Laser Cutting as a Method of Creating Perforations in Engineering a Regenerative Biomaterial for the Periodontal Ligament

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INTRODUCTION

Significance & Background

- Periodontitis is a common (~70% of US adults 65+ [1,2]) and progressive disease that leads to destruction of the periodontal ligament (PDL) and eventual tooth loss
- The PDL is important for mechanical stability, absorbing forces associated with mastication, and providing sensory input
- PDL structure is composed of parallel 30-50 μm thick fibers spaced about 50-100 μm apart [1,3]
- Current therapies focus on stopping the progression of periodontitis rather than the regeneration of the tissues
- A biomaterial scaffold offers the ability to promote regeneration of the PDL [1,3,4]
- Laser cutting previously done for a different application [5]

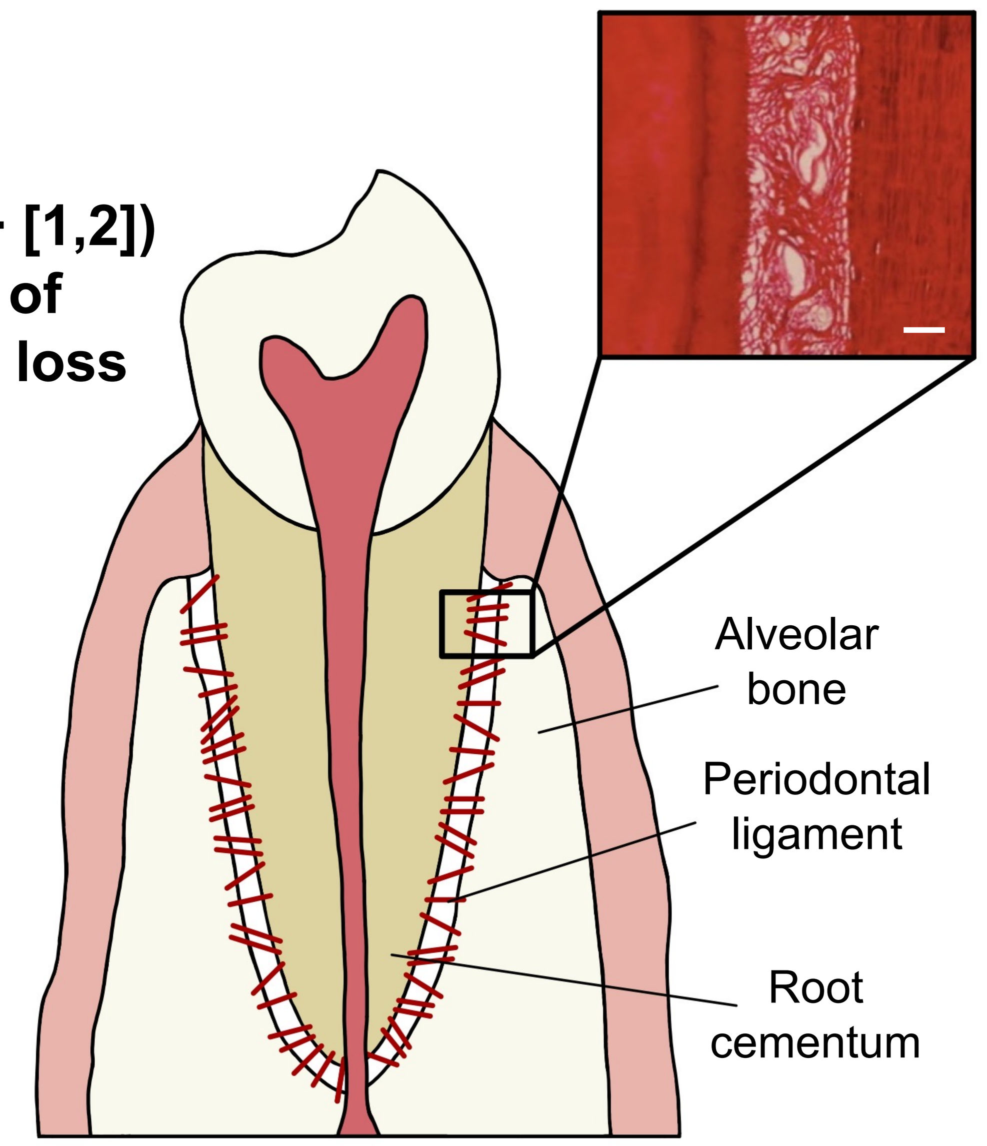


Fig. 1: Periodontium (Microscopy image adapted from [3]. Scale: 100 μm).

Objective & Hypothesis

- Creating perforations in the scaffold to increase the accuracy of the biomimetic design; perforations would recapitulate the spacing and fiber bundles in the native PDL region

MATERIALS & METHODS

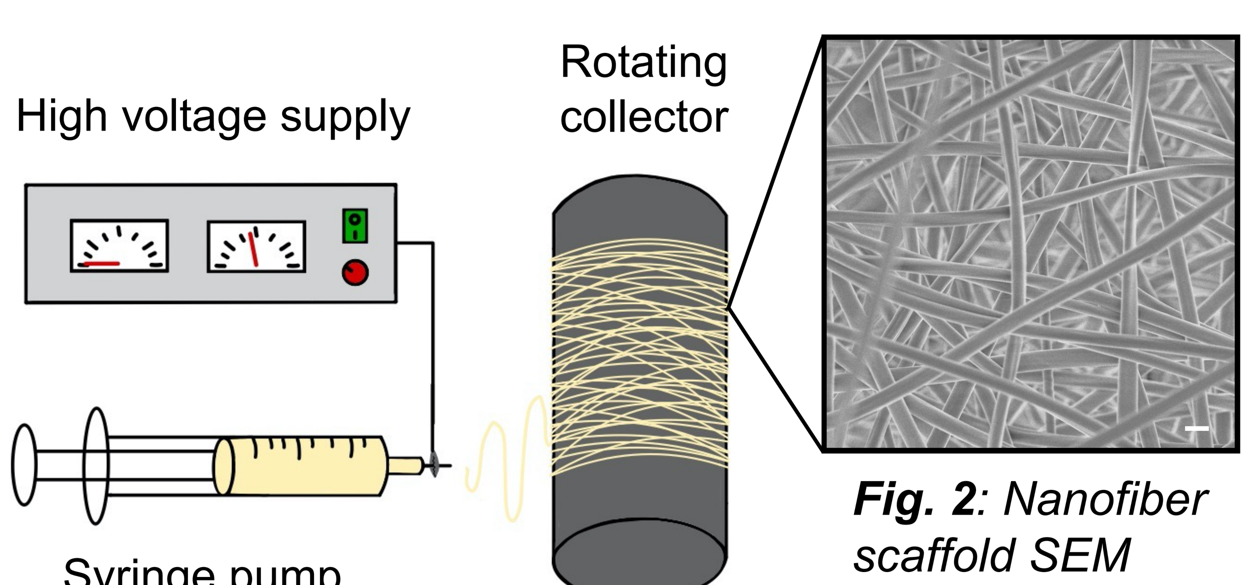
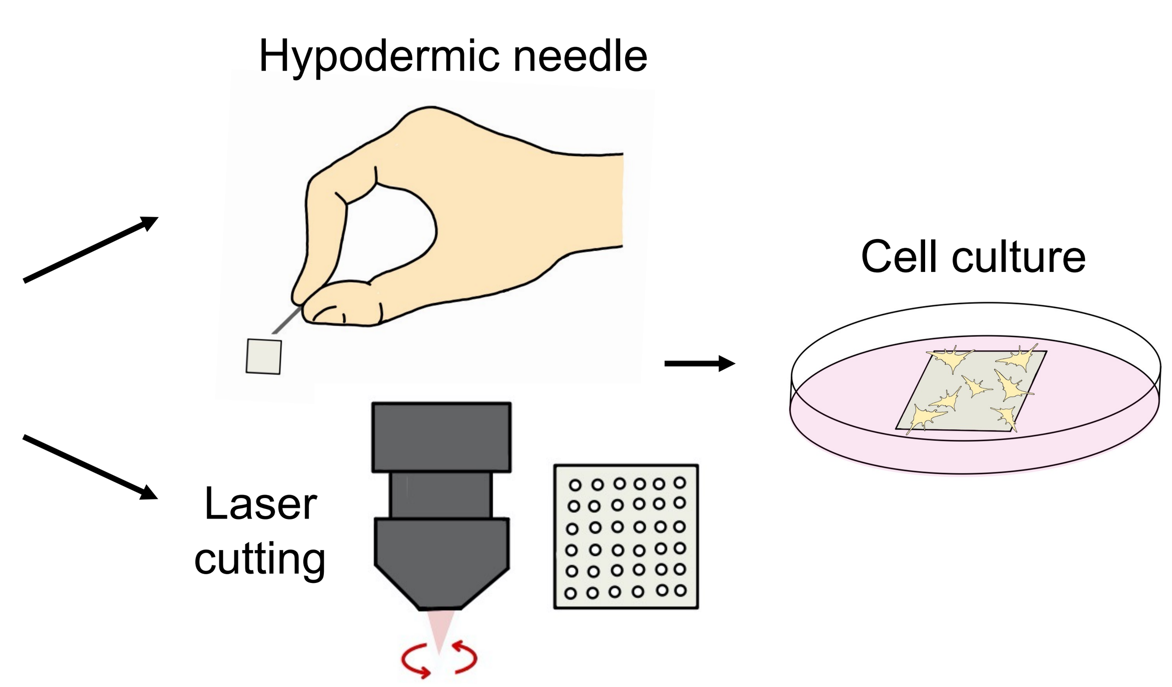


Fig. 2: Nanofiber scaffold SEM (Scale: 1 μm).



Nanofiber Scaffold Fabrication

- Nanofibrous scaffolds were created by using an electrospun gelatin mix in an unaligned fiber orientation
- Gelatin scaffold functions as ECM analog [4]

Hypodermic Needle Perforations

- Created perforations by hand via hypodermic needle

Laser Cutting Perforations

- Created perforations via laser cutting on an acrylic backboard on settings of power: 20% and speed: 100%
- AutoCAD to design template: 200 μm diameter and 400 μm center to center

Cell Culture

- PDL fibroblasts (passage 4) cultured at 50,000 cells/cm^2

RESULTS

Perforation Method

- Hypodermic needle creates tears versus actual holes with irregular sizing (Fig 3a,b; Fig 4)
- Laser cutting allows for much greater control of size and spacing (Fig 4)

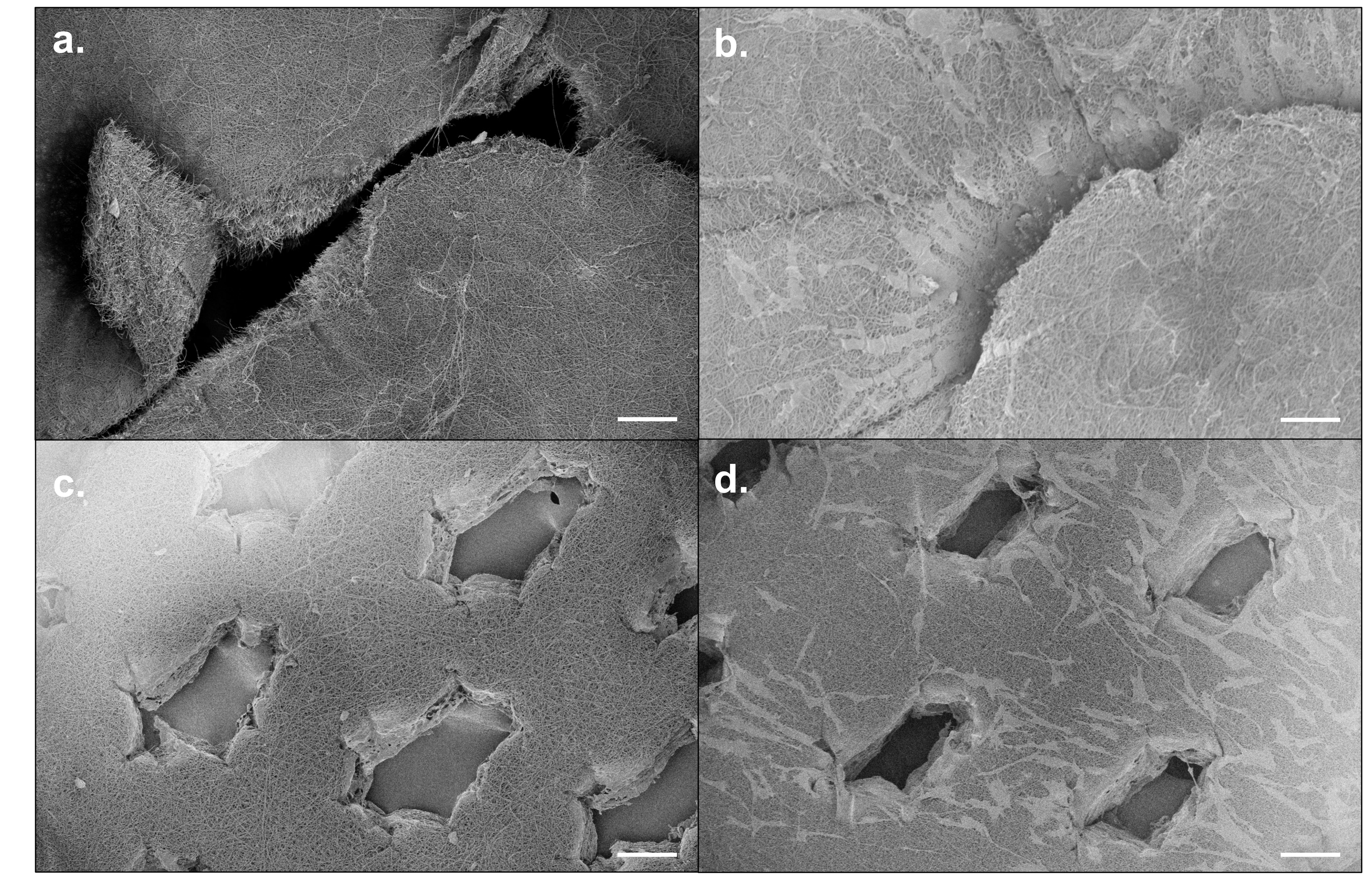


*Fig. 3: SEM imaging of perforations pre- and post-cell culture. **a/b.** 18G hypodermic needle. **c/d.** Laser cut (200 μm diameter). Scale: 100 μm.*

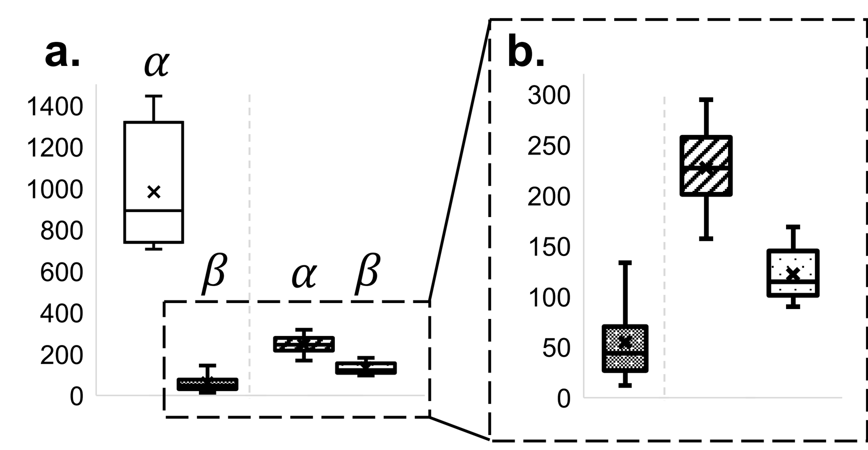


*Fig. 4: **a.** Range of perforation sizes in μm; left: 26G, right: laser cut (α = major axis, β = minor axis). **b.** Zoomed-in view of boxed region.*

Cellular Response to Perforated Scaffold

- Greater cell density surrounding perforations (Fig 3b,d; Fig 5a)
- Presence of perforations and seeding method have no effect on cell viability (Fig 5b)

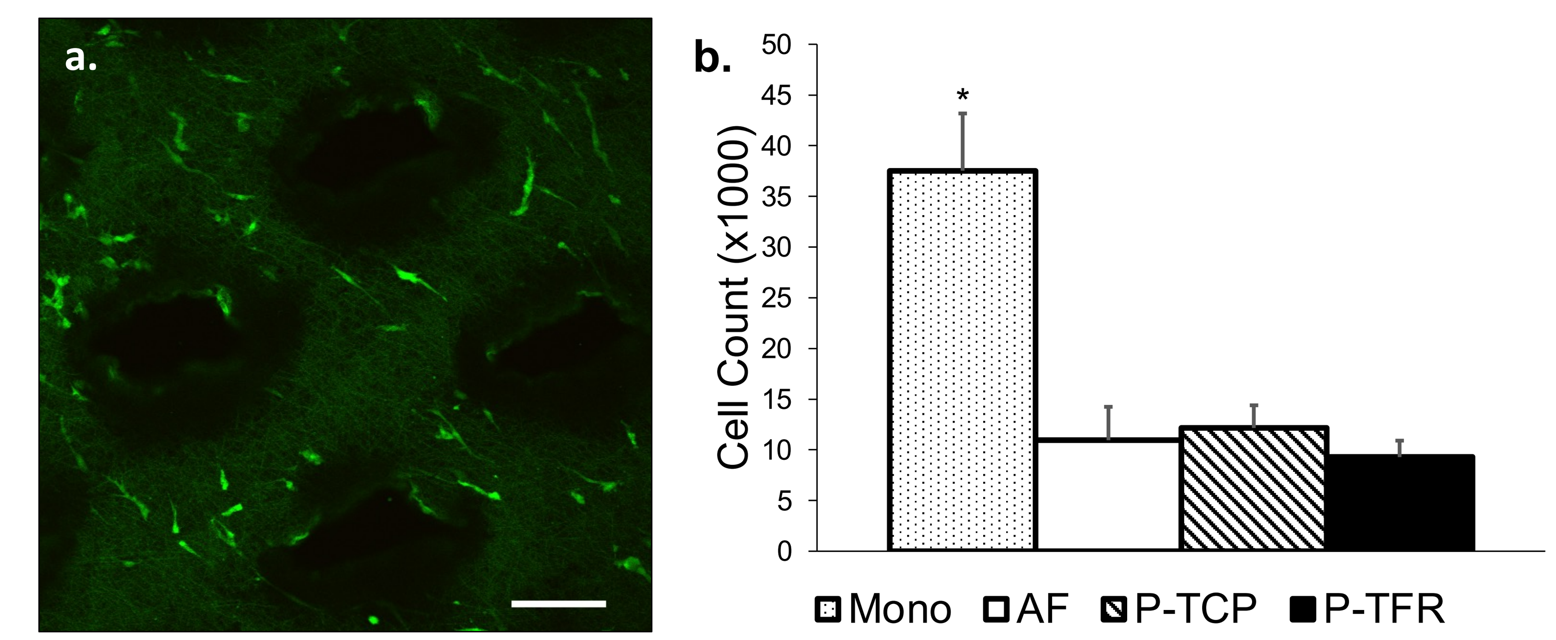


*Fig. 5: **a.** Live/Dead fluorescence of perforation localized PDL fibroblasts (Scale: 200 μm). **b.** Cell proliferation measured by dsDNA (*=p<0.05, AF = as fabricated, P-TCP = perforated and seeded on tissue-culture treated plastic (TCP), P-TFR = perforated and seeded on non-TCP).*

DISCUSSION & CONCLUSIONS

- Hypodermic needle is inconsistent with hole sizes and creates tears as opposed to actual perforations
- Results suggest laser cutting as a reasonable way to introduce perforations due to the ability for control and uniformity of perforation diameters and spacings
  - Method presents no apparent impact on cell proliferation
  - Cells localize around perforations
- Future studies to experiment with more laser cutting parameters (diameters, spacings, point cutting versus circle cutting)
- In the long term, incorporate into an engineered triphasic scaffold that is biomimetic of the periodontium

REFERENCES [1] T. de Jong, et al. J Pdl Rsch 2017; [2] P.I Eke, et al. CDC 2012; [3] N.M Lee, ProQuest 2017; [4] H.N Woo, et al. Bio Matl 2021; (6):3328-3342; [5] B. Kong, et al. Sci Rep 2017

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