TECH REPORT

118:

CellSoft®

Soft Substrate Culture Ware

From brain to bone!

Soft substrates that mimic tissue stiffness.

07-23-21

Culturing Cells in a Mechanically Active Environment™
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PRODUCT DETAILS:
CellSoft® 100mm round dishes
CellSoft® polystyrene 6-well plates
CellSoft® BIOFLEX® flexible-bottom plates

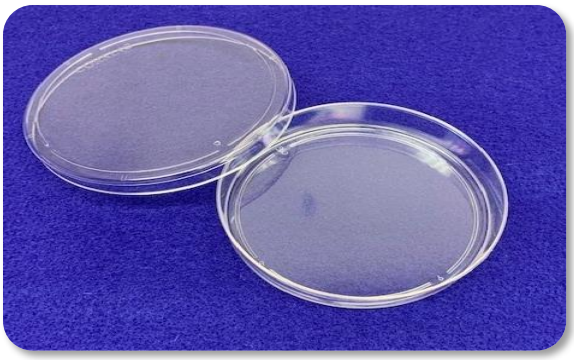

Figure 1. *CellSoft® 100mm round dish*

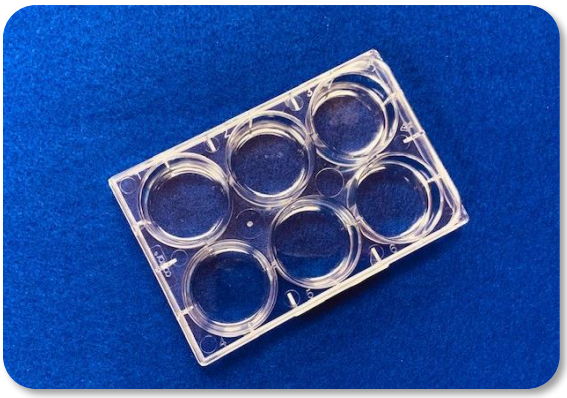

Figure 2. *CellSoft® polystyrene-bottom plate*

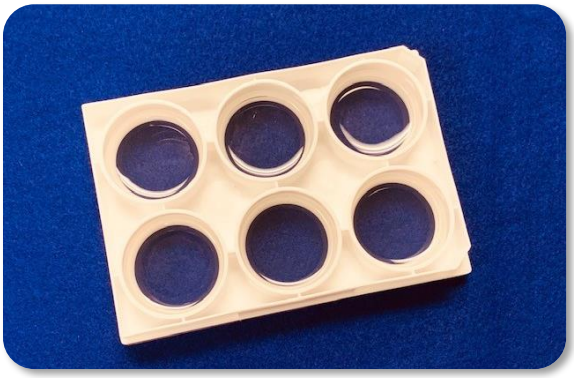

Figure 3. *CellSoft® BioFlex® flexible-bottom plate*

Matric Coatings:
Collagen type I, Untreated

Substrate Stiffness:
1, 5, 10, 20, 40, 60, 80 kPa

INTRODUCTION

Conventional cell culture labware designed to grow cells in vitro, is comprised of polystyrene growth surfaces that are either gas plasma-treated or coated with matrix attachment proteins to allow cell attachment. The polystyrene surface alone is hydrophobic and must be treated to sustain cell attachment. Moreover, a typical polystyrene dish growth surface is a replicate of the steel mold from which it was made. That means whatever surface roughness was in the mold gets transferred to the lab dish surface. Another facet of virgin, grade A polystyrene is that it is quite hard and stiff, about 1 GigaPascal in stiffness as measured by indentation (1 GPa). Tissue stiffnesses range from 1-5 GigaPascals (1-5 GPa) for bone or calcified cartilage (Hou et al., 1998), to less than 1 kiloPascal (1 kpa) for fat, brain and even a cell itself (Pratt et al., 2020).

Cells have been grown on “soft” substrates for many years, often without the investigator including a control for growth surface stiffness in the experimental protocol. For instance, early embryologists grew cells and even organs on fibrin clots, denatured collagen (gelatin material) or even in or on agarose (Baitsell, 1915). Virologists used neutral-red laden, agarose overlays, *in vitro* as a method to slow the diffusion of viruses from infected cells, Thereby, enumerating the number of viral plaques in a viral

dilution (clear areas of dead cells that could no longer pump out the neutral red dye) and hence, the number of infectious particles present (Baer and Kehn-Hall, 2014).

In today's 3D cell culture world, including engineered tissues, cell growth on synthetic biomaterials such as vicryl, nylon, silk, cotton, Gortex®, poly-lactic acid and a myriad of other materials, directs the investigator to consider how growth with substrate stiffness, as well as chemistry, affect a cell's response to its environment. Therefore, labware culture plates with substrate stiffnesses that match that of a cell's native tissue of origin or cellular niche, represent a more natural growth environment and should be considered in experimental procedures.

Data indicate that stem cells of various types, grown on soft, 2D substrates (<1 kPa to 5 kPa) maintain their phenotype for a longer period than those grown on stiff, polystyrene substrates (Disher et al., 2005; Engler et al., 2006; Buxbaum et al., 2010; Vertelov et al., 2016; Gerardo et al., 2019; Pagliari et al., 2021).

Flexcell International Corp. was the first commercial company to offer "soft substrates" with the Flex I and Flex II products in 1988. It was recommended that the user culture their cells on the control, Flex II surface that had a cast silicone elastomer surface covalently treated with Collagen type 1 peptides. The intent was that the user should grow their cells on the same, soft elastomer surface as that which would then be used to mechanically stimulate the cells on a Flex I flexible-bottom plate (according to a particular strain regimen with controlled magnitude, duration and frequency with a Flexcell® Strain Unit). The point was to avoid a "growth substrate shock" by transferring cells from a stiff, polystyrene substrate to a soft, silicone elastomer substrate. This cell shock in substrate stiffness shift is like the carbon source shift that bacteria experience when shifted from glucose to galactose, for instance. Bacteria upregulate the enzymes needed to take up and metabolize the new carbon source. Likewise, cells grown on soft substrates tend to have less robust actin cytoskeletons and integrins with which to attach to the substratum (Pagliarai et al., 2021). Discher and coworkers popularized the use of "soft substrates" when they published the effects of growing cells on controlled stiffness substrates in polyacrylamide gel materials (Engler et l., 2006).

References:

1. Hou et al., J. Biomech. 31: 1009-1015, 1998.
2. Cooper et al., J. Neurotrauma 37:494–506, 2020.
3. Alan Baer, Kylene Kehn-Hall JOV 2014.
4. Engler et al., Cell 126:677-689, 2006.
5. Discher et al., Science 310: 1139-1143, 2005.
6. Buxbaum et al., J Phys Condens Matter 22(19): 1-19, 2010.
7. Gerardo et al., Sci Rep 9:9086, 2019.
8. Vertelov et al., Sci rep 6:3411, 2016.
9. Pagliari et al., Cell death and Diff. 28:1193-1207, 2021.
10. Pratt et al., PNAS 117: 26008-26019, 2020

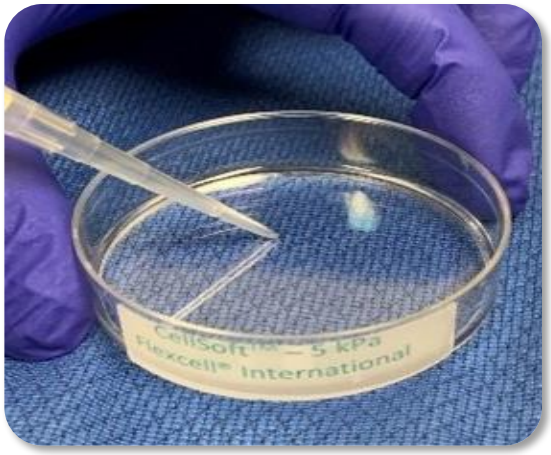

Figure 4. *CellSoft® substrate (5 kPa stiffness silicone elastomer polymer) in the 100 mm diameter polystyrene dish Panel A.*

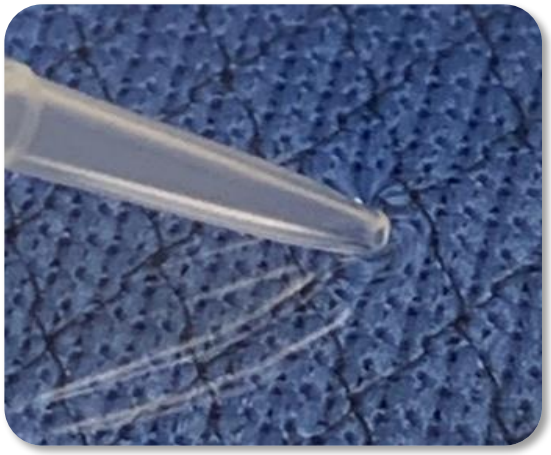

Figure 5. *Image shows a pipette tip deforming the surface of the growth substrate with wrinkles in the substrate Panel B.*

Product Description:

CellSoft® cell culture labware products are designed to match the stiffness of tissue niches in which cells reside in vivo. CellSoft® labware products consist of proprietary mixtures of silicone elastomer polymers to yield consistent, thickness and stiffness growth surfaces of high optical clarity, low auto-fluorescence and reproducible cell attachment and growth qualities.

The select stiffnesses are 1, 5, 10, 20, 40, 60 and 80 kPa, as measured by indentation with a Piuma instrument (Piuma model, Optics 11 Inc. Netherlands). CellSoft® labware comes in 100 mm diameter culture dishes, 6 well polystyrene plates and 6 well Bioflex® flexible-bottom plates with CellSoft® polymer substrates. CellSoft® products are either non-coated or covalently bonded with whole chain bovine, collagen type 1.

Quality Assurance:

Each batch of CellSoft® labware products is tested for physical properties by indentation testing with a Piuma nanoindentor (Optics11 Life, Amsterdam, The Netherlands).

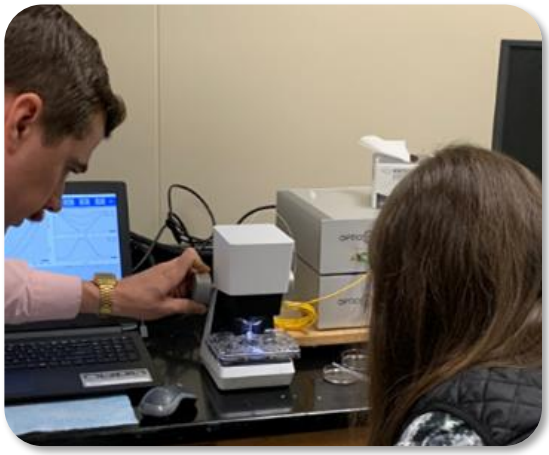

Figure 6. CellSoft® soft substrate product under test conditions for stiffness determination.

A minimum of 9 indentations are performed at unique positions on the sample plate's surface using a probe with 0.5 N/m stiffness and a 50 µm radius spherical tip.

The Young's modulus (E) is calculated from each indentation curve using the Optics11 Dataviewer software program (V2.4), applying the Hertzian model to fit the data to 1.5 µm indentation depth.

Figure 7. *Base instrument holding the specimen*

The Young's modulus results for all indentations are averaged and recorded in the batch's quality record.

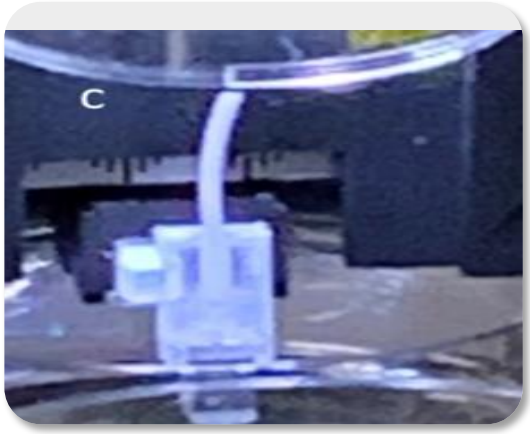

Figure 8. *Nanoindentor holder with cantilever probe contacting the substrate surface for a measurement*

Cell attachment properties:

Each batch of CellSoft® labware products is tested for cell attachment and growth with three different cell lines (MC3T3E1 osteoblast-like cells, CH3T10 ½ stem cell -like cells, and C2C12 muscle-like cells).

CellSoft® 100 mm diameter, polystyrene, round dishes: CellSoft® polymer is robotically deposited in each 100 mm diameter round dish (56.7 cm^2 growth surface area (GSA)) and allowed to cure in a clean room environment. The cured polymer is then derivatized with collagen type 1 or other peptides, using Flexcell's proprietary, covalent bonding protocol (Banes 1988, USPTO). Stiffness is verified with a Piuma indentation protocol. Product is then packaged, gamma sterilized and shipped. Shelf life is one year from date of manufacture stored at 4C protected from light. The product can be stored at 25C protected from light.

CellSoft® 6 well, 35 mm diameter per well, polystyrene culture plates (10 cm^2 GSA/well). CellSoft® is dispensed robotically to each well, then growth surfaces are derivatized with collagen type 1 or other peptides, using Flexcell's proprietary, covalent bonding protocol (Banes 1988, USPTO). Stiffness is verified with a Piuma indentation protocol. Product is then packaged, gamma sterilized and shipped. Shelf life is one year from date of manufacture stored at 4C protected from light. The product can be stored at 25C protected from light.

CellSoft® in Bioflex®, flexible bottom culture plates (10 cm^2 GSA per well). CellSoft® is dispensed robotically to each well, then growth surfaces are derivatized with collagen type 1 or other peptides, using Flexcell's proprietary, covalent bonding protocol (Banes 1988, USPTO). Stiffness is verified with a Piuma indentation protocol. Product is then packaged, gamma sterilized and shipped. Shelf life is one year from date of manufacture stored at 4C protected from light. The product can be stored at 25C protected from light.

Cell Culture Methods:

Dissociating cells and plating cells on CellSoft® culture surfaces:

1. Wash cells with PBS pH 7.2, 5 min. Add 1 ml dissociation fluid per 100 mm CellSoft® culture dish and scale volume for other CellSoft® culture dish formats (6 well (0.5 ml dissociation reagent) or 24 well (0.2 ml). Dissociate cells using 0.025-0.25% trypsin or 0.1-0.5% collagenase in balanced salt solution as per your usual protocol for your cell type. The covalently bonded collagen (or other peptide surface) surface on CellSoft® growth surfaces is compatible with the use of standard trypsinsinization or collagenase treatment procedures.
2. Incubate cells for 5 min in sterile PBS pH 7.2, or other like solution at RT or 37C. Aspirate wash fluid from the edge of the dish or well to waste. Be careful to cant the dish and touch the pipet tip at the edge of the polystyrene wall of the culture dish, so as not to apply vacuum to the soft substrate. Vacuuming the soft silicone elastomer will plug the aspiration pipet.
3. Add the appropriate amount of dissociation reagent to each dish or well (as above). One normally would add 1 ml of 0.25% trypsin to a 100 mm diameter dish to coat cells with trypsin, for instance. You should add 2 ml of trypsin and 2-3 ml PBS or one ml trypsin and 2-3 ml PBS so that the cell sheet is covered with fluid. The CellSoft® surface requires additional fluid to completely cover the growth surface. Incubate 5-10 min at 37C to allow reagent to act on protein to release cells from the substrate and each other.
4. Add 5 ml PBS to 100 mm diameter dish. Cant the dish back and forth to move the overlying fluid and shear the cells from the

substratum. Pipet the cells and fluid up and down in the dish to free cells, then transfer to a sterile 15 ml, conical tube and sediment at 200 x g to pellet the cells.

5. Post-centrifugation, decant overlying fluid to a waste cup and reconstitute cell pellet in the appropriate volume of growth medium to plate your cells at 5-25 k cells/cm^2 growth surface area. For instance, use 15 ml growth fluid medium in a 100 mm diameter culture dish. Count cells using your method of choice (hemocytometer, electronic cell counter).

6. To dispense your cells to a CellSoft® surface, add fluid at the edge of the culture dish or well to avoid penetrating the CellSoft® surface. 1 kPa surfaces are more susceptible to deformation than 5-60 kPa surfaces. Soft surfaces are elastic and will resume their shape after penetrating the surface.

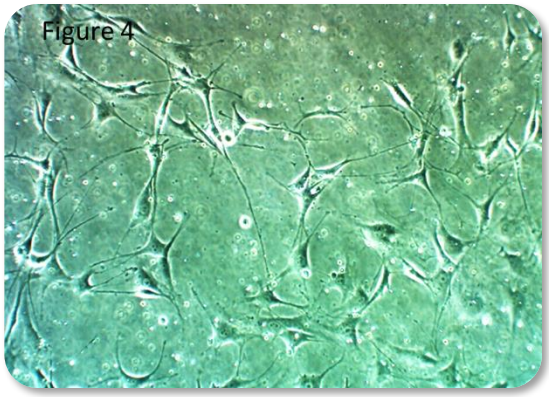

Figure 9. C3HT101/2 cells replated from CellSoft® 5 kpa to CellSoft® 5 kpa culture dish (10x phase contrast).

Notes:

1. Cells attached to CellSoft® substrates of the softest categories (1-5 kPa) have a lesser diameter initially than the same cells attached to a stiff substrate (polystyrene culture surface or a stiffer, silicone elastomer substrate (10-100 kPa).

2. Cells can be enzymatically removed from CellSoft® substrates more easily than from polystyrene surfaces. Less trypsin or collagenase may be used for a briefer period of treatment time for most cells grown on CellSoft® substrates than on polystyrene substrates, even with collagen treatment.

3. CellSoft® substrates can be re-plated with your cells 2-3X before the surface will no longer sustain cell attachment.

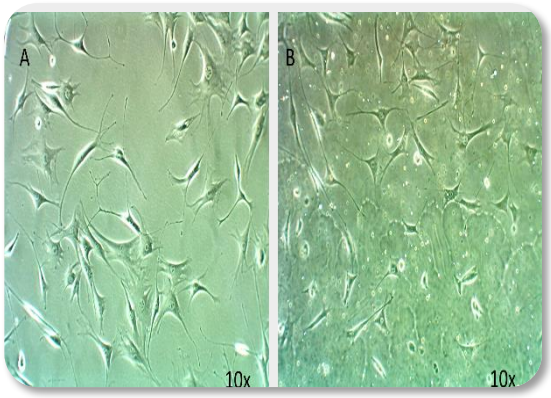

Figure 10. a *CH3T101/2 stem cell-like cells on Corning polystyrene culture surface (24h 10x phase contrast). Figure 9b CH3T101/2 cells on 5 kPa CellSoft® surface bonded with type 1 collagen. (10X, phase contrast).*

Note the CellSoft® surface shows small air bubbles as refractive spots in the background.