Preparing the paper scaffolds

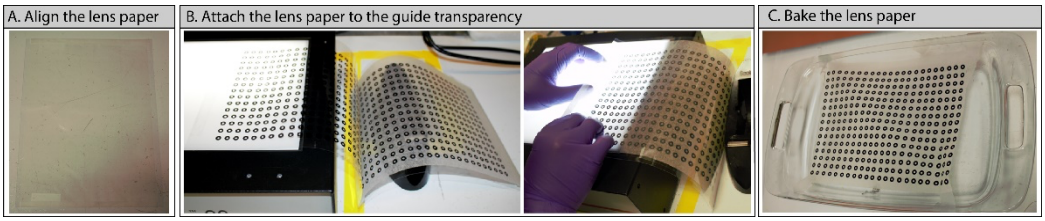

Figure 1. Pictorial overview of the scaffold-making process. A) First, a sheet of 105 Grade lens tissue is A) aligned and taped to a transparency. After printing the pattern of interest to the paper, B) the printed paper is attached to a new transparency in which the mirrored image of the pattern was printed. We align and tape the transparency and lens tissue together with a bend to mimic the stretching of the paper in the printer. Once the 105 Grade lens tissue has been patterned on both sides, it is C) suspended in a Pyrex baking dish and baked to ensure the wax is distributed throughout the scaffold. Finally, the wax-patterned scaffolds are cut from the sheet and sterilized before use.

***Materials**:*

70% (v/v) ethanol (See recipe)

3-quart Pyrex baking dish (Amazon, no. B01IUKIPYY)
Biological safety cabinet
Double-sided tape
Drying oven
Desktop trace box
Ethylene oxide chamber (e.g., Anderson Anprolene Gas Sterilizer AN74i)
Lens cleaning tissue paper, Grade 105 (Sigma-Aldrich, no. WHA2105862)
Printer paper
Single-sided tape
Stainless steel, precision non-magnetic tweezers (Amazon, no. B00FZPEWI6)
Stainless steel surgical scissors (Amazon, no. B07Y96T1NZ)
Swingline 1 hole punch (Amazon, no. B00FBQM010)
Utility knife (Amazon, no. B08QQZMZQY)
Vector-based drawing software (e.g., Adobe Illustrator)
Wax printer (e.g., Xerox ColorQube 8570)
Wide-mouth jar (Thermo Scientific, no. 2116-0250)
Write-on transparency film (Amazon no. VWO100C-BE)

***Steps**:*

1. Wipe down the work area with 70% ethanol.

2. Align a sheet of lens paper and transparency. The paper should extend beyond the length of the transparency by approximately 13 mm on both ends.

3. Fold the overhangs over and tape to the transparency with the single-sided tape, ensuring there are no wrinkles or folds in the lens paper (Figure 1A).

4. Open the vector-based file containing the paper scaffolds.

5. Place the transparency-lens paper combination into the individual paper loading section of the printer, filter tissue side down and print the pattern.

6. In the software, reflect the image across a vertical axis.

    Note. If using Adobe Illustrator, select object > transform > reflect.

7. Print the reflected image on a new, clean transparency, to be used as a guide transparency.

8. Place a line of double-sided tape the top of the guide transparency.

    Note. Place the double-sided tape as close to the top of the transparency as possible to reduce the chances of the printer jamming.

9. Align the transparency-lens paper combination and the guide transparency, the wax patterns should be touching each other.

10. Once aligned stick the lens paper to the double-sided tape.

11. To help replicate the stretching and bending the paper experiences during the printing process, move the now taped guide transparency and transparency-lens paper combination, so it is hanging off the lightbox. The already taped side should be touching the table the lightbox is sitting on. (Figure 1B) This incorporates a bend into the lens paper and transparency and makes it easier to align, while also helping to get a more complete sheet of aligned scaffolds.

12. Place a second piece of double-sided tape on the bottom of the guide transparency and attach the lens paper (Figure 1C).

13. Using a razor blade, cut the lens paper off the original transparency, at the edges.

14. Ensure there are no wrinkles or folds in the lens paper.

15. Place the new guide transparency-lens paper combination on the printer, paper side down, and print again.

16. Inspect the sheet. The patterns should be aligned, and the center regions of each pattern free of wax. If there is a significant overlap between the wax borders and the culture regions, restart from step 1.

17. Using the box cutter, free the lens paper from the transparency.

18. Using the single-sided tape, suspend the lens paper sheet in the Pyrex dish. The printed lens paper should not be touching the dish, rather it should be “floating” above the glass dish (Figure 1D).

    Note. Suspending the paper ensures the wax will not melt to the Pyrex dish, causing it to smear or distort the patterns.

19. Place the Pyrex dish in a 100 ^{o}C oven for 15 min.

20. Remove from the oven and allow to cool for 15 min.

21. Using single-sided tape, tape the lens paper to a sheet of printer paper.

    Note. This step can be skipped, although some people find it easier to cut out the scaffolds with them taped to a sheet of printer paper.

22. Use the hole punch to remove the small single zones from the sheet of Whatman 105 lens paper. Cut out the large single zones with surgical scissors. Place the scaffolds in a non-sterile, clean Nalgene container or Petri dish.

23. Sterilize the scaffolds using an ethylene oxide chamber or UV light. Do not use an autoclave or ethanol to sterilize the paper scaffolds.

    Note. If using an ethylene oxide chamber, follow the manufacturer’s instructions using a 12-hour cycle. If using UV light, expose the scaffolds to UVa and UVb light for 30 minutes. The UV source in most tissue culture hoods is capable of emitting photons in both regions of the electromagnetic spectrum.