OSTEOPROMOTION OF MANDIBULAR DISTRACTION OSTEOGENESIS USING STEM CELLS FROM HUMAN DECIDUOUS TEETH AND IN BIPHASIC CALCIUM PHOSPHATE SCAFFOLD IN RABBIT MODEL

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by

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LIST OF ABBREVIATIONS

BCP	Biphasic calcium phosphate
MBCP	Macroporous biphasic calcium phosphate
BMPs	Bone morphogenetic proteins
BMMSCs	Bone marrow mesenchymal stem cell
CT	Computed tomography
ECM	Extra cellular matrix
ESCs	Embryonic stem cells
FBS	Fetal bovine serum
FGF-2	Fibroblast growth factor
IGF-1	Insulin-like growth factor-1
MSCs	Mesenchymal stem cells
MTT	Dimethylthiazol diphen
OC	Osteocalcin
ON	Osteonectin

KAJIAN PENGGALAKKAN PERTUMBUHAN TULANG MENGGUNAKAN SEL STEM DARIPADA GIGI DESIDUS DAN SEL STEM BERSAMA KERANGKA BIPHASIC KALSIUM PHOSFAT DWIFASA DI DALAM OSTEOGENESIS REGANGAN MANDIBEL MENGGUNAKAN MODEL ARNAB

ABSTRAK

Osteogenesis Regangan (OR) yang digambarkan sebagai kejuruteraan tisu tulang dalaman telah menjadi semakin popular di tahun-tahun kebelakangan ini dan teknik penggunaan telah mengalih perhatian kepada rangka kraniofasial yang telah mengembangkan bilangan rawatan alternatif untuk pesakit yang tidak normal dan kekurangan maksilofasial. Ia telah digunakan pertama kali dalam pembedahan ortopedik

untuk pembetulan kerencatan pertumbuhan anggota badan, dan seterusnya telah digunakan dalam rawatan microsomia kraniosfasial dan kecacatan tulang. Dalam OR, pembentukan tulang yang baru adalah didorong oleh pemisahan secara beransur-ansur segmen bertulang selepas osteotomi atau kortikotomi semasa proses biasa penyembuhan patah yang diganggu oleh penggunaan daya tarikan secara beransur-ansur terhadap kalus lembut. Walaupun OR mempunyai beberapa kelebihan yang nyata berbanding kaedah pembedahan tradisional terutamanya kedua-dua tisu yang lembut dan keras yang akan memanjang pada masa yang sama, namun kelemahan utama adalah rawatan yang lama yang kadangkala tidak dapat diterima oleh pesakit dan boleh dikaitkan dengan komplikasi bertulang dan tisu lembut dan kemungkinan

penyatuan berserabut atau ketidaksatuan kekal sebagai batasan utama yang menghalang amalan klinikal yang selanjutnya. Banyak kajian telah memberi tumpuan kepada promosi pembentukan tulang baru untuk memendekkan siri gangguan osteogenesis. Cara fizikal telah dimasukkan ke medan elektromagnetik berdenyut, keamatan ultrabunyi rendah dan rangsangan elektrik. Kaedah intervensi seperti pemindahan daripada sel-sel seperti osteoblast atau sumsum tulang ke bahagian gangguan, suntikan faktor pertumbuhan atau plasma platelet yang padat dan terapi gen juga telah digunakan untuk mempercepatkan kematangan tulang yang dihasilkan. Beberapa kaedah bukan intervensi, seperti penggunaan asid kalsitonin, alendronate dan zoledronik juga telah menampakkan kejayaan awal. Kejuruteraan tisu (KT) adalah satu bidang baru yang sangat menjanjikan rekonstruktif yang menarik dalam kemajuan terkini dalam bidang perubatan dan pembedahan. Tiga elemen utama KT adalah sel

stem, randuk dan faktor pertumbuhan. Sel stem dari gigi daun luruh exfoliatif (SHED) telah terbukti mampu dibezakan kepada osteoblas dan kondrosit-kondrosit dalam pembentukan tulang vitro dan in vivo dan menjadi sumber sel utama bagi kejuruteraan tisu tulang. Ia telah digunakan dalam pembinaan semula kecacatan saiz kritikal.Penggunaan SHED semasa gangguan osteogenesis untuk menggalakkan pertumbuhan tulang baru belum dilaporkan. Microporus dwifasa kalsium fosfat seramik (MDKF) merupakan biomaterial yang digunakan sebagai pengisi tulang dan sebagai tangga-tangga dalam kejuruteraan tisu tulang. Tujuan utama kajian ini adalah untuk menguji sama ada penambahan SHED dan komposit yang terdiri daripada pilihan dalam granul MDKF dalam osteotomy sebagai kejuruteraan tisu membina potensi peningkatan osteogenik dalam menjana semula OR. MBCP disintesiskan dengan ciri-ciri yang diingini, dicirikan dan diuji dalam cytotoxicity vitro. SHD adalah terpencil, berkembang dan dicirikan. Lapan belas arnab putih New Zealand telah dibahagikan kepada 3 kumpulan. Kumpulan A merupakan kumpulan kawalan (tiada intervensi dalam potongan osteotomy), kumpulan B, 6 juta sel-sel (SHD) telah dipindahkan dan kumpulan C, (SHD / BCP membina) telah dipindahkan ke dalam potongan osteotomy. Janaan semula itu dinilai dalam 3 tempoh; 2,4 dan 6 minggu secara postoperatif menggunakan X-ray konvensional, bahagian histologi dan ukuran histomorfometrik. Jumlah tulang yang baru ditentukan kuantitinya menggunakan penganalisis imej Hasilnya menunjukkan bahawa kedua-dua SHD dan SHD / MBCP membina meningkatkan pembentukan tulang. Peratusan tulang yang baru tumbuh pada 2 minggu kawalan, SHD dan SHD /MBCP adalah 18,41, 41,53 dan 57,28, pada minggu 4 31,68, 59,78 DAN 66,49 DAN DALAM MINGGU 6 52.34,60.24 dan 72,98%. Kesimpulannya, kajian ini boleh memberikan maklumat tambahan dan bukti daripada pelbagai mekanisme tindakan SHD

dalam pembentukan tulang yang mungkin berguna dalam memilih tulang yang berkesan menggalakkan binaan untuk pembentukan tulang. Seperti yang telah ditunjukkan, kedua-dua SHD dan SHD / MBCP mempunyai aktiviti osteopromosi dan potensi binaan pembentukan tulang. Kajian lanjut tentang kesan SHD dan SHD / MBCP kekal penting kerana ia akan merentas laluan dalam membangunkan binaan osteopromotik untuk regangan osteopromosi manusia. MDKF disintesiskan dengan ciri-ciri yang diingini, diberi ciri-ciri dan diuji dalam cytotoxicity vitro. SHD adalah terpencil, berkembang dan diberi ciri-ciri. Lapan belas Neozeanald putih arnab telah dibahagikan kepada tiga kumpulan. Kumpulan A kawalan (campur tangan tidak digunakan dalam pemotongan osteotomy, kumpulan B, 6 juta sel-sel (SHD) telah dipindahkan dan kumpulan C, SHD / MBCP membina telah dipindahkan ke dalam potongan osteotomy.Penjanaan semula itu dinilai dalam 3 tempoh 2,4 dan 6 minggu secara post operatif menggunakan Xray konvensional, bahagian histologi dan ukuran histomorfometrik. Jumlah tulang yang baru dibilang menggunakan penganalisis imej. Hasilnya menunjukkan bahawa kedua-dua SHD dan SHD/ MBCP membantu meningkatkan pembentukan tulang. Peratusan tulang yang baru dibentuk pada 2 minggu kawalan, SHD l dan SHD / MBCP 18,41, 35.97 dan 57,28, pada minggu 4 31,68, 59,78 dan 66,49 dan dalam minggu 6 52.34,60.24 dan 72,98%. Kesimpulannya, kajian kami boleh memberikan maklumat tambahan dan bukti daripada pelbagai mekanisma tindakan SHD dalam pembentukan tulang yang mungkin berguna dalam memilih tulang yang berkesan menggalakkan pembinaan untuk pembentukan tulang. Seperti yang telah ditunjukkan kedua-dua SHD dan SHD / MBCP mempunyai aktiviti osteopromosi dan tulang yang berpotensi membentuk pembinaan.Kajian lanjut tentang kesan SHD kedua-dua SHD/ BCP kekal penting kerana

kita boleh membangunkannya sebagai pembinaan osteopromotik untuk gangguan osteogenesis manusia.

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ABSTRACT

Distraction osteogenesis (DO) is described as endogenous bone tissue engineering has become increasingly popular in recent years and the application of distraction technique to the craniofacial skeleton has expanded the number of treatment alternatives for patients with maxillofacial abnormalities and deficiencies. It is applied first in orthopedic surgery for correction of limb length discrepancies, and subsequently has been utilized in the treatment of craniofacial microsomia and bony defect. In DO, new bone formation is induced by gradual separation of bony segments after an osteotomy or corticotomy during which the normal process of fracture healing is interrupted by the application of gradual traction to the soft callus. DO has some distinct advantages over

traditional surgical methods which involve the elongation of both hard and soft tissue at the same time. The major disadvantage is the long treatment time which is some time not well tolerated by patients and the chance of associated bony and soft-tissue complications with possible fibrous union or nonunion remains as major limitations impeding its further clinical application. Many studies have focused on the promotion of new bone formation to shorten the course of DO. Physical means have included pulsed electromagnetic fields, low- intensity ultrasound and electrical stimulation, interventional methods such as transplantation of osteoblast-like cells or bone marrow to the distraction site. Bone formation *in vivo* has become the main cell source for bone tissue engineering, it has been used in critical size defect reconstruction. The application of stem cells from exfoliative deciduous teeth (SHED) during distraction, injection of growth factors or platelet rich plasma and gene therapy have also been applied to accelerate the maturation of the regenerated bone. Some non interventional methods, such as administration of calcitonin, alendronate and zoledronic acid have also shown promising results. Tissue engineering (TE) is a new highly promising field of reconstruction that is drawing attention in recent advances in medicine and surgery. The main 3 element of TE are stem cells, scaffold and growth factor. SHED has been proven to be capable of differentiating into osteoblasts and chondrocytes *in vitro*. The usage of stem cells from human deciduous teeth (SHD) to promote new bone formation in DO has yet not been reported. Macroporous biphasic calcium phosphate ceramic (MBCP) is biomaterial used as bone filler and as a scaffold in bone tissue engineering. The main aim of this study was to test whether the addition of SHD and a composite consisting of SHD seeded in MBCP granules in osteotomy as a tissue engineering construct increases osteogenic potential in DO. MBCP was synthesized with desirable properties, Calicum /

Phosphate ratio, micro and macro porosities and particle size. The material was characterized using x ray diffraction, scanning electron microscope and particle size analyzer. *In vitro* cytotoxicity was performed to test the biocompatibility of the synthesized macroporous biphasic calcium phosphate. Stem cells were isolated from human pulp of deciduous teeth, expanded *in vitro* and characterized using 2 antibodies CD 166 and 105. The *in vivo* study was performed using rabbit's model, Eighteen New Zealand white rabbits were divided into 3 groups. Group A underwent DO without addition of materials as control, group B had 6 million cells (SHD) transplanted in osteotomy gap and group C had SHD/BCP construct consisted of 6 million cells in 50 mg MBCP transplantation. DO protocol was 4 days latency period, 6 days

distraction period 1mm/day and assessed in 3 consolidation periods 3, 18 and 32 days. The regenerate was evaluated in 3 intervals of 2, 4 and 6 weeks postoperative periods, clinically, radiographically using conventional X ray, significance of callus formation, formation of bone cortex and bone marrow cavity were evaluated to compare between groups. Histological sections for new bone formation, blood vessels, cartilage and fibrous tissue were assessed. Quantitative histomorphometric measurements were carried out using Zeiss image analysis system to quantify the amount of bone formed, cartilage and fibrous tissue ruminants. The samples were also analyzed histomorphometrically for the grade of osseous regeneration using an established numerical scoring system for the assessment of bone healing. Serial sections were scored for 2 independent bone-forming indices stage of bone union and grade of bone maturity. The result demonstrated that MBCP synthesized with Ca/P ratio of 1.52 confirmed by XRD, micro and macro porosity of 200-400 µm was confirmed by SEM. *In vitro* cytotoxicty showed that MBCP is free of toxicity, Mann-Whitney test detected that the

optical absorbance (OD) median of SHD for all MBCP concentrations was statically higher than control using 7 days extraction, P value < 0.001 for each. SHD were successfully isolated by enzyme digestion method. Early cell cultures revealed typical fibroblast-like spindle shaped cells arranged in colonies. Results of flow cytometry showed expression of CD 105 and 166, >42 and >95% respectively. The *in vivo* study showed that both SHD and SHD/MBCP construct enhance bone formation in all time points 2, 4 and 6 weeks postoperatively. Radiographic examination revealed presence of radiological evidence of DO healing in all treatment groups with more bone formation in the transplanted one. Histologically, intramembrance ossification was evidence, the highest bony formation was seen in SHD/MBCP group followed by SHD group and the least amount was in control group. Histomorphometric measurement detected that the percentage of newly formed bone in 2 weeks control, SHD and SHD/BCP were 18.41, 35.97 and 57.28% respectively, in week 4 were 31.68, 59.78 and 66.49% and in week 6 were 52.34, 60.24 and 72.98% respectively. Non parametric ANOVA (Kruskal Whalis Test) showed significant difference between groups P value = 0.003. Bone-forming indices such as stage of bone union and grade of bone maturity were highest in SHD/BCP group and lowest in control group, Kruskal Whalis Test showed significant difference between groups P value = 0.001 and 0.002 respectively. In conclusion, this study may provide additional information and evidence of the various mechanisms of action of SHD in bone formation which may be useful in selecting effective bone promoting construct for bone formation. As we have shown both SHD and SHD/BCP have osteopromoting activities and potential bone forming construct. The osteopromoting effect is better with combination of SHD and BCP scaffold. Further studies on the effect of both SHD and SHD/BCP remain important as it may lead to

developing an osteopromotic construct for human distraction osteogenesis and to know whether SHD differentiate directly to form osteoblast or indirectly promote the bone formation in DO.

CHAPTER ONE

INTRODUCTION

1.1 Background

Reconstruction of bone deficiency such as craniofacial anomalies and complex trauma can pose serious problems in clinical surgery, including orthopaedic, plastic/ reconstructive and craniofacial surgery. Successful bone repair depends on a sequential interaction of a tissue matrix scaffold with pluripotent cells and growth factors within the local environment. Only autogenous cancellous bone graft possesses all the important qualities of osteoinduction, osteoconduction, osteointegration and osteogenesis. Even though the healing of the autologous cancellous bone graft can be unpredictable, it is still considered the best material available to repair significant bone defects (Bauer and Muschler, 2000). Bone autograft is a graft of own cancellous bone tissue. However, the harvest of an autograft has several limitations, it requires a separate surgical approach with all its potential complications including prolonged surgery and anaesthetic time, donor site morbidity and pain, inadequate amount and quality of autograft (Ahlmann *et al.*, 2002; Boone, 2003).