I. INTRODUCTION

The cornea is the anterior transparent window of the eye which acts as a physical barrier between the optical and external environment. The major function of cornea is to refract light into the retina together with lens. It is made up of five layers - epithelium, Bowman’s layer, stroma, Descemet’s membrane and endothelium. The epithelium is the outer most layer of cornea. It is 4 - 6 layers thick and is composed of non-keratinized, stratified epithelium, wing cells and basal columnar cells. Each of these cell layers has unique structural features contributing to the transparency and refraction of light. The corneal epithelium is regenerated from a distinct population of corneal epithelial stem cells (CESCs) - small sized, slow-cycling cells residing in the basal epithelial layer of the limbus at the corneoscleral junction (Arpitha et al., 2008; Limb and Daniels, 2008). These adult SCs reside in a specialized microenvironment or niche, which regulates their maintenance, self-renewal, activation and proliferation. SCs leave their niche, proliferate, migrate centripetally and undergo terminal differentiation reaching the superficial layers of cornea to maintain homeostasis (Schlotzer-Schrehardt et al., 2007).

Dysfunction or deficiency of the CESC can disrupt the delicate balance of corneal epithelial homeostasis, leading to conjunctivalization, neovascularization, and eventually to blindness which in common medical parlance called as limbal stem cell deficiency (LSCD). Based on etiology, it can be broadly classified into two categories – (I) secondary LSCD caused by mechanical, thermal or chemical trauma, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, multiple surgeries, cryotherapies, contact lens wear, extensive microbial infection and (II) primary LSCD resulting from gradual loss of SC functions over time, probably due to malfunctioning of limbal stroma such as aniridia, congenital erythrokeratodermia, keratitis associated with multiple endocrine deficiencies, neurotrophic (neural and ischaemic) keratopathy and chronic limbitis (Puangsricharern and Tseng, 1995; Dua et al., 2000). LSCD may be partial (part of the limbal circumference is damaged) or total (entire limbal circumference is damaged). Based on eyes involved, LSCD can be unilateral (involving one eye) or bilateral (involving both eyes). Many efforts have been made to improve treatment of LSCD like conjunctival limbal autografting (Kenyon and Tseng, 1989), transplantation of autologous cultured limbal epithelium (CLET) (Pellegrini et al., 1997), buccal mucosal epithelium (COMET) (Nakamura et al., 2003; Priya et al., 2011) and more recently direct use of multiple limbal autografts (SLET) (Sangwan et al., 2012).
The success of such treatment depends upon the stabilization of physiological conditions of the eye and providing adequate quantity of stem cells for survival of the epithelial graft. However, the nature of damage to limbal stroma in LSCD is not clearly understood. Thus exploring limbal microenvironment is essential to understand the full potential of transplanted epithelial graft (Bakthiari et al., 2010).

Within the limbal region of the cornea, the CESC niche is thought to be located within the palisades of Vogt (Secker and Daniels, 2008) and is identified by the presence of rete folds, melanocytes (infra basal compartment of limbus), dendritic Langerhan’s cells, nerves and underlying blood vessels (Du Toit and Page, 2010). The basement membrane (BM) of limbus is undulating with papillae of stroma and is different from corneal BM composition (Tuori et al., 1996; Schlotzer- Schrehardt et al., 2007). These anatomical features suggest that CESC are closely interacting with cells in the underlying stroma.

Besides this anatomical structure, limbal stroma is known to control epithelial proliferation, differentiation and apoptosis thus maintaining CESC in its undifferentiated state, while the corneal stroma supports epithelial differentiation (Espana et al., 2003). Limbal stroma has been thought to provide a unique environment composed of (1) the blood vessels which aids in better signaling, (2) supporting cells of mesenchymal origin and (3) the BM and extracellular matrix (ECM) composed of collagen, laminin, fibronectin etc. Further, the high level of expression of SC markers in groups of cells in limbal basal layer (Chen et al., 2004) indicate that CESC interact with the unique components in the niche that might regulate SC distribution, release of various cytokines and growth factors (Schlotzer- Schrehardt et al., 2007).

Recent studies highlight the importance of anterior limbal stroma and its cellular components in the maintenance of CESC. The presence of anterior limbal stroma along with epithelium in limbal explants or after enzymatic separation has been demonstrated to be essential for better expansion of CESC (Mariappan et al., 2010; Chen et al., 2011; Gonzalez et al., 2013; Li et al., 2014). Upon culturing, these limbal stromal cells expressing markers for embryonic stem cells (ESCs), mesenchymal stem cells (MSCs) and angiogenesis (CD34, CD31, Flk-1, VWF) supported holoclone formation (Polisetty et al., 2008, Lim et al., 2012, Branch et al., 2012; Li et al., 2012). Further, several studies in animal models showed that transplanted MSCs engrafted to injured corneal surface (Ye et al., 2006; Ma et al., 2006; Wu et al., 2007; Reinhagen et al., 2009) or secreted factors from MSCs such as TSG-6 promoted wound healing and reconstruction of the damaged
corneal surface (Oh et al., 2010; Roddy et al., 2011). Recent reports have also indicated the importance of MSCs in relation to the niche and biology of other adult SCs (Lee and Kim, 2012). All these studies emphasize MSCs’ role in homeostasis of adult SCs specifically CESC. Hence it is necessary to understand the limbal niche components that influence the CESC.

Despite the possible clinical importance of underlying limbal stroma as a niche for CESC, its microarchitecture has not been examined. It is of significance to identify and characterize these in vivo microstructures of the limbal stroma in the region of the palisades of Vogt. With the advent of in vivo laser scanning confocal microscopy (IVCM), live imaging of the corneolimbal epithelial architecture in both normal (Patel et al., 2006; Miri et al., 2012) and LSCD patients (Miri et al., 2012; Nubile et al., 2013) became possible. Thus, clarification of the identity of limbal stromal niche cells in vitro and in vivo, its influence in modulating the epithelial stem cell fate and understanding the factors secreted by them required to be explored for developing strategy for better prognosis and treatment for patients with LSCD.

A. Hypothesis

CESCs are maintained by intrinsic and extrinsic factors in their niche. Niche is located in the limbus closer to the epithelium at the palisades of Vogt. It is composed of limbal ECM, particularly basement membrane, cell- matrix and cell- cell interactions, underlying limbal stroma with blood vessels, and cells of mesenchymal origin that provide various soluble trophic factors. It is responsible for regulating and maintaining stemness of CESC.

B. Aim and Objective

- **To identify the niche cells essential for the maintenance of stemness.**
  - Structural, cellular and functional characterization of niche cells in the human limbal stroma.
- **To identify and characterize the limbal stromal niche in vivo.**
  - Identification of limbal stromal niche by live imaging of the limbus in healthy individuals and in patients with LSCD.
- **To elucidate the protein profile of the limbal niche.**
  - Secretome analysis of limbal stromal cells using mass spectrometry.