3D-printed wound chambers: a novel splint system for wound healing research

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Healing in humans is driven by re-epithelialisation and granulation tissue formation. We know that rodents heal primarily through strong skin contraction (Chen et al, 2015), complicating its clinical translatability as a wound model. Splint systems have attempted to minimise wound contraction, but are associated with severe limitations, such as frailty, the inability to retain liquids, and high costs and weights. To overcome these drawbacks, we designed a 3D-printed polylactic acid (PLA) wound chamber. This chamber can inhibit wound contraction, retain liquids, be customised, and act as an *in vivo* incubator. Thus, this chamber can serve as a simple, cost-effective platform for *in vivo* wound studies.

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Skin and soft tissue wounds affect tens of millions of people and contribute to a conservative estimate of >$30 billion in US healthcare expenditure as a result of acute and chronic injuries, ulcers and infections (Nussbaum et al, 2018). These numbers have risen as a byproduct of the aging population alongside increases in comorbidities, such as obesity and diabetes (Sen et al, 2009). As a result, extensive research has been conducted to develop novel treatment options for surface wounds, with a common experimental avenue being *in vivo* studies.

However, one of the biggest challenges of using a murine model to study the pathophysiology of wound healing is that wound closure occurs through contraction forces in mice (Ren et al, 2012). In contrast, compromised human epithelium is repaired via the action of fibroblasts that secrete a collagenous matrix, leading to the formation of granulation tissue. This is then followed by re-epithelialisation of the wound space (Evans et al, 2013).

In mice, however, the contraction of the panniculus carnosus (a thin layer of striated muscle) is the primary mechanism of wound closure, which decreases the amount of granulation tissue formation and epidermal resurfacing. This discrepancy in the natural wound-healing process between the two species is a major limitation to the applicability of the mouse model in experiments pertaining to human wound repair. This is especially unfortunate as rodents are abundantly available, easy to handle and inexpensive, which would make them an excellent candidate otherwise (Chen et al, 1999).

It is for this reason that devices designed to inhibit skin contraction have been used in rodent wound models to closely replicate the human healing process. With varying levels of success, silicone rings, toothed metal rings and titanium chambers have demonstrated inhibition of wound contraction (Carlson et al, 2003; Galiano et al, 2004; Nuutila et al, 2016).

However, these interventions are either unable to retain therapeutic solutions, do not fully inhibit contraction, or pose a significant financial burden to researchers (Ren et al, 2012; Junker et al, 2013). To overcome this, the authors developed a 3D printable chamber made of polylactic acid (PLA), which effectively inhibits wound contraction and serves as an *in vivo* incubator for various treatments, sampling and gross observation.

Materials and methods

3D Printed Chambers

Computer-aided design (CAD) software

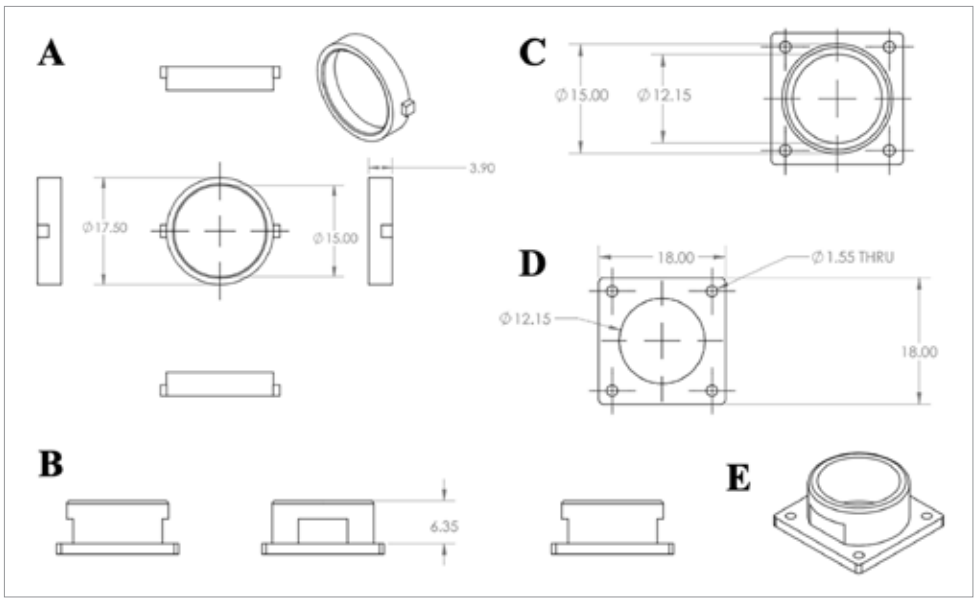

Figure 1. Renderings of the 3D-printed wound chamber. Depictions include measurements for the open cylinder, square base, and snap-on lid. Images represent (A) lid (bottom, side, quarter view), (B) chamber side view, (C) chamber top-down view, (D) chamber bottom-up view, and (E) chamber quarter angle view. All numbers are reported in millimeters (mm).

(Solidworks, Waltham, MA, US) was used to design the two-piece chamber set that consists of an open cylinder with a square base and a snap-on lid.

Structurally, the chamber has a square base that is 18 mm in length and 1.55 mm in thickness, which is connected to a cylinder with an inner diameter of 12.15 mm, an outer diameter of 15 mm and a height of 6.35 mm. The lid has an inner diameter of 15 mm and a height of 3.9 mm with two thumb tabs for easy manipulation. The chamber's outer diameter and the lid's inner diameter were both set to 15 mm to ensure a tight seal *[Figure 1]*. Four small holes in each corner of the base allow for suture placement. All the contacting surfaces on the chamber's outer lip and the lid's inner lip were recessed for ease of fit. The thermoplastic 3D printer settings were set to extrude PLA 0.06 mm in layer height with 100% infill out of a 0.4 mm nozzle. A chamber set was generated in less than 1.5 hours.

Animals and surgical procedure

All the experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of California, Irvine, and were consistent with federal guidelines. The experiment was aimed to assess the ease of use and functionality of the wound chamber. Four male Sprague Dawley rats (6–8 weeks old) (Charles River: Wilmington, MA, US) were anaesthetised using isoflurane and oxygen at 5% for induction and 2%–3% for maintenance of anaesthesia. The dorsa were shaved with electric hair clippers and depilatory cream was applied. The rats were transferred to a sterile field, cleaned with betadine and the dorsum of each rat was surgically draped. Two skin island wounds were created along the spine of each rat (one posterior and one anterior), following the procedure described by Nuutila et al (2016). A 4 mm biopsy punch (Robbins Instruments: Chatham, NJ, US) was used to imprint the skin and a full-thickness excisional wound was completed using iris scissors. Subsequently, a 10 mm full-thickness incision was created concentric to the excisional wound using a 10 mm biopsy punch and iris scissors.

Special caution was exercised so as to not undermine the tissue connecting the skin island and the underlying tissue. The PLA chambers were implanted under the skin using the previous 10 mm incision and were secured in place using four interrupted 4-0 nylon sutures. In four separate rats, titanium chambers were implanted following the same procedure. The wounds were irrigated with normal saline and secured with the lid. Each rat was implanted with two chambers. Thus, eight chambers of each material were assessed. The rats were then transferred to their respective cages and were observed until the anesthesia wore off. Fluids were aspirated daily and replaced with fresh saline. Over the course of 15 days, photographs were taken daily to record gross observations of contraction, re-epithelialisation and granulation tissue deposition.

Results

Chamber weight

The standard-sized (12.15 mm diameter) wound chamber has a square base that weights 0.77 grams and a cap that weights 0.54 grams.

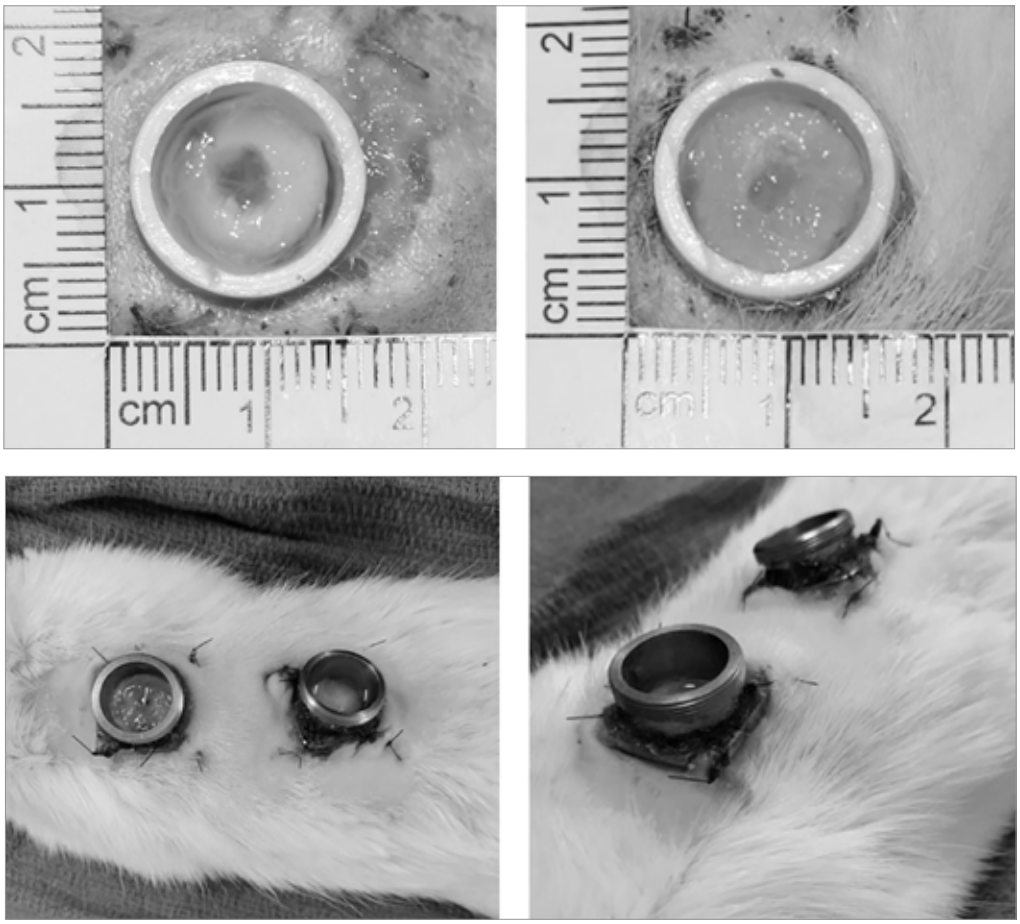

Figure 2. Gross observations of rat models. (A) Macroscopic images of rat wounds with PLA chambers. The images were taken of the wounds at 4 and 9 days for the upper left and right images, respectively. Wound contracture was inhibited, and epithelialization and granulation tissue formation were observed. (B) Representation of the dislodged titanium chambers used in a previous experiment (chamber caps not included). The images were from the same animal (12 days post-implantation) taken at different angles.

Once placed together, the entire splint system only weighs 1.31 grams. This is approximately 3x lighter than similarly sized titanium chambers (3.7 grams) (Nuutila et al, 2016).

Gross observations

In our study, the PLA chambers effectively prevented wound contraction and independent granulation tissue deposition and re-epithelialisation was observed *[Figure 2a]*. The chambers successfully held saline, maintained levels close to the original volume and preserved a moist environment with no adverse reactions. For the entire duration of the study, none of the PLA chambers dislodged from their respective wound sites, which was in contrast to the titanium chambers *[Figure 2b]*.

Discussion

Rodents provide the most practical model to study the pathophysiology of wound healing and how various pharmacologic agents affect the wound microenvironment. However, healing by wound contraction in these animals prohibits them from mimicking human wound healing by secondary intention. Thus, several strategies have been devised to support the clinical translatability of animal studies by allowing for mechanical fixation of the skin. Galiano et al (2004) described a method to minimise wound contraction where a silicone ring was placed on top of a circular wound and was secured with instant bonding adhesive and nylon sutures (Galiano et al, 2004). They observed independent granulation and epithelialisation.

Unfortunately, this approach had several drawbacks, such as the insubstantiality of the adhesive, the detachment of sutures due to the formation of dry eschar, and the difficulty of re-suturing as a result of partial wound contraction (Ren et al, 2012). The subcutaneous implantation of the rings, as demonstrated by Ren et al (2012), addresses the concerns of ring loss due to the motion and activity of animals. However, because of the frailty of the silicone rings, wound contraction is not completely inhibited.

An alternative option reported in the literature is metal rings that have toothed edges to allow for easy suture placement and a more secure adherence (Carlson et al, 2003). Like the silicone rings presented by Galiano et al (2004), these toothed rings inhibit wound contraction and successfully promote granulation tissue

in vivo (Carlson et al, 2003). However, neither the silicone rings nor the toothed metal rings successfully retain liquids, such as saline. This restricts wound care dressings to topical gel treatments and limits the clinical translatability of the model. Consequently, polyurethane chambers have been developed to retain liquids over the wound bed. These chambers contain an adhesive rim and an injection port, allowing for fluids to be added and removed while maintaining closure of the system (Junker et al, 2013). Yet, although these chambers retain liquids, they do not prevent wound contraction.

Recently, titanium chambers have gained popularity for their ability to fulfill all of the aforementioned criteria and act as an *in vivo* incubator, with minimal leakage of fluids (Junker et al, 2013; Kruse et al, 2018). These chambers are more secure than silicone rings and do not elicit a foreign body reaction. Moreover, their use has demonstrated deceleration in wound contraction and a simultaneous increase in re-epithelisation, compared to controls without titanium chambers (Nuutila et al, 2016).

In our study, the titanium chambers maintained a tight seal and retained fluids after implantation in the dorsum of our rodent models. However, over the duration of our study, the weight of the titanium ultimately forced the chambers to dislodge, which not only released the treatment fluids, but also damaged the granulation tissue *[Figure 2b]*. It is for this very reason that we sought to design wound chambers similar to the titanium models, albeit much lighter.

Our 3D-printed chambers are made of PLA, a biocompatible, biodegradable and eco-friendly polymer that has been used for diverse biomedical applications, including medical implants, orthopedic devices, tissue engineering and drug delivery (Singhvi et al, 2019). The non-toxic nature of PLA allows it to be implanted under the skin of mice, without generating an immune response. Our observations demonstrate an initial efficacy of 3D-printed PLA chambers in a rat model. The authors visualised granulation tissue deposition and re-epithelialisation, mimicking the benefits of other splinting techniques (Galiano et al, 2004; Nuutila et al, 2016; Kruse et al, 2018). In addition, the 3D-printed chambers weigh only a third of the titanium chambers, and as a result, are less likely to dislodge. This reduces the risk of damaging the wound site, inducing animal discomfort and compromising the study results.

Financial considerations

These 3D-printed chambers are also remarkably more affordable given that PLA materials can be purchased at $30.00 per kilogram. This means that 763 chambers can be created at a total cost of four cents per standard-sized (12.15 mm) chamber set. Because 3D printing technologies are becoming more accessible and ubiquitous in recent years, PLA-based 3D-printed chambers can be easily available to researchers everywhere at a low cost.

Wound chamber adaptability

3D printing technologies allow researchers to manufacture wound chambers in various shapes and sizes according to their experimental needs. Whether an experiment requires chambers to be suitable for various animals or wounds of different shapes and sizes, the dimensions of the chambers can be easily manipulated *[Figure 3]*. To do so, the blueprint model for the chambers can be adjusted using any CAD software, which is a simple task to learn. Customisations include altering the chamber diameter, width, height, the number of tactile grips on the lid cap, or even the number of suture holes. Although titanium chambers and other alternatives may include this adaptability, their high cost limits this benefit.

Potential uses

A mechanical chamber that covers the entirety of the wound is certainly a more secure option that enables experiments to be performed in a controlled, protected environment. This allows for researchers to evaluate the effects of growth factors, transplanted cells, and other bioactive materials on wound healing (Zhang et al, 2012). It

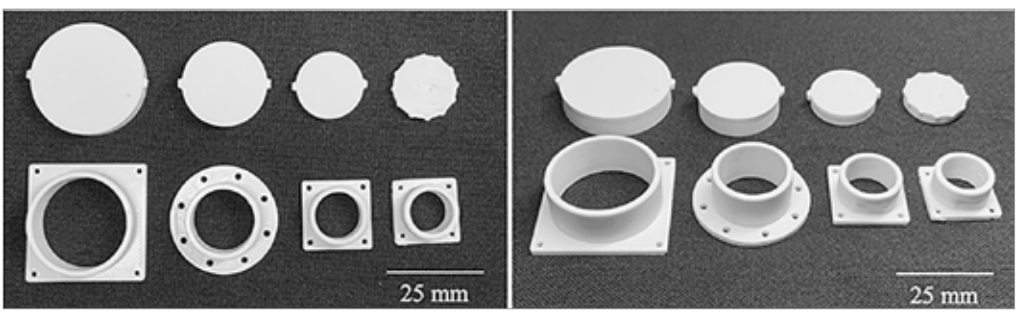

Figure 3. Images of 3D-printed chambers. The two images are different angles of the same chambers. The chambers from left to right are as follows: 22.5 mm-diameter chamber with a standard lid; 16 mm-diameter chamber with additional suture holes and a standard lid; 12.5 mm-diameter chamber with a standard lid; and 12.5 mm-diameter chamber with a modified lid.

also provides a means for studying the biological molecules that play a critical role in tissue repair by monitoring and sampling the wound microenvironment (Junker et al, 2013).

Moreover, the large aperture of the chamber allows for the introduction of a variety of experimental materials, including scaffolds, cells, micrografts, antibiotics, saline and oxygen loaded micro-nanobubbles into wound sites in order to augment healing and negate scarring (Kangesu et al, 1993; Zhan et al, 2015; Hartmann-Fritsch and Biedermann, 2019). These conditions not only allow for the study of novel techniques, but can further the translatability of the animal model by replicating clinical pathologies.

Conclusion

3D-printable PLA chambers serve a low-cost alternative platform for successfully inhibiting murine wound contraction and to allow a process that more accurately models human epithelial repair. The chambers are designed with a lightweight polymer that allows for secure subcutaneous installment with minimal dislodgement.

Moreover, the minimal production cost of approximately four cents makes them accessible to most institutions. This cost efficiency contributes to the reproducibility of the chambers, as CAD software allows the chambers to be adaptable to any study. Together, these benefits demonstrate that our chambers can be highly useful for *in vivo* wound studies in the future. WINT

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Ethical Principles

This manuscript is an original work and is being submitted only to Wounds International; it will not be submitted elsewhere while under consideration. Should it be published in Wounds International, it will not be published elsewhere, either in similar form or verbatim, without permission of the editor.

Conflict of interest

The authors have no conflicts of interest to disclose.

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