UNIVERSITI PUTRA MALAYSIA

STRUCTURAL AND ULTRASTRUCTURAL STUDIES OF TISSUE ENGINEERED CORNEA

SITI SALEHA BINTI MASRUDIN

FPSK(m) 2010 15

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By

SITI SALEHA BINTI MASRUDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2010

I would like to dedicate this thesis to my parents and family for their unconditional love and sacrifices, and forbearing with me during the writing of this thesis and bring joy and happiness to my heart

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

STRUCTURAL AND ULTRASTRUCTURAL STUDIES OF TISSUE ENGINEERED CORNEA

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SITI SALEHA BINTI MASRUDIN

December 2010

Chairman : Professor Fauziah Othman, PhD

Faculty : Faculty of Medicine and Health Sciences

This study was carried out to evaluate corneal organisation and regeneration after transplantation of bilayer *in vitro* cornea construct (BICC) into the New Zealand White Strain rabbit's eye. Studies were conducted to investigate the structural and ultrastructural features after corneal regeneration 90 days post-transplantation. The epithelial cells and keratocytes were isolated from the limbus of the rabbit and then cultured *in vitro* in 5 mL tissue culture flasks. BICC and fibrin without seeded cell construct (FWCC) were produced by mixing the epithelial cells and keratocytes with rabbit fibrin and calcium chloride ($CaCl_2$) and maintained in culture media. The cornea was subjected to lamellar keratectomy before BICC and FWCC were implanted into the defect area. The transplanted corneas were harvested after 90 days post-transplantation for microscopic analysis and immunolabelling studies for

cytokeratin 3. Slit lamp microscopic analysis revealed that engineered cornea (EC) showed good corneal regeneration with no significant difference in cornea transparency to normal cornea (NC). However, for fibrin cornea (FC), the cornea was opaque compared to NC. In addition, the defect cornea (DC), which was cornea without implantation after lamellar keratectomy, showed a transparent cornea similar to NC. Morphometric analysis of the corneal thickness was done by using Least Significant Difference (LSD) test and Analysis of Co-Variance, showed that EC was capable of regenerative similar thickness functional cornea as NC when compared to FC and DC ($p<0.05$). Scanning electron microscope (SEM) analysis demonstrated that epithelial surface of EC showed significantly similar features to NC compared to FC and DC ($p<0.05$). Transmission electron microscope (TEM) analysis showed that the basal lamina development of EC was similar to NC with the establishment of cell junction compared to FC and DC. Furthermore, the EC showed a compact stromal organization with homogenous collagen fibrils diameter similar to NC ($p<0.05$). However, FC and DC showed a loose stromal organization with heterogenous fibrils diameter, with FC fibrils diameter were bigger than that of NC; while for DC, the fibrils diameter was smaller than NC ($p<0.05$). Confocal microscopy analysis confirmed that the regenerated epithelial cells in all groups were corneal epithelial cells by using corneal differentiation marker, cytokeratin 3 (CK3). As a conclusion, the EC demonstrated excellent regenerative ability of cornea and better wound healing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KAJIAN TERHADAP STRUKTUR DAN STRUKTUR ULTRA TISU KORNEA YANG TELAH DIJURUTERAKAN

Oleh

SITI SALEHA BINTI MASRUDIN

Disember 2010

Pengerusi : Professor Fauziah Othman, PhD

Fakulti : Fakulti Perubatan dan Sains Kesihatan

Kajian ini telah dijalankan bagi menilai organisasi kornea dan penjanaan semula selepas pemindahan dwilapisan konstruk kornea *in vitro* (BICC) kepada mata arnab strain New Zealand White. Kajian ini telah dijalankan bagi menyiasat ciri-ciri struktur dan ultrastruktur selepas penjanaan semula kornea 90 hari selepas implantasi. Sel epitelium dan keratosit telah diasingkan daripada limbus arnab dan kemudian dikultur secara *in vitro* dalam 5 mL kelalang kultur tisu. BICC dan fibrin konstruk tanpa sel (FWCC) telah dihasilkan dengan mencampur sel epitelium dan keratosit dengan fibrin arnab dan kalsium klorida ($CaCl_2$) dan disimpan di dalam media kultur. Kornea arnab melalui proses keratektomi lamela sebelum BICC dan FWCC ditransplan ke dalam kawasan dicacatkan. Selepas 90 hari, kornea yang telah ditransplan, dituai untuk analisis mikroskopi dan kajian

perlabelan immuno untuk cytokeratin 3 (CK3). Analisis 'mikroskop lampu celahan' mendedahkan bahawa kornea yang dijuruterakan (EC) menunjukkan penjanaan semula kornea yang baik dengan transparensi kornea menyerupai kornea normal (NC). Namun, untuk kornea fibrin (FC), kornea adalah legap berbanding NC. Seperkara lagi, kornea yang dicacatkan (DC), iaitu kornea tanpa pengimplanan selepas keratektomi lamela, menunjukkan kornea telus dan menyerupai NC tanpa sebarang ciri-ciri nekrotik. Analisis morfometri bagi ketebalan kornea telah dibuat dengan menggunakan ujian perbezaan minima bererti (LSD) dan ANCOVA, menunjukkan bahawa EC mampu meregenerasi dengan baik untuk menghasilkan ketebalan yang serupa dengan NC berbanding dengan FC dan DC ($p<0.05$). Analisis mikroskop elektron imbasan (SEM) pada permukaan epitelium EC membuktikan ia mempunyai ciri-ciri signifikan yang menyerupai NC berbanding dengan FC dan DC ($p<0.05$). Analisis daripada mikroskop elektron pancaran (TEM) menunjukan bahawa pembangunan basal lamina dalam EC menyerupai NC dengan penubuhan simpang sel hemidesmosom, lamina lusida, lamina densa dan rangkaian fibril berlabuh berbanding FC dan DC. Tambahan pula, organisasi stroma EC menunjukkan satu persamaan dengan NC yang mempunyai satu persamaan susunan corak tenun reraga bungkusan kolagen yang padat, dan kolagen fibril EC mempunyai garis pusat yang seragam dan secara signifikan menyerupai NC ($p<0.05$). Namun begitu, kornea FC dan DC menunjukkan satu susunan yang longgar dan menunjukkan garis pusat yang berbeza dan pelbagai, di

mana garis pusat fibril FC lebih besar apabila dibandingkan dengan NC, manakala untuk DC; garis pusat fibril ternyata lebih kecil daripada NC ($p<0.05$). Analisis mikroskop konfokal mengesahkan sel epitelium yang telah diregenerasikan dalam semua kumpulan adalah sel epitelium kornea dengan menggunakan penanda pembezaan kornea, cytokeratin 3 (CK3). Kesimpulannya, kejuruteraan semula kornea dan kemajuan penyembuhan adalah sangat baik serta mampu memberikan harapan baru kepada kaedah yang sedia ada.

ACKNOWLEDGEMENTS

All praise to Allah S.W.T., the Almighty God and the Lord of the Universe. Firstly, I am grateful to my supervisor, Prof. Dr. Fauziah Othman, who inspired me throughout my study, financially, physically and spiritually. Without her continuous support and interest, I will not be able to complete this research. I am also grateful to the support and mentoring of my co-supervisors, Prof. Dr. Asmah Rahmat and Prof. Dr. Ruszymah Haji Idrus for their guidance and encouragement on me throughout this project.

Secondly, I would like to thank my research collaborator Dr. Norzana Abdul Ghafar, for her cooperation and valuable advice during sample preparation for this study. I would also like to acknowledge the support of my lab mates and friends, especially Anushia, Zolkapli, Norhayati, Syafini, Phang, Maslina, Lee Fong and Zarida for providing me with happiness especially in times of sorrow. Not forgetting the staff of Microscopy Unit, Institute of Bioscience, Universiti Putra Malaysia for their excellence services and help during microscopy imaging.

I would like to thank my parents and siblings for their understanding, support, and unconditional love. Their love and support have been and will

continue to be my inspiration, and I am so blessed to have such a caring and supporting family to enable me to complete this research.

Last but not the least; I would like to thank Ministry of Science and Technology (MOSTI) for sponsoring the research project.

Thank you.

I certify that a Thesis Examination Committee has met on 29 December 2010 to conduct the final examination of Siti Saleha Binti Masrudin on her thesis entitled 'Structural and Ultrastructural Studies of Tissue Engineered Cornea' in accordance with the Universities and Universiti College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institution.

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SITI SALEHA BINTI MASRUDIN

Date: 29 December 2010

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