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Using the Millifluidic Device V.1

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Abstract

Sterile manufacture of indented PDMS base, agar sheet and peripherals for agar perfusion device, along with protocols for operation in sterile conditions

1 DEVICE SUPPORT TEMPLATE

Need:

- 3D printer and printing resin capable of withstanding prolonged temperatures up to **80 °C** without deformation (used Formlabs standard resin in the Form 3 3D printer)

1. Design a template for the device support using CAD software (e.g. AutoCAD) to the required specification, with minimum **8 mm** high walls and **1 mm** channel dimensions for optimal device performance.
2. Print according to manufacturer instructions. Bake the template at **70 °C** for **12 hours** to prepare for use.

- **The first time a template is used, the PDMS may not set properly - if so, bake the template for a further 12 hours.**

2 DEVICE HOLDER

Need:

- Nunc™ OmniTray™ Single-Well Plate
- A cutting tool (used flame-heated **1.2 mm** gauge needle)

1. Cut full-height slots out of each side of the OmniTray base corresponding to inlet and outlet holes in the device, such that an assembled device with tubing can be placed inside.

- **Wider slots can be made by cutting two vertical slits and pulling the area between the slits until it snaps off.**

3 DEVICE ASSEMBLY

Need:

- Poly di-methyl siloxane (PDMS) curing kit (Used Sylgard 184)
- 1 mm biopsy punch
- Device template from **Step 1**
- Razor blade
- Autoclavable tubing with **1.0 mm** OD and **0.5 mm** ID (used https://uk.vwr.com/store/product/577475/tubing-ptfe)
- Borosilicate glass screw top bottles with polypropylene or self-sealing silicone lids (used **100 mL** vials)
- Flexible autoclavable tubing capable of tightly fitting over the **1.0 mm** OD tube

1. Fill the support template with PDMS (1:10 curing agent to base) to a thickness of approx. **6 mm** (**45-50 mL** is enough for a device of 80 x 110 mm).
2. Leave at room temperature until bubbles are dispersed before baking for **1 hour** at **70 °C** to cure.
3. Remove the support from the template and place smooth side up on a cutting board. Cut the inlet and outlet walls with the razor blade at a **45° angle** outwards, starting **2 mm** from the channel ends.

4. Turn the support over and punch holes at inlets and outlets with the biopsy punch, pushing down on the slanted walls for diagonal tube entry.
5. Cut two **30 cm** and one **15 cm** length of the tubing for each device channel. Connect one **30 cm** tube to the channel outlet and the **15 cm** tube to the channel inlet. Cut a **2 cm** section of the flexible tubing and use to connect the **15 cm** inlet tube to the second **30 cm** tube.
6. Thread each inlet tube through a bottle lid until it reaches the bottom of the bottle (If using polypropylene lids, puncture first with a heated **0.8 mm** needle or drill to make a hole slightly smaller than the tube diameter).
7. Label bottle lids and outlet tubes with the channel number, and tape the bottles together.
8. Wrap the device support securely in foil, leaving the tubes and bottles free. Seal the outlet tubes with tape.
9. Secure the bottles to the device, ensure the lids are tight and autoclave the setup at **121 °C** for **15 minutes** to sterilise. Leave the setup in a drying oven until no liquid is visible in the tubing.

- **Dried sterile devices can be stored indefinitely at room temperature.**

4 EXPERIMENTAL SETUP

Need:

- Diffuse light source (used https://www.amazon.co.uk/gp/product/B088GGG9S2/)
- Raspberry Pi with camera module (used a Pi 4B connected to a Pi Camera Module 2 by a **1 metre** ribbon cable)
- Stable camera mount (used **https://www.speedgraphic.co.uk/copy_stands/cs500__mediu/25946_p.htmlm_**)
- Syringe drivers (capable of withdrawing and multiple steps)
- A humidity controller system with transparent chamber large enough to fit the device holder

1. Assemble the camera mount in a large incubator set to **37 °C**. Plug in the Raspberry Pi and attach the camera module to the mount pointing downwards to image from a bird's eye view. The focus can be adjusted as necessary by twisting the camera lens.
2. Place the diffuse light source under the mounted camera.
3. Set the Raspberry Pi to take and save images to an appropriate location after a set time interval (used 30 mins).
4. Set the syringe drivers in reverse mode with looped alternating periods of fast (**0.3 mL** at **10 mL/hr**) and slow (**1.5 mL** at **0.75 mL/hr**) flow.
5. Set up the humidity chamber at **90%** relative humidity.

5 DRIED AGAR SHEETS

Need:

- Molten **1.5% w/v** agar
- Sterile Petri dishes larger than the device base (used https://www.fishersci.co.uk/shop/products/borosilicate-glass-petri-dishes-1/12941408 in **140 mm**, autoclaved at **121 °C** for **15 minutes**)

1. In a sterile environment, evenly add agar to the Petri dishes to **1.8 mm** thickness (**11 mL** for 90 mm plate/ **28 mL** for 140 mm plate) and allow to set.

2. Seal the Petri dish and dry at **37 °C**, for approx. **8 hours** if the dish is vented, or **24 hours** otherwise. Can be stored at **7 °C** for up to a month if not using immediately.

- **If using agar without nutrients, add nutrient media to one of the dishes to verify sterility.**

6 EXPERIMENTAL PROCEDURE - STREAK

Need:

- Overnight culture (**MADE PREVIOUS DAY**)
- Sterile **250 mL** conical flask
- Nutrient media
- Assembled device
- **60 mL** syringe per channel
- **25G** needle per channel
- Dried agar sheet
- Razor blade
- Flat spatula
- Device holder
- Sterile cotton swab

1. In a sterile environment, set up a culture by inoculating **50 mL** of medium with **1 mL** overnight culture in the conical flask. Agitate at **37 °C** until required.
2. Connect the syringes to the needles, then connect to the device outlet tubes, taking care to avoid scratching the inside of the tubes. Detach the media bottles and unwrap the tin foil to expose the device base.
3. Open the dish containing the agar sheet and slide the spatula under the edge of the sheet, pulling it away from the dish. Grasp the edge by hand, quickly separate it from the dish and place it flat onto the device base. Use the razor blade to trim overhanging agar, then separate the device from the tin foil and place into the device holder.
4. When the culture is in exponential phase (optical density of **0.15 - 0.45**), use the swab to apply a streak of culture to the agar surface (may be helpful to use a ruler to guide). Cover the holder and tape up the open sides.
5. Carefully unscrew each media bottle and fill with an appropriate amount of media before replacing the lid loosely.
6. Move the device to the experimental set-up from **Step 2**, place in the camera view and cover with the humidity chamber. Loosen the reservoir bottle lids to equalise the pressure.
7. Connect the syringes to the syringe driver and pull the syringes manually until the channels and tubing are full, before starting the driver program. Cover with the opaque box to block light.

- **If using multiple syringe drivers, fully set-up and start each driver before moving on to the next, to keep channel pressure constant.**

7 EXPERIMENTAL PROCEDURE - LAWN

Need:

- Overnight culture (**MADE PREVIOUS DAY**)
- Molten **0.7% w/v** agar (cooled to **50 °C**)

- Dried agar sheet
- Assembled device
- **60 mL** syringe per channel
- **25G** needle per channel
- Razor blade
- Flat spatula
- Device holder
- Nutrient media

1. In a sterile environment, add **0.2 mL** of the overnight culture to the agar sheet, then top agar to **0.3 mm** thickness (**2 mL** for 90 mm plate/ **5 mL** for 140 mm plate). Distribute evenly across the sheet and leave to dry, then incubate at **37 °C** for approx. 1 hour.
2. Connect the syringes to the needles, then connect to the device outlet tubes, taking care to avoid scratching the inside of the tubes. Detach the media bottles and unwrap the tin foil to expose the device base.
3. Open the dish containing the agar sheet and slide the spatula under the edge of the sheet, pulling it away from the dish. Grasp the edge by hand, quickly separate it from the dish and place it flat onto the device base.
4. Use the razor blade to trim overhanging agar, then separate the device from the tin foil and place into the device holder. Cover the holder and tape up the open sides.
5. Carefully unscrew each reservoir bottle and fill with an appropriate amount of media before replacing the lid.
6. Move the device to the experimental set-up from **Step 2,** place in the camera view and cover with the humidity chamber. Loosen the reservoir bottle lids to equalise the pressure.
7. Connect the syringes to the syringe driver and pull the syringes manually until the channels and tubing are full, before starting the driver program. Cover with the opaque box to block light.

- **If using multiple syringe drivers, fully set-up and start each driver before moving on to the next, to keep channel pressure constant.**

8 DISASSEMBLY

1. When finished, disassemble set-up, discarding the agar sheet, needles and syringes. Detach the media bottles from the tubing.
2. Clean the device and flush the tubing with 70% ethanol using a syringe. Replace the media bottles and re-sterilise, then repeat from step 5 for new experiments.

- **All parts of the assembly can be reused for further experiments, but the device base will become yellow and less flexible over time and should be replaced after approx. 3 experiments.**