Cultivation of Limb Stem Cells Using Non-Polymeric Scaffolds[1]

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ABSTRACT

Introduction: *The limbal stem cells maintain the ongoing regeneration of the corneal epithelium throughout life (LSCs).*

Aim of the study: *the main aim of the study is to Cultivation of Limb Stem Cells Using Non-Polymeric Scaffolds*

Material and method: *The prawn shells were obtained from the neighbourhood fish market and thoroughly cleaned with distilled water. Now, the experimental process for chitin isolation can be broken down into three stages.*

Conclusion: *it is concluded that Chitosan is one of the most promising biopolymers for cell treatment, tissue engineering, and gene therapy as a result of its new features.*

Keywords : *Limb Stem Cells, Polymeric, Chitosan matrix*

INTRODUCTION

The sense of seeing is perhaps the most crucial. More than 80% of the information we get from the outside world comes to us through visual sources. These factors all contribute to the phenomenon of good vision. The preservation of its clarity is its fundamental physiological need. It is not an independent tissue.

In addition to providing structural support, the connective tissue in all three areas also acts as a conduit for nutrients and fluids and is home to support cells that help to maintain the matrix and underlying epithelium. The cornea has thus gotten the greatest attention in studies of its structure, function, and disease because of its crucially essential roles in light refraction and transmission. The adjacent limbal and conjunctival areas, which serve in part as corneal support tissues, have recently received increasing focus. This review focuses on the clinical use of limbal stem cell research.

LITERATURE REVIEW

Ruan, Yue & Jiang, Subao & Musayeva (2021) When deciding on a treatment for acquired forms of limbal stem cell insufficiency, it is important to take into account whether the condition is unilateral or bilateral. There are now procedures for transplanting both autologous and allogenic stem cells. Limbal transplants, limbal cell expansion in vitro, and even small biopsies have all been used to restore the limbus. This review will discuss the pathophysiology of LSCD, the physiology of the corneal epithelium, and current treatment options.

Tong, C Maya & He, Bonnie & Iovieno, Alfonso & Yeung, Sonia (2021) Corneal limbal stem cell insufficiency is a complex, vision-threatening condition that poses significant diagnostic and

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therapeutic challenges. This article discusses normal limbal stem cell function in the cornea, the causes and diagnosis of limbal stem cell deficit, and the medication and surgical treatment options available for this condition. The diagnosis, management, and design of collaborative studies for this condition will be helped by the recent consensus recommendations for limbal stem cell insufficiency. However, these treatments may come with a significant risk of morbidity and may only be a temporary solution. Regenerative medicine advances, such as ex vivo cell culture and keratoprosthesis implants, may improve outcomes for individuals with bilateral limbal stem cell deficit.

Bonzano, Chiara & CANCIANI, BARBARA & Olivari (2019) The 10 pairs of fresh, healthy human corneas used in this study were donated by individuals who had recently passed away. After seven days of digital fluorescence tracking, the rate of cell migration was determined. Cells in corneas that were tagged with CFSE were tracked. An examination revealed that temporal sequences rotated centripetally. Every day, CFSE-labeled LSCs moved an average of 0.0730.01 cm.

Yazdanpanah, Ghasem & Haq (2019) The epithelial cells that make up the cornea's outermost layer provide a protective barrier while also maintaining the cornea's transparency. Located in a unique niche called the limbal stroma, limbal epithelial stem cells (LESCs) contribute to the rapid and effective turnover of these epithelial cells. Defects in LESCs and the limbal niche have been related to a wide variety of conditions that prevent the corneal epithelium from regenerating normally.

MATERIAL AND METHOD

Use of Non-Polymeric Scaffolds in Cultivation of Limbal Stem Cells

1. Design of experiments:

- **Preparation of chitosan matrix:**

The prawn shells were obtained from the neighbourhood fish market and thoroughly cleaned with distilled water. Now, the experimental process for chitin isolation can be broken down into three stages.

Deproteinization:

Proteins, inorganic substances—primarily CaCO3 colours and liquids—and chitin are all found in prawn shells. The deproteinization process included immersing the cleaned, washed prawn shells in a 5% NaOH solution, followed by refluxing for two to three hours. The pH of the NaOH solution was raised to 7 by decanting it and washing it with distilled water.

Protein + chitin + NaOH → Amino acid + dipeptide + tripeptide + chitin

Demineralization:

The shells obtained from the deproteinization step were demineralized by soaking them in 2N HCl for four hours. The pH of the HCl solution was raised to 7 by decanting it and washing it with distilled water.

$CaCO3 + HCl \rightarrow CaCl2 + H2O + CO2$

- **Characterization of the chitosan scaffold:**

Tensile Test:

Tensile testing was performed using an Instron universal material testing machine at room temperature, with a gauge length of 10 mm and a crosshead speed of 5 mm/min (model 5567). The property values shown here are the averaged outcomes of at least six samples.

Scanning electron microscopy:

Figure 3.2 displays a scanning electron microscopic image of the chitosan matrix from studies on surface morphology using a scanning electron microscope (SEM model JSM 5300).

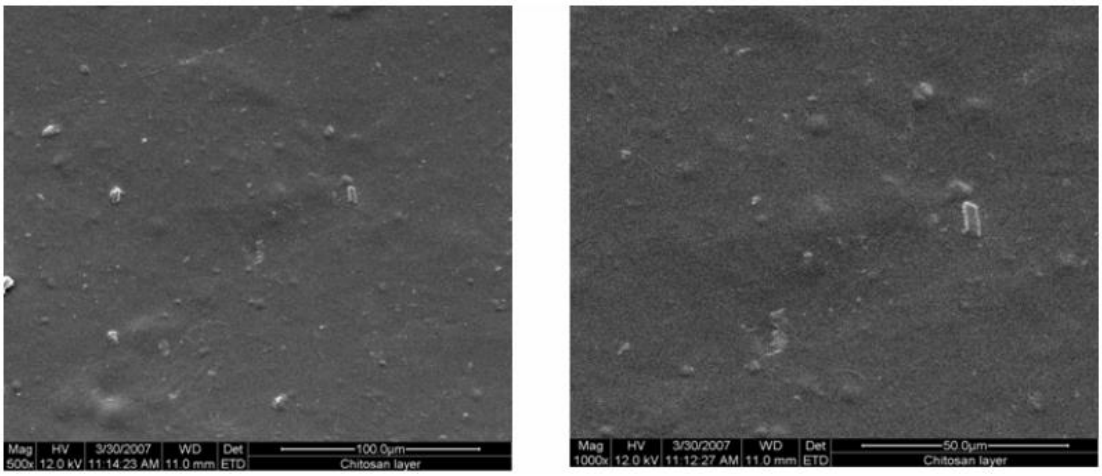

Figure 3.2 *Scanning Electron Microscopic picture of the chitosan sheet*

RESULTS

Cellular Morphology of the Chitosan and Chitosan Fabricated scaffolds:

By the end of the second day, limbal explants had begun to grow on the various scaffolds from the explant, and by the end of the twenty-first day, they had formed a monolayer. Figure 4.1 displays the morphology of the cells developed on the various scaffolds. Cell outgrowth was observed using an inverted phase contrast microscope (Optiphot, Nikon, Japan), and a photograph was taken with a Nikon cool pix digital camera. There is no discernible cytotoxic effect on the cells cultured over these scaffolds, and all of the scaffolds easily supported the growth of the corneal limbal epithelial cells. While it is a little slower on the chitosan with silver, the growth rate on chitosan and chitosan + gold is almost identical, with an average reaching about 150mm2. The rate of cell growth in chitosan scaffolds and its derivatives is depicted in Figure 4.1. The H&E image of the cells expanded over the chitosan scaffold is shown in Figure 4.2.

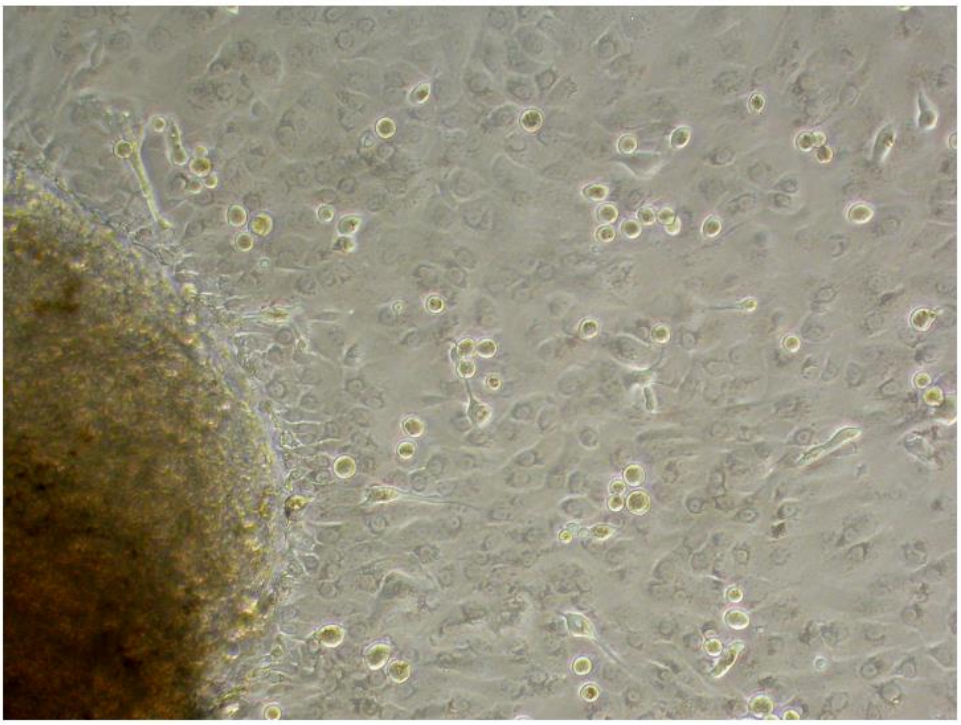

Figure 4.1 *Growth of the cells on the chitosan matrix*

After two weeks of incubation, the limbal epithelial cells from the explant have formed a confluent growth, and the magnification in this figure is less than 100X.

Viability of cells by trypan blue dye exclusion test:

The results are shown in Figure 4.2, where the percentage of viability ranged from 95 to 60% and all scaffolds supported the growth of the cells up to the 21st day of incubation.

Expression of corneal stem cell associated markers and differentiation markers on the various scaffolds using semiquantitative RT-PCR:

At the end of the 21st day, cells were taken from the various scaffolds and subjected to semiquantitative RT-PCR. Connexin 43, K3/K12, p63 (a putative marker for keratinocyte stem cells), and ABCG2 (a putative marker for stem cells) were all weakly expressed on the cells expanded on the Chitosan, Chitosan-gold scaffold, but ABCG2 expression was completely absent on the cells expanded on the Chitosan, Chitosan-silver scaffold. The expression of the markers on the cells taken from the chitosan matrix and its derivatives at the end of two weeks is shown in Figure 4.4.

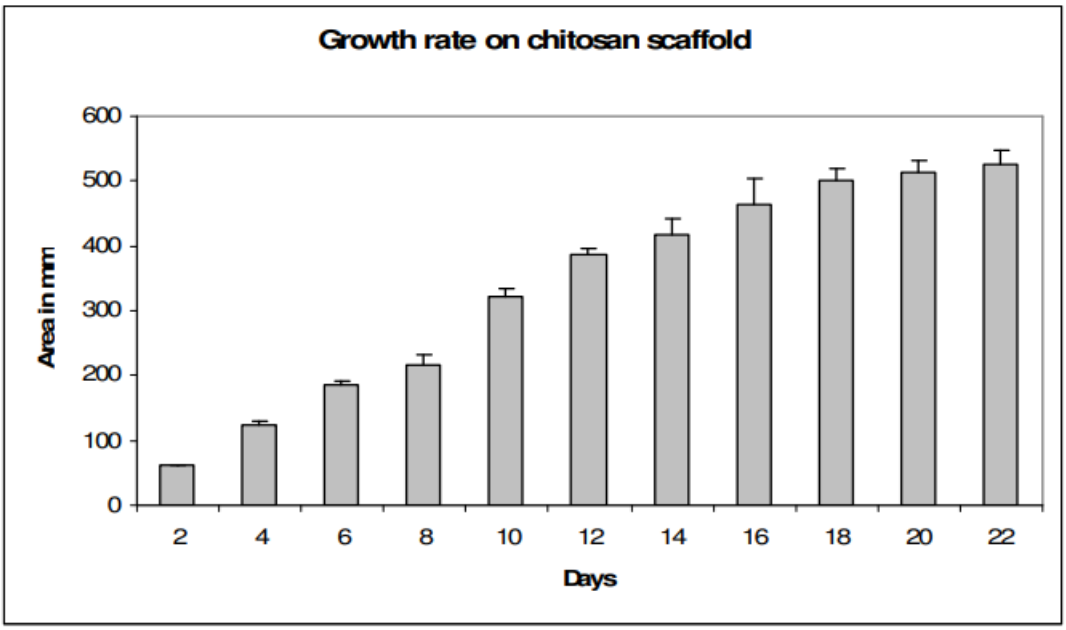

Figure 4.2 *Rate of growth on the chitosan scaffold*

The growth rate of limbal epithelial cells on the chitosan scaffold is depicted in this figure. On the six-well tissue culture plate, the growth started by the end of day 2 and nearly reached confluence by the end of day 21.

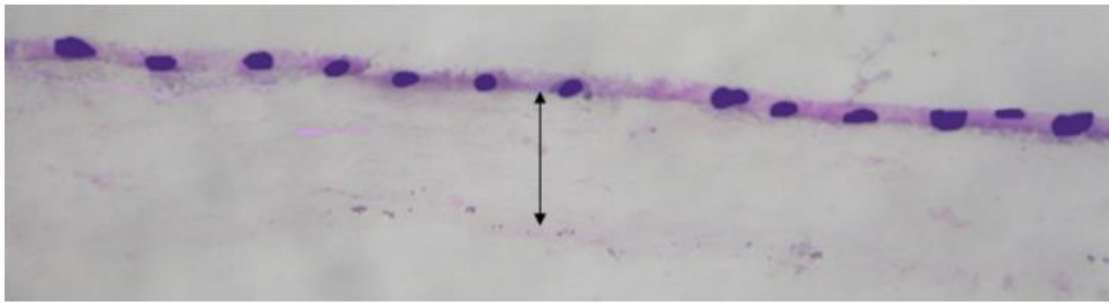

Figure 4.3 *H & E shows the growth of the limbal epithelium on the chitosan scaffold*

After two weeks of incubation, the monolayer of limbal epithelial cells grown on the chitosan matrix is depicted in this figure. The chitosan matrix was taken out in its entirety, and a frozen section was taken and stained with hematoxylin and eosin.

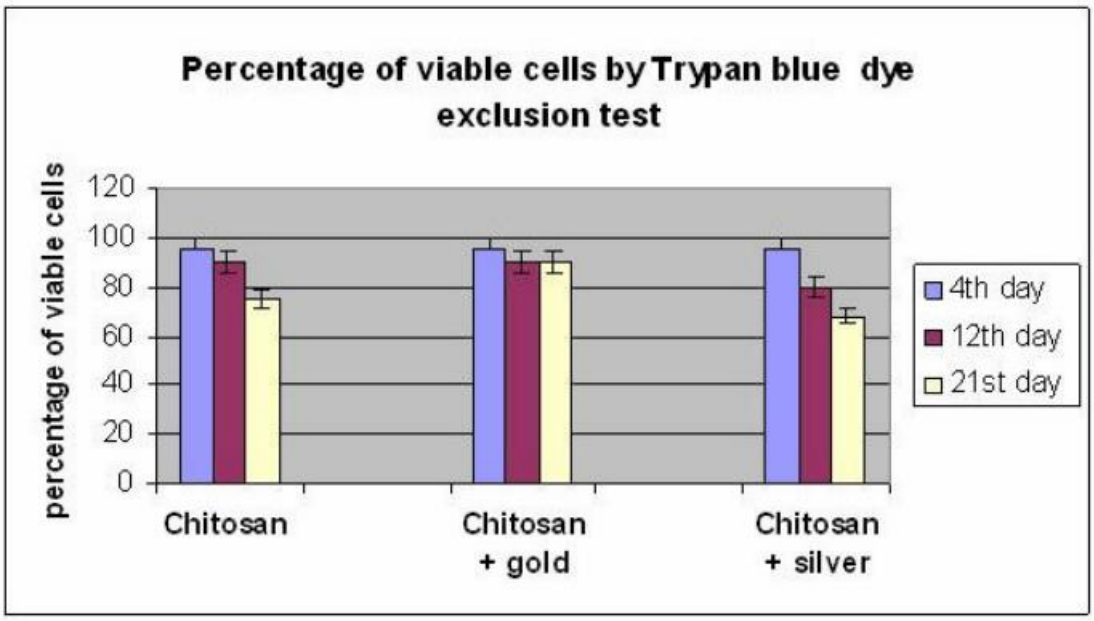

Figure 4.4 *Percentage of viable cells by trypan dye exclusion method on the cells cultured over the chitosan matrix and its derivatives*

The percentage of viable cells present at the end of days 4, 12, and 21 is depicted in this graph. On day 4, all of the cells cultured over the scaffolds had 100% viability. The cells cultured over chitosan and chitosan + gold had the highest viability on day 21.

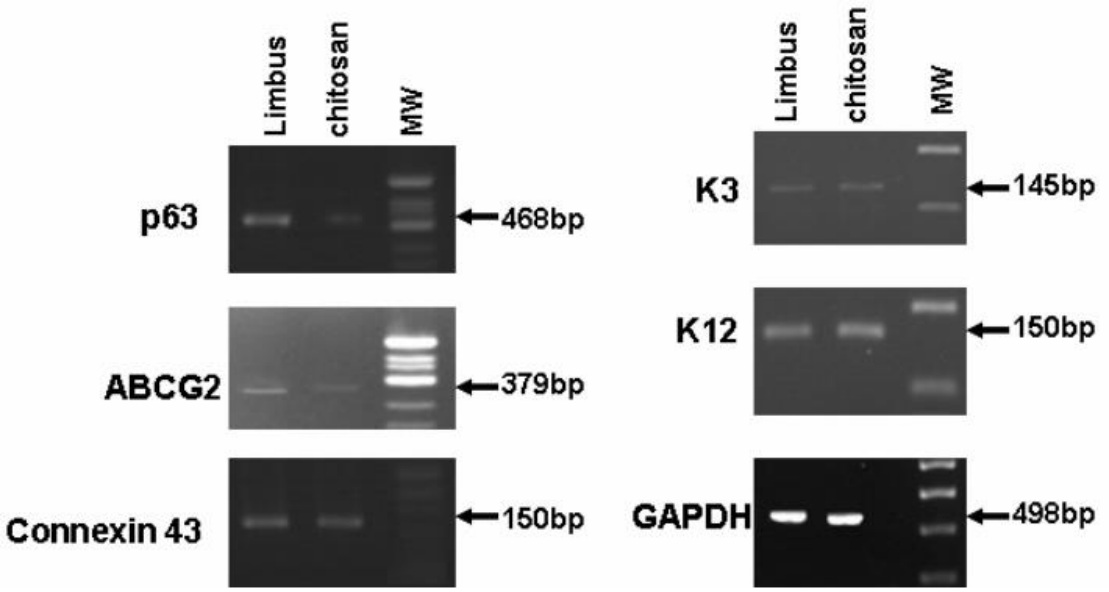

Figure 4.5 *The cells were cultured over a chitosan matrix, and the electrophoretogram demonstrates the expression of stem cell-associated and differentiation markers on those cells.*

CONCLUSION

In the future, it is anticipated that these many regenerative medicine treatments would go from "bench to bedside." However, for this kind of application, particularly for the rebuilding of the ocular surface, attempts to enhance the mechanical characteristics of chitosan-based composite biomaterials are crucial. Before we go further with the clinical application, other concerns about transparency and tensile strength need to be thoroughly investigated.

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Conflict of Interest: None

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