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Assembly of Chambers for Stable Long-Term Imaging of Live Xenopus Tissue

Hye Young Kim[1] and **Lance A. Davidson**[1,2,*]

[1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15213

[2]Department of Developmental Biology, University of Pittsburgh, Pittsburgh, PA 15213

Understanding the molecular mechanisms and cellular mechanics that drive morphogenesis in Xenopus laevis embryos requires high-resolution quantitative imaging of cell movements and protein dynamics within multicellular tissues. To perform long-term tissue culture and stable imaging of live Xenopus tissue, we have developed a simple acrylic chamber that can be sealed and reused. The explants can be cultured on an adhesive substrate or within a nonadhesive microenvironment. We have developed a method for sandwiching isolated marginal zone (MZ) explants between thin agarose sheets. To manipulate the stiffness of the substrate or to measure cell traction generated by cells in an intact tissue, a fibronectin-conjugated polyacrylamide gel (FN-PAG) substrate can be used. The mechanical properties of this substrate can be easily modulated by varying the ratio of acrylamide to bis-acrylamide and the transparent nature of the PAG can allow observation of intracellular dynamics through the gel. Cell tractions are detected by following movements of fluorescent beads embedded in the gel.

a. Acrylic chambers

To carry out long term tissue culture and stable imaging of live Xenopus tissue, we developed a simple acrylic chamber which can be sealed and reused. The chamber is milled from a 25 by 50 by 6 mm (1 × 2 × 1/4 inch) acrylic plate to include one or two wells. The chamber is assembled using silicon grease to affix a large cover glass to the bottom and sealed with small coverslip over the top. The two well design minimizes the volume required for multiple samples in each well and is compatible with the addition of small molecule inhibitors. To provide adhesive substrate for Xenopus tissue, 25μg human plasma fibronectin diluted in 1 ml 1/3X MBS is added to a chamber covering the lower glass substrate and allow to coat overnight at 4°C. Large glass coverslips are best prepared for adhesive substrates by washing briefly in alkaline ethanol and dried by flame.

b. Agarose pads (non-adhesive)

To culture the explants within a non-adhesive micro-environment, isolated MZ explants can be sandwiched between thin agarose sheets held in place by a small coverslip fragment and silicon grease. Agarose sheets are prepared in advance by casting 1% agarose between two

*Author for correspondence: lad43@pitt.edu, (phone) 412-383-5820.

glass coverslips. Sheets are then cut with a scalpel and transferred into the culture chamber. Assembling the agarose-explant-agarose sandwich can be tricky since the thin sheets of agarose are nearly transparent. Low-angle side illumination with a fiber-optic lamp can provide the contrast needed. Explants sandwiched between agarose sheets undergo rapid convergence and extension often making it difficult to track cells at high magnification without vigilant repositioning.

c. Polyacrylamide Gels

To manipulate substrate stiffness or measure cell traction generated by cells in an intact tissue a fibronectin conjugated polyacrylamide gel (FN-PAG) substrate can be used (Leach et al., 2007). Substrate mechanical properties can be easily modulated by varying the ratio of acrylamide to bis-acrylamide and the transparent nature of the PAG can allow observation of intracellular dynamics through the gel (Beningo et al., 2002). Cell tractions are detected by following movements of fluorescent beads embedded in the gel. To construct a FN-PAG, first assemble a pre-mix solution of 5% Acrylamide, 0.05% Bis-acrylamide, 1 μg/μl bovine plasma fibronectin and dark red beads (43 nm diameter FluoSpheres; Invitrogen, Carlsbad CA) in phosphate-buffered saline (PBS). The pre-mix solution is de-aerated for 20 minutes, then N,N,N_,N_-Tetramethylethylenediamine (TEMED, Sigma-Aldrich), 0.01 μg/μl acrylic acid N-hydroxysuccinimide (Sigma-Aldrich), and freshly made 0.4 ng/μl ammonium persulfate (APS, Sigma-Aldrich) are added to begin the polymerization process. A small volume of the polymer mix is dropped on the clean cover glass and covered by a 7 by 11mm coverslip fragment. The volume of the drop and the dimensions of the coverslip fragment determine the thickness of the FN-PAG. Immediately invert the cover glass to deposit beads on the surface of the gel and polymerize in a humid nitrogen chamber for 40 minutes at room temperature. Once the FN-PAG has polymerized the coverslip gel assembly can be immersed in 1/3X MBS and the top coverslip fragment removed. The FN-PAG can be used immediately or stored at 4° C overnight.

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