Combinatorial Development of Biomaterials for Clonal Growth of Human Pluripotent Stem Cells

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Supplementary Methods

***Combinatorial Array Preparation*:** Before coating with proteins, the chips are sterilized by UV for 30 min for each side, and then washed with PBS twice for 15 min to remove the residue monomer or solvent. The chips were then coated with either: 20% FBS (v/v, Hyclone) at room temperature for 15 min, BSA (1 mg/mL, Sigma) at room temperature for 1 hr, laminin (4 μg/mL, Sigma) at 37^{o}C for 2 hr, human fibronectin (25 μg/mL, Sigma) or human vitronectin (Invitrogen; 1-3 μg/mL in DMEM) at 37^{o}C for 1 hr, or 20% human serum (v/v, Sigma) at room temperature for 15 min. These surfaces were then washed once with cell culture medium before cell seeding.

Surface Roughness Measurements: The automated acquisition of height and phase measurements for all polymer spots on the primary array was achieved by calculating the coordinates of each polymer spot and inputting these values into the programmed move feature of Nanoscope 5.31R1 software. Measurements were taken in both air and fluid. In air, silicon tips with a resonant frequency of approximately 300 kHz and a force constant of 40 N/m were used (Tap300Al, Budget Sensors). In fluid, silicon nitride tips with a resonant frequency of approximately 7 kHz and a force constant of 0.58 N/m were used (DNP-S, Veeco). Tapping mode was achieved using Z-modulation. Solutions used were either Milli-Q water or DMEM (GIBCO) containing 25% FBS (Hyclone) and supplemented with non-essential amino acids and L-Glutamine. Samples were incubated with the solution for 24 hours before AFM measurements were conducted and were kept in solution until all polymer spots were sampled. 5 μm regions of the polymer were taken and the root mean square (RMS) roughness was measured across this region. Image processing was conducted using SPIP V3.3.6.0 software.

Elastic Modulus Measurements: Instrumented indentation was conducted on the primary array, comprising polymer spots with an average diameter of 300 μm, height of ~15 μm with center-to-center distance of 740 μm, using a pendulum-based instrumented nanoindenter (NanoTest, Micro Materials Ltd.). The array glass slide was mounted on an aluminum support with a thin layer of cyanoacrylate. Experiments were conducted in ambient air, as well as upon full immersion and hydration of the array in PBS at room temperature. Hydrated arrays were immersed in PBS for at least 12 hours prior to indentation to achieve equilibrium hydration, and maintained in this hydrated state throughout the experiment using a modified platform for *in situ* liquid experiments.[52] Samples were indented with a spherical ruby indenter of radius R = 500 μm, (n=3 locations for each polymer spot), with loading and unloading rates of 0.5 mN/s, dwell of 10 s and a maximum load of 3 mN or a maximum depth of 600 nm, depending on which limit was attained first. This condition was chosen such that the average strains imposed on the polymer spots (estimated as a/R, where a is the contact radius) was less than 5% on all samples and the ratio of maximum indentation depth h_{max} to polymer spot thickness t was maintained less than 4% on all samples; this low h_{max}/t minimized contributions from the stiff glass support to the measured elastic response, and data were not corrected for finite thickness because the hydrated thickness was not measurable with high accuracy for all polymers.

Water Contact Angle Measurements: The piezo-doser on the Kruss DSA 100 apparatus allowed small ultra pure water droplets (110 pL) to be deposited onto the polymer spots. Sample positions and data acquisition were automated, with droplet side profiles being recorded (a dual camera system was used, one to record a spot's side profile and the other to record a bird's eye view to ensure that the water droplet was deposited at the centre of each spot) for data analysis. WCA calculations were performed using a circle segment function as required for small water droplets.

Cell Culture: hES cell lines BG01 (National Institutes of Health [NIH] code: BG01; BresaGen, Inc., Athens, GA) and WIBR33 (Whitehead Institute) were maintained on mitomycin C (MMC)-inactivated mouse embryonic fibroblast feeder (mEFs) layers in hES cell medium (Dulbecco's modified Eagle's medium DMEM/F12 [Invitrogen] supplemented with 15% FBS [Hyclone], 5% KnockOut Serum Replacement [Invitrogen], 1 mM glutamine [Invitrogen], 1% nonessential amino acids [Invitrogen], 0.1 mM β-mercaptoethanol [Sigma], penicillin/streptomycin [Invitrogen], and 4 ng/ml FGF2 [R&D Systems]). Cultures were grown at 37ºC in 5%O_2 and were passaged every 5 to 7 days either manually or enzymatically with collagenase type IV (Invitrogen; 1 mg/ml for 10 min). At the time of this study, hiPS C1 cells had been cultured for 5-15 passages on mEFs.

hES BG01-Oct4-GFP cells were made by introducing a Oct4-GFP-puro construct into hES cells.[31] In this construct, the GFP reporter gene is expressed from the human Oct4 promoter that is active when cells are in an undifferentiated state. Upon differentiation, the Oct4 promoter is gradually inactivated and therefore the GFP reporter is down-regulated (Supplementary Figure S2). At the time of this study, this BG01-Oct4-GFP line had been cultured over 30-95 passages with 46XY normal karyotype. This line expresses all pluripotent stem cell markers and forms teratomas after being grafted into severe combined immunodeficient mice (SCID) (Figure 6; unpublished data).

For EB-induced differentiation, hES cell colonies were harvested with 1 mg/ml collagenase type IV (Invitrogen), separated from the mEF cells by gravity, gently triturated, and cultured for 13 days in nonadherent suspension culture dishes (Corning) in DMEM supplemented with 15% FBS.

For FACS sorting, cells were harvested enzymatically with collagenase type IV (Invitrogen; 1 mg/ml), and then with 0.05% trypsin / ethylenediaminetetraacetic acid (EDTA) solution (Invitrogen) for 5 minutes at 37°C. hiPS cells were labeled with immunostained using SSEA4 (mouse monoclonal, Developmental Studies Hybridoma Bank; 1:10 supernatant dilution in mTeSR1 media for 10-15 min at 4ºC) and Molecular Probes AlexaFluor 647 dye–conjugated secondary antibodies (Invitrogen; 1:50 for 10 min at 4ºC).

Biological assays: For teratoma formation, hES cells were collected by collagenase treatment (1 mg/ml for 10 min) and separated from feeder cells by subsequent washes with medium and sedimentation by gravity. hES cell aggregates were collected by centrifugation and resuspended in 250 μL of PBS. hES cells were injected subcutaneously in the back of SCID mice (Taconic). Tumors generally developed within 4-8 weeks and animals were sacrificed before tumor size exceeded 1.5 cm in diameter. Teratomas were isolated after sacrificing the mice and fixed in formalin.

For immunocytochemistry, cells were fixed in 4% paraformaldehyde in PBS and immunostained according to standard protocols using the following primary antibodies: SSEA4 (mouse monoclonal, Developmental Studies Hybridoma Bank); Tra 1-60, (mouse monoclonal, Chemicon International); hSOX2 (goat polyclonal, R&D Systems); Oct-3/4 (mouse monoclonal, Santa Cruz Biotechnology); hNANOG (goat polyclonal R&D Systems); appropriate Molecular Probes Alexa Fluor® dye conjugated secondary antibodies (Invitrogen) were used. When necessary, cells were permeabilized with 1% Triton X-100 in PBS for 10 mins, and then stained. The chips were washed with PBS and water to remove the salts, and air dried. The chips were imaged with iCys laser scanning cytometry.

Numerical analysis: A "leave one out" cross validation method was used for the PLS analysis. Both ToF-SIMS and hES cell data were mean-centered before analysis. The Root Mean Square Error of Prediction (RMSPE) was calculated to quantify how well each model predicted the training set or test set polymers. The individual peak intensity was normalized to the total secondary ion count to remove the effect of primary ion beam fluctuation. The positive and negative ion intensity data was arranged into one concatenated data matrix. 181 positive and 43 negative ions were selected from a group of polymers from the array containing all 22 monomers to form the peak lists. The PLS model constructed from the training polymer samples produced a set of regression coefficients for each secondary ion. These regression coefficients were used to predict the hES cell colony formation on the test samples using their SIMS spectra. Due to variations in ion intensity, predicted frequencies were normalized.

Efficiency measurements: For efficiency experiments on TCPS, single cells were sorted individually (1 cell/well) directly into each well of a 96 well plate (Corning) coated with human vitronectin (Invitrogen; 1-3 μg/mL in DMEM), FBS (20% in DMEM), 20% human serum (v/v, Sigma), or matrigel (Invitrogen; using supplier's thin gel method).