Proposal for NUS PhD Project

Title of project: Immortalisation of human Meibomian gland derived cells for the purpose of lipid replacement therapy in dysfunctional tear syndrome

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Co-supervisor: Dr Louis Tong, Consultant and clinician scientist, Singapore National Eye Center.

Background: Dysfunctional tear syndrome or dry eye is a very common human ocular surface disease with huge morbidity and socioeconomic impact. Unfortunately current therapy is unsatisfactory, and replacement with artificial tears did not provide long lasting relief against inflammation and cannot correct tear instability problems. There is no medication which can replace the natural lipids in human tears.

Objective: To evaluate the following hypothesis,
That using immortalized cells cultured from human Meibomian glands, it is possible to extract and store a mixture of natural lipids which can be used to reconstitute human tears in dysfunctional tear syndrome.

Methodology
Patient undergoing entropion or other lid resection surgery at the Singapore National Eye Center by Prof Seah Lay Leng will have lid segments retained and prepared to explant cell culture. Institutional review board approval has been obtained and funding from NMRC for this project is current available for 3 years. Under previously established protocols, cells will be grown and lipid inclusions identified by Oil Red staining. Using the pBabe-neo-hTert vector (which is lentivirus compatible), a stable, immortalized cell line will be constructed and propagated. Selection of stably transfected cells will be achieved by using an appropriate concentration of G418 in the culture media for all progeny of these cells. Analysis of lipid components (polar and neutral lipids) will be performed for various cell inclusions for different progeny of the immortalized cells. Briefly, various liquid chromatography/mass spectrometry systems will be applied for characterisation and quantitation of various lipids. In addition, this project might also include (1) comparison of lipid types and categories to lipids extracted from Meibum secretion of humans, and (2) purification of lipids, and testing of lipid stability in aqueous emulsions. This may be repeated for preparations stored for different durations.

Potential importance
Lipids may be added as an emulsion for therapeutic uses in humans in the form of eyedrops, and tear function and symptomatology can be evaluated eventually, in human trials.