Abstract: "Developing a Novel Bio-Compatible Scaffold for Pulp-Dentin Regeneration Following Trauma to the Immature Permanent Teeth"

There is a critical need for a new approach to replace the current treatment options for traumatized immature permanent teeth of children. Because children possess thin and underdeveloped dental structures, conventional treatments are risky, painful, and cause the loss of the natural tooth. The goal of this research is to meet this medical need by developing a novel thin film composite scaffold, with materials approved for use in the mouth, which can enhance the regeneration potential of dental pulp stem cells (DPSCs) of the traumatized tooth to biologically regenerate a pulp-dentin complex, an indicator of consequent tooth formation. Scaffold surface topography, chemistry, and mechanical properties were manipulated to understand their effects on DPSC proliferation and differentiation. Varying concentrations of titanium dioxide nanoparticles and mineral trioxide aggregate (MTA) were spun-cast with polyisoprene (PI), a biocompatible and malleable polymer. Of all eight created scaffolds, the one of 15 mg/mL PI combined with 5 mg/mL MTA and exposed to the signaling molecule dexamethasone induced a faster doubling time and more differentiated odontoblasts. Hydrophobic and bumpier scaffold surfaces exposed to dexamethasone stimulated the greatest DPSC differentiation and proliferation, and successfully regenerated lost dental tissues, providing a new and promising direction for future treatment possibilities.

Executive Summary

There is a critical need for a new approach to replace the current treatment options for traumatized immature permanent teeth of children. Trauma includes injuries that expose the dental pulp of the tooth. Because children possess thin and underdeveloped dental structures, conventional treatments are risky, painful, and cause the loss of the natural tooth. Furthermore, the immature permanent tooth has an enormous capacity for healing due to its extremely vital dental pulp. The goal of this research is to meet this significant medical need by developing a novel thin film composite scaffold, with materials approved for use in the mouth, which can enhance the regeneration potential of dental pulp stem cells (DPSCs) of the traumatized tooth to biologically regenerate a pulp-dentin complex, an indicator of consequent tooth formation. Scaffold surface topography, chemistry, and mechanical properties were manipulated to understand their effects on DPSC proliferation and differentiation. Varying concentrations of titanium dioxide nanoparticles and mineral trioxide aggregate were imbedded in polyisoprene (PI), a biocompatible and malleable polymer, through spin casting procedures to create thin polymer films and the equal distribution of additives. Physical properties of scaffolds were characterized using optical microscopy. Proliferation induction was analyzed though cell culture and counting, and differentiation induction was analyzed through scanning electron microscopy. Hydrophobic and bumpier scaffold surfaces exposed to the signaling molecule dexamethasone (DEX) stimulated the greatest DPSC proliferation and differentiation, with faster doubling times and more differentiated odontoblast cells present. The created scaffold, 15 mg/mL PI combined with 5 mg/mL MTA and exposed to DEX, successfully restored lost dental tissues. Our research provides a new and promising direction for future research.

I. Introduction

Currently, the conventional treatment options for immature permanent teeth that have sustained trauma which exposes the pulp predominately include root canal surgery and dental cement plugging.[1] However, in children, the root canal system is difficult to debride and the thin dentinal walls increase the risk of fracture. Hence, both methods are risky, painful, and cause the loss of the natural tooth.[6] This is a significant unmet medical need in this field, particularly when trauma to the anterior teeth accounts for one third of all traumatic injuries in boys and one fourth in girls.[1]

Thus, there is a critical need to develop a biologically based treatment for tooth structure repair and regeneration directly in the patient's mouth, to both avoid surgical procedures and preserve the natural tooth. In fact, the bone regeneration potential of the immature permanent tooth is especially promising, as the young dental pulp tissue is extremely vital.[5] Scientists have begun experimenting with tooth regeneration from dental pulp stem cells (DPSCs), multipotent stem cells found in the pulp tissue, in laboratory settings.[9] However, little progress has been made in attempting to regenerate and repair teeth directly in the oral cavity, retaining the natural tooth.[13] The goal of this research is to develop a novel thin film composite scaffold, with materials approved for use in the mouth, which can induce the exposed DPSCs of the traumatized tooth to proliferate and differentiate to biologically regenerate a pulp-dentin complex, an indicator of consequent tooth formation.

The dental pulp, the innermost living tissue of the tooth, is characterized by its abundance of DPSCs.[9] DPSCs are multipotent stem cells capable of self-renewal, proliferation, and differentiation into a number of somatic cells including odontoblasts, cementoblasts, and fibroblasts.[5] Odontoblasts are cells that comprise the outer surface of the dental pulp, and

differentiate to form the dentin of the tooth. Dentin formation, or the formation of a pulp-dentin complex, is the first identifiable and necessary stage of tooth development.[2] Cementoblasts are cells around the root of the tooth, and proliferate to form the cementum of the tooth.[3] Fibroblasts are cells from the dental follicle that proliferate to form the periodontal ligament of the tooth. Both the cementum and the periodontal ligament form after the dentin.[11]

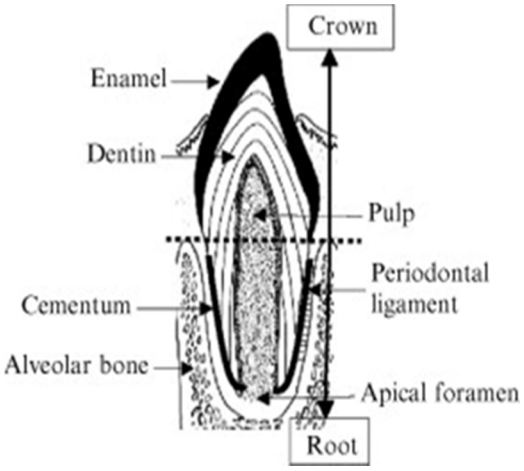

Figure 1: *Diagram of the tooth structure.* [9]

Stem cell proliferation, or growth, and differentiation are dependent upon three main factors: the type of stem cell, the underlying scaffold, and the signaling molecules added.[10] Ideal scaffolds provide a foundation and initial support for cells to adhere, signal, proliferate, and differentiate. Because scaffold surface topography, surface chemistry, and mechanical properties can influence cell behavior, it is essential to manipulate such factors and understand their effects to create a bioactive scaffold.[4]

To ensure safety in the oral cavity, all constituents of the scaffold needed to be FDA approved for use in the mouth. Polyisoprene (PI), a hydrophobic, biocompatible, and malleable polymer found in root canal filling materials, was chosen as the base substance of the scaffold.[14] The surface hydrophobicity of biomaterials is an important mechanical property of cell response, and previous studies have shown that hydrophobic surfaces can promote cell adhesion and attachment.[16] Material surface topography, or surface roughness, which can affect cell proliferation and differentiation, was altered with the imbedding of varying concentrations of titanium dioxide nanoparticles (TiO_2 NPs) and mineral trioxide aggregate (MTA).[7] TiO_2 NPs,

20-35 nanometer long particles found in toothpaste, can be utilized to generate surface roughness.[12] MTA, a unique dental material composed of various minerals and oxides, possesses antimicrobial qualities. It is biocompatible with tissues of the tooth, non-cytotoxic to cells, and antimicrobial to bacterial.[15]

Additionally, because signaling molecules are capable of stimulating cellular differentiation, it is likely that they play major roles in guiding the development of cells in regenerating dental tissue.[16] In fact, they have also been shown to have an important role in the signaling reparative processes in the dentin and the pulp.[17] Dexamethasone is a promising signaling molecule, and its effects were tested on DPSC differentiation.[4] Composite material scaffolds may possess the optimal surface properties to stimulate DPSC proliferation and differentiation, and ultimately, tooth structure regeneration.

II. Materials & Methods

This research was divided into two parts to effectively distinguish the induced proliferation and differentiation capabilities of each created scaffold. The first part evaluated DPSC proliferation on each test scaffold using an 8-day proliferation experiment. The second part evaluated DPSC differentiation on each test scaffold using a 28-day differentiation experiment. Proper care (lab coat, nitrile gloves, and safety goggles) was exercised whenever in the laboratory and whenever handling chemicals and bacteria. All experiments were performed under a fume hood for contamination prevention and safety precautions.

Part 1: Proliferation

Test scaffolds were engineered and tested to determine the one with the most favorable proliferation capabilities. Eight scaffold conditions differing in additives and concentrations were created:

Material	Test scaffold							
	1	2	3	4	5	6	7	8
Polyisoprene (PI)	15mg/mL	15mg/mL	15mg/mL	15mg/mL	15mg/mL	15mg/mL	15mg/mL	15mg/mL
Anatase TiO_2 NP		0.1mg/mL	0.5mg/mL					
Rutile TiO_2 NP				0.1mg/mL	0.5mg/mL			
MTA						0.5mg/mL	5mg/mL	10mg/mL

Figure 2*: Table displaying different combinations of PI, TiO_2 NP, and MTA.*

PI was acquired from Scientific Polymer Products Incorporated, anatase and rutile TiO_2 NP (20-35 nm diameter) were acquired from Sigma-Aldrich, and MTA was acquired from Tulsa Dental Specialties.

Eight 20 mL scintillation vials were prepared. Toulene, a monodispersed solvent used to effectively blend PI with the varying additives, was added to all vials. The working test volume of each vial was 3 mL; therefore, 45 mg of PI was added to toluene to achieve a final concentration of 15 mg/mL. Depending on the allocated concentrations of anatase TiO_2 NP, rutile TiO_2 NP, and MTA, masses were calculated, measured, and added to vials. All eight vials were placed in a sonicator bath for 15 minutes at 30^{o}C for increased dispersion of all constituents.

Cleaned and treated 1 cm^2 silicon wafers were utilized in spinning solutions into thin film surfaces. In order to significantly reduce the organic contaminants and metallic residue on the surface of the silicon wafers, wafers were submerged into Base Piranha solution, a combination of ammonium hydroxide, hydrogen peroxide, and deionized water in a 1:1:5 ratio, and brought to a boil using a hotplate. After 15 minutes, the Base Piranha solution was then removed and the

silicon wafers were rinsed with deionized water 2-3 times. The wafers were then immersed into a hydrofluoric acid solution, which reacts with the OH^- groups on the surfaces of the silicon wafers, to make the surfaces hydrophobic and thus able to affix with the hydrophobic PI.

Using a plastic 1 mL pipette, two drops of each solution were used to coat the surface of the silicon wafers. The silicon wafers were then placed on the chuck of a Headway Research Incorporated spin casting machine (model PWM32) and spun cast at 2500 revolutions per minute (RPM) for 30 seconds. As the silicon wafer is spun, toluene quickly evaporates, leaving a thin film of PI and additive on its surface. Spun cast silicon wafers were then placed into a 275 convectron gauge Granville-Phillips Hotpack vacuum oven for 24 hours at 10^{-3} torr and 150°C in order to anneal, or release the stress between polymer chains of the thin film scaffolds. The test scaffold of solely 15 mg/mL PI was analyzed using ellipsometry (SOPRA GES5E Spectroscopic Ellipsometer), a device that measures the way polymers change the angle of light, to measure the thin film thickness of all test scaffolds. Scaffolds were then examined using optical microscopy (Nikon Eclipse Ni-U Optical Microscope) to analyze surface morphology.

While the silicon wafers were annealing, DPSCs were cultured for an 8-day proliferation experiment. For every test scaffold condition, nine physical thin films were created for triplicate counting at three time points. AX3 Dental Pulp Stem Cells (P7) were grown in a culture flask and nourished with alpha-MEM medium (Invitrogen) with 10% fetal bovine serum, 200 mol/L L-ascorbic acid 2-phosphate 2 mM Lgluatamine, 100 unit/mL penicillin, 100 μg/mL streptomycin, and 10 mM beta-glycerophosphate. Silicon wafers with thin film scaffolds were placed in 24-well plates. After standard cell culturing and detaching procedures, 40 μL DPSCs were seeded onto each thin film scaffolds for a cell confluence of 10,000 cells/cm^2. 1 mL of cell medium was added to each well, and 24-well plates were put into a Steri-Cult 200 incubator

(Forma Scientific) at 30ºC. DPSCs were seeded on "Day 1." On days 3, 5, and 8, DPSCs grown on each test scaffold triplicate were cultured, detached, and counted using a hemoctyometer to create a growth curve.

On Day 8, one sample out of each triplicate was prepared for confocal microscopy to visualize DPSC proliferation. 1 mL 10% formalin was added to each well for 15 minutes to fix cells and stop further cell growth and insure an accurate representation of the cell confluence on each test scaffold. DPSCs were then stained with two dyes: Alexa Fluor 488, a green dye that stains the actin fibers of the cell to outline cell structure, and Propidium Iodide, a red dye that stains the nucleus of the cell. Samples were then observed using confocal microscopy.

Part 2: Differentiation

Depending upon the results from the 8-day proliferation experiment, the test scaffolds with the most favorable proliferation capabilities, and a differentiation control of pure PI, were engineered and tested for differentiation capabilities. Four scaffold conditions differing in additives and concentrations were created: pure 15 mg/mL PI, PI combined with 0.5 mg/mL anatase TiO_2 NP, PI combined with 0.5 mg/mL rutile TiO_2 NP, and PI combined with 5 mg/mL MTA.

Three 20 mL scintillation vials were prepared. Following the same procedure as in *Part 1*, solutions were measured and mixed for each test scaffold condition. Solutions were then spun cast onto cleaned and treated silicon wafers, and the wafers were annealed in a vacuum oven.

DPSCs were then cultured for a 28-day differentiation experiment. For every test scaffold condition, two physical thin films were created to test scaffold induced differentiation capability with and without dexamethasone. AX3 Dental Pulp Stem Cells (P7) were grown in a culture

flask and nourished with alpha-MEM medium (Invitrogen) with 10% fetal bovine serum, 200 mol/L L-ascorbic acid 2-phosphate 2 mM Lgluatamine, 100 unit/mL penicillin, 100 μg/mL streptomycin, and 10 mM beta-glycerophosphate. Silicon wafers with thin film scaffolds were placed in 24-well plates. After standard cell culturing and detaching procedures, 40 μL DPSCs were seeded onto each thin film scaffolds for a cell confluence of 10,000 cells/cm^2. 1 mL of cell medium was added to each well, and after 24 hours, 5 μL of dexamethasone was added to half of the wells. 24-well plates were then put into an incubator at 30°C. Subsequently, the culture flasks were left undisturbed for 4–5 days to promote cell attachment. After this time, the non-adherent cells were removed by replacing the medium and dexamethasone every day.

After 28 days, DPSCs grown on each test scaffold were prefixed in a solution containing 1% paraformaldeyde, 1.5% glutaraldehyde in 0.15 M cacodylate buffer for 2 hours at room temperature. Samples were then dehydrated with acetone, and left to dry for 24 hours and observed with a Hitachi S4000 FE scanning electron microscope.

III. Results & Illustrations

Because surface topography was expected to affect scaffold proliferation and differentiation capabilities, test scaffolds were examined and photographed using optical microscopy to ensure additive encapsulation and discern and analyze topographical differences. Pictures were taken using a Nikon Eclipse Ni-U Optical Microscope under 10X magnification (*Fig. 3*).

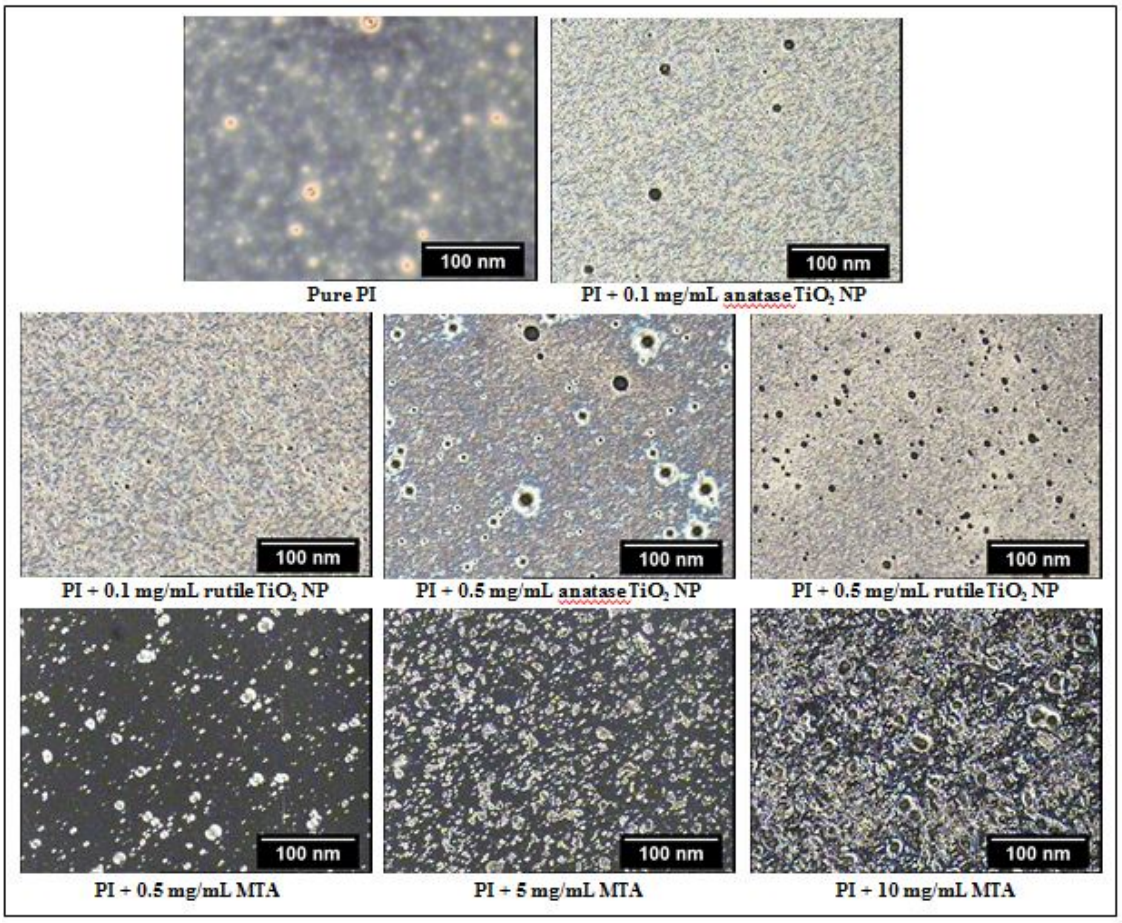

***Figure 3**: Optical microscope images of spun cast thin film scaffolds on silicon wafers. (Upper row: pure PI, PI combined with 0.1 mg/mL anatase TiO_2 NP; Middle row: PI combined with 0.1 mg/mL rutile TiO_2 NP, PI combined with 0.5 mg/mL anatase TiO_2 NP, PI combined with 0.5 mg/mL rutile TiO_2 NP; Lower row: PI combined with 0.5 mg/mL MTA, PI combined with 5 mg/mL MTA, PI combined with 10 mg/mL MTA).*

The greater the concentration of TiO_2 NP added to PI was, the bumpier the surface of the scaffold became. Scaffolds spun with 0.5 mg/mL TiO_2 NP displayed significantly larger and more frequently occurring nanoparticle aggregations that those spun with 0.1 mg/mL TiO_2 NP. More specifically, anatase TiO_2 NP was more inclined towards aggregation than rutile TiO2 NP, thus creating larger but less frequently occurring clusters. Following a similar pattern, the greater the concentration of MTA added to PI was, the bumpier the surface of the scaffold became. In the higher concentrations of MTA scaffolds, especially the one containing 10 mg/mL MTA,

particles covered the entire scaffold surface. Sizes of aggregated MTA particles were slightly smaller in size compared to aggregated anatase TiO_2 NP particles, but more frequently occurring.

Thin film thickness of test scaffolds was measured using ellipsometry (Plasmos SD-4000 Ellipsometer), an optical technique that measures the way a polymer changes the angle of light. The average thickness of scaffolds was 1184.67 angstroms, equivalent to 118.467 nanometers.

Part 1: Proliferation

Two growth curves were created with cell counting data, the first involving scaffolds with TiO_2 and the second involving those with MTA. For the first growth curve, linear averages of six test conditions (Pure PI, PI combined with 0.1 mg/mL anatase TiO_2 NP, PI combined with 0.1 mg/mL rutile TiO_2 NP, PI combined with 0.5 mg/mL anatase TiO_2 NP, PI combined with 0.5 mg/mL rutile TiO_2, and tissue culture plastic control) were plotted against each other (*Fig. 4*).

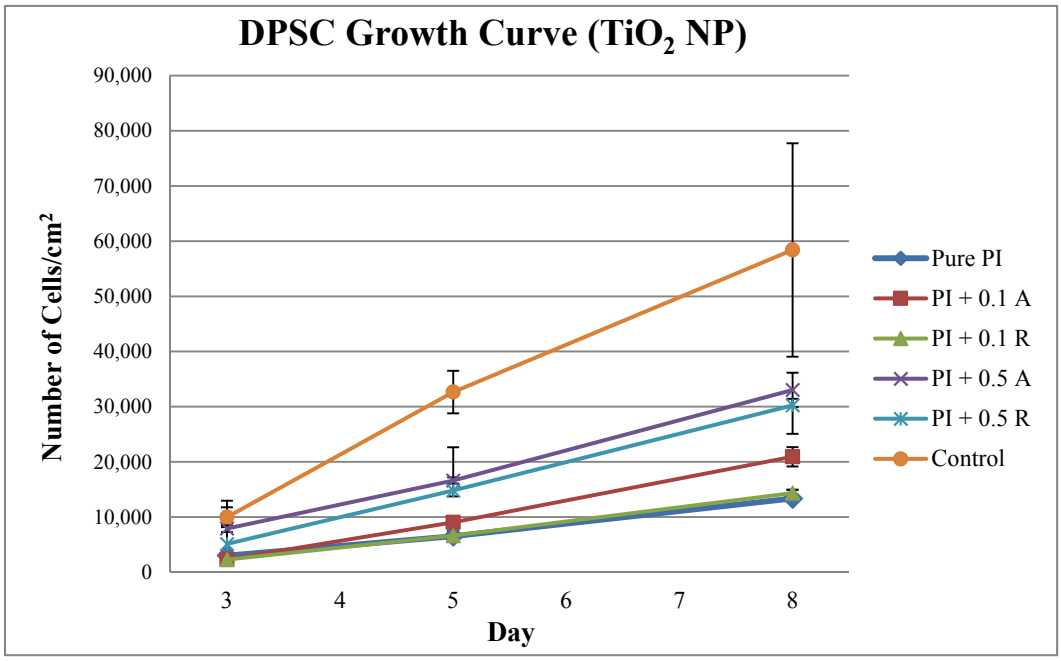

Figure 4: *Points were plotted with cell counting data on Days 3, 5, and 8. Error bars represent standard error of triplicates for each test scaffold condition.*

Scaffolds spun with higher concentrations of TiO_2, which had bumpier surfaces, induced greater DPSC proliferation, stimulating the greater number of cells on Day 8. More specifically, the test scaffold condition of PI combined with 0.5 mg/mL anatase TiO_2 induced the greatest cell proliferation, notwithstanding cell proliferation on the tissue culture plastic control. Furthermore, scaffolds containing anatase TiO_2 induced greater cell proliferation for both 0.1 mg/mL and 0.5 mg/mL concentrations than those seeded on scaffolds containing rutile TiO_2. The pure PI scaffold, with its relatively smooth surface, performed the worst.

The second growth curve involved scaffolds spun with MTA. Linear averages of five test conditions (Pure PI, PI combined with 0.5 mg/mL MTA, PI combined with 5 mg/mL MTA, PI combined with 10 mg/mL MTA, and tissue culture plastic control) were plotted against each other (*Fig. 5*).

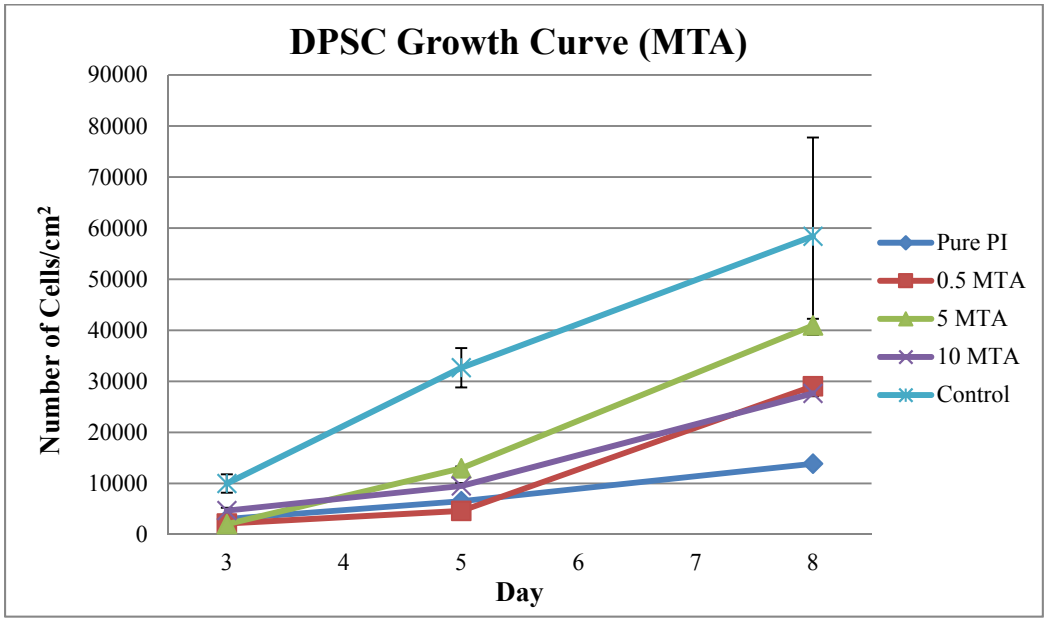

Figure 5*: Points were plotted with cell counting data on Days 3, 5, and 8. Error bars represent standard error of triplicates for each test scaffold.*

The PI combined with 5 mg/mL MTA test scaffold induced the greatest DPSC proliferation out of both MTA concentrations and TiO_2 concentrations. It stimulated the greatest

number of cells on Day 8 (around 40,000), notwithstanding cell proliferation on the tissue culture plastic control. The pure PI scaffold performed the worst.

Cell proliferation data of DPSCs seeded on eight test scaffolds and tissue culture plastic was also represented using calculated cell doubling times (*Fig. 6*). Cell doubling time was calculated by taking the logarithm of the data points (averages of Day 3, 5, and 8) of each test condition and making a linear regression line The slope (k) of each line was then used to calculate doubling time (t) through the derived formula: t = (log2)/(k).

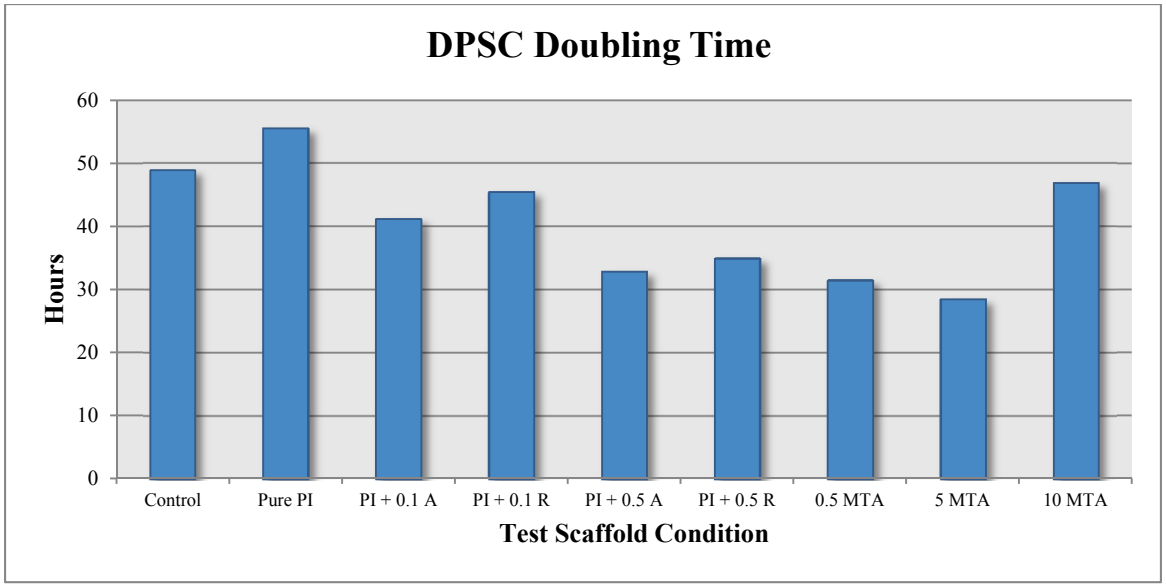

Figure 6*: Bars represent doubling times of DPSCs seeded on different test scaffold conditions over an 8-day proliferation period.*

Trends displayed by both growth curves were verified, with the exception of that of the tissue culture plastic control. Although in both growth curves, more DPCSs were present on Day 8 on the control, doubling time data indicates that with more time, the amount of DPSCs on almost every test scaffold would have exceeded the amount on the control. DPSCs seeded on the test scaffold of PI combined with 5 mg/mL MTA had the fastest doubling time while DPSCs seeded on the test scaffold of pure PI had the slowest doubling time. Similar to trends indicated by the growth curve, DPSCs seeded on scaffolds containing a greater concentration of TiO_2 NP

doubled faster than those seeded on scaffolds containing a lower concentration of TiO_2 NP. Furthermore, DPSCs seeded on scaffolds containing anatase TiO_2 doubled faster for both 0.1 mg/mL and 0.5 mg/mL concentrations than those seeded on scaffolds containing rutile TiO_2. On Day 8, DPSCs on each test scaffold were stained and examined through confocal microscopy. Pictures were taken using an Olympus Spinning Disk BX51 Confocal Microscope under 20X magnification (*Fig. 7*).

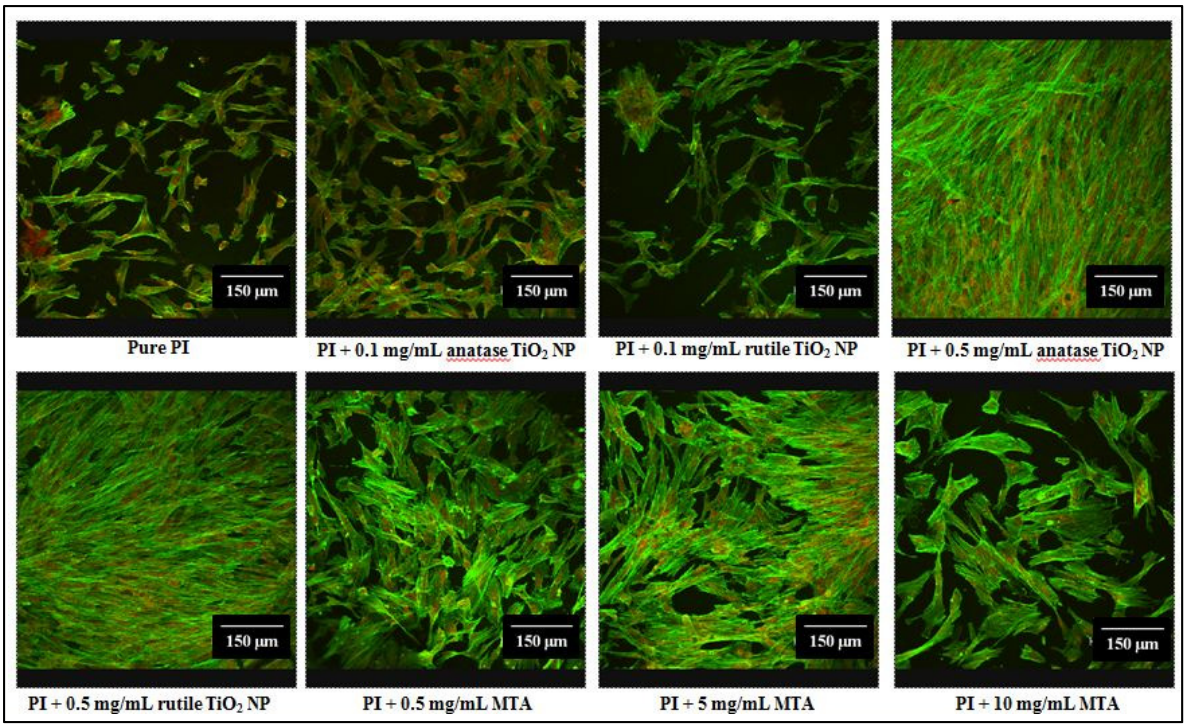

***Figure 7**: Confocal images of Day 8 DPSCs seeded on test scaffolds. (Upper row: pure PI, PI combined with 0.1 mg/mL anatase TiO_2 NP, PI combined with 0.1 mg/mL rutile TiO_2 NP, PI combined with 0.5 mg/mL anatase TiO_2 NP; Lower row: PI combined with 0.5 mg/mL rutile TiO_2 NP, PI combined with 0.5 mg/mL MTA, PI combined with 5 mg/mL MTA, PI combined with 10 mg/mL MTA).*

Trends displayed by growth curves and cell doubling time data were further verified. DPSCs seeded on the pure PI scaffold showed the lowest confluency, while DPSCs seeded on increasing concentrations of TiO_2 NP imbedded test scaffolds showed higher cell confluency. As concentrations of TiO_2 NP in test scaffolds increased, DPSC growth increased as well. Furthermore, anatase TiO_2 NP test scaffolds induced greater cell proliferation than rutile TiO_2

NP scaffolds, a trend supported by earlier growth curve and cell doubling time data. In test scaffolds containing MTA, cell confluence was greatest on the 5 mg/mL MTA test scaffold and lowest on the 10 mg/mL MTA test scaffold.

The three test scaffolds inducing the greatest DPSC proliferation- 0.5 mg/mL anatase TiO_2 NP, 0.5 mg/mL rutile TiO_2, and 5 mg/mL MTA- were then tested for differentiation.

Part 2: Differentiation

DPSCs were plated on test scaffolds selected from the 8-day proliferation experiment (pure PI, PI combined with 0.5 mg/mL anatase TiO_2 NP, PI combined with 0.5 mg/mL rutile TiO_2, and PI combined with 5 mg/mL MTA) for a 28-day differentiation experiment. After 28 days, scanning electron microscopy (SEM) was utilized to analyze DPSC osteocalcin bio-mineralization, or differentiation into odontoblasts. For each test scaffold condition, two physical scaffolds were created: one with exposure to dexamethasone (DEX), and one without exposure.

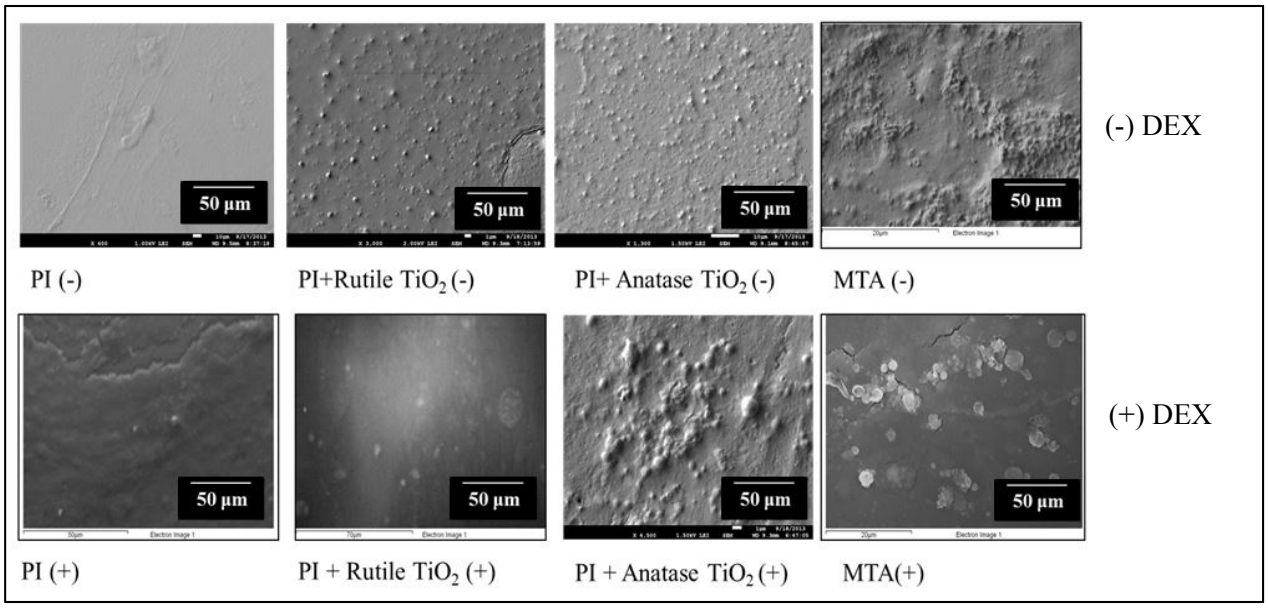

Figure 8: *SEM images of Day 28 DPSCs plated on test scaffolds with/without DEX. Test scaffold combinations: pure PI; PI combined with 0.5 mg/mL rutile TiO_2 NP; PI combined with 0.5 mg/mL anatase TiO_2 NP; or PI combined with 0.5 mg/mL MTA.*

The SEM images of pure PI samples with and without DEX displayed little to no cell differentiation. In contrast, the DPSCs seeded on the scaffold containing 5 mg/mL MTA induced the greatest cell differentiation in both with and without DEX experiments. In fact, with exposure to DEX, the DPSCs on the 5 mg/mL MTA scaffold showed clear differentiation into odontoblast cells of the dentin, as indicated by the cellular shapes. Overall, DPSCs with exposure to DEX had an increase in differentiation as compared to those without exposure.

Energy Dispersive X-Ray Spectroscopy was used to analyze the elemental composition of the samples in order to determine the presence of calcium and phosphorous, indicators of bio-mineralization and differentiation, within the DPSCs seeded onto the test scaffolds.

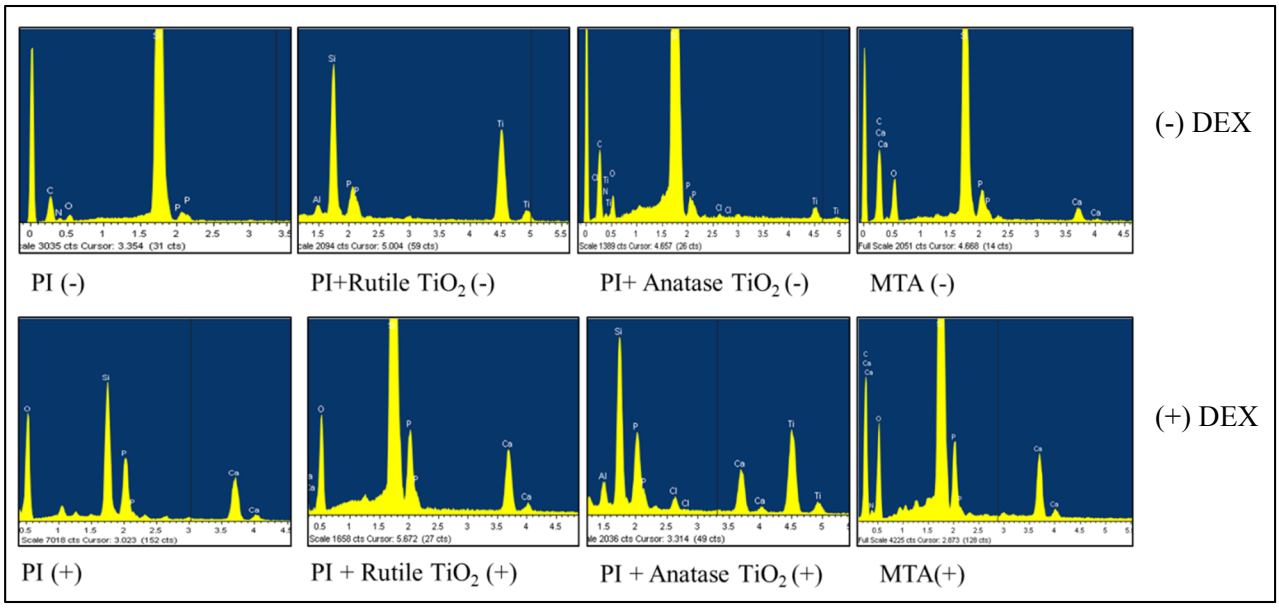

Figure 9: *EDX spectra of Day 28 DPSCs plated on test scaffolds with/without DEX. Peaks represent relative quantity of elements present in sample. Test scaffold combinations: pure PI; PI combined with 0.5 mg/mL rutile TiO_2 NP; PI combined with 0.5 mg/mL anatase TiO_2 NP; or PI combined with 0.5 mg/mL MTA.*

The high peak of silicon in each image is a result of emissions from the silicon wafer each scaffold was spun on, and is not indicative of differentiation. The pure PI scaffold without DEX had the lowest amount of bio-mineralization and differentiation as seen by the absence of

significant calcium (Ca^{+2}) or phosphorus (P) peaks. The scaffold containing 0.5 mg/mL anatase TiO_2 NP had the most prominent Ca^{+2} and P peaks, which indicate a relatively high induced DPSC differentiation. In general, the scaffolds and DPSCs exposed to DEX induced more bio-mineralization and differentiation than those without it, confirming previous SEM studies (*Fig. 7*).

IV. Discussion

This study sought to develop a biocompatible thin film scaffold possessing beneficial topographical and mechanical properties to increase the potency of DPSCs and thus regenerate lost dental tissues of the immature permanent tooth.

Part 1: Proliferation

Scaffold surface topography and mechanical properties had a pronounced effect on DPSC proliferation capabilities. Of the test scaffolds imbedded with TiO_2 NPs, DPSCs seeded on scaffolds imbedded with higher concentrations proliferated better that those seeded on scaffolds imbedded with lower concentrations, with faster doubling times and more cells present on Day 8. Higher concentrations of TiO_2 NPs created more frequent bumps on the surfaces of scaffolds; hence, DPSCs proliferated better on test scaffolds with bumpier surfaces. More specifically, test scaffolds spun with anatase TiO_2 NPs induced greater DPSC proliferation that those spun with rutile TiO_2 NPs, with the scaffold containing 0.5 mg/mL anatase TiO_2 NP inducing the greatest cell proliferation. Scaffolds spun with anatase TiO_2 NP were more inclined towards aggregation than rutile TiO_2 NP, creating larger and more prominent clusters (*Fig. 3*). The test scaffold comprised of pure PI maintained a relatively smooth surface, and induced the least DPSC proliferation. Thus, scaffold surface topography is directly related to DPSC proliferation

capabilities, as DPSCs proliferated better on test scaffolds with larger and more prominent superficial bumps.

The surfaces of test scaffolds imbedded with MTA showed similar, but slightly smaller, particle aggregation sizes to the surfaces of test scaffolds imbedded with anatase TiO_2 NP (*Fig 3*). Of the test scaffolds imbedded with MTA, the scaffold combined with 5 mg/mL MTA induced the greatest DPSC proliferation and the scaffold imbedded with 10 mg/mL MTA induced the least DPSC proliferation. These results imply that although DPSCs proliferation is indeed affected and enhanced by a bumpier and rougher scaffold surface, too high of a bump coverage per unit surface area ratio (10 mg/mL MTA) can hinder proliferation. Furthermore, the anti-microbial properties of MTA seem to be beneficial to DPSC proliferation. These findings are consistent with past studies on material surface roughness, in which cells grown on rough surfaces proliferated better compared to those grown on smooth surfaces. [7]

Furthermore, the hydrophobicity of PI seems to enhance the properties of topography, as previous studies have predicted.[4] Based on 8-day growth curve data (*Fig. 4-6*) and confocal images (*Fig. 7*), the test scaffolds imbedded with 0.5 mg/mL anatase TiO_2 NP, 0.5 mg/mL rutile TiO_2 NP, and 5 mg/mL MTA induced the greatest DPSC proliferation. Thus, the DPSCs seeded on these test scaffolds have begun to regenerate the pulp of the tooth, and have initiated the start of regeneration of the dentin-pup complex.

Part 2: Differentiation

Scaffold surface topography and mechanical properties also had a marked effect on the differentiation of DPSCs. The larger and more frequent bumps on the surfaces of the 0.5 mg/mL anatase TiO_2 NP and 5 mg/mL MTA scaffolds induced greater differentiation as compared to

pure PI and 0.5 mg/mL rutile TiO_2 NP. The difference in differentiation can be seen by the correlation between the textured surface morphology of the 0.5 mg/mL anatase TiO_2 NP and 5 mg/mL MTA samples displayed by the optical microscopy images (*Fig. 3*) and notably higher Ca^{+2} and P peaks of the elemental composition spectra created via EDX spectroscopy, which indicate the presence of increased bio-mineralization and differentiation (*Fig. 8,9*). Specifically, the scaffold imbedded with 5 mg/mL MTA and exposed to DEX induced the greatest DPSC differentiation.

The effect of DEX was pronounced. The samples not exposed to DEX had less distinct Ca^{+2} and P peaks while the all of the samples exposed to DEX, especially the 5 mg/mL MTA scaffold, had much higher Ca^{+2} and P peaks (*Fig. 9*). Thus, DEX aids in inducing DPSC differentiation, an observation consistent with prior studies.[2] All test scaffolds and DPSCs exposed to DEX had a distinct increase in differentiation, which confirms prior studies[2] on the effects of DEX on stem cell differentiation. The increased bio-mineralization and differentiation in the DEX scaffolds indicates an association between the usage of DEX and the differentiation of DPSCs.

The scaffold embedded with 5 mg/mL MTA and exposed to DEX most successfully induced DPSC differentiation into odontoblast cells, an initiator of dentin formation.

V. Conclusions and Future Work

Previous studies [1, 2, 13] have delineated scaffolds that induce tooth regeneration in laboratory environments. However, none have addressed a more natural process of regrowth: creating a thin film scaffold, with FDA approved materials, that induces proliferation and differentiation directly on the traumatized immature tooth in the oral cavity. By manipulating

surface topography and chemistry, and mechanical properties, a novel scaffold was created that induced DPSCs to become more potent, and thus restoring lost dental tissues.

Out of the original eight test scaffold conditions, the scaffold of 15 mg/mL PI combined with 5 mg/mL MTA and exposed to DEX was the most successful at inducing both proliferation and differentiation of DPSCs, with a faster doubling time and more differentiated odontoblasts present. The scaffold induced an increased proliferation of DPSCs, a crucial component of the dental pulp. The scaffold also induced an increased bio-mineralization and differentiation of DPSCs into osteoblasts, an initiator of dentin formation. The composite scaffold's particularly bumpy and hydrophobic surface, anti-microbial properties from MTA, and enhanced signaling properties from DEX successfully initiated the regrowth of a dentin-pulp complex, and restored and regenerated lost dental tissues.

In this research, scaffolds were tested on 2D models of DPSCs. Future work includes testing the scaffolds on 3D models of teeth to simulate more accurate real-life conditions. Furthermore, other future work will consist of utilizing real-time polymerase chain reaction on differentiated cells, which will indicate their gene expression. Gene expression can further confirm the presence of differentiation, and whether other cells besides odontoblasts have formed. Moreover, further experiments include expanding scaffold development to increase potency in DPSCS in the mature permanent teeth as well.

The results from this research demonstrate a promising potential of developing more advanced regenerative approaches to dentistry. This research can be applied and expanded upon to target and treat a wide spectrum of other dental injuries and diseases- including caries and other traumas- to clinically develop a less risky, less painful, and more naturally restorative method of treatment.

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