49th ECLSO
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Novotel Tour Eiffel
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EUROPEAN CONTACT LENS SOCIETY OF OPHTHALMOLOGISTS

Limbal Explant Culture on Temperature Sensitive Smart Polymeric Surface Coated Contact Lenses

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Limbal Stem Cell Deficiency (LSCD)

- Loss or deficiency of the stem cells in the limbus that are vital for re-population of the corneal epithelium and to the barrier function of the limbus
- Epithelial breakdown and persistent epithelial defects, corneal conjunctivalization and neovascularization, corneal scarring, and chronic inflammation.

- Loss of corneal clarity, potential vision loss, chronic pain, photophobia, and keratoplasty failure.

Ahmad S, Osei-Bempong C, Dana R, Jurkunas U. The culture and transplantation of human limbal stem cells. Journal of cellular physiology 2010;225(1):15-9.
Sangwan VS. Limbal stem cells in health and disease. Bioscience reports 2001;21(6):385-405.

Etiology

Traumatic, iatrogenic, and malignant causes (more favorable)	Inflammatory, hereditary, and neuropathic causes (unfavorable)
• **Chemical or thermal burn** • **Multiple surgeries** • Radiation • Medical treatments (antimetabolites) • **Extended contact lens wear** • Infections (bacterial, fungal, or protozoal) • Neoplasia (BCC, SCC, MM)	• Stevens-Johnson syndrome • **Vernal keratoconjunctivitis** • Mucous membrane pemphigoid • Chronic bullous keratopathy • Neurotrophic keratopathy (trigeminal neuralgia, DM, herpes simplex, and zoster) • Aniridia • Epithelial dysplasia

Treatment Options

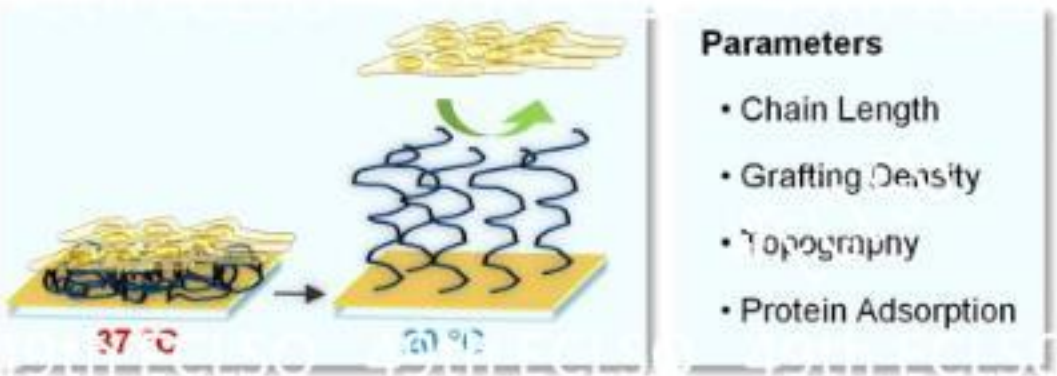
- Autologous limbal stem cell transplantation (LSCT)
- Aiiogeneic LSCT (from reiative)
- Allogeneic keratolimbal transplantation (from cadaver)
- Simple limbal epitheliai transplantation (SLET)
- Cultivated Limbal epithelial transplantation (CLET)

- **Corneal Regenerative Medicine**

CULTURE CONDITIONS	
Substrate	Medium and Supplement
• 3T3 Feeder Layer • Human Amniotic Membrane (HAM) • **Thermo-responsive surface (PIPAAM)**	• DMEM • Modified DMEM with various growth factors • Fetal Bovine Serum (FBS) • Serum free conditions

XENOBIOTIC-FREE HUMANIZED CULTURE SUBSTRATE AND MEDIUM SUPPLEMENTED WITH HUMAN SERUM

What is Thermoresponsive Surface?

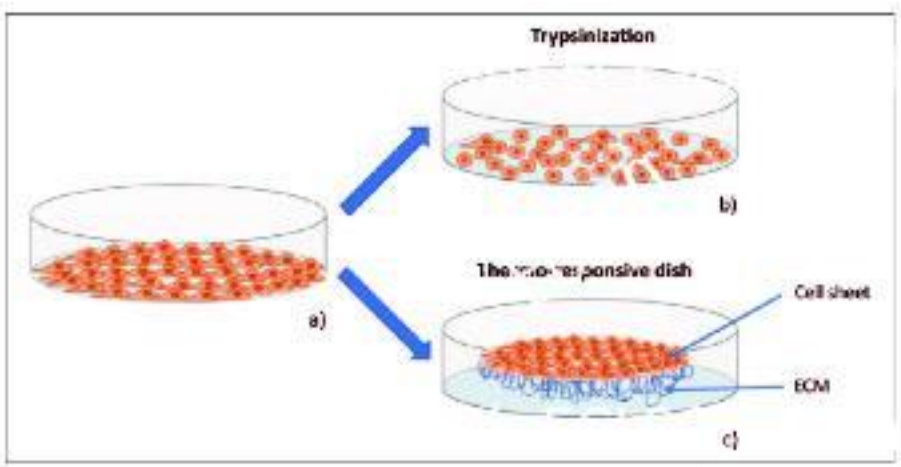


- Thermoresponsive polymers exhibit a drastic and discontinuous change of their physical properties with temperature.
- The chains of the polymers grafted onto the tissue culture substrate either collapse or extend as a consequence of phase transition near its' lower critical solution temperature (32°C).
- During the conventional cell culture at 37 °C, where the chains are collapsed, resulting in enhanced hydrophobicity and cell adhesion. Under the lower critical solution temperature (~ 25°C) the polymer is hydrated and the chains extend themselves causing the spontaneous detachment of cell monolayer from the substrate.

poly(Nisopropylacrylamide) (pNIPAm)
poly 2-(dimethylamino) ethyl methacrylate (pDMAEMA)

Cell Sheet Technology

- An intact confluent monolayer cell sheet can be obtained without the utilization of harmful digestion of enzymes (e.g. trypsin) and the preserved cell-to-cell interactions allow the survival of the cell sheet in the transplanted area.



Altomare, L., Bonetti, L., Campiglio, C. E., De Nardo, L., Draghi, L., Tana, F., & Farè, S. (2018). Biopolymer-based strategies in the design of smart medical devices and artificial organs. *The International Journal of Artificial Organs*, *41*(6), 337-359.

Aim

- The cell sheet technology has not yet been integrated to the surface of a contact lens!
- We use the commenced chemical vapor deposition (iCVD) approach, we coated the concave surface of the contact lens with pDMAEMA hydrogels and developed a thermo-responsive concave surface for cell culture.

ICVD is a free-radical polymerisation technique in which monomer and initiator vapours are given to the polymerisation surface in a vapour phase. The monomer molecules attach quickly on the refrigerated sample surface, whilst the initiator molecules are thermally destroyed in the deposition chamber, resulting in initiator radicals. The thin films created by CVD are of interest because they can grow on a variety of substrates and are not confined to flat surfaces or specific substrate materials. The risk of solvent-stimulated degradation, swelling, or dissolution of polymeric substrates is eliminated with CVD. As a result, CVD coating of arcuated polymeric lens surfaces is a viable option. The surface engineering of contact lenses via smart hydrogels has led our efforts to produce and characterize a novel cell sheet platform for the production of preserved cell layers during transplantation.

Aim

- At a filament temperature of 260°C and a stage temperature of 20°C, films were deposited. For all depositions, the vacuum chamber's total pressure was held at 0.6 Torr. To monitor layer growth and deposit appropriate thicknesses on substrates, a laser source (JDS Uniphase) was employed.

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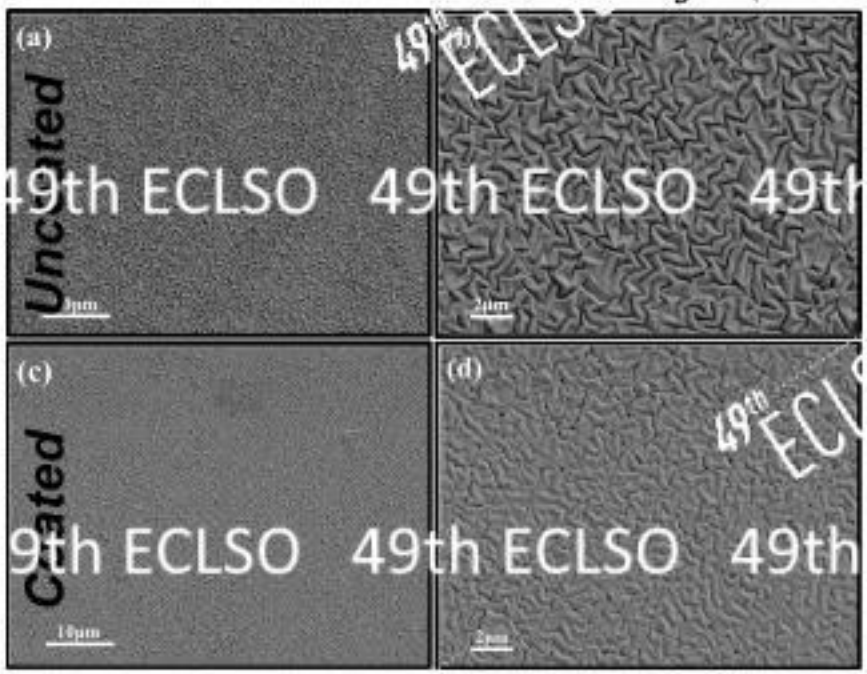
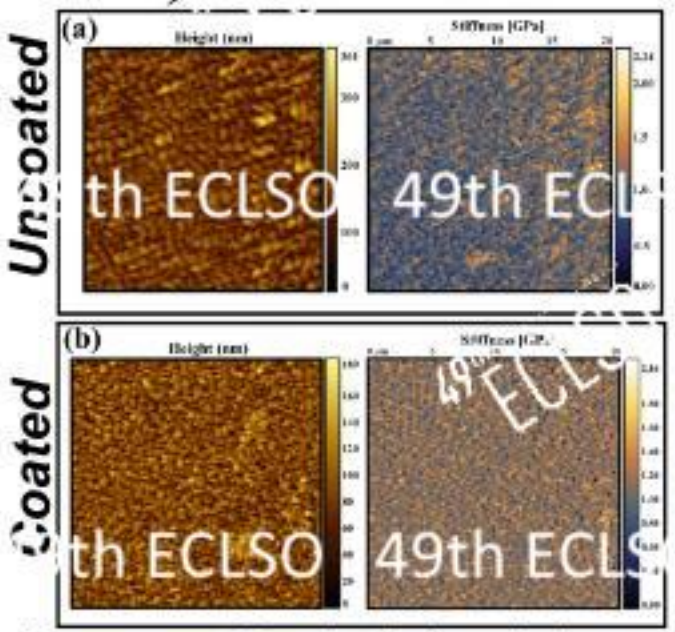
Characterization of P(DMAEMA-co-EGDMA) coated contact lenses



Uncoated contact lenses exhibit uniform roughness across the surface, but coated lenses appear to be smoother due to the presence of the thin film.



The rooth mean square is found to be lower in the coated lenses confirming the smoothing effect of the thin film. The average elastic modulus values was higher in the coated contact lenses due to the softer polymer coating. So, the roughness is lower and stiffness is higher in the coated contact lens surfaces.

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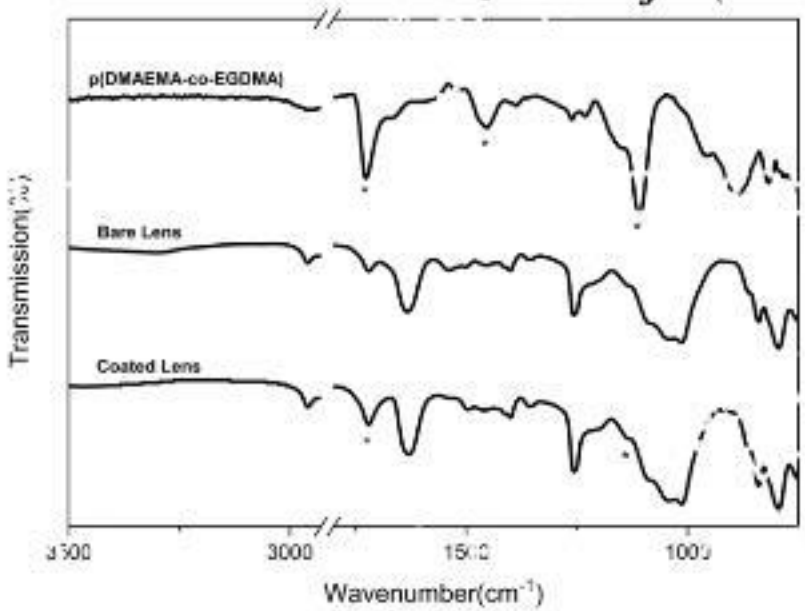
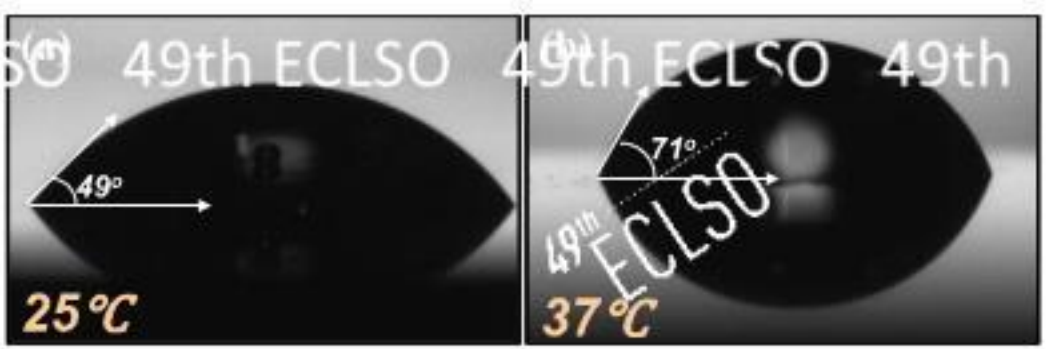


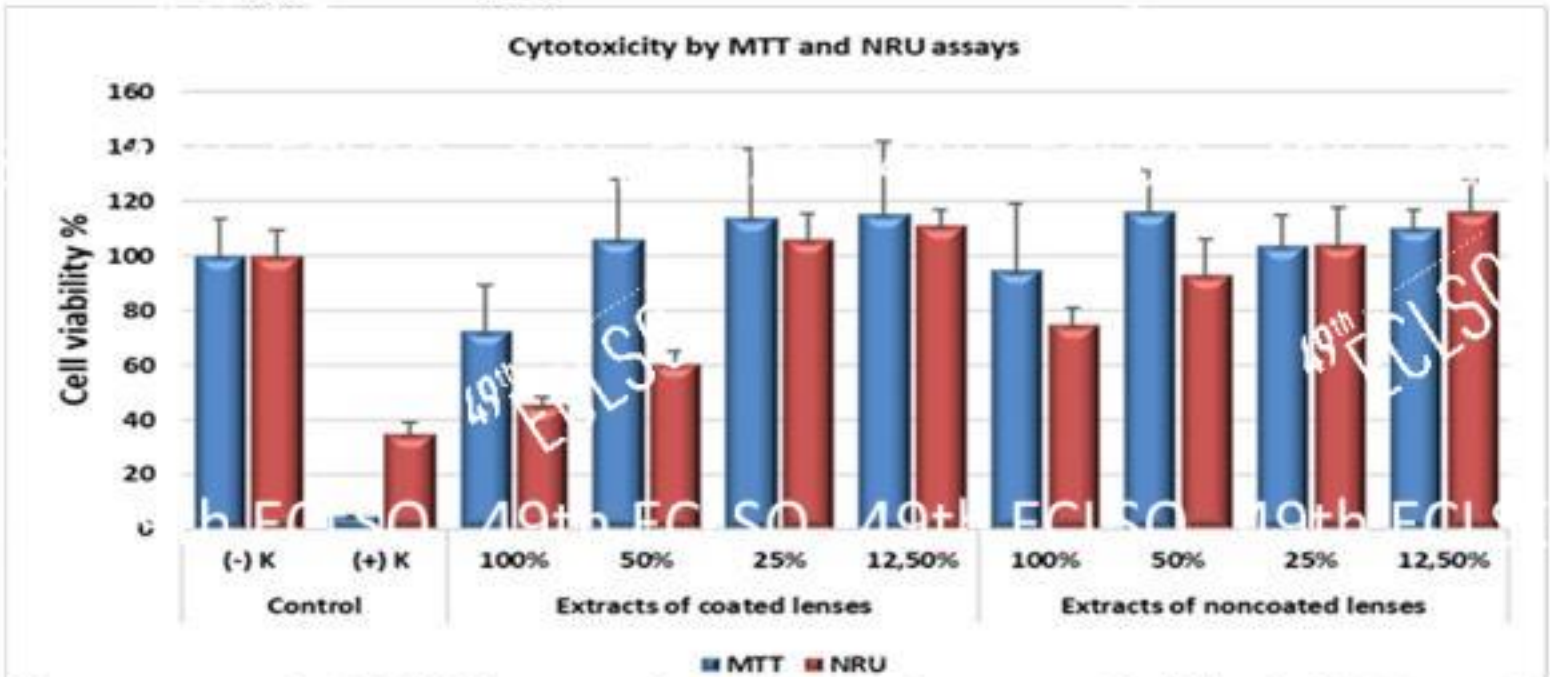
The chemical composition of the polymer coating is characterized by FTIR analysis. Unlike the bare lens, the absorbance peaks were similar with the polymeric composition in the coated contact lenses.

Coated Contact Lens



Increased hydrophilicity with lowering temperature

Cytotoxic effects of pDMAEMA coated contact lenses



Cell viability of the group treated with 100% extract of coated contact lenses was 72.4% in the MTT assay. The NRU assay was correlated with the MTT assay. So, low concentrations of extracts were determined as not cytotoxic.

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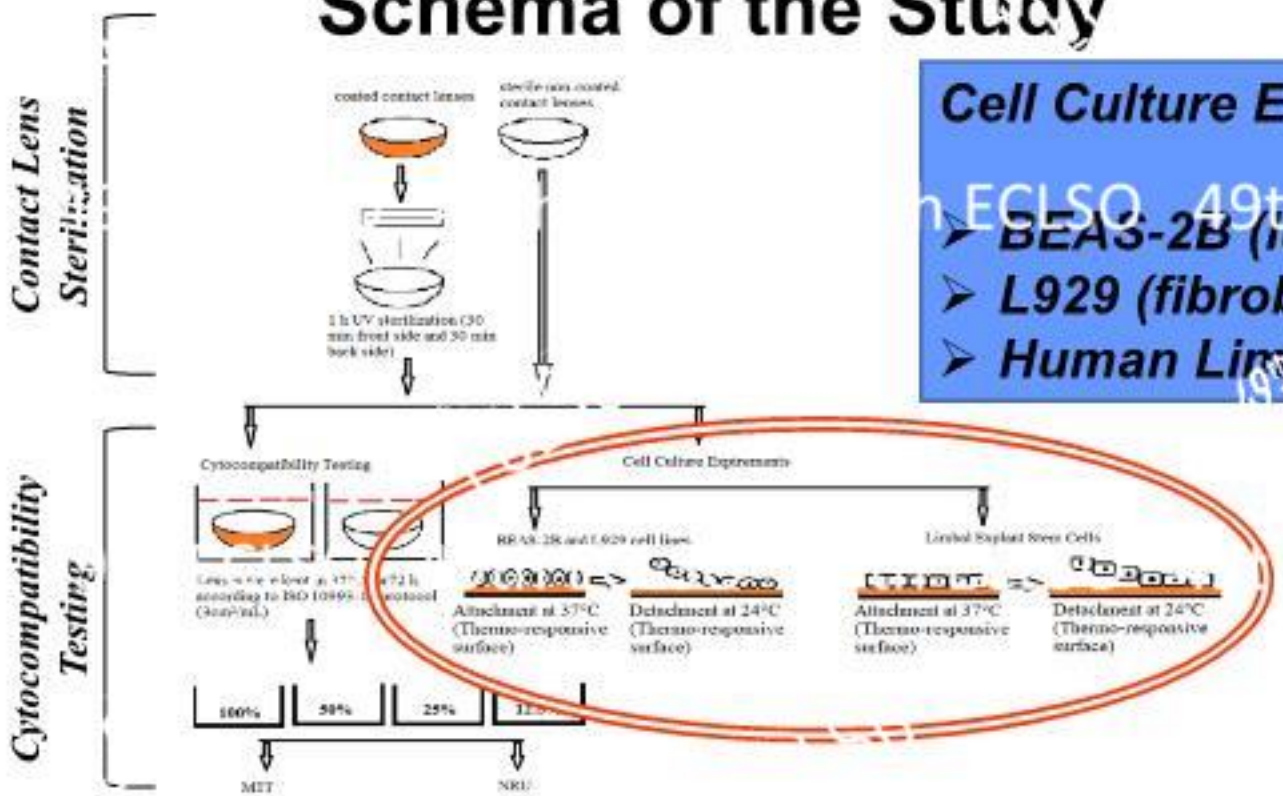
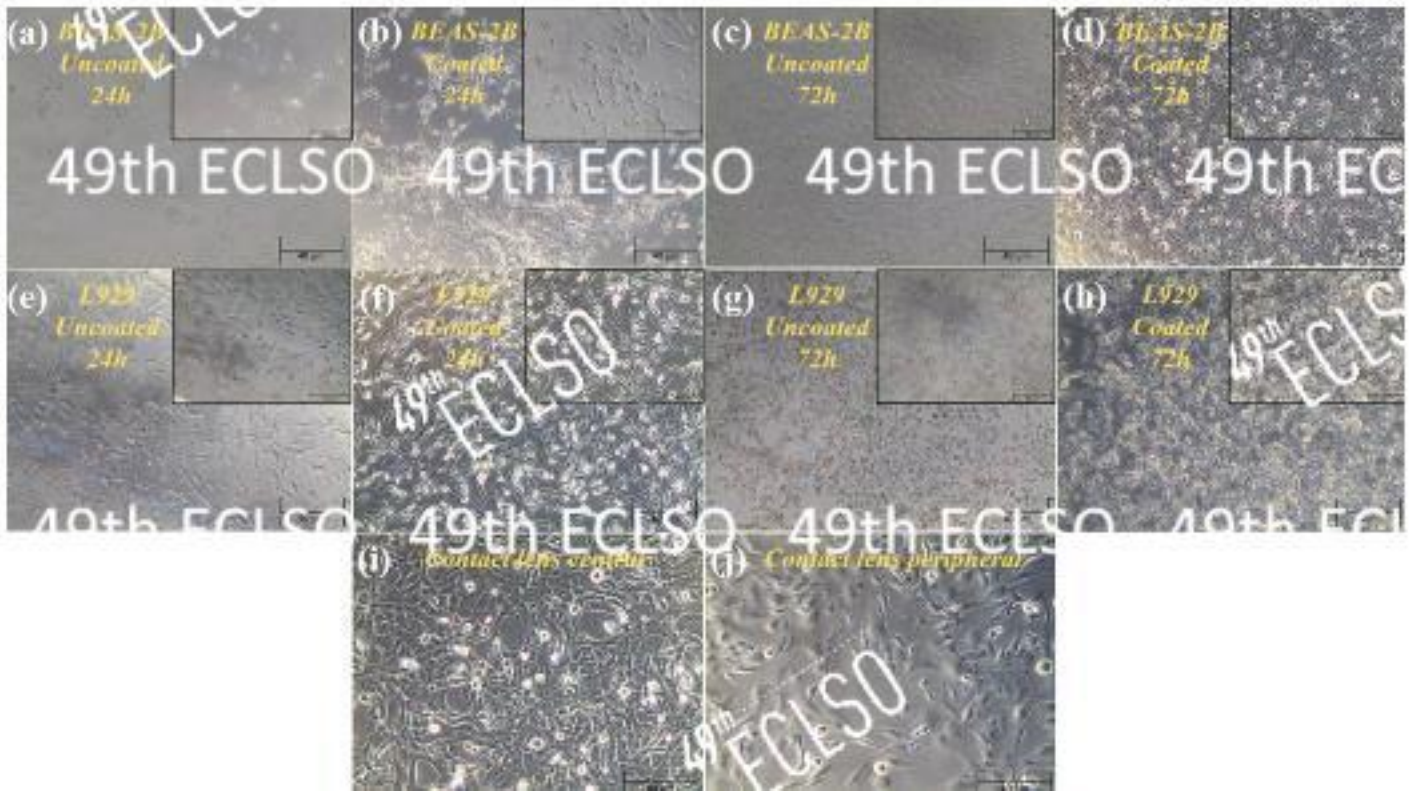
Schema of the Study



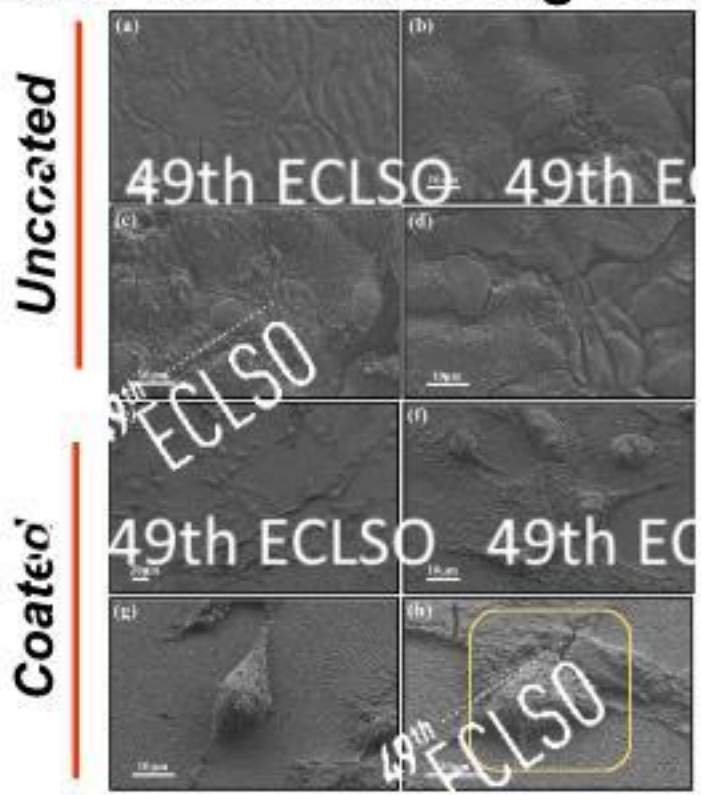
Cell Culture Experiments

- BEAS-2B (lung epithelium)
- L929 (fibroblast)
- Human Limbal explants

BEAS-2B and L929 cells Phase Contrast Microscope



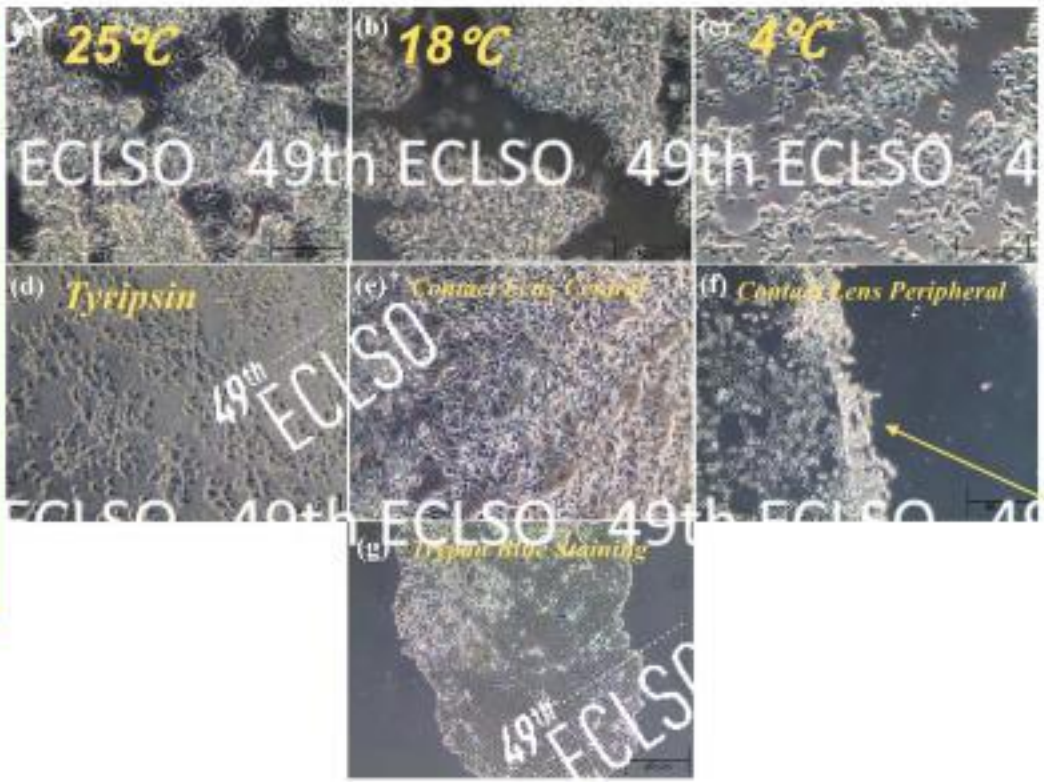
BEAS-2B and L929 cells Scanning Electrone Microscope



The SEM images confirmed better cell adhesion in the coated contact lens group.

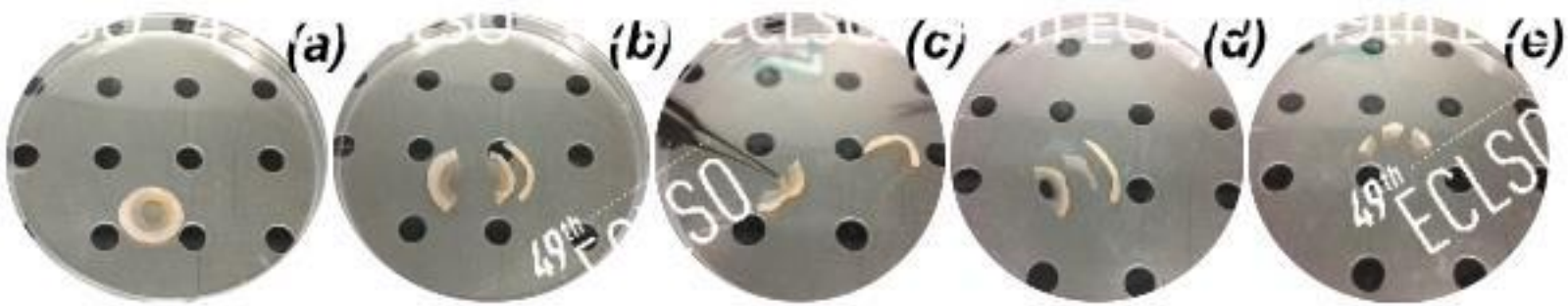
Detachment of BEAS-2B and L929 cell sheets

When we set the temperature under the lower critical solution temperature (25°C) we observed the cell sheet lift from the surface within 30 minutes. The cells break up and rise as the temperature decreases (18°C and 4°C) like trypsin treatment.



Peripheral roll of the cell sheet

The Preparation of Limbal Explants



Finally we used human limbal explants obtained from cadaver which was also used for keratoplasty.

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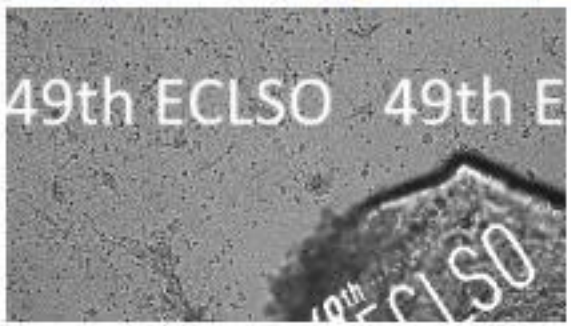
Limbal explants on coated contact lenses

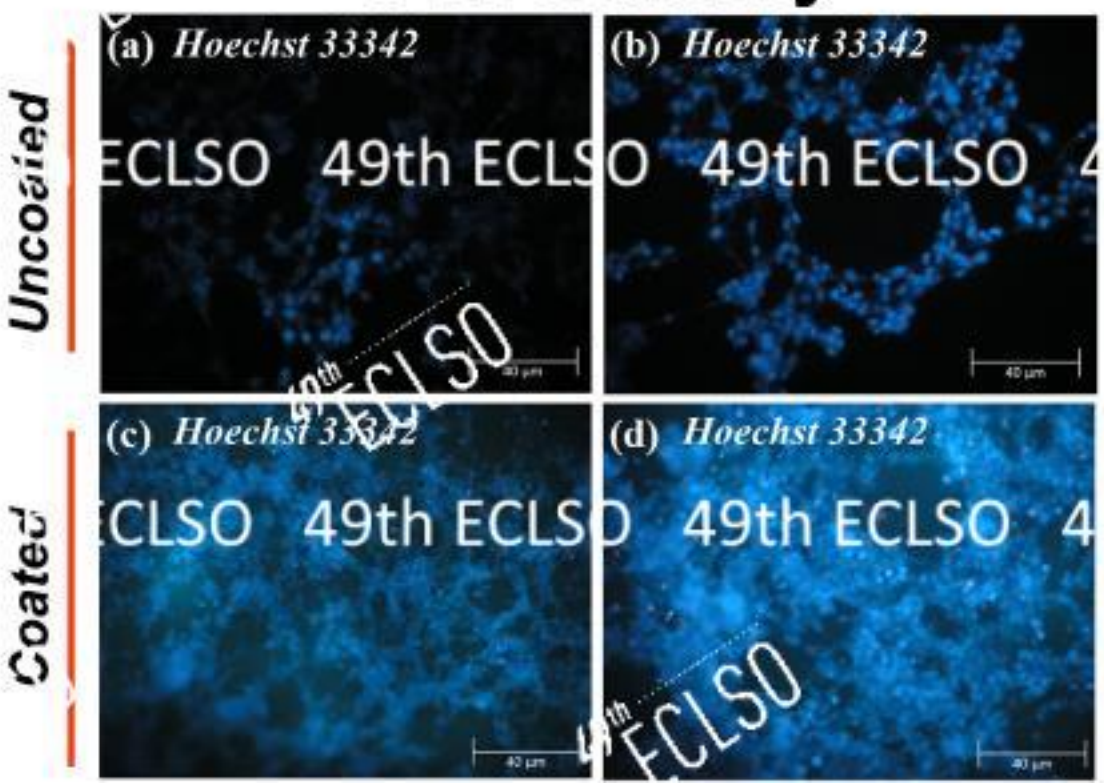


Stem cell morpnology

Cobblestone morphology

Limbal explant cell sheet removal

Cell Viability



Increased cell viability was correlated with the higher cell adhesion and proliferation rates on the coated contact lenses.

Conclusion

After cultivating patients' LESCs on the desired contact lens, we can easily locate the contact lens on the corneal surface to regenerate the ocular surface.

This will not require an additional surgical intervention.

Following the cell sheet detachment from the contact lens-attachment to the corneal surface period, we can remove the contact lens from the ocular surface.

During this period, we can also observe the patient with slit-lamp biomicroscopy behind the clear contact lens, and we can easily interfere to the corneal recovery process.

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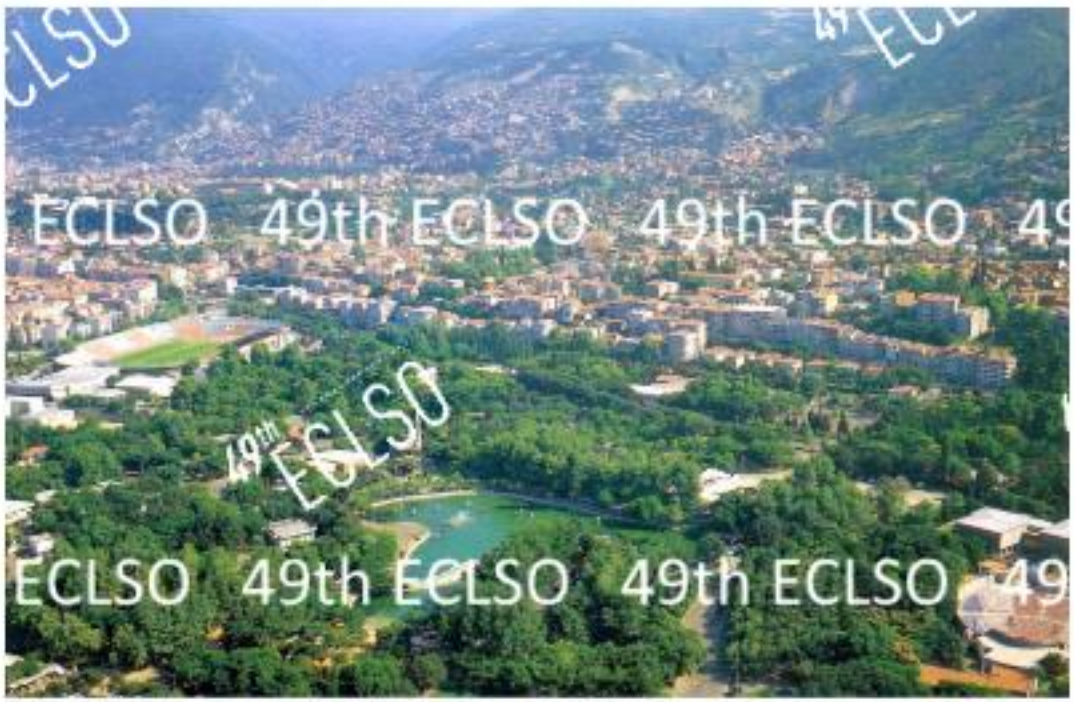

Bursa, Turkiye