makes it possible for cells to affect their own environment [12, 20].

By definition, hydrogels are gels of great abilities to be swollen in aqueous environment, and are composed of hydrophilic material components cross-linked in a three-dimensional network [21]. They are regarded as deformable viscoelastic materials as the cross-links give a dimensional stability while they have fluid-like

transport abilities due to the high content of solvent. With all the properties, hydrogels play a variety of roles in tissue engineering. They are used as scaffolds that organize the formation of cells to a demanded tissue, as molecule delivery vehicles, or simply as space occupying materials. Based on the different mechanism of cross-linking, hydrogels can be divided into three types, which are:
1) Chemically cross-linked hydrogels forming covalent bonds, 2) physically cross-linked hydrogels which has chain entanglements, association bonds such as hydrogen bonds, Van der Waals interactions or ionic interactions [22], and 3) enzymatically cross-linked hydrogels in which covalent bonds dominate [23].

1.2 Objective and methodology

As a basic study of hydrogel, this thesis is aimed to investigate the behavior of cells and virus on hydrogels with a changing parameter of gel stiffness.

In order to obtain hydrogels of different stiffness, we made the amount of enzyme used vary when preparing the enzymatically cross-linked gelatin-mTG hydrogels. Then, a rheometer was used to measure the elastic modulus of hydrogels prepared. To obtain the growth curve of cells and infectivity of viruses, confocal fluorescence microscopy was used to image the specimens which were stained.

1.3 Thesis Structure

Chapter 2 presents the details of how we made hydrogels of five different stiffness. The method of measuring the elastic modulus of hydrogels are also introduced in

this chapter.

Chapter 3 is focused on how cells grew on these hydrogels. By the work done in this chapter, we mean to find a stiffness of hydrogel most suitable for RK13 cell to be cultured on, which may potentially have applications in tissue engineering field. In addition, this chapter reveals the methods we used to observe the cells and analyze the data.

In chapter 4, virus infection of cells on hydrogels is studied to evaluate if there is a potential threat to use hydrogels as drug delivery vehicles or tissue reconstruction scaffolds in certain conditions for there might be a possibility that hydrogels promote the infection of virus.

Chapter 2

Preparation of Enzymatically Cross-linked Gelatin Hydrogels With Different Elastic Modulus

2.1 Experimental Materials

Porcine type A gelatin with a gel strength of 300 g Bloom from Sigma Aldrich was used as a source of polymer to prepare hydrogel.

Microbial transglutaminase (mTG) enzyme was used as a cross-linker.

2.2 Experimental Equipments

Filters with a pore size of 0.2 μm along with 5 ml syringes from BD were used to sterilize both hydrogel and mTG solution, as most fungi and bacteria are not likely to be smaller than 1 μm in scale.

A Bohlin Gemini HR nano rheometer from Malvern Instruments was used to carry out oscillatory shear rheometry tests to obtain the elastic modulus of the hydrogels.

2.3 Experimental Steps

2.3.1 Preparation of Solutions

mTG was dissolved in deionized (DI) water with a concentration of 0.1 g/ml (10%(w/v)), and was kept frozen for storage.

2.3.2 Preparation of Hydrogels

Hydrogels were made by dissolving gelatin in DI water with a concentration of 0.1 g/ml (10%(w/v)), followed by sterile filtration through the filter at a temperature of 60-70 °C. These hydrogels are in solid gel state at room temperature, but thermally reversible as they will change into liquid state when heated.

What we did next was mixing the mTG solution with gelatin solution at the temperature about 40 °C. The gelain-mTG hydrogels were then put in a cell culture incubator at 37 °C for 24 hr during which crosslinking reaction took place. The amount of mTG and gelatin solution used when mixing is shown in Table 2.1. With the increasing of gelatin-mTG ratio, the hydrogels are graded from hard to soft. Hydrogels with a gelatin-mTG ratio of 3:1 become the hardest while 125:1 indicates the most soft ones. Hydrogels were then heated in a rocker at 65 °C for 10 minutes to deactivate mTG. These hydrogels are thermally stable and will stay in solid state when being heated.

2.3.3 Dynamic Rheological Characterization

By using the rheometer, a stress sweep was done on the hydrogel at a constant temperature of 37 °C and frequency of 1 Hz while the shear stress was increasing from 1 to 5000 Pa. In this test, the shear stress applied on hydrogel was controlled in every cycle while the corresponding strain was measured to obtain the elastic modulus as:

$$Elastic\ modulus = Stress/Strain \tag{2.1}$$

A constant elastic modulus can be clearly observed in the result graph page of the test till it reaches the yield point of the material which leads to a downhill in the graph. The elastic modulus decreases rapidly after the yield point as the structure of hydrogel is about to break down.

2.4 Results and Discussion

Plots of elastic modulus are shown in Figure 2.1 which represents the stiffness and yield stress of each hydrogel. Figure 2.1 shows that hydrogels, from hard to soft, have elastic moduli of 7.5 KPa, 6.3 KPa, 5.2 KPa, 3.2 KPa and 2.4 KPa. As observed from the plots, elastic modulus remains constant before the yield point, which illustrates the structural stability of the hydrogel under these conditions. A yield point represents the highest shear stress hydrogel can take before being structurally which will finally lead to the breaking of bonds between chains.

It is obvious that hydrogel which had a higher mTG enzyme concentration maintains a higher cross-linking rate, making the chains more strongly connected.

Therefore, under the same shear stress, hydrogel with a lower gelatin-mTG ratio performs less strain which leads to a higher elastic modulus.

2.5 Conclusion

In this step, enzymatically cross-linked hydrogels were successfully prepared. We controlled the concentration of mTG enzyme to obtain hydrogels of different stiffness and quantified the stiffness in elastic modulus through rheometry tests for further studies in this thesis.

2.6 Figures and Tables

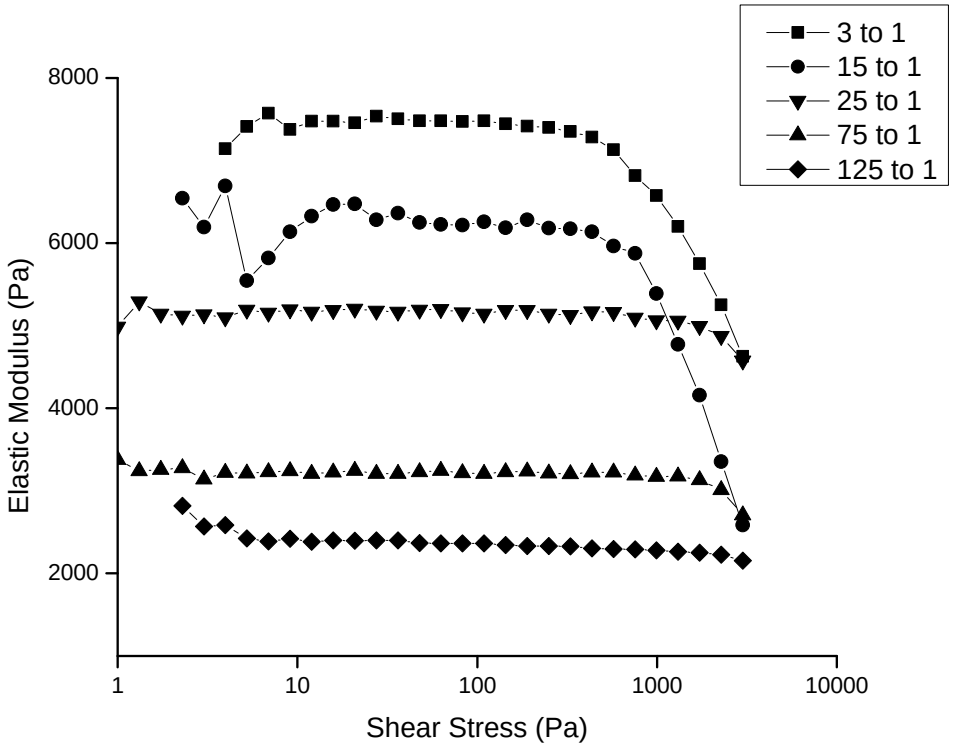

Figure 2.1: Elastic modulus of hydrogels with different gelatin-mTG ratio.

Gelatin-mTG Proportion	Amount of Gelatin Solution (μl)	Amount of mTG Solution (μl)
3:1	900	300
15:1	1125	75
25:1	1153	46
75:1	1184	15.8
125:1	1190	9.52

Table 2.1: Amount of materials used in making hydrogels of different stiffness.

Chapter 3

Growth Rate of Rabbit Kidney Cells Cultured on Enzymatically Cross-linked Hydrogels

3.1 Experimental Materials

The cells that we used in this study were rabbit kidney cells (RK 13) obtained from the ATCC with ATCC number of CCL-37.

Standard Dulbecco's modified eagle medium (DMEM) with catalog number of 11965-092 from GIBCO was used as cell growth medium.

Fetal bovine serum (FBS) was used to provide essential proteins for growth of cells while penicillin/streptomycin (Pen/Strep) was used to prevent the cells and medium from bacterial contamination. Both of them were obtained form GIBCO.

Dulbecco's Phosphate-Buffered Saline (DPBS) with catalog number of 14190-144 was obtained from GIBCO.

0.05% Trypsin- EDTA (1X) solution from GIBCO was used to trypsinize cells from flasks and dishes.

Triton X-100 obtained from Sigma was used to get the cell permeabilized for staining.

Other materials such as propidium iodide (PI), formaldehyde were used in this study as well.

3.2 Experimental Equipments

A TCS SP2 sectral confocal & multiphoton system from Leica was used to observe stained cells.

IEC HN-SII centrifuge from Damon/IEC for centrifugation.

3.3 Experimental Steps

3.3.1 Preparation of Solutions

Cell growth medium was prepared by adding 10% of FBS and 1% of Pen/Strep into DMEM.

0.4% Triton solution was prepared by dissolving Triton X-100 in DPBS.

3.7% formaldehyde solution was made by diluting 37% formaldehyde solution in DPBS.

PI with an original concentration of 1 mg/ml was diluted by DPBS into $5*10^{-3}$ mg/ml.

3.3.2 Cell Culture and Seeding

RK 13 cells were cultured in cell growth medium in a humidified incubator with 5% CO_2 at 37 °C for 3 days, during which the medium was changed once. The cells were then harvested after treated with 0.05% trypsin-EDTA solution before centrifuged under 800 rpm for 10 minutes, and finally put in a 15 ml tube with growth medium ready for seeding. After being counted, 0.4 M of cells were seeded onto enzymatically cross-linked hydrogels in six-well dishes with growth medium. Cells had been plated directly on plastic dishes as well, as control groups.

3.3.3 Cell Fixing and Staining

In this part of the study, cells prepared in different dishes in day 0 had been fixed and stained every day in a roll of 3 days. To fix, cells were soaked in 3.7% formaldehyde solution for 15 minutes before rinsed by DPBS twice. After being fixed, they were permeabilized with 0.4% Triton solution for 7.5 minutes before rinsed twice by DPBS, and then stained by $5*10^{-3}$ mg/ml PI solution for 3 minutes, followed by being rinsed twice by DPBS.

3.3.4 Confocal Laser Scanning Microscopy

After being stained by PI, the cells were imaged using a confocal microscopy with a 10X lens. Cells in 6 conditions including 5 cultured on hydrogels of 5 different stiffness and 1 plated on plastic dishes directly, each with a number of 3 samples, were being imaged. A total number of 150 pictures of cells were taken from each sample. The pictures were then being analyzed using a software called ImageJ to

obtain the densities of cells cultured on each hydrogels. A picture of cells being analyzed by ImageJ can be seen in Figure 3.1. ImageJ would automatically counting the number of pixels in the dark, which stand for cells, and calculate the number of cells after we determined the pixel numbers of each cell manually.

3.4 Results

After being analyzed, cell densities of each sample from day 1 to day 3 had been calculated. The density of cells increases with the increasing of hydrogel stiffness, varies from 14928 cell/cm^2 to 29701 cell/cm^2 in day 1, and from 32280 cell/cm^2 to 69199 cell/cm^2 in day3, excludes the control group which has a 2 fold higher number then others in average. Growth curves of the cells are plotted in Figure 3.2. Cells different growing days under microscopy are shown in Figure 3.3, Figure 3.4, and Figure 3.5.

Based of the growth curves, doubling times of cells have been calculated which is shown in Table 3.1. Cells on the stiffest hydrogel had a doubling time of 1.3 days while the doubling time of cells on the softest hydrogel was 2.64 days. It has been indicated that the softer the hydrogel is the longer doubling time the cells have.

3.5 Discussion

Hydrogels have been widely studied in tissue engineering field as great tissue scaffolds. Not only in tissue engineering do hydrogels have a dominant position, but also in basic studies like this, hydrogels have advantages over normal culture sur-

faces. For instance, there is no need to trypsinize to remove cells from a fixed surface to another. It can be easily done by transferring the whole hydrogel substrate with cells. However, back in the 1970s, hydrogels had been found unsuitable for cell adhesion and growth as substrates [24]. C. F. Newton et al. found that it is because of the lack of collagen or other proteins which can support cell attachment and growth. Many kinds of mammalian cells, including RK 13 used in this study, will not grow if they cannot become attached to a proper surface. This phenomenon is termed "anchorage-dependent" [25]. As most of the cells being studied in the laboratories are mammalian cells, there is quite a considerable possibility for them to be anchorage-dependent. However, as hydrogels are cross-linked and have a good ability to absorb water, there are many "water pockets", which are able to carry macromolecules, between the monomers . It makes hydrogels possible to be "programmed" when employed in cell culture by embedding desired macromolecules. This makes hydrogel even more desirable as scaffolds in tissue engineering [26–28]. As gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen, it is reasonable for RK 13 cells to be attached and proliferate on gelatin hydrogels as observed in this study.

As can be seen in Figure 3.2, cells, cultured on hydrogels, have lower growth rates than the cells plated on plastic dishes. Contemporarily, cells cultured on softer hydrogels have lower growth rates as is mentioned in section 3.4. This property of cell growing on hydrogel can be utilized in certain circumstances when the growth rate of cells need to be controlled. For example, it can be used to obtain an appropriate cell growth rate in order to coordinate with a curtain chemical releasing rate. However, there is a possibility that the chemical releasing rate is also related to the

stiffness of hydrogels. In this case, more studies have to be done to determine the variables.

In advanced tissue engineering field, hydrogels are usually to be patterned to guide cells to grow in certain 3-D structures by colloidal templating or interference lithography [29]. But the scale and shape of the 3-D hydrogel structure are limited. A technology called direct-write assembly breaks the limitation which allows the hydrogel to be made in nearly any shapes and dimensions by forming the scaffolds layer by layer, while hydrogels are patterned in both planar and 3-D forms [30].

Based on this basic study of cell growth on hydrogels, advanced studies such as interactions between cells and the structure of hydrogel scaffolds could be involved in further study.

3.6 Conclusion

In this study, we cultured cells on hydrogels of different stiffness. We imaged the cells under a confocal microscope, plotted the growth curves and calculated the doubling times of each group of cells. We found that cells grow slower on hydrogels than they do on plastic dishes. And the stiffness of hydrogels plays an important role in controlling cell growth rate. The growth rate attends to be slower as cells are cultured on softer hydrogels. Moreover, hydrogels patterned into 3-D structures can be further studied.

3.7 Figures and Tables

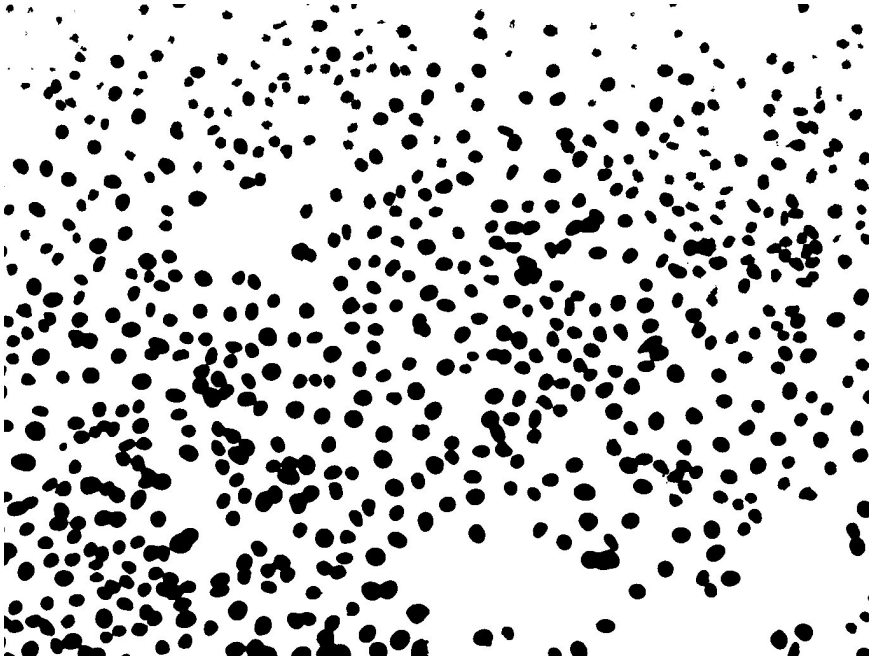

Figure 3.1: Cells Being Analyzed by ImageJ.

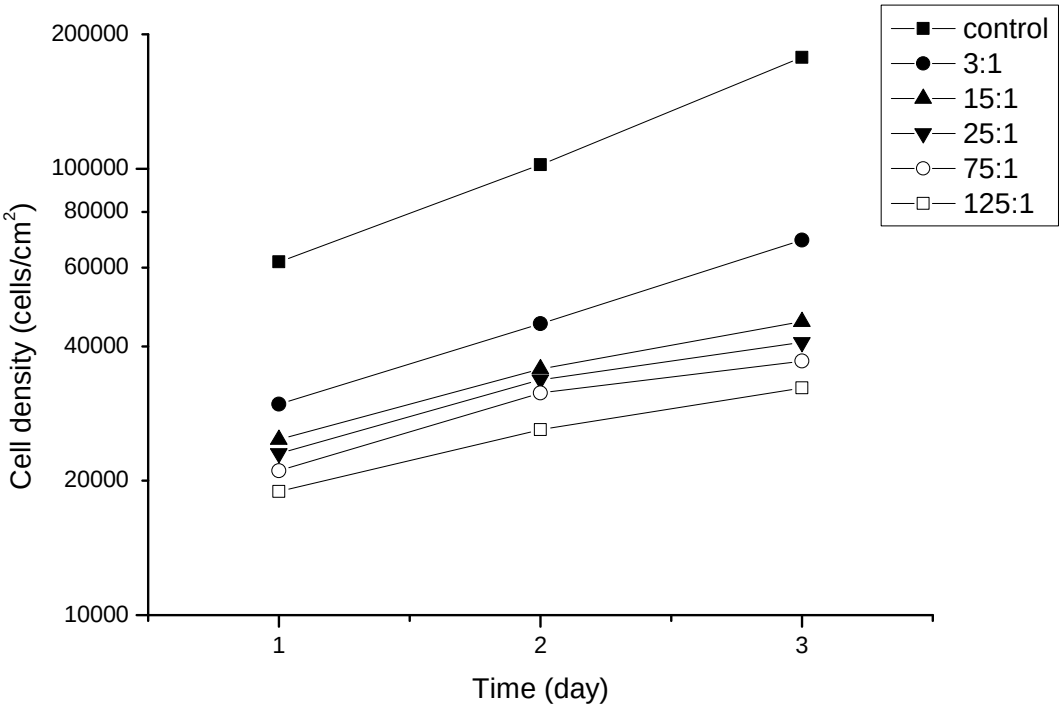

Figure 3.2: Growth Curves of Cells on Hydrogels of Different Gelatin-mTG Ratio.

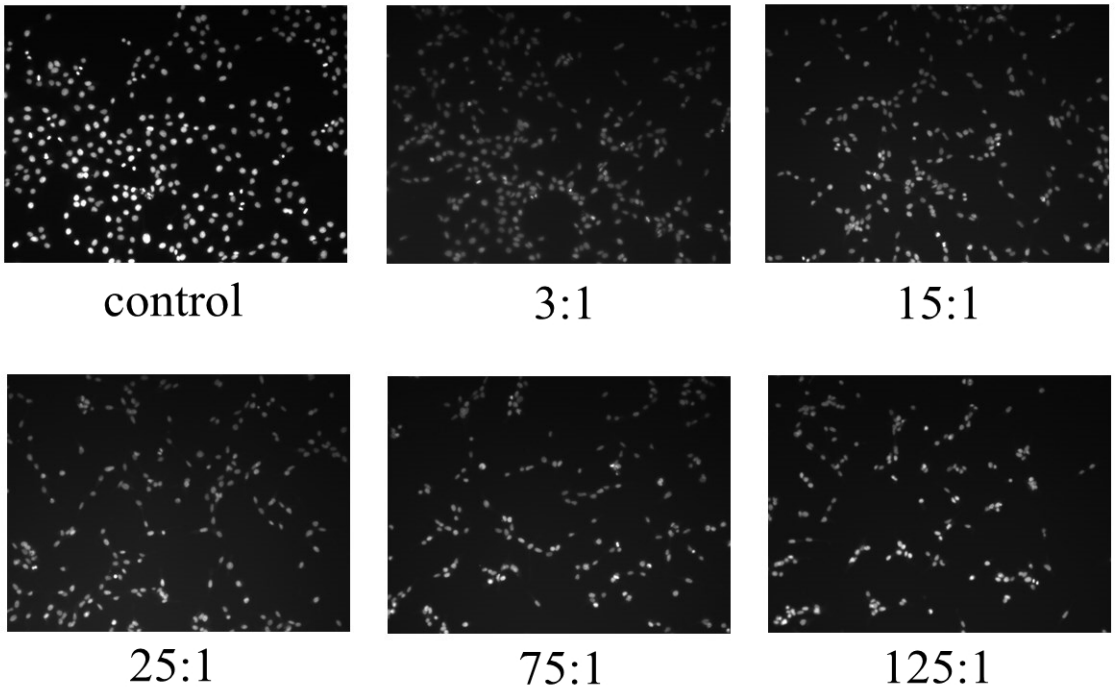

Figure 3.3: Cells From Day 1 Under Fluorescence Microscope.

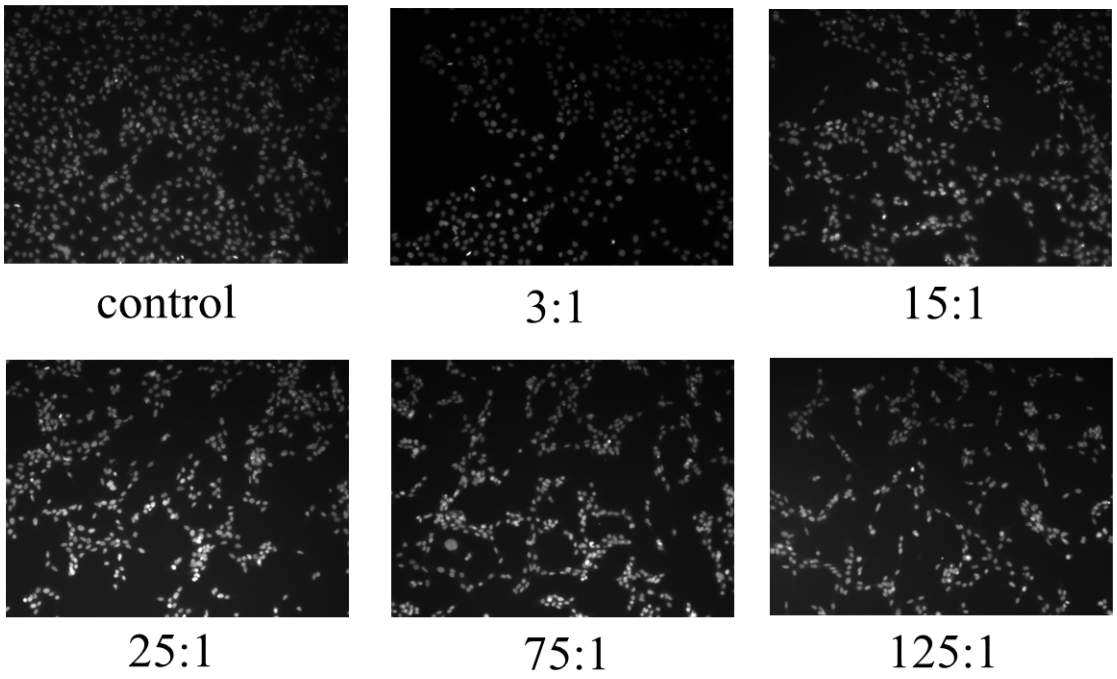

Figure 3.4: Cells From Day 2 Under Fluorescence Microscope.

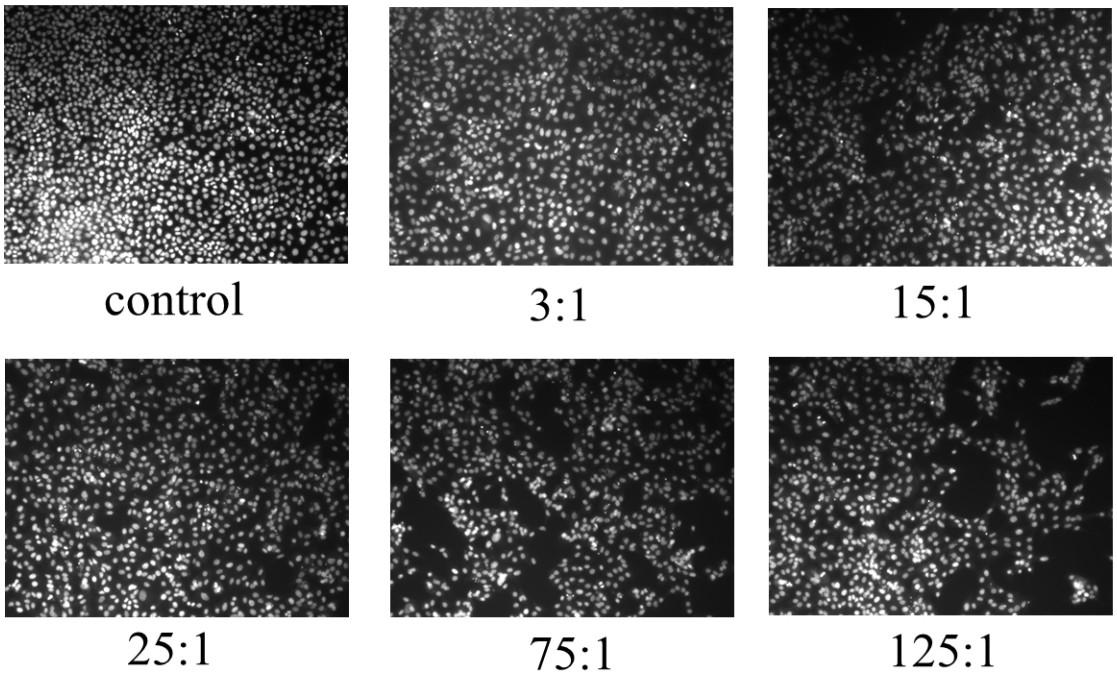

Figure 3.5: Cells From Day 3 Under Fluorescence Microscope.

Gelatin-mTG Proportion	Doubling Time (day)
control	1.3
3:1	1.63
15:1	2.33
25:1	2.5
75:1	2.56
125:1	2.64

Table 3.1: Doubling Times of Cells on Hydrogels of Different Gelatin-mTG Ratio.

Chapter 4

Infectivity of Pseudorabies Virus on RK 13 Cells Cultured on Enzymatically Cross-linked Gelatin Hydrogels

4.1 Experimental Materials

The virus we used in this study was Pseudorabies Virus (PRV), which is an unpurified, crude sample generically modified and labeled with green fluorescent protein (GFP) to become PRV K26-GFP. This genetic modification was performed in Albert Einstein College of Medicine. The original concentration of the virus was $2.6*10^9$ Plaque Forming Units (PFU)/ml. It is storaged in a -80 °C freezer.

Rabbit kidney cells (RK 13) obtained from the ATCC with ATCC number of CCL-37.

Standard Dulbecco's modified eagle medium (DMEM) with catalog number of 11965-092 from GIBCO.

CO_2 idependent medium obtained form GIBCO was used to let the cells be put in

an incubator which has no CO_2 supplied.

Fetal bovine serum (FBS) and penicillin/streptomycin (Pen/Strep) obtained form GIBCO.

Dulbecco's Phosphate-Buffered Saline (DPBS) with catalog number of 14190-144 obtained from GIBCO.

0.05% Trypsin- EDTA (1X) solution from GIBCO was used to trypsinize cells from flasks and dishes.

Triton X-100 obtained from Sigma was used to get the cell permeabilized for staining.

Other materials as propidium iodide (PI), formaldehyde were used in this study as well.

4.2 Experimental Equipments

A TCS SP2 sectral confocal & multiphoton system from Leica was used to image the stained cells along with the virus in which fluorescent proteins are engineered.

IEC HN-SII centrifuge from Damon/IEC for centrifugation.

4.3 Experimental Steps

4.3.1 Preparation of Solutions

Cell growth medium was prepared by adding 10% of FBS and 1% of Pen/Strep into DMEM.

Another growth medium was prepared by adding 10% of FBS and 1% of Pen/Strep into CO_2 independent medium which was used when cells were infected by virus, for the dishes containing cells and virus needed to be put in another incubator which has no supply of CO_2.

0.4% Triton solution was prepared by dissolving Triton X-100 in DPBS.

3.7% formaldehyde solution was made by diluting 37% formaldehyde solution in DPBS.

PI with an original concentration of 1 mg/ml was diluted by DPBS into $5*10^{-3}$ mg/ml.

4.3.2 Cell Seeding

RK 13 cells were first cultured, harvested and calculated ready for seeding in the same process as mentioned in section 3.3.2.

A 6-well dish was prepared by filling each well with an amount of 1.2 ml hydrogel of different stiffness. As we made hydrogels of 5 stiffness, 5 wells were padded by hydrogels while leaving one well blank as a control group. Each well was added with 3 ml of DMEM growth medium. Three dishes were prepared at the same.

RK 13 cells were then plated in the dishes with a seeding density of 0.6 M cells per well before put in the incubator with 5% CO_2 at 37 °C for 24 hr for cells to be attached and grow.

4.3.3 Virus Infection

Amount of virus added to each well was determined by the number of cells in well which was calculated based on the growth rate obtained in section 3.3.2. We add virus with a multiplicity of infection (MOI) of 0.1 after changing the growth medium into CO_2 independent one. To prevent virus contamination, we had to put the dishes which contained virus in another incubator with no CO_2 supplied, away from other samples. Arrangement of a 6-well dish is illustrated in Figure 4.1.

We let the viruses find their host cells for 1 hr. After that, medium was changed to remove excess viruses. Based on a former study, we put the dishes back to the incubator and let the infection take place for another 16 hr to get a considerable infection rate.

4.3.4 Confocal Laser Scanning Microscopy

Cells and virus were fixed by 3.7% formaldehyde for 15 minutes, and stained by PI for 3 minutes after permeabilized with 0.4% Triton solution for 7.5 minutes as introduced in section 3.3.3.

Confocal imaging was then processed using a 20X water lens. Stained cells under confocal microscope appeared red and viruses were formerly engineered to be green as demonstrated in Figure 4.2.

A number of 150 pictures of cells were taken for each well. To get the infectivity, we calculated the number of cells which were infected in every picture which are shown in Table 4.1.

4.4 Results

The numbers of cells infected in each well are presented in Table 4.1. However, due to the different growth rate of cells on hydrogels of different stiffness, these numbers cannot be compared as infectivity directly. Thus, we have to integrate the numbers based on the growth data obtained in chapter 3 as:

$$Integrated\ number = (Oringinal\ number / Cell\ density\ from\ day\ 1) * 30000 \quad (4.1)$$

We multiplied by 30000 here to make the numbers on the same order of the original ones. Table 4.2 shows the numbers of cells infected after being integrated. Control group has a total number of 1174, while cells on hydrogels have numbers varying from 2086 to 2527 in sum. Comparisons among the infectivities is shown in Figure 4.3. It is interesting that viruses have greater infectivities on cells cultured on hydrogels than those grew on plastic dishes. Meanwhile, the infectivity of PRV in RK 13 cells on medium-soft hydrogels seems to be a bit larger than others.

4.5 Discussion

Pseudorabies Virus is a member of herpesviridae family and imposed a lifelong infection on its host, sometimes remaining latent for extended periods of time [31]. Typically, PRV infects swine and can also infect other domestic and wild mammals such as cattle, sheep, cats, and dogs. Hosts experience various symptoms including nervous system complications, respiratory difficulties, and extreme pruritus often originating at the infection site. The virus begins by infecting in the mucosal mem-

branes of the nose and proceeds to infect the central nervous system from where it is disseminated throughout the body [32]. As it is nervous system that PRV mainly infects, many studies of PRV infection of animal neurons have been accomplished. J. P. Card et al. have discovered that PRV replicates within a population of neurons which are linked by synapses, and that the spreads of viruses from compromised infected cells are impeded by glia and macrophages which means that the viruses are not likely to spread through extracellular space [33]. This is believed to be the explanation for what we discovered in last section that virus has a greater infectivity on cells cultured on hydrogels than those grew directly on plastic dishes.

As hydrogels could not affect the behavior of virus directly, the infectivity of PRV is related to the properties of the cell. When cells were imaged under the fluorescent microscope, we found that the shapes of cells between those plated on plastic dishes and cells cultured on hydrogels appeared to be different. The light spots in Figure 4.4 indicate cell nucleuses. It can be obviously observed that nucleuses in Figure 4.4 (b) had narrower shapes. Although the cytoplasm and membranes were not fluorescent, we are still able to observe them dimly with effort. Not alike the cells plated on plastic dishes, isolated cells cultured on hydrogels seemed to be stretched forming strip-like shapes. Arrows in Figure 4.4 (b) point out the ”strips” which elongate the length of cells making it easier for them to be connected with others, thus, leading to a higher ratio of PRV infection. Despite patterning, cell shapes are affected by adhesiveness of the substrates. As the adhesiveness increases, the shapes of cells change from spherical to flat [34]. The adhesiveness of hydrogels made in this study was not examined. However, cell shapes affected by the stiffness of hydrogel substrates turns out to be an interesting subject which

could be included in further studies. Some methods used in this study might not be of good preciseness such as counting the number of cells infected by hand. Thus, it is not unreasonable to assume that the infectivity of PRV remains the same in cells cultured on hydrogels of different stiffness taking experimental error into consideration. Moreover, we found that it extends the experimental error when integrating the numbers of the infected cells using two sets of data both with error. It should be avoided in the further studies, and more accurate methods such as plaque assay should be used to determine the infectivities to get date with more preciseness. For we have discovered that gelatin-mTG hydrogel promotes the infection of PRV on RK 13 cells which means a potential threat exists in using this kind of hydrogel as tissue reconstruction scaffolds, study in more types of viruses and cells cultured on different kind of hydrogels turns to be of great significance in the field of tissue engineering.

4.6 Conclusion

In this study, we made hydrogels of five stiffness as substrates for cell culturing. We add virus after plating cells on the hydrogels to study the infectivity in cells on variable substrates. As a result, we found that infectivity of PRV nearly remains the same in cells cultured on hydrogels of different stiffness while it approximately doubled compared by that in cells plated directly on plastic. Therefore, we believe that there is a potential threat for gelatin-mTG hydrogel to be used as tissue reconstruction scaffolds when subject is infected by Pseudorabies virus.

4.7 Figures and Tables

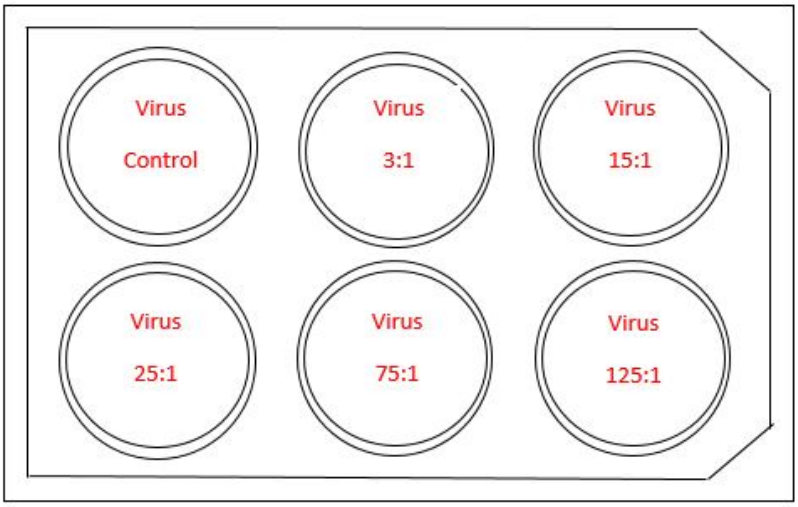

Figure 4.1: 6-well Dish for Virus Infection.

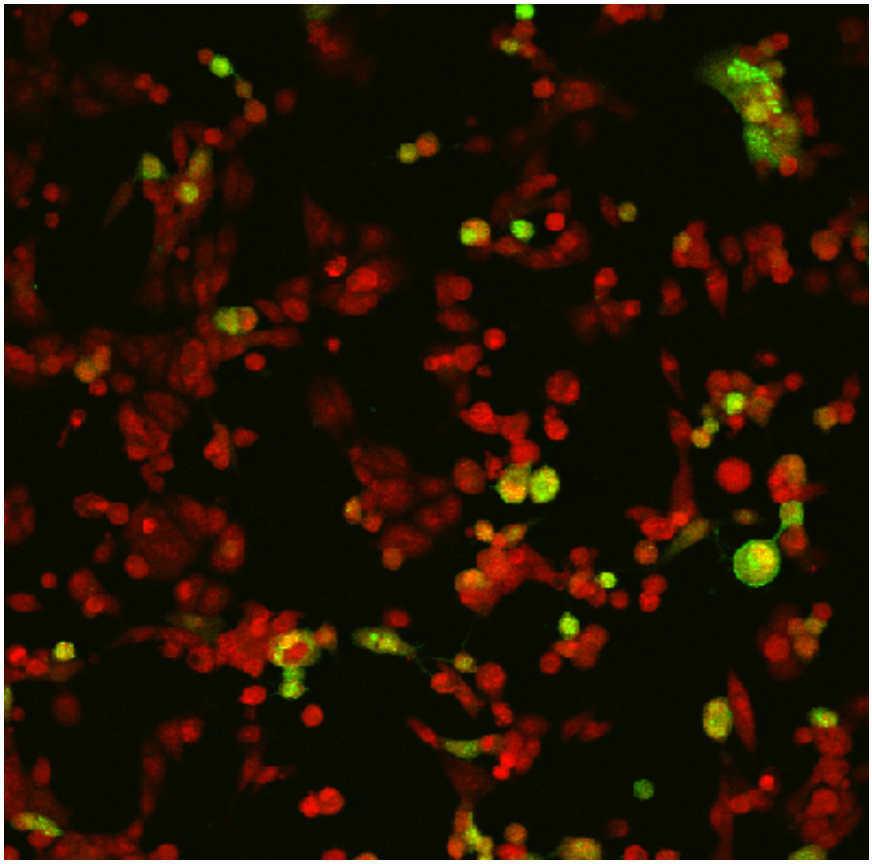

Figure 4.2: RK 13 Cells Infected by PRV Under Confocal Microscope.

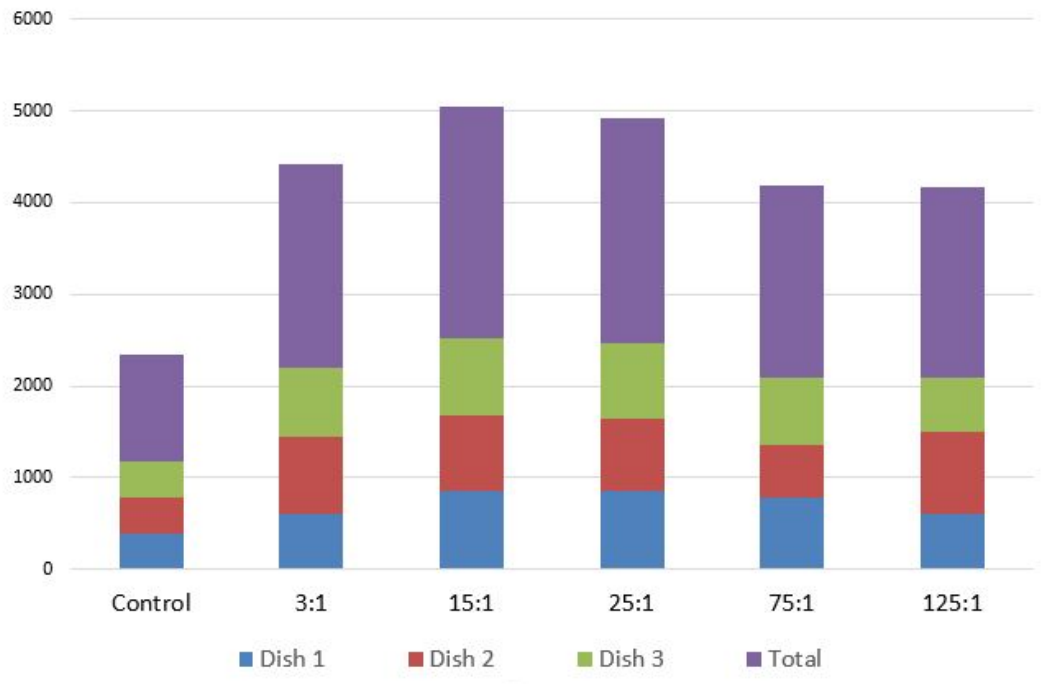

Figure 4.3: Relative Infectivities of Virus in Cells on Hydrogels of Different Gelatin-mTG Ratio.

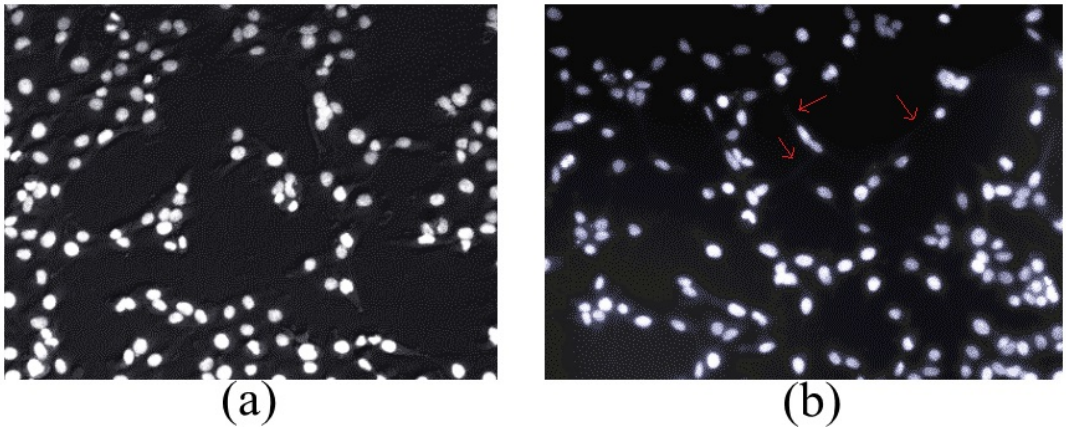

Figure 4.4: Different Shapes of Cells Between Those Plated on Plastic Dishes (a) and Cells Cultured on Hydrogels (b).

	Dish 1	Dish 2	Dish 3	Total
Control	798	820	800	2418
3:1	598	830	756	2184
15:1	710	671	701	2082
25:1	655	599	635	1889
75:1	550	398	520	1468
125:1	379	568	369	1316

Table 4.1: Number of Cells Infected on Hydrogels of Different Gelatin-mTG Ratio.

	Dish 1	Dish 2	Dish 3	Total
Control	387	398	388	1174
3:1	604	838	764	2206
15:1	862	814	851	2527
25:1	854	781	828	2462
75:1	783	567	740	2090
125:1	601	900	585	2086

Table 4.2: Integrated Number of Cells Infected on Hydrogels of Different Gelatin-mTG Ratio.

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