Smart Thermo-responsive Surfaces for Cell Culture

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Non invasive lift off of cells growing on thermo-responsive surface

Loss of cell receptors, gap junctions and underlying ECM

Non invasive cell removal, adhesive proteins and cell-cell junctions are maintained, ECM is also perfectly preserved

37°C

25°C
Photo immobilization of poly (N-isopropylacrylamide) [PNIPAAm]

ISurTec® proprietary crosslinker, ISurLite™, generates thin, transparent, uniform and durable thermo-responsive coatings.

- Flat surfaces (multiwell dishes and T-Flasks)
- Porous Surfaces (PET membranes)
- Nanofibrillar surfaces
- Microcarriers (polystyrene microcarriers)
Comparison of three different cell dissociation methods

BAEC cells plated on TCPS and stained with anti-Laminin

A
Low temp lift off

B
Trypsin

C
Mechanical dissociation

A'

B'

C'

Detached BAEC cell sheets maintain the gap junctions.

A, B: cell sheets

C, D: the intact cell sheets readily reattach on fresh surfaces and maintain gap junctions.

Connexin 43 staining for gap junctions
Coatings are non-cytotoxic

Phase image of BAEC cells

Cells stained with calcein and ethydium. Note all the cells are stained positive with calcein which is a live cell permeant.
Thermo-responsive polymer coating on flat TCPS surface

BAEC

T47-D

PC12

Thermo-responsive coated

Uncoated TCPS
Thermo-responsive polymer coating on porous substrates (PET, 0.2 µm pore size)

BAEC = cell detachment starts as early as 7 minutes. Complete detachment = 30 minutes

T47D cells = cell detachment starts at 11 minutes. Complete detachment = 40 minutes
Thermo-responsive polymer coating on nanofibrillar surfaces

- Nanofiber surfaces mimics the native ECM surface and thus provide a more physiologically relevant surface. The coatings do not mask the nanofibrillar features.

- The high porosity of nanofiber surface offers better hydration of the polymer chains resulting in rapid cell release.

T47D cells
F actin staining
A: LIVE/DEAD cytotoxicity assay of BAEC cells growing on thermo-responsive polymer coated polystyrene microcarriers (20X).

B: 40X magnification of BAEC cells growing on microcarriers.

C: Low temperature removal of BAEC cells from the microcarriers.

D: Released cells after the temperature reduction for 20 minutes.

E: Released cells replated on fresh surfaces.
Comparison with commercially available thermo-responsive surfaces

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<th>ISurTec®- Thermo-responsive</th>
<th>Commercial surfaces</th>
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<td>25 minutes</td>
<td>25 minutes</td>
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<tr>
<td>T47-D</td>
<td>30 minutes</td>
<td>120 minutes</td>
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<tr>
<td>PC12</td>
<td>33 minutes</td>
<td>120 minutes</td>
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Conclusion

- ISurTec’s high performance thermo-responsive coatings have been applied with success to a variety of cell culture surfaces of different geometries.

- The immobilization strategy is fast and simple without the use of expensive equipment, hazardous chemicals or high energy source in the process.

- The coatings are non cytotoxic to all the cell lines tested.

- This noninvasive method of cell recovery has considerable value for applications in standard cell culture, tissue engineering, cell based therapies, extracellular matrix studies and cell tissue function.
Acknowledgment

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