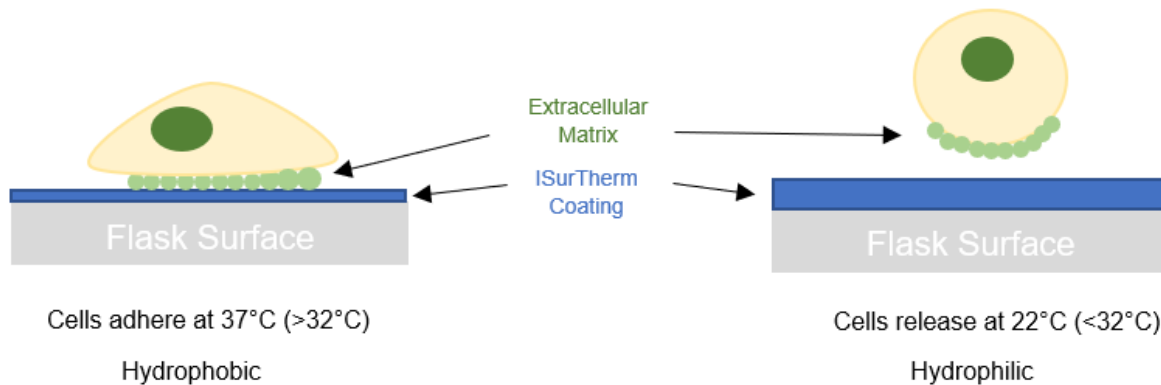


ISurTherm Thermoresponsive Cell Culture Surfaces

The thermoresponsive T25 ISurTherm® flasks from ISurTec® allow users to harvest strongly adherent cells without enzymatic digestion. The harvested cells retain high viability and intact cell junction proteins. Without the need for trypsin or other enzymes for passaging, cost and contamination risk are greatly reduced. ISurTherm is designed for use with strongly adherent cell lines such as human mesenchymal stem cells (hMSC) as well as typical adherent cell types, including fibroblast and endothelial cells.



No enzyme is needed. The cells release within 30 minutes when brought to room temperature. The thermoresponsive coating is hydrophobic at 37°C but becomes hydrophilic below 32°C. This causes the cells to release with their extracellular matrix (ECM) and cell-to-cell junctions intact. Furthermore, disruption of transmembrane signaling complexes is avoided. Depending on the degree of confluence when brought to room temperature, the cells can be released as single cells, small clusters, or cell sheets.

Advantages

- Quick and easy cell harvests
- No enzymatic digestion or cell scraping
- ECM and cell junctions remain intact
- Lower contamination risk
- Retention of cell signaling protein complexes
- Release hMSC cells and other strongly adherent cell lines
- Harvest cells as single cells, clusters, or sheets
- Fast recovery and reattachment for propagation

Quality

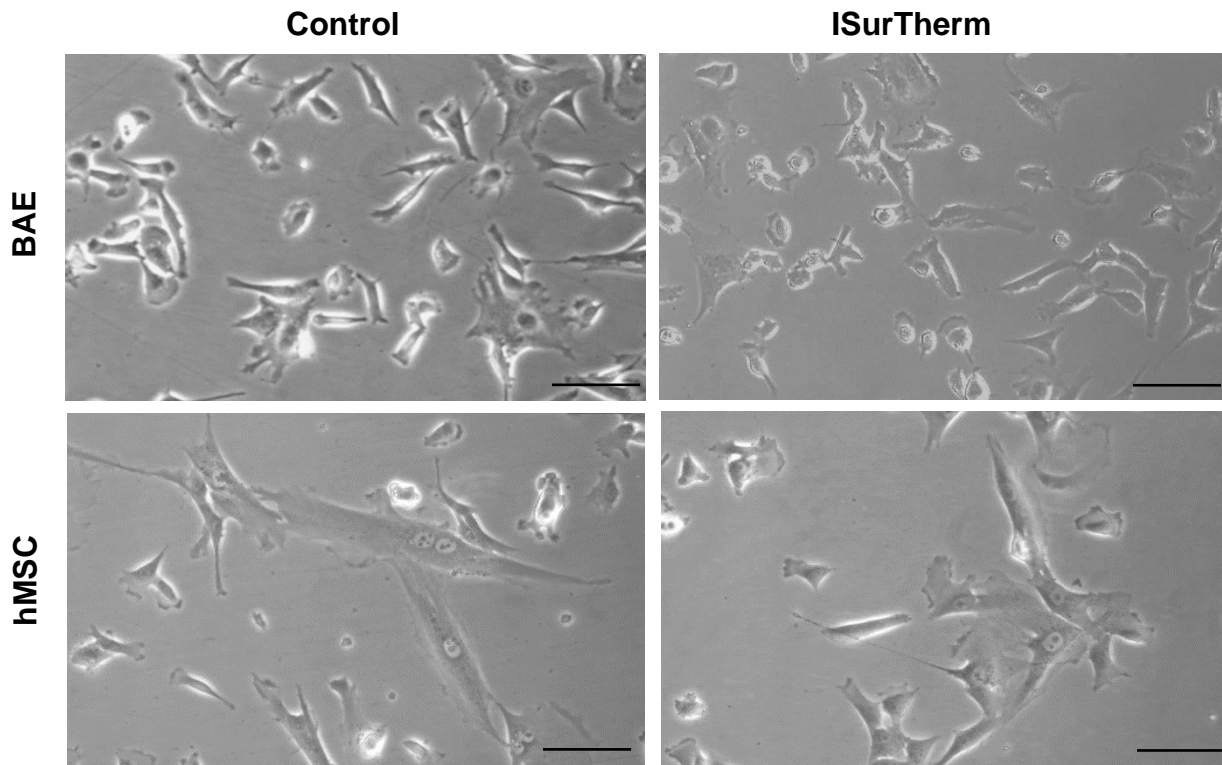
Each lot is tested for cell confluence and detachment with human dermal fibroblast cells (hDF). The minimal acceptance level is 80% confluence and 80% detachment.

ISurTherm surfaces have greater than 90% cell viability determined by viability stains (Invitrogen, Molecular Probes). The endotoxin level is below 0.1 EU/mL (GenScript).

ISurTherm flasks are sterilized via ethylene oxide gas (Andersen Products) to a sterility assurance level (SAL) of 10^{-6} .

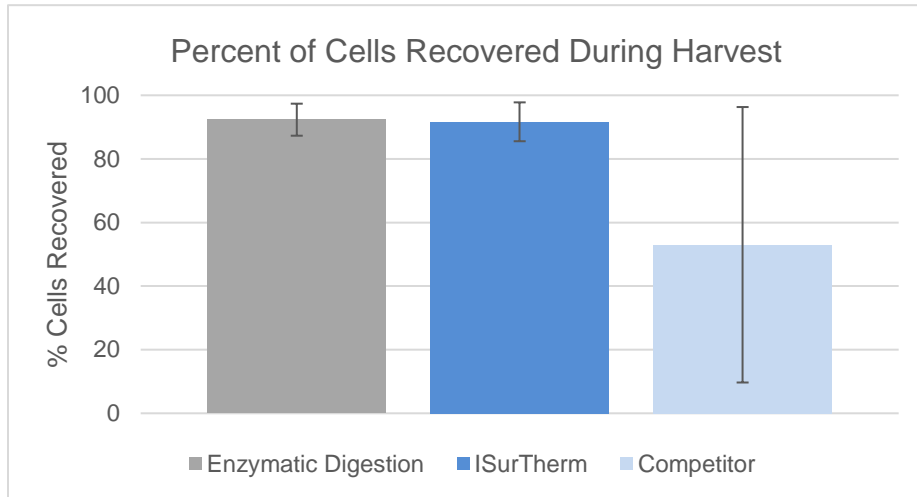
Comparison to uncoated and competitor thermoresponsive surfaces

hMSC and bovine aortic endothelial cells (BAE) were cultured for 48-hrs on ISurTherm, uncoated TCPS flasks, and a competitor's thermoresponsive surface in MSC Gro low serum media (Vitro Biopharma) or 10% fetal bovine serum in DMEM (Gibco), respectively. The cell morphology was observed to be the same across the surfaces.



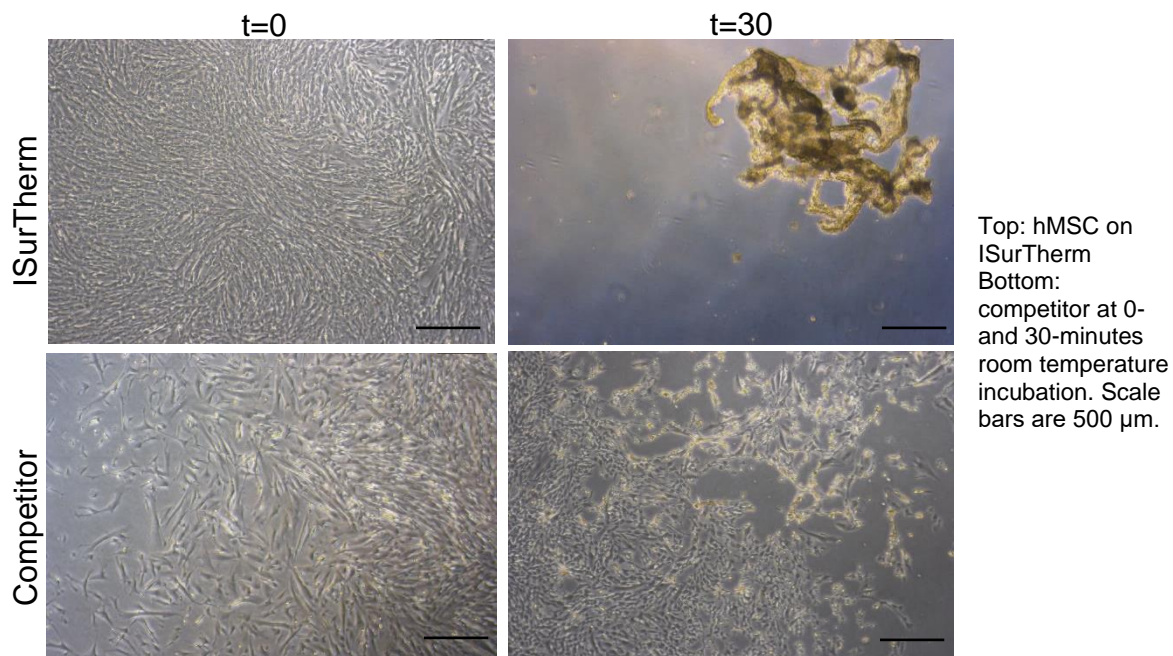
Control surface (left) and ISurTherm (right) surfaces after 48-hrs in culture; top BAE cells, bottom: hMSC. Scale bars 100 μ m.

When the ISurTherm flasks and multiwell plate were brought to room temperature for 30 minutes and the control flask treated with an enzyme, the ratio of cells released versus unreleased was determined via a metabolic assay (alamarBlue™ ThermoFisher) and by cell counts (ImageJ). The hMSC data is shown below. The ISurTherm flask released more cells and had less variability in release than the competitor. The number of cells released from ISurTherm was similar to the standard enzymatic digestion method.

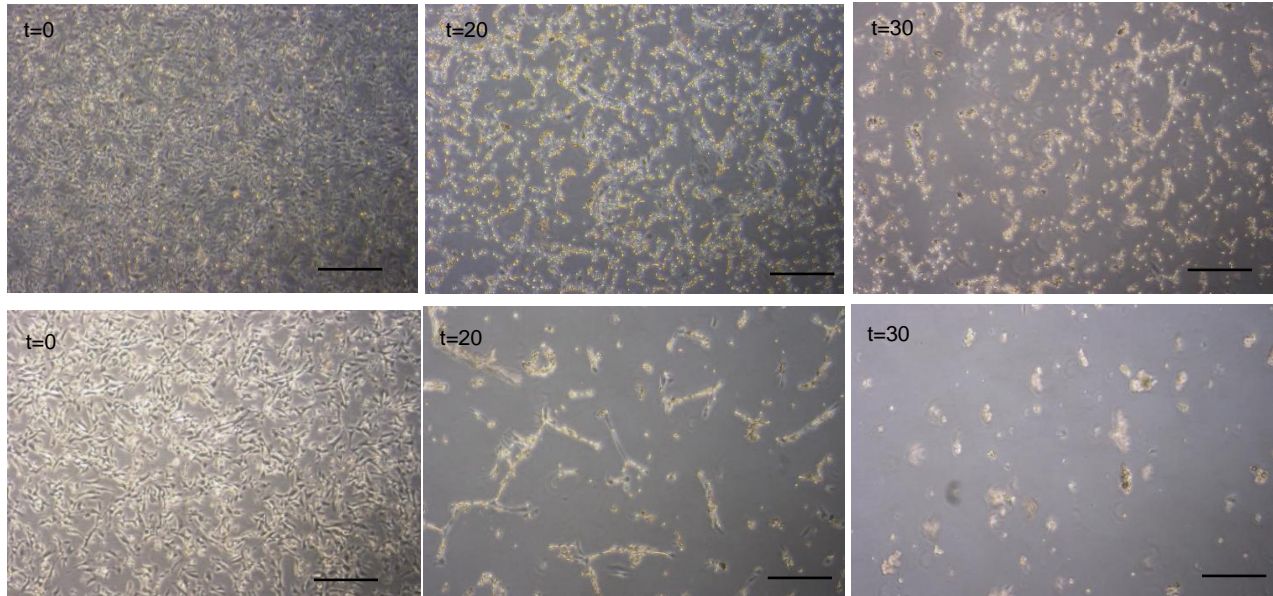


hMSC were counted via ImageJ before and after release with enzymatic digestion, ISurTherm T25 flask, and a competitor's thermoresponsive surface. The experiment was done in triplicate with cell counts taken in triplicate in random locations across the surfaces.

In comparison with the commercial thermoresponsive multiwell plate option, ISurTherm released hMSC faster and more completely after 30 minutes at room temperature.



ISurTherm releases strongly adherent cells within 30 minutes:



BAE (top) and hMSC (bottom) released from ISurTherm T25 flask at 0, 20, and 30 minutes at room temperature. All scale bars 500 μ m. More cells from release are in suspension out of the image focal plane.

ISurTherm cell sheet release:

Cell sheets can also be created on ISurTherm surfaces. hDF cells were grown to confluence in fibroblast growth media with 2% FBS (ATCC, Gibco). For harvesting, the flask incubated for 30 minutes at room temperature and a cell sheet released from the ISurTherm surface.

The cell-to-cell junctions in released cell sheets are maintained with ISurTherm when stained for the gap junction protein Connexin-43.



hDF cells at 0 (left) and 30 min (middle) at room temperature. Right: a cell sheet released from ISurTherm stained with gap-junction stain Connexin-43. All scale bars 500 μ m.