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AgarSqueezer

Questions & Answers

Q/ What are the benefits of using an agarose-based instead of a PDMS-based compression system?

- **Physiological relevance**: the agarose hydrogel rigidity is tunable and reproduces the stiffness of the *in vivo* microenvironment (1-150 kPa), contrary to PDMS which rigidity is several orders of magnitude larger (MPa).
- **No reduction of the available drug dose**: agarose hydrogels overcome drug adsorption issues associated with PDMS.
- **Passive medium renewal & oxygen diffusion**: the porous nature of agarose hydrogels allows free diffusion of oxygen, salts and small molecules and therefore minimize nutrient depletion, allowing for long-term confinement studies.

Q/ Which types of cells has AgarSqueezer been tested for?

The Agarsqueezer device has been successfully used to study compression of adherent and non-adherent cells, including:

Human cells	Fibrosarcoma (HT-1080) Osteosarcoma (U-2 OS) Colorectal adenocarcinoma (HT29 & HCT116) Prostate cancer (PC-3 & DU 145) Breast cancer (MDA-MB-231) Leukemia (TF1 & ML2) Megakaryocytes Fibroblasts (HS27A) Breast cells (MCF10A) Primary T-lymphocytes
Murine cells	Osteocyte-like cells (MLO-Y4) Primary dendritic cells Primary muscle cells
Plant cells	Arabidopsis Thaliana root cells
3D cell culture	Mice gastruloids

Q/ Which types of analysis can I carry out with AgarSqueezer?

The system is fully compatible with live imaging and time-lapse microscopy and all immunostaining steps can be performed *in situ*. Alternatively, cells can be collected for classical biochemical and molecular analysis (qPCR, Western Blot, flow cytometry, etc).

Q/ How long can cells be cultured in the AgarSqueezer device for?

The device has been designed for long-term confinement studies and the porous nature of agarose enables passive medium renewal as well as oxygen diffusion. You can safely grow cells up to 10 days in the AgarSqueezer device.

Q/ Can I stimulate my cells with drugs or other compounds during confinement studies?

Yes, the use of agarose allows free diffusion of small molecules (size <30 nm in 2% agarose). You can easily add drugs, antibodies or other compounds at any point during cell confinement studies thanks to an open access to the reservoir. Drug availability and activity within the system has been validated using *in situ* addition of the tyrosine kinase inhibitor imatinib, and diffusion experiments showed that 3 hours are required for the diffusion of small molecules such as BSA or FITC.

Q/ Which pillar heights are available, and which one should I choose?

The currently available pillar heights are:

- 2.5µm for inducing highly confined conditions
- 5µm for inducing moderately confined conditions
- 30µm to serve as an unconfined control
- 100µm for inducing confinement of 3D cellular objects

Those dimensions have been determined based on the size of most classical human cell lines. The resulting level of compression applied will depend on your sample type, size and the use of mono- or multi-cell layers.

Q/ How can I tune the matrix stiffness?

You can easily modulate the matrix stiffness by tuning the type and concentration of agarose used to mold the pillars. Using a standard agarose at the recommended 2% concentration will result in a storage modulus within the range of 50-200kPa. To accentuate the matrix stiffness and the resulting confinement applied, the agarose concentration can be increased. For example, a 3% agarose was found to be more efficient at confining cell cultures from

Arabidopsis roots. If you wish to decrease the matrix stiffness, we recommend using a “low melting point” or “ultra-low gelling” temperature agarose to yield a storage modulus in the range of ~1kPa. For a similar stiffness on top and bottom walls, the coverslip can be coated with an additional soft agarose layer.

Q/ How can I tune the matrix composition?

To analyze the role of various ECM proteins on cell response to mechanical confinement, you can coat the coverslip with adhesive proteins (fibronectin, collagen, Matrigel, etc). In addition, the agarose can be added with collagen, PEGDA with covalently immobilized RGD peptides or silk.

Q/ What is included in the AgarSqueezer kit? Is there anything I need that is not provided?

Our AgarSqueezer kits contain all elements making up the compression device, a wafer to mold your agarose gel, some needles specifically designed to form your entry ports in the agarose and a microscope stage adapter to mount up to 2 Agarsqueezers for imaging.

All you need to have on your side is:

- Agarose powder
- Distilled water
- Round 22 or 30 mm coverslips
- A screwdriver
- For imaging: an *Okolab* imaging chamber, or any compatible system, to fit our holder dimensions. If not available, a standard petri dish holder can be used but we cannot guarantee a correct distance with objectives depending on the microscope used.

Q/ Can I re-use the AgarSqueezer device?

Yes, the AgarSqueezer device can be re-used as long as it is thoroughly sterilized by autoclaving before each experiment. Simply keep in mind that the agarose solution should be prepared no more than one day before your experiment.

A Q&A updated in August 2023.