Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for:

A microfluidic bubble perfusion device for brain slice culture

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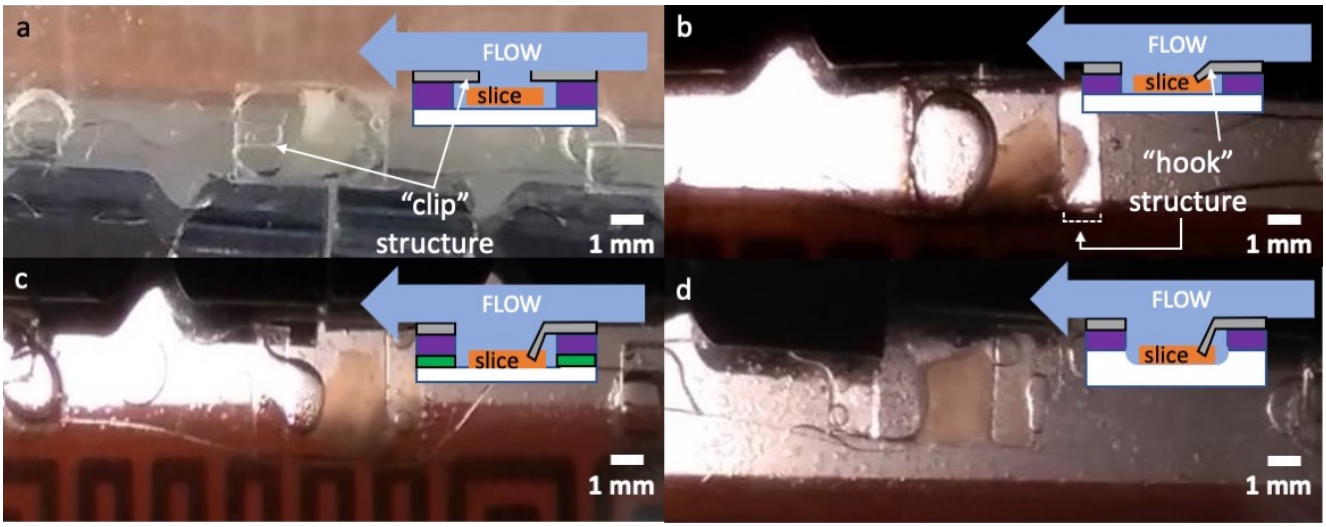

Figure S-1: Tissue immobilization via mechanical structures. **a.** A "clip" structure was used to trap the tissue slice in a well without strong physical contact with the slice. **b.** A "hook" structure is shown, similar to the clip structure but with the substrate material bent to mechanically clamp the tissue slice in place. c. and d. The well holding the tissue slice was deepened by additiona of a PDMS layer (c), or by etching the underlying glass substrate (d). In every case, bubble trapping and droplet breakup are evident in the tissue chamber. Insets show the cross-sectional schematic of the respective chamber structures. Materials are color coded as follows: white = glass, purple = VHB adhesive, gray = GelBond film, green = PDMS, blue = perfused culture media.

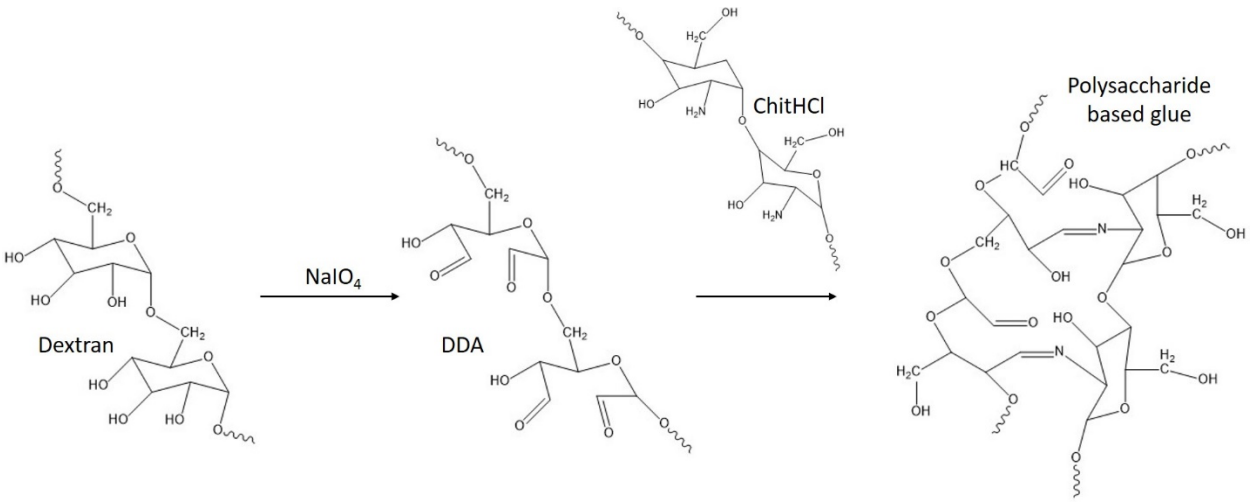

Figure S-2: Reaction scheme of Chitosan-DDA glue

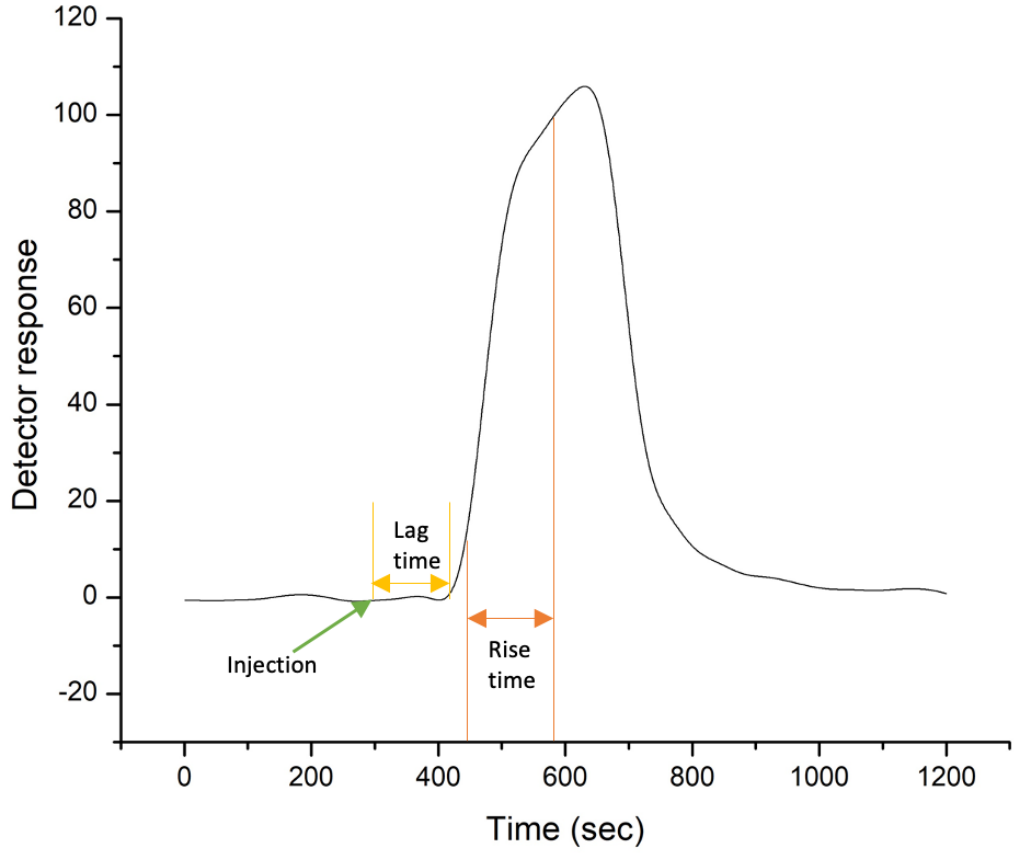

Figure S-3: Characterization of lag and rise time for stimulus delivery
Fluorescent profile of a typical lag time/rise time determination. Lag time is the time between stimulus injection and the initial response observed at the tissue chamber. Rise time describes the time needed for stimulus concentration to increase from 10% to 90% of maximum observed signal.

Standard operating procedure for the synthesis of Chitosan-Dextran tissue glue

Part A: Preparation of Chit-HCl (chitosan hydrochloride)

1. Weigh 10 g of high molecular weight chitosan and transfer to a 250 mL beaker
2. Slowly mix 60 mL of HCl into 40 mL of ethanol in a fume hood
3. Carefully pour the HCl-ethanol mixture into the 250 mL beaker containing chitosan
4. Put a stir bar in the beaker and gently stir (100 rpm) the mixture for 3 hours at room temperature.
5. After 3 hours filter off the Chit-HCl salt using a gravity filter
6. Make a 6:2 acetone-water mixture
7. Wash the Chit-HCl salt extensively with acetone-water mixture while still on the filter paper
8. Put the Chit-HCl salt inside a dialysis tubing (MWCO 12 KDa)
9. Tie both ends of the dialysis tubing firmly to prevent any leaks
10. Put the dialysis tubing in a 1000 mL beaker in such a manner so that the bottom of the tubing does not touch the floor of the beaker. You may use binder clips to hold both ends of the tubing on the wall of the beaker, essentially making a U-shape for the tubing
11. Fill the 1000 mL beaker with ultra-pure water (>14.1 MΩ) and put a stir bar in the beaker
12. Gently stir (100 rpm) the water in the 1000 mL beaker for 2 hours
13. Change the water in the beaker and repeat steps 11-13 for 3 times
14. Pour 2 mL water from the 1000 mL beaker into a test tube and add a few drops of 1 wt% $AgNO_3$(aq) solution. If white precipitate appears due to presence of HCl in the water, repeat steps 11-14 until no precipitate appears.
15. Transfer the solution from inside of the dialysis tubing to a glass vial.
16. Freeze dry the solution
17. Store the freeze-dried Chitosan-HCl at 4˚C until use

Part B: Preparation of DDA (dextran dialdehyde)

1. Weigh 5 g of dextran and transfer to a 250 mL beaker
2. Weigh 4.5 g of sodium metaperiodate and transfer to the same beaker

3. Add 100 mL of ultra-pure water (>14.1 MΩ) to the beaker
4. Add a stir bar in the beaker
5. Cover the beaker with aluminum foil to prevent exposure of light
6. Stir gently (100 rpm) the mixture in the beaker for 6 hours
7. Take 5 mL of the solution and transfer it inside a dialysis tube (MWCO 12 KDa)
8. Tie both ends of the dialysis tubing firmly to prevent any leaks
9. Put the dialysis tubing in a 1000 mL beaker in such a manner so that the bottom of the tubing does not touch the floor of the beaker. You may use binder clips to hold both ends the tubing on the wall of the beaker essentially making a U-shape for the tubing
10. Fill the 1000 mL beaker with ultra-pure water (>14.1 MΩ) and put a stir bar in the beaker
11. Gently stir (100 rpm) the water in the 1000 mL beaker for 2 hours
12. Change the water in the beaker and repeat steps 10-12 for 3 times
13. Pour 2 mL water from the 1000 mL beaker into a test tube and add a few drops of 1 wt% $AgNO_3$(aq) solution. If white precipitate appears due to presence of I^- in the water, repeat steps 10-13 until no precipitate appears.
14. Transfer the solution from inside of the dialysis tubing to a glass vial.
15. Freeze dry the solution
16. Store the freeze-dried DDA at 4˚C until use

Part C: Formation of the two-part hydrogel glue

1. Obtain phosphate buffer saline (PBS)
2. Hydrate Chit-HCl with PBS to form a 5 wt% solution
3. Hydrate DDA with PBS to form a 10 wt% solution
4. Apply 2 µL of each solution on the hydrophilic side of a GelBond film to adhere a typical rodent SCN slice. The volume of each part may vary for different dimension of tissue slices.