

**Laser Welding of Porcine Skeletal Muscle Tissue Using Near Infra-red Irradiation with Gold
Nanorod Biosolders**

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

The composition of the GNR-collagen disc and the hyaluronic acid hydrogel are found in references 10 and 13, respectively. Procedures for their synthesis were adapted and changed.

Kai Tian Zhang contributed to the literature review, conceptualization, methodology, software, investigation, analysis and writing of all draft manuscripts.

Prof. Michael Mayer assisted with the development of graphing and curve-fitting programs.

Profs. Michael Mayer and Norman Zhou contributed to supervision, project administration, funding acquisition, conceptualization, writing, reviewing, and editing of all draft manuscripts.

Prof. Vivian Choh assisted with the methodology and training of the cryostat.

Jansen Zhou and Michelle Hyunh assisted with investigation and tool development.

Abstract

Laser tissue welding is an efficient and quick technique used to join tissues for wound repair in the event of injury or surgery. Compared to traditional joining methods such as suturing, optimized laser tissue welding offers a simpler procedure, reduced operator skill requirements, and improved healing. With the use of laser light, exogenous chromophores such as gold nanorods can be added in addition to localize thermal energy and streamline the welding process. The focus of this research is to build upon current understandings of laser tissue welding, using near-infrared irradiation and exogenous chromophores, performed on ex vivo porcine skeletal muscle tissue as a model.

To accomplish this goal, laser interactions with porcine skeletal muscle tissue was studied using a parametric approach. By manipulating laser power (0-30 W) and lasing duration (0-20 s), the thermal effects of laser irradiation (Ex. coagulation) on biological tissue was modeled. It produced a threshold whereby laser parameters that cause irreversible tissue damage can be estimated with the model. This information guided welding efforts and may facilitate the safe development of laser-muscle treatments for chronic muscle pain and muscle regeneration through non-thermal effects.

Laser welding of porcine skeletal muscle tissue was performed using a continuous wave, near-infrared (1070 nm) fiber laser. A tensile test revealed a 46.7 % recovery in tensile strength. Optical and scanning electron microscopy revealed gaps at the interface which suggests the potential for procedural or parametric changes to improve the weld quality.

Exogenous chromophores (gold nanorods) were introduced to the laser tissue welding procedure and was delivered to the tissue in two ways, using a biocompatible hydrogel comprised of hyaluronic acid, and a solid collagen disc. The gold nanorod within these biosolders had an absorbance maximum that was attuned to the continuous wave fiber-coupled diode laser (808 nm) used. A complete seal of an artificial incision on porcine skeletal muscle tissue using the hyaluronic acid hydrogel was achieved within 6 minutes of irradiation time with a power density of 4.7 W/cm². In addition, welds achieved with the GNR-collagen disc (1.9 W/cm²) with an irradiation time of 4 minutes revealed a 48 % recovery in tensile strength.

These results highlight the simplicity of the procedure and strength of the bonds achieved using laser tissue welding. It also showcases the advantages of using gold nanorod-infused biosolders. This study demonstrates their ease of application, versatility with different tissues, and speed. This is evidence that laser tissue welding is a strong alternative to traditional joining techniques and may be useful in optimization of this technique for increased use in surgical scenarios.

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Chapter 1: Introduction

A laser is a device that produces a directed beam of mono-colored light. It functions as a tool for various applications including the bonding of materials by applying thermal energy that originates from the absorption of laser light by the materials undergoing laser irradiation. Laser light is distinguished by its monochromatic, coherent, minimally divergent nature. These unique characteristics allows lasers to deliver energy in a precise, concentrated, and controllable manner, which makes it particularly valuable for welding [1].

1.1 Laser Fundamentals and Applications for Joining

To generate laser light, the initial step involves the excitation of atoms in a lasing medium, achieved through electrical or chemical methods. Lasing media are diverse, and they can be found in either solid, liquid, or gaseous states to generate a laser beam [1,4]. Examples include neodymium-doped yttrium aluminum garnet (solid), dye solution (liquid), and CO₂ (gas) [2,3]. The specific medium determines the wavelength of laser light produced and it ranges from ultraviolet (200 – 400 nm) to the far infrared (10600 nm) regions of the electromagnetic spectrum [1]. After energizing the medium, atoms transition to a higher energy state, releasing a photon through spontaneous emission [4]. Subsequently, when another excited atom encounters this photon, it releases a second photon with identical wavelength and phase through stimulated emission [4]. Mirrors guide photons back-and-forth through the medium, amplifying this reaction to ultimately generate laser light that leaves the medium through a partially reflective mirror. Collimating and focusing lens can be used to concentrate the emitted light [1].

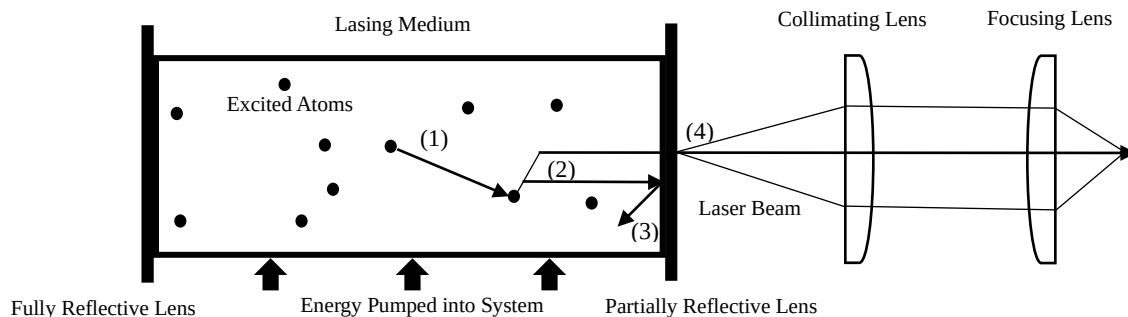


Figure 1.1. Diagram of laser operating principle. Photons are generated via spontaneous emission from excited atoms in the lasing medium (1). Photon collision with another excited atom produces a second photon identical in wavelength, direction, and phase as the incident photon (2). Photons are reflected back into the medium to exponentially amplify this process (3), eventually building up a beam that exits through the partially reflective lens (4).

This energy is applied in laser welding, a joining technique conventionally for metals [5]. The process begins with laser irradiation at the interface between two metal components. The thermal energy, generated as the material absorbs laser light, creates a narrow but deep zone of melted material referred to as a keyhole [6]. As the laser moves along the interface, the melted material cools and joins the two components at the interface.

1.2 Literature review

An alternative application of laser welding is in the joining of biological tissues, operating under a similar principle where components in biological tissues, such as melanin or retinol, absorb thermal energy from laser light [4]. This method, known as laser tissue welding (LTW), is applicable in the medical field as a surgical technique, aimed to join tissues more efficiently.

Traditional tissue joining methods involve materials such as sutures, staples, metal brackets, and adhesives [7]. However, these devices risk contamination and lead to additional tissue trauma [7]. Alternatively, LTW relies solely on light as the bonding agent, which eliminates the need to introduce foreign material and reduces the risk of infection [8]. In comparison to traditional methods, LTW offers a simpler procedure, requires less operator skill and minimizes procedural risks [9-11]. In addition, LTW can result in improved wound recovery and the creation of a water-tight seal, a feat that traditional techniques struggle to obtain [9-11]. This becomes crucial in sealing internal organs such as the small intestine, where the risk of bacterial infection due to leakage can be severe and potentially life threatening [10,11]. The potential for LTW to enhance the quality of medical care as an efficient and uncomplicated method for wound repair inspired this study.

The first experiment involving LTW traces back to the 1970's and it was used to join small blood vessels (0.3 – 1.0 mm in diameter) in rats [8]. Since then, LTW has been used to successfully join many tissues, such as nerves and internal organs like the liver and small intestine [10-12]. Advancements of this technology include the application of exogenous chromophores and the addition of biocompatible materials to be used as solders. This allows the laser to be operated at lower powers, to a point where laser light alone is not strong enough to affect the tissue. Exogenous chromophores are added to the intended area to absorb laser light and to generate thermal energy to achieve fusion [13]. When performed in this manner, it reduces unnecessary damage as laser light harmlessly passes through regular tissues, and only generates thermal energy locally in intended areas [14]. This elevated the accuracy and feasibility of this technology as a surgical technique that minimizes unnecessary damage as well as achieving tissue fusion in a simple and effective manner.

Examples of exogenous chromophores used in LTW include organic dyes such as indocyanine green (ICG) and gold nanorods (GNRs) [10,11,14]. GNRs are a more versatile tool since their absorption spectra can be controlled by manipulating its aspect ratio during its synthesis [4]. This versatility is crucial in addressing the diversity in optical behaviour of biological tissue.

These chromophores are delivered to the tissue interface using liquid, semi-solid, or solid vehicles. For example, Matteini et al. (Ref. 13) used hyaluronic acid, a biocompatible polysaccharide that forms a viscous hydrogel when mixed with water. This paste-like material can be easily applied onto tissue, effectively depositing gold nanorods at the interface to generate thermal energy that drives tissue fusion upon laser irradiation [13]. This material was used in the successful welding of rabbit carotid arteries [13]. Huang et al. (Ref. 11) embedded gold nanorods in a solid protein disc, acting as a solder-like material to weld small intestine. The weld created a water-tight seal while the protein solder remained malleable and elastic to accommodate the movement and stretching that is common in the small intestine [11]. The laser welding of tissues such as the carotid artery and small intestine is clinically significant. It offers a simple and effective way to join tissues with advantages such as reducing the risk for leakage [10,11]. LTW studies identified an optimal temperature range of 60 – 80 °C for tissue joining [11,14]. At this range, proteins denature and fuses with other proteins across the interface that drives joining [11].

1.3 Laser Tissue Interactions

1.3.1 Optical Properties

As laser irradiation reaches the surface of biological tissues, several phenomena occur and the extent to which they do so vary based on the type of biological tissue and laser light. Absorption of photons can take place as common chromophores such as melanin and retinol absorb light and produce thermal energy. The absorption of biological tissue can be modeled using the equation

$$\mu_a = \sum c_a \sigma_a \quad (1)$$

where c_a is the concentration of the absorber species (cm^{-3}), σ_a is the absorption cross section of the absorber species, and μ_a is the absorption coefficient [4]. Since there are many different molecules capable of light absorption in biological tissues, μ_a is a sum of all absorber species involved [4]. As light passes through a layer of tissue, the light intensity that exits (I_T) can be calculated with the equation from the Bouguer-Lambert law

$$I_T = I_0 \exp(-\mu_a d). \quad (2)$$

where I_0 is the intensity of incident light, and d is the thickness of the tissue layer through which the light travels (cm) [4].

Photons can also be scattered by biomolecules and by structures such as mitochondria and membranes. Scattering is represented in an identical manner as seen in equation 1 [4]. Scattering allows for reemission and for light to be transmitted through tissue layers [4]. In addition, incident laser light is reflected at the air-tissue interface due to the change in medium [4].

1.3.2 Thermal Properties

With the absorption of laser light energy into thermal energy by chromophores within biological tissues, it causes a localized temperature increase within the area or volume of irradiation. The maximum temperature increase experienced by the tissue is governed by the laser beam intensity [4]. The thermal energy within the irradiated volume will flow into adjacent, colder regions via thermal conduction [4]. This correlates with the temperature gradient as well as the cross-sectional area of heat flow. Thermal conductivity (λ) for biological tissues rest between the range of $0.3 - 0.5 \text{ Wm}^{-1}\text{K}^{-1}$ depending on tissue composition [4]. In comparison, conductive materials such as copper have a much higher thermal conductivity ($418 \text{ Wm}^{-1}\text{K}^{-1}$) [4]. Biological tissues also possess a specific heat capacity that measures the amount of heat needed to raise the temperature of a certain unit mass by 1 K. These factors directly affect the energy that is required from the laser to bring tissues to a high enough temperature for joining.

1.3.3 Thermal and Non-Thermal Effects

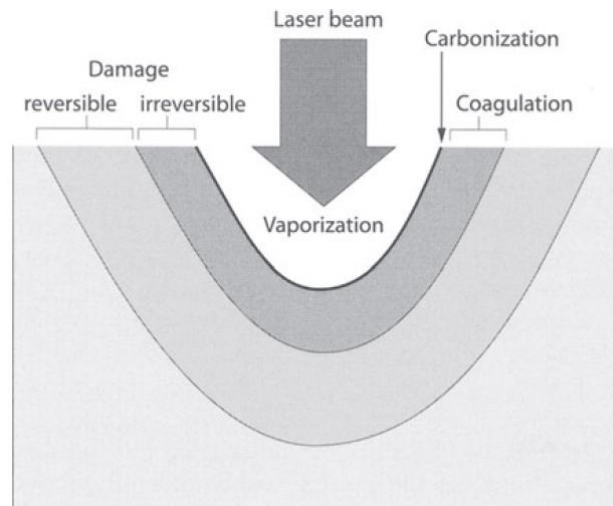
As tissues experience a localized increase in temperature from laser irradiation, it will have different reactions to the thermal energy as outlined in Table 1.

Table 1. Tissue reactions to different temperature ranges as a result of laser irradiation. (Adapted from Ref. 4 with permission)

Temperature (°C)	Tissue Effects
< 37	No irreversible effects
40 – 45	Enzyme production, cell death, membrane disintegration
60	Protein denaturation, coagulation, necrosis
80	Collagen denaturation
100	Desiccation
> 150	Carbonization
> 300	Vaporization

From table 1, irreversible effects begin at above the normal human body temperature of 37°C. Protein coagulation begins at 60 °C and coincides with the ideal joining temperature determined in multiple studies. At temperatures above 100 °C, tissues experience desiccation, carbonization, and vaporization [4].

From Fig. 1.2, vaporization creates a trough of ablated material and a thin-black film of carbonized organic matter covers it. This severely damages the tissue and is ideally avoided to increase the effectiveness of LTW and to develop it into a surgical technique to join wounds in high-risk scenarios. In addition, the optical and thermal properties of tissues change throughout laser irradiation as they are transformed by the elevated temperature. Examples include tissues blackening by carbonization, and whitening (bleaching) via coagulation as thermal energy changes the optical properties of the tissue [4]. This creates a dynamic system where tissue behaviors change depending on its state during the laser



irradiation process.

Figure 1.2. Zones of temperature-dependent tissue effects during laser irradiation [4]. (Replicated with permission)

In addition, laser irradiation produces photochemical effects that are independent from thermal energy, altering the structures of certain biomolecules. Light alone can cause isomerization, dissociation of molecular bonds, and photo-induced synthesis of compounds, such as UV-induced melanin production (tanning). The breadth of photochemical effects that can occur has some therapeutic potential [4].

Ref. 20 found that low-level laser irradiation reduces chronic muscle pain in skeletal muscles via photochemical effects. Other research has found that low-level laser irradiation increases adenosine triphosphate (a molecule providing chemical energy harnessed by cells) synthesis in muscle tissue, as well as improve the proliferation of stem cells [21,22]. Thus, laser-muscle interactions may have the potential for improving muscle regeneration and athletic performance, leading to major developments in sports medicine and therapeutic treatments. Administering these advantageous photochemical effects without damage from thermal effects is an avenue of exploration for this study.

1.4 Tissue Joining Mechanism

Tissue joining results occurs through a combination of electrostatic (secondary, Van der Waals) and covalent interactions between structural proteins across the interface [4,11,14]. These proteins are primarily located in the extracellular matrix of connective tissue [14]. One essential structural protein is fibrillar collagen, which plays a key role in tissue fusion in addition to proteoglycan by engaging in non-covalent interactions (Ex. hydrogen bonding) [14,23]. Proteoglycans are globular proteins with a glycosaminoglycan (GAG) tail which bind non-covalently to fibrillar collagen through their protein component at specific intervals. Proteoglycans connect collagen fibers to each other via non-covalent interactions between their sugar tails [23]. These molecular interactions connect and organize different strands of fibrillar collagen in a parallel and orderly manner that contributes to the structural integrity of biological tissues [23]. Fig. 1.3. summarizes the joining process. When subjected to low-temperature heating via LTW, electrostatic interactions between GAG tails are destroyed which unlocks the collagen fibers. This allows strands of fibrillar collagen from across the wound to interact with each other and reform electrostatic interactions upon cooling, resulting in tissue fusion.

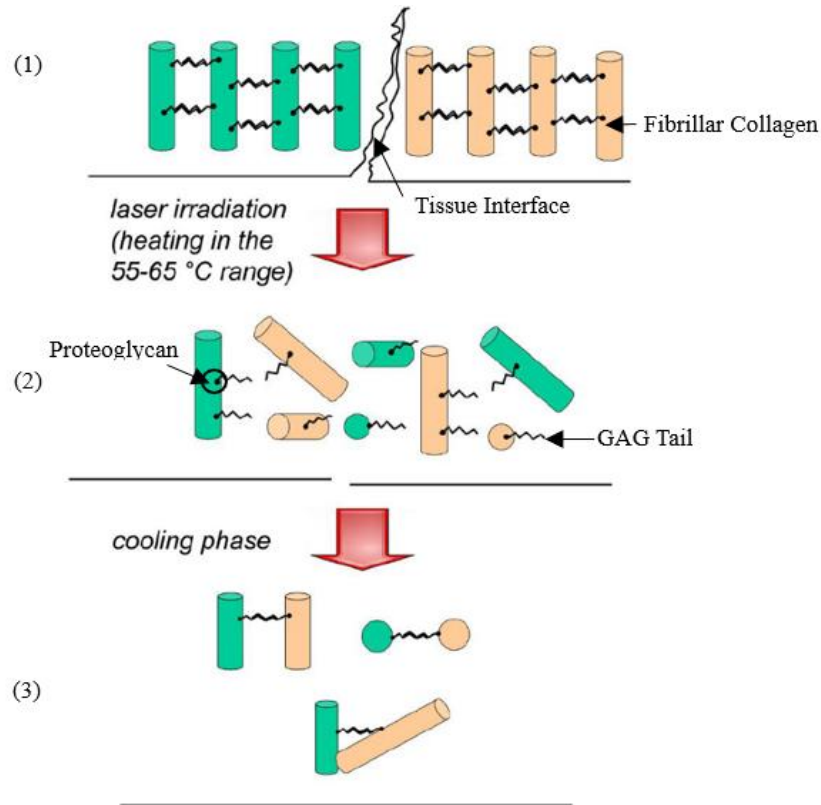


Figure 1.3. Schematic of laser welding mechanism using fibrillar collagen as a model. Collagen is organized in a parallel fashion in tissues normally (1) and are “unlocked” upon heating (2). This allows collagen fibers to interact with their counterpart across the wound, resulting in tissue fusion as temperature cools and intermolecular bonding is re-established (3) [23]. (Adapted from Ref. 23 with permission)

Furthermore, proteins other than collagen participate in the joining process but they are unknown [24]. To summarize, tissue joining is a combination of covalent bonding and electrostatic forces between proteins in the ECM, caused by laser-induced heating, resulting in the interdigitation of structural proteins.

LTW is categorized into two distinct models [14]. The morphology of collagen proteins after laser irradiation are used for categorization since it is the main structural protein in biological tissues and is an important participant in joining [14]. The first model is homogenization. It refers to the process where collagen loses its distinct fibrous structure when welding and homogenizes into a uniform material [14]. An example is shown in Fig. 1.4. Both scanning electron microscopy (SEM) images depict dura mater, a structural tissue found in the brain [25]. Fig. 1.4 (A) depicts collagen fibers in their natural state, unaffected by laser irradiation. After laser welding, the loss of the fibrous structure and homogenization into a fused, globular material is seen in Fig. 1.4 (B).

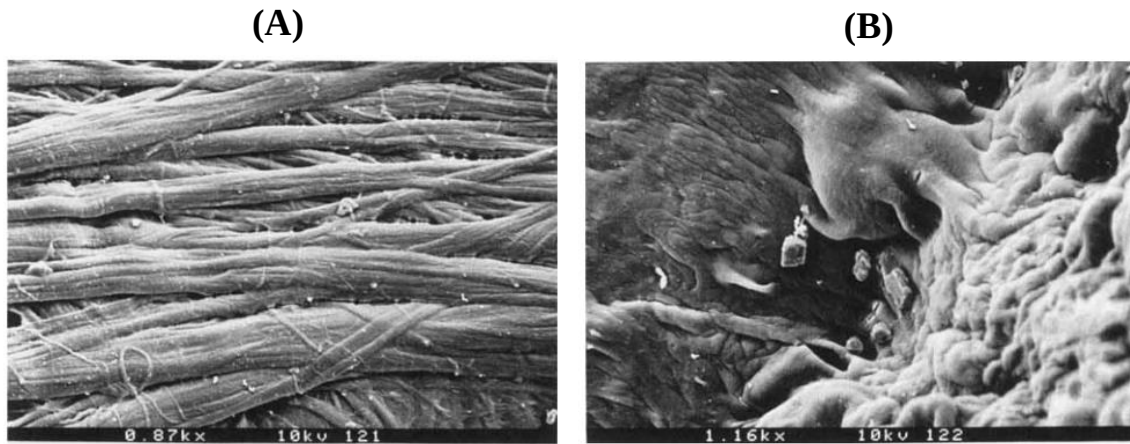


Figure 1.4. SEM images of dura mater (brain tissue) before laser welding depicting distinct fibers of collagen (A). Dura mater after laser welding with globular and homogenized protein (B) [25]. (Replicated with permission)

The second model of bonding is interdigitation. As displayed in Fig. 1.5 (A), fibrous collagen fibers are organized into layers that provide structural integrity [26]. As LTW generates heat, collagen fibers dissociate from their natural configuration but remain intact [26].

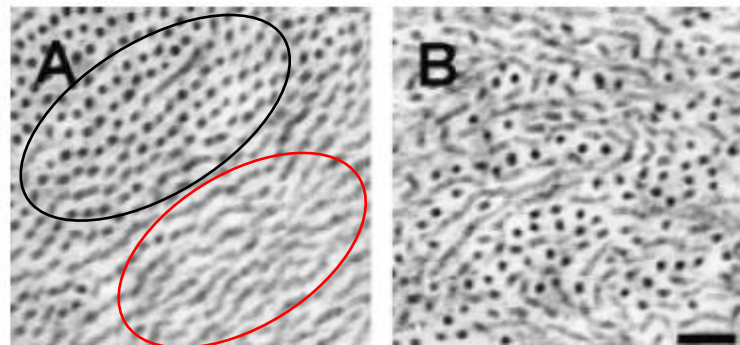


Figure 1.5. TEM image of cornea tissue before (A) and after (B) laser welding. Two distinct layers of collagen fibers are seen, with cross-sectioned fibers (circled in black) and longitudinally sectioned fibers (circled in red) that are orthogonal to each other (A). After laser welding, bi-layer structure was disrupted but collagen fibers remain intact, with some orthogonal layering. (Adapted from Ref. 26 with permission)

After laser irradiation imaged in Fig. 1.5B, collagen fibers can still be observed but the layered structure is lost [26]. They have dispersed but maintained an orthogonal orientation, resembling interlocking fingers, hence interdigitation.

1.5 Factors Affecting Tissue Joining

Several factors affect the joining of biological tissues. Important ones include those that affect the consistency of energy delivery to the tissue, which affects the quality or strength of joining.

1.5.1 Laser Parameters

The temperature at the site of LTW directly correlates with the power density of the laser. It is crucial to control power density to bring the volume of irradiated tissue to an optimal temperature range for welding. Power density is influenced by the spot size of a laser, which depends on parameters such as focal length. Other factors such as average power in continuous wave operation or pulse duration for pulsed wave operation are also necessary to calculate power density, typically measured in watts (W) per unit area. LTW has been successfully performed with power densities as low as 2.50 W/cm^2 [11]. The welding speed also affects power density. Longer irradiation time delivers more power to the tissue, resulting in increased temperature and affecting tissue joining.

Wavelength is another critical laser parameter with two common approaches. One method involves using laser light to directly heat tissues without using exogenous chromophores. For instance, CO_2 lasers generate light at around 10 microns in wavelength, coinciding with an absorption peak of water. It thereby aims to generate heat through the absorption of laser light by the water held ubiquitously within biological tissues. This method is highly dependant on tissue characteristics and is less adjustable.

The other method prefers laser light in the near-infrared range (NIR) because biological tissues exhibit low absorbance for light at this wavelength. This enables laser light to pass through tissues without significant impact, allowing for higher transmittance. Exogenous chromophores are coupled with this method and applied to the tissue interface to absorb laser light and produce thermal energy to drive fusion. These chromophores can be deposited deep into tissue and retain its functionality since NIR light can still reach it due to the tissue's low absorbance. This allows for deeper welds to be achieved, joining more tissues for better recovery and structural integrity. Furthermore, the absorption spectra of chromophores, especially gold nanorods, can be controlled, enhancing the technique's versatility. Moreover, research has demonstrated therapeutic benefits of NIR light on skeletal muscle, making it an ideal wavelength for experimentation.

1.5.2 Surface Topography

The surface of biological tissues can have irregularities that affect laser energy delivery and thus bonding.

As depicted in Fig. 1.6, when the laser is focused onto the surface of a tissue but encounters uneven areas (irregularities), the beam diameter increases. Consequently, this leads to a decrease in power density, potentially causing a transient reduction in local temperature which in turn may weaken the bond at the site of the irregularity.

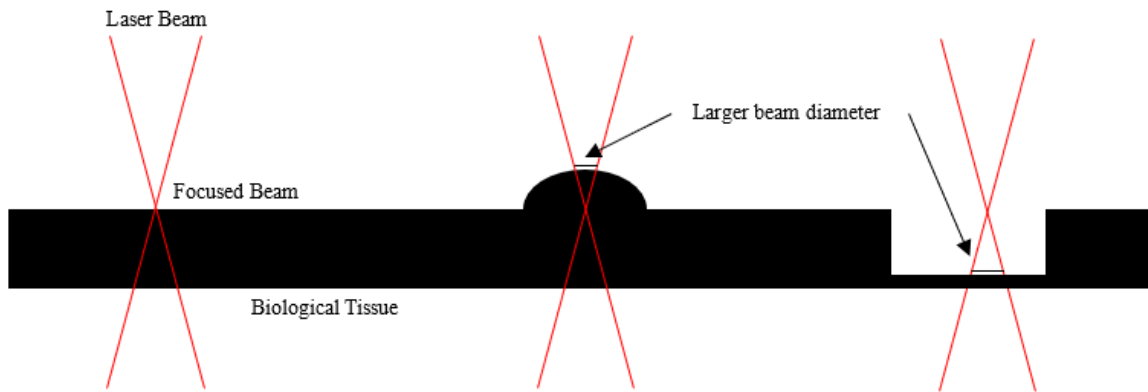


Figure 1.6. A schematic depicting biological tissue with surface irregularities and how it affects beam diameter.

Moreover, in the process of joining two pieces of biological tissue, the contact area becomes crucial, particularly in the presence of surface irregularities. Maximizing contact area is essential to facilitate the fusion of proteins across the interface as illustrated in Fig. 1.3. Applying sufficient force to bring the two pieces together is necessary to increase contact area, made feasible by the soft and malleable nature of biological tissues.

1.5.3 Environmental Factors

Ambient temperature plays a significant role in the welding process as it influences the rate at which heat dissipates into the surroundings. For instance, when welding in colder conditions, a greater amount of heat will be lost to the environment due to the large temperature gradient, potentially impacting weld quality. Conversely, in warmer environments, this heat loss may be less pronounced. Similarly, the temperature of the tissue itself can have a similar effect on the welding process.

Furthermore, humidity is another critical factor to consider, primarily because water has a high specific heat capacity. Higher levels of moisture may require the use of more laser power to achieve the optimal temperature required for effective welding. On the other hand, dry or desiccated tissues will have a lower capacity to conduct heat [4]. Therefore, increasing the accumulation of thermal energy at the point of irradiation, potentially resulting in higher-than-expected temperatures at the weld site [4].

1.5.4 In vivo vs. Ex vivo

“In vivo” denotes experiments performed on living organisms, while “ex vivo” refers to experiments conducted outside of a living organism (I.e. using dead tissue). Utilizing an in vivo model offers several advantages. It allows for insights into the wound repair process after LTW and allows for observations or chronic and acute responses. Conversely, an ex vivo model neglects some important factors. The temperature of the tissue sample will be different as it lacks the heat contribution from metabolic

activities. It also cannot replicate heat dispersion from the welding site via blood flow. Therefore, an in vivo model can be more representative and informative, but it is important to note that it entails more procedural and ethical considerations.

1.5.5 Other Factors

Other factors affecting joining in LTW is the hydration level of the tissue. Similar to humidity, tissues with high water content will demand more energy to reach required temperatures while low water content may reach higher temperatures.

Furthermore, biological tissues exhibit non-uniform characteristics. When welding tissues like the small intestine, the organ constitutes multiple layers of morphologically different tissues. Therefore, they have differences in optical behaviour that give rise to variability and unexpected behaviour when welding.

1.6 Chromophores

Chromophores can be organic molecules such as ICG or metal nanoparticles such as GNRs. The latter is preferred because it fosters a lower diffusivity through the tissue matrix, a higher photochemical stability, and converts light into heat energy more efficiently [15-19].

In LTW, GNRs absorb laser light energy and convert to thermal energy via the localized surface plasmonic resonance (LSPR) effect; a phenomenon whereby heat is produced by the collective oscillation of conducting electrons on a metal surface when irradiated with light that matches their wavelength [4]. GNRs with peak absorbances in the near-infrared (NIR) region are preferred due to the low absorbance of biological material for light at that wavelength, allowing NIR laser light to pass through tissue without causing significant heat damage, while GNRs drive tissue fusion in applied areas via heat energy from LSPR [10, 11]. Localized heat generated in this manner ensures efficiency and minimizes extraneous damage. GNRs are delivered to the weld site using biocompatible vehicles such as hyaluronic hydrogels or protein-based solders.

1.7 Scope and Objectives

This work aims to build upon the current understandings of LTW with exogenous chromophores to elevate this technology into a fast and uncomplicated method of wound repair for use in the medical field using ex vivo porcine skeletal muscle tissue as a model. Skeletal muscle tissue has received little attention in the field of LTW and this work aims to investigate its role in this domain. The research will encompass a wide breadth including its interactions with NIR laser light, and welding with an emphasis on GNR usage.

The specific objectives of this study are:

1. Characterize the effects of NIR on porcine skeletal muscle tissue at different lasing parameters (E.g. laser power, irradiation time).
2. Achieve bonds using laser irradiation alone and determine optimal parameters to do so.
3. Evaluate bond quality with tensile testing and imaging techniques.

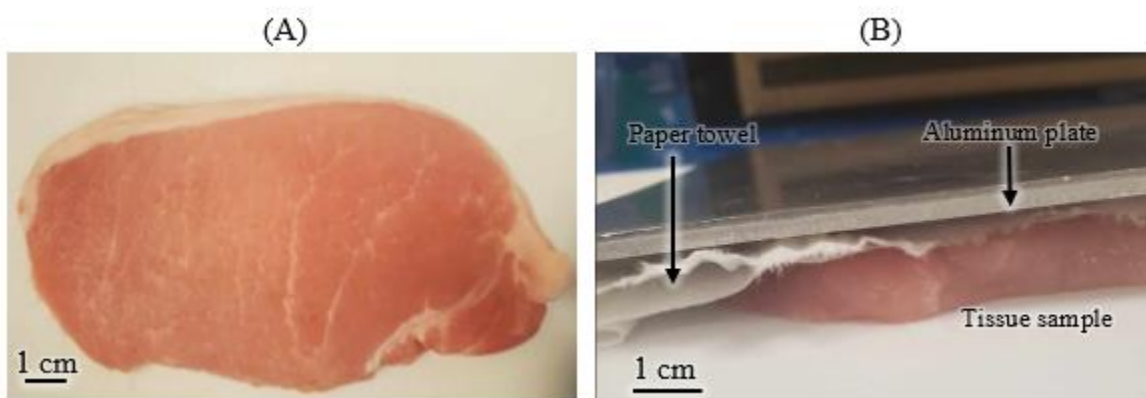
4. Develop biocompatible materials (e.g. protein solders) for application of GNRs as exogenous chromophores to assist in welding.
5. Produce bonds of porcine skeletal muscle tissue using exogenous chromophores and biocompatible materials and assess bonding strength.

Chapter 2: Effects of NIR Radiation on Porcine Skeletal Muscle Tissue

The content of this chapter is included in its entirety in a manuscript [28] that has been submitted for publication in a peer-reviewed journal. This chapter details a parametric study that models the threshold of coagulation by manipulating laser power and irradiation time. With this model, laser parameters can be accurately identified to cause photochemical effects that have potential therapeutic effects on muscle. Or to cause a high enough temperature for joining (coagulation).

2.1 Sample Preparation

Six pork loin skeletal tissue specimens, each approximately 10 mm thick with a surface area of at least 6 cm², were used for the experiments. These specimens were packaged in plastic and used for experimentation within one day after purchase. Prior to experimentation, each specimen was removed from refrigeration and allowed to equilibrate to room temperature (21 °C) for 2 hours before removing its packaging. Standardized procedures were employed to control the water content at the site of laser irradiation. The surface of the sample was dried using a paper towel to eliminate excess water, thereby reducing variability in heat distribution upon laser irradiation. An example specimen after drying is shown in Fig. 2.1 (A). Concurrently, the specimen was flattened for 2 min using gravitational force from a flat 207 g aluminum plate that is larger than the specimens, as shown in Fig. 2.1 (B). Specimen flattening caused a less than 0.7 mm reduction in thickness in all instances (N=6). Flattening surface topography aims to minimize variation in specimen surface height. Fewer variations allow a single height adjustment to focus the laser to the top of the specimen and are expected to achieve a more consistent power density



during the experiment.

Figure 2.1. (A) Image of a pork loin muscle tissue sample after surface drying and flattening. (B) Image of setup for drying and flattening of porcine skeletal muscle tissue.

2.2 Lasing Procedure and Power Parameters

A diode-pumped continuous wave (CW) ytterbium fiber laser (IPG Photonics; YLR-30-Y14) at a wavelength of 1070 nm was used to irradiate each pork skeletal muscle specimen at 21 locations in a 3×7

grid. These locations are spaced at least 0.635 cm apart vertically and 1.27 cm apart horizontally. Table 2 presents the lasing parameters used for this study.

Table 2. Laser parameters used for coagulation threshold study

Laser Power (W)	Lasing Duration (s)
0 (control)	3
0.3	5
0.7	7
2.2	10
3.8	15
5.4	20
7.0	
8.6	
10.2	
11.8	
13.5	
15.2	
16.8	
18.5	
20.2	
21.9	
23.5	
25.3	
27.1	
29.0	
30.5	

Each of the 21 locations on a single muscle tissue specimen was subjected to one of the laser powers in Table 2 in a randomized order. Furthermore, the time the laser dwelled on any single location (lasing duration) was the same for the one muscle specimen but differed between the 6 specimens prepared. Lasing duration is limited to 20 s to prevent excessive carbonization. The movement pattern of the laser is shown in Fig. 2.2.

Detailed diagrams of the experimental setup are shown in Figures 2.3 (A) and (B). An example result of an irradiated specimen cross-sectioned using a scalpel after the experimental procedure is shown in 2.3 (C).

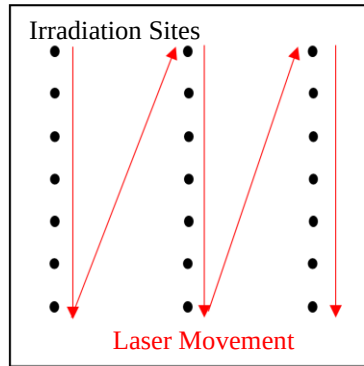


Figure 2.2. Diagram of lasing procedure depicting laser movement (red) and sites of irradiation in a 3x7 grid.

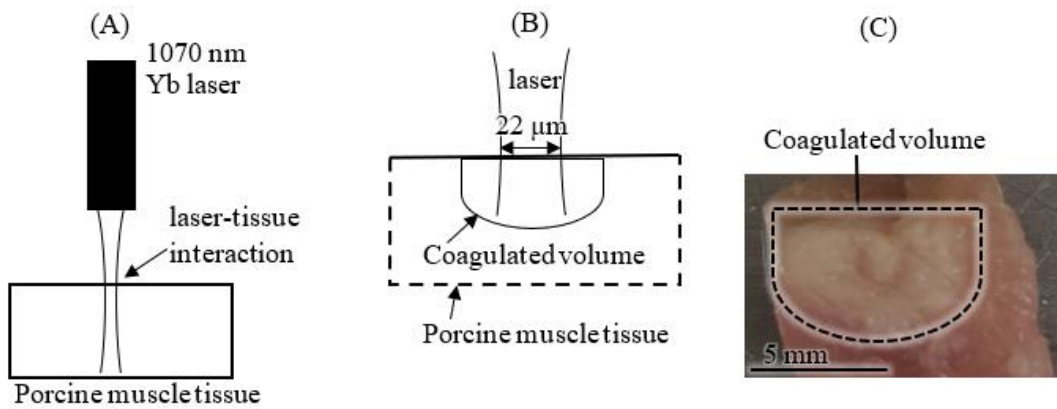


Figure 2.3. (A) Diagram of the laser setup showing positioning of the laser relative to the porcine skeletal muscle tissue sample. (B) Enlarged diagram of laser-tissue interaction leading to coagulated volume. The laser focal point (22 μm) was focused to the top of porcine muscle specimens. (C) Photograph of the cross-sectioned coagulated volume. The surface of the coagulated volume exhibits color change.

2.3 Data Acquisition from Tissue Specimens After Laser Irradiation

The patterns of laser-induced color change on the meat surfaces were imaged under consistent lighting conditions and camera angles. The camera was held 9 – 10 inches directly over the sample with a lamp projecting light from the right side of the camera's field of view to eliminate shadows. The diameter of the heat-affected area (whitened areas), denoted as D , was accurately determined using the Canny edge detection algorithm within MATLAB, using the code "Canny_Edge_Detection.m" in Appendix A. This algorithm identifies local maxima of the image gradient and by applying the derivative of a Gaussian filter. It employed two thresholds to detect strong and weak edges (set to 0.16 and 0.18), and a sigma term (0.8) to define the filter's standard deviation. These parameters were selected because they produced the most

accurate fit, determined through experimentation. The resulting images depicted the coagulation zones outlined in white, and their diameters were measured in pixels using image manipulation software (GIMP). These measurements were converted to millimeters using conversion scales derived from images of a ruler taken under the same lighting and camera conditions.

For each irradiation time, a plot of diameter versus power was made and a square-root modeled curve was fitted onto the data to identify the x-intercept. The x-intercept identifies the threshold power at which coagulation appears.

2.4 Optical and Scanning Electron Microscopy Imaging

At areas of laser irradiation, thermal damage was determined visually by the colour change (bleaching) that accompanies tissue coagulation shown in Fig. 2.4 (A). The effects observed include circular zones of coagulation and instances carbonization. Carbonization is characterized by small, circular, and blackened zones with a central indentation. The coagulation area increases with both irradiation time and power. Non-coagulated laser-irradiated zones do not display any visible changes. Fig. 2.4 (B) displays the results of applying the Canny edge detection algorithm. It is an accurate fit as all coagulation zones were accurately identified by the algorithm, although it outlines irrelevant textures as well.

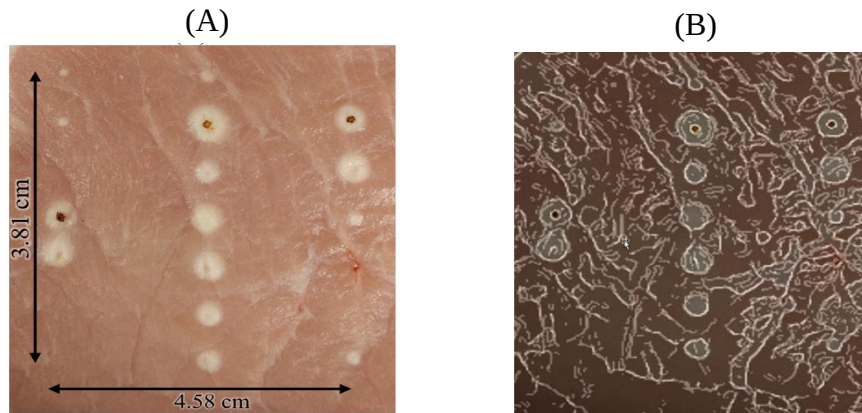


Figure 2.4. Photograph of coagulated areas and instances of black-coloured coagulation. Specimens were irradiated in a 3 x 7 grid and not all locations display visible effects. Laser power was randomized for each location using values from an exhaustive list (A). Image processed using the Canny edge detection algorithm to determine the diameter of circular heat-affected and whitened areas (B).

The whitening observed is a result of alterations in tissue microstructure and changes in the content of light-absorbing chromophores due to laser-induced heating. These microstructural alterations can stem from the thermal coagulation of proteins and organelles such as mitochondria within muscle cells, forming granules that contribute to light scattering and absorbance [27]. Figures 2.5 (A) and 2.5 (B) shows the presence of granules or nodular formations, indicating the loss of fibrous appearance of muscle cells. This evidence of coagulation aligns with other observations of thermally coagulated tissues [25].

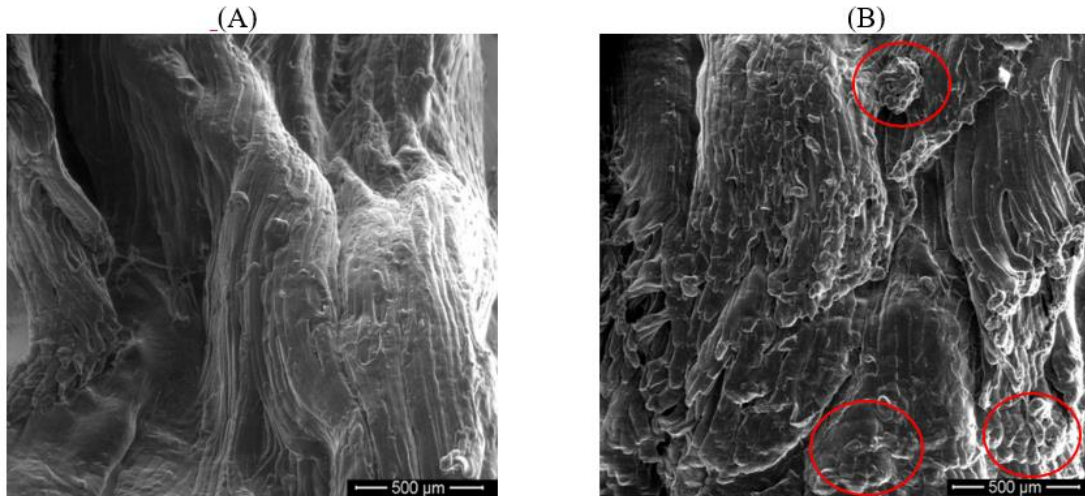


Figure 2.5. SEM images of porcine skeletal muscle tissue before (A) and after (B) laser treatment which displays coagulation through nodular and contracted appearance of the muscle fibers (circled in red) that occurred during laser irradiation. Samples were not coated with gold and imaged under low vacuum using the Quanta FEG ESEM.

Coagulation is a thermal effect initiated by laser irradiation, which typically begins at 60 °C and is accompanied by thermal necrosis of the tissue [4]. The bleaching effect seen in coagulated tissues serves as an indicator for irreversible thermal damage [4]. Laser parameters too low to induce coagulation are expected to result in an intended photochemical effect in irradiated tissues, while undesired optical or mechanical alterations are avoided due to insufficient thermal energy from low laser power or thermal diffusion [4].

2.5 Data Manipulation and Coagulation Threshold

Fig. 2.6 illustrates the relationship between the diameter of coagulated areas and laser power, based on data collected from an irradiation time of 7 s. The x-intercept was identified by fitting a square-root equation to the dataset using the code “Curve_Plot.m” in Appendix A. Coagulation or irreversible thermal damage are observed using parameters higher than the intercept, while for lower power levels, therapeutic photochemical effects prevail.

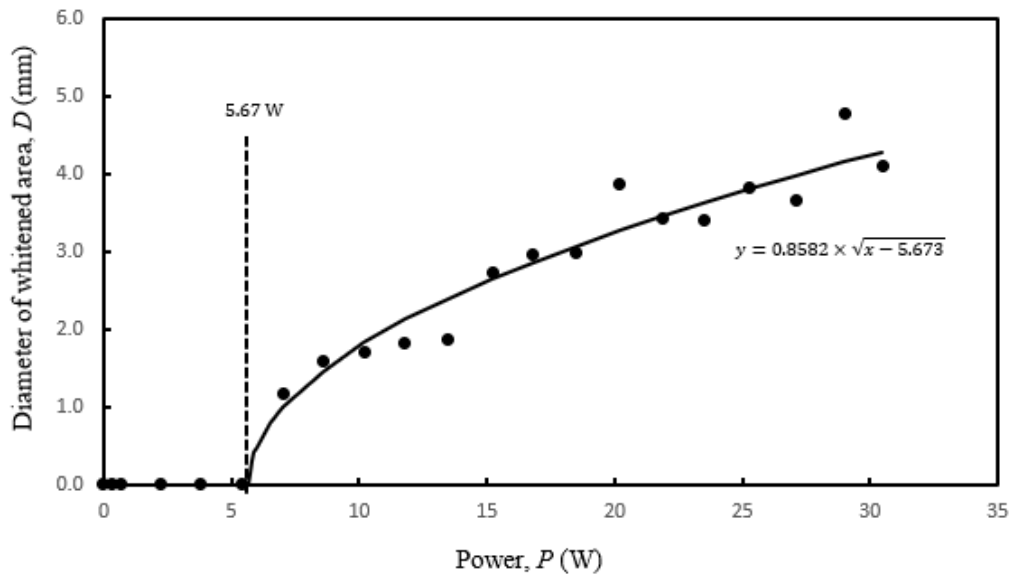


Figure 2.6. Scatterplot of the diameter of whitened (coagulated) areas at 7 s of irradiation time versus laser power, with a square-root curve fitted to the data to find the power threshold (5.67 W) at which coagulation occurs.

Data from 6 different irradiation times (see Section 2.2) are compiled into a contour plot (Fig 2.7) that outlines the threshold at which thermal damage is observed, as a function of irradiation time and laser power. This threshold identifies parameters at which 1070 nm laser light does not cause thermal damage to the tissue. This may be useful to optimize laser-muscle therapies that rely on photochemical mechanisms.

In Fig 2.7, the threshold line approximates the threshold where no coagulation is expected for process settings to its left. To the right of the line, coagulation or whitening occurs within a range of 3.8 – 7.5 W for irradiation time of up to 20 s. This threshold line was determined by identifying the x-intercept as shown in Fig. 2.6 as the threshold, for all irradiation times. The threshold values predictably trended to higher power levels as irradiation time decreased. The coagulation diameter increases with irradiation time and laser power. All isolines of this contour plot exhibit a nonlinear trend that curves towards higher laser powers as it approaches the x-axis.

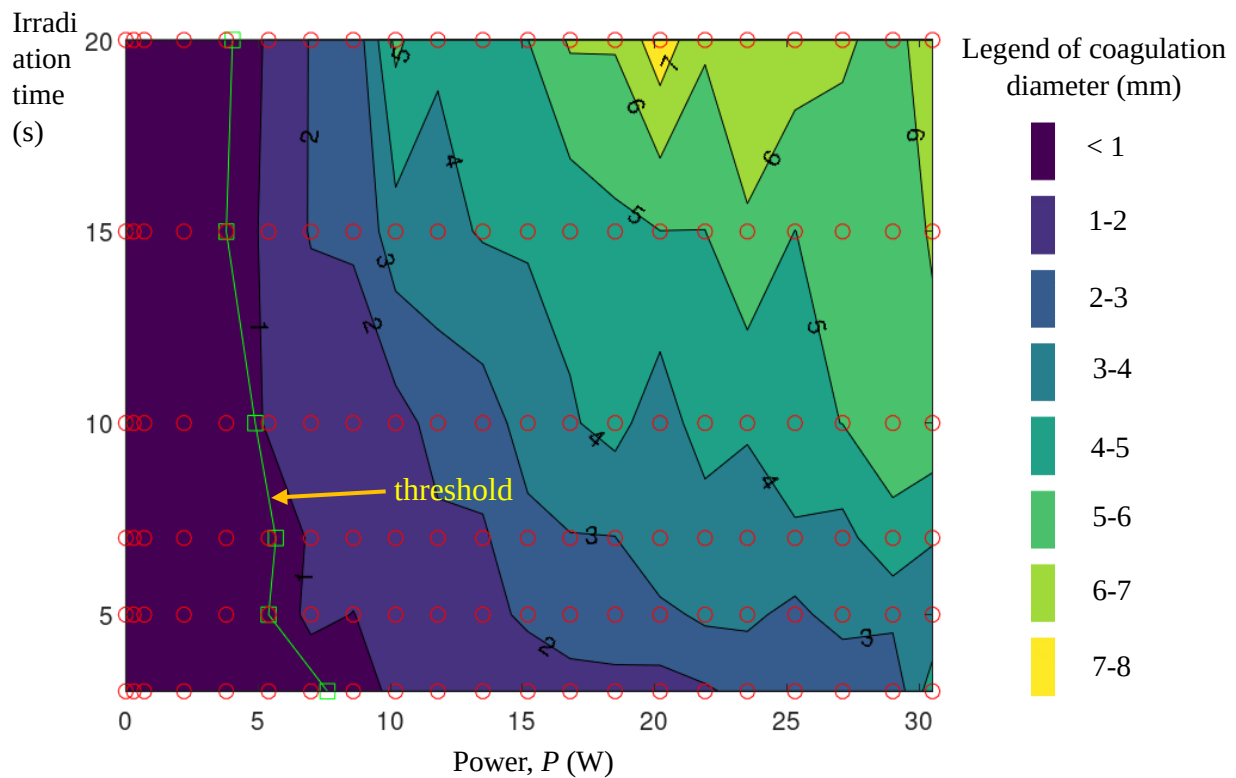


Figure 2.7. Contour plot of diameter (mm) of whitened heat-affected areas after laser irradiation for various values of power and irradiation time factors. Red circles represent experimental laser power and irradiation time parameter combinations. Green border line labeled “threshold” outlines coagulation threshold from curve fit data, dividing instances where no coagulation was observed (left of border line) and parameters that show evidence of coagulation (right of border line).

All conditions of this contour plot were tested once. To study the variability of the whitening from the laser irradiation, Fig 2.8 offers data on variability by repeating the procedure using the same lasing conditions. Data from (N=5) repeated trials was compiled with error bars. The value for the x-intercept was 5.39 W, which is 5 % smaller than that from the (N=1) procedure shown in Fig 2.6.

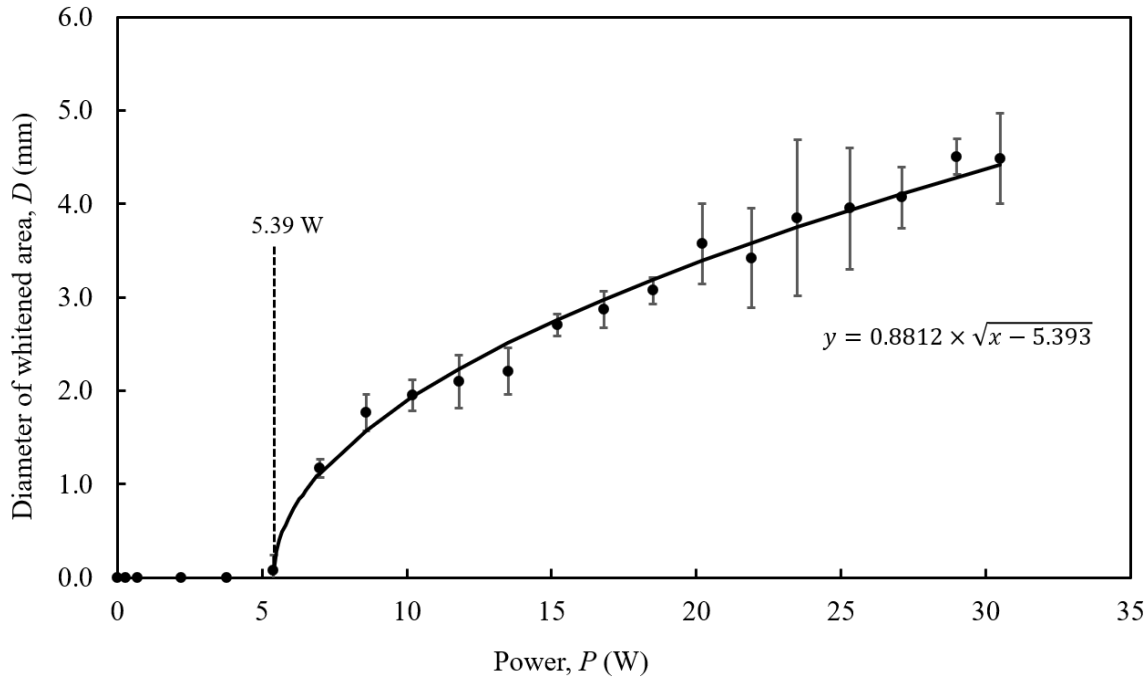


Figure 2.8. Scatterplot of coagulation data based on 5 repeated trials at 7 s irradiation time to assess replicability of the experiment with error bars showing standard deviation.

There is less variation in the diameter of the coagulated zone at lower powers and the variation increases with laser power. The threshold value obtained from an average of repeated trials in Fig 2.8 remains roughly consistent with the one identified in Fig. 2.7. Out of 5 trials, only one was inconsistent where the first instance of coagulation was observed at 25 % power (5.4 W). The observed variation is smaller than the characteristics of the main curve, allowing the threshold power to be determined with sufficient precision. The shape of the fitted curve is similar to other research that outlines the relationship between temperature (laser power) and irreversible tissue effects (coagulation) [4].

2.6 Summary of Chapter 2

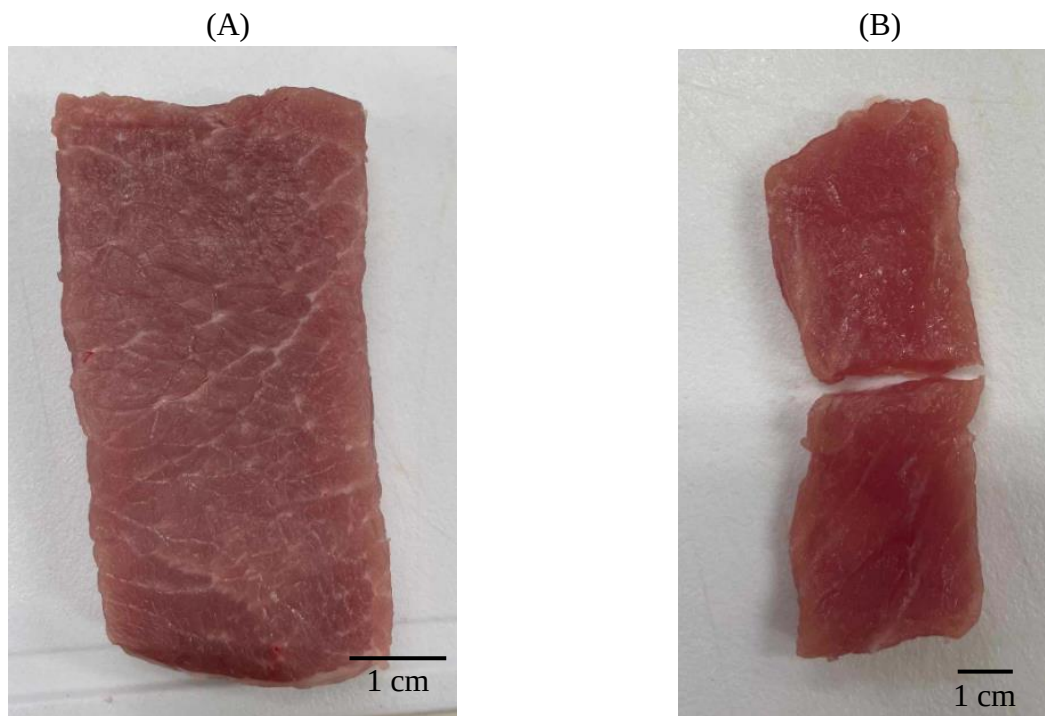
Using porcine skeletal muscle tissue as a model and applying a popular therapeutic wavelength (NIR), a coagulation threshold as a function of laser power and irradiation time was successfully defined. This threshold identifies a safe upper limit of laser irradiation, mitigating irreversible thermal effects such as coagulation. These results provide parametric knowledge to prevent tissue damage and may facilitate the safe and effective development of laser-muscle treatments for chronic muscle pain and muscle regeneration through non-thermal effects outlined in Section 1.3.3. This completes the first objective outlined in Section 1.7.

Chapter 3: Laser Welding of Porcine Skeletal Muscle Tissue

This chapter covers an experiment that welds porcine skeletal muscle tissue specimens using an NIR (1070 nm) alone. A tensile strength was conducted to assess the strength of bonding achieved.

3.1 Sample Preparation

Pork loin skeletal muscle tissue was cut using a scalpel to produce specimens with a rectangular shape (approximately 60 mm by 30 mm) with a thickness of approximately 5-6 mm as shown in Fig. 3.1 (A). The tissue was packaged in plastic and used for experimentation not more than one day after purchase. The tissue was equilibrated to room temperature, dried, and flattened using procedures described in



Section 2.1. The specimen was bisected for welding as shown in Fig. 3.1 (B).

Figure 3.1. Image of an uncut pork loin skeletal tissue specimen (A) and a specimen bisected using a scalpel (B).

Fig. 3.2 depicts a specimen held within a custom plastic device designed to hold the two pieces in close proximity for laser welding. The position of the device is secured using nuts and bolts and six needles poking into the specimen to apply clamping pressure between the two specimen pieces.

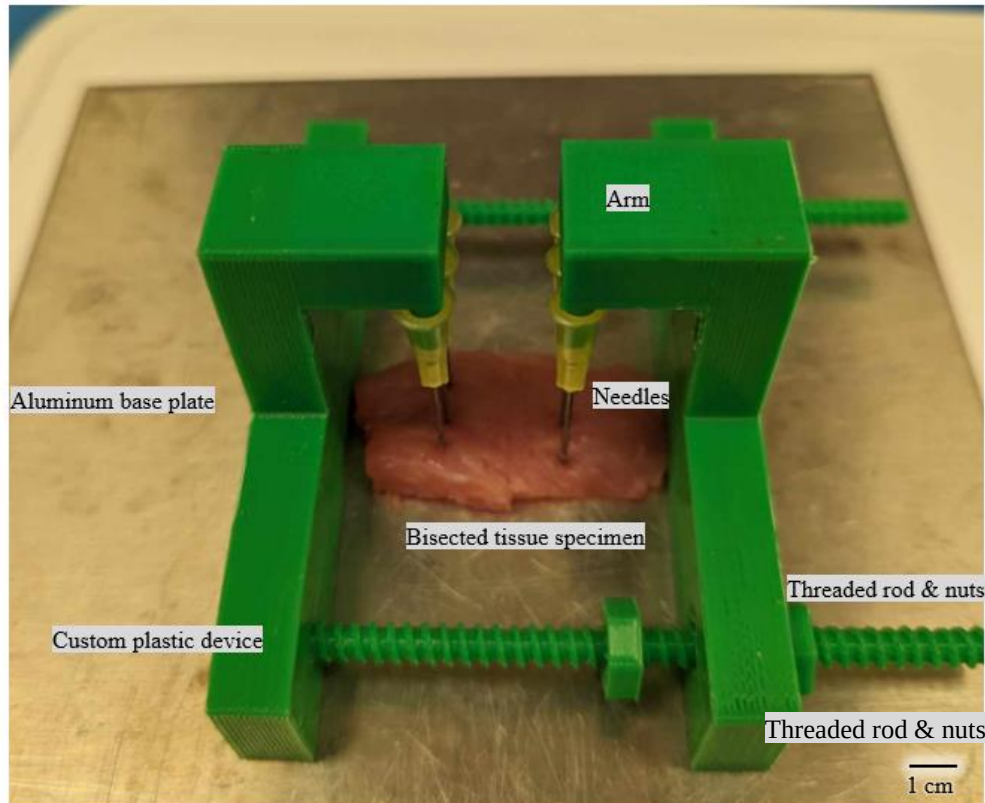


Figure 3.2. Image of laser welding setup using custom 3-D printed plastic device. Tissue specimen placed in center of device and bringing arms close together applies compressive force on both sides. Position of arms is secured by fastening nuts. Needles conduct compressive force to specimen.

3.2 Laser Welding

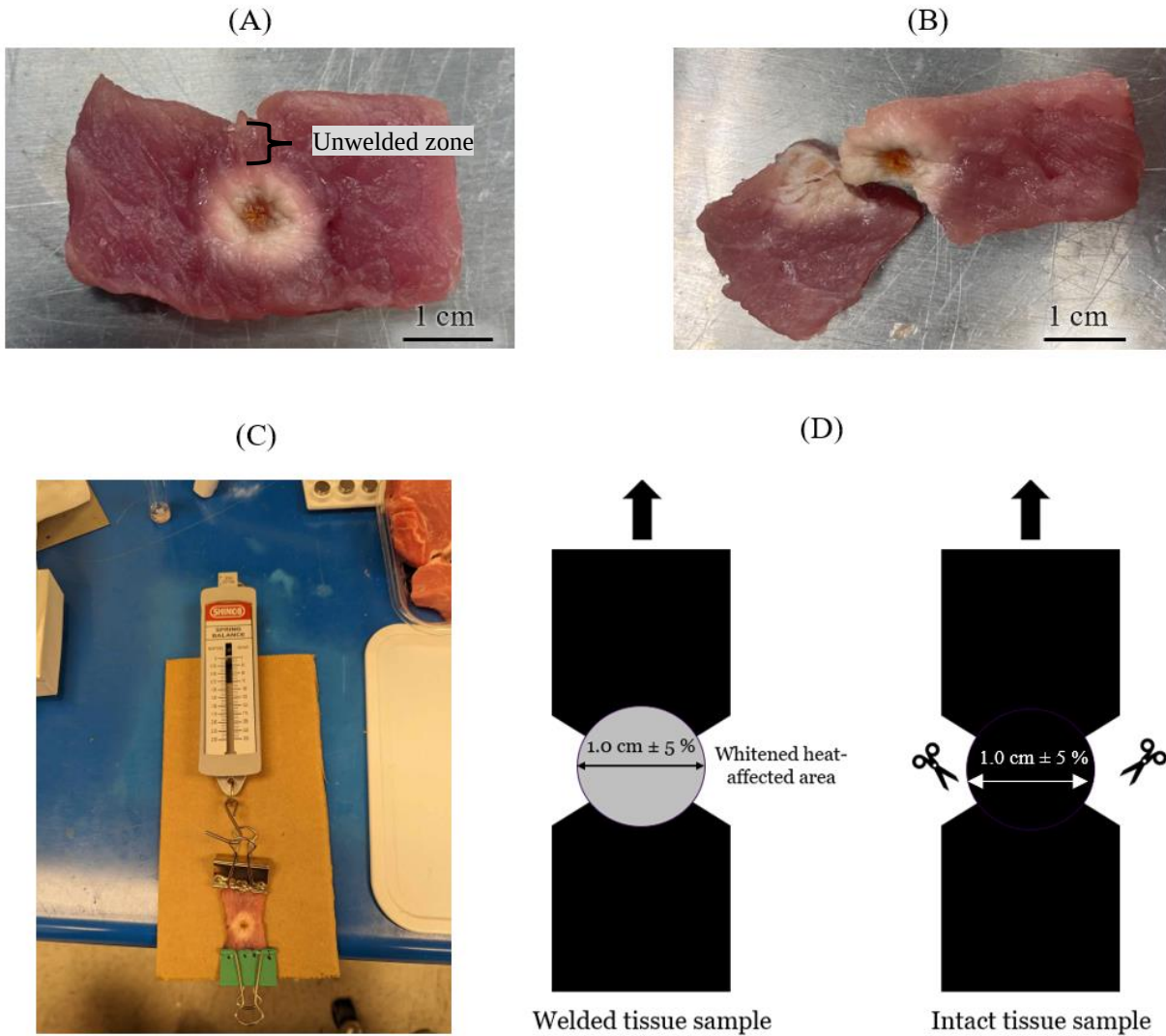
The same laser used in Chapter 2 is used here. A diode-pumped continuous wave (CW) ytterbium fiber laser (IPG Photonics; YLR-30-Y14) at a wavelength of 1070 nm was used for the welding studies. After focusing the laser to the surface of the specimen manually and to the interface of the bisected specimen, it was moved 1-inch upwards in the z-direction to lower energy density. The laser was operated at a power of 16.5 W without movement for a duration of 2 min to achieve joining. The irradiation time and power density align with other research on laser tissue joining [10, 11]. Schematics of the laser interaction are similar to Fig 2.3 (A) and (B), except that the beam diameter is larger. An example for a welded specimen is shown in Fig. 3.3 (A).

Fig. 3.3 (B) depicts a welded sample post-failure as well as the fracture surface. The zone of coagulation exhibits a typical whitened appearance with an average diameter of 1.0 cm which corresponds to the size of the weld. In addition, there is an additional zone of reddish discoloration within the center of the coagulated area. This is the area immediately under the laser beam and discoloration is caused by dehydration. This also hardens the tissue which explains how the fracture in Fig 3.3 (B) avoids this zone. Fig. 3.3 (C) shows a spring balance utilized for measuring tensile strength of the specimen. One end of the sample was fixed, while the other end was attached to the spring balance and subjected to a tensile

force. A detailed diagram of the experimental setup is shown in Fig. 3.3 (D). The spring balance was video recorded to accurately determine the failure point.

3.3 Tensile testing

Tensile tests were conducted on welded specimens. In the welded samples, there exist regions at the interface where welding was incomplete (Fig. 3.5 (A)). To replicate this condition for intact samples,



artificial incisions (1 cm on each side) were made when measuring its tensile strength.

Figure 3.3. Image of porcine skeletal muscle tissue specimen after LTW (A) and image of a specimen after tensile testing (B). Image of tensile testing procedure depicting shape of specimen as well as fixtures using perforated metal clips (C). Diagram of tensile testing procedure depicting the direction of tensile force. Scissor icons indicate artificial cuts (1 cm in length) (D).

Fig 3.4 is a box and whisker plot that presents data from tensile strength testing of intact and welded samples. The data shows that samples welded using these laser parameters recovered 46.7 % of the intact sample's tensile strength on average. This tensile strength recovery is considered typical when compared against instances of LTW on other tissues. For instance, Huang et al. (2013) performed laser welding on small intestine using a polypeptide nanocomposite solder and achieved approximately 47 % tensile strength recovery as well [10]. Recovery may be improved with parametric studies to investigate the effects of factors such as lasing duration and laser power in welding of porcine skeletal muscle tissue. Lasing mode may be significant in producing better tensile strength recovery. But work done by Urie et al. (2015) in the welding of porcine small intestine did not yield a significant difference between continuous wave and pulsed wave lasing modes.

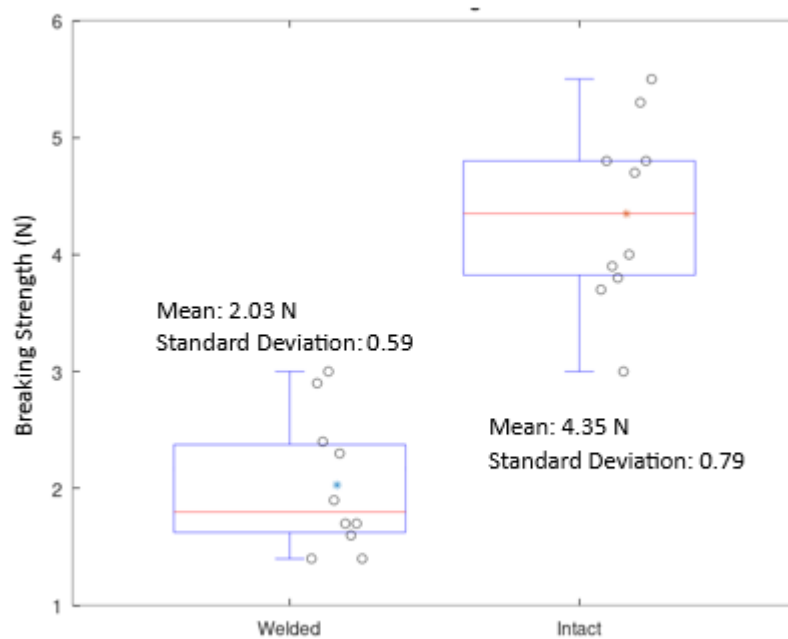
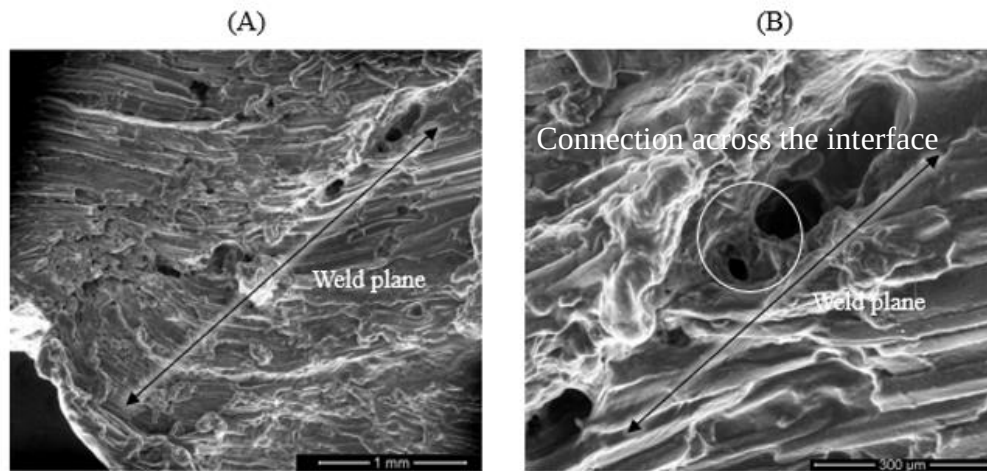


Figure 3.4. Box and whisker plot of tensile strength data from welded porcine skeletal muscle samples (N = 10) and unwelded/intact samples (N = 10). * Represents mean value.

3.4 Electron and Optical Microscopy of the Bonded Interface

The SEM images of a cross-sectioned weld (shown in Fig. 3.5 (A)) reveals the weld plane formed at the interface between two tissue pieces. The plane includes gaps, suggesting the potential for further strengthening of the weld. This may be accomplished procedurally by improving the contact area between two pieces at the interface, or by manipulating laser parameters such as irradiation time and laser power. Using exogenous chromophores such as ICG or GNR (see Section 1.2) for the targeted delivery of thermal energy may also improve weld quality through localized delivery of thermal energy.

Fig. 3.5 (B) shows connections across the weld plane in more detail. Although its composition cannot be definitively identified through SEM or XRD analysis, it possibly depicts the joining of fibrillar collagen across the interface. Alternatively, it possibly is the result of thermal coagulation of other proteins found abundantly in skeletal muscle fibers such as myosin and actin. Further investigation is needed to identify the proteins involved in joining, as well as how they have changed through coagulation using techniques



such as a Western blot [29].

Figure 3.5. SEM images of a cross-sectional view of welded porcine skeletal muscle specimen (A) and a magnified image depicting connections across weld plane (B).

Figure 3.6 shows a micrograph of the welded interface where individual muscle fibers are visible. Gaps also exist here, and they are larger in comparison to Fig. 3.5. This may be caused by shearing artifacts produced from cryostat slicing. Nevertheless, there are connections between muscle fibers on both sides of the interface accompanied by some discolouration near the weld plane that are indicative of coagulation. There is significantly less coagulation observed along the interface when compared to that observed on the surface of the specimen in Fig. 3.5 (A). This presents an ideal joint where coagulation is kept to a minimum while still achieving a weld.

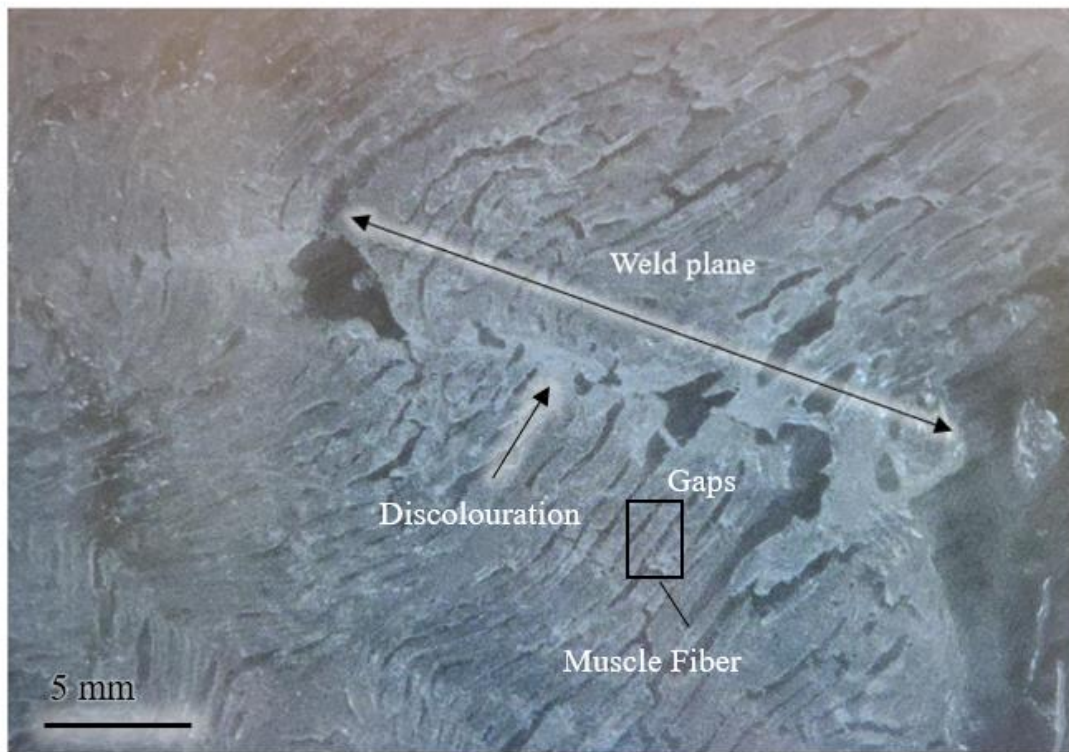


Figure 3.6. Optical microscope image of a cross-sectional view of a welded porcine skeletal muscle specimen obtained from cryostat, displaying individual muscle fibers and connections that reach across the interface.

3.5 Summary of Chapter 3

This chapter presented a method to join skeletal muscle tissue using a porcine model. This adds to literature as there are not any reports known to the author on the laser welding of skeletal muscle. Laser parameters commonly found in other LTW studies were used to inform parameters for this study. Using a method that defocuses the laser to provide a weaker power density over a larger area, and with irradiation time on the scale of minutes, a bond was achieved with a quality consistent to other works on laser tissue welding. The joined interface was imaged which displayed connections across the weld plane with the presence of a significant number of gaps. Therefore, despite the typical recovery in tensile strength, the bond can be improved further by manipulating laser parameters or with the assistance of exogenous chromophores. This chapter completes objectives 2 and 3 outlined in Section 1.7.

Chapter 4: Creation of Gold Nanorod Biosolders and its Application in Laser Tissue Welding

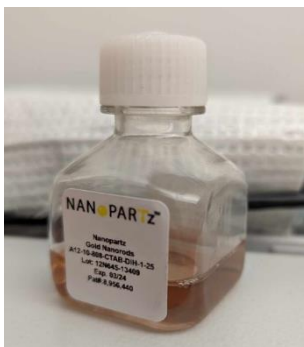
This chapter covers the creation of two biosolders containing GNRs to assist in LTW. One is a hydrogel comprised of (3 % w/v) hyaluronic acid (HA) and forms a paste with physical properties (Ex. viscosity) ideal for tissue application. To assess its applicability in LTW, a standardized amount of paste was applied over an artificial incision. The lasing procedure uses a fiber-coupled diode laser (808 nm) and forceps to manipulate the tissue during irradiation. The artificial incision was successfully sealed in its entirety within a 6 min irradiation time, although with significant carbonization. The second biosolder is a solid collagen disc, applied over the surface of a bisected skeletal muscle specimen like a patch. The area of laser irradiation as fixed and over an irradiation time of 4 min, a bond was formed. A tensile test using intact specimens as a control revealed a 48 % tensile strength recovery.

4.1 Gold Nanorods

Gold nanorods (GNRs) are useful in LTW because they are biocompatible, and they efficiently convert laser light energy to thermal energy which drives tissue fusion [30]. In addition, using GNRs allow for safer wavelengths (Ex. NIR has low absorptivity by tissues) at lower powers because of its efficient heat conversion, thereby minimizing extraneous damage caused to the tissue by the laser. Their absorbance maxima can also be manipulated to match the variety of lasers used for medical procedures.

For GNRs to be useful in laser welding, it cannot be applied onto tissue in its native aqueous form as it does not localize to the welding area. This chapter considers two methods of delivery by holding GNRs in a hydrogel and in a solid form using biocompatible molecules. This type of material is dubbed “biosolder” and its behaviour in LTW using porcine skeletal muscle tissue as a model is be assessed.

Gold nanorods used for this study were purchased from Nanopartz™ (part number: A12-10-808-CTAB-DIH-1-25, Nanopartz™, Loveland, USA). These nanorods possess a 4.1 aspect ratio (diameter = 10 nm, length = 41 nm) with an absorbance peak at 800 nm and optical density = 1.1 (no variation data available). The concentration of the gold nanorods in a solution using cetyltrimethylammonium bromide as a capping agent is 1.126 nM. It is a pale pink liquid solution with a viscosity consistent with water as



shown in Fig. 4.1.

Figure 4.1. Image of gold nanorod liquid solution purchased from Nanopartz™ with a peak absorbance at 800nm (A).

4.2 Biosolder Development: Hydrogel Formulation

The use of 3 % w/v HA to create a hydrogel is inspired by Matteini et al. (Ref. 13). The hydrogel consists of two components. One is the GNR liquid solution, and the other is a 3 % w/v inclusion of hyaluronic acid (HA). HA is a linear polysaccharide chain and is a major component of the extracellular matrix of tissues such as the skin and eyes [31]. Its role is to attract water, expanding its volume to provide structural support, an ideal ingredient for a hydrogel [31]. This molecule is identical across different species and thus would avoid an immune response, making it a biocompatible material that fits the purposes of LTW [31]. In its pure form, it exists as white, grainy powder (Hyaluronic acid sodium salt from *Streptococcus equi*, molecular weight: 1.5-1.8 x 10⁶ Da, product number: 53747, from Sigma Aldrich, Oakville, Canada). The creation of this hydrogel is simple and is summarized into two steps:

1. Obtain/measure a desired volume of the GNR solution.
2. Add 3 % w/v HA to the solution and stir every 10 minutes for one hour, or leave overnight.

The resulting product is a clear, pinkish, and viscous paste with a consistency similar to hair gel shown in Fig. 4.2. It is ideally stored at 4°C for GNR stability and sealed to prevent water loss [32].



Figure 4.2. Image of gold nanorod hydrogel formulation using 3 % w/v hyaluronic acid. It forms a viscous, pinkish paste.

4.3 Study to Seal Artificial Wound in Pork Skeletal Muscle Tissue

An investigation was conducted with this hydrogel formulation to seal an artificial wound using pork loin skeletal muscle tissue.

4.3.1 Sample Preparation

Skeletal muscle tissue was cut using a scalpel to create specimens for this study as shown in Fig 4.3 (A). The dimensions of the specimens ranged approximately from 4 – 5 cm in width and length, and 2 – 3 cm in height. The specific dimensions of the specimens were enough to accommodate an artificial incision

intended to evaluate the effectiveness of this formulation on muscle tissue. This incision was standardized to 1 cm in length and depth. Figs 4.3 (A) and (B) shows images of an example specimen with an incision before and after the application of hydrogel.

The tissue was packaged in plastic and used for experimentation not more than one day after purchase. Before experimentation, the tissue was equilibrated to room temperature, dried, and flattened using procedures described in Section 2.1. Figs 4.4 (A)-(C) illustrate the application procedure of the hydrogel. In Fig 4.4 (A), a uniform application of 10 mg of the GNR-HA formulation (blue) was administered inside the incision. Subsequently, the incision was subjected to a compressive force to minimize the gap at the interface. This leaves some hydrogel at the wound interfaced and causes some excess hydrogel to emerge from the incision shown in Fig. 4.4 (B). Subsequently, this excess hydrogel was evenly spread across the surface of the specimen, shown in Fig. 4.4 (C).

An image of a specimen processed in this way is shown in Fig 4.3 (B). The incision is hardly visible after compression and the hydrogel appears more transparent when used at little amounts. On the specimen, the hydrogel exhibited uniform spread and maintained its shape while adhering to the tissue.

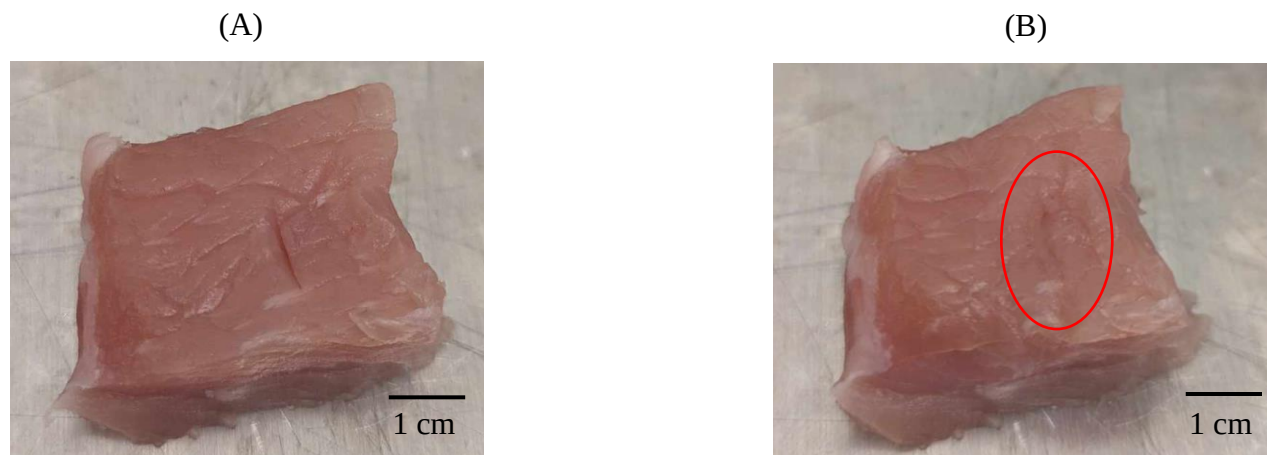


Figure 4.3. Image of pork loin skeletal muscle specimen after an artificial incision (1 cm length and depth) was made using a scalpel (A). Image of specimen after application of hydrogel and compression of the incision (B). Compressed incision (circled in red) is hardly visible. Paste appeared transparent.

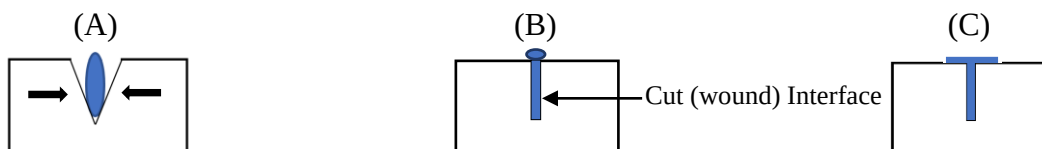


Figure 4.4. Schematic of application procedure for gold nanorod hydrogel formulation (blue) on skeletal muscle tissue (black rectangle). In (A), 10 mg of hydrogel was placed inside incision and a compressive force (black arrows) was exerted to close it. Some hydrogel remains at the wound interface while some accumulates at the surface of the specimen due to the compressive force (B). This accumulation was spread evenly across the surface of the specimen with a spatula (C).

4.3.2 Lasing Procedure

A continuous wave diode laser (808 nm, 5 W maximum power output) coupled to a fiber from Box Optonics Tech (Shenzhen, PR China) was used for this study (Model number: BLD-F808-05-22SMA). The fiber is coupled to a lens tube consisting of a single collimating lens with a beam diameter of 2 mm and a divergence angle of 7.5 mrad (focal length: 6.2 mm, numerical aperture: 0.40, working distance: 1.6 mm) from Thorlabs, Saint-Laurent, Canada (part number: C110TMD-B).

Fig. 4.4 shows the structure of the output optics of the laser. The black cylinder is a lens tube that is threaded on the inside to accommodate lens, adapters, or spacers. Two adapters and a collimating lens are housed inside the tube. The collimating lens is positioned at a distance of 6.4 mm from the laser output which is also its focal length.

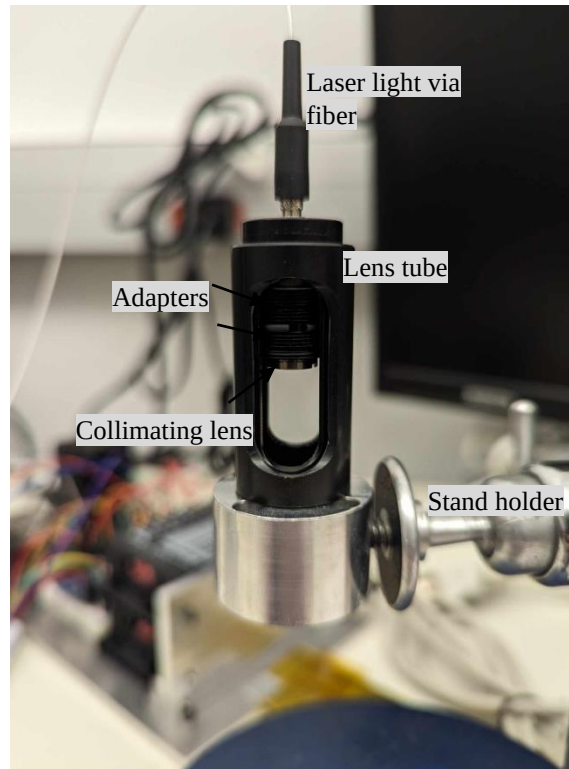


Figure 4.4. Image of output for the diode-pumped fiber laser. A lens tube (black cylinder) houses two adapters for the laser fiber input and a collimating lens. Positioned downwards using a stand holder.

Figure 4.5 shows the experimental setup. The lens tube/laser output is securely fixed to a stand using a holder and positioned directly above the sample. The distance between the laser output and the surface of the specimen is 105 mm. Due to the divergence angle, the beam diameter expands from 2 mm to 3.6 mm. Operating at 3 W, the laser produces a power density of 4.7 W/cm^2 on the specimen. The specimen can be moved along the x-y plane to change irradiation area by moving an aluminum plate placed underneath the specimen.

To replicate a surgical scenario, the device shown in Fig. 3.2 that was designed to conduct a compressive force to the specimen was not used. Instead, pressure was applied using two forceps to keep the wound tightly closed together throughout the experiment. Additionally, the sample was moved along the length (1 cm) of the artificial incision so that the laser can reach across the entire cut in an attempt to fully seal it. A camera was positioned in front of the sample to monitor the laser beam during the lasing process as shown in Fig. 4.5. Unlike in other studies, there was no standardized irradiation time. Instead, observations were noted along with approximate timeframes. Laser irradiation was stopped when a bond was produced after approximately 5.5 min, determined via testing with forceps. A control was conducted by irradiating tissue without hydrogel using the same laser for 5 min, resulted in no observable effects.

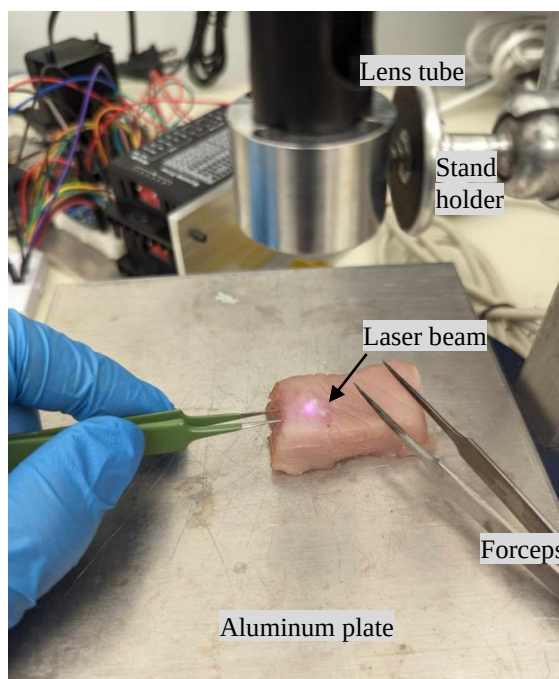


Figure 4.5. Image of experiment setup, taken from the camera used to visualize laser beam. Laser output is positioned directly above the sample and fixed using a stand holder. Two pairs of forceps are used to manipulate the sample (Ex. Applying compressive force) during laser irradiation. Aluminum plate can be moved to reposition the irradiated area. Phone camera was used to visualize the laser beam.

4.3.3 Observations and Discussion

Two stages were observed during the irradiation process. The first stage begins with the visible layer of GNR-HA hydrogel on the surface of the tissue. During this phase, laser irradiation induces coagulation/whitening on the tissue surface beneath the gel. A phenomenon that consistently occurred in all trials (N=5) was hydrogel carbonization. This appears as small black specks of carbonized material on the gel, consisting of organic material (HA). Fig. 4.6 shows an image of this material after laser irradiation of the hydrogel alone. Devoid of the tissue sample and other variables, it supports the conclusion that the composition of the carbonized material is HA in the hydrogel. If unaddressed, these

black specks absorb more laser light, leading to excessive and uncontrollable heating. Therefore, carbonized hydrogel is promptly removed. No joining (assessed by pulling with forceps) was ever documented at this stage across five trials.



Figure 4.6. Image of 3 % w/v hyaluronic acid hydrogel on aluminum plate after laser irradiation at 7.8 W/cm² for 10 s. Carbonized material (black speck) appears on the surface of the hydrogel.

The subsequent stage begins after approximately 3 min when hydrogel is no longer visible on the specimen surface. Since the hydrogel material is 97 % water, it disappears because the water is evaporated due to thermal energy from the laser/GNR. Coagulated tissue from the first stage become more susceptible to carbonization during this stage. With irradiation times as short as 15 s, coagulated tissue can undergo carbonization. Carbonization is an unwanted phenomenon because it is an indicator for excessive damage, occurring at temperatures above 150 °C. For LTW to be a safe and effective method of wound repair, damage should be minimized.

Ease of carbonization at this stage is expected as the departure of water from the system reduces heat conductivity at the irradiation site. This stage is crucial for tissue joining, as the evaporation of water at the cut interface (see Fig. 4.4 (B)), allows for closer contact. In all trials (N=5), the artificial incision was acceptably sealed using this procedure as shown in Fig 4.7 (A). Acceptability here is an arbitrary measurement of bond strength, assessed by gently pulling on the bond using forceps. The weld is considered acceptable if this force is insufficient to break it. The time required to produce an acceptable weld ranged from 4.5 to 6 min.

Fig. 4.7 (A) shows an image of the weld after laser irradiation. Carbonization is evident and proved necessary for tissue joining, which is a notable deviation from welding results presented in Chapter 3. While coagulated areas on the incision can be separated by forceps throughout irradiation, carbonized areas could not. Carbonization resulted in a slight depression on the specimen's surface due to destroyed material as seen in the photo of a cross-section of the welded material shown in Fig 4.7 (B). In contrast to the welds achieved in Chapter 3 the coagulation depth here is notably shorter (see Fig. 3.6). This discrepancy results from the difference in power density, with a higher density employed in Chapter 3.

Despite the presence of GNRs, the thermal energy remains comparatively lower, resulting in a shorter coagulation depth.

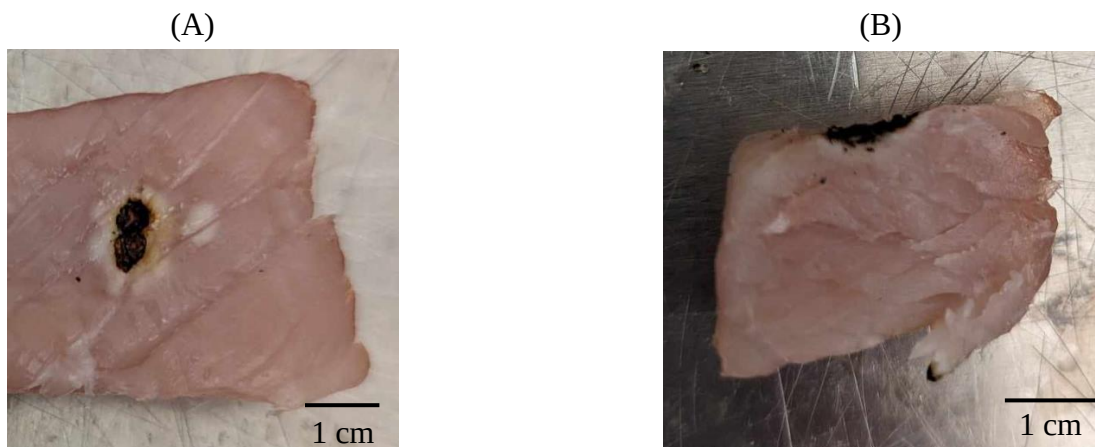


Figure 4.7. Image of porcine skeletal muscle specimen after application of 10 mg hydrogel formulation onto a 1 cm incision with 5.75 min irradiation time (A). Additional coagulated area to the right of the incision was a result of procedural error. A cross-sectional view of the weld displays a layer of carbonization followed by a layer of coagulation underneath (B).

Chapter 3 demonstrated the potential for robust welds without resorting to carbonization. Therefore, better outcomes are expected here with increased operator efficiency, refined technique, and the ability to quickly adjust laser power. Currently, laser power output is regulated by uploading code to the control chip. A more efficient and fast approach could involve a knob for adjusting laser power. Such improvements are also practical, should a similar procedure be adapted for surgical applications.

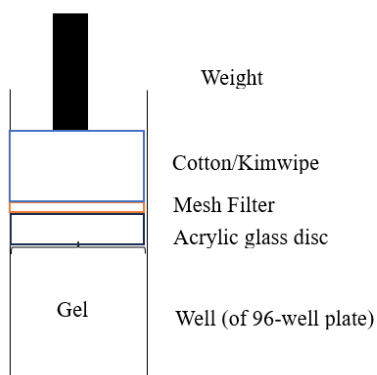
4.3.4 Conclusion

The hydrogel appears to be an effective vehicle for delivering GNRs to targeted surfaces on muscle tissue due to its spreadability, adhesive properties, and ability to retain its shape. However, during LTW, water from the hydrogel becomes a barrier as it resides at the wound interface, preventing complete contact. Evaporation of water is necessary during the first stage of LTW when using this material to enable tissue joining. Since joining without carbonization is possible, excessive carbonization encountered in the second stage of LTW can be controlled with better tools and techniques. With these improvements, this biosolder may become a promising option for wound sealing in muscle tissues.

4.4 Biosolder Development: Collagen Discs

The second method to deliver GNRs to target tissues uses a solid collagen disc, inspired by Ref. 10. With the application of thermal energy, collagen acts as a solder that can adhere to tissues and assist with joining (see Section 1.4).

The GNR-collagen disk is manufactured using a liquid solution of collagen (Rat tail collagen, Type 1, 4 mg/ml from Advanced Biomatrix, Carlsbad, USA). Collagen fibers were cross-linked by increasing basicity using a neutralization solution to form a hydrogel. For cross-linking to occur, collagen solution and the neutralization solution was mixed using a 9:1 ratio. To create a solid disc, liquid was removed via compression. Fig. 4.8 shows a schematic of the compression process that takes place in a plastic well (6 mm diameter). A flat acrylic glass disc is placed on top of the gel to conduct the compressive force. A mesh filter (stainless steel, from Amazon) and absorbent materials were placed above to absorb and remove water. A compressive force is administered via the gravitational force of a weight (1-2 g). Figs. 4.9 (A)-(D) show images of the tools involved with this procedure. A detailed protocol can be found in



Appendix B.

Figure 4.8. Diagram showing the procedure to compress collagen hydrogels into solid discs. Wells from a 96-well plate are filled with the collagen hydrogel. An acrylic glass disc is used to conduct the compressive force from the gravitational force of a cylindrical weight. Absorbent materials and the mesh filter assist in removing the water that was evacuated from the hydrogel.

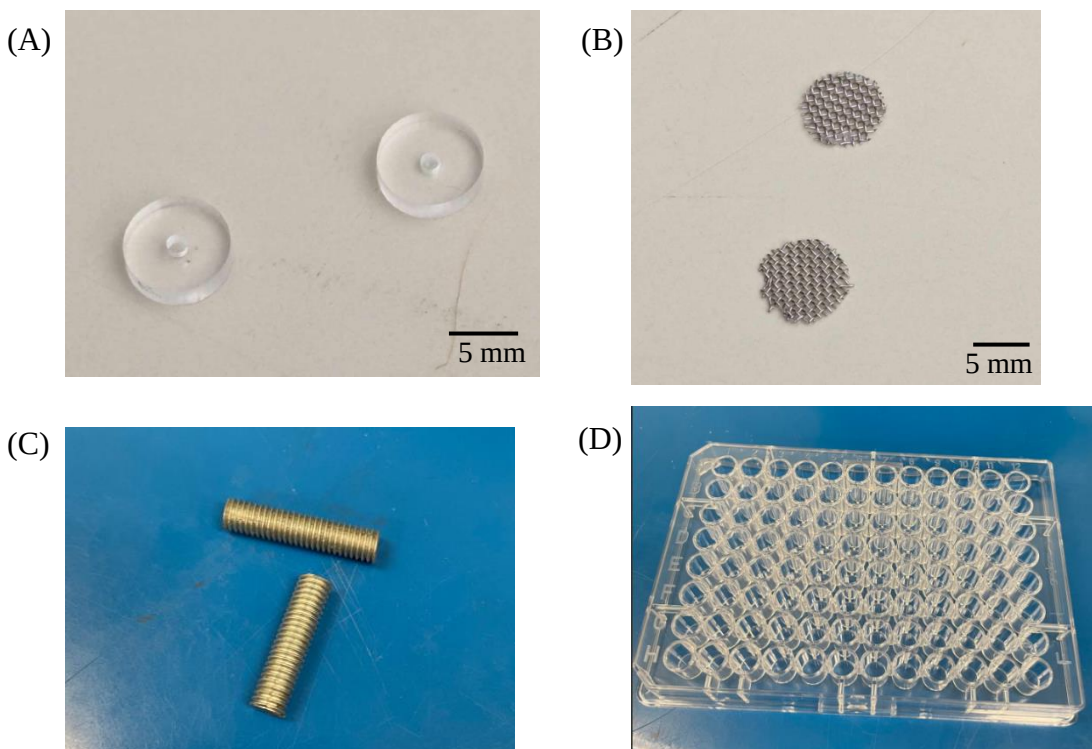


Figure 4.9. Images of materials used in the creation of GNR-collagen discs. Acrylic discs (A), mesh filter (B), weight (C), 96-well plate (D).

The resulting product is a white, opaque, circular patch (6.5 mm diameter, 1 mm thickness) that can be easily deformed as shown in Fig. 4.10 (A). The patch material shown in the image is not completely flat, contributing to the different degrees of opacity observed in the photo. During the procedure, the patch was laid completely flat onto the surface of an artificial incision using opacity as a measure. Prior to laser irradiation, discs were incubated in a GNR solution (see Section 4.1) at a temperature of 4 °C overnight to allow GNRs to enter the material. Fig. 4.10 (A) shows an example of a GNR-collagen disc before incubation. After incubation, the disc experiences a colour change, becoming darker with a faint pink hue, as seen in Fig. 4.10 (B). This image shows some inconsistencies (circled in red) that can arise the production stage. These were easily removed due to their malleable nature prior to experimentation for the purpose of consistency.

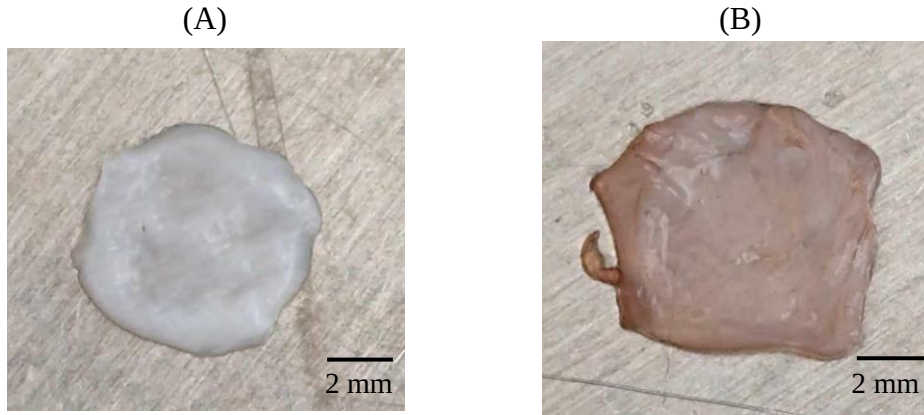


Figure 4.10. Images of a GNR-collagen disc after compression, it is opaque and white in colour (A). After incubation with GNR solution at 4°C, it developed a darker and pinkish color. No change in other properties was observed after incubation.

4.4.1 Sample Preparation and Lasing Procedure

Skeletal muscle tissue specimens were prepared using the same procedure outlined in Section 3.1 and bisected in the same fashion. Except that the specimens were to a thickness of approximately 2.5-3.5 mm. A different thickness was chosen here since preliminary studies showed a lower coagulation depth. Therefore, the thickness was decreased so that it may be more comparable to the intact control condition, while remaining feasible to consistently cut by hand. The muscle tissue and GNR-collagen discs were removed from incubation and given 2-3 hours to equilibrate to room temperature. Before use, excess fluid was drained from the disc using a paper towel. The disc was placed at the center of the specimen, on the surface of the bisected sample. Fig. 4.11 shows a specimen before laser welding.



Figure 4.11. Image of a bisected specimen with a GNR-collagen disc positioned at the center of the cutline.

Using Ref. 10 as a guideline, lasing was conducted for a duration of 5 min using the same laser and setup identified in Section 4.2.2. The power density delivered by the laser was 1.9 W/cm^2 . The laser was fixed to the center of the disk and not moved throughout the procedure.

4.4.2 Observations and Tensile Test

Fig. 4.12 (A) shows the image of a specimen undergoing laser irradiation. Carbonization is found at the center of the disc. This center area is under direct laser irradiation which experiences a higher concentration of thermal energy, thus resulting in carbonization. Surrounding areas were indirectly heated and thus experienced only coagulation. Bubbling was observed during irradiation and it was an expected phenomenon caused by water within and under the disc escaping upon heating. There was evidence of carbonization in all trials (N=10). Fig. 4.13 shows an image of the setup during laser irradiation, showing the camera used to visualize the laser beam throughout the procedure.

Fig. 4.12 (B) shows the depth of carbonization with a cross-sectional view, typically 1 mm deep. The carbonized material consists of the GNR-collagen disc but it is unclear if the carbonization effect extended downwards to damage the tissue. Fig. 4.12 (C) shows an image of the sample post-failure and a fracture analysis of the surface. The observations are similar to results presented in Section 3.3 (B) where the fracture is in the coagulated region, avoiding the center of the irradiated tissue. This is because that this center zone was hardened via carbonization and solidification of the GNR-collagen disc.

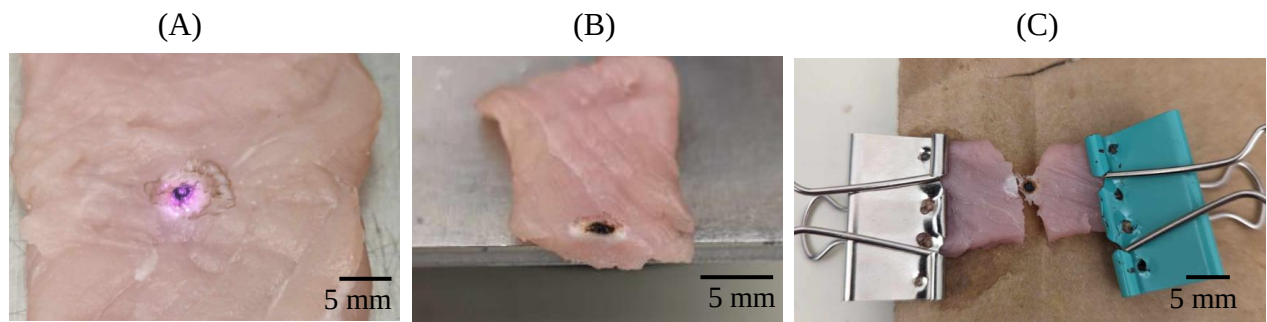


Figure 4.12. Image of bisected porcine skeletal muscle specimen undergoing laser irradiation (beam in purple) with power density of 1.9 W/cm^2 for 5 min (A). Photo taken after approximately 3 min. Cross-sectional view of a welded specimen exhibiting layers of carbonization and coagulation (B). Image of specimen post-failure from tensile test (C).

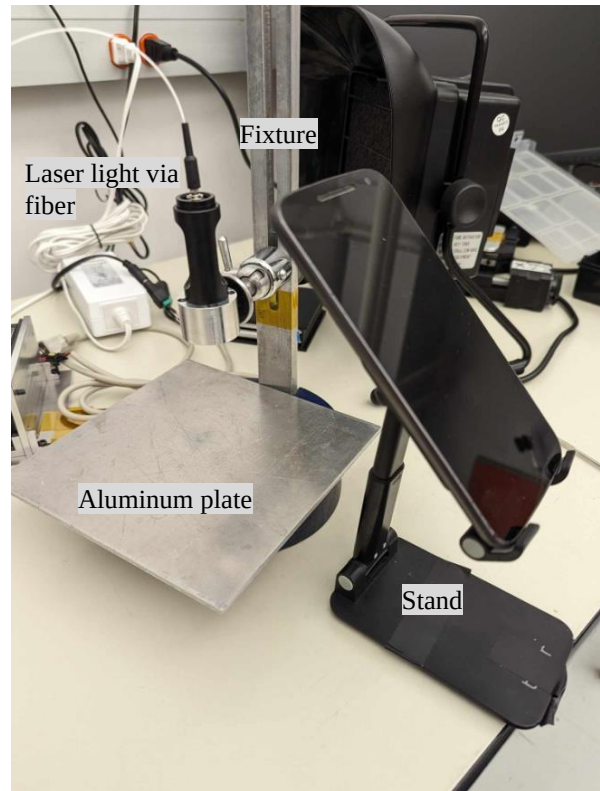


Figure 4.13. Image of laser irradiation setup for LTW using GNR-collagen discs. A stand fixes the output optics of the laser at a specific height (105 mm) over the tissue specimen which was placed on the aluminum plate. A camera with a stand was used to take photos and observe the laser beam and site of irradiation during the procedure.

After subjecting the specimen to laser irradiation, a tensile test was performed using the same procedure and setup outlined in Section 3.3. Initially, discs possessed a diameter of 6.5 mm. However, they experienced shrinkage post-irradiation, resulting in an average diameter (N=10) of 4.5 mm. To evaluate the tensile strength of intact samples, artificial incisions were made on both sides of the specimen, leaving an intact zone measuring 4.5 mm in length (see Fig. 3.3).

Figure 4.14 presents tensile test data obtained from welded (N=10) and intact (N=10) conditions in a box and whisker plot. Comparing the mean tensile strengths reveals a 48 % recovery using a GNR-collagen disc with 6 mm diameter. Ref. 11 utilized the disc and achieved a 68.3 % tensile strength recovery on small intestinal tissue. The disparity in tensile strength recovery is likely a result of the differences in tissue morphology. In Ref. 11, the patch was applied on the surface of small intestinal tissue which a naturally grown epithelial layer, whereas in this study, the patch was applied onto a cut surface of a tissue comprised of interlocking fibers. This allows for a greater degree of surface roughness which may result in a lower tensile strength recovery as observed.

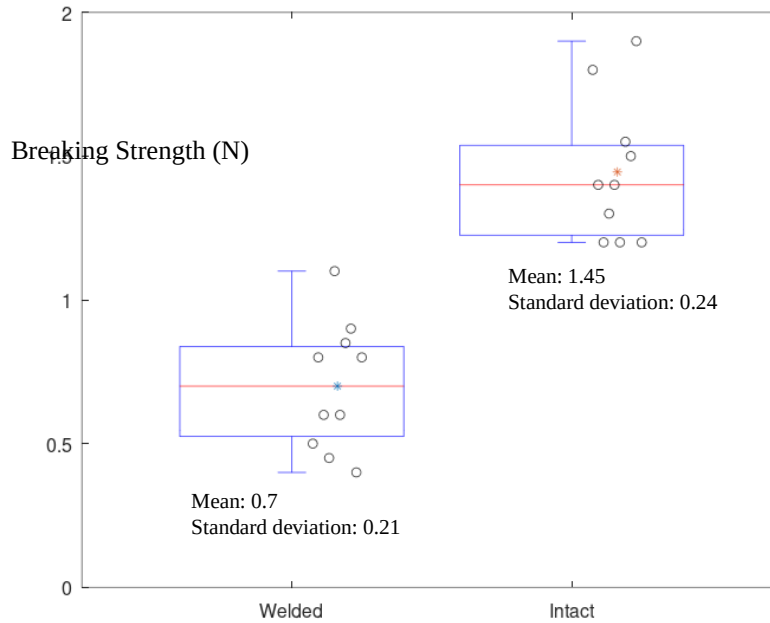


Figure 4.14. Box and whisker plot of tensile strength data from welded porcine skeletal muscle samples using GNR-collage discs (N = 10) and unwelded/intact samples (N = 10). * Represents mean value.

The weldability of the GNR-collagen discs to skeletal muscle fibers is notable. Previous studies have demonstrated the weldability of collagen discs to the small intestine, an elastic organ comprised of layers of connective tissue and smooth muscle [10, 33]. There is a substantial difference in collagen content between skeletal muscle tissue and the small intestine. Collagen can account for 1-10% of dry muscle mass [34]. Total collagen content in a similar organ, the large intestine, can vary widely from 12 - 74 % depending on the layer [35]. Therefore, the disparity in collagen content of target tissues may account for the difference in tensile strength recovery, considering the mechanism outlined in Section 1.4. In addition, despite the relatively low collagen content in skeletal muscle tissue, an approximate 50 % recovery in tensile strength was observed, and is comparable to the recovery that was reported in Ref. 10. This study highlights the versatility of this material in LTW applications, which is a significant advantage given the large variation in tissue types and composition. Compared to results in Chapter 3, a lower laser power density produced a stronger weld using GNR-infused biosolders. This speaks to the efficiency of GNRs in generating thermal energy, allowing for lower and safer laser powers to be used in surgery. In addition, by comparing the coagulation area around the weld imaged in Chapter 3 and 4, the ability of GNRs to localize thermal energy is apparent. This is a desirable trait in a surgical scenario as it minimizes extraneous damage to the tissue.

4.5 Summary of Chapter 4

This chapter presented two biosolders and used them to achieve joining using porcine skeletal muscle tissue as a model. One option involved a hydrogel, where hyaluronic acid was used to thicken a GNR solution, resulting a paste-like product with 97% water content. The paste proved highly versatile, given its ease of application at the wound interface due to its spreadability and ability to maintain its shape. However, its high-water content proved to be a barrier, as joining occurred only after the complete

evaporation of water from the irradiation site. The viscous nature of this material allows for a wider range of applications, compared to the other biosolder presented in this chapter, a solid collagen disc with GNRs. This disc was applied atop of a simulated wound like a patch and with laser irradiation, achieved a 48 % recovery in tensile strength. While the disc was successfully applied to small intestinal tissue in a previous report, it was shown in this work to be applicable also to skeletal muscle, highlighting the versatility of this material. In both cases, a lower laser power density, relative to results from Chapter 3, was used to achieve joining. This highlights the efficiency of GNRs in generating thermal energy and shows advantages in surgical applications as safer and lower-powered lasers can be used to achieve welding. In addition, welds achieved with GNR biosolders possess a smaller coagulation zone with showcases its ability to reduce extraneous damage to surrounding tissues. This chapter completes objectives 4 and 5 outlined in Section 1.7.

Chapter 5: Conclusions and Future Work

A widespread acceptance of LTW as a method of muscle tissue wound repair in clinical applications requires advancements in the understanding of the mechanism, efficiency, and versatility of the technique. To this end, effectiveness of LTW using ex vivo porcine skeletal muscle tissue as a model was studied. A parametric study using a NIR laser was conducted to determine the threshold of coagulation. By manipulating laser power and lasing duration, a threshold was determined which defines the safe upper limit of laser irradiation. This prevents tissue damage in the form of coagulation and allows for the identification of safe laser parameters to engage therapeutic photochemical effects. This research contributes towards the development of laser-muscle treatments for chronic pain and muscle regeneration. It also holds relevance for welding studies by elucidating parameters that can induce coagulation.

Furthermore, the joining of porcine skeletal muscle specimens was achieved using NIR laser irradiation alone, resulting in a 46.7 % percent recovery in tensile strength, a result consistent to existing literature. Analysis of the weld interface using optical and SEM microscopy revealed gaps, which suggests the potential for optimization by conducting parametric studies in the future to improve weld quality.

Biosolders containing GNRs were developed using biocompatible materials and was applied onto artificial wounds in ex vivo porcine skeletal muscle tissue. A hyaluronic acid hydrogel was produced which possess ideal characteristics such as viscosity and adhesion for applications with biological tissue. It effectively sealed wounds under a simulated surgical environment. Moreover, a solid collagen disc biosolder was incubated with GNRs and used as a patch, to be applied to the surface of a cut. A joint was produced with this material that resulted in a 48 % recovery in tensile strength. These studies highlight the applicability of these biosolders and simplicity. It also demonstrates their versatility for different tissue types as it was used to successfully join porcine skeletal muscle tissue.

Carbonization from welding studies with GNR biosolders remain an issue as it represents excessive heating and a form of irreversible damage. Further studies can conduct parametric investigations to optimize the welding process to prevent carbonization. In addition to laser parameters such as power or irradiation time, properties of biosolders can be controlled as well. For example, the diameter, width, and GNR concentration of GNR-collagen discs. These are potential variables that can be optimized to improve weld quality and to minimize tissue damage (carbonization) for better recovery in a living model. Furthermore, tools such as an adjustable power laser output or temperature control during laser irradiation possibly can serve as useful tools to eliminate carbonization and to improve bonding.

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Appendices

Appendix A: Code Used for Edge Detection

```
1 x=imread('Trial 4.jpg');
2
3 %Coverts the read image to grayscale
4 x1=rgb2gray(x);
5
6 %Resizes the image to a lower resolution so Canny works better
7 x2=imresize(x1,[320, NaN]);
8
9 %Increasing contrast
10 px=localcontrast(x2, 0.2, 0.8);
11 %adjust brightness
12 px1=px-15;
13 px2=edge(px, 'canny',[0.16 0.18],0.8);
14
15
16
17
18 %overlay
19 dx=localcontrast(x2, 0.2, 0.8);
20 dx1=dx-15;
21 dx2=edge(dx1, 'canny', 0.2,2.5);
22 dx3=imresize(dx2,[2988,NaN]);
23
24 %px3=imrotate(px2,270);
25 px4=imresize(px2,[2988,NaN]);
26
27
28 composite=imfuse(px4,dx3, 'blend');
29 imshow(composite)
30
31
32 imshowpair(composite,x, 'blend')
33 imsave
```

Figure A-1. Code used for Canny edge detection to consistently measure diameter of coagulated areas on pork loin skeletal muscle specimens. Filename: Canny_Edge_Detection.m

```

1 clear all;close all;
2 % raw data pasted from spreadsheet
3 d=[
4 ];x=d(:,1)';P=x; % power values [W]
5 dwell_s=[3 5 7 10 15 20]; % s
6 dwell=[]; % data used to be filled later
7 Plim=dwell-dwell; % threshold power to be filled later
8 diam=[]; % diameter values to be filled later
9
10
11 % loop over data for each duration time experiment (columns of spreadsheet)
12 for j=2:length(d(1,:));
13     % take next dataset from array
14     y=d(:,j)';dwell=[dwell dwell_s(j-1)];diam=[diam; y];
15
16     % plot experimental data on new figure
17     figure;plot(x, y, 'k.',x, y, 'ko');title(disp(num2str(j-1)));hold on;drawnow
18
19     %Define the model for minimization (least squares):
20     % our model: model_y=real(sqrt(x-p(1)).*p(2)) with p(1) & p(2) for fitting
21     % for fitting we minimize the sum of the squares of the differences of
22     % experimental and model values:
23     my_fun = @(p) sum((y- real(sqrt(x-p(1)).*p(2)) ).^2);
24
25     %Initial guesses
26     p0 = [5.5,.46];
27
28     %Nonlinear fit to improve on the initial guess p0; extract threshold "th"
29     result = fminsearch(my_fun, p0); th=result(1); mf=result(2);
30
31     % new x values for evaluating the model
32     xf=[min(x):.005*range(x):max(x)];
33     %Function to evaluate the model
34     fun_eval = @(p, t) real(sqrt(t-p(1)).*p(2));
35
36     % outputting the threshold value "result(1)" (P_lim)
37     disp([num2str(j-1) ' : Dwell = ' num2str(dwell_s(j-1)) ' s; threshold = ' num2str(th,4) ' W; mult.-factor = ' num2str(mf,4) ' mm/sqrt(W)'])
38
39     % add a plot of the result of fitting
40     yf=fun_eval(result, xf); plot(xf, yf, 'r',xf, yf, 'k.', [th th],[min(yf) max(yf)]);
41     title(['Dwell = ' num2str(dwell_s(j-1)) ' s; Fit: y = ' num2str(result(2),4) '*sqrt(x - ' num2str(th,4) ' ')]
42     xlabel('Power [W]');ylabel('Diameter [mm]'); Plim(j-1)=result(1);
43     text(th,max(yf),[num2str(th) 'W'])
44 end
45
46 % visualize threshold data
47 z=dwell;figure ;
48 [C,h] =contourf(P,z,diam,[min(min(diam)):max(max(diam))]);
49 clabel(C,h); hold on;title(['Diameter [mm]']);
50 xlabel('Power [W]'); ylabel('Dwell Time [s]')
51 plot(Plim,dwell,'g',Plim,dwell,'gs');hold on
52 for j=1:length(x);
53     for k=1:length(z); plot(x(j),z(k),'ro'); end
54 end
55 print -dpng contplot_Kai.png;
56 %%%% end of code

```

Figure A-2. Code used to plot coagulation diameter data and to model a fitted curve. Code was written by Prof. Michael Mayer. Filename: Curve_Plot.m

Appendix B: SOP for the Creation of GNR-Collagen Discs

Materials

- Rat Collagen 4 mg/ml (from Advanced Biomatrix)
- Neutralization solution (from Advanced Biomatrix)
- 96-well plate
- Weights (pieces of a threaded rod)
- Circular mesh filter
- Acrylic glass disc
- Cotton
- Kimwipe
- Needle
- Micropipette
- Tweezers

Procedure

1. Synthesis is carried out in the 96-well plate. Each well has a maximum volume of 350 μ L. Determine the amount of gel you wish to make (no more than 350 μ L); Recommended amount is 300 μ L.
2. Each well makes one collagen disc. Determine how many you want to make. Then, to each well, add rat collagen and neutralization solution in a 9:1 ratio (Ex. When making 250 μ L of gel, add 225 μ L of rat collagen 4 mg/ml and 25 μ L of neutralizing solution)
3. Stir well using needle.
4. Incubate the 96-well plate in 37°C for 2 hours or at room temperature for 5 hours.
5. Remove from incubation, double check that the solution in the well is gelled by gently agitating it with a needle and observing that the colour is white and opaque.
6. Add on top of each well in order, the acrylic glass disc (using tweezers), circular mesh filter, cotton/kimwipe, weight.
7. Press down gently on to the weight to create some pressure and release some water from the gel.
8. Replace the cotton/kimwipe with new ones.
9. Repeat steps 7-8 every couple of minutes, or when the cotton/kimwipe becomes wet.
10. When the cotton/kimwipe is no longer wet, remove components from the well and retrieve the collagen disc from the bottom of the well using tweezers.