Human dental pulp stem cells in ceramic; accelerate bone formation during mandibular distraction osteogenesis in rabbit model

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Introduction

Distraction Osteogenesis (DO) is a non invasive surgical technique to lengthen bone by creating corticotomy, placing a rigid distractor across the bone cut and gradually activating the device (1). It is used to treat congenital and acquired bone defects however; the major problems of DO is the length of time required for the treatment which may lead to higher possibility of complications such as infection(2), therefore shortening the distraction period would be of great benefit. Several attempts were done to promote bone healing during DO such as Electrical stimulation, Ultrasound, Low-level laser therapy and recently tissue engineering concept (3,4,5). Cell based strategy of bone tissue engineering is to deliver osteoprogenitor cells with a vehicle (scaffold) to the sites for regeneration to build an alternative or equivalent to autograft (6). A possible alternative to accelerate bone regeneration at the distracted gap (7). **SHED** is stem cell from human exfoliated/extracted deciduous teeth, population of highly proliferative postnatal stem cells can be differentiate into odontoblasts, adipocytes, neural and osteogenic cells. Easily isolated and expanded *in vitro* (8) Bone formation from mesenchymal stem cells (MSC) needs a three-dimensional scaffold The biological importance of a tissue-engineered scaffold is to mimic the extracellular matrix architecture of the native tissue(9). Biphasic calcium phosphate is biocompatible, bioactive, biodegradable and osteoinductive ceramic, used as a bone graft as its chemical composition is similar to that of bone mineral, drug delivery such as; antibiotics , anti -osteoporotics, anticancer drugs, steroid hormones , etc.) and scaffolding in bone tissue engineering to ensure that stem cells remain in the recipient site.

Objectives

The main aim was to study osteogenesis using tissue engineering construct consist of SHD seeded in biphasic calcium phosphate scaffold and SHD alone transplanted in mandibular distraction osteogenesis gap in comparison with normal DO

Materials & Methods

Part I: Synthesis of BCP with desired properties, controlled Ca/P ratio, granules size, micro and macroporosity with characterization. Wet precipitation with titration and heating method was used. CaCO3 and H3PO4 as a starting materials.The material was charachterized using X-ray diffraction (XRD), Scanning electron microscope (SEM).

Part II: ***In vitro*** **isolation, expansion and characterization of SHD** as usual, using CD 105&166

Part III: ***In vitro*** **cytotoxicity test** of MBCP. MTT test was used in this study.

Part IV: ***In vivo*** **study**: A randomized controlled trial was conducted in School of Dental Sciences Universti Sains Malaysia between *January & November 2012. Eighteen* white New Zealand rabbits divided in to 3 groups with 6 animal in each.

Six animals with SHD/MBCP as group A, 6 in SHD as group B and 6 in group C as control. The first 2 groups were regarded as an experimental . The experimental protocol was approved by the animal ethics committee of the Universiti Sains Malaysia(Number:USM/AnimalEthics Approval/2010/ (58)(226).

Surgical Procedure : Skin incision was done,the lateral aspect of mandible was exposed. Osteotomy cut was made immediately anterior to the first premolar root and distraction device was fixed in each side of osteotomy(Figure 1)

Fig 1:distractor in place

Fig 2: SHD and SHD+BCP

Transplantation protocol

Group A : 6×10^6 SHD seeded in 0.05mg MBCP was transplanted in to osteotomy gap

Group B: 6×10^6 SHD transplanted in osteotomy gap

Group C : No transplantation

Evaluation

Histologically, histomorphometrically and biochemically

Statistical analysis

Kruskal-Wallis test was used for data analysis, and P < 0.05 was considered statistically significant

Results

Study 1:Synthesis and characterization of MBCP

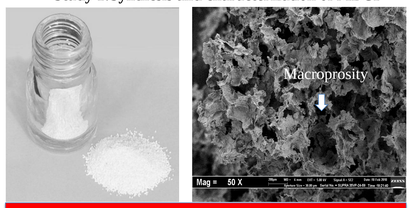


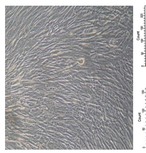
Fig 3 &4 BCP and Macroporosity

Study II: Biocompatibility of MBCP

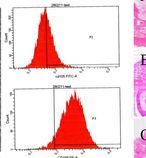
MBCP Concentration	Mean OD	RGR
100 %	0.326	301
50%	0.358	331
25%	0.355	328
12.5%	0.366	338
0% - ive control	0.108	100

OD = Optical absorbance
RGR = Relative growth rate

Study III: Isolation & Characterization of SHD

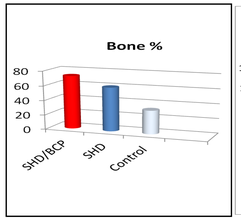


Stem cells (SHD), fibroblast cell like, spindle shape

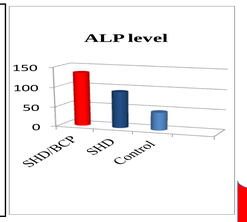


SHD, positive to CD 105 &166,95 and 43% respectively

Study IV: *in vivo* osteopromotion



Bone % of the 3 groups



ALP % of the 3 groups

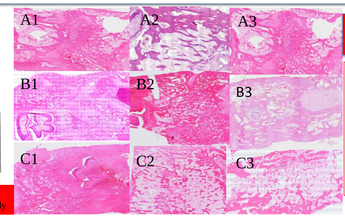


Fig 7: Histological features of the 3 groups showing bony trabiculi : A1,A2&A3: SHD/MBCP,2,4&6 weeks Po B1,B2&B3: SHD, 2,4 &6 weeks PoC1, C2 & C3 : Control 2,4 &6 weeks Po

Statistical analysis

Bone% & ALP
Significant difference between groups P < 0.001 for both

Conclusions

The bioengineered constructs of MBCP/ SHED demonstrated successful bone formation in the DO of mandible at selected time points

Acknowledgment

I would like to acknowledge all the organization committee of this conference for giving me this voluble chance to share this conference , hope I can attend the next event. Please pray to my country Iraq

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