Biocompatibility of Gentamicin-Coated Hydroxyapatite (Ha) with Osteoblast

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SUMMARY

This study was carried out to compare the surface morphological changes and cell-to-cell attachment with different concentrations of gentamicin-coated hydroxyapatite (HA) on the osteoblast cell lines. Osteoblast cell lines were cultured and maintained in complete medium, 1:1 HAM's F12 Medium Dulbecco's modified Eagle's medium without phenol red (DMEM) and incubated at 37^{o}C in 5% CO_2. The cell lines were treated with gentamicin-coated HA and undergone the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. After 72 hours treatment, the gentamicin-coated HA was processed for viewing the morphological changes by using scanning electron microscopy. The MTT assay results indicated that varying concentrations of gentamicin coated hydroxyapatite do not significantly affect viability of osteoblast. The osteoblast-like cells attached and spread well on the surface of hydroxyapatite and grew into the pores. In conclusion, the gentamicin-coated HA is biocompatible towards osteoblast.

INTRODUCTION

Biomaterials have been widely used for the tissue engineering of bone, teeth and liver and as medical devices. A common complication with the use of medical implants is biofilm formation. Biofilms are adherent, multi-layered colonies of bacteria that are more resistance to the host immune response [2].and antimicrobial therapy [3]. Biofilm formation can lead to systemic infection and cause device failure. In orthopaedics, parenteral administration of antibiotics does not provide good local bone response due to poor vascularisation of bone tissue and low drug penetration [5]. As an alternative, antibiotic-incorporated bone cement beads specifically designed and directly implant to the infected bone to combat infection localized to bone and bone tissue [4]. Thus, the purpose of this study was to determine the biocompatibility of gentamicin-coated HA with osteoblast.

MATERIALS AND METHODS

The porous bone graft substitute known as BoniPor® is provided by Malaysian Nuclear Agency (MNA). The irregular chips of HA were coated with gentamicin using the immersion technique to form gentamicin-coated HA. Human fetal osteoblastic cell line (hFOB 1.19) was cultured and maintained in 1:1 mixture of HAM's F12 Medium Dulbecco's modified Eagle's medium without phenol red (DMEM, Hyclone) and incubated in a humidified atmosphere of 5% CO_2 at 37°C. The cell suspension was dispatched in 96-well plates with 100µl of 10^5 cell/ml of cell suspension and cultivated overnight at 37^{o}C and 5% CO_2 incubator. The cell lines were treated with various concentration of gentamicin-hydroxyapatite in the range of 0.01 mg/ml to 10 mg/ml. After inoculation for 72 hours, the proliferation of osteoblast on gentamicin-coated hydroxyapatite was determined using the MTT (3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and optical density was evaluated using a micro-ELISA auto-reader (UVM 340, ASYS) at 570 nm. Finally, gentamicin-coated hydroxyapatite was processed for morphology analysis by scanning electron microscopy.

RESULTS AND DISCUSSION

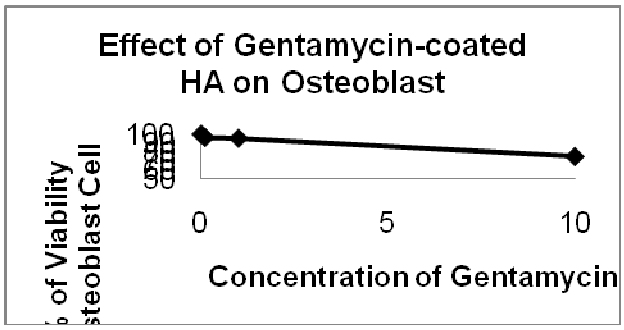
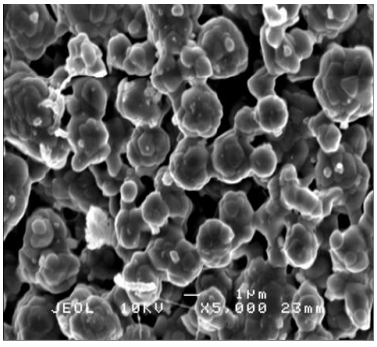
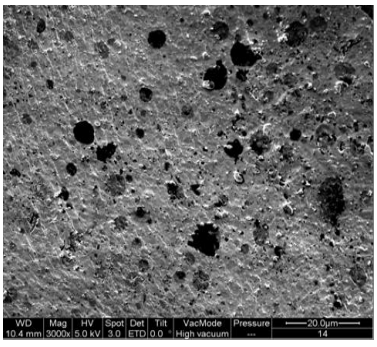


Fig 1. Percentage of osteoblasts viability treated with different concentrations of gentamicin-coated HA.

From MTT assays result, no inhibitory concentration of 50 (IC_{50}) of gentamicin-coated HA on osteoblasts was shown and no adverse effect was observed. Hence, HA was assessed as possible potential scaffold material in bone tissue engineering [1].





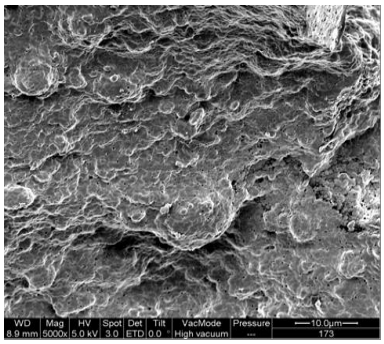


Figure 2. Scanning electron micrographs of HA. (A) The surface of HA showed various pore sizes (arrows) without soaking in gentamicin solution (3000x). (B) The surface of HA covered partially (arrows) after soaking in gentamicin solution (3000x). (C) The surface of HA was covered by osteoblast (3000x).

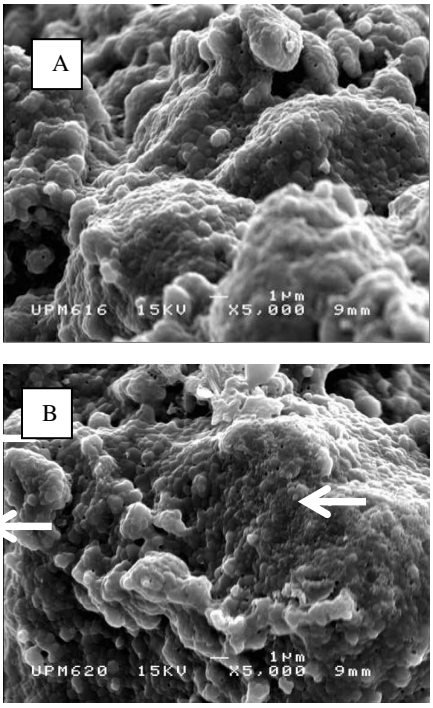


Fig 3. Scanning electron micrographs of gentamicin-coated HA with osteoblasts. (A) The osteoblasts attached on 0.1mg/ml concentration gentamicin-coated HA (5000x). (B) The osteoblasts shrinked and some cells sloughed off (arrow) from HA coated with 10.0mg/ml gentamicin (5000x).

The tested HA has the feature of high porosity (~80%) and the pore sizes (100 - 500 µm) of HA is large enough to allow osteoblasts to migrate into the scaffolds and it has enough space to deliver nutrient, waste removal and exclusion of materials. The osteoblast attached, spread and proliferate well on the gentamicin-coated HA surfaces. Thus, gentamicin-coated HA is biocompatible and able to support the osteoblast to grow.

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Effects of Hyaluronan on Mesenchymal Stem Cells

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SUMMARY

Hyaluronan (HA) and mesenchymal stem cells (MSC) are two very promising tools and hold great potential in regenerative medicine. This study aims to investigate the effects of hyaluronan in gel form on mesenchymal stem cells *in vitro*. Our results showed that hyaluronan did not affect the characteristics and properties of MSC in culture.

INTRODUCTION

In this new era of tissue engineering, several biomaterials have played an important role in assisting the implementation of new exciting cellular therapies. Hyaluronan (HA) have been used widely as scaffold [1] and lately as fillers for injectable applications[2]. Our objective in this study is to investigate the effects of HA on Mesenchymal Stem Cells (MSC). MSC is a promising cell source for cellular therapies due to their differentiation properties and immunosuppressive characteristics[3,4]. The hypothesis for this study is HA does not affect the properties and characteristics of MSC in culture.

MATERIALS AND METHODS

HA gel (10mg/mL) was coated onto 24-well plates 1-2 hours before seeding of MSC (replate at 5000 cells/ well). Quantitative and qualitative analyses of cultured hMSC in HA were performed. These include (a) plastic adherent assessment; (b) cell viability and proliferation assessment (using CellTiter Glo® Luminescent Cell Viability Assay; Promega); (c) morphology observation under inverted microscope on a daily basis; (d) surface antigen expression using flow cytometry to measure the expression of CD29, CD73 and CD90 and (e) multipotent differentiation potential assessment for *in vitro* differentiation to adipocytes and osteocytes[(5)].

RESULTS

Our results indicated that HA did not affect MSC characteristics when cultured together. The morphology of MSC remains spindle-shaped and adheres onto the plastic flask similar to the control group (Figure 1). Proliferation assay showed increased proliferation over time in all groups. The increase appeared more marked in the experiment group (715%) compared to control (561%) (Figure 2).

However, the experiment group showed a lower cell number. This may be due to the cross-linking of the HA particles in the gel and the molecular weight of HA. Flow cytometry results on surface antigen expression showed expression of CD29 (97.9%), CD73 (99.4%) and CD90 (99.1%) similar to control MSC. Multipotent differentiation potential of MSC was not affected by HA as shown in Figure 3. MSC in HA-coated wells could still differentiate into adipocytes and osteocytes, which were stained positively by Oil Red O and Alizarin Red Solution respectively.

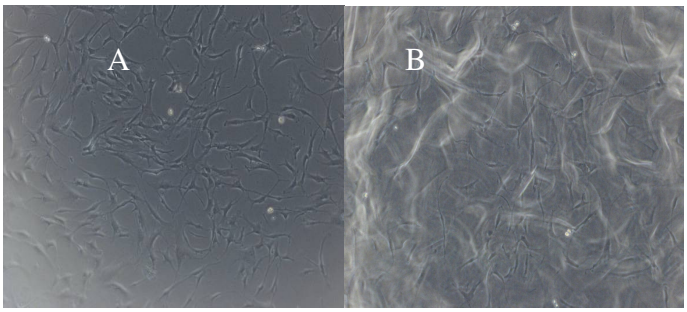

Fig 1. Morphology of MSC in culture at Day 3. (A) MSC in medium without HA (control). (B) MSC cultured with HA (Magnification 50x)

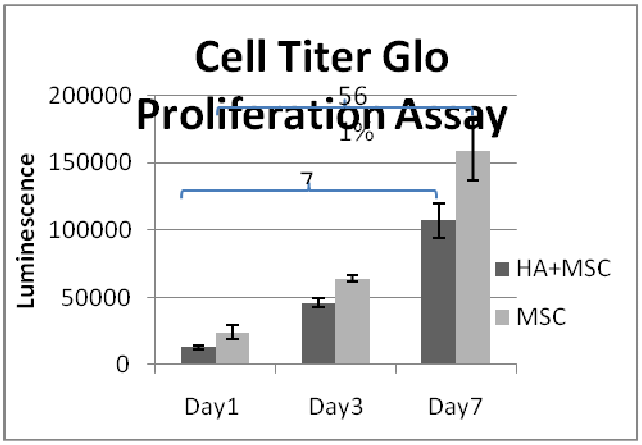

Fig 2. Utilizing luminescence signal in proliferation assay to study the propagation of MSC alone and MSC with HA. Error bars showed the standard deviation of the data.

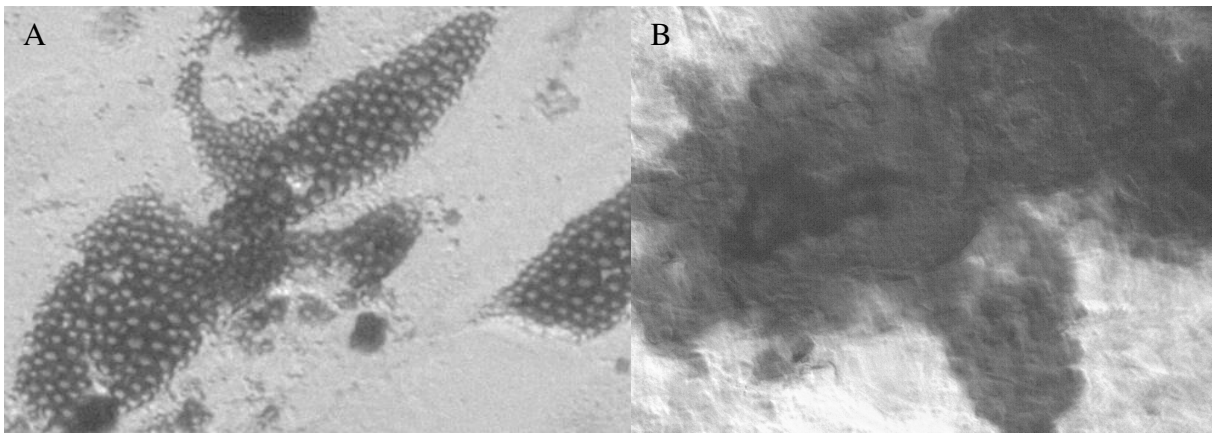

Figure 3. Differentiation of MSC on HA coated wells. (A) Differentiation of MSC into adipocytes as determined by oil droplet and positively stained by Oil Red O (magnification x400) and (B) differentiation of MSC into osteocytes as indicated by calcium deposition, which was stained positive by Alizarin Red Solution (magnification x200).

DISCUSSION

HA is one of the main contributors of extracellular matrix and our study showed that HA did not affect the characteristic of MSC. The proliferation result suggested that HA regulated the expansion of MSC, preventing over growth of cell. Thus, HA could serve as a good cell carrier for therapeutic usage.

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Growth Factor Supplementation Does Not Maintain Hair Cell Specific Genes in Cochlear Culture

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SUMMARY

Several approaches are being considered for hair cell regeneration. Stimulating potential cells from the cochlear or encouraging auditory progenitor cells to proliferate will create many chances to help deaf individual. The aim of this study was to culture auditory progenitor cells from the cochlear in growth factors supplementation and compared to the normal culture medium. The cells were evaluated via morphology, growth kinetics, genes and protein expression. The addition of growth factors gave an immune-positive result for hair cells specific genes: Myosin VIIa and Calretinin comparable to the normal medium. However, these genes are not maintained as the cells were passage in the growth factor supplemented medium. Cells in the normal medium expressed higher gene expression and maintained the hair cells specific genes. In conclusion, addition of growth factors is not beneficial for hair cells maintenance.

INTRODUCTION

Most hearing loss is caused by deterioration of the hair cells in the cochlea. Potential therapeutic implications have spurred investigation of inner ear progenitor cells and several approaches are being considered for hair cell regeneration [1]. It seems most likely that stem cells or precursor cells endogenous to the inner ear are primed to differentiate into hair cells more readily than other stem cell types. A potential cure for hearing loss would be to regenerate hair cells by stimulating cells of the damaged inner ear sensory epithelia or encourage auditory progenitor cells to proliferate and differentiate into hair cells [2]. The aim of this study was to culture auditory progenitor cells from the cochlear in growth factors supplementation and compared to the normal culture medium in terms of maintaining the hair cells specific genes.

MATERIALS AND METHODS

Adult mice cochlea tissue was harvested surgically. In-situ digestion of the cochlea tissue was carried out with 500 ul of 0.3 % Collagenase type 1 at 37^{0}C. After 1 hour, the digestive enzyme was neutralized by

adding 2 ml of growth medium (equal mix Ham's F: 12 and DMEM) supplemented with 10 % FBS without removing the digestive buffer. Other supplements added into the growth medium were 20 ng/ml EGF, 50 ng/ml IGF, 20 ng/ml b-FGF, B27 and N2 supplements. Cultured auditory progenitor cells were serially passaged at 1, 2 and 3. Auditory progenitor cell morphology was examined daily with cell count and viability recorded at each passage. Auditory progenitor cell specific protein was verified by immunocytochemistry using hair cell specific markers (Myosin 7a and Calretinin). Quantitative RT-PCR was used to evaluate the cultured cell characteristic.

RESULTS

The results showed auditory progenitor cells isolated from cochlea membrane were heterogenous in morphology. Cultured auditory progenitor cells exhibited a better proliferative activity in the medium supplemented with growth factors. Under specific hair cell markers identification with fluorescence microscope, the cultured cells for both culture conditions were immune-positive for Myosin VIIa in the cytoplasm (Fig. 1A) and Calretinin for hair cell soma and stereocilia label (Fig. 1B)

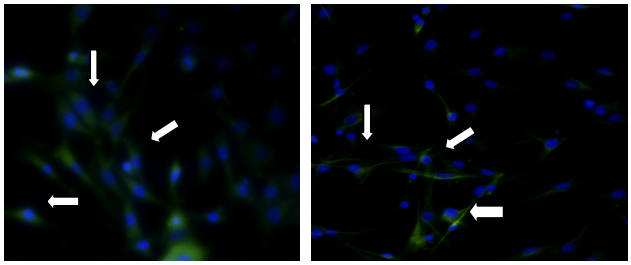

Fig. 1A **Fig. 1B**

In quantitative RTPCR gene expression, the present data showed that hair cell specific genes (Myosin VIIa and Calretinin) were still expressed in both:

addition of growth factors and non-addition of growth factors (Fig. 2A and Fig. 2B).

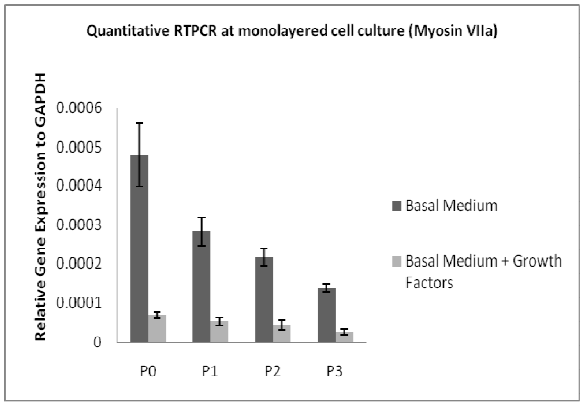

Figure 2A: The Hair Cell specific gene expression of Myosin VIIa (P0, P1, P2 & P3).

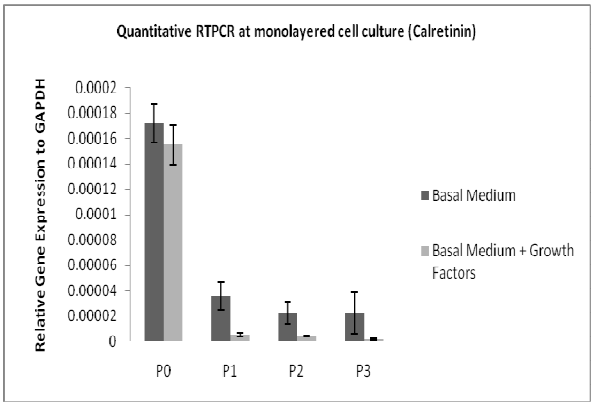

Figure 2B: The Hair Cell specific gene expression of Calretinin (P0, P1, P2 & P3).

DISCUSSION AND CONCLUSIONS

This study showed that growth factors supplementation decrease the hair cells specific genes compare to the basal medium. Basal medium increased the gene expression but the level decline with further passages. Basal medium is a better culture medium to maintain hair cells specific genes in cochlear culture.

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Histological Analysis of Tissue Engineered Cartilage Made from Microtic Auricular Chondrocytes

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SUMMARY

Chondrocytes were isolated from microtic human auricular cartilage after ear surgery and subsequently cultured and expanded until passage 4. Construct was made with the help of fibrin in the concentration of 30-million cells/ml fibrin. Constructs were then implanted at the dorsal part of nude mice, subcutaneously for 8 weeks. The tissue explanted was subjected to histological analysis using Haematoxylin & Eosin (H&E) staining, Safranin O staining and immunohistochemistry analysis (IHC) with Collagen type I & II antibody. H&E and Safranin O staining showed that there are homogeneous cartilage with abundant ECM and mature lacuna. IHC analysis showed presence of collagen type II in its extracellular matrix (ECM). Thus, from this preliminary study, we can conclude that tissue formed from microtic chondrocytes has the same properties as tissue formed from native tissue and thus has the potential to be used as a cell source in the reconstruction of human pinna in the future.

INTRODUCTION

Microtia is a malformation of the ear with extreme variability of expression that may range from hypoplasia with minimal structural abnormalities to the total malformation of the ear or its complete absence (anotia) [1]. This condition can exist in unilateral or bilateral form and this condition occurs in 1 out of about 10000 births. Ear reconstruction for congenital microtia or auricular traumatic amputations remains one of the most difficult challenges in reconstructive surgery. Although the ear represents a small part of the human body, it is one of the most complex and difficult three-dimensional structures to be developed [2]. The main source for microtia reconstruction is rib fibrocartilage. Brent [3,4] showed good long-term durability and cosmetics results by using sculptured autologous costal cartilage graft. This comes with a risk, thus, the need of finding alternative sources of cartilage is of great importance. The objective of this study is to reconstruct tissue engineered cartilage from microtic chondrocytes.

MATERIALS AND METHODS

Samples of human chondrocytes were obtained from excessive tissue during ear reconstruction surgeries. Chondrocytes were cultured and expanded until passage 4. Upon confluence, cells were trypsinized and construct was made with fibrin in the concentration of 30-million cells/ml fibrin in a cylindrical shape. Construct was later incubated for 24 hours before implantation. Afterwards, constructs were implanted at the dorsal part of nude mice, subcutaneously for 8 weeks. The explanted construct was later processed and subjected to histological analysis by using Haematoxylin & Eosin (H&E) staining, Safranin O staining and immunohistochemistry analysis (IHC) with Collagen type I & II antibody. This antibodies was detected by using DAKO Cytomation Immunohistochemistry staining kit protocol. All slides prepared and stained were visualized using a light microscope.

RESULTS

Histological sections were stained positive with safranin O (orange-red colour). Significant cartilage formation with high cell density was noted. Cells were located within a typical lacunar space surrounded by its extracellular matrix. The cells resembled well-differentiated chondrocytes embedded within cartilaginous matrix similar to native tissue. Immunohistological staining with collagen type I and II also showed abundance of collagen type II and low type I deposition.

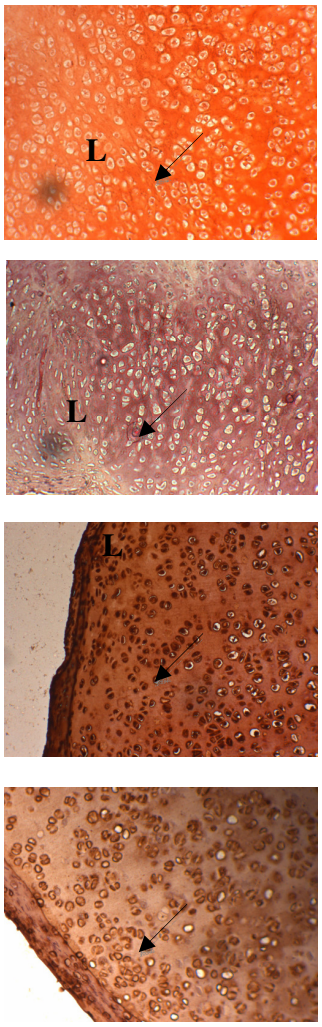

Figure 1**:** Histological examination using safranin O (1a) and haematoxylin and eosin (1b) staining. There was significant cartilage formation with high cell density (L) (magnification × 50). The histological sections stained positive with safranin O, indicating the presence of proteoglycan cartilage-rich matrix. Immunohistochemical localization of collagen Type II (1c) is positive presented as a dark brown precipitation around the pericellular matrix of the lacunae and deposited throughout the matrix [×40] whilst immunohistochemical localization of collagen Type I (1d) revealed a sparse distribution and light brown precipitation throughout the extracellular matrix [×40]. Arrows indicates chondrocytes in its lacunae. (Magnification X100).

DISCUSSION AND CONCLUSION

Safranin O staining (orange-red colour) was used to indicate the presence of accumulated proteoglycan-rich matrix. The distribution of the cells is homogenous and compact indicating mature cartilage formation. Assessment using immunohistochemical localizations with specific human monoclonal antibodies proved that in vivo tissue-engineered cartilage creates an accumulated pericellular matrix composed of newly synthesized collagen Type II around the lacunae with sparse distribution of collagen Type I throughout the section. Constructs formed from chondrocytes derived from microtic cartilage exhibit similar cells arrangement, distribution and expression of collagen type II in its ECM as of native cartilage. This showed that microtic choncrocytes may serve as a potential cell source in reconstructing autologous human pinna for future clinical application.

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SEM Evaluation of Acellularized Muscle Stuffed Vein Construct Seeded with Human Neuro Trans-Differentiated Mesenchymal Stem Cells: *In Vitro* and *In Vivo* Study

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SUMMARY

We fabricate an acellular muscle stuffed vein containing viable human neuro transdifferentiated mesenchymal stem cells (MSCs) as a substitute for nerve autograft. Human vein were decellularized in liquid nitrogen while the muscle were decellularized in liquid nitrogen and subsequently hydrolyzed by soaking in HCL acid. Cells were seeded on the pre-treated muscle and this muscle was stuffed into the pre-treated vein. Scanning electron micrographs showed that the cell maintained cell morphology with elongated processes and a high number of cells attached on the surface of the pre-treated muscle 3 days of culture. Post implantation showed cells actively secreted matrix vesicles which then were breakdown into fibers laying dense extracellular matrix throughout the entire construct. After 8 weeks implantation, the matrix became more dense and compact with mature cells embedded within a dense homogenous matrix. These results revealed succesful delivery of neuro-transdifferentiated cells into the vein cavity throught cellular attachment on muscle fibres and a tissue structure of the construct resembling that of natural nerve.

INTRODUCTION

In peripheral nerve injury, if the gap is very short the proximal and distal stump can be directly sutured by neurosurgical operation. However, injury with a longer gap cannot be repaired by this method. Microsurgical autografting is the treatment of choice that avaiable for bridging long gaps with improved functional outcome[1]. However it is harmful to take another part of the peripheral nervous sytem out of the patient's body since it will causing sensory deficits and a significant risk of neuroma formation at the donor site. Therefore, various type of nerve conduits have been studied as an alternative to nerve autografts. These conduits act to guide and support the re-growth of axons along the damage nerve fibre to the target organ in a very optimum environment as well as to keep the space for the proper re-growth of axons from being blocked by scar tissues formed after inflammation. Thus, this study is to fabricate a biological nerve graft from human muscle and vein seeded with human neuro transdifferentiated MSCs as a substitute for nerve autografts.

METHODS

Approximate measurements of the vein length is 20mm, diameter 3mm and muscle dimensions of 20 mm x 2 mm x 2 mm. The vein and muscle were washed three times with PBS to wash out remaining blood. The vein were decellularized by liquid nitrogen immersion (10 sec, 3X) while the muscle were decellularized by liquid nitrogen immersion and subsequently hydrolyzed by soaking in 8% HCl for 24 hours. Adult human bone marrow was harvested from orthopaedic femoral interlocking nail procedures following informed consent. Human mesenchymal stem cells (MSCs) were cultured in alpha-minimum essential medium (α-MEM) containing 10% fetal bovine serum (FBS) at 37^{O}C, 5% CO_2. MSCs were subjected to a series of induction into neuro-trans-differentiated mesenchymal stem cells[2]. Upon confluency, cells were trypsinized and seeded at a density of 3 x 10^6 cells on the pre-treated muscle via static seeding and immersed in culture media at 37^{o}C, 5% CO_2. After 24 hours, cell-seeded muscle was stuffed into the pre-treated vein. The muscle stuffed vein construct was further incubate for 3 days for in vitro assessment. The prepared construct were also implanted muscularly into the atymic mice and harvested after 2, 4 and 8 weeks for in vivo assessment.

RESULTS

Ultra structural observation demostrated the attachment and a random distribution of the cells on the surface of the acellular muscles by 3 days of culture (Figure 1). Cells were found attached to the surface of the acellular muscle in a desired position allowing further proliferation. There was no influence of the prepared scaffolds on the morphology of the cells, suggesting good biocompatibility of muscle and vein. After 2 weeks implantaion, the cells were

attached to the surface of the acellular muscle andshowed cells actively secreted matrix vesicles. These vesicles were then breakdown into fibers laying dense extracellular matrix throughout the entire construct. Finally, after 8 weeks implantation, the matrix became more dense and compact with mature cells embedded within a dense homogenous matrix (Figure 2).

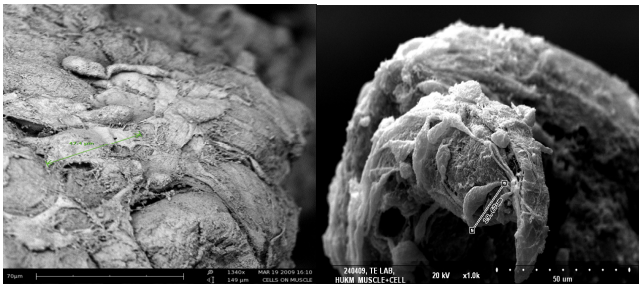

Figure 1: SEM view of the biocompatibility of neuro trans-differentaited MSCs on the acellular musclestarted to secrete the matrix surrounding it. 4 weeks implantation

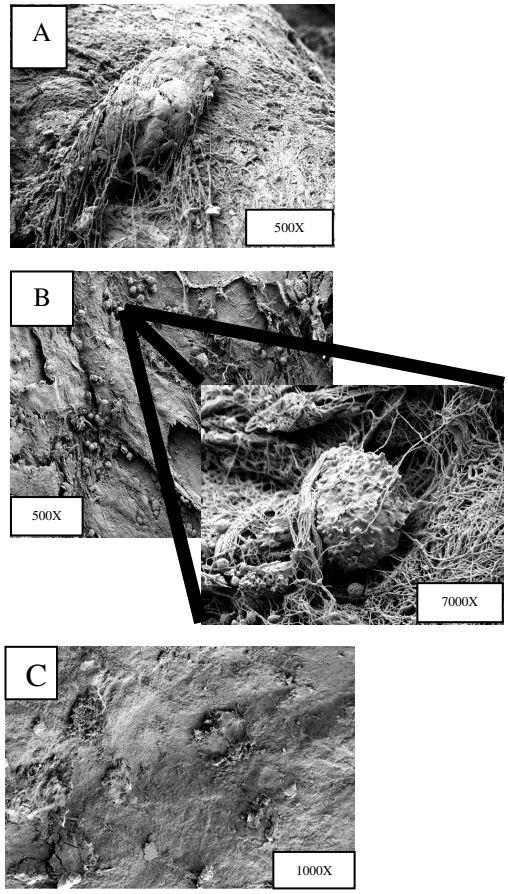

Figure 2: SEM micrographs of muscle stuffed vein construct after 2 (A), 4 (B), and 8 (C) weeks post implantation.

DISCUSSION AND CONCLUSIONS

The process of hydrolyzation in the preparation of the acellularized muscle is important to loosen the fiber bundles of the muscles. The muscle matrix itself gives an optimum microenvironment for further proliferation of the cells. At the end of the process, the structures of the acellular muscle resemble closely the structure of the nerve fibers. The SEM micrographs showed a random distribution of the cells on the surface of the muscles with no influence on the morphology of the cells, suggesting good biocompatibility of the muscle and vein materials although after treated with hydrochloride acid. Post implantation showed the proliferation of the cells and as evidence by the production of matrix vesicles which then were breakdown into fibers laying extracellular matrix throughout the entire construct. This study proved the feasibility of the muscle stuffed vein construct as a suitable carrier for the seeded cells in a nerve tissue engineering purpose. Electron microscopy revealed succesful delivery of neuro-transdifferentiated cells into the vein cavity throught cellular attachment on muscle fibres and a tissue structure of the construct resembling that of natural nerve.

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5-Azacytidine Failed to Up-Regulate Cardiogenic Genes Expression in Long-Term Culture Human Adipose-Derived Stem Cells

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SUMMARY

Adipose tissue is a source of multipotent adult stem cells and it has the ability to differentiate into several types of cell lineages such as neuron cells, osteogenic cells and adipogenic cells. Several reports have shown that adipose-derived stem cells (hADSCs) have the ability to undergo cardiomyogenic differentiation. However, most of these studies were carried out at the early passage. In this study, we evaluated the ability of long-term culture hADSCs on the cardiomyogenic differentiation by determining the expression level of cardiomyogenic genes i.e. GATA4, MLC-2v, MLC-2a, NKX2.5, β-MHC, α-MHC, Atrial natriuretic peptide (ANP), Connexin 43, Cardiac Troponin C, Cardiac Troponin I and myocyte enhancer factor (MEF2C) at P5 and P10. Our data demonstrated the decline of cardiomyogenic genes expression after induction in cardiogenic medium, which contained 5-Azacytidine. This showed that 5-Azacytidine may not be suitable to induce long-term culture hADSCs differentiation towards cardiomyogenesis.

INTRODUCTION

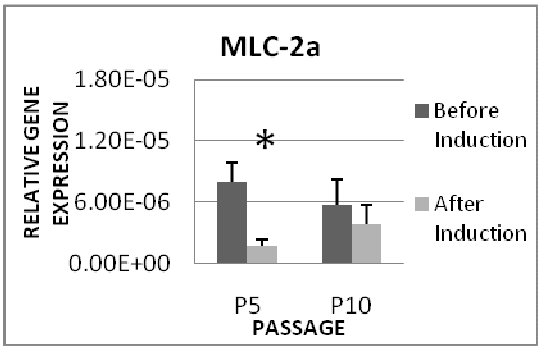
Myocardial infarction results in the death of cardiomyocytes and these cells cannot regenerate as they are terminally differentiated. Dead cardiomyocytes are normally replaced with scar tissue. Human adipose-derived stem cells have been shown to demonstrate cardiomyocytes differentiation under appropriate stimuli. Several reports illustrated the use of 5-Azacytidine to induce bone marrow stem cells [3] and hADSCs [2] to differentiate into cardiomyocytes. Therefore, the aim of this study was to investigate the ability of long-term culture of hADSCs to undergo cardiomyogenesis using 5-Azacytidine as an induction agent.

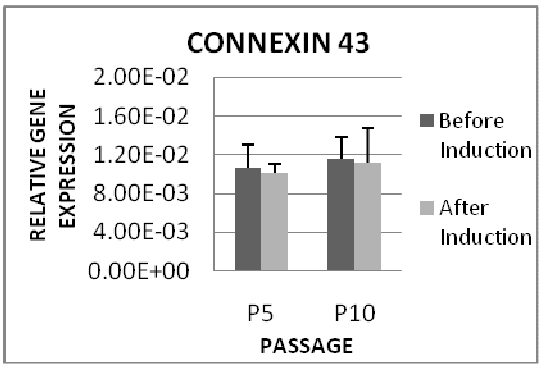
MATERIALS AND METHODS

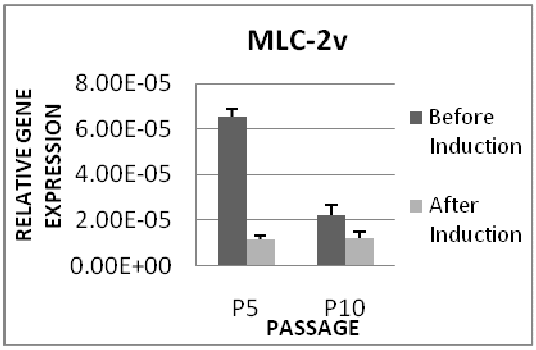
Human ADSCs were culture-expanded in Ham's F12:Dulbecco's Modified Eagle Medium (1:1). The cells were induced in cardiogenic medium containing 10uM 5-Azacytidine for 24 hours and maintained in normal medium for 3 weeks. Total RNA from induced ADSCs at P5 and P10 was extracted. The cardiogenic genes expression was evaluated by quantitative RT-PCR: GATA4, MLC-2v, MLC-2a, NKX2.5, Atrial natriuretic peptide (ANP), Connexin 43, Cardiac Troponin C, and Cardiac Troponin I. All primers were designed by using Primer 3 software based on the published GeneBank database sequences. PCR reaction was carried out using SYBR Green as an indicator in Bio-Rad iCycler instrument. The specificity of the primers and PCR protocol were confirmed with melting curve analysis and further verified by agarose gel electrophoresis. Expression level of each targeted gene was then normalized to GAPDH. Student t-test was used for statistical analysis to compare the gene expression level before and after the induction at P5 and P10.

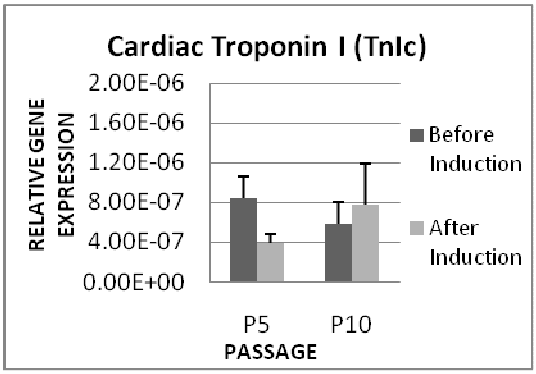
RESULTS

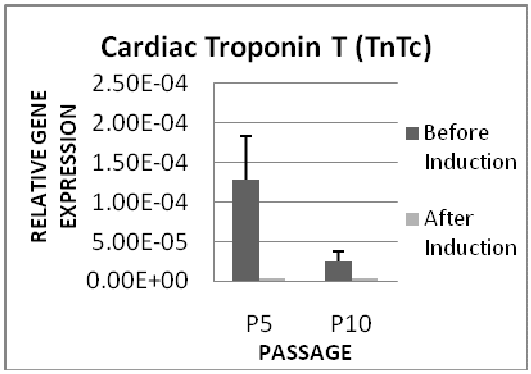
Quantitative RT-PCR analysis showed that the cardiac specific genes i.e. MLC-2a, MLC-2v, ANP, Connexin 43, Cardiac Troponin T decreased in expression at P5 and P10 after being induced with cardiogenic medium containing 5-Azacytidine (Fig. 1). However, there was a modest increase in GATA-4 and Nkx2.5 expressions at P5 as well as cardiac troponin I (TnIc) expression at P10 after induction.

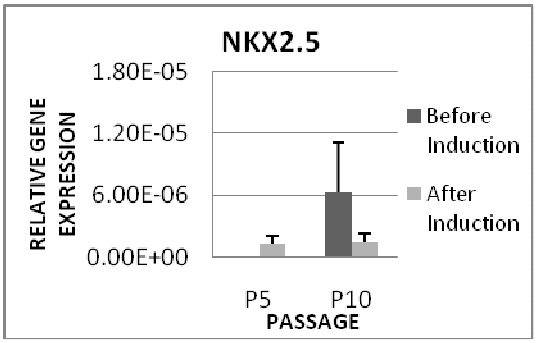












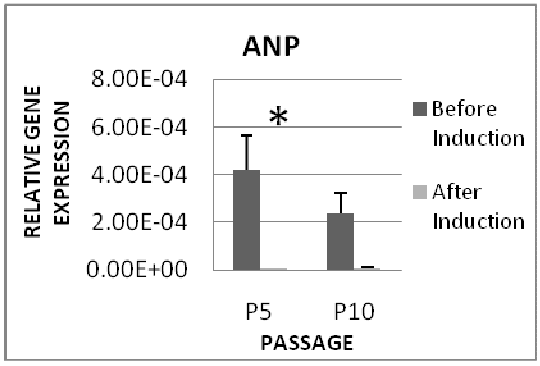


Figure 1: The expression level of cardiomyogenic genes in hADSCs at P5 and P10 before and after induction. (* P<0.05 when compared to before induction)

DISCUSSION

In this study, the cardiogenic medium containing 5-Azacytidine failed to induce hADSCs into cardiomyogenic lineage. This result is contrary to the previous studies by Xu et al 2004 [3] and Rangappa et al 2003 [2]. However, Liu et al reported that rat bone marrow stromal cells cannot be differentiated into cardiomyogenic cells using 5-Azacytidine unless they were immortalised [1]. While Zhang et al 2005 reported that the potentiality of bone marrow derived stem cells to differentiate into cardiomyocytes was proliferation related and passage-restricted with 5-Azacytidine [4]. Cardiomyogenic differentiation was associated with growth-arrest in stem cells. Perhaps, a stronger stimulus is needed to stimulate the cells into the growth arrest state and induce hADSCs into cardiomyogenic cells.

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