

Video Article

A Chitosan Based, Laser Activated Thin Film Surgical Adhesive, 'SurgiLux': Preparation and Demonstration

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URL: <https://www.jove.com/video/3527>

DOI: [doi:10.3791/3527](https://doi.org/10.3791/3527)

Keywords: Bioengineering, Issue 68, Chitosan, Infra-red Laser, Indocyanine Green, Biomaterial, SurgiLux, Surgical Adhesive

Date Published: 10/23/2012

Citation: Foster, L.J., Karsten, E. A Chitosan Based, Laser Activated Thin Film Surgical Adhesive, 'SurgiLux': Preparation and Demonstration. *J. Vis. Exp.* (68), e3527, doi:10.3791/3527 (2012).

Abstract

Sutures are a 4,000 year old technology that remain the 'gold-standard' for wound closure by virtue of their repair strength (~100 KPa). However, sutures can act as a nidus for infection and in many procedures are unable to effect wound repair or interfere with functional tissue regeneration.¹ Surgical glues and adhesives, such as those based on fibrin and cyanoacrylates, have been developed as alternatives to sutures for the repair of such wounds. However, current commercial adhesives also have significant disadvantages, ranging from viral and prion transfer and a lack of repair strength as with the fibrin glues, to tissue toxicity and a lack of biocompatibility for the cyanoacrylate based adhesives. Furthermore, currently available surgical adhesives tend to be gel-based and can have extended curing times which limit their application.² Similarly, the use of UV lasers to facilitate cross-linking mechanisms in protein-based or albumin 'solders' can lead to DNA damage while laser tissue welding (LTW) predisposes thermal damage to tissues.³ Despite their disadvantages, adhesives and LTW have captured approximately 30% of the wound closure market reported to be in excess of US \$5 billion per annum, a significant testament to the need for sutureless technology.⁴

In the pursuit of sutureless technology we have utilized chitosan as a biomaterial for the development of a flexible, thin film, laser-activated surgical adhesive termed 'SurgiLux'. This novel bioadhesive uses a unique combination of biomaterials and photonics that are FDA approved and successfully used in a variety of biomedical applications and products. SurgiLux overcomes all the disadvantages associated with sutures and current surgical adhesives (see **Table 1**).

In this presentation we report the relatively simple protocol for the fabrication of SurgiLux and demonstrate its laser activation and tissue weld strength. SurgiLux films adhere to collagenous tissue without chemical modification such as cross-linking and through irradiation using a comparatively low-powered (120 mW) infrared laser instead of UV light. Chitosan films have a natural but weak adhesive attraction to collagen (~3 KPa), laser activation of the chitosan based SurgiLux films emphasizes the strength of this adhesion through polymer chain interactions as a consequence of transient thermal expansion.⁵ Without this 'activation' process, SurgiLux films are readily removed.⁶⁻⁹ SurgiLux has been tested both *in vitro* and *in vivo* on a variety of tissues including nerve, intestine, dura mater and cornea. In all cases it demonstrated good biocompatibility and negligible thermal damage as a consequence of irradiation.⁶⁻¹⁰

Video Link

The video component of this article can be found at <https://www.jove.com/video/3527/>

Protocol

1. Preparation of SurgiLux Solution

1. Prepare a 2% (v/v) solution of acetic acid using deionized water in a clean glass beaker; use a laminar flow hood to avoid contamination.
2. Weigh 0.02% (w/v) of the chromophore, indocyanine green, ICG, in a sterile Eppendorf tube; ensure the tube is wrapped in silver foil to prevent any light penetration.
3. Using a clean, disposable pipette, transfer approximately 1 ml of the dilute acetic acid solution to the tube to dissolve the dye, shake gently and keep wrapped in foil.
4. Transfer the solubilized ICG into the beaker and add 2% (w/v) of chitosan powder before adding a sterile magnetic stirrer.
5. Cover the beaker with Parafilm then wrap in silver foil, before mixing the contents at about 125 rpm for 72 hr at room temperature in a laminar flow hood.
6. Transfer the contents into clean centrifuge tubes and centrifuge at 15,000 x g for 15 min at 4 °C to remove any particulate matter.
7. Carefully transfer the green SurgiLux solution into a clean glass beaker, cover using Parafilm then wrap in silver foil, before storing in a refrigerator for 12 hr to increase the viscosity of the solution.

2. Casting of SurgiLux Films

1. Using a sterile syringe, dispense 8 ml of the cold SurgiLux solution into a clean, Petri dish of 95 mm diameter, and gently tilt the plate to ensure complete coverage by the solution. Varying the ratio of solution volume to casting area permits control of the film thickness, see **Figure 1**.
2. Remove any visible bubbles in the solution using the tip of a sterile needle. Cover the dish in silver foil and place in a refrigerator to remove any residual micron-sized bubbles.
3. After 20 min carefully remove the Petri dish from the refrigerator, place in a laminar flow hood, cover with silver foil and leave the solution to evaporate for 3 weeks.
4. After complete evaporation, score the outer edges of the clear green SurgiLux film in the Petri dish and gently 'peel' the film away from the dish surface.
5. The SurgiLux film should be flexible and readily manipulated without tearing or breaking.
6. Store the circular SurgiLux films in the Petri dish wrapped in silver foil under dry conditions until ready for use.

3. Laser Activation of SurgiLux Adhesive Films

1. To demonstrate the laser activation process we will use a piece of bovine tissue such as steak cut to a size of 15 mm wide and 20 mm length. Dissect the tissue in a straight line using a Number 10 surgical blade, to produce 2 pieces of 15 by 10 mm.
2. Approximate the two pieces of tissue so that their edges are touching but not overlapping, and using a cotton swab or gauze, gently absorb any excess fluid.
3. Next, cut a piece of SurgiLux film 7 x 9 mm and carefully place the film lengthways across the bisected piece of tissue; then press down gently with a dry cotton swab.
4. SurgiLux films are activated using an infra-red diode laser at a setting of 120 mW. As this is a class IIIB laser, appropriate safety measures should be taken, including the use of appropriate safety glasses for all personnel.
5. Starting in the corner, irradiate the SurgiLux with an infra-red laser set at 120 mW and a beam spot size of 1 mm diameter. Pass the beam spot over the green film at a rate of approximately 1 mm per second. Repeat the irradiation process two more times.

4. Strength of the Repair

1. Carefully secure the ends of the tissue in the clamps of a tensile testing instrument. We are using an Instron Mini55 system with a 50 Newton load cell. The maximum load, tensile strength, and extension at break were calculated using Bluehill computer software (USA). Means from at least 10 samples were determined ($n = 10$).
2. Take up the 'slack' and then separate the tissue pieces at a rate of 1 mm per second, until the two pieces of tissue held together by the SurgiLux strip separate completely.

Representative Results

Centrifugation leads to a transparent green solution, which increases viscosity after storage at 4-6 °C. After standing for 3 weeks, the green solution is converted into a transparent green SurgiLux film approximately 20 microns thick and, as demonstrated in the video, is readily flexible.

Upon irradiation with the laser, the SurgiLux film bonds to the tissue. This can be observed at the edges of the film where the tissue appears to contract as the laser beam passes over the film (**Figure 2**). No charring or ablation of the tissue and film should be observed. The bonding strength of SurgiLux to the tissue should be sufficient to lift the bisected pieces of tissue, and when the tensile strength is measured should be approximately 15 KPa for the test reported here.

	Fibrin Glues (Gel)	Cyanoacrylate Glues (Gel)	SurgiLux Adhesive (Thin Film)
Tissue bond strength (KPa)	~4	~70	~15
Material toxicity	No	Yes	No
Potential for viral Infection	Yes	No	No
Solubility	Yes	No	No
Ease of Application	No	No	Yes
Biocompatible & Biodegradable	Yes	No	Yes
Post-operative complications	Yes	Yes	Under investigation

Table 1. Comparison of properties for proposed SurgiLux system and commercially available fibrin and cyanoacrylate surgical adhesives.

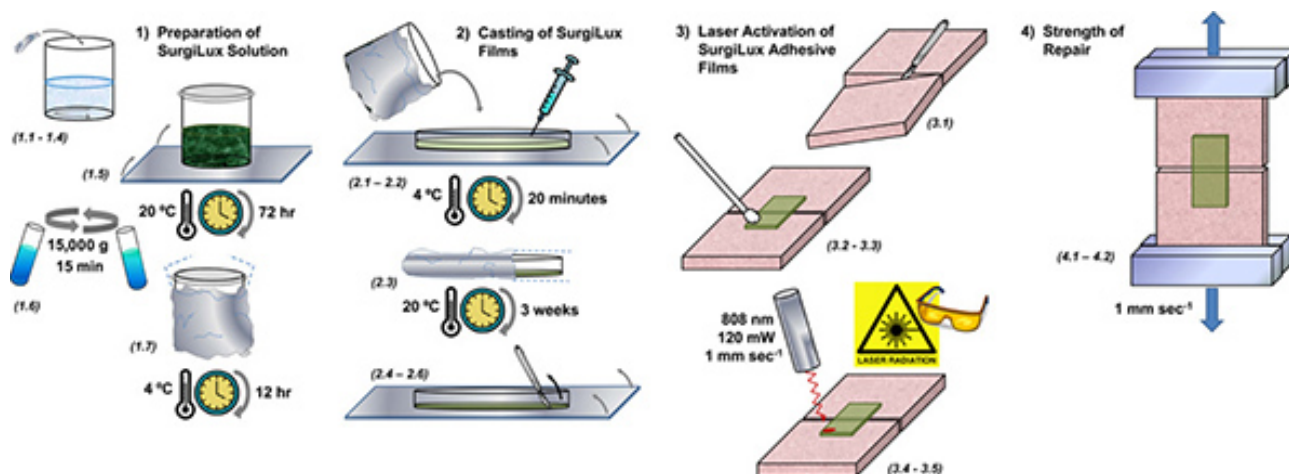


Figure 1. Diagrammatic illustration of the SurgiLux thin film adhesive fabrication and activation process; corresponding to the protocol text. [Click here to view larger figure.](#)

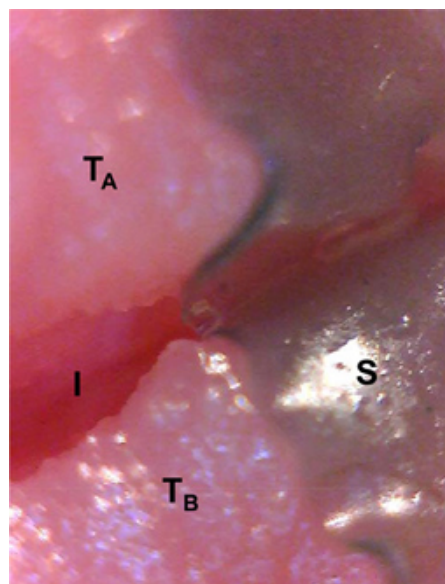


Figure 2. Photograph (x20) showing the thin SurgiLux film after laser activation adhered to bovine intestine tissue and 'contracting' into the tissue incision (T_A, T_B: separate pieces of tissue, I: incision, S: SurgiLux film).

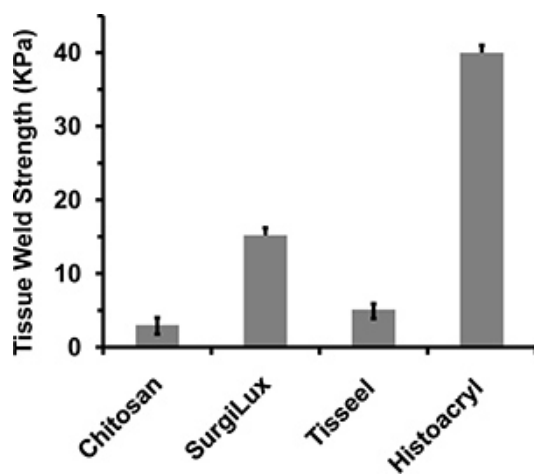


Figure 3. Graph showing tissue adhesion strengths for various adhesives: Chitosan and SurgiLux films applied on tissue, Tisseel (fibrin) and Histoacryl gels (cyanoacrylate) applied on tissue.

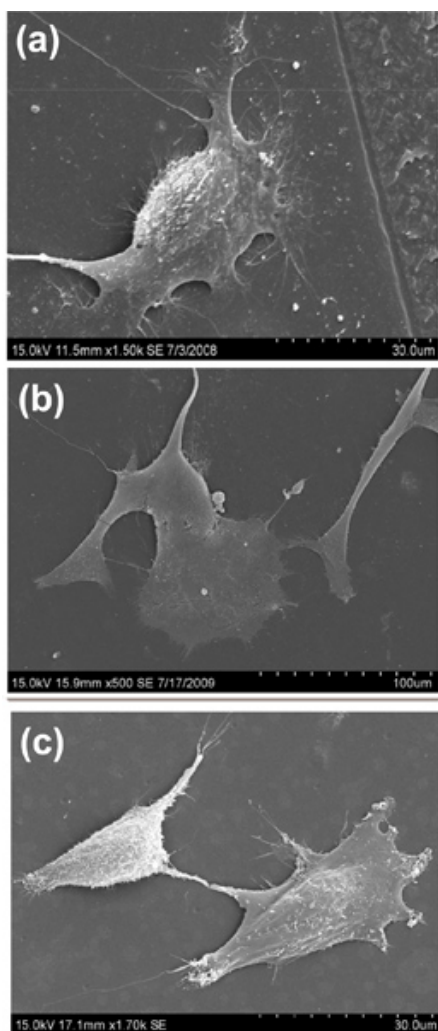


Figure 4. Scanning Electron Micrographs (SEMS) illustrating cells attached to SurgiLux films; human cell lineages (a) olfactory ensheathing cells [x1.5k], (b) stromal fibroblasts [x500], and (c) skeletal muscle derived satellite stem cells [x1.7k]. [Click here to view larger figure.](#)

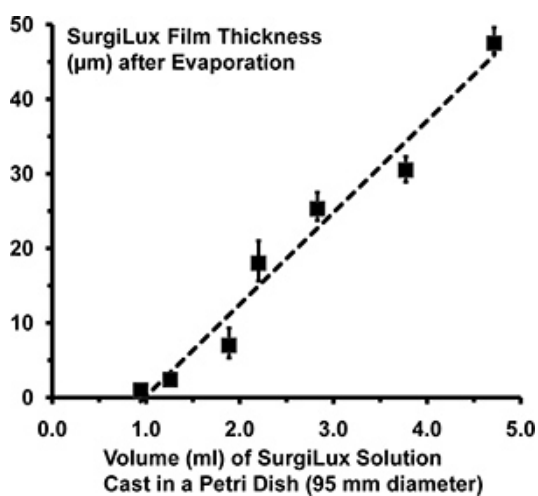


Figure 5. Graph showing the change in film thickness with increasing SurgiLux solution casting volume (ml) and a constant casting area ($7.09 \times 10^3 \text{ mm}^2$).

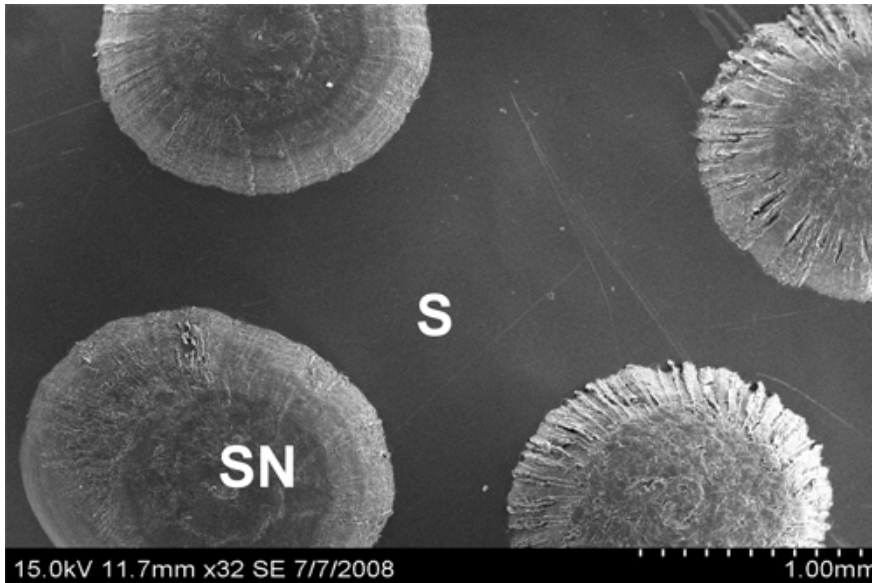


Figure 6. Scanning Electron Micrograph (SEM) of film showing the presence of 'nipples' (SN) protruding from the surface (S).

Discussion

Chitosan can be obtained in a variety of molecular weights and with different degrees of deacetylation (DDA). Variations in chitosan purity may lead to the presence of particulates in the SurgiLux solution; centrifugation is used to eliminate these and should result in a transparent green solution. However, filtration can also be used as an added or alternative fabrication step. As with any materials processing, variations, such as chitosan DDA and molecular weight, have implications for the physiochemical, biological and material properties of the resulting SurgiLux films, including the strength of its bonding to tissue.

The fabrication process for SurgiLux allows for considerable variation. For example, changes to the ratio of solution volume to casting surface area (ml:mm^2) can be utilized to adjust the film thickness. **Figure 5** shows a linear increase in the thickness of final SurgiLux films as the volume of solution poured into the Petri dish was increased. Similarly, modifications to the casting surface can be used to modify the film's surface morphology. **Figure 6** shows the presence of micron-sized 'nipples' on the surface of SurgiLux film. Such templating techniques can be utilized to produce various surfaces to improve tissue adhesion, prevent microbial cell attachment and promote tissue reintegration.¹¹ Furthermore, various biologically active agents can be incorporated into the fabrication process to produce an adhesive film for regional drug delivery.¹⁰

Table 1 summarizes the advantages of this SurgiLux thin film adhesive system compared to conventional fibrin and cyanoacrylate adhesives. While the strength of tissue repair is less than sutures, SurgiLux avoids the numerous disadvantages of this traditional wound closure technique as well as the current favored commercial surgical adhesives.

The ability of SurgiLux to bond various collagenous tissues combined with its material flexibility suggest its potential in laparoscopy, while the versatility of the fabrication process promotes its further development for applications in tissue engineering and regenerative medicine.

Disclosures

No conflicts of interest declared.

Acknowledgements

The authors acknowledge a grant from the National Health and Medical Research Council of Australia (NHMRC #1000674) to L.J.R. Foster.

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