Photochemical Tissue Bonding of Amnion Allograft Membranes for Peripheral Nerve Repair: A Biomechanical Analysis

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Abstract

Background Photochemical tissue bonding (PTB) is a technique for peripheral nerve repair in which a collagenous membrane is bonded around approximated nerve ends. Studies using PTB with cryopreserved human amnion have shown promising results in a rat sciatic nerve transection model including a more rapid and complete return of function, larger axon size, and thicker myelination than suture repair. Commercial collagen membranes, such as dehydrated amnion allograft, are readily available, offer ease of storage, and have no risk of disease transmission or tissue rejection. However, the biomechanical properties of these membranes using PTB are currently unknown in comparison to PTB of cryopreserved human amnion and suture neurorrhaphy.

Methods Rat sciatic nerves ($n = 10$ per group) were transected and repaired using either suture neurorrhaphy or PTB with one of the following membranes: cryopreserved human amnion, monolayer human amnion allograft (crosslinked and noncrosslinked), trilayer human amnion/chorion allograft (crosslinked and noncrosslinked), or swine submucosa. Repaired nerves were subjected to mechanical testing.

Results During ultimate stress testing, the repair groups that withstood the greatest strain increases were suture neurorrhaphy ($69 \pm 14\%$), PTB with crosslinked trilayer amnion ($52 \pm 10\%$), and PTB with cryopreserved human amnion ($46 \pm 20\%$), although the differences between these groups were not statistically significant. Neurorrhaphy repairs had a maximum load (0.98 ± 0.30 N) significantly greater than all other repair groups except for noncrosslinked trilayer amnion (0.51 ± 0.27 N). During fatigue testing, all samples repaired with suture, or PTBs with either crosslinked or noncrosslinked trilayer amnion were able to withstand strain increases of at least 50%.

Conclusion PTB repairs with commercial noncrosslinked amnion allograft membranes can withstand physiological strain and have comparable performance to repairs with human amnion, which has demonstrated efficacy in vivo. These results indicate the need for further testing of these membranes using in vivo animal model repairs.

Keywords
► nerve
► photochemical tissue bonding
► amnion
► allograft
► neurorrhaphy

Peripheral nerve injuries cause an often-debilitating reduction in quality of life for patients and present a significant challenge for medical providers. Despite recent technological advancements, surgical repair via suture neurorrhaphy remains the gold standard of treatment. However, this is often unable to restore full motor and sensory function, contributing to negative physical, psychological, and socioeconomic outcomes.[1,2] Developing alternate techniques for nerve repair may enhance axonal regeneration, addressing this deficiency in treatment outcomes. One emerging technique is photochemical tissue bonding (PTB), in which photoactivated dye and light are used to induce collagen crosslinking between tissues in a nonthermal process, with demonstrated effect in the repair of peripheral nerves, as well as other tissues such as skin, blood vessels, tendons, and colon.[3–7] In PTB repair of transected nerves, amnion is wrapped around approximated nerve ends and then bonded to the nerve epineurium; in addition to creating a physical connection between nerve ends, the resulting seal has been shown to reduce perineural adhesions and is theorized to retain growth factors, prevent axonal escape from the neurorrhaphy, and exclude scar tissue formation.[7–9] Studies using animal models have demonstrated enhanced functional recovery between nerves repaired with PTB and suture neurorrhaphy, further supporting the use of this technique.[8,10]

Despite the successful use of PTB for in vivo nerve repairs, the biomechanical properties of these repairs have yet to be characterized. Moreover, de-epithelialized human amnion—which has been used for animal model studies of PTB—has several disadvantages for clinical use including difficulty of storage, low availability, and risk of infectious disease transmission. Dehydrated collagenous membranes from amnion with and without chorion, as well as those from nonplacental sources, may provide a viable alternative, but it is unknown what types of collagen membranes are most suitable for PTB nerve repair.

The aim of this study is to characterize the biomechanical properties of PTB nerve repairs using a variety of commercial collagen membranes from both placental and nonplacental sources and to compare their performance against cryopreserved human amnion, which has demonstrated efficacy in vivo.

Methods

Sciatic nerves were harvested from male Lewis rats and stored at −80°C until testing. For each experimental group ($n = 10$), nerves were transected and then repaired using one of the following methods: neurorrhaphy using six 10–0 epineurial stay sutures, PTB with human amnion, or PTB with one of five commercial collagen membranes. The composition of the five membranes was as follows: monolayer human amnion (AmnioExcel, Integra LifeSciences, Princeton, NJ), crosslinked monolayer amnion (Dryflex, Integra), tri-layer amnion–chorion–amnion (Amnio Excel Plus, Integra), crosslinked trilayer amnion–chorion–amnion (G3, Integra), and swine intestinal submucosa (Oasis, Smith and Nephew, London, United Kingdom). An additional group of nerves was not transected and utilized as a control group. Mechanical testing was performed on the nerves to characterize strain at

fracture, maximum load, and behavior under repeated strain.

Membrane Preparation

Human amnion was manually separated from human placenta, de-epithelialized, and stored in a solution of 50:50 Dulbecco's Modified Eagle Medium and glycerol with 1.5% antibiotic/antimycotic (Sigma A5955, Sigma Aldrich, St. Louis, MO) at −80°C. Commercial collagen membranes were rehydrated in Dulbecco's phosphate-buffered saline (Sigma Aldrich) for 60 seconds prior to use. All membranes were cut into rectangular pieces with dimensions 1 cm × 0.7 cm. Immediately prior to nerve repair, each piece of membrane was fully covered with 1 mg/mL Rose Bengal solution using a cotton-tipped applicator, then shielded from light for 4 minutes to allow the dye to saturate the membrane without premature photoactivation of the dye. Finally, any excess dye was removed from the membrane using a cotton-tipped applicator.

Photochemical Tissue Bonding

The sciatic nerve was thawed, transected using a scalpel, and the nerve ends approximated. The dyed membrane was wrapped around the nerve ends ensuring that (1) the 1-cm edge was lengthwise along the nerve, (2) there was a tight and uniform connection to the nerve surface, and (3) after wrapping the membrane had a 25 to 50% overlap with itself (any excess membrane was removed using a scalpel) (►**Fig. 1**). Finally, laser light with wavelength 532 nm and intensity 0.5 W/cm^2 was applied to the nerve surface for 60 seconds, a total of three times, with the nerve being rotated 120 degrees between each lasering (►**Fig. 1**). This process was repeated for all specimens and types of membranes.

Note that in vivo PTB repairs in prior studies have used two stay sutures to approximate the nerve ends before the membrane is wrapped around the nerve ends; however, no stay sutures were used in these in vitro repairs to characterize the PTB repair properties in isolation.

Suture Neurorrhaphy

In the euthanized rat, the sciatic nerve was transected using a scalpel and the nerve ends approximated. Six evenly spaced 10–0 nylon sutures (Ethicon, Cincinnati, OH) were used to perform a standard epineurial neurorrhaphy. The nerve was then excised from the rat carcass. These repairs were performed by a general surgeon with training in microsurgery. The nerves were frozen at −80°C until day of testing.

Mechanical Testing

To prevent premature fracture of the nerve samples by the tensiometer clamps, a scaffold was created to hold each nerve during mechanical testing. After testing several scaffolds, it was found that the following design was able to withstand the highest load without causing rupture at the nerve–scaffold interface, thereby permitting an accurate measurement of the strength of the nerve repair: 4–0 silk suture (Ethicon) was passed through each nerve end and tied in place, and then a drop of cyanoacrylate adhesive (Elmer's,
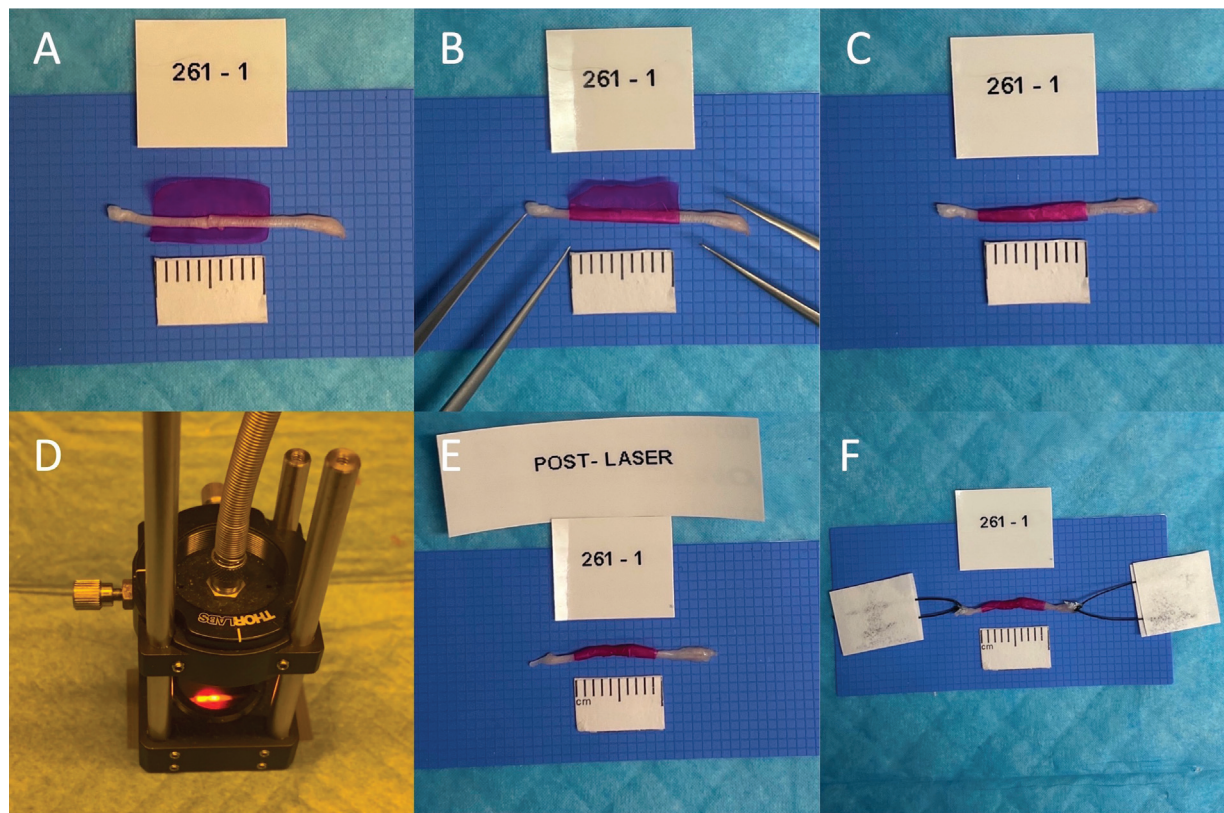
Fig. 1 (**A–C**) Nerve repair using photochemical tissue bonding of collagenous wraps. Membranes are saturated in 0.1% Rose Bengal dye, then wrapped around approximated nerve ends ensuring a tight and even connection to the nerve surface. If a membrane has more than 25 to 50% overlap with itself, then excess membrane is excised. (**D**) Laser light with wavelength 532 nm and intensity 0.5 W/cm² is applied to the nerve surface for 60 seconds, a total of three times, with the nerve being rotated 120 degrees between each lasering. (**E**) The membrane may darken in color after lasering. (**F**) A scaffold is created to hold the nerve in the tensiometer clamps using 4–0 silk suture tied at the nerve ends and with a drop of glue placed on the knot.

Columbus, OH) was placed on the knot (►**Fig. 1**). The suture ends were glued to pieces of paper that could be placed directly in the tensiometer clamps.

The cross-sectional area of each nerve repair was estimated as the product of the width and thickness of each nerve measured at the site of repair using calipers. The length of each nerve was measured as the distance between insertion points into the silk suture scaffold.

Data from the tensiometer (ADMET, Norwood, MA) was analyzed in RStudio (Posit, Boston, MA). Statistical tests were performed in RStudio.

Linear Strain Testing

Half of each repair group ($n = 5$ nerves) was subjected to linear strain testing. Each nerve was pulled apart at a constant rate of 2 mm/min, with the load and position recorded by the tensiometer. The strain at fracture was taken to be the strain at maximum load.

Fatigue Testing

The other half of each repair group ($n = 5$ nerves) was subjected to stepped fatigue testing meant to mimic the repeated strain experienced by a nerve in vivo. Each nerve was pulled out to a 10% strain increase at 6 mm/min and held for 30 seconds, returned to its original length at 6 mm/min

and held for 15 seconds, pulled out again to 10% strain at 6 mm/min and held for 30 seconds, then returned to its original length at 6 mm/min and held for 15 seconds. This cycle was repeated for strain increases of 20, 30, 40, 50, and 60%, after which testing was stopped. Fracture was considered to be the point where upon re-extension to the same or greater strain the nerve demonstrated a 50% or greater reduction in load. A Kimtech wipe dampened with phosphate-buffered saline was draped over the nerve during testing to prevent its premature dehydration.

Results

Linear Strain Testing

Upon a linear pull to fracture, suture-repaired nerves were able to withstand the highest average strain, or relative increase in length, before fracture ($69 \pm 14\%$, comparable to native nerves) (►**Fig. 2A**). Of the PTB-repaired nerves, those using crosslinked trilayer amnion (G3) withstood the highest strain ($52 \pm 10\%$), followed by cryopreserved human amnion ($46 \pm 20\%$) and monolayer allograft amnion ($44 \pm 10\%$, AmnioExcel). The only statistically significant difference between groups was that suture neurorrhaphy repairs had significantly higher strains at failure than trilayer amnion ($35 \pm 3\%$, AmnioExcel Plus) and submucosa repairs
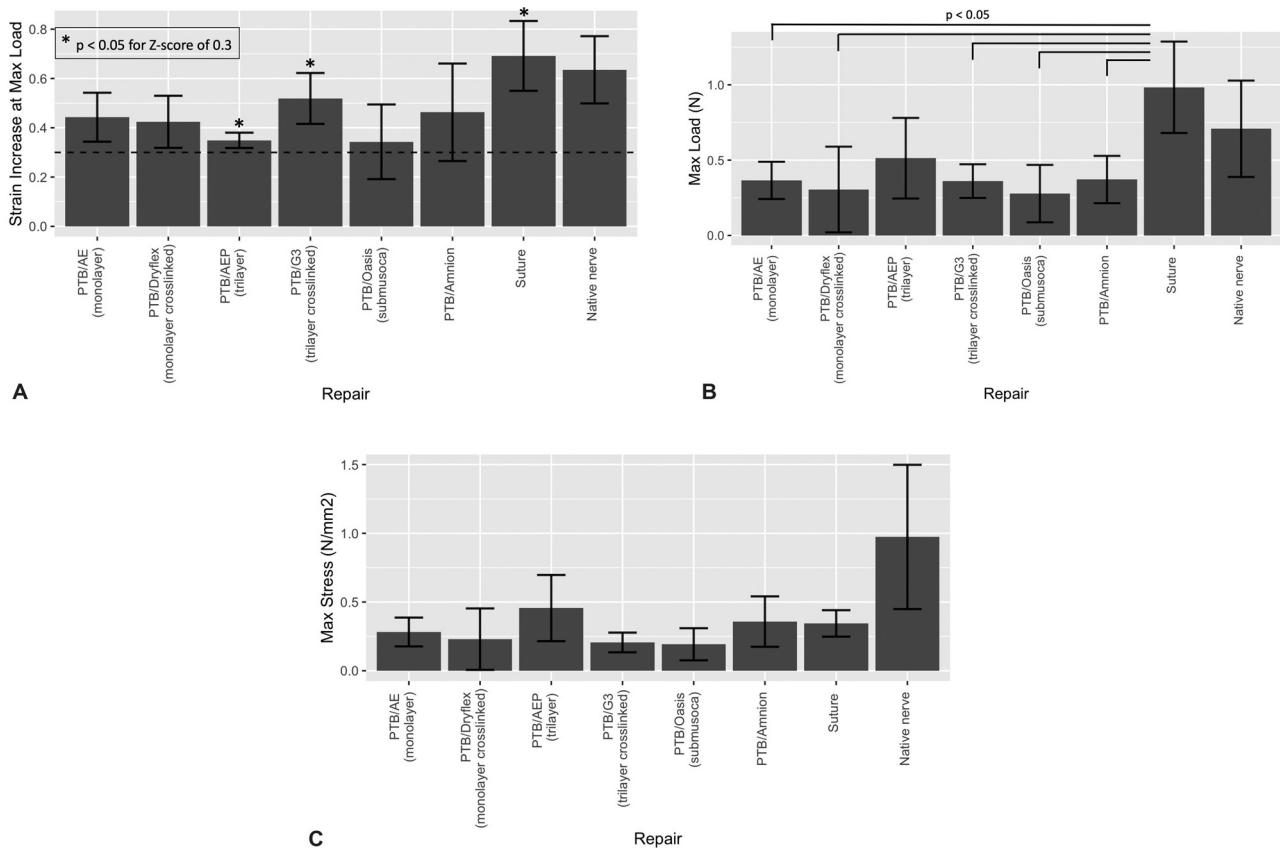
Fig. 2 (**A**) Linear strain testing: average increase in strain at fracture by repair group, with standard deviations shown in brackets. Samples were loaded into the tensiometer and pulled apart at 2 mm/min until fracture. The horizontal dotted line at 0.3 represents maximum physiological strain increase of 30%.[11] *Of the repair groups, suture (69 ± 14%), PTB with trilayer amnion (35 ± 3%, AmnioExcel Plus), and PTB with crosslinked trilayer amnion (52 ± 10%, G3) had strain increases significantly higher than 30% ($p < 0.05$ for Z-score of 0.3 in the log-transformed normal distribution). (**B**) Linear strain testing: average maximum load by repair group, with standard deviations shown in brackets. Suture repairs had a significantly higher maximum load than all other repair groups except for trilayer amnion (AmnioExcel Plus) ($p < 0.05$ for *t*-tests with Bonferroni correction). (**C**) Linear strain testing: average maximum stress by repair group, with standard deviations shown in brackets. There were no statistically significant differences between repair groups. PTB, photochemical tissue bonding.

(34 ± 15%, Oasis) ($p < 0.05$ for ANOVA [analysis of variance] and *t*-tests with Bonferroni correction).

Previous studies have determined that the maximum strain a nerve experiences in vivo is ∼30%, experienced by the ulnar nerve during elbow flexion.[11] Nerves repaired with both trilayer amnion membranes (AmnioExcel Plus and G3) and suture had a strain at fracture significantly higher than 30% ($p < 0.05$ for log-transformed normal distribution with Z-score of 0.3).

Linear strain testing also revealed that of all repair types, those with suture had the highest maximum load before failure (0.98 ± 0.30N). Of the PTB repairs, those with trilayer amnion (AmnioExcel Plus) withstood the highest load (0.51 ± 0.27N), while the other PTB repairs ranged from 0.28 to 0.37N (►**Fig. 2B**). Suture repairs were able to withstand significantly higher loads than all other repair groups except trilayer amnion.

It is worth noting that the suture neurorrhaphy repair sites had a greater cross-sectional area than the PTB repair sites due to bunching of the epineurium or perineurium during suturing. Therefore when normalizing maximum load by cross-sectional area of the repair sites – in other

words, calculating maximum stress – the significant differences between repair groups disappear (►**Fig. 2C**).

Fatigue Testing

To mimic in vivo conditions nerves were repeatedly pulled apart and then returned to baseline, starting at 10% strain and increasing by steps of 10% up to 60% strain. The results of this fatigue testing are shown in ►**Fig. 3A**.

All repairs with suture were able to withstand full fatigue testing up to 60% strain, and all repairs with noncrosslinked monolayer amnion (AmnioExcel) and trilayer amnion (AmnioExcel Plus) were able to withstand testing at least up to 50% strain. Of the other groups, only crosslinked trilayer amnion (G3) had a sample that fractured before 30% strain.

To estimate the force applied at the repair site as the nerve is stretched, the elastic modulus was calculated for each sample (►**Fig. 3B**). This was done by finding the slope of the linear section of the stress-strain curve for the first 30% strain step during fatigue testing. The elastic modulus could not be calculated for one G3 sample that fractured at 20% strain increase. Native nerve had a significantly larger elastic modulus (7.83 ± 3.07 MPa) than all repair groups ($p < 0.05$ for
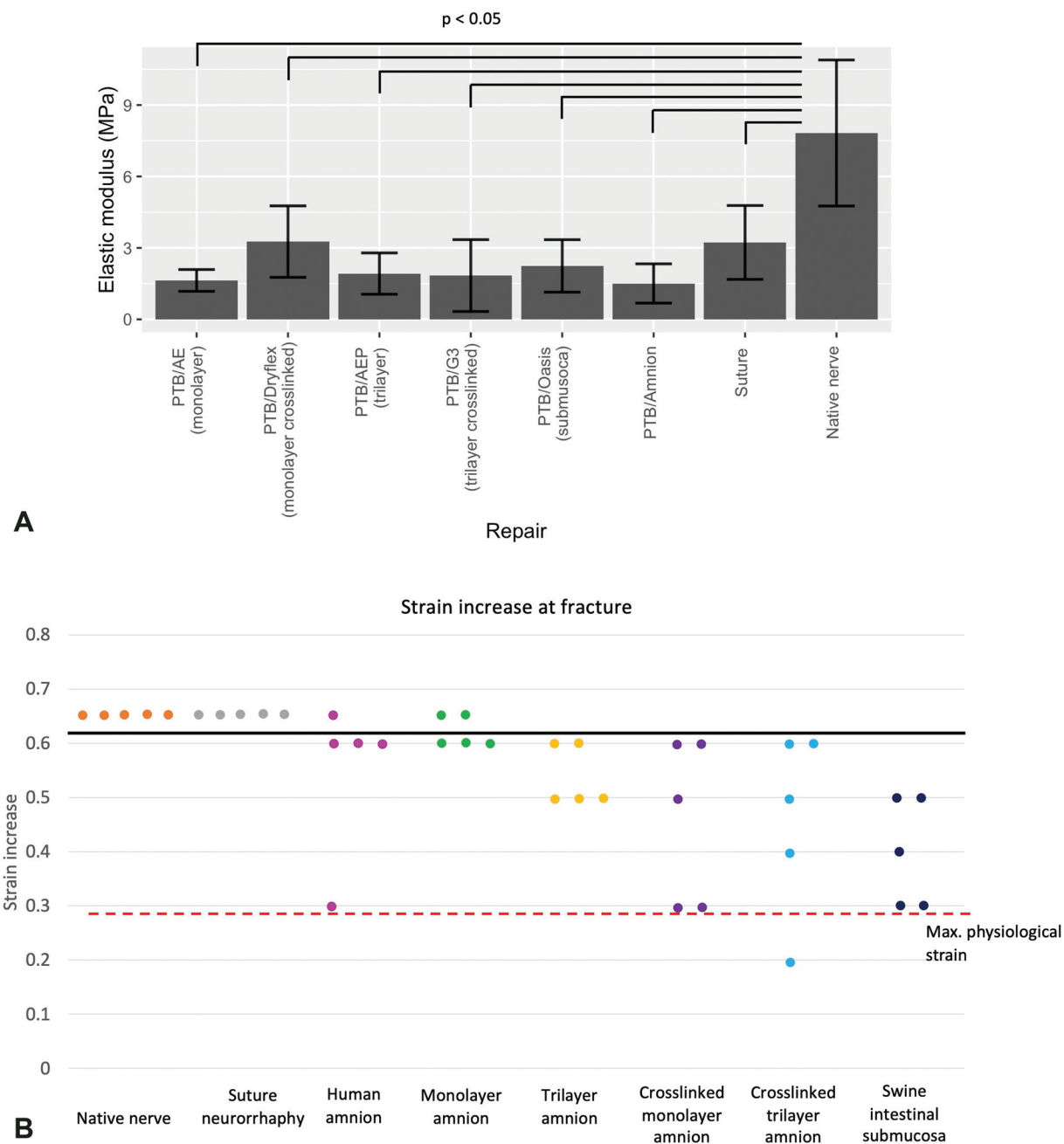
Fig. 3 (A) Fatigue testing: strain increase of samples at fracture, by repair group. Samples were loaded into the tensiometer and pulled apart at 6 mm/min to strain increases of 10, 20, 30, 40, 50, and 60% (twice for each step, and returned to baseline between each increase). The plot shows the strains at which samples fractured, organized by repair group. The red line shows maximum physiological strain increase of 30%, and the horizontal black line shows the 60% strain increase at which testing was stopped. (B) Fatigue testing: average elastic modulus at first 30% strain increase by repair group, with standard deviations in brackets. Native nerve had a significantly higher modulus than all repair groups ($p < 0.05$ for ANOVA and t-test with Bonferroni correction). There were no significant differences between PTB repair groups. PTB, photochemical tissue bonding.

ANOVA and t-test with Bonferroni correction), out of which crosslinked monolayer amnion (3.26 ± 1.50 MPa, Dryflex) and suture neurorrhaphy (3.23 ± 1.55 MPa) repairs had the largest modulus. Repairs with cryopreserved human amnion (1.51 ± 0.82 MPa) and monolayer allograft amnion (1.63 ± 0.46 MPa, AmnioExcel) had the lowest modulus, indicating that repairs with these membranes experienced the smallest increase in force as the nerve was stretched. However, these differences in elastic modulus between repair groups were not statistically significant. It is worth noting that repair sites with suture neurorrhaphy were wider than PTB repair sites due to bunching of the epineurium or perineurium during the repair process; therefore, the true elastic modulus of nerve repaired with suture may be higher than calculated here.

Discussion

One of the goals of this study was to compare the mechanical properties of PTB nerve repairs using a variety of collagenous membranes, with the goal of understanding what properties make a collagenous membrane ideal for use in PTB nerve repair. All PTB repairs failed via the nerve "slipping out" of the membrane wrap rather than the membrane wrap tearing in half or unravelling, indicating that the limiting factor in the strength of these repairs is the amount of collagen crosslinking between the membrane and epineurium rather than the intrinsic strength of the membrane. The main factors required for effective bonding between a membrane and epineurium are (1) ability of a membrane to contour to irregularities in the epineurium surface, (2) ability of light to penetrate through the membrane to the epineurium surface to induce collagen crosslinking, and (3) collagen composition that allows for a high number of covalent bonds to form.

The mechanical testing results suggest that whether an allograft amnion membrane is trilayer or monolayer has minimal impact on the behavior of its PTB repairs. Linear strain testing showed no statistically significant differences in maximum strain, load, or stress between allograft repair groups (►Fig. 2). Likewise, fatigue testing showed no significant differences between membranes on the basis of being monolayer or trilayer (►Fig. 3A). It was hypothesized that trilayer amnion membranes may not permit sufficient light to reach the epineurium–membrane interface, or that their stiffness may prevent them from contouring to variations in the epineurium surface; however, it appears that neither of these factors significantly altered the strength of trilayer amnion repairs.

In contrast, the mechanical testing results suggest that prior crosslinking of an allograft amnion membrane negatively effects the resulting PTB repair. During fatigue testing, all samples in the two noncrosslinked allograft groups fractured at strains of 50% or higher, while the two crosslinked allograft groups contained samples that fractured at strains of 30% or lower (►Fig. 3A). Crosslinking has been shown to increase the stiffness of collagen films, and it is possible that the crosslinked membranes were less able to adapt to variations in the epineurial surface, leading to decreased crosslinking during PTB.[12,13] These stiffer membranes may also have experienced higher stress on the membrane–epineurium interface, which became apparent during cyclical fatigue testing. Lastly, the chemical crosslinking of these membranes during production may have decreased the number of sites available for subsequent crosslinking during PTB.

The intestinal submucosa (Oasis) membrane also performed worse than the noncrosslinked amnion groups during fatigue testing, as the samples fractured on average at lower strain (►Fig. 3A). This may be due to differences in collagen composition between submucosa and amnion, but further studies are needed to confirm this.

Another goal of this study was to compare PTB repairs with commercially available collagenous membranes to those with cryopreserved human amnion, as human amnion has demonstrated efficacy in previous animal model studies.[10] Both the linear strain and fatigue testing results suggest that noncrosslinked allograft amnion membranes and cryopreserved human amnion are able to withstand comparable load and strain (►Figs. 2 and 3A). These results suggest that further studies on the performance of these allograft membranes in in vivo animal model repairs are warranted.

This study also included a suture neurorrhaphy repair group for comparison with PTB repairs. It is important to note that these in vitro PTB repairs did not use stay sutures to approximate nerve ends as have been done in vivo, and that therefore the PTB repairs in this study are likely less robust than their in vivo counterparts. Linear strain testing showed that suture repairs tended to have higher maximum strain and load than PTB repairs (►Fig. 2). Notably, differences in maximum load disappeared when normalized by the cross-sectional area of the repair site (►Fig. 2C), but this was likely due in part to an overestimation of the cross-sectional area of the suture repair group from bunching of epineurium and bulging of the endoneurial contents during neurorrhaphy and does not represent a true equalization of stress between repairs. Suture also performed well during fatigue testing, with all repairs able to withstand strain increases of 60% (►Fig. 3A). These results indicate that suture repairs are likely able to withstand higher load and strain than PTB repairs without stay sutures. However, the fact that PTB repairs with several membranes were able to withstand higher-than-physiological strain makes it unclear whether the greater strength of neurorrhaphy repairs provides additional benefit in vivo. This is further supported by the fact that in vivo PTB repairs using stay sutures have had better outcomes than suture repairs.[8,10] Furthermore, studies suggest that transected nerves regain significant strength in as little as 1 week after primary repair.[14] Although further studies would be needed, it is not unreasonable to expect that the difference in strength between PTB and suture-repaired nerves would diminish over time, especially given that PTB repairs are able to accelerate regeneration.[10,15] Another consideration in regard to the interpretation of the biomechanical properties of the PTB repairs is where the strain is placed along the nerve during stressing of suture and PTB repairs. In suture repairs, the nerve directly attached to either side of the repair site (<1 mm) is stretched when the entire nerve is stretched. In contrast, in PTB repairs the length of nerve covered by the 1-cm membrane (e.g., the repair site) is only stretched to the degree permitted by the (presumably stiffer) membrane wrap. This preserves a relatively constant amount of tension, or lack thereof, at the repair site, to the level determined during the initial repair. Studies suggest that tensionless repairs generally have superior outcomes, and PTB may be able to ensure a low-tension environment for the site of axon regeneration despite stretch of the nerve as a whole.[16–18]

This study has several limitations. One limitation of the mechanical testing setup is that the fracture load of the tensiometer scaffold is lower than the fracture load for a native (unaltered) nerve. Therefore, the strain and load at failure for

native nerves likely represent a failure of the nerve–scaffold interface rather than a true fracture of the nerve (►**Fig. 2A, B**). However, all PTB-repaired nerves fractured at the site of repair; this was confirmed visually. Another limitation of this study is that it did not detect gapping between nerve ends, which occurs before absolute failure of a repair and likely has a negative effect on axon regeneration.[14,19] It was not possible to directly observe gapping in PTB repairs because membrane wraps covered the repair site.

Conclusion

In conclusion, the results of this study suggest that PTB nerve repairs with noncrosslinked amnion allograft membranes can withstand physiological strain and have comparable performance to repairs with cryopreserved human amnion, which have demonstrated efficacy in vivo. Although suture repairs withstood higher strain and load than PTB repairs, the strength of PTB repairs for the majority of groups tested demonstrated a range which would withstand normal physiologic stress. These results suggest that PTB repair with amnion allograft membranes merits in vivo animal model study to assess its effects on nerve regeneration.

References

1 Lad SP, Nathan JK, Schubert RD, Boakye M. Trends in median, ulnar, radial, and brachioplexus nerve injuries in the United States. Neurosurgery 2010;66(05):953–960

2 Stonner MM, Mackinnon SE, Kaskutas V. Predictors of functional outcome after peripheral nerve injury and compression. J Hand Ther 2021;34(03):369–375

3 Kamegaya Y, Farinelli WA, Vila Echague AV, et al. Evaluation of photochemical tissue bonding for closure of skin incisions and excisions. Lasers Surg Med 2005;37(04):264–270

4 O'Neill AC, Winograd JM, Zeballos JL, et al. Microvascular anastomosis using a photochemical tissue bonding technique. Lasers Surg Med 2007;39(09):716–722

5 Ding B, Wang X, Yao M. Photochemical tissue bonding technique for improving healing of hand tendon injury. Surg Innov 2019;26(02):153–161

6 Scott BB, Wang Y, Wu RC, Randolph MA, Redmond RW. Light-activated photosealing with human amniotic membrane strengthens bowel anastomosis in a hypotensive, trauma-relevant swine model. Lasers Surg Med 2022;54(03):407–417

7 Fairbairn NG, Ng-Glazier J, Meppelink AM, et al. Light-activated sealing of acellular nerve allografts following nerve gap injury. J Reconstr Microsurg 2016;32(06):421–430

8 Johnson TS, O'Neill AC, Motarjem PM, et al. Photochemical tissue bonding: a promising technique for peripheral nerve repair. J Surg Res 2007;143(02):224–229

9 Kim SS, Sohn SK, Lee KY, Lee MJ, Roh MS, Kim CH. Use of human amniotic membrane wrap in reducing perineural adhesions in a rabbit model of ulnar nerve neurorrhaphy. J Hand Surg Eur Vol 2010;35(03):214–219

10 Fairbairn NG, Ng-Glazier J, Meppelink AM, Randolph MA, Winograd JM, Redmond RW. Improving outcomes in immediate and delayed nerve grafting of peripheral nerve gaps using light-activated sealing of neurorrhaphy sites with human amnion wraps. Plast Reconstr Surg 2016;137(03):887–895

11 Topp KS, Boyd BS. Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. Phys Ther 2006;86(01):92–109

12 Grover CN, Gwynne JH, Pugh N, et al. Crosslinking and composition influence the surface properties, mechanical stiffness and cell reactivity of collagen-based films. Acta Biomater 2012;8(08):3080–3090

13 Kang AH, Gross J. Relationship between the intra and intermolecular cross-links of collagen. Proc Natl Acad Sci U S A 1970;67(03):1307–1314

14 Temple CL, Ross DC, Dunning CE, Johnson JA, King GJ. Tensile strength of healing peripheral nerves. J Reconstr Microsurg 2003;19(07):483–488

15 Scott BB, Wu RC, Nietlispach V, Randolph MA, Redmond RW. A photosealed cap prevents disorganized axonal regeneration and neuroma following nerve transection in rats. Plast Reconstr Surg Glob Open 2022;10(03):e4168

16 Flores AJ, Lavernia CJ, Owens PW. Anatomy and physiology of peripheral nerve injury and repair. Am J Orthop 2000;29(03):167–173

17 M F G, M M, S H, Khan WS. Peripheral nerve injury: principles for repair and regeneration. Open Orthop J 2014;8:199–203

18 Millesi H. Reappraisal of nerve repair. Surg Clin North Am 1981;61(02):321–340

19 Temple CLF, Ross DC, Dunning CE, Johnson JA. Resistance to disruption and gapping of peripheral nerve repairs: an in vitro biomechanical assessment of techniques. J Reconstr Microsurg 2004;20(08):645–650