INFLAMMATORY CD8+ T CELLS ARE REDUCED BY APPLYING FTY-720 TO ACTIVATE SPHINGOSINE-1-PHOSPHATE RECEPTOR SIGNALING

A Thesis
Presented to
The Academic Faculty

by

Dailu Hou

In Partial Fulfillment
of the Requirements for the Degree
Bachelors of Science in the
School of Biomedical Engineering

Georgia Institute of Technology
May 2018

INFLAMMATORY CD8+ T CELLS ARE REDUCED BY APPLYING FTY-720 TO ACTIVATE SPHINGOSINE-1-PHOSPHATE RECEPTOR SIGNALING

Approved by:

Dr. Edward Botchwey, Advisor
School of Biomedical Engineering
Georgia Institute of Technology

Dr. Johnna Temenoff
School of Biomedical Engineering
Georgia Institute of Technology

Date Approved: April 25, 2018

ACKNOWLEDGEMENTS

First of all I would like to thank the all mighty for the gift of knowledge for enabling me to make it throughout the study. Secondly I would like to thank my family for the continued support that they offered during the study making it a success. Finally I will take this great chance to acknowledge my research mentor Dr. Molly Ogle and principal investigator Dr. Edward Botchwey that have greatly guided me on this research and showed me the way forward making this project a success.

TABLE OF CONTENTS

LIST OF FIGURES

SUMMARY

Biomaterials generally induce a natural inflammatory response which can be altered by adding molecules to influence immune cell recruitment. Sphingosine-1-phosphatase (S1P) is a biological signaling lipid which performs the function of immunity regulation and controls the transportation of immune cells through the blood and body tissues. There are five S1P receptors (S1PRs), in which case are all G-protein coupled receptors that attach to and mediate most of the functions that the S1P performs. FTY720 is an agonist of 4 out of the 5 S1PRs and is known as immunomodulatory compound due to its ability to control the trafficking of immune cells. Biomaterial loaded with FTY720 were implanted into the injury skin of mice. Flow cytometry was used to analyze T-cell population in the digested tissues. This study shows that the application of FTY720, reduces inflammatory CD8+ T-cells by using a localized presentation of S1P which also shows how S1P signaling affects the distribution of harmful and helpful T-cells after injury. For the successful application of S1P in a localized tissue, it is important to consider the possible effects of its interaction with the environment. In an injury, with high levels of CD8+ T cells have been shown to block healing by increasing inflammatory cytokines (Reinke et al., 2013). In our study showed that local FTY720 reduces inflammatory CD8+ T-cells that helps the early anti-inflammation healing environment.

CHAPTER 1

INTRODUCTION

Sphingosine-1-phosphatase (S1P) is a lipid signaling molecule, which regulates immune cell trafficking, and is derived from sphingolipid of the cell membrane (Maceyka, 2012). S1P signals through receptors to regulate immunity and the transportation of cells through the blood and body tissues. There are five separate S1P receptors, which are all G-protein coupled, and mediate most of the functions that S1P has to offer (Awojoodu et al., 2013). FTY720 is an agonist of four out of the five S1P receptors and known as an immunomodulatory compound for its ability to control immune cell trafficking. According to our previous research, the localized application of FTY720 in an inflammatory wound stimulates S1P receptors to promote selective recruitment of monocytes to the tissue, promoting healing of wounds and vascular remodeling (Awojoodu et al., 2013 & Ogle et al., 2017). S1P receptors are also expressed on T-cells and are critical for their trafficking between blood and tissues (Garris, 2014). T cells play many beneficial and harmful functions in tissue healing and therefore this study seeks to investigate how implantation of a biomaterial releasing FTY720 be able alter the T-cells population and identify the role of different types of T-cells within tissue injury contexts.

Biomaterial PLGA Nanofibers

Biomaterials generally induce a natural inflammatory response which can be altered by adding molecules to influence immune cell recruitment (Ogle et al., 2016). Poly (lactic-co-glycolic) acid (PLGA) has attracted much attention as a base material for many biomedical applications, due to its clinical acceptance by the FDA. This is mostly due to its biocompatibility and suitability for export including to countries whose culture is not synonymous with animal-derived products (Ogle et al., 2016). Its biodegradation rate can be tailored by changing the

molecular weight and copolymer ratios. Immunomodulatory drugs in PLGA materials can alter the immune response to the material and the injury to assist in better healing (Ogle et al., 2016). In this study, we included FTY720 into PLGA nanofibers to test the adaptive immune response to this material during injury. To understand how FTY720 alters local T-cell responses an understanding of different T-cell types and their functions in inflammation and regeneration is necessary.

Types of T-Cells

The release of FTY720 from PLGA materials creates a gradient of the molecule with in the injured tissue. Local drug delivery can change the inflammatory cells that respond to injury, without causing systemic changes. T-cells consist of several types which all play different roles during regeneration and response to biomaterials. These types of T-cells include gamma-delta T-cells, CD8+, CD4+, Th17 T-cells, as well as Regulatory T-cells (Treg). Gamma-delta T-cells have the role of up-regulating positive co-stimulatory ligands that further produce pro-inflammatory cytokines to activate both innate and adaptive immune cells (Liu, 2010). CD8+ cells are the "cytotoxic" T cell sublineage that participates in innate immunity defense against intracellular pathogens. Furthermore, CD4+ "helper" T-cells play a role in antigen-specific, cognate immunity. CD4+ cells also carry out multiple purposes that range from activation of the cells of the innate immune system to nonimmune cells as it plays a critical mission in the immune suppression reaction. Tregs are a subpopulation of CD4 T-cells that assist in the prevention of other immune cells from attacking tissues of the same body as well as other harmful foreign materials in the environment. CD4+, CD8+, and Th17 T-cells have the function of recognizing peptide antigens. They primarily coordinate and promote several activities of

different inflammatory cells. In the context of regulation, an increase in Tregs or a decrease in CD8+ T-cells would be desirable to enhance healing and reduce total inflammation.

Regulatory T-cells (Treg)

Treg cells are classifiable into two broad classes: natural and adaptive commonly known as induced Treg. The natural Treg cells are characterized as CD4+ CD25+ T-cells that develop and emigrate from the thymus to assist in performing their primary role in immune homeostasis (Tao, 2013). The adaptive class of Treg cells includes CD4+ T-cells that acquires CD25 (IL-2R alpha) expression away from the thymus (Tao, 2013). The recruitment of Tregs assist in self-renewal and the ability of the body to produce all types of cells made in the body's tissues. The depletion of Tregs during the process of repair and regeneration prolongs the pro-inflammatory infiltrate and repair of impaired muscle (Burzyn, 2013). The initial step of differentiation of the active cells during the healing process remains to be the stimulation of antigen which occurs because of the interaction of CD4 as a co-receptor with antigen major histocompatibility II (MHC II) complex (Artyomov, 2010).

Cytotoxic T-cell (CD8+ T-cell)

CD8+ T-cells play an important role as a body defense mechanism against intracellular pathogens such as the bacteria and virus. Moreover, these cells act as a surveillance for tumor, and help in controlling or killing the cancer cells. (Germain, 2012). These cells produce and release the cytotoxic granules which contain the proteins granzyme and perforin. The perforin attaches on the target cell membrane and forms a pore. The pore allows the granzymes to enter into the affected cell and this helps in shutting down the cell from producing the viral proteins. FTY720 has been shown to reduce the recruitment of CD8+ T-cells to peripheral injuries, supporting the potential to treat ongoing T-cell-medicated immunopathologic disease

(Pinschewer, 2000). One study shows that fracture healing is inversely correlated to the level of the terminally differentiated CD8+ T cell in peripheral blood, suggesting that CD8+ T-cells block healing (Reinke et al., 2013).

The development of different biomaterials to recruit endogenous pro-regenerative cells assist in the generation of tissues while decreasing reliance on exogenous tissues and organ transplantation (Rooney, 2014). Implantation of FTY720 releasing biomaterials in an injury area to recruit pro-regenerative immune cell macrophage and monocytes to repair the injury tissue; however the effect on T-cells is unknown (Olingy, 2017). This study explored the change in T-cell populations after injury and biomaterial implantation. We hypothesized that local biomaterial release of FTY720 will alter T-cell populations after injury.

CHAPTER 2

METHOD

Animal model

The dorsal skin fold window chamber model was used as inflammatory injury model as previously described (Awojoodu, 2013). Mice (C57B/6) were obtained from Charles River Laboratories, and they were of ages 6-8 weeks. The mice were anesthetized with inhaled isoflurane for surgery and randomly divided into three groups: no implant, blank PLGA/PCL (polycaprolactone) nanofiber (NF) implants and FTY720 NF implant. Biomaterials were implanted into the injured skin within a titanium frame mounted on the top side of skinfold. Mice were euthanized by the use of CO2 asphyxiation after 24 hours. Skin was harvested and digested into single cells with collagenase A1. Cells were then prepared for analysis by flow cytometry.

T-Cells enumeration by flow cytometry

The obtained cells from the injured skin tissue were washed in phosphate-buffered saline (PBS) and spun down to remove supernatant fluid. Conjugated primary antibody was added into each sample in 3% fetal bovine serum (FBS) in PBS. Cells were incubated in room temperature for 30 minutes in dark environment. After incubation, each sample was washed and resuspended in PBS. The following conjugated primary antibodies were used: PE/Cy7-conjugated anti CD3+, BV605-conjugated anti-CD4+, BV800-conjugated anti-CD8+ and PE/FITC-conjugated anti-CD25+Foxp3+. Cells were analyzed by flow cytometry. CD3+ were staining for all T-cell population, CD3+CD4+ were staining for CD4 “helper” T-cells, CD3+CD8+ were staining for CD8 “cytotoxic” T-cells and CD3+CD4+CD25+Foxp3+were staining for Treg cells. Flow

cytometry allowed for phenotyping of T cells as well as their quantification so that the immune status of the injured skin tissue on the mice could be easily monitored.

Statistical Analysis

The cell frequency from flow cytometry was analyzed by one-way ANOVA and Tukey's Post hoc tests which made comparison in no implant, blank implant and FTY720 NF implant group. The result considered to be significant with $p<0.05$.

CHAPTER 3

RESULTS

Implantation of FTY720 NF showed no significant recruitment of CD3+T-cells

Recruitment of T-cells with various implants were measured with flow cytometry. The blank or unloaded PLGA biomaterial implanted into the wound on the mice caused the highest T-cells recruited to wound as compared to the no implant state, however this was not statistically significant (Figure 1). On adding a biomaterial with FTY720 to the wounded mice; T-cells inflammation showed a trend of decrease as compared to the blank biomaterial. (Figure 1)

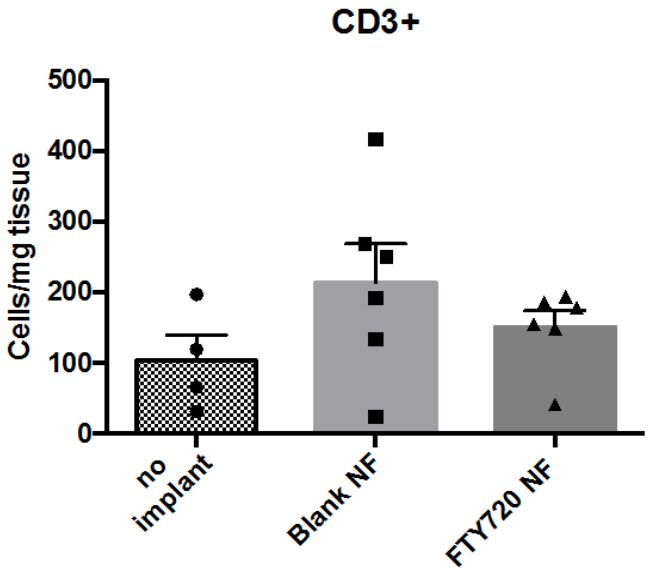

Figure 1. Implantation of FTY720 NF showed no significant recruitment of CD3+T-cells. Injuried mice were treated with no implant, blank biomaterial or biomaterial loaded with FTY720. Digested tissues were test by flow cytometry. No significant difference by One-way ANOVA statistical analysis.

CD4+ T-cells showed no significant change by various implant

To investigate the recruitment of CD4+ T-cell population on FTY720 loaded NF, we performed a surgical injury model in *vivo*, and analyzed digested tissues with flow cytometry after 24 hours. No statistically significant differences were observed in CD4+ T-cells, but

FTY720 and no implant had a lower mean as compared to blank NF(Figure 2). The repeating examination is needed for further discussion.

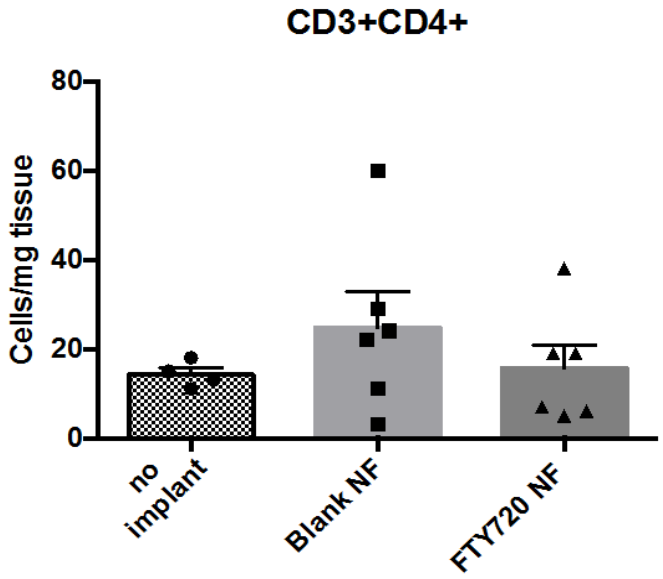

Figure 2. CD4+ T-cells showed no significant change by various implant. Wounded mice were treated in three groups, no implant, blank biomaterial or biomaterial loaded with FTY720. Digested tissues were test by flow cytometry. No significant difference by One-way ANOVA statistical analysis.

Implantation of FTY720 releasing biomaterial does not significantly increase Treg Cells

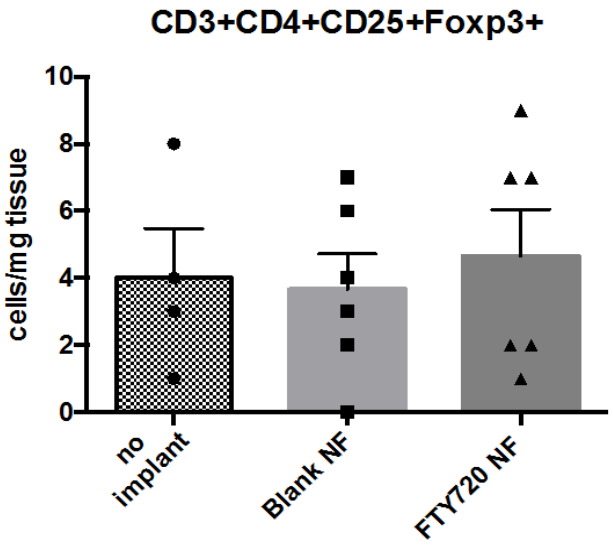

Figure 3. Implantation of FTY720 releasing biomaterials does not significantly increase Treg Cells. Wounded mice were treated with no implant, blank biomaterial or biomaterial loaded with FTY720. Digested tissues were test by flow cytometry. The statistical analysis showed no significant difference by One-way ANOVA.

Few Tregs were present in the injured tissue and no significant changes were found between group. (Figure 3) Longer time points should be examined.

Cytotoxic CD8+ T-cells are reduced by FTY720 Implants

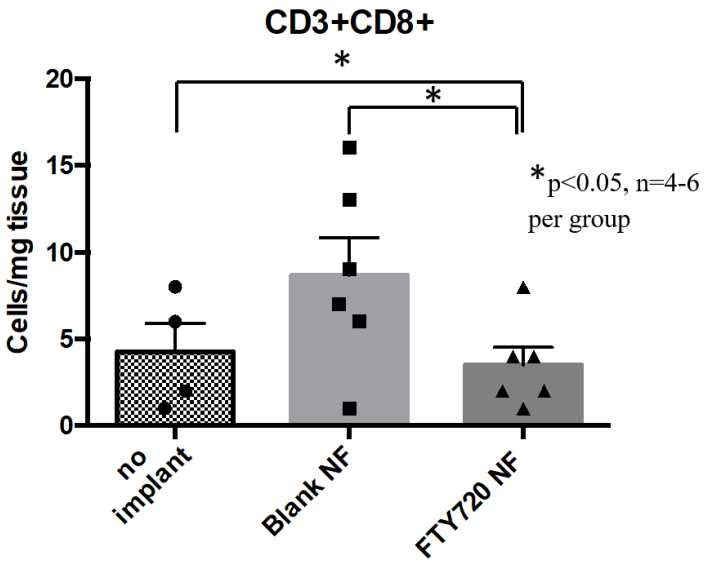

Figure 4. Cytotoxic CD8+ T-cells are reduced by FTY720 Implants. Wounded mice were treated with no implant, blank biomaterial or biomaterial loaded with FTY720. CD8+ T-cells were recruited significantly more with blank biomaterial compare to no implant and FTY720 loaded biomaterial. One-way ANOVA and Tukey's multiple comparison post hoc test were used in statistical analysis.

CD8+ T-cells, which can negatively impact healing, (Reinke, 2013) were recruited with blank NF(Figure 4). Treatment with FTY720 significnatly decreased CD8+ T-cells. There has a growing evidence that the endogenous regeneration processes are contributed by the adaptive immunity. For example, previous work shows the level of the terminally differentiated CD8+T cell in peripheral blood is directly correlated to the delayed fracture healing (Reinke et al., 2013). This is very important as it opens opportunity strategies for the early and the targeted intervention. Our data suggest that FTY720 modulates the CD8+ T-cells response to inflammatory injury.

CHAPTER 4
DISCUSSION

In this study, we have shown that CD8+ cytotoxic cells are increased in biomaterial induced inflammation in the dorsal skinfold window chamber injury model. The addition of FTY720 to the PLGA nanofibers reduces CD8+ T-cells; however, there were no changes in CD4+ T-cells or Tregs.

CD8+ T-cells are a powerful group of immune cells and are important for fighting infectious disease; however, uncontrolled CD8+ cells can impair regeneration. CD8+ T-cells play an important role in the destruction of intercellular infections in addition to control of many other diseases. CD8+ T-cells have a particularly destructive capability which can be harnessed to help in cancer eradication (Ding et al. 2017). However, the cells have adverse effects on the human body for them contribute to rejection of organ transplant, for example, hepatic transplant (Mazzola et al. 2015). Also, CD8+ T-cells have an inhibitory effect on wound healing in terms of collagen deposition and wound breaking strength (Chen, 2014). Furthermore, the CD8+ T-cells causes a massive reduction in essential body cells, especially once used in fighting diseases. During injury, high levels of CD8+ T-cells have been shown to block healing by increasing inflammatory cytokines such as interferon-gamma (IFN) (Reinke et al., 2013). Here we have shown that local FTY720 reduces inflammatory CD8+ T-cells that helps the early anti-inflammation healing environment.

FTY720 is a sphingolipid analog that signals through S1P receptors. S1P signals via five G protein-coupled receptors, termed S1P1–5. Each of the receptors couples to several G proteins eliciting different downstream events including migration of the immune cells. Figure 5 shows

the biological functions of S1P receptor signaling. Previously, our lab have shown that FTY720 stimulates the S1P receptors to signal selective recruitment of anti-inflammatory monocytes to the tissue (Awojoodu, et al., 2013). This recruitment initiates healing of wounds and vascular remodeling (Awojoodu, et al., 2013). S1P receptors are also expressed on T-cells and are critical for their trafficking between blood and tissues (Garris, 2014). FTY720 has a multi-targeted mode of action, and its CD8+ T-cell suppressive effects are initiated by its phosphorylation to an S1P analog (FTY720-P) that causes S1P receptor 1 internalization that sequesters T-cells in the lymph node (Ntranos, 2014). FTY720 phosphate activates all S1P receptors except S1P2. S1P and FTY720 phosphate are generated by intracellular phosphorylation of sphingosine and FTY270, respectively and transported outwards to elicit signaling via S1P receptors. Characteristically, FTY720 phosphate leads to agonist-induced receptor internalization of the S1P1 receptor. Our lab has shown that local FTY720 is found in the blood in low levels within the first day of material implantation (Ogle et al. 2014) and therefore may cause CD8+ cell activity in the blood.

However, this study was made at early timepoints, at later timepoints FTY720 could have more significant effects on T-cell populations regeneration. FTY720 did not show changes on Tregs, the reason could be the amount of Tregs population are to low so the effects are hard to be seen.

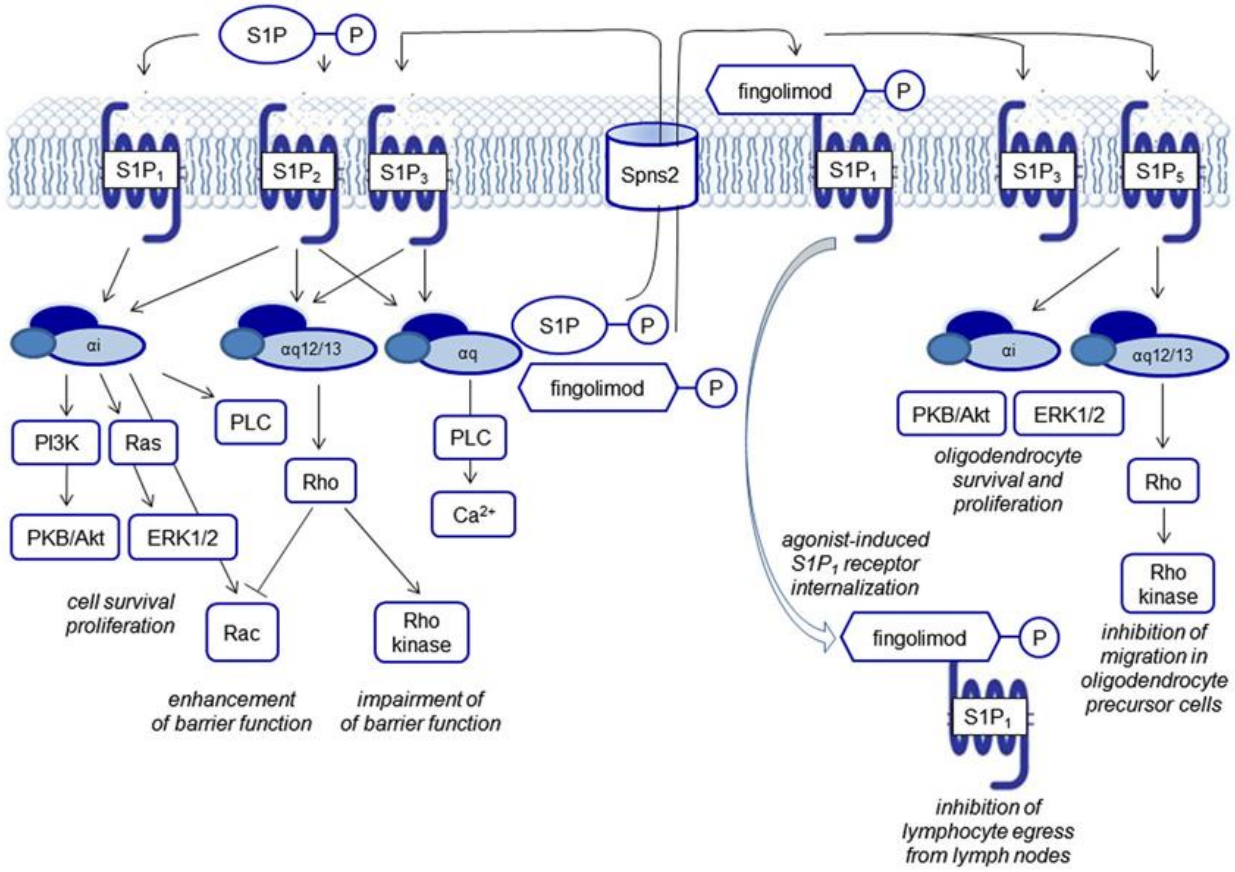

Figure 5. Biological functions of S1P receptor signaling. (Brunkhorst, 2014)

CHAPTER 5
CONCLUSION AND FUTURE WORK

The presence of CD8+ cytotoxic cells around the blank biomaterial indicates increased inflammation, while FTY720 reduced CD8+ T-cells. This result support the hypothesis, FTY720 can alter T-cell population. Application of FTY720 with biomaterials in an inflammatory wound can active the S1P receptors signaling which recruit the immune cells to the tissues necessary promote vascular remodeling. FTY720 released within the injury creates a highly localized vascular repair environment (Ogle et al., 2016). As the understanding of the role of the immune system in repair continues to grow, the opportunities also arise to solve the problems of engineering the strategies for regeneration of utilizing immune regenerate. As a drug delivery system, biomaterials have been more preferred due to its advantage in coordinating the immune response system. Moreover, the temporal control of drug delivery is very critical especially in repair and it also targets the specific phase in inflammation. Our biomaterial composition could be changed to alter the release kinetics.

In the future studies, we will look at the CD8+ T-cell negatively effective on regeneration and repeat this study with more timepoints to investigate if FTY720 be able to alter T-cell populations other than CD8+T-cells.

REFERENCES

1. Arpaia, N., Green, J. A., Moltedo, B., Arvey, A., Hemmers, S., Yuan, S., Treuting, P. M. Rudensky, A. Y. (2015) *A Distinct Function of Regulatory T Cells in Tissue Protection, Cell*.162, 1078-89.
2. Artyomov, M. N., Lis, M., Devadas, S., Davis, M. M., & Chakraborty, A. K. (2010). CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(39), 16916–16921. http://doi.org/10.1073/pnas.1010568107
3. Awojoodu, A. O., Ogle, M. E., Sefcik, L. S., Bowers, D. T., Martin, K., Brayman, K. L., . . . Botchwey, E. (2013). Sphingosine 1-phosphate receptor 3 regulates recruitment of anti-inflammatory monocytes to microvessels during implant arteriogenesis. *Proceedings of the National Academy of Sciences,110*(34), 13785-13790. doi:10.1073/pnas.1221309110
4. Ba, H., Wang, D., & Li, C. (2016). MicroRNA profiling of antler stem cells in potentiated and dormant states and their potential roles in antler regeneration. Molecular Genetics and Genomics, 291(2), 943-955. http://dx.doi.org/10.1007/s00438-015-1158-8
5. Brunkhorst, R., Vutukuri, R., & Pfeilschifter, W. (2014). Fingolimod for the treatment of neurological diseasesâ€"state of play and future perspectives. *Frontiers in Cellular Neuroscience,8*. doi:10.3389/fncel.2014.00283
6. Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J. L., Cerletti, M., Jang, Y., … Mathis, D. (2013). A Special Population of Regulatory T Cells Potentiates Muscle Repair. *Cell*, *155*(6), 1282–1295. http://doi.org/10.1016/j.cell.2013.10.054

7. Castiglioni, A., Corna, G., Rigamonti, E., Basso, V., Vezzoli, M., Monno, A., Almada, A. E.,Mondino, A., Wagers, A. J., Manfredi, A. A. & Rovere-Querini, P. (2015) FOXP3+ T Cells Recruited to Sites of Sterile Skeletal Muscle Injury Regulate the Fate of Satellite Cells and Guide Effective Tissue Regeneration, PLoS One. 10, e0128094.
8. Cerletti, M., Jang, Y. C., Finley, L. W., Haigis, M. C., & Wagers, A. J. (2012). Short-term calorie restriction enhances skeletal muscle stem cell function. *Cell stem cell*, *10*(5), 515-519.
9. Chen, L., Mehta, N. D., Zhao, Y., & DiPietro, L. A. (2014). Absence of CD4 or CD8 lymphocytes changes infiltration of inflammatory cells and profiles of cytokine expression in skin wounds, but does not impair healing. *Experimental Dermatology*, *23*(3), 189–194. http://doi.org/10.1111/exd.12346
10. Cottler, Shayn Peirce, (2010). "Anatomical model and method for medical training." U.S. Patent No. 7,850,455.
11. Dawson, J. &Oreffo, R. (2013). Clay: New Opportunities for Tissue Regeneration and BiomaterialDesign. Adv. Mater., 25(30), 4069-4086. http://dx.doi.org/10.1002/adma.201301034
12. Ding, K., Kirchartz, T., Bittkau, K., Lambertz, A., Smirnov, V., Hüpkes, J., & Rau, U. (2017). Photovoltaics: Nanoengineered Materials and Their Functionality in Solar Cells. *Nanotechnology for Energy Sustainability*, 181-206.
13. Fehervari, Z. (2015). Treg cell wiring. Nature Immunology, 17(1), 47-47. http://dx.doi.org/10.1038/ni.3358
14. Fuller, A. & Qian, L. (2014). MiRiad Roles for MicroRNAs in Cardiac Development and Regeneration. Cells, 3(3), 724-750. http://dx.doi.org/10.3390/cells3030724

15. Garris, C. S., Blaho, V. A., Hla, T., & Han, M. H. (2014). Sphingosine-1-phosphate receptor 1 signalling in T cells: trafficking and beyond. *Immunology*, *142*(3), 347–353. http://doi.org/10.1111/imm.12272

16. Germain, R., & Kastenmuller, W. (2012). Faculty of 1000 evaluation for The alarmin interleukin-33 drives protective antiviral CD8^{+} T cell responses. *F1000 - Post-publication Peer Review of the Biomedical Literature*. doi:10.3410/f.13988961.15478088

17. Kinsey, G. R., & Okusa, M. D. (2014). Expanding role of T cells in acute kidney injury. *Current Opinion in Nephrology and Hypertension*, *23*(1), 9–16. http://doi.org/10.1097/01.mnh.0000436695.29173.de

18. Liu, G., Zhang, L., & Zhao, Y. (2010). Modulation of immune responses through direct activation of Toll-like receptors to T cells. *Clinical and Experimental Immunology*, *160*(2), 168–175. http://doi.org/10.1111/j.1365-2249.2010.04091.x

19. Maceyka, M., Harikumar, K. B., Milstien, S., & Spiegel, S. (2012). SPHINGOSINE-1-PHOSPHATE SIGNALING AND ITS ROLE IN DISEASE.*Trends in Cell Biology*, *22*(1), 50–60. http://doi.org/10.1016/j.tcb.2011.09.003

20. Mazzola, M. A., Aly, L., Griffin, R., Nejad, P., Weiner, H., & Gandhi, R. (2015). Immune regulatory effects of FTY720 on Dendritic cells (P1. 142). *Neurology*, *84*(14 Supplement), P1-142.

21. Ntranos, A., Hall, O., Robinson, D. P., Grishkan, I. V., Schott, J. T., Tosi, D. M., . . . Gocke, A. R. (2014). FTY720 impairs CD8 T-cell function independently of the sphingosine-1-phosphate pathway. *Journal of Neuroimmunology*,*270*(1-2), 13-21. doi:10.1016/j.jneuroim.2014.03.007

22. Ogle, M. E., Krieger, J. R., Tellier, L. E., Mcfaline-Figueroa, J., Temenoff, J. S., & Botchwey, E. A. (2017). Dual Affinity Heparin-Based Hydrogels Achieve Pro-Regenerative Immunomodulation and Microvascular Remodeling. *ACS Biomaterials Science & Engineering,4*(4), 1241-1250. doi:10.1021/acsbiomaterials.6b00706

23. Ogle, M. E., Sefcik, L. S., Awojoodu, A. O., Chiappa, N. F., Lynch, K., Peirce-Cottler, S., & Botchwey, E. A. (2014). Engineering *in vivo* gradients of sphingosine-1-phosphate receptor ligands for localized microvascular remodeling and inflammatory cell positioning. *Acta Biomaterialia, 10*(11), 4704–4714. http://doi.org/10.1016/j.actbio.2014.08.007

24. Ogle, M. E., Segar, C. E., Sridhar, S., s& Botchwey, E. A. (2016). Monocytes and macrophages in tissue repair: Implications for immunoregenerative biomaterial design. *Experimental Biology and Medicine*, *241*(10), 1084-1097.

25. Olingy, C. E., San Emeterio, C. L., Ogle, M. E., Krieger, J. R., Bruce, A. C., Pfau, D. D., … Botchwey, E. A. (2017). Non-classical monocytes are biased progenitors of wound healing macrophages during soft tissue injury. *Scientific Reports, 7*, 447. http://doi.org/10.1038/s41598-017-00477-1

26. Osherovich, L. (2011). Raising a Treg cell army. Science-Business Exchange, 4(23). http://dx.doi.org/10.1038/scibx.2011.646

27. Pinschewer, D. D., Ochsenbein, A. F., Odermatt, B., Brinkmann, V., Hengartner, H., & Zinkernagel, R. M. (2000). FTY720 Immunosuppression Impairs Effector T Cell Peripheral Homing Without Affecting Induction, Expansion, and Memory. *The Journal of Immunology,164*(11), 5761-5770. doi:10.4049/jimmunol.164.11.5761

28. Reinke, S., Geissler, S., Taylor, W. R., Schmidt-Bleek, K., Juelke, K., Schwachmeyer, V., ... & Unterwalder, N. (2013). Terminally differentiated CD8+ T cells negatively affect bone regeneration in humans. *Science translational medicine*, *5*(177), 177ra36-177ra36

29. Rooney, C. (2014). Can Treg elimination enhance NK cell therapy for AML?. Blood, 123(25), 3848-3849. http://dx.doi.org/10.1182/blood-2014-05-570291

30. Schultz, L., & Mackall, C. (2018). Immunotherapy with Genetically Modified T Cells. *Immunotherapy in Translational Cancer Research*, 101-114.

31. Sreejit, P. &Verma, R. (2013). Natural ECM as Biomaterial for Scaffold Based Cardiac Regeneration Using Adult Bone Marrow Derived Stem Cells. Stem Cell Rev and Rep, 9(2), 158-171. http://dx.doi.org/10.1007/s12015-013-9427-6

32. Tao, L. (2013). High CCR6/CCR7 expression and Foxp3+ Treg cell number are positively related to the progression of laryngeal squamous cell carcinoma. Oncology Reports. http://dx.doi.org/10.3892/or.2013.2603

33. Usui, Y. (2013). The roles of non-T-cells in infectious uveitis. Inflammation and Regeneration, 33(5), 269-273. http://dx.doi.org/10.2492/inflammregen.33.269

34. Young, F., Sloan, A., & Song, B. (2013). Dental pulp stem cells and their potential roles in central nervous system regeneration and repair. Journal of Neuroscience Research, 91(11), 1383-1393. http://dx.doi.org/10.1002/jnr.23250