Supplementary Information

On-chip immobilization of planarians for *in vivo* imaging

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Supplementary figures:

Fig. S1

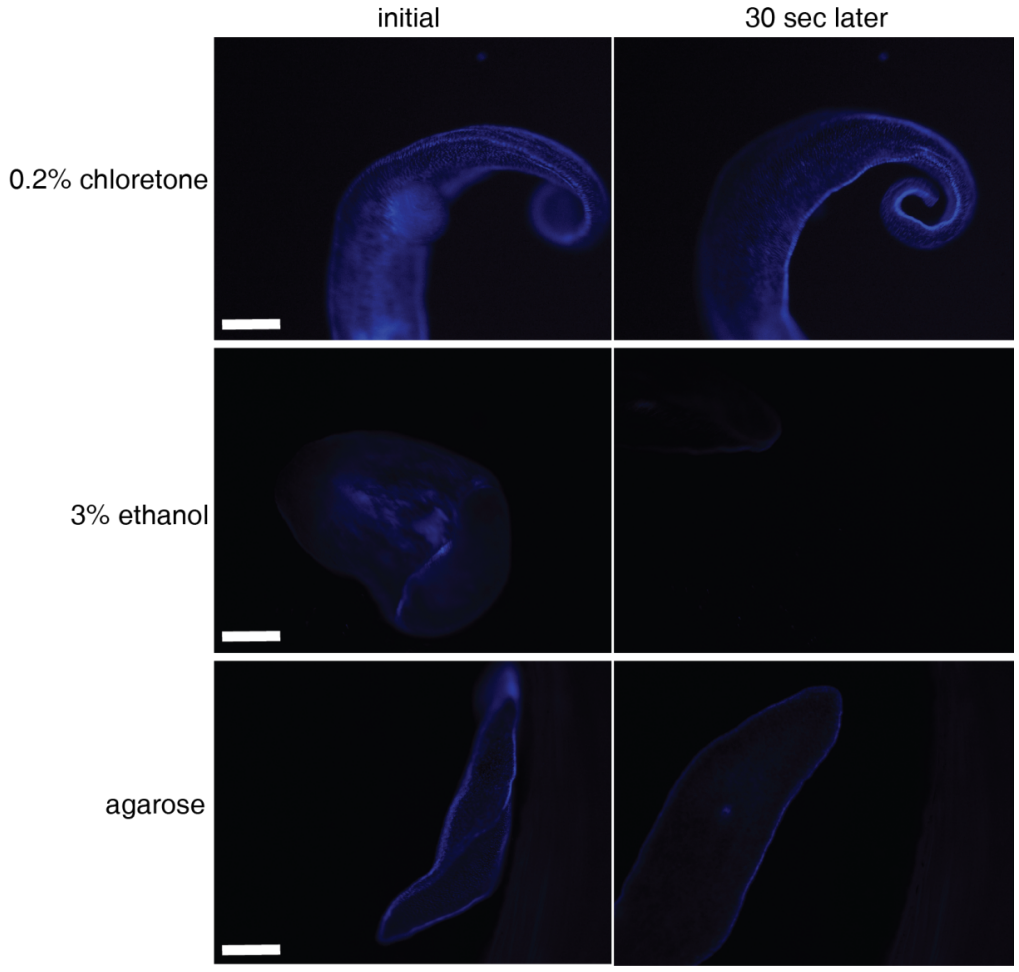

Fig. S1 Fluorescence imaging of planarians using standard immobilization procedures. Sample 4x

images of planarians stained with Hoechst 33342 and immobilized using 0.2% chloretone, 3% ethanol, or embedding in agarose. Images were taken immediately after focusing on the worm (left column) and 30 seconds later (right column), with no adjustments made in between. The anesthetized worms curled up into distorted shapes. Poor focusing and blurriness is due to worm movement. Scale bars: 100 µm.

Fig. S2

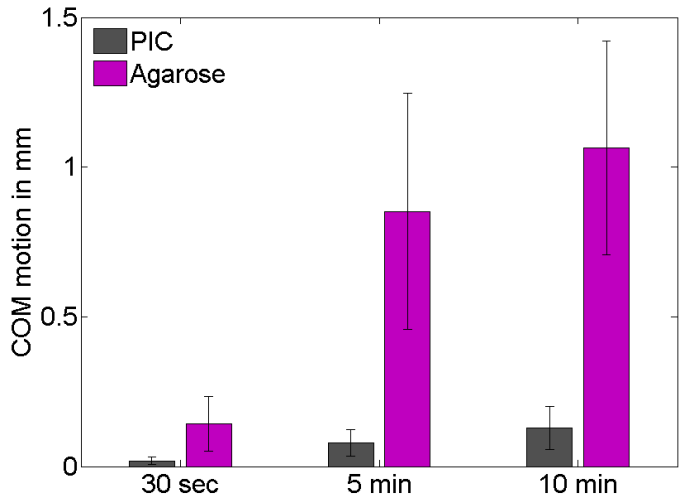

Fig. S2 Change in the movement of the worms' center of mass (COM). Histogram of the individual data points shown in Fig. 1F in the main text (n=5 for PIC and n=7 for agarose). Error bars denote the SEM.

Fig. S3

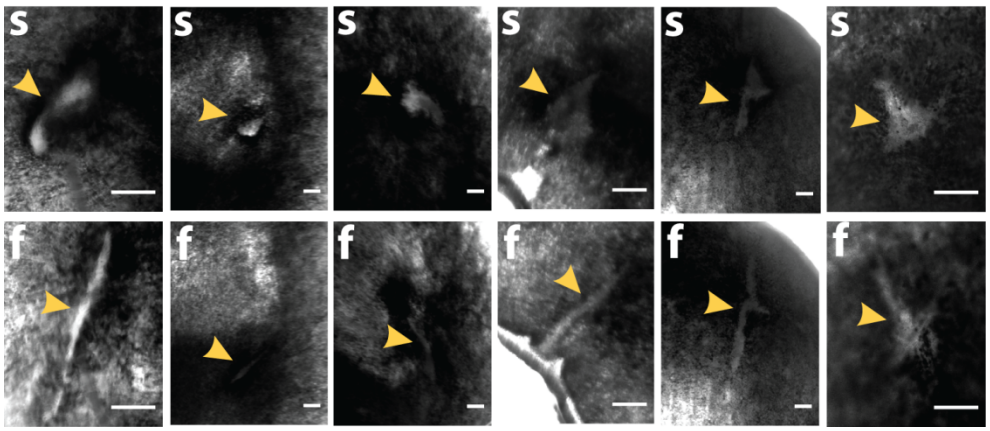

Fig. S3 Wound closure images. The start (s) and final (f) frames of the 6 wound closure movies analyzed in Fig. 5 in the main text are shown. The yellow arrows indicate wounds. Scale bars: 100 µm.

Fig. S4

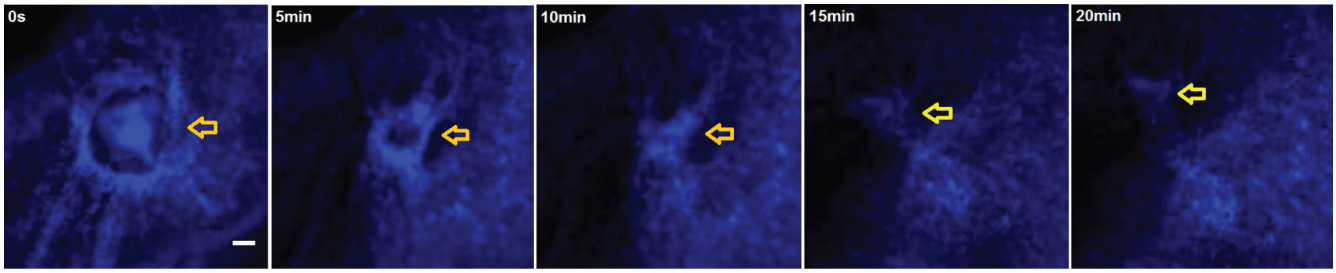

Fig. S4 Dynamics of wound closure. Individual frames of Supplemental Movie S8, which illustrates wound closure dynamics of a planarian stained with Hoechst 33342. The yellow arrows indicate wounds. Scale bar: 100 μm.

Supplemental protocol for PIC fabrication and use:

Part I: Fabrication

A: Fabrication of the PDMS "handle"

Step 1

Combine the PDMS base and curing agent in a 10:1 (or 20:1) ratio by weight. 50 g of the PDMS mixture is sufficient to make approximately 5 PICs.

Step 2

Mix the base and curing agent using a metal spatula.

CRTICAL STEP: Thorough mixing is required for the PDMS to cure properly. The components should be mixed vigorously for at least 3-5 min.

Step 3

Place a metal or plastic block inside a small tissue culture dish; this will create a hole in the handle which will later be covered by the PDMS membrane. Pour the PDMS mixture into the dish.

Step 4

Place the dish in a dessicator, and apply vacuum for 15-20 min, or until all bubbles in the PDMS are removed.

Step 5

Cure the de-gassed PDMS in an oven until firm. There are multiple curing protocols, including 30 min at 90 °C or 1 hr at 70 °C, are acceptable.

Step 6

After curing, cut the plastic sides of the dish and peel the solid PDMS off the bottom. Push on the top of the PDMS until the metal or plastic block "pops" out.

B: Fabrication of the PDMS membrane and assembly of the PIC

Step 7

Prepare a thin PDMS membrane (~0.2 mm) by spreading 1mL of liquid 20:1 (base: curing agent) PDMS on a plastic plate with compressed air. Heat the plate for 5 min at 70 °C to partially cure the PDMS. Stick the PDMS handle to the membrane with the opening oriented down, and cure together at 70 °C for several hours or overnight.

Step 8

Peel the membrane (with the handle bonded to it) off the plastic plate to obtain the final device. As needed, trim away parts of the membrane extending beyond the handle.

CRITICAL STEP: It is easy to tear the membrane during this process, which renders the chip unusable. Peeling should therefore be done gradually and with care.

Part II: Worm immobilization

Step 1

Orient the PIC so that the PDMS membrane is facing up. Transfer a small drop of 2% liquid agarose (~100 uM) to the center of the membrane, and spread the drop with the tip of a pipette so that the membrane is coated uniformly with agarose.

Step 2

After the agarose has solidified, transfer the worm to the membrane using a plastic Pasteur pipette. Once the planarian is positioned near the center of the agarose, thoroughly dry the surface with a rolled-up Kimwipe. All water transferred with the worm should be removed.

CRITICAL STEP: A dry surface is essential to stable immobilization, as residual liquid can enable the worm to move between the agarose-coated membrane and the plastic film. It is also important, however, to avoid injury to the worm from excessive prodding with the Kimwipe.

Step 3

After drying the surface, place a piece of the gas-permeable film on top of the membrane. Press down on the film with a pipette tip so that it sticks to the agarose. To prevent injury, do not push directly on the worm. The specimen is now ready to be imaged.

Step 4

To image on an inverted microscope, place the loaded PIC membrane-down on a glass coverslip. After imaging, gently peel away the plastic film. Squirt the worm with liquid, and transfer it using a plastic Pasteur pipette to a dish containing planarian water for recovery.

List of supplemental movies:

Movie S1
Protocol. Video protocol detailing the procedures used in device fabrication and the correct technique for worm immobilization.

Movie S2
Tracking movie 1. Sample tracking movie (on-chip immobilization, brightfield illumination). 10 min duration, frames taken every 10 sec.

Movie S3
Tracking movie 2. Sample tracking movie (agarose immobilization, brightfield illumination). 10 min duration, frames taken every 10 sec.

Movie S4
Tracking movie 3. Sample tracking movie (on-chip immobilization, fluorescence illumination). 10 min duration, frames taken every 30 sec.

Movie S5
Tracking movie 4. Sample tracking movie (agarose immobilization, fluorescence illumination). 10 min duration, frames taken every 30 sec.

Movie S6
Fluorescence imaging of the gut. Confocal z-stack showing the planarian gut labeled with fluorescein and rhodamine and acquired at 10x alternating the blue-green excitation channels.

Movie S7
Sample wound healing movie. Sample brightfield movie showing wound closure over 3 hrs in an injured planarian. Two of the images in Fig. 4 (b) are the initial and final frames of this movie. 135 min duration, frames taken every 30 sec.

Movie S8
Sample fluorescence wound healing movie. Sample fluorescence movie showing wound closure over 20min in an injured planarian. Frames were taken every 30 sec.