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OSTEOGENIC POTENTIAL OF HUMAN ADIPOSE DERIVED STEM CELL CO-CULTURE WITH HUMAN OSTEOBLAST ON TITANIUM DIOXIDE NANOFIBROUS SURFACE

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ABSTRACT

The study aims to evaluate the osteogenic potential of human adipose derived stem cell (HADSC) co-culture with human osteoblast (HOB) using selected HADSC/HOB ratio of 2:1, 1:1 and 1:2, respectively. The HADSC/HOB was seeded on Titanium dioxide (TiO_2) coated with or without nanofibre substrate. The non-coated TiO_2 was used as control. The effects of TiO_2 based scaffolds on cell adhesion were characterized by scanning electron microscopy (SEM). Cell viability, differentiation and mineralization were assessed by Alamar Blue, alkaline phosphatase (ALP) and Alizarin Red assays, respectively. The combination of HADSC/HOB, 2:1 ratio, seeded on nanofibrous-coated TiO_2 showed better cell adhesion, viability, differentiation and mineralization than the other groups. This study offers opportunity to assess in vitro cellular development of HADSC through direct cell to cell contact with HOB. This study indicates that the co-cultured HADSC/HOB seeded on TiO_2 based scaffolds may serve as a promising approach to facilitate osteogenic differentiation activity.

1.0 Introduction

In tissue engineering principles, cell-cell interactions are considered to play an important role to regenerate a quality tissue (1). Human osteoblasts (HOB) are known as large bone cells that responsible for the synthesis and mineralization of bone. The processes occur during both initial bone formation and later bone remodelling (1). Human adipose derived stem cell (HADSC) is one of the most viable stem cell sources for skeletal tissue regeneration (2) and contributes to the understanding of stem cell biology. Numerous studies have employed HADSC or HOB as a single layer or monolayer culture (2). However, monolayer culture system cannot provide adequate evaluation on cell-cell interactions between HADSC and HOB. To overcome these inadequacies, many researches have now employed co-culture system to study cell-cell interactions in vitro (3). Hence in this study, co-culture compositions of HADSC and HOB using various HADSC/HOB ratios were established to evaluate direct cell-cell interactions. The HADSC/HOB was also seeded on TiO_2 based scaffolds. The scaffolds were meant to provide three dimensional (3D) microenvironment and to direct cells growth. Results on cell adhesion, viability, differentiation and mineralization are presented in this paper.

2.0 Materials and Method

With the approval of the UKM Research and Ethical Committee, HADSC was co-cultured with HOB using selected HADSC/HOB ratios of 2:1, 1:1 and 1:2, respectively. They were cultured in an equal mixture of F12 Nutrient Mixture and Dulbecco's Modified Eagle Medium (F12/DMEM; Gibco) supplemented with 10% foetal bovine serum (FBS; Gibco), 1% antibiotic–antimycotic (Invitrogen,

Carlsbad, CA), 1% glutamax (Invitrogen) and 1% vitamin C (Sigma-Aldrich). The HADSC/HOB was then seeded on TiO_2 coated with or without nanofibre substrate. Cell adhesion were characterized by field emission SEM, cell viability using Alamar Blue assay, cell differentiation by alkaline phosphatase (ALP) assay and cell mineralization by Alizarin Red Assay. All evaluations were carried out at each time point of Day-1, -7 and -14.

3.0 Results

The HADSC/HOB (2:1) seeded on nanofibre-coated TiO_2 showed significantly higher cell viability ($p=0.011$) on day 14 and displayed significantly higher production of alkaline phosphatase activity ($p= 0.014$) and mineralization ($p=0.000$) compared to the other groups. Field emission SEM also showed better cell adhesion, migration and morphology of HADSC/HOB (2:1) seeded on nanofibre-coated TiO_2 when compared to other groups.

4.0 Discussion and Conclusion

Most of the previous studies assessing osteogenic differentiation of HADSCs into osteoblastic lineage have focused on modifying of osteogenic factors added to the media or the treatment with an osteogenic media (4,5) which are in contrast to this study. The outcome of this study suggested that the nanofibre-coated TiO_2 scaffold seeded with HADSC/HOB (2:1) serves as a better technique to promote osteogenic capacity. Other than holding the advantage of evaluating cell-cell interactions by having the co-culture system, the presence of the in vitro 3D microenvironment has facilitated cell growth and differentiation. Special emphases on this synthetic biology system are clearly necessary to understand various biological phenomenon as well as to develop its full potential for tissue engineering and regenerative applications. The method may be useful for generating osteoblasts or bone cells derived from other mesenchymal stem cells for skeletal tissue regeneration or repair.

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