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• 計畫中文名稱	探討不同分子量片段之眼角膜專一啓動子在超微粒基因傳送與調控表現(III)
• 計畫英文名稱	Evaluation of Different Size Fragments of Specific-Promoters with Polymeric Micelles on Cornea Transfection and Expression (III)
• 主管機關	行政院國家科學委員會
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• 中文關鍵字	上皮細胞、實質、專一啓動子、聚合載體、穿透性
• 英文關鍵字	Epithelial, Stroma, Tissue-specific plasmid, Polymeric micelles, Permeability
• 中文摘要	本計畫主要目的爲評估利用眼角膜組織專一性之啓動子與聚合載體，進行角膜眼藥水之基因(keratin 12 與 keratocan3.2)傳遞；同時了解此劑型進入角膜之機制探討。所使用三種基因 pCMV-Lac Z, pK12-Lac Z and pKera3.2-Lac Z；並均含有 the Lac Z 之報告 gene。而在傳遞基因後，其基因在角膜表現則利用 X-Gal 染色、beta-Gal 酵素測試與 real-time PCR analysis. 並利用 EDTA and RGDpeptide 二種方式探討進入角膜之機制。 結果發現在投與針對角膜上皮細胞之 pK12-Lac Z 基因 6 個劑量後，在兔子與老鼠之眼角膜上皮細胞組織中，在 beta-Gal 酵素測試均可有效發現基因表現。而針對角膜實質組織下，則需以先前處理 5mM EDTA 後，角膜實質也會有基因表現出現。對於 pK12-Lac Z 與 pKera3.2-Lac Z 基因在角膜組織表現中，則均會受到 RGD 物質所抑制，並產生降低組織專一性基因表現量，因此推測此專一性組織基因傳遞劑型受包飲作用所影響。本計畫在利用專一性引子進行眼藥水基因傳遞下，均可以在眼角膜不同組織部位表現。
• 英文摘要	PURPOSE. This study evaluates the eye drop delivery of gene with cornea-specific promoters, i.e., keratin 12 (K12) and keratocan (Kera3.2) promoters, by non-ionic polymeric micelles (PM) to mouse and rabbit eyes, and investigates the underlying mechanisms. METHODS. Three PM-formulated plasmids (pCMV-Lac Z, pK12-Lac Z and pKera3.2-Lac Z) containing the Lac Z gene for beta-Galactosidase (beta-Gal) whose expression was driven by either cytomegalovirus early gene, keratin 12 gene, or keratocan gene. Transgene expression in ocular tissue after gene delivery was analyzed by 5-bromo-4-chloro-3-indolyl-beta-D-galactoside (X-Gal) color staining, 1,2-dioxetane beta-Gal enzymatic activity measurement, and real-time PCR analysis. The delivery mechanisms of

plasmid-PM on mouse and rabbit corneas were evaluated by EDTA and RGD peptide. RESULTS. The sizes of the three plasmid-PM complexes were around 150-200 nm with unimodal distribution. Enhanced stability was found for three plasmid-PM formulations after DNase I treatment. After six doses of eye drop delivery of pK12-Lac Z-PM 3 times a day, beta-Gal activity was significantly increased in both mouse and rabbit corneas. Stroma-specific Lac Z expression was only found in pKera3.2-Lac Z-PM treated animals with pretreatment of 5 mM EDTA, a junctional opener. Lac Z gene expression in both pK12-Lac Z-PM and pKera3.2-Lac Z-PM delivery was inhibited by RGD peptide, a receptor-mediated transport motif. Conclusions. Cornea epithelium- and stroma-specific gene expression could be achieved using cornea-specific promoters of keratin 12 and keratocan genes, and the gene was delivered with PM formulation through non-invasive, eye drop on mice and rabbits. The transfection mechanism of plasmid-PM may involve endocytosis and particle size dependent-paracellular transport.