



SDI Review Form 1.6

Journal Name:	Ophthalmology Research: An international Journal
Manuscript Number:	2014_OR_12138
Title of the Manuscript:	Apoptosis of corneal stromal cells induced by telopeptides: An <i>invitro</i> study
Type of the Article	

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	1. Please explain why C-terminal telopeptide is indicator of collagen degradation not N-terminal telopeptide? 2. The main weaknesses of the study are a) the authors used just one donor cornea and also b) the authors did not characterized stromal cells after isolation. 3. Use of serum in culture will change keratocytes to fibroblasts, therefore this study does not evaluate the effect of telopeptides on primary keratocytes. 4. The amount of telopeptide that cause apoptosis is way higher than clinical measurements; therefore the conclusion is not valid clinically. 5. Please explain why collagen degradation will start in first place in keratoconus.	The comments and the revision of the reviewer is well accepted. Following are the response to the reviewers comments: 1. Proteolytic cleavage of type 1 collagen results in release of carboxy-terminal and amino-terminal cross-linked telopeptide (CTX and NTX). The half life of NTX is 11 hours and CTX is 1 hour. Though both are produced, studies have been performed with CTX for ocular samples. Since ready reference values are available for CTX (Abalain <i>et al</i>) the current study was undertaken to study the apoptosis with CTX. 2. A) Five donor corneas were used for growing the stromal cells. Though all had shown outgrowth, from only one which showed good growth of stromal cells was used for the experiment. The results were reproducible. B) The P1 cells were characterized using reverse transcriptase PCR for the expression of keratocan and the cells showed detectable levels of expression of keratocan. 3. Study performed by Zhou X <i>et al</i> (Local anesthetic lidocaine induces apoptosis in human corneal stromal cells <i>in vitro</i>. Int J Ophthalmol. 2013; 6(6): 766–771) 10% Fetal calf serum was used to cultivate human corneal stromal cells <i>in vitro</i>. In the current study P1 cultures were used for the study. The P1 cells were characterized and were found to express keratocan at detectable levels. Thus the study can be correlated with the effect of telopeptide on primary keratocytes. 4. According to Abalain <i>et al</i> the physiological concentrations of telopeptides obtained was 8.11µg for 10 µl of the tear ie 811 µg/ml. The present study shows that even minimum concentrations of telopeptides (94µg, 47 µg/ml) can induce apoptosis. 5. Production of collagenolytic enzymes is the widely quoted reason for the degradation and pathogenesis of keratoconus. Weak stromal results in keratoconus which is an eventual degradative effect of the proteolytic enzymes. Matrix metalloproteinases (MMPs) have been studied extensively and has been concluded that they are responsible for progression of keratoconus. The cornea is 70% collagen and the reduced collagen composition of keratoconus cornea is suggestive of degradation. (Collier SA. Is the corneal degradation in keratoconus caused by matrix metalloproteinases? Clin Experiment Ophthalmol. 2001; 29(6):340-4).



SDI Review Form 1.6

	6. Please mention which passage was used at page 4, line 88. 7. Page 8, line 150-152 should be in method section not discussion.	6. Telopeptide treatment was performed on passage 1(P1). And it is included in the text in the revised manuscript . 7. The lines 150-152 was removed from discussion part and included under method section.
<u>Minor</u> REVISION comments	Page 1, line 1: please change invitro to 'in vitro' Page 1. Line 7: please change the order of concentration from low to high and also in line 14 Page 2, line 37: please change is not studied to has not been studied	The reviewer's comments are well accepted and the changes are incorporated in the text and are highlighted.
<u>Optional/General</u> comments		