4-25-2022

Comparison of PLGA and PCL Ratios in Kartogenin-Containing Nano-Scaffolds Produced Via the Electrospinning Process

John Graham Roberson
Mississippi State University

Comparison of PLGA and PCL Ratios in Kartogenin-Containing Nano-Scaffolds Produced Via the Electrospinning Process

By

John Graham Roberson

Steve Elder

Professor

Agricultural and Biological Engineering

(Director of Thesis)

David Van den Heever

Associate Professor

Agricultural and Biological Engineering

(Committee Member)

Anastasia Elder

Associate Dean

Shackouls Honors College

(Shackouls Honors College Representative)

Honors Thesis submitted to Shackouls Honors College, Mississippi State University, Spring 2022

Comparison of PLGA and PCL Ratios in Kartogenin-Containing Nano-Scaffolds Produced Via the Electrospinning Process

By

John Graham Roberson

Mississippi State University

Honors Thesis

04/25/2022

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to those who have helped me reach this point and provided the means to make this study possible:

I owe the most to Dr. Steve Elder, for agreeing to serve as Thesis Director on my defense committee, for strongly encouraging me to undertake the Undergraduate Honors Thesis journey, for his guidance over the past three semesters across multiple research projects, and most of all, for being a mentor to me.

To Lamar and Tracy Conerly for the funding of this study through the Presidential Endowed Scholars Program.

To Dr. Anastasia Elder, for serving as the Honors College representative on my defense committee.

To Dr. David Van den Heever, for his willingness to serve on my defense committee as the Agricultural and Biological Engineering department representative.

To Dr. Lauren Priddy, for the use of her Electrospinner

To Luke Tucker, for his knowledge and instruction on using the Electrospinner.

To I^2AT for the use of their Scanning Electron Microscope and assistance in capturing the SEM images used in this study.

ABSTRACT

Microfracture surgery is the most common procedure to treat osteochondral lesions in the knee. However, instead of developing the original hyaline cartilage at the site of treatment, this procedure leads to the development of fibrocartilage, which lacks the durability and function of hyaline cartilage. A small molecule compound, Kartogenin (KGN), is known to promote the differentiation of STEM cells into chondrocytes and can be incorporated into a polymer nano-scaffold via the electrospinning technique. The scaffold could serve as a release mechanism for the KGN as the polymer degrades in the body. This study implemented the use of two different polymers, poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL), and compared the characteristics of the electrospun scaffolds when different ratios of the polymers were used. Four scaffolds were electrospun with PCL to PLGA ratios of 1:0, 3:1, 1:3, and 0:1. Three samples were taken from each scaffold for a total of twelve samples. The samples were soaked in PBS for thirty days, during which the amount of KGN released was measured periodically. After soaking in PBS for thirty days, one sample from each scaffold was observed under SEM. The release data indicated that a heavy PCL concentration causes an initial burst release of KGN and a slow release rate following, while a heavy PLGA concentration causes no initial burst release, and a steady release rate. The SEM images displayed a loss in structure and swelling of the scaffolds with higher PLGA concentrations. This study concluded that combining PCL and PLGA is viable, and in order to optimize the characteristics of both polymers, some PCL is needed to maintain the structure of the scaffold, while, in order to take advantage of better release kinetics, the majority of the polymer component is to be PLGA.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 Limitations of Microfracture Surgery

Osteochondral lesions in the knee, shown in Figure 1, are tears, or defects, in the articular cartilage, most commonly on the medial femoral condyle. They are usually caused by trauma or osteoarthritis and most often occur during the ages of 15-30 and above the age of 50. Lesion symptoms include pain and tenderness in the knee, locking, swelling due to a lack of shock absorber functions, and sometimes even fever. Over-the-counter pain relievers, therapy, and corticosteroid injections do not often provide long-term solutions.[1] Surgery is typically needed to repair the articular cartilage and prevent the further development of osteoarthritis.[2]

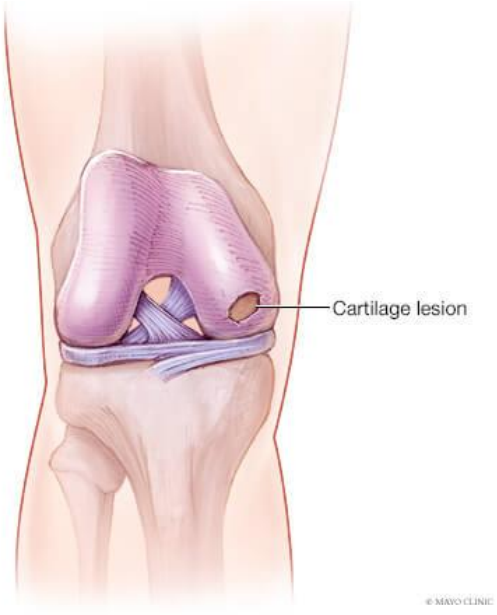

Figure 1. Depiction of an Articular Cartilage Lesion on the Medial Femoral Chondyle.[1]

To treat severe osteochondral lesions, microfracture surgery, an arthroscopically performed surgery, is the most efficient solution. According to one insurance database, in the United States, about 32.5 million adults are affected by osteoarthritis, and around 78,000

microfracture surgeries are performed every year.[3] In this procedure, shown in Figure 2, the area of damaged articular cartilage and all stray fragments of cartilage are cut away, exposing the surface of the cancellous bone and leaving only healthy hyaline cartilage lining the site. Lesions additionally leave a layer of calcified cartilage behind, which is also removed. This exposed bone allows a space for a clot to form. Then, tiny holes, or "microfractures," are drilled 4mm deep into the surface of the bone, allowing the blood and bone marrow to flow up out of the bone, fill and attach at the site, and form a "super clot". The marrow is rich in pluripotent mesenchymal stem cells, which, when in contact with the chondrogenic cell-signaling factors on site, differentiate into chondrocytes.[4] However, once articular cartilage is damaged, there is a small chance that the original, healthy hyaline cartilage forms upon repair. The cartilage does repair, but it heals as fibrocartilage. Fibrocartilage, which is high in type I collagen, lacks the durability and function of the original hyaline cartilage, composed mainly of type II collagen. Because of its mechanical inferiority to perform tasks meant for hyaline cartilage, fibrocartilage leads to long-term complications in the knee.[5]

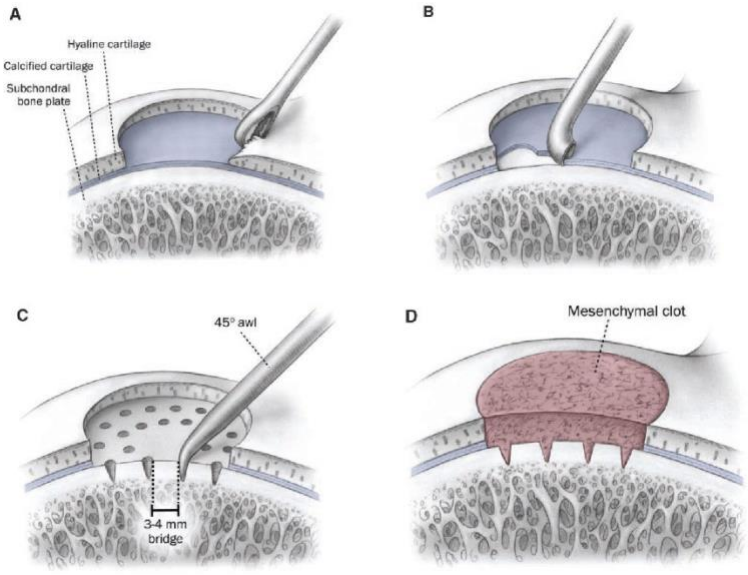

Figure 2. Steps to a Microfracture Procedure.[6]

1.2 Kartogenin

One potential way to increase the repair of cartilage after microfracture surgery is to implement the use of Kartogenin (KGN). KGN is a recently discovered, synthetic, bioactive, small-molecule compound which facilitates cartilage repair. KGN up-regulates the amount of collagen II and aggrecan that are specific to hyaline cartilage, but does not up-regulate type X collagen, which is a marker for undesirable hypertrophic chondrocyte differentiation. As a chondrogenic and chondroprotective agent, KGN is additionally used in tendon-bone healing, wound healing, and limb development.[7] KGN works by binding with filamin A, which in turn disrupts filamin A's interaction with core-binding factor β (CBFβ), a transcription factor. KGN then regulates the CBFβ-RUNX1 transcriptional program, inducing chondrogenesis.[8]

In one study, an *in vivo* analysis was conducted on New Zealand White rabbits, where cartilage defects were created in the patellar grooves of the right femur. Half of the animals were injected intraarticularly with 10μM KGN while the other half were left untreated. After 4 weeks the rabbits injected with KGN showed signs of early healing and at 12 weeks hyaline-cartilage had formed. At both 4 weeks and 12 weeks, proteoglycan staining showed new tissue had developed as well as an increased production of collagen II and a decrease in collagen I. Histology revealed the formation of fibrotic tissue in the untreated rabbits, whereas no fibrotic tissue was formed in the KGN treated rabbits. This study showed that KGN was a useful agent in cartilage repair.[9]

1.3 The Process of Electrospinning

Electrospinning is a technique used to electrostatically produce nano-fibers to be used for tissue engineering, drug delivery, filtration, wound dressings, and many other applications. Electrospinning was the choice method of scaffold fabrication due to the nanoscale fiber size,

which provides a high surface area for mesenchymal stem cell (MSC) attachment, migration, and proliferation. Furthermore, the scaffolds can be electrospun using FDA approved biodegradable polymers, such as PCL and PLGA, which are optimal for drug delivery.[10] The process involves a dissolved polymer solution in a syringe, which is slowly being pushed out by a syringe pump. As the solution is being expelled, a high voltage, typically in the range of 12kV-20kV, is sent through the solution in the needle. The electric forces overcome the surface tension in the emerging droplet of solution, and a charged jet of solution shoots out from the droplet. As the jet leaves the droplet, the surrounding electrical, surface, and molecular forces become unstable, causing the jet to stretch and decrease in diameter. This allows the solvent to evaporate and the remaining jet of polymer to expand, bend, and solidify into a fiber. The fibers are attracted to and gather on a metal collecting plate a short distance away and together form a polymer nano-scaffold.[11] Figure 3 shows the process below.

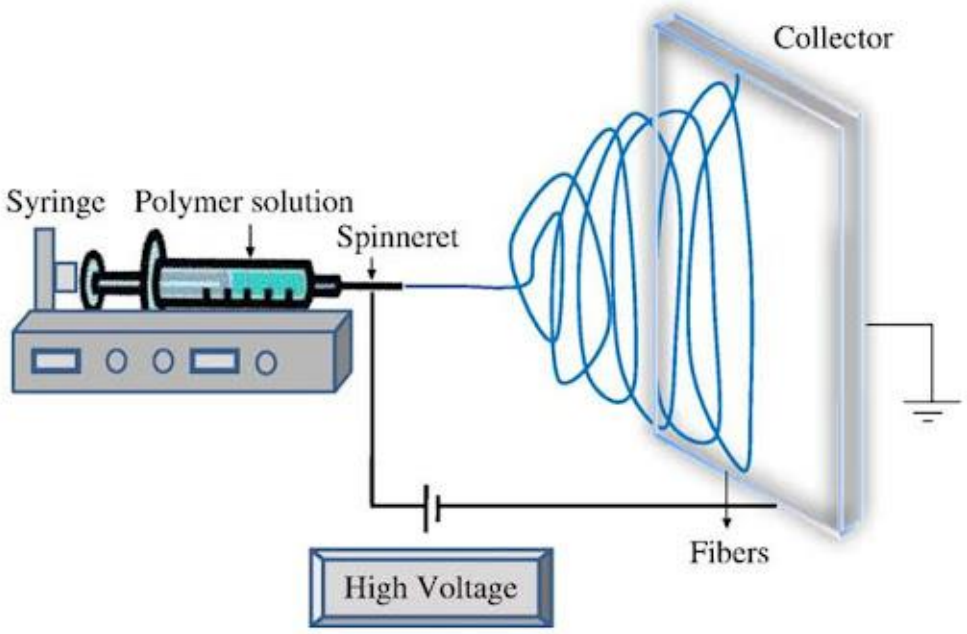

Figure 3. Diagram of the Electrospinning Process.[12]

Nano-scaffolds are used in tissue engineering applications to serve as a supportive structure for cell adhesion and proliferation. In time, as the cells grow and become self-supporting, filling the pores within the scaffold, the scaffold degrades leaving behind healthy cells.[12]

The electrospun scaffolds are also widely used as release mechanisms in drug delivery. Drugs can be dissolved into the electrospinning solution along with the polymer, so that when the solution is spun, the drug is encapsulated within the polymer fibers. As the polymer degrades within the body, the drug is released. Due to the size of the nano-fibers, the scaffold has a large surface area, making it ideal for drug release. Depending on the polymer or combination of polymers chosen, the degradation rate can be slow, quick, immediate, or varied.[12]

1.4 PCL and PLGA

Polycaprolactone (PCL) and poly(lactic-*co*-glycolic acid) (PLGA) are two polymers frequently used in tissue engineering applications. They are commonly used as components of polymer scaffolds in vivo due to their biocompatibility and ability to undergo bulk degradation by hydrolysis over a period of weeks or months, making them a choice drug-release mechanism.[13] PLGA is broken down into lactic acid and glycolic acid monomers, which are natural by-products of certain metabolic pathways that take place within the body and are removed from the body by the same treatment.[14] PCL is likewise excreted from the body once it has been bioresorbed.[15] As the polymers break down, they release the drug at a steady rate into the body.[13] The main difference between PCL and PLGA relative to this study is the rate at which hydrolysis occurs. PLGA degrades in vivo over a period of around 12 weeks, which is much faster than PCL's 24 month period.[15] This indicates that PLGA is more hydrophilic than PCL and enables it to release a drug faster. PCL has a Young's modulus of 330-360 MPa whereas PLGA has a Young's modulus of around 26 MPa, indicating PCL is much stiffer than PLGA.[13,16] This would benefit a scaffold by

ensuring the maintenance of structure throughout the degradation process, allowing cells to continue to adhere to and proliferate in the scaffold without collapse occurring.

1.5 Study Objectives

This study builds on a previous study conducted in the Elder laboratory, where KGN was incorporated into a 15% PLGA solution and electrospun into a nanofibrous scaffold. In the study, two scaffolds, one with and one without KGN were analyzed. It established that firstly, the KGN had minimal effect on the mechanical properties of the scaffold, secondly, the KGN was still chemically intact after the electrospinning process, thirdly, when the scaffold soaked in saline, there was a sustained release, and lastly, that cell adhesion and proliferation took place on the scaffold under the presence of KGN.

This study aims to compare the characteristics of an electrospun scaffold when different ratios of PCL and PLGA are used. The primary characteristics that will be analyzed are the KGN release profile and the physical structure of the scaffolds. Through this analysis, the ratio of PCL to PLGA that is optimal for KGN release and chondrogenic differentiation will be determined. In an optimal release profile, there would be a sustained release for at least two months, at which point all the drug would be released. This would allow time for complete stem cell differentiation as well as matrix synthesis and maturation. A burst release is not harmful, but it would prohibit efficient chondrogenesis from taking place because the process takes several months. KGN would need to be released at a steady rate to ensure that no opportunity to influence differentiation would be lost.

CHAPTER II

METHODS

2.1 Producing a Solution to Electrospin

When performing the electrospinning process, first a solution was produced. In this study, a combination of 1,1,1,3,3,3-hexafluro-2-propanol (HFP) and dimethylformamide (DMF) in the ratio of 9:1 was used as the solvent for the polymers and KGN. First, a 15mL solution was created to allow ample solution for each sample. 1.5mL of DMF was placed into a centrifuge tube. To maximize the KGN within the scaffold, the KGN was dissolved in the DMF at a ratio of 40mg/mL. At this ratio, 60mg of KGN was dissolved in the DMF. To finish creating the solvent, 13.5mL of HFP was placed into a 100mL beaker along with a stir bar and placed on a magnetic stirrer in the fume hood. The stirrer was placed on low and the DMF/KGN solution was added dropwise to the HFP using a 200μL-1000μL micropipette. The solution was stirred until the DMF/KGN was dissolved in the HFP, completing the solvent.

The solvent was split into two scintillation vials with 7.5mL in each. In one, the PLGA was dissolved, and in the other, the PCL was dissolved. The polymers had to be dissolved separately before they were mixed so that they did not separate when combined. 1125mg of PLGA was added to the first vial to create a 15% polymer concentration. 1125mg of PCL was added to the second vial to create a 15% polymer concentration. The two vials were then capped and laid on their sides on a rocking platform. The vials were rocked for 12 hours until the solutions homogenized.

To compare the performance of different combinations of PLGA and PCL within the same scaffold, four samples were prepared. The first sample was prepared by separating 3mL of the 15% PCL concentration solution into a vial. For the second sample, 2.25mL of the original PCL solution and 0.75mL of the original 15% PLGA concentration solution were placed in a second

vial to create a solution of PCL and PLGA in a ratio of 3:1. For the third sample, 0.75mL of the original PCL solution and 2.25mL of the original PLGA solution were placed into a third vial to create a solution of PCL and PLGA in a ratio of 1:3. For the fourth and final sample, 3mL of the original 15% PLGA concentration solution was placed in a fourth vial. All four samples contained a consistent polymer concentration of 15% and contained a consistent amount of KGN at 12mg in each sample. Samples 2 and 3 (PCL to PLGA ratios of 3:1 and 1:3 respectively) were placed on the rocker for 12 hours until they were homogenized.

2.2 Performing the Electrospinning Technique

To spin the solution, a wire was run from the positive terminal of the high voltage power supply to the spinneret, or needle, of the syringe, which was resting in the syringe pump. The negative terminal of the high voltage power supply was connected by a wire to a screw embedded in the rear of a square copper collecting plate, as seen in Figure 4. The electrospinning process for each sample was the same. The sample was loaded into a 5mL syringe, and a 22G blunt needle was fastened to the end. The syringe was secured onto the syringe pump, pointing toward the collector plate, and the pump was set to a flow rate of 0.75mL per hour. A sheet of aluminum, 12cm in width and length, was taped onto the collector plate, which was positioned 20cm directly in front of the syringe needle. The spinning process began with the syringe pump being turned on. As soon as the first drop of solution began to stick out of the end of the needle, the high voltage supply power was turned on to 11kV. The voltage was varied between 11kV and 18kV to maintain a steady Taylor cone. With such an intense electric field around the droplet, it is deformed into a cone shape, called a Taylor cone, shown in Figure 4, where the jet of solution is ejected from the peak of the cone. A steady Taylor cone is formed when the flow rate of the solution out of the syringe is equal to the flow rate of the jet from the droplet outside the needle.[17]

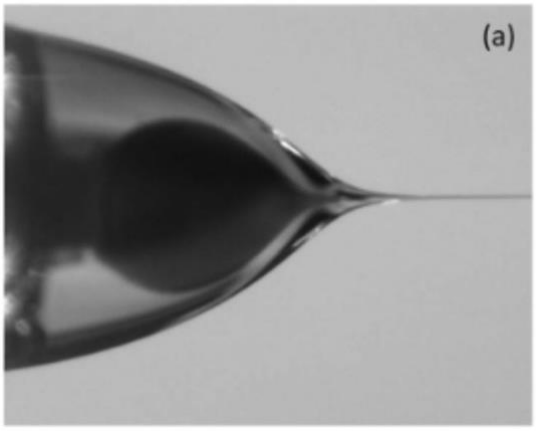

Figure 4. Taylor cone formation of a solution being ejected from a needle during the electrospinning process.[17]

When the syringe pump had completely ejected the solution, the voltage was turned off, and the aluminum sheet containing the newly-spun nano-scaffold was removed off the collector plate. This process was repeated three more times, with each iteration containing 3mL of the tested sample solution.

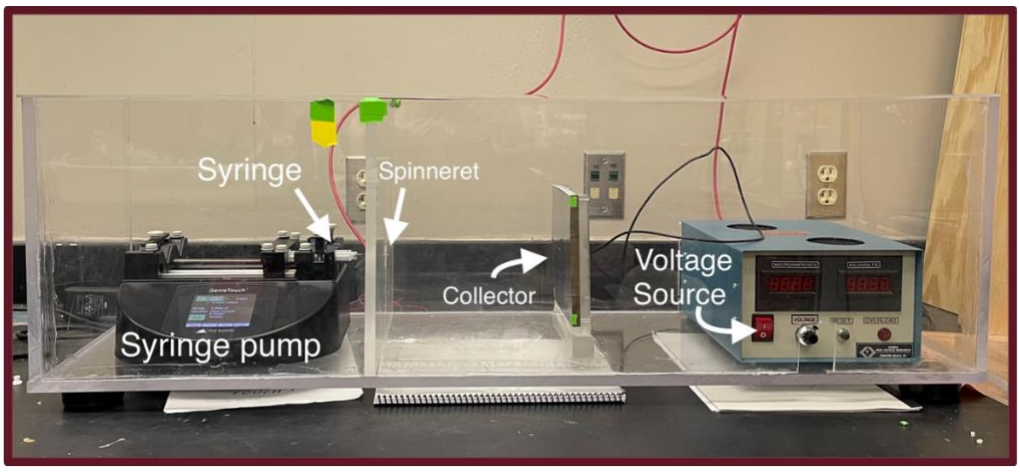

Figure 5. Electrospinning set up as used in this study.

2.3 KGN Release Testing

To determine the rate of KGN release of the four nano-scaffold samples, sample cuts from the scaffolds were placed in phosphate-buffered saline (PBS) so that the polymer component of the scaffold hydrolyzed, releasing the KGN into the saline solution. To measure the amount of

KGN released, the absorbance of the PBS was measured using the UV-Vis setting of the NanoDrop 2000c Spectrophotometer.

First, three samples, each weighing 4.2mg, were cut out of a scaffold. This was repeated for each scaffold yielding twelve total sample cuts. Next, each sample was placed in a scintillation vial with 2 mL of PBS. The samples were placed into a ThermoScientific MaxQ 4000 incubator shaker and set to 37°C at 100 rpm for one month. After each of the first five days, the absorbance levels of the samples were tested at a wavelength of 284nm to record the presence of KGN in the PBS. Each time the absorbance was recorded, the PBS was replaced. After the first four days, the absorbance was recorded every three to four days until one month had gone by.

2.4 SEM Imaging

At the end of the month, the samples were dried by soaking in a series of increasing concentrations of ethanol in distilled water. First, they were soaked in a 10% ethanol concentration, followed by 25%, 50%, 75%, 90%, and 100%. One sample from each of the four groups, was analyzed using a JOEL 6500F Field Emission Scanning Electron Microscope (SEM). The samples were sputter coated with platinum and prepared for SEM imaging. Each sample's image was captured at x10000 magnification. Once the images were taken, they were analyzed using Matlab SIMPoly, an image analysis tool used to measure fiber diameters of electrospun scaffolds.

CHAPTER III

RESULTS AND DISCUSSION

3.1 KGN Release Results

To determine the amount of KGN released from the four scaffold samples, a curve, shown in Figure 6, depicting the relationship between the UV absorbance value at a wavelength of 284nm and the concentration of KGN in mg/ml was used.

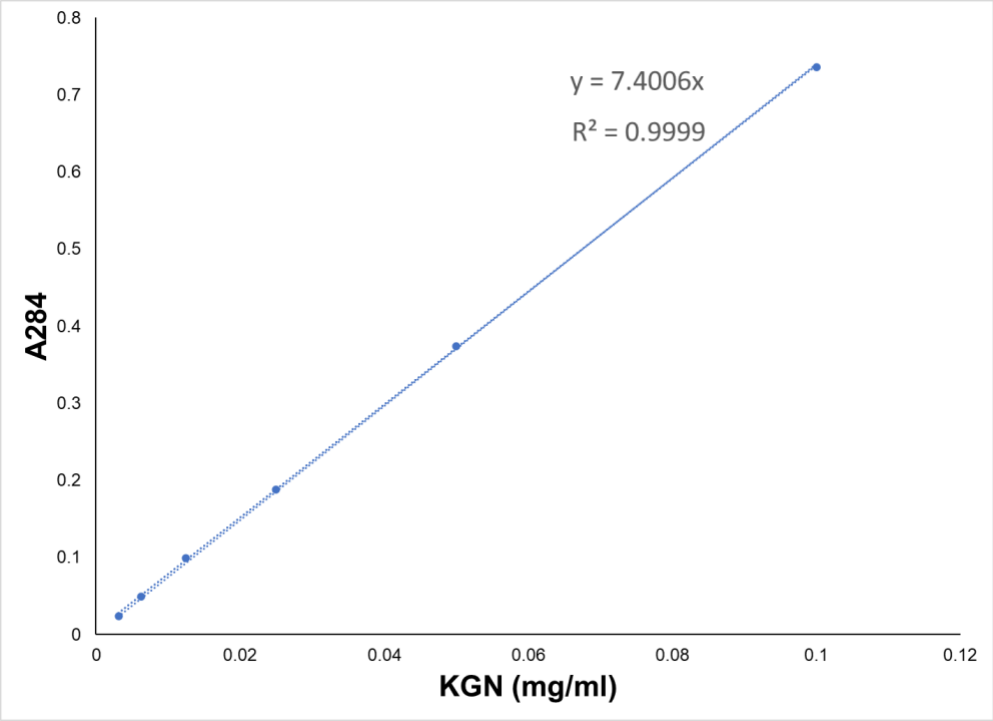

Figure 6. Relationship between the measured absorbance at 284nm and the concentration of KGN in mg/ml.

Using the known ratio of mass of KGN to mass of polymer in each scaffold (12 to 450), and knowing the starting weight of each sample from the scaffolds (4.2mg), the starting mass of KGN in each sample was found to be 0.112mg. Using these starting mass values and the standard curve in Figure 6, the percentage of total KGN released over the course of the thirty days was recorded as shown in Figure 7. The release data of each group of three samples from each scaffold was averaged to produce one release curve for each scaffold.

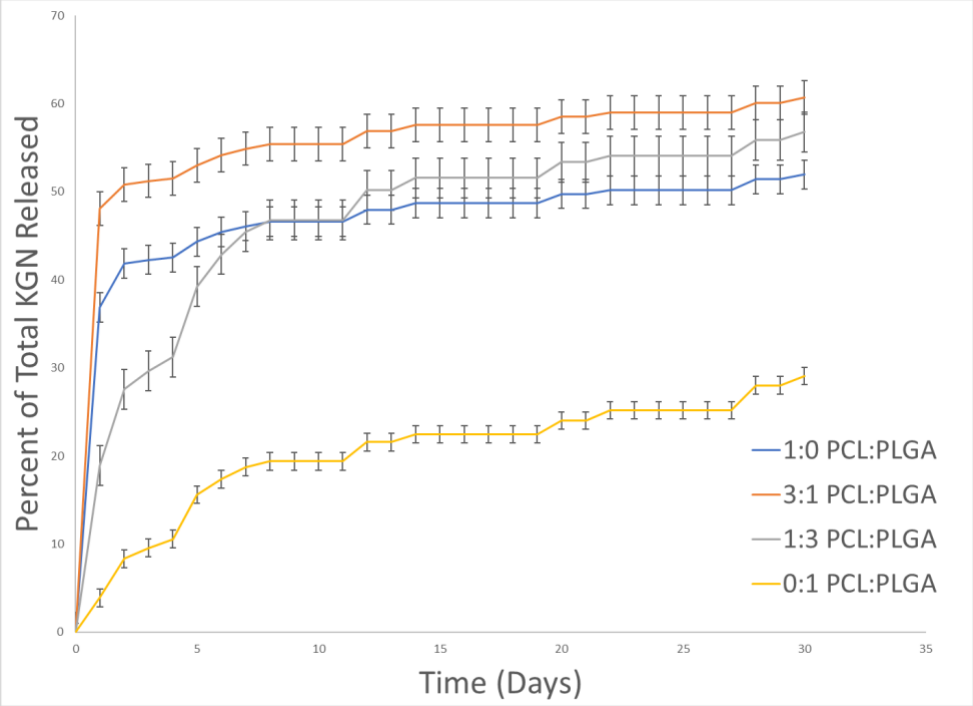

Figure 7. Percent of KGN released from the scaffold samples over the course of thirty days.

The 1:0 PCL:PLGA sample experienced a burst release of KGN over the course of the first two days in PBS. During that time, approximately 41.8% of the total KGN within the scaffold was released. Over the next four weeks, only an additional 10.1% was released for a total release of 51.9% over thirty days. The 3:1 blend experienced a burst release of 50.8% during the first two days, with an additional 9.9% release during the following four weeks for a total release of 60.7%. The 1:3 blend experienced a minimal burst release of 45.6% over the first seven days. An additional 11.2% was released over the next three weeks for a total release of 56.8%. The 0:1 PCL:PLGA blend had no significant burst release and, over the course of the thirty days, had a total release of 29.1% of the total KGN.

The initial burst release of KGN happened within 48 hours, indicating the release did not occur from hydrolysis of the polymers. Instead, it was from an initial diffusion of the KGN out of the scaffold. The scaffolds composed mostly of PLGA (1:3 and 0:1 blends), did not undergo a

burst release, while the scaffolds containing a majority of PCL (1:0 and 3:1 blends) did. Therefore, there is a higher initial rate of diffusion of KGN out from PCL than out of PLGA. Once the burst release took place in the 1:0 and 3:1 blends, the rate of release that followed over the next four weeks was much slower than the rate of release of the 1:3 and 0:1 blends. This confirms that PCL undergoes hydrolysis at a lower rate than PLGA in PBS. Because of the low rate of hydrolysis and slow release of KGN, a scaffold composed only of PCL is not an efficient release mechanism for KGN. Too much of the KGN is initially released in the burst release, leaving insufficient amounts of KGN to be released over the course of the cartilage healing process. That insufficient amount of remaining KGN is then spread thin over the course of a slow hydrolysis process.

In a comparable past study conducted by Nikhita Joy and Satyavrata Samavedi,[18] the release of a hydrophobic glucocorticoid dexamethasone (DEX) drug from electrospun polymer scaffolds in PBS was tested over the course of 28 days. Five sample scaffolds were used, four of which were spun with different polymers: PCL, 50:50 PLGA, 85:15 PLGA and PLLA. The release profiles of each were recorded, shown below in Figure 8. As seen in the results of the current study, the DEX release study supports the findings that a PCL scaffold undergoes a large burst release within the first 24-48 hour period whereas a PLGA scaffold experiences no burst release and holds a consistent release rate throughout the 28 days.

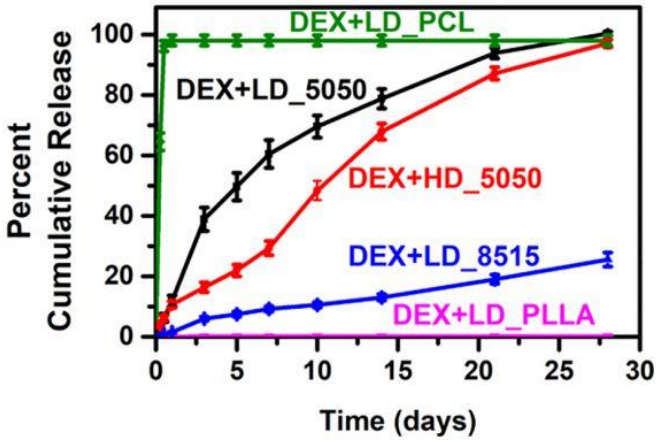

Figure 8. Percent cumulative release of DEX from electrospun meshes over 28 days.[18]

3.2 SEM Image Results

The images in Figure 9 were analyzed in the Matlab SIMPoly program. The 1:0, 3:1, and 1:3 PCL:PLGA blends had average fiber diameters of 0.159nm, 0.278nm, and 0.314nm respectively, with standard deviations of 0.041, 0.073, and 0.112. The average fiber diameter of the 0:1 blend was unmeasurable.

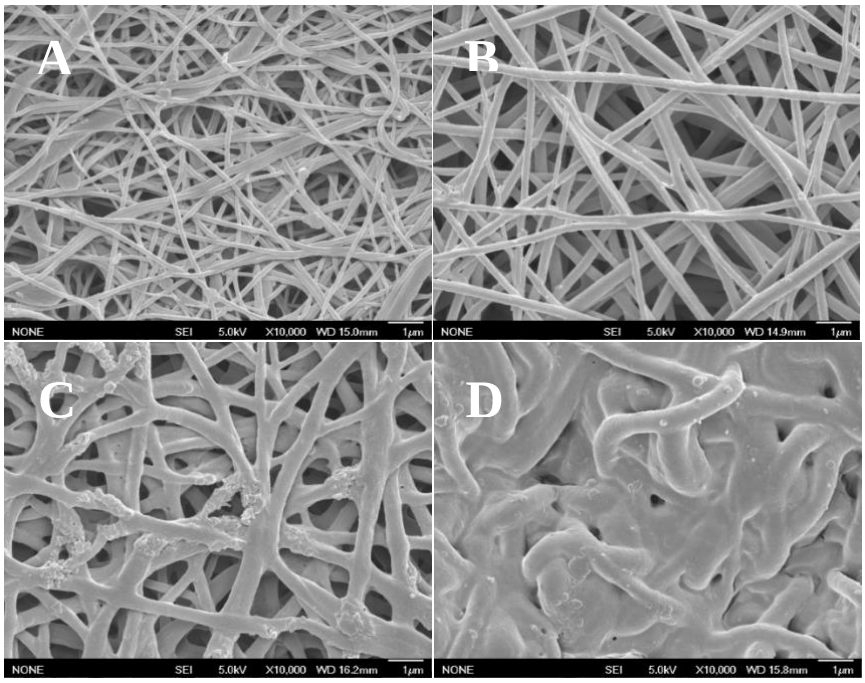

Figure 9. SEM images of the polymer scaffolds at x10000 magnification. Samples include PCL:PLGA ratios of (A) 1:0, (B) 3:1, (C) 1:3, (D) 0:1.

As the scaffolds experienced hydrolysis in the PBS, the fibers swelled, and over time, lost their structure. As bulk degradation occurred, and KGN left the fibers, spaces opened within the fibers, allowing water to enter and cause swelling. Image D, shown in Figure 9, displays a scaffold with a polymer composition of purely PLGA. Its average fiber diameter was unmeasurable due to the amount of hydrolysis that had taken place. The fibers had swelled, lost their structure, and fused together in some places. Because PLGA hydrolyzes at a faster rate, the 1:3 and 0:1 blends swelled and lost their structure at a faster rate. The scaffold's loss in structure limits the potential for cell adhesion and growth due to a lack of space within the scaffold. The scaffolds in Image A and Image B of Figure 9 are structurally intact. The 1:3 blend scaffold in Image C is intact, but it has begun swelling, making it less than ideal for cell growth as there is less space for cells and ECM to occupy.

In Joy and Samavedi's study,[18] SEM images were acquired for the scaffolds after 1, 3, 7, and 28 days of soaking in PBS. As seen in Figure 10, the PCL scaffold retained its structure after 28 days, whereas the PLGA scaffolds collapsed. However, differing ratios of lactic acid to glycolic acid components of the PLGA altered the degree to which collapse took place. Seeing that the 50:50 PLGA scaffold had a higher degree of collapse and PLLA underwent minimal collapse, the more glycolic acid component within the scaffold, the higher the degree of collapse of the scaffold in PBS. This supports the findings of the current study.

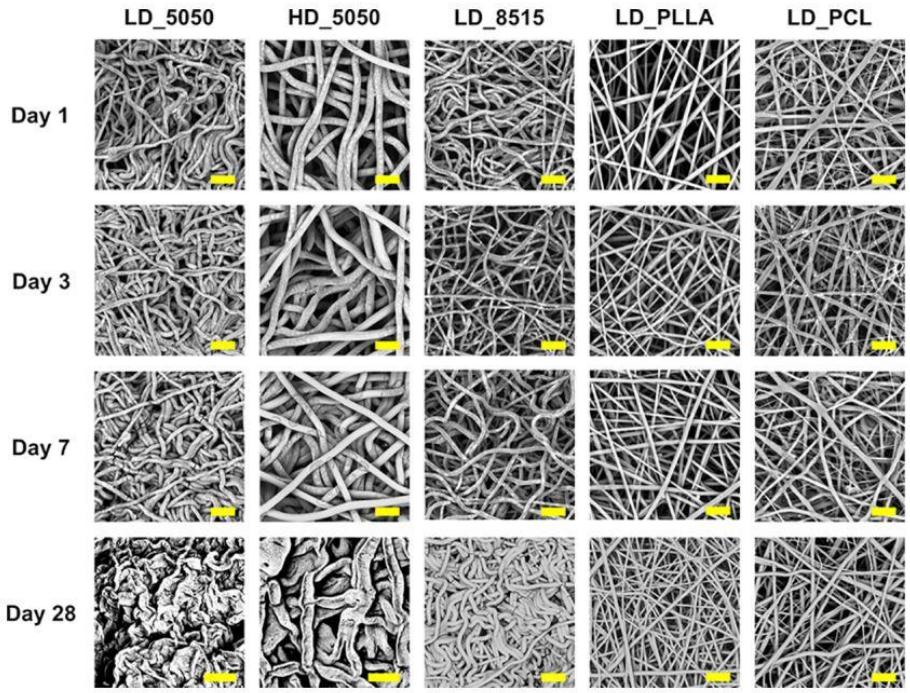

Figure 10. SEM micrographs of electrospun samples immersed in PBS at 37 °C for 1, 3, 7, and 28 days.[18]

CHAPTER IV

CONCLUSION

This study presented valuable findings on the use of PCL and PLGA as a drug release mechanism for KGN in the form of an electrospun nano-scaffold. The use of PCL as the sole polymer led to a burst release of KGN followed by too slow of a release rate for efficient chondrogenic differentiation to occur. The use of PLGA as the sole polymer resulted in a collapse of the scaffold structure, which prevents the growth of cells within the scaffold. Using a blend of PCL and PLGA in a ratio of 3:1 resulted in too high of an initial release, but the scaffold remained intact. Using a blend of PCL and PLGA in a ratio of 1:3 resulted in a desired release rate but was accompanied by some undesirable fiber swelling. Therefore, a 1:2 or 1:1 ratio of PCL to PLGA would likely be the most effective pairing of the two polymers. With this ratio, the burst release would be decreased, while the release rate would be high enough for efficient chondrogenic differentiation to occur throughout the healing process.

Electrospun KGN-containing scaffolds have the potential to advance lesion repair to a new level of efficiency as well as provide a long-term solution for it. When implementing the scaffolds onto the target site, due to the flexibility of the electrospun mesh, these scaffolds could easily be used in arthroscopic surgeries by feeding them through the small incisions before opening them back up in the body and putting them in place within the joint. The use of these scaffolds could minimize the need for more invasive procedures, such as arthroplasty, a bone resurfacing procedure, because an efficient cartilage repair process would be an option. As an alternative to arthroplasty, the scaffolds could be implanted arthroscopically which would additionally avoid a more invasive procedure.

REFERENCES

1. Mayo Foundation for Medical Education and Research. (2019, October 23). *Knee Cartilage Lesions - Mayo Clinic Orthopedics & Sports Medicine*. Mayo Clinic. Retrieved April 20, 2022, from https://sportsmedicine.mayoclinic.org/condition/cartilage-lesions/
2. Buda, R., Vannini, F., Cavallo, M., Grigolo, B., Cenacchi, A., & Giannini, S. (2010). Osteochondral lesions of the knee: A new one-step repair technique with bone-marrow-derived cells. *Journal of Bone and Joint Surgery*, *92*(Supplement_2), 2–11. doi:10.2106/jbjs.j.00813
3. Song, S. J., & Park, C. H. (2019). Microfracture for cartilage repair in the knee: Current concepts and limitations of systematic reviews. Annals of Translational Medicine, 7(S3). doi:10.21037/atm.2019.05.11
4. Steadman, J. R., Rodkey, W. G., Singleton, S. B., & Briggs, K. K. (1997). Microfracture technique forfull-thickness chondral defects: Technique and clinical results. *Operative Techniques in Orthopaedics*, *7*(4), 300–304. doi:10.1016/s1048-6666(97)80033-x
5. Armiento, A. R., Alini, M., & Stoddart, M. J. (2019). Articular fibrocartilage - why does hyaline cartilage fail to repair? *Advanced Drug Delivery Reviews*, *146*, 289–305. doi:10.1016/j.addr.2018.12.015
6. Mithoefer, K., Williams, R. J., Warren, R. F., Potter, H. G., Spock, C. R., Jones, E. C., Wickiewicz, T. L., & Marx, R. G. (2006). Chondral Resurfacing of Articular Cartilage Defects in the Knee with the Microfracture Technique. *The Journal of Bone and Joint Surgery, Incorporated*, *88*(1), 294–304. doi:10.2106/jbjs.f.00292

7. Cai, J.-yu, Zhang, L., Chen, J., & Chen, S.-yi. (2019). Kartogenin and its application in Regenerative Medicine. *Current Medical Science*, *39*(1), 16–20. doi:10.1007/s11596-019-1994-6
8. Johnson, K. A. (2013). A stem cell-based approach to cartilage repair. *Osteoarthritis and Cartilage*, *21*. doi:10.1016/j.joca.2013.02.019
9. Bhatti, F.-U.-R. (2016). Kartogenin induced chondrogenesis of stem cells and cartilage repair. *International Journal of Stem Cell Research & Therapy*, *3*(2). Doi:10.23937/2469-570x/1410036
10. Agarwal, S., Wendorff, J. H., & Greiner, A. (2008). Use of electrospinning technique for biomedical applications. *Polymer*, *49*(26), 5603–5621. https://doi.org/10.1016/j.polymer.2008.09.014
11. Chase, G. G., Varabhas, J. S., & Reneker, D. H. (2011). New methods to Electrospin nanofibers. *Journal of Engineered Fibers and Fabrics*, *6*(3), 155892501100600. doi:10.1177/155892501100600304
12. Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. *Biotechnology Advances*, *28*(3), 325–347. doi:10.1016/j.biotechadv.2010.01.004
13. Wang, L., Abedalwafa, M., Wang, F., & Li, C. (2013). Biodegradable poly-epsilon-caprolactone (PCL) for tissue engineering applications: a review. *Rev. Adv. Mater. Sci*, *34*, 123-140.
14. Makadia, H. K., & Siegel, S. J. (2011). Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers*, *3*(3), 1377–1397. doi:10.3390/polym3031377

15. Azimi, B., Nourpanah, P., Rabiee, M., & Arbab, S. (2014). Poly (∈-caprolactone) fiber: An overview. *Journal of Engineered Fibers and Fabrics*, *9*(3), 155892501400900. doi:10.1177/155892501400900309

16. Choi, S. H., & Park, T. G. (2002). Synthesis and characterization of elastic PLGA/PCL/PLGA tri-block copolymers. *Journal of Biomaterials Science, Polymer Edition*, *13*(10), 1163–1173. doi:10.1163/156856202320813864

17. Gañán-Calvo, A. M., López-Herrera, J. M., Herrada, M. A., Ramos, A., & Montanero, J. M. (2018). Review on the physics of Electrospray: From electrokinetics to the operating conditions of single and coaxial Taylor Cone-jets, and AC Electrospray. *Journal of Aerosol Science*, *125*, 32–56. doi:10.1016/j.jaerosci.2018.05.002

18. Joy, N., & Samavedi, S. (2020). Identifying specific combinations of matrix properties that promote controlled and sustained release of a hydrophobic drug from electrospun meshes. *ACS Omega*, *5*(26), 15865–15876. doi:10.1021/acsomega.0c00954