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Review article

Native and synthetic scaffolds for limbal epithelial stem cell transplantation

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ABSTRACT

Limbal stem cell deficiency (LSCD) is a complex blinding disease of the cornea, which cannot be treated with conventional corneal transplants. Instead, a stem cell (SC) graft is required to replenish the limbal epithelial stem cell (LESC) reservoir, which is ultimately responsible for regenerating the corneal epithelium. Current therapies utilize limbal tissue biopsies that harbor LESCs as well as tissue culture expanded cells. Typically, this tissue is placed on a scaffold that supports the formation of corneal epithelial cell sheets, which are then transferred to diseased eyes. A wide range of biological and synthetic materials have been identified as carrier substrates for LESC, some of which have been used in the clinic, including amniotic membrane, fibrin, and silicon hydrogel contact lenses, each with their own advantages and limitations. This review will provide a brief background of LSCD, focusing on bio-scaffolds that have been utilized in limbal stem cell transplantation (LSCT) and materials that are being developed as potentially novel therapeutics for patients with this disease.

Statement of Significance

The outcome of patients with corneal blindness that receive stem cell grafts to restore eye health and correct vision varies considerably and may be due to the different biological and synthetic scaffolds used to deliver these cells to the ocular surface. This review will highlight the positive attributes and limitations of the myriad of carriers developed for clinical use as well as those that are being trialled in pre-clinical models. The overall focus is on developing a standardized therapy for patients, however due to the multiple causes of corneal blindness, a personal regenerative medicine approach may be the best option.

Contents

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1. Introduction

Vision impairment is estimated to affect >400 million people worldwide, ~40 million of whom are blind [1]. Of these individuals, blindness due to corneal dysfunction from injury or disease affects 10 million, with more than 1 million new cases reported annually [2]. Corneal diseases cause pain and discomfort, and are a burden on the economy [3,4]. Limbal stem cell deficiency (LSCD) represents a complex and debilitating corneal disease primarily affecting a working age population. Due to the various aetiologies and lack of a clinical framework for diagnosis, the incidence of LSCD remains elusive. Extrapolating data from the most common causes, Shortt and colleagues (2011) estimated an incidence of 240 cases per annum in the United Kingdom [5]. We recently conducted a surveillance study in Australia and New Zealand, the first to directly investigate the incidence of severe LSCD [6]. Notably our study suffered from several limitations that likely resulted in underestimating the incidence of this disease. Nonetheless, an expected incidence of 63 new cases per annum was extrapolated from our data [6].

LSCD cannot be treated with a conventional donor corneal graft, instead a specialized stem cell (SC) therapy is required to restore eye health and eye sight [7]. Stem cell therapies for patients with LSCD are generally successful in the short to mid-term, while in the long-term seem to be less efficacious. Failures are likely influenced by disease stage, aetiology, duration, co-existent ocular disease and therapies, accompanying systemic diseases, level of inflammation, genetics, method of SC transplantation, and source of SCs (autologous vs allogeneic). The other variable to consider when assessing outcomes, is the material used to nurture and carry these cells to the ocular surface; hence the recent focus on identifying and developing better scaffolds that support corneolimbal epithelial growth, maintain 'stemness', promote wound-healing, and integrate into the cornea while possessing sufficient mechanical strength and biocompatibility [8]. Notably, no study has thus far been conducted to compare outcomes of different scaffolds in patients with LSCD patients. There are however numerous *in vitro* and preclinical investigations that compare cell growth characteristics, phenotype, and gene expression profiles between the same [9] and different [10–12].

A major limitation of current therapies for patients with LSCD is the lack of understanding on precisely how transplanted donor cells restore corneal epithelial integrity. To this end, researchers have reported varying durations of donor cell survival in recipient eyes, speculating that transplanted cells stimulate proliferation of remaining resident SCs via paracrine effects rather than directly replenishing the SC pool [13]. Moreover, where on the recipient cornea grafted SCs nestle is not known as permanent marking and real-time *in vivo* tracking of these cells has not been possible. Our recent studies have begun to address these shortcomings with highly innovative technologies. For example, we have led the field in devising a novel therapy that uses contact lenses (CLs) to successfully deliver a payload of SCs to diseased corneas of patients with LSCD [7,14]. To address the limitation of being unable to track the fate and kinetics of transplanted cells, we recently developed a transgenic mouse model that allows us to monitor the activity of multi-colored limbal epithelial SCs (LESCs) and their clones before, during and after grafting in real-time in live animals using minimally-invasive microscopy platforms [15,16]. The purpose of this article is to review the advances made in the treatment of patients with severe ocular surface disease, focusing on the scaffolds that have been trialled in the clinic as well as new biomaterials that are being developed and tested *in vitro* and in preclinical models.

1.1. The cornea and its stem cells

The cornea is the window to the eye, responsible for two-thirds of its refractive power. It consists of three cellular layers: a non-keratinized stratified squamous epithelium, a keratocyte-containing collagen-rich stroma, and a posterior monolayer of specialized endothelial cells, separated by Bowman's layer and Descemet's membrane respectively [7,17]. Even in the absence of disease, corneal epithelial cells are continually lost through aging, environmentally-induced dryness, and by the simple act of blinking [18,19]. Corneal epithelial SCs function to replenish and maintain an intact corneal surface, which is essential for unobstructed vision.

The putative location of corneal epithelial SCs is thought to be the limbus; a 1–2 mm wide transition zone that separates the cornea from conjunctiva. Phenotypic and functional studies have provided compelling evidence that the epithelial SCs of the cornea are located almost exclusively in the basal layer of the limbus, hence termed limbal epithelial stem cells [20–23]. Only a small proportion (<10%) of basal limbal epithelial cells (LECs) are considered to be SCs [19].

While the limbal niche is a protective microenvironment, it is also a nurturing habitat for these specialized cells as it provides soluble nutrients and matrix-associate factors. This is obvious in studies that have identified differences in protein composition between the limbal and corneal basement membrane (BM) [24]. Other characteristics that distinguish these cells from surrounding epithelia include their small size, primitive morphology (high nuclear:cytoplasm ratio) [19], capacity for unlimited self-

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