PENILAIAN DAN PENJANAAN SEMULA KONDUIT SARAF YANG DISEMAI DENGAN SEL STEM MESENKIMA SUM-SUM TULANG YANG DIARUH KEPADA FENOTIP SEL SARAF

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PENGAKUAN

Saya akui karya ini adalah hasil kerja saya sendiri kecuali nukilan dan ringkasan yang tiap-tiap satunya telah saya jelaskan sumbernya.

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ABSTRAK

Penyambungan saraf yang terputus menggunakan saraf penderma autologous adalah rawatan piawai untuk pesakit yang mengalami kecederaan saraf periferi. Namun, rawatan yang digunakan ini mempunyai kelemahan seperti perbezaan saiz saraf penderma dengan saraf penerima, memerlukan pembedahan kedua untuk memperolehi saraf penderma dan risiko neuroma di kawasan pembedahan kedua. Untuk mengatasi masalah tersebut, kajian ini dijalankan untuk menjana saraf gantian melalui teknik kejuruteraan tisu menggunakan sel stem mesenkima daripada sum-sum tulang manusia. Sel stem mesenkima (MSCs) manusia diperolehi daripada lebihan sum-sum tulang pesakit yang menjalani pembedahan tulang yang patah. MSCs dikultur dalam media α-Minimum Essential (α-MEM) sebelum diaruh menjadi sel saraf. Analisa immunositokimia dan aliran sitometri mengesahkan kejayaan proses diferensiasi MSCs kepada sel saraf. Sel yang telah menjalani proses diferensiasi ini turut mengekalkan profil kitaran sel normal. Salur vena dan tisu otot ternyah sel digunakan sebagai bahan kerangka yang menyerupai struktur saraf normal. Bagi menghasilkan konstruk saraf yang hidup, sel MSCs yang diaruh kepada fenotip sel saraf disemai ke atas tisu otot semalaman dan dipadatkan ke dalam salur vena pada keesokkan harinya. Konstruk saraf yang dihasilkan akan diinkubasi dalam media α-MEM selama 3 hari untuk analisa in vitro dan diimplantasi pada mencit athymik untuk analisa in vivo. Kontruk saraf di kedua-dua peringkat in vitro dan in vivo menunjukkan kadar proliferasi sel yang baik. Analisa histologi pada minggu kedua menunjukkan tisu otot mula mengalami proses degradasi dan perlahan-lahan diganti dengan matrik luar sel yang dihasilkan oleh sel yang disemai. Selepas lapan minggu implantasi, kesemua tisu otot telah mengalami proses degradasi dan diganti sepenuhnya dengan matrik luar sel. Salur vena masih utuh walaupun selepas lapan minggu implantasi. Analisa struktur ultra menunjukkan sel yang disemai menghasilkan matrik luar sel dengan merembeskan vasikel matrik secara aktif. Kesimpulannya, tisu otot ternyah sel yang disemai dengan sel MSCs yang diaruh kepada fenotip sel saraf dan dipadatkan ke dalam salur vena menghasilkan konstruk saraf yang stabil dan berpotensi untuk menggantikan penggunaan saraf autologus di masa hadapan.

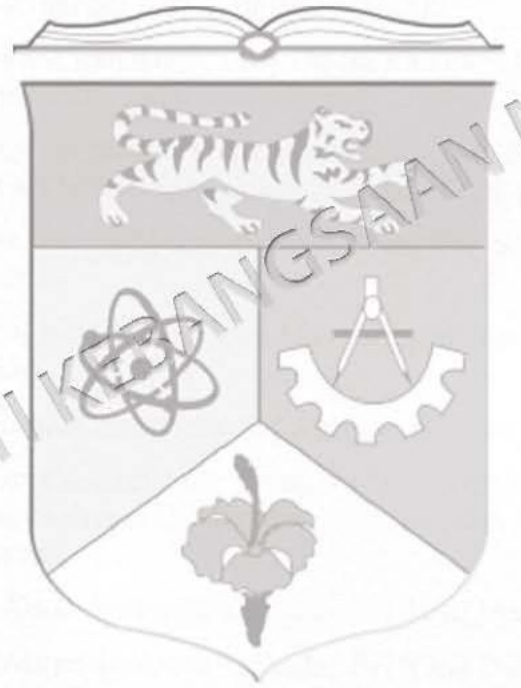
DEVELOPMENT AND EVALUATION OF NEURAL CONDUIT SEEDED WITH NEURO DIFFERENTIATED BONE MARROW MESENCHYMAL STEM CELLS

ABSTRACT

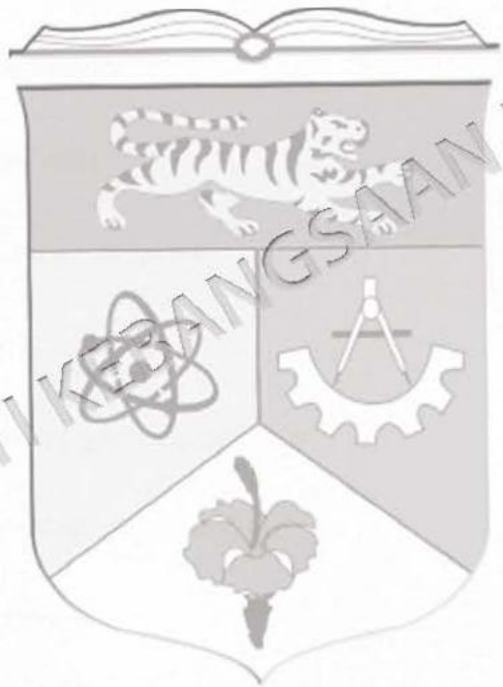
Bridging nerve gaps using autologous donor nerve is the treatment of choice for patients with peripheral nerve injury. However, it has disadvantages such as size mismatch between donor and recipient nerve, requirement of a second surgery for the extraction of donor nerve and risk of neuroma. To overcome these problems, this study aimed to construct a human nerve substitute via tissue engineering technique using mesenchymal stem cells (MSCs) derived from human bone marrow. Excess bone marrow was derived from patients who underwent a routine fracture management procedure called the IM nailing procedure where a nail was inserted into the medullary canal of a fractured long bone. The acquired MSCs were cultured in α-Minimum Essential Medium (α-MEM) followed by induction with a neuro differentiation protocol. Immunocytochemistry and flow cytometry analysis confirmed the successful neuro differentiation of the MSCs. These cells retained the normal cell cycle profile. Human muscles and veins were decellularized and used to form a nerve conduit that mimicked native nerve structure. To form a living nerve conduit, neuro differentiated MSCs were seeded on decellularized and hydrolyzed muscles overnight and then stuffed into a decellularized vein the next day. The nerve construct was incubated in α-MEM for 3 days for in vitro analysis and implanted into athymic mice for in vivo analysis. Both in vitro and in vivo analyses showed good cell proliferation within the conduit. Histology analysis demonstrated that at two week post implantation, these muscles started to degrade and gradually became replaced by extracellular matrix produced by the seeded cells. At eight weeks post implantation, the muscles were fully degraded and completely replaced by extracellular matrix while the veins remained intact. Ultra structural analysis further revealed that these seeded cells actively produced extracellular matrix via secretion of matrix vesicles. In conclusion, decellularized muscle stuffed vein seeded with neuro differentiated MSCs forms a stable neural conduit and has the potential to replace autologous nerve in future.

KANDUNGAN

SENARAI JADUAL



SENARAI RAJAH



SENARAI SINGKATAN

kg	Kilogram
cm	sentimeter
mm	milimeter
%	peratus
µm	mikron
µl	mikroliter
ml	mililiter
cm^2	sentimeter persegi
µM	mikro molar
µg/ml	mikro gram per mililiter
ng/mL	nano gram per mililiter
N	Normal
MSCs	Sel Stem Mesenkima
HSCs	Sel Stem Hematopoitik
SCs	Sel Schwann
IL-1	Interleukin 1

BAB I

PENGENALAN

1.1 KEJURUTERAAN TISU

Kejuruteraan tisu adalah satu bidang bioteknologi yang berkembang dengan cepat dan menggabungkan pelbagai disiplin ilmu yang melibatkan biologi, perubatan dan kejuruteraan yang merevolusikan atau mengubah sepenuhnya cara kita mempertingkatkan tahap kesihatan dan kualiti kehidupan berjuta manusia di dunia, dengan cara memulih, mengekalkan atau meningkatkan fungsi tisu atau organ. Dalam erti kata lain, matlamat utama bidang kejuruteraan tisu berdasarkan konsep rawatan ialah untuk menggantikan atau mengembalikan struktur anatomi dan fungsi tubuh yang hilang akibat kecederaan, kerosakkan atau kehilangan tisu atau organ berikutan kemalangan mahupun proses patologi dalam tubuh. Matlamat ini cuba dicapai dengan cara menggabungkan sel, biobahan, faktor biokima dan fisio-kimia bersama faktor pertumbuhan yang tertentu dalam satu persekitaran mikro yang sesuai bagi setiap pertumbuhan tisu atau organ (Ibarra et al 2000; Atala 2000; Lanza 2000).

Definisi lain turut diberikan oleh Charles A. Vacanti dan Langer R. (1993) dalam artikel bertajuk *Tissue Engineering*, dalam jurnal *Science*. Namun begitu, walau bagaimana pun ayatnya diolah, ianya tetap memberikan pengertian dan matlamat yang sama iaitu mengubati penyakit. Menurut CA Vacanti dan Langer R. (1993) kejuruteraan tisu merupakan bidang interdisiplin yang menggabungkan prinsip kejuruteraan dan sains kehidupan dalam aplikasi ke arah pembentukkan bahan ganti biologi yang lebih memfokus kepada penciptaan, pemeliharaan atau pengembalian fungsi organ yang rosak. Selain menyediakan platform untuk mengubati penyakit, di mana tisu tumbuh di dalam badan pesakit atau di luar badan pesakit dan kemudiannya

ditransplantasi semula kepada pesakit, kejuruteraan tisu juga menyediakan platform menggunakan tisu yang dihasilkan secara in vitro untuk kajian metabolisma dan penyerapan dadah, kajian ketoksikkan dan kajian ke atas penyebab penyakit. Pengasasan kejuruteraan tisu untuk aplikasi rawatan atau aplikasi diagnostik adalah untuk memanfaatkan sel hidup dalam pelbagai cara.

Dalam bidang kejuruteraan tisu, sumber sel autologus merupakan sumber terbaik kerana dapat mengelakkan risiko penolakkan imunisasi, risiko penularan penyakit dan masalah mencari penderma yang sesuai. Sel yang digunakan juga adalah sel yang hidup dan berupaya untuk terus berproliferasi. Selain itu, penggunaan biobahan yang menjadi struktur sokongan utama bagi memberi bentuk kepada tisu yang dijana, perlu diselidiki dari segi penerimaan sel terhadap biobahan tersebut dan kesesuaiannya dengan pengguna kerana biobahan yang tidak sesuai akan menyumbang kepada kegagalan graf. Maka dengan itu, biobahan dari sumber biologi dan autologus adalah pilihan terbaik supaya dapat mengembalikan fungsi dan berintegrasi dengan tubuh penerima. Biobahan yang digunakan juga selamat kepada pengguna apabila proses degradasi berlaku tanpa mengakibatkan mudarat kepada pengguna.

Bidang kejuruteraan tisu sudah mula bertapak di negara kita sejak beberapa tahun yang lepas selepas hampir 35 tahun penyelidikan berkaitan kejuruteraan tisu telah dijalankan oleh penyelidik luar (Saim et al. 2000). Ini dibuktikan daripada kajian yang dilaporkan oleh Chick et al. pada tahun 1975 berkenaan kejayaan mereka menjana satu sistem yang melibatkan sel pankreas di simpan di dalam membran separa telap untuk membantu proses pengawalan paras glukosa pesakit diabetes melitus. Namun begitu, sejarah kejuruteraan tisu sebenarnya telah bermula seawal 1970-an oleh pakar tulang kanak-kanak dari Children's Hospital, W. T. Green, M.D yang menjalankan kajian untuk menjana tisu rawan menggunakan sel kondrosit yang disemai di tulang spikul dan diimplan ke atas mencit atymik seperti mana yang dilaporkan oleh Charles A. Vacanti dalam penulisannya yang bertajuk '*The history of tissue engineering*'.

Walaupun kajian ini tidak berjaya, namun ia dapat mencetuskan idea dan membuka mata para penyelidik lain dalam meningkatkan mutu dan kualiti penyembuhan penyakit. Beberapa tahun kemudian, penyelidik daripada Massachusetts General Hospital and M.I.T bergabung menjalankan penyelidikan berkaitan kejuruteraan tisu kulit menggunakan matriks kolagen untuk menyokong pertumbuhan sel fibroblast. Pada tahun yang sama juga ujian klinikal dijalankan ke atas pesakit yang tercedera akibat kebakaran dengan menampal kepingan nipis sel keratonosit di kawasan yang melecur. Bermula dari saat itu, lebih banyak kajian dilakukan terhadap pelbagai jenis tisu seperti telinga (Aminuddin et al. 2003; Cao et al. 1997; Saim et al. 2000), tendon (Cao 2002), kornea (Li et al. 2003; Shimura et al. 2003; Tsai et al. 2003), saraf periferi (Lundborg & Richard 2003; Scherman et al. 2001; Evans et al. 2002; Fansa & Keilhoff 2004; Fawcett & Keynes 1990) dan lain-lain lagi. Selepas pada itu, bermula pula episod penggunaan sel stem dari sumber seperti sum-sum tulang (Pittenger et al. 1999; Pittenger et al. 2000; Prockop 1997; Azizi et al. 1998) dan tisu adipos (Kokai et al. 2005; Prunet-Marcassus et al. 2006; Safford & Rice 2005; Zuk et al. 2001; Strem et al. 2005) yang dipercayai boleh diaruh kepada sel yang dikehendaki. Hingga kini, pelbagai jenis kajian berkaitan kejuruteraan tisu sedang giat dijalankan di seluruh dunia dengan satu matlamat iaitu untuk meningkatkan tahap kesihatan dan kualiti kehidupan berjuta manusia di dunia.

1.2 PERMULAAN RAWATAN KECEDERAAN TISU SARAF PERIFERI

Usaha awal merawat kecederaan tisu saraf telah bermula seawal tahun 1608. Akan tetapi sehingga abad kesembilan belas barulah pakar saraf mula memfokus kepada usaha pembaikan yang lebih berkesan. Malangnya, kebanyakkan teknik pembedahan yang digunakan untuk menjana semula saraf periferi yang tercedera memberi keputusan pemulihan fungsi yang tidak baik. Oleh itu, alternatif lain diambil bagi mengatasi masalah ini, termasuk menggunakan tulang, tiub logam dan gauze (Gregory 2003). Sehinggalah pada awal tahun 1960an, Millesi memperkenalkan konsep mikrosurgeri yang mana setiap fasikel saraf boleh disambung secara lurus dari hujung distal dan proksimal menggunakan saraf lain sebagai penyambung, dan teknik ini meningkatkan tahap regenerasi saraf dengan berkesan pada waktu itu (Millesi 1990a; Millesi 1990b).

Namun begitu, masa silih berganti, teknik yang diperkenalkan ini menimbulkan beberapa masalah seperti memerlukan pembedahan kedua untuk memperolehi saraf lain sebagai penyambung, menimbulkan rasa sakit atau risiko neuroma pada tempat pembedahan kedua dilakukan, kekurangan bekalan saraf yang sihat dan hanya boleh diaplikasi sekiranya jarak antara dua hujung yang terputus adalah pendek (Bae & Chung; terzis et al. 1997; Oliveira et al. 2004; Trumble & Shon 2000). Sekali lagi membuka mata para penyelidik dunia mencari sumber alternatif dalam mengatasi masalah ini. Dengan itu, pelbagai kajian dilakukan bagi menggantikan saraf yang rosak selain mengembalikan fungsinya. Sehingga kini, masih tiada jalan penyelesaian yang konkrit diperolehi kerana setiap langkah yang digunakan memiliki kebaikan dan keburukkan yang tersendiri. Sebagai contoh, terdapat beberapa kajian mendapati dinding salur vena yang digunakan untuk menyambung dua hujung saraf runtuh walaupun jarak antara dua hujung adalah 2 cm (Tang 1995; Brunelli et al. 2005; Tang 2005). Hasil yang sama juga diperolehi jika hujung disambung menggunakan tisu otot sahaja (Brunelli 2005), walaupun persekitaran di dalam salur vena dan basal lamina tisu otot dipercayai sangat sesuai untuk regenerasi akson.

Oleh itu, kajian ini berfokus kepada penjanaan semula tisu saraf periferi menggunakan salur vena dan tisu otot manusia sebagai biobahan dan sel stem mesenkima yang diaruh menjadi sel saraf sebagai sumber sel melalui kaedah kejuruteraan tisu. Ini adalah langkah permulaan dalam penjanaan tisu saraf periferi bagi merawat kecederaan saraf periferi, yang diharapkan kajian ini dapat diperdalam pada suatu masa kelak agar lebih banyak informasi diperolehi sebelum menapak kepada kajian klinikal .

1.3 OBJEKTIF KAJIAN

1.3.1 Objektif Umum Kajian

Objektif umum kajian ini ialah untuk menjana konstruk saraf menggunakan salur vena yang disumbat dengan tisu otot ternyah sel yang disemai dengan sel stem mesenkima yang diaruh kepada fenotip sel saraf melalui kaedah kejuruteraan tisu.

1.3.2 Objektif Khusus Kajian

1. Mengenalpasti morfologi dan profil pertumbuhan sel stem mesenkima manusia dan sel sel stem mesenkima manusia yang diaruh teraruh kepada fenotip sel saraf secara mengukur peratusan viabiliti, masa penggandaan dan analisa kitaran sel.
2. Menilai ciri-ciri sel stem mesenkima manusia dan sel stem mesenkima manusia yang diaruh kepada fenotip sel saraf menggunakan penanda sel Schwann dengan mengesan protein permukaan sel seperti S100b, reseptor p75 Faktor Pertumbuhan Saraf (NGF), Protein Glia Fibril Berasid (GFAP) dan Nestin melalui teknik imunofloresen dan menilai secara kuantitatif protein permukaan sel melalui teknik aliran sitometri.
3. Menilai bahan kerangka salur vena manusia yang dipadatkan dengan tisu otot manusia ternyah sel dan disemai dengan sel stem mesenkima manusia yang diaruh kepada fenotip sel saraf berdasarkan analisa histologi dan struktur ultra (SEM).
4. Menilai konstruk tisu saraf selepas implantasi pada lapisan otot mencit athymik (in vivo) secara analisa histologi dan struktur ultra (SEM).

1.4 HIPOTESIS

1 Sel stem mesenkima boleh dikultur di dalam media pengkulturan α-MEM dan profil pertumbuhan sel selepas proses diferensiasi tidak terganggu.

2 Sel stem mesenkima boleh diaruh kepada sel yang memiliki fenotip sel saraf secara in vitro dan mempamerkan penanda protein permukaan sel Schwann yang dikaji seperti S100b, Nestin, GFAP dan reseptor p75NGF.

3 Salur vena dan tisu otot ternyah sel merupakan bahan yang berpotensi untuk digunakan sebagai bahan kerangka dalam penghasilan konstruk saraf dalam menggalakkan perlekatan sel walaupun selepas menjalani siri rawatan menggunakan larutan kimia.

4 Konstruk saraf yang disemai dengan sel neuro diferensiasi sel stem mesenkima selepas implantasi menggalakkan proliferasi sel dan penghasilan matrik luar sel oleh sel yang disemai.

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