عنوان مقاله:

Isolation, Culture, Optimization and Validation of Human Corneal Stromal Keratocytes from Discarded Corneal Tissue

محل انتشار:

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خلاصه مقاله:

Introduction: Keratocytes are the major components of the human corneal stromal cell. Cell therapy by keratocytes can be used in some corneal diseases. Because keratocytes are mitotically quiescent; therefore, the cultivation of these cells is associated with challenges. The present study aimed to isolate, culture, and validate keratocyte cells from discarded corneal tissue based on optimizing some cultivation conditions.Materials and Methods: In this experimental study, keratocytes were isolated from discarded corneal tissue. Different culture medium composition such as amniotic membrane extract, time, and the role of coating scaffolds was evaluated. Real-time PCR of specific genes were used to confirm the primary keratocyte cells compared to corneal epithelial cells. The specific genes were keratocan, lumicane, aldehyde dehydrogenase three members of family A۱ (ALDH۳A۱), and CD۳۴. In addition, immunocytochemistry (ICC) was used to confirm the expression of specific keratocan and lumican markers.Results: Keratocytes was isolated and cultured in the culture medium containing amniotic membrane extract. Based on analyses, keratocan, lumicane, ALDH۳A۱, and CD۳۴ gene expression in keratocytes was significantly higher than in the epithelial cells. Moreover, keratocan and lumican expression was detected in ۹۲.۵% and ۹۱.۱% of the cells, respectively. According to the results, the addition of amniotic membrane extract significantly increased the growth of keratocytes.Conclusions: Our findings in this study showed that discarded corneal tissue can be used as a suitable source for obtaining keratocyte cells needed in corneal tissue engineering.

کلمات کلیدی:

Primary cell culture, Corneal Keratocytes, Amniotic Membrane Extract, Tissue engineering