WP.4 DEVELOPMENT OF AN ENGINEERED MATRIX TO IMPROVE CORNEAL REGENERATION

In this WP, we will engineer a hydrogel-based matrix for the release of selected pharmacological modulators (identified in WP3) and to accelerate the regenerative potential of LSCs for cellular transplantation via the SLET procedure.

4.1 Engineered biomatrix design, functionalization and production

To study the mechanism and improve the regeneration of LESCs for cellular transplantation via the SLET procedure, we will design a hyaluronic acid (HA) based supportive matrix able to specifically encapsulate and interact with limbal cells. The matrix will control and guide cells differentiation. The biomatrix will encapsulate the pharmacological modulators (identified in WP3) and release these at the required therapeutic dose. A small library of hydrogel-based bioengineered matrices, varying in chemical and physical properties, will be used to encapsulate cells and obtain 3D in vitro models. 3D models will be used to assess the response of LSCs to the matrix properties, as well as the molecules used, the dose released and their release kinetic. Data obtained from this model will be used to select the biomatrix to be further used for clinical applications and for cornea-on-chip screening models.

The workflow, from modification of materials to their fabrication, will be designed and optimized for ease scaling-up and a faster transition to the clinic.

HA is selected as it is commercially available as medical-grade product, and FDA approved for eye disease treatment. Moreover, high molecular weight (> 100 kDa) HA exhibits anti-angiogenic and anti-inflammatory properties, ideal properties for corneal regeneration.

Bio-active components (e.g. pharmacological modulators selected in WP3) will be conjugated or physically loaded into HA-base hydrogels and further released with known kinetics. A HA-based 3D bioengineered matrix will be tested in co-culture with relevant cell lines (cells seeded on top and encapsulated) allowing cell-cell and cell-matrix interactions.

Besides HA, silk fibroin (SF) (extracted from Bombyx mori silk cocoon) will be also included as matrix components to improve the bioactivity [38-39] and mechanical properties.

HA engineered matrices will be fabricated on the basis of our previous studies. Some preliminary experiments of HA-based biomatrix were already performed.

HA crosslinking (CXL) was obtained by using enzymes (HRP and H_2O_2) and linked to SF. Mechanical properties and their degradation rate can be tuned and tested in vitro to match the requirements set to support corneal regeneration.

4.2 In vitro tests and translatability

Preliminary cytotoxicity tests confirmed biocompatibility of engineered hydrogels, as well as removal of any toxic chemical reagents used. Preliminary biological tests with human limbal epithelial cells (HCLE) on HA-based hydrogels available from the Research Unit 2, showed fast cell adhesion and proliferation up to 10 days of culture.

Viability of encapsulated cells was also tested using human lung fibroblast cell line (MRC5) up to 7 days of culture.

If required, a further optimization of the HA-SF hydrogels library will be performed to guarantee printability and preserve cells viability. This will allow use of bioprinting technologies for the fabrication of patient-specific 3D geometries from patient scan, progressing towards the customization (patient specific) of implants for the regeneration of the corneal tissue.

The optimization steps are identified as: selection of CXL agent, fast CXL, and cytocompatibility. Biomaterials, cells, and biomolecules, will be selected in compliance with clinical requirements, for a quick translation of tested products.

Firstly, carboxylic groups of HA will be modified by click chemistry to improve the CXL efficiency, as well as allowing few CXL methods (e.g. UV photo-crosslinking). Bioinks formulation, viscosity and

its impact on cell viability will be also considered during the optimization step. Printed products will vary geometries to provide required oxygen permeability for cell encapsulation. To mimic

3. Project development, with identification of the role of each research unit, with regards to related
MUR - BANDO 2022
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cell-ECM interaction in a regenerative context, engineered matrix bioactivity will be specifically designed by adding the identified modulator molecules (WP3) and the mechanisms underpinning drug related impact will be investigated in in vitro 3D model(s). Spatial and temporal molecule release will be controlled by means of matrix formulation and properties, by purpose drug release system that will be incorporated into the matrix.
Based on the physico-chemical properties of the pharmacological modulators, appropriate nanocarriers will be formulated and characterized. The potentiality of solid nanoparticles will be evaluated to prolong the release kinetics up to 1-2 weeks.
Among the possible materials, the encapsulation will be performed to preserve pharmacological modulators integrity and protect them from biological hazards, for example limiting exposure to acidic degradation byproducts, such as phospholipids and poly-caprolactone, or their combinations.
Efficiency of the drug releasing system will be evaluated in terms of spatial-temporal and release kinetic, in order to control the dose delivered to cells.
Matrices physico-chemical (including transparency and adhesiveness on wet surfaces) and mechanical properties will be evaluated in physiological conditions.
In addition, cytotoxicity tests will be performed to excluder any cytotoxic effects on encapsulated cells.
Expected results and SWOT: in this WP, we expect to obtain the optimized formulation and standardized processing protocols for biomatrix fabrication and molecule/s controlled releasing suitable for SLET procedure. The described techniques are already in use in the laboratories of the Research Unit 2. Therefore, no unforeseen technical risks are expected.