Evaluating the Effect of Laser Angle on Laser Therapies for Burns

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Thesis Committee

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Abstract

Burns account for a significant number of injuries worldwide. Scarring caused by burns lead to cosmetic issues and reduce the quality of life for the patient. To improve scarring, several methods are used clinically, including compression, laser therapy, and anti-fibrotic injections. Laser treatment is currently heavily utilized despite definitive evidence to support its efficacy. It has been hypothesized that the ablative wells created by fractional carbon dioxide (FXCO₂) laser therapy causes tissue relaxation and tissue remodeling leading to more pliable, smoother scars. In an effort to induce maximum tissue relaxation, we investigated the role of laser angle on tissue mechanics in vitro and in vivo. Findings from a porcine animal study show that laser treatment was not effective in healing burn scars and that laser angle showed no differentiation in healing. This experiment looks at the efficacy of laser treatment in an in-vitro setting. The experiment creates a more controlled environment and can help prove if laser treatment works in a more ideal setting. Aligned collagen scaffolds are inoculated with fibroblasts to create similar conditions to that of skin and the ECM that forms upon healing. Laser treatments on these scaffolds, similar to that of previous experimentation will determine the potential for laser treatment to be used on scars. Overall, non-lasered samples reported higher linear stiffness than lasered samples by about 33%. Between lasered groups, there was no difference in stiffness found. These findings confirm results found in the animal study, showing that laser angle does not have an effect on wound healing for burns.
Acknowledgements

I would like to thank Dr. Powell for being my primary advisor throughout this project. Your support and guidance have allowed me to grow as a researcher over the course of my undergraduate studies and has fostered my passion for research in biomaterials and tissue engineering.

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Introduction

Background

The American Burn Association estimates that nearly half a million people experience burns serious enough to seek medical treatment. Scarring that occurs at burn sights not only cause cosmetic problems for the patient, but also cause pain, itching, and reduction of movement surrounding the burn area. One of the most common forms of scarring for burn patients is hypertrophic scars, or HTS. HTS is a thick and raised scar that is formed by extra connective tissue that develops one to two months after injury (Hypertrophic).

In order to limit HTS and other scarring, several different treatments are used including laser therapy, compression, and anti-fibrotic agents (Ogawa). One of the most effective treatments to reduce scarring has proven to be compression. However, patient adherence to compression treatment plans is low, and therefore other treatment options must be explored. Laser treatment has become common clinical practice to reduce scarring, however little data has been collected to show the efficacy of the treatment. Laser treatment works by ablating the scarred skin, with hope to relax the stiff hypertrophic scarring and eventually create smooth and fully healed skin. Overall, laser treatment is costly, occupies operating rooms, and requires the patient to undergo anesthesia, which could lead to unnecessary complications for the patient. The Powell lab works to quantify and optimize laser treatment to give the patient the best outcome possible for treatment. Previous studies show that scar areas are not significantly altered by FXCO2 laser treatment, which is a cause of concern for the efficacy of laser treatment as a whole (Baumann).
**Previous Work**

In an effort to discover why FXCO2 laser treatment does not significantly improve scarring, a porcine animal study was done focusing on the angle of which the laser treatment was performed. 45°, 60°, and 90° laser angles were used as well as a control group without laser treatment. Figure 1 shows pictures of the wounds at each condition 18 weeks post injury and laser treatment. The images do not show any differences in healing between laser treated groups, and do not show any differences between samples that were and were not laser treated.

![Figure 1: Images of wounds at different laser angles and control.](image)

*Figure 2: Pig wounds 18 weeks post treatment*

Early findings show that there is not a significant difference in scarring between laser angles and that there is not a significant alteration in scarring by any form of FXCO2 laser treatment. Histology data also matches observations made above. Scar anatomy was not significantly altered by laser therapy at any angle with similar ECM density in all groups (Figure 2).
Figure 2 shows no visible differences in the layers of skin between time points and laser angles.

No visible changes in the composition of skin leads to a developing hypothesis that laser treatment is not influenced by angle and in addition laser treatment shows little change compared to sections that were not treated with the laser. Figure 3 shows the cross-sectional area of the porcine skin with Picrosirius Red stain at 18 weeks post laser treatment with various laser angles. Picrosirius red stains the collagen fibers of the sample. As in Figure 2, these images do not show any significant differences in the cross-sectional area based on laser angle and laser treatment.

Figure 3: H&E Stained Porcine Skin after Laser Treatment at Various Angles

Figure 4: Cross Section of Porcine Skin After Laser Treatment with Picro-Sirius Red Staining
While early studies show that laser treatment is not effective in scar healing for the porcine model, there is still a question of the effectiveness of laser treatment under ideal conditions. In order to answer this question, in-vitro experimentation can be done using collagen scaffolds inoculated with fibroblasts to determine if laser angle, and laser treatment is effective in reducing scars.

**Significance**

This project is significant in determining if laser treatment is effective on scar wounds caused by burning. In-vitro experimentation will allow for a more focused and controlled experiment in order to discover if laser treatment can ideally work in healing scars. Since there is little systematic knowledge on the efficacy of laser treatment, this study holds great significance. If laser treatment is shown to be ineffective in in-vitro work, this will raise a greater question of the overall efficacy of laser treatment on scars and whether it is necessary for patients to have these treatments. This knowledge can be used to focus future studies on other sources of scar treatment that may be more effective than laser treatment. However, if laser treatment is proven to be effective, other factors must be considered to determine why this work does not translate to experimentation on the porcine model.

**Research Goals**

The main objective of this experiment is to determine the efficacy of laser treatment, and to determine the importance of laser angle on the treatment. While it has been shown that laser treatment and the differentiation of laser angles does not improve scarring in pigs, there is still a question if it would work in an in-vitro experiment. Collagen scaffolds inoculated with fibroblasts will be strained to create an alignment of fibers. This will create a biological
environment similar to that of the aligned extracellular matrix that forms upon healing. Samples will be assessed for stiffness after laser treatment. A decrease in stiffness after laser treatment will help prove that laser therapy is effective, and the group with the lowest stiffness will prove to be the most optimal angle for laser treatment.
Methodology

Electrospinning Collagen Scaffolds

A 10 wt./vol.% collagen type I in HFP, hexafluoropropylene, solution was made mixed on a stir plate for 48 hours at 320 rpm. After 48 hours of stirring, the collagen solution is taken up using a 10mL syringe and is secured to the syringe pump. The syringe pump is then set at 4.2 mL/hr. Aluminum foil is wrapped around a 9x9 metal collector plate and secured and centered approximately 16cm from the syringe tip. Two alligator clips are secured to both the collector plate and the syringe tip and the power source operates between 30-32kW (Figure 4).

![Randomly Aligned Collagen Scaffold Electrospinning Set Up](image)

The collector plate is rotated 90 degrees for each quarter (1.25mL, 2.50mL, 3.75mL) of solution that is dispensed in to create random collagen fibers in the scaffold. Once electrospinning is complete, the aluminum foil is removed from the collector plate with the collagen scaffold and is placed in a DTC oven at 165 degrees Celsius for 24 hours. After drying, the scaffold is bagged and placed in humidity-controlled chambers until ready for use.
Cell Culture

The collagen scaffolds are carefully removed from the aluminum foil and placed in a dish. A crosslinking solution of 100% EtOH and 5mM of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) is created and poured into the dish until the scaffold is submerged. After 24 hours, the scaffold is moved to a sterile dish and is submerged in sterile 70% EtOH for an additional 24 hours. Next, the scaffold is submerged in two changes of sterile PBS (Phosphate Buffered Saline) for 24 hours each. The collagen scaffold is then submerged in 5 changes of HBS (HEPES Buffered Saline) for 5 minutes each and then is placed in DME and incubated overnight. Cell fibroblasts that have been cultured in DME for six days are now ready to be inoculated onto the collagen scaffolds at 500,000 cells/cm². Figure 5 shows a visual timeline for the cell culture procedures used in this experiment.

Figure 6: Timeline of Cell Culture Process
Straining and Testing

The day after inoculation, scaffolds are cut into 2cm x 4cm rectangles and placed into strain devices. The scaffolds are strained 5% each day for a total of 5 consecutive days. Collagen scaffolds are then removed from strain devices and cut into small dog bone shapes. The scaffolds are organized by control, 45-degree laser angle, 60-degree laser angle, and 90-degree laser angle. A Lumenis Ultra Pulse FXCO2 laser, as seen in Figure 6 is set to a square laser shape, with a 10mm spot size, a pulse density of 25% with 10mJ of power.

![Lumenis Ultra Pulse FXCO2 Laser](image)

Three different laser heads are used that direct the laser to a 45°, 60°, or 90° angle. Once a scaffold is lasered, it is immediately tested in the mechanical tester at a rate of 2.2 mm/sec until failure. Data from the mechanical tester is analyzed by graphing the Load (N) vs. Position (mm) and the stiffness of the sample is calculated by finding the linear slope of the graph before failure.
Results

Data collected from the mechanical tester was plotted on a Load (N) vs. Position (mm) graph as shown in Figure 7. The portion of the graph highlighted in grey was given a linear line of best fit, and the slope of this line is the stiffness of the sample in N/mm.

![Figure 7: Load vs Position graph](image)

Table 1 gives the average stiffness of each condition. Overall, the controlled group had stiffness levels approximately 33% higher than lasered samples. Between each laser treated sample group, there is very little variation in stiffness.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>45°</th>
<th>60°</th>
<th>90°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values (N/mm)</td>
<td>0.003082</td>
<td>0.001829</td>
<td>0.002205</td>
<td>0.001833</td>
</tr>
</tbody>
</table>

*Figure 8: Load vs Position graph from a 90° lasered sample with linear fit and slope*

*Table 1: Average Stiffness Values (N/mm) for control, 45°, 60°, and 90° lasered samples*
Statistical analysis was performed for each group, and results show that the 60° group had data that was insignificant, and therefore it will not be considered in any conclusions or analysis for the experiment. Figure 8 shows the linear stiffness values with the non-laser treated control group with the highest stiffness, and the 45° and 90° lasered samples with lower stiffness values.

Figure 9: Linear Stiffness (N/mm) as a function of laser ablation. Laser ablation at both 90 and 45 degrees significantly reduced the stiffness of the engineered dermis with more substantial reductions observed with the 45 degree group. ***p < 0.001, *p < 0.01


**Discussion**

Linear stiffness values show a 33% decrease in stiffness between lasered and non-lasered samples. These findings show that laser treatment does influence the stiffness of a wound. This confirms that ablation of the wound site does help relax the tissue, which could help regain range of motion for the patient. Based on prior in-vivo experimentation, this change in stiffness may still not be significant enough to show an observable change in wound healing and the reduction of scarring.

Linear stiffness values between 45° and 90° lasered samples were essentially the same. No change between these groups proves that the angle at which the laser is pointed at the skin does not have an effect on the efficacy of the treatment. While different laser angles may change the size and placement of ablation, it is not significant enough to make a change in the overall stiffness of the sample. These findings correspond with the in-vivo experiment.

This experiment generally supports all findings from the in-vivo porcine study, and therefore the effectiveness of laser treatment is still in question. While other factors may influence laser treatment, the angle of the laser treatment does not change the treatment.
Future Work

Despite many clinical studies claiming marked improvement following laser therapy, our current research shows no benefit in a clinically relevant porcine model and little reduction in tissue stiffness in vitro. It is possible that the change in angle and the associated beam spreading induces increased inflammation in vivo hindering any scar reduction. In a clinical setting, laser therapy is commonly repeated in 5-6 sessions spaced 4-6 weeks apart. It is possible that there were not enough sessions to yield significant reductions in scarring.

For the in vitro studies, repeated testing and optimization of the experimental set up is needed. We plan to examine more robust collagen scaffolds and test not only engineered dermis but also engineered skin to examine the role of each tissue component in the response to laser therapy. Additionally, we aim to increase the overall sample size of the experiment. More complete data will allow for a more confident conclusion regarding the role of laser angle on tissue relaxation.
Conclusions

Hundreds of thousands of patients are affected by burn injuries serious enough to require medical attention. Scarring from burn sites are uncomfortable for the patient, limit range of motion, and cause cosmetic problems. Many treatment options are available for patients, however, data collected on the efficacy of each of these treatments is insufficient. This study focuses specifically on laser treatment, which is a common form of treatment for patients. Laser treatment is expensive and requires patients to undergo anesthesia repeatedly over the course of treatment. In-vivo experimentation on pigs has repeatedly showed that laser treatment does not have a significant effect on wound healing. In a study focusing on laser angle, these findings were shown once again, with no difference between 45º, 60º, and 90º lasered samples. This in-vitro study calculated the linear stiffness values of samples lasered at 45º, 60º, and 90º and the linear stiffness of samples without laser treatment. Overall, laser treated samples showed a 33% lower stiffness than samples that did not have laser treatment. However, between 45º and 90º samples, stiffness values are the same. These findings contribute to doubts that laser treatment is effective in treating hypertrophic scarring. More work should be done to confirm these findings, with the goal of optimizing overall treatment for burn victims. Additionally, these findings prove that a difference in laser angle ablation does not change the stiffness of the sample, and therefore does not have an effect on laser treatment for patients.
References


