Basic information

ISI Cat. No.

0101, 0102, 0103, 0104, 0105, 0106, 0107, 0201, 0202, 0203, 0204, 0205, 0206, 0207, 0301, 0001

Protein coating

Human fibronectin

Storage conditions

Store BioWrite micropatterned substrates at 4 C in a dry environment. The pattern is stable up to 1 month at 4 C.

Sterility

BioWrite substrates are not sterile and are not intended for long-term culture. Sterilization procedures are under development. Please contact Intelligent Substrates for more information.

Pattern orientation

The BioWrite micropattern is on the side of the substrate facing up (in the single sample packs) or on the side of the substrate facing the 'ISI' cutout on the 10-substrate package. The patterns are in the center of the substrate.

Handling

BioWrite substrates are made of fragile glass. Handle each substrate with care, and wear protective gloves and/or safety goggles. Use forceps to grasp and handle the substrate. Take care to grasp the substrate by the edge. Contact with the patterned surface may damage the pattern.

Plating Swiss-3T3 fibroblasts

Introduction

This protocol describes the steps required to plate and grow Swiss-3T3 fibroblasts on a BioWrite micropatterned fibronectin substrate. The procedure has been tested and verified on Swiss-3T3 cells. Other cell types, proteins, and substrates may require modifications to this procedure. Contact Intelligent Substrates for recommendations.

Materials

- BioWrite micropatterned glass coverslip
- 35 mm Petri dish or similar container
- Casein from bovine milk (Sigma, Cat. No. C5890)
- 1x Phosphate buffered saline (PBS)
- Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, Cat. No. D5796 with L-glutamine)
- Swiss-3T3 fibroblasts (American Type Culture Collection, Cat. No. CCL-92)

Procedure

1. Block the BioWrite micropatterned substrate with 1% of casein* in PBS by gently adding 200 µl or more of the blocking solution onto the middle area of substrate. Be careful not to touch the surface of the substrate with the pipette tip -- contact with the tip may damage the pattern.
2. Aspirate the blocking solution in a laminar flow hood and wash the substrate three times with the medium used for cell seeding. In the case of Swiss-3T3 cells, for a short-term experiment (2-4 h) use serum-free DMEM with 1% penicillin/ streptomycin. For long-term experiments, use medium with a low concentration of serum.
3. In a laminar flow hood, place the substrate into the 35 mm dish with the patterned side facing up.
4. Seed 5×10^4 cells per ml in total volume of 2 ml of DMEM on the substrate (for the 35 mm dish) and incubate for 2 h at 37°C. Cell responses to the micropatterns may be observed by phase microscopy during the incubation period (Fig. 1).
5. Aspirate medium.
6. Fix and stain cells following the protocol below.

*Casein in PBS is usually a colloidal suspension of low solubility. We recommend using strong agitation and filtering the solution through a 0.2 µm filter before use.

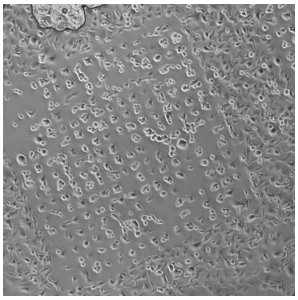

Figure 1: Phase image of Swiss 3T3 cells conforming to a pattern of 50 µm fibronectin squares.

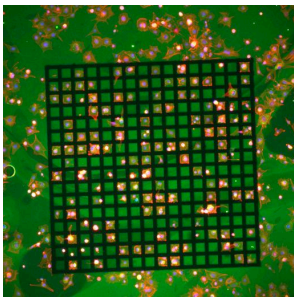

Figure 2: Immunofluorescence image of Swiss 3T3 cells on 50 µm squares of fibronectin as described below.

Staining BioWrite micropatterned substrates

Introduction

This protocol describes the steps required to fix and stain cells plated and grown on a BioWrite micropatterned fibronectin substrate. The fibronectin in the pattern will be observable in the fluorescein channel of a fluorescence microscope, the actin cytoskeleton of the cells will be in the rhodamine channel, and the nucleus will be in the Hoechst/ DAPI channel.

This procedure has been tested and verified on Swiss-3T3 cells grown on BioWrite fibronectin substrates. Other cell types, proteins, and substrates may require modifications to this procedure.

Materials

- BioWrite micropatterned substrate plated and incubated with cells
- 3% Paraformaldehyde (PFA) with 0.1% Triton®-X in PBS
- 3% PFA in PBS
- 0.1% bovine serum albumin (BSA) in PBS
- Anti-fibronectin IgG antibody from rabbit (diluted 1:100 from stock, AbCam Cat. No. Ab299)**
- Fluorescently labeled secondary antibody (for example, FITC-labeled anti-rabbit from goat (Invitrogen, Cat. No. A 11008) diluted 1:100 from stock)
- Rhodamine-labeled phalloidin (5 µL in 200 µL of 0.1 % BSA in PBS), (Invitrogen, Cat. No. R415)**
- Nuclear stain such as Hoechst (1:3000 dilution, Invitrogen Cat. No. 33342)
- Mounting medium (i.e., FluorSave™ Reagent, CalBioChem, Cat.No. 345789)

Procedure

1. Aspirate cell media from BioWrite substrate
2. Add to the BioWrite substrate 3% PFA with 0.1 % Triton X (Triton X in PBS).
3. Aspirate the solution off the BioWrite substrate.
4. Add 3% PFA (without Triton-X), and incubate for 20 min.
5. Wash 3 times with 0.1 % BSA in PBS.
6. To stain for actin, add ~30-50 µL of rhodamine-labeled phalloidin, and incubate for 20 min in darkness**.
7. Wash 3 times with 0.1 % of BSA in PBS.
8. To stain the fibronectin (FN) pattern, add ~30-50 µL of anti-FN IgG antibody, and incubate for 20 min in darkness to avoid photobleaching the labeled actin**.
9. Wash 3 times with 0.1 % of BSA in PBS.
10. Add ~30-50 µL of secondary antibody for FN, FITC-labeled anti-rabbit from goat, and incubate for 20 min in darkness.
11. Wash 3 times with 0.1 % of BSA in PBS.
12. To stain nucleus, add Hoechst stain and incubate for 10 min in darkness.
13. Wash 3 times with 0.1 % of BSA in PBS.
14. Mount with FluoroSave or similar mounting medium, and image with a fluorescence microscope (Fig. 2).

** The primary antibody for fibronectin and the labeled phalloidin can be mixed to stain for fibronectin and actin simultaneously. Prepare a 1:100 solution of anti-FN antibody as described above. Add 5 ul of phalloidin to 200 µl of the anti-FN solution. Apply 30-50 µl of the mixture per substrate, and incubate for 20 min in darkness.

Contact information

For more information about Intelligent Substrates' products, please visit www.IntelligentSubstrates.com.

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