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CREATING CORNEAL STEM CELLS WITH A THERMOREVERSIBLE GELATION POLYMER

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ABSTRACT

Introduction: Recent technological advancements in single-cell RNA sequencing and single-cell quantitative real-time PCR have enabled researchers to conduct single-cell analyses of limbal epithelial cells.

Aim of the study: the main aim of the study is to Cultivating corneal stem cells with a thermoreversible gelation polymer

Material and method: Each human limbal biopsy were placed in 1 mL of TC medium containing 3% FCS, antibiotics (penicillin, gentamicin, and amphotericin B), and DMEM, and then transported to a cell biology lab.

Conclusion: The thermoreversible gelation polymer facilitates the growth of limbal epithelial cells. The limbal characteristic could still be seen in the grown cells.

Keywords: *stem cells; thermoreversible; gelation; polymer*

INTRODUCTION

1. LIMBAL EPITHELIAL STEM CELLS

Researchers presented their findings on the differences between central corneal cells and limbal cells in the early 1940s. These preliminary experiments used mitotic figure counts and irradiated thymidine to show an increase in the number of mitoses in the peripheral cornea's basal layer. Melanin-expressing cells migrated in a centripetal pattern from the limbus into the corneas of both rabbits and humans, suggesting that this is where new corneal cells are generated. Multiple studies since then have used the unique properties of this cell type to pinpoint the limbus as the location of corneal epithelial stem cells:

1.1. Slow cell turnover rate

DNA label-retention experiments suggest that the limbus is home to cells that are either undergoing a delayed cell cycle or are in a growth-arrested condition. Human limbal explant cultures, complete cornea organ cultures, and limbal cells of mice corneas in vivo have all been shown to retain radiated thymidine or 5-bromo-2'-deoxyuridine (BrdU). In these tests, the DNA label remained intact for as long as nine weeks. The labelling index, or percentage of BrdU-retaining cells, varied from 1% to 4% in mice corneas and was similar to that observed in human limbal explant cultures. The nuclear label gradually diminished as the tagged cells moved towards the centre cornea, showing

greater cell division during centripetal migration.

1.2 Clonogenicity and proliferative potential

Any stratified epithelium must have a self-renewing stem cell reservoir, asymmetric precursor cell division, and a quick proliferative response in order to maintain itself over its lifetime. These characteristics appear to be specific to the limbal cell population, according to studies.

LITERATURE REVIEW

Sharma, Ashok & Sharma, Rajan (2020) Conjunctivitis, corneal vascularization, recurrent erosions, and corneal opacification are all symptoms of LSCD. The most effective treatment for LSCD is surgery, including limbal stem cell transplantation (LSCT). Autologous LSCT is used to treat unilateral LSCD, and live-related LSCT or cadaveric LSCT is used to treat bilateral LSCD. Recently, injured ocular surfaces have been repaired using cultured limbal stem cell transplantation (CLSCT). The ocular surface has also been effectively rebuilt using grown oral mucosal epithelial cells. Simple limbal epithelial cell transplantation (SLET), a more recent treatment option for LSCD, has been developed. The method is easy, affordable, and doesn't call for a complex laboratory setup. Dry eye, glaucoma, abnormalities of the eyelids, and ocular surface inflammation should be treated after LSCT. Inflammation threatens LSC's ability to survive. In order to prevent excessive ocular surface inflammation, use immune suppressants or topical or systemic corticosteroids.

Tur, Veronica & AlMaazmi, Amna & AlSaadi (2019) This study set out to identify an early LSCD symptom that is both consistent and unique across a number of LSCD causes. Methods The study included 17 eyes from 11 consecutive patients who had LSCD from a variety of known causes. Clinical examinations of the patients included in vivo confocal microscopy, fluorescein staining, and slit lamp examinations. Slit lamp cameras were used to take pictures of each cornea. Exams using in vivo confocal microscopy were performed on seven patients. Although the cause of this manifestation is unknown, it might be connected to the architecture of the palisade's haphazard dysfunction or loss.

Sharma, Ashok & Sharma, Rajan (2018) Limbal stem cell deficit (LSCD) may develop as a result of hereditary conditions, repetitive limbus surgeries,

immune-mediated illnesses, and trauma. Chemical eye damage continues to be the most common reason for LSCD. Conjunctivitis, corneal vascularization, recurrent erosions, and corneal opacification are all symptoms of LSCD. Bilateral LSCD is treated with live-related LSCT or cadaveric LSCT, while uniocular LSCD is treated with autologous LSCT. Recently, damaged ocular surfaces have been repaired using cultivated limball stem cell transplants (CLSCT). The ocular surface has also been effectively rebuilt using grown oral mucosal epithelial cells. Simple limbal epithelial cell transplantation (SLET), a more modern treatment option for LSCD, has been developed. The method is easy, affordable, and doesn't call for a complex laboratory setup. Dry eye, glaucoma, anomalies of the eyelids, and ocular surface irritation should be treated after LSCT. Inflammation threatens LSC's ability to survive. In order to prevent excessive ocular surface inflammation, use immune suppressants or topical or systemic corticosteroids.

Notara, Maria & Lentzsch, A. & Coroneo, Minas & Cursiefen, Claus (2018) The cornea serves as our window to the outside world since it is a clean structure devoid of lymphatic and blood arteries. Defects in limbal epithelial stem cells have been linked to symptoms like pain, discomfort, blurred vision, and the development of goblet cells, chronic inflammation, conjunctivalization, epithelial abnormalities, breaks in Bowman's membrane, persistent epithelial defects and ulceration, ocular surface squamous neoplasia, lipid keratopathy, and a lack of goblet cells. It is believed that pterygium is an example of regional limbal insufficiency. Previous studies have shown alterations in the limbal epithelium, suggesting a link between stem cell injury and pterygium development. Pterygium, which is characterised by lymphangiogenesis and the development of blood vessels in the cornea, thus serves as a model illness for studying the effects of ultraviolet (UV) light on stem cells. In this study, we will examine the role that epithelial cells of the cornea and limbus play in maintaining avascularity and corneal immune privilege and how UV radiation may cause these characteristics to become dysregulated. Here, we present a synopsis of the related PUBMED literature and present results from our own laboratories.

Wang, Li & Wang, Bowen & Ouyang, Hong (2018) The cornea acts as the eye's primary light-reflecting organ and partial front barrier. For proper eye function, the corneal surface must be intact. The milieu of limbal epithelial stem cells (LESCs), in

particular, might be significantly damaged by injuries or congenital disorders, which would ultimately impair corneal regeneration and reduce LESC numbers. We seek to explain the characteristics and market niche of LESCs in this study and address many facets of their use in cornea surface restoration.

MATERIAL AND METHOD

1. USE THERMOREVERSIBLE GELATION POLYMER IN CULTIVATION OF CORNEAL STEM CELL

1.1 Collection of Human limbal tissues

Within 3-6 hours of death, 24 human limbal biopsies were taken from the superior and inferior portions of the limbus of human donor eyes from respective sources. All tissue samples were treated in accordance with the Helsinki Declaration.

1.2 Preparation of Mebiol Gel-tissue culture growth medium mixture

From Mebiol Inc., a flask containing 10 mL of lyophilized and sterilised Mebiol gel was bought. When dissolved in 10 ml of 2X strength TC growth media, which is DMEM + Ham F-12 with 10% FCS at pH 7.0, Mebiol Gel-TC medium mixture (gel-TC medium mixture) becomes a viscous transparent liquid devoid of air bubbles that may be utilised in the studies.

1.3 Cultivation of Corneal limbal tissue embedded within the Mebiol Gel:

Careful dissection of the limbal epithelium from the stroma allowed for three washes in TC growth media before the tissue was sliced into 0.5- to 1-mm pieces and grown in Mebiol gel for explants. A drop of the liquid (4-8°C) gel-TC medium combination was placed in the centre of the 24-well TC plate, and the plate was incubated at 37°C for around 20-30 minutes to allow the mixture to solidify. The explant piece of tissue was placed within the gel, and then another drop of the cold liquefied gel-Tissue culture media mixture was put on top. The tissue explant was then positioned atop the gel. The sequential steps for growing limbal biopsy within the mebiol gel are shown in Figure 3.4. A 10% CO2 atmosphere was used to incubate the tissue culture plates at 37°C.

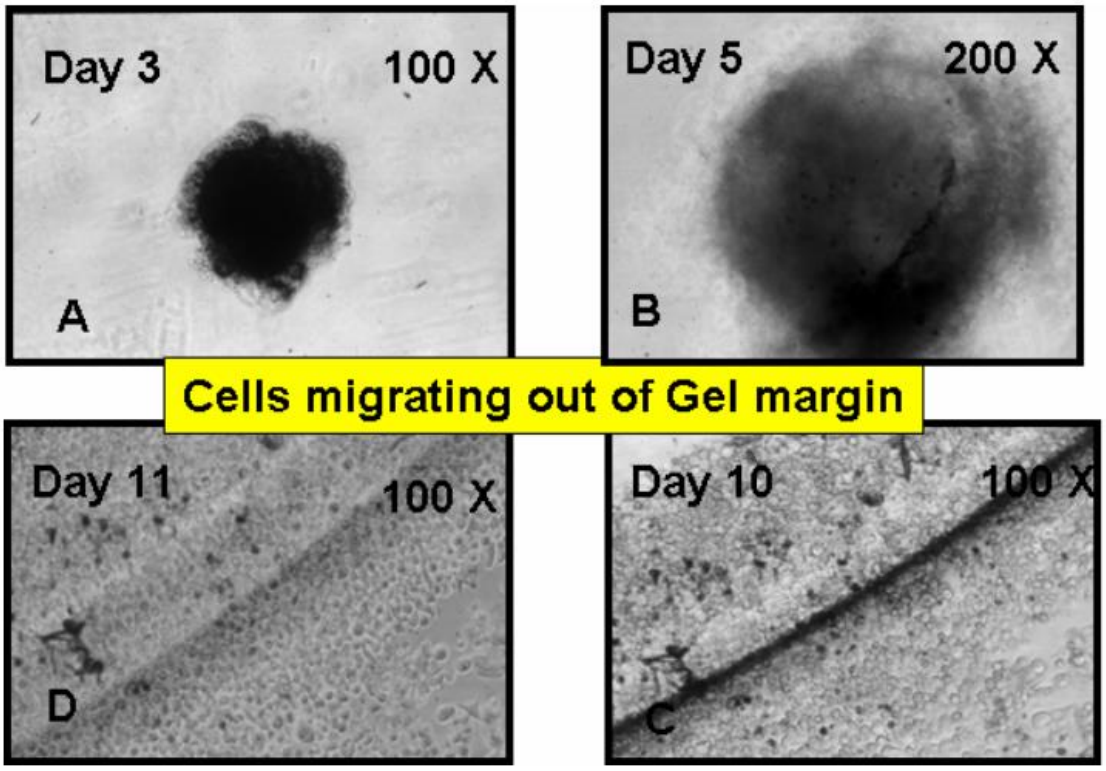

Figure 4.1 *Figs. The successive three-dimensional (3-D) growth in size of the HCLT biopsy tissue implanted inside the Mebiol Gel is shown in images A, B, C, and D. The expansion as of day three is shown in Fig. A, along with a 3-D rise in tissue size and cell proliferation. The Fig, B On day 10, image C illustrates the movement of growing cells through Mebiol Gel's edge. Fig. On day 11, the proliferating cells outside the Mebiol Gel boundary are seen in D creating a monolayer. Mag. performed photomicrography on all figures. 100X*

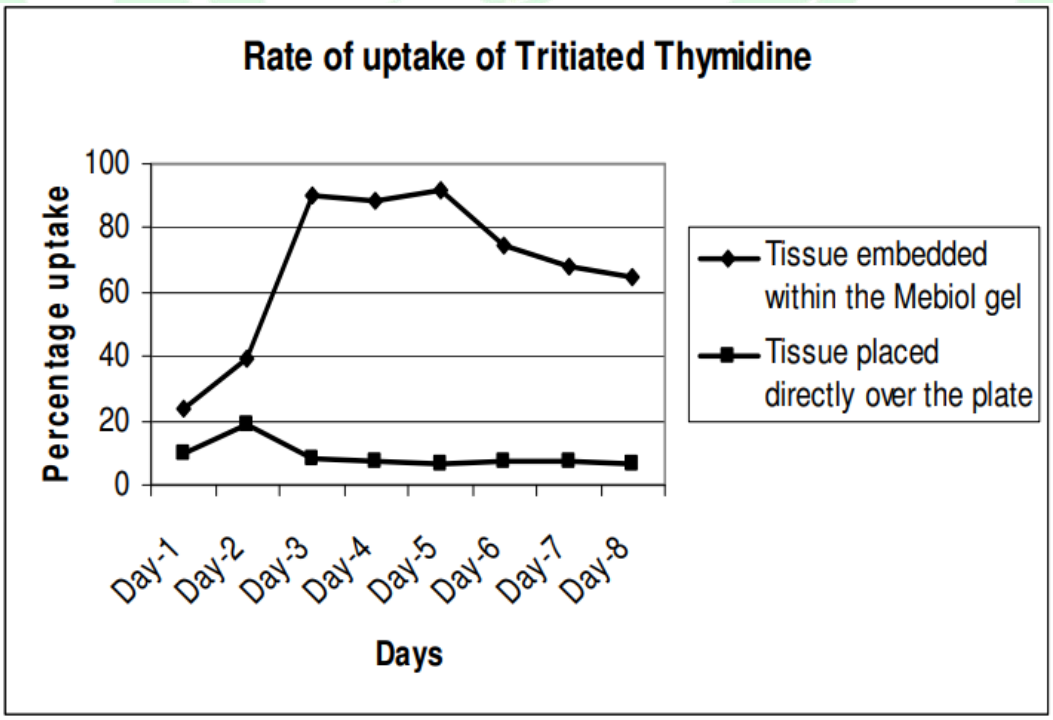

Figure 4.2

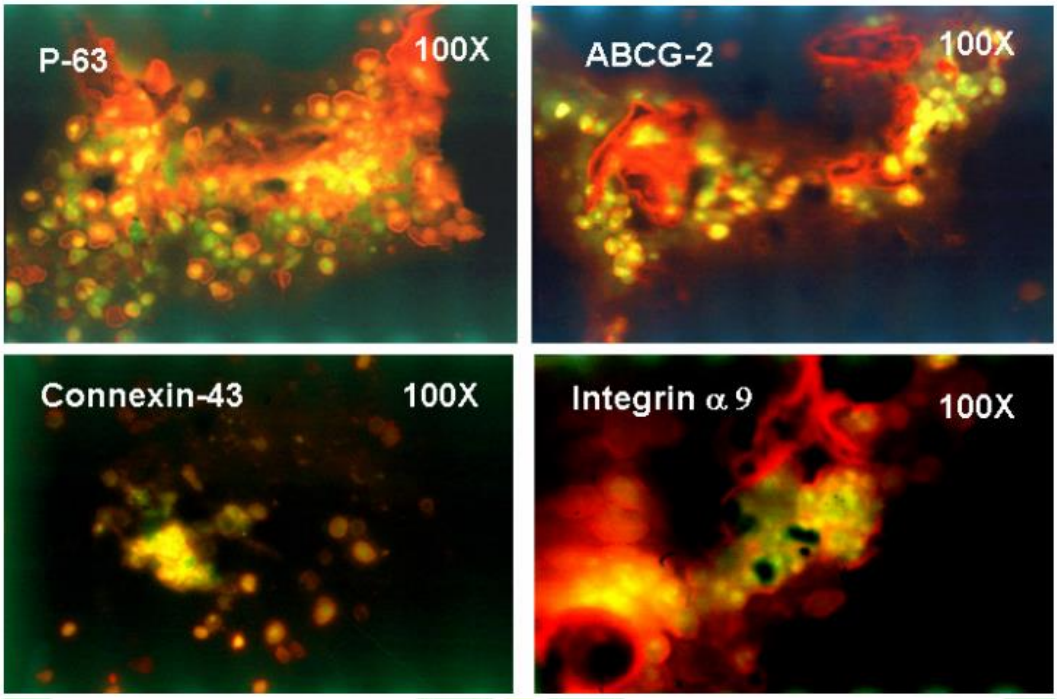

Figure 4.3 *Immunofluorescence staining*

This image displays the expression of p63, ABCG2 (a marker for stem cells), connexin 43, and integrin. By using the immunofluorescence method, green fluorescence demonstrates the positive expression.

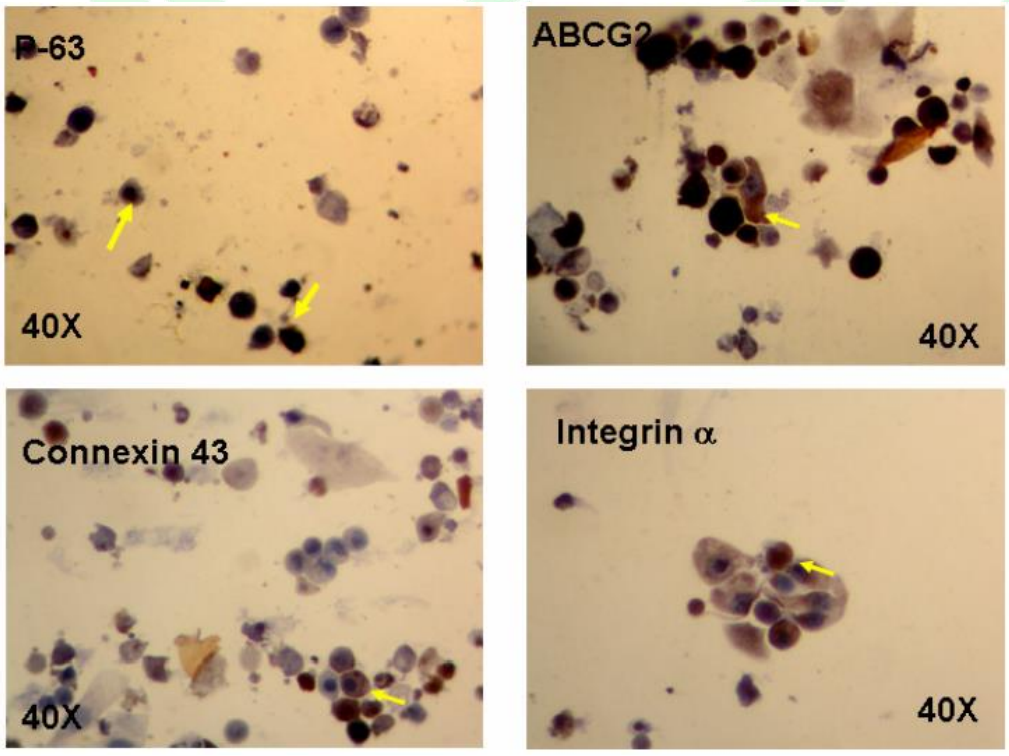

Figure 4.4 *Immunocytochemistry*

The immunocytochemistry on the cytospin smear preparation of the gel-extracted cells is shown in this image. The cells were collected, washed, and cytospun for five minutes at a speed of 1000 rpm before being stained with the appropriate antibodies. Positive cells are indicated with a yellow arrow.

CONCLUSION

The thermoreversible gelation polymer facilitates the growth of limbal epithelial cells. The limbal characteristic could still be seen in the grown cells. According to the rabbit model's findings, unilateral LSCD in eyes may be treated with autologous limbal epithelial cells cultured in thermo-reversible gel polymer to restore a virtually normal ocular epithelial surface.

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