



## Why the dish makes a difference: Quantitative comparison of polystyrene culture surfaces



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### ABSTRACT

There is wide anecdotal recognition that biological cell viability and behavior can vary significantly as a function of the source of commercial tissue culture polystyrene (TCPS) culture vessels to which those cells adhere. However, this marked material dependency is typically resolved by selecting and then consistently using the same manufacturer's product – following protocol – rather than by investigating the material properties that may be responsible for such experimental variation. Here, we quantified several physical properties of TCPS surfaces obtained from a wide range of commercial sources and processing steps, through the use of atomic force microscopy (AFM)-based imaging and analysis, goniometry and protein adsorption quantification. We identify qualitative differences in surface features, as well as quantitative differences in surface roughness and wettability that cannot be attributed solely to differences in surface chemistry. We also find significant differences in cell morphology and proliferation among cells cultured on different TCPS surfaces, and resolve a correlation between nanoscale surface roughness and cell proliferation rate for both cell types considered. Interestingly, AFM images of living adherent cells on these nanotextured surfaces demonstrate direct interactions between cellular protrusions and topographically distinct features. These results illustrate and quantify the significant differences in material surface properties among these ubiquitous materials, allowing us to better understand why the dish can make a difference in biological experiments.

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### 1. Introduction

Researchers studying biological systems attempt to mimic physiological conditions by systematically controlling specific aspects of *in vitro* culture. These parameters include solution pH, culture media nutrient composition, oxygen tension, frequency of media exchange and the number of cells per volume of liquid media. It is widely appreciated from practical experience, and strict adherence to established cell culture protocols, that each individual feature can significantly influence cell behavior [1–3]. Even the choice of modern culture surfaces, including the source of tissue culture polystyrene (TCPS), is known anecdotally to play a key role in repeatability of cell culture observations though the reasons remain unclear. Here, we explore and quantify variations in physical properties among such culture surfaces, as well as the corresponding effects on protein adsorption and cell behaviors.

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Through the 1950s, borosilicate glass was the culture surface of choice [4]. So-called tissue culture polystyrene was accidentally discovered when researchers at Falcon Plastics Company were attempting to coat plastic with glass to create cultureware. Instead, the oxygen plasma treatment of the polystyrene produced an optimal surface without the glass [5]. Today, TCPS is one of the most commonly used surfaces in mammalian cell biology [5]. Numerous commercial sources of TCPS are available, creating a wide variety of manufacturers, surface chemistries and culture formats [6]. Polystyrene manufactured for tissue culture uses a special resin formulation with tightly controlled molecular weight distribution. The tissue culture formats (flasks, dishes, multi-well plates, etc.) are created using high speed, hot runner injection molding [5]. After molding, polystyrene plates and flasks are placed in a vacuum chamber and undergo reactive gas plasma and secondary treatments to render the surfaces hydrophilic, enhancing cell–substrate adhesion [5]. Many manufacturers offer special proprietary techniques and qualification standards, the details of which are seldom published [7], such as the Nunclon<sup>®</sup> Delta or Greiner Bio-One Advanced TC<sup>™</sup> brands, to certify the consistency of their manufacturing processes and repeatable surface characteristics. Despite these

assurances by various manufacturers, inconsistent or irreproducible results within a given set of *in vitro* experiments are often blamed on the culture dish. A common “solution” is to then change the format or brand of dish [8,9]. However, given that TCPS is a material that is a means to an end in most biological and biomaterials investigations, the source(s) of variation among cell culture responses to different types and sources of TCPS typically remain uninvestigated.

We considered whether the largely overlooked differences in material surface features and properties among commercial TCPS sources contribute strongly to such discrepancies among *in vitro* culture results for ostensibly identical experiments. Researchers increasingly appreciate that surface features such as roughness and mechanical stiffness, independent of surface chemistry and media conditions, can play a significant role in guiding cell behavior [10–12]. Substratum topography can alter organization of the cytoskeleton, and influence attendant properties such as adhesion, proliferation, migration and differentiation potential [13–16]. Measurable effects of surface roughness on cell behavior have been demonstrated for polystyrene, as well as for hydroxyapatite, polydimethylsiloxane, polymethyl methacrylate and even titanium [17–20]. The size of these features can also play a significant role in cellular response [21,22]. In this study, we considered numerous samples of commonly used TCPS culture vessels to quantify variations in surface topography and other physical features as a function of commercial source and of culture vessel formats. We then investigated whether these differences affected cell morphology and behavior. The aim of this study was not to identify or claim that one TCPS source is “better” than others, but rather to understand how the material processing and surface properties varied and correlated with protein adsorption and cell responses.

## 2. Methods

### 2.1. Atomic force microscopy

Samples were prepared from tissue culture surfaces found in Table 1 and characterized via atomic force microscopy (AFM; MFP-3D Asylum Research, Santa Barbara, CA) within an inverted optical microscope (IX51, Olympus America, Inc.) and imaged in air using AFM cantilevers of nominal spring constant  $k = 0.035 \text{ N m}^{-1}$  and probe radius  $R = 25 \text{ nm}$  (MLCT, Veeco, Malvern, PA). At least five surfaces were analyzed for each sample type and manufacturer. Root mean squared (RMS) roughness values were extracted from height trace images using the scientific computing software Igor Pro (Wavemetrics Portland, OR) and reported as reported as mean  $\pm$  standard error of measurement.

### 2.2. Atomic force microscopy of cells on dishes

NIH 3T3 fibroblasts were cultured on Falcon Petri dishes of 35 mm diameter (P35) at a density of  $\sim 15,000 \text{ cells cm}^{-2}$  in 10% bovine calf serum (BCS) in Dulbecco's modified Eagle's medium (DMEM). After 1 day of incubation, media was aspirated and cells were incubated for 15 min at room temperature in a 4% paraformaldehyde (AlfaAesar 43368 Ward Hill, MA) solution in phosphate

buffered saline (PBS). Five rapid washes in PBS + 0.05% Tween-20 (Teknova P1176 Hollister, CA) were performed before imaging via AFM under contact mode, as described above, in  $1 \times$  PBS.

### 2.3. Contact angle measurements

Approaching contact angle measurements were taken with a VCA 2000 Video Contact Angle System (AST Inc.) goniometer. Contact angles were measured by dropping a single droplet of double deionized water ( $\text{ddH}_2\text{O}$ ), DMEM or DMEM + 10% BCS onto samples prepared from the tissue culture side of Celltreat<sup>®</sup>, Corning<sup>®</sup>, Cyto One<sup>®</sup>, Falcon<sup>™</sup>, Greiner Bio One Cellstar<sup>™</sup>, Nunclon<sup>™</sup>, Sarstedt, 75  $\text{cm}^2$  TCPS culture flasks (see Table 1) and compared to a 100 mm diameter VWR non-tissue culture Petri dish (#25384-088). VCA OptimaXE (AST Inc.) software was used to estimate angles on the left and right sides of contact.

### 2.4. Immunocytochemistry

To assay orientation of vinculin and F-actin, NIH 3T3 murine fibroblasts were fixed using 4% paraformaldehyde (AlfaAesar 43368 Ward Hill, MA) in PBS for 15 min at room temperature after 24 h. Following fixation, cells were washed briefly with PBS containing 0.05% Tween-20 and permeabilized for 3 min at room temperature with 0.1% Triton X-100 (Fluka 93443, Switzerland). To minimize non-specific binding, cells were treated with 3% bovine serum albumin (BSA; Sigma, A7906) in PBS for 30 min before staining. Cells were incubated at room temperature with relevant primary antibodies in 3% BSA for monoclonal anti-mouse vinculin (Sigma V4505, 1:200). Cells were then incubated with secondary antibody goat anti-mouse IgG (Abcam6785, 1:400). Cells were also double labeled with Alexa Fluor 555 Phalloidin (Molecular Probes, A34055, 1:1000) for 60 min. Cells were rinsed three times (10 min each) with PBS and imaged by fluorescence microscopy (IX-81, Olympus America, Inc.) and captured using Slidebook 5.0 (Intelligent Imaging Innovations, Inc., Denver, CO). Cell nuclei were also counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Millipore 90229, 1:2000).

### 2.5. Cell area

Human bone-marrow-derived mesenchymal stromal or “stem” cells (hMSCs; ReachBio Seattle, WA) were expanded until passages 4–6, and plated onto P35 TCPS dishes at low density ( $\sim 5000 \text{ cells cm}^{-2}$ ) to maintain subconfluent culture conditions. NIH 3T3 murine fibroblasts were seeded in P35 dishes from each manufacturer at a density of  $\sim 15,000 \text{ cells cm}^{-2}$ . hMSCs were cultured in typical basal MSC culture media consisting of complete MesenCult medium (MesenCult basal plus with 20% MesenCult Supplemental; StemCell Technologies, Vancouver, BC) and  $2 \mu\text{M}$  L-glutamine,  $100 \text{ units ml}^{-1}$  penicillin and  $100 \mu\text{l ml}^{-1}$  streptomycin (Invitrogen, 15140-163, Carlsbad, CA). hMSCs and NIH 3T3 fibroblasts were fixed and immunocytochemistry was conducted, as described above. Cells were imaged by fluorescence microscopy (IX-81, Olympus America, Inc.) and captured using Slidebook 5.0 (Intelligent Imaging Innovations, Inc., Denver, CO). Cell areas were

**Table 1**  
Manufacturer-specific catalog numbers for TCPS samples analyzed in this study.

	Celltreat <sup>®</sup>	Corning <sup>®</sup>	CytoOne <sup>®</sup>	Falcon <sup>™</sup>	Cellstar <sup>™</sup>	Nunclon <sup>™</sup>	Sarstedt
75 $\text{cm}^2$ flask	229341	430641	CC7682-4875	353135	658175	178905	83.1813.302
35 mm Petri dish	229635	430165	CC7682-3340	353001	627160	153066	83.1800
60 mm Petri dish	229660	430166	CC7682-3359	353002	628160	150228	83.1801
Six-well plate	229106	3516	CC7682-7506	353046	657165	140675	83.1839
96-well plate	229196	3598	CC7682-7596	353072	655162	160004	83.1835

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