Keratoconus is a common degenerative disorder of the cornea where the collagen is weakened as a result of uncontrolled degradation. Increased activities of proteases have been shown in keratoconic corneas. Keratoconic corneas show signs of increased activity of proteases. In our laboratory, we undertook research to study the role of telopeptides, which are released during the degradation of collagen of the cornea. Human corneal stromal cell line was established and was incubated in varying concentrations of synthetic C-terminal telopeptides as follows: 3.012, 6.125, 12.25, 12.25, 23.5, 47 and 94 µg. The rate of death of cells was measured by TUNEL assay where DNA fragments released upon death of cells are detected. The difference between the number of viable cells present in the treated and untreated cells was considered for the analysis.

Interestingly, the results showed that primary corneal stromal cells treated with varying concentrations of synthetic telopeptides at 24 and 48 hours had no morphological or apoptotic changes, and the viability remained 100%, whereas the percentage viability measured at 72 hours of incubation with the synthetic telopeptide concentrations of 47 and 94 µg/ml showed considerable decrease in the cell viability ($p < 0.05$, t-test) (Figure 1). Thus, it is hypothesized that the telopeptides lead to the death of keratocytes, which are essential for the stromal structural maintenance.1

In yet another study, the effect of UV rays on corneal stem cells was evaluated as collagen cross-linking is performed to strengthen the cornea in keratoconus. The process called riboflavin–UV-A collagen cross-linking is used as (CXL) a standard procedure for keratoconus treatment. The study included 30 freshly enucleated human cadaveric eyeballs. The eye balls were subjected to a CXL procedure, mimicking the clinical protocol and during the UV-A exposure, one half of the limbus (sector A) was left unprotected, whereas the other half (sector B) was covered with a metal shield. Limbal biopsies were taken from both sectors before and after the procedure for analysis. Each strip of tissue divided into three segments was subjected, for cell count of viable cells, for cultivation on human amniotic membrane (HAM) (Figure 2) and for stem cell and differentiated corneal epithelial cell marker studies using reverse transcriptase–polymerase chain reaction. The total cell count of cells was drastically reduced upon exposure to UV rays, and stem cells were damaged.2

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The protective effect of metallic and PMMA rings on stem cells from UV rays was studied by carrying a similar kind of experiment mentioned above, but upon exposure one set of eye balls was protected by covering the limbal region with a metallic ring, and another set of eyes was shielded with a PMMA ring. The total cell count and stem cell markers were studied. It was observed that covering with metallic ring offered complete protection from UV damage compared to PMMA ring.3

References