School of Dentistry

FACULTY OF MEDICINE AND HEALTH

UNIVERSITY OF LEEDS

Bone Regeneration Using Periodontal Ligament Cells Seeded on Silk Scaffold

F Al-Dabbagh[1,2], JG Hardy,[3], V Clerehugh[4], and XB Yang[1]

[1] Division of Oral Biology, Leeds Dental School, University of Leeds, UK. [2] College of Dentistry, University of Mosul, Iraq. [3] Department of Chemistry and Materials Science Institute, Lancaster University, Lancaster, UK. [4] Leeds School of Dentistry, UK |

Email: ml13fmad@leeds.ac.uk

1553

INTRODUCTION

Bone replacement is one of the critical steps for management of oral bone defects. Many methods have been introduced to restore such traumatic or pathological defects. However, biocompatibility and increasing donor site morbidity are still among the main challenges. The current study aims to investigate the potential of using human periodontal ligament cells (HPDLCs) for bone formation, with the use Bombyx mori fibroin (BMF) silk as a scaffold material.

METHODOLOGY

Isolation of HPDLCs:

Human periodontal ligament tissues were isolated from healthy, extracted molar teeth, which obtained from Leeds Dental Research Tissue Bank, according to the ethical approval authorised by Leeds Dental School. The harvested tissues were digested using collagenase and filtered to obtain the cells suspension.

Examining the presence of progenitor cells:

The presences of progenitor cells were investigated using Colony Forming Unit- fibroblast (CFU-F) Assay, where cells obtained from three different biological donors and cultured for 10 days at density of 100 cells/35 mm diameter petri dish in basal medium (alpha minimum essential medium (αMEM) supplemented with 10% fetal bovine serum.

Monolayer and 3D seeding of HPDLCs:

Cells were culture as a monolayer in 24-well plate; as well as on silk scaffolds. These foam scaffolds were fabricated from Bombyx mori Fibroin silk in cylindrical shapes. Sterilised scaffolds were subjected to surface modification with 20% Fetal bovine serum (FBS). Calcein fluorescence staining was used to label the cells before being seeded dynamically ($4x10^5$ cell/scaffold). For all experiments, the samples were incubated in osteogenic medium for 21 days with other group of cells being culture in Basal medium. Furthermore, group of scaffolds were placed in diffusion chambers and implanted in peritoneal space of CD1 nude mice to evaluate the cells' growth in-vivo.

Evaluation of HPDLCs' osteogenic activity:

Alkaline phosphatase (ALP) activity of these cells was examined both qualitatively (ALP stain) and quantitatively (ALP non-specific Activity assay). The phosphate and calcium accumulation within the extracellular matrixes was detected by Von Kossa and Alizarin red stains respectively. Immunocytochemical stains were used to confirm the expression of osteogenic markers (e.g. osteocalcin, osteopontin, and collagen type-I).

RESULTS

Characteristics of isolated HPDLCs:

The isolated cells showed the spindle-like shaped cell body with prominent nucleus, figure 1.

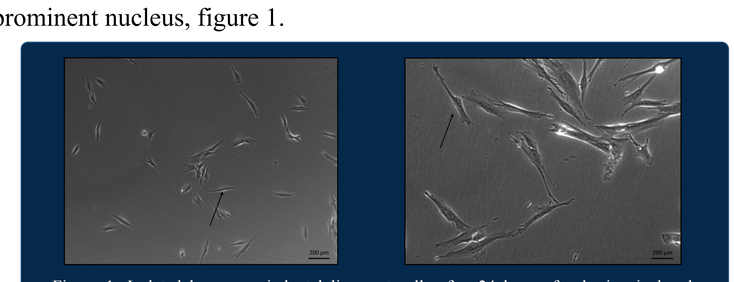

Figure 1: Isolated human periodontal ligament cells after 24 hour of culturing in basal medium.

Progenitor cells in HPDLCs population:

Results of CFU-f assay demonstrated the ability of subpopulation of HPDLCs to adhere to the vessels' plastic surface and proliferate, forming colonies of more than 50 cells, see figure (2-a). However, the number of colonies formed is variable between the various donors (figure2-b).

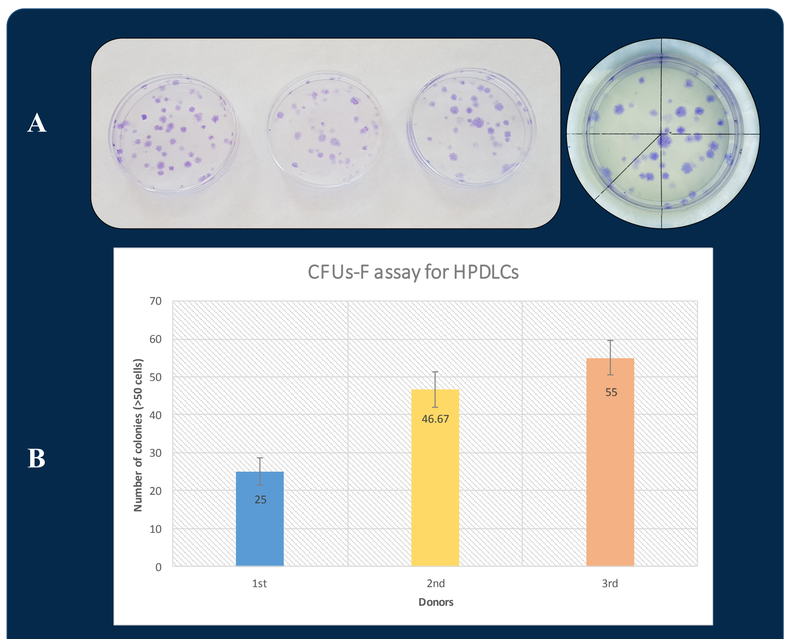

Figure 2: Colony forming unit Assay for HPDLCs obtained from three donors after ten days of culture: a. CFU-F assay for HPDLCs. Violet circles represent the colonies with each of > 50 cells. b. Number of HPDLCs colonies from three donors .

Human periodontal ligament cells' osteogenic capacity:

After 21 days of monolayer culturing in osteogenic differentiation medium, the cell morphology demonstrated more cuboidal-like shaped, with a large nucleus and numerous cytoplasmic processes. Furthermore, those cells were stained positively with ALP stain compared with the cells in basal medium culture. This result was confirmed by ALP non-specific activity assay. Figure (3-a, 3-b). Moreover, monolayered HPDLCs cultured in osteogenic media showed stronger brown and red stains for von Kossa and Alizarin red stains respectively, in comparison to those cells cultured in basal media. These results indicated phosphate and calcium deposition within the matrices. Figures (3-c) and (4).

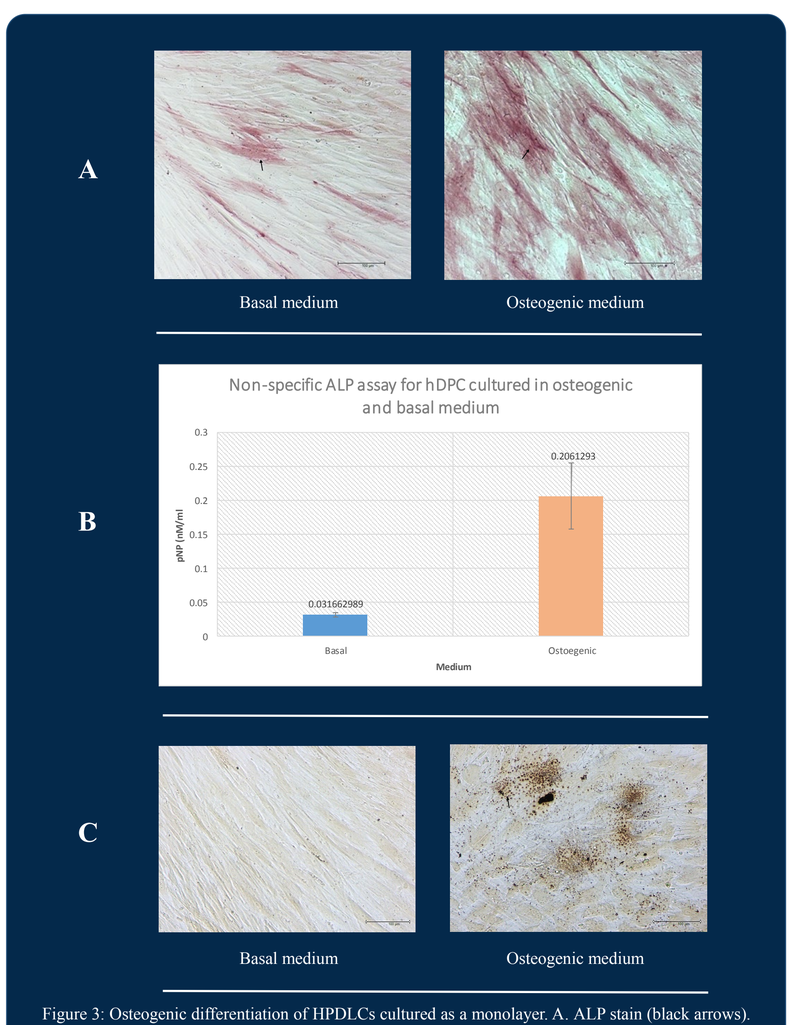

Figure 3: Osteogenic differentiation of HPDLCs cultured as a monolayer. A. ALP stain (black arrows). B. Non-specific ALP assay to compare the ALP levels of HPDLCs cultured in basal and osteogenic media. C. Extracellular mineral deposition (black arrows) after 21 days of inducing osteogenic differentiation.

Immunohistochemistry staining of monolayer cells culture demonstrates the expression of collagen type-I (Col-1), osteopontin (OPN) and osteocalcin (OCN) proteins at a higher level than in basal media group (Figures 5-a, 5-b and 5-c).

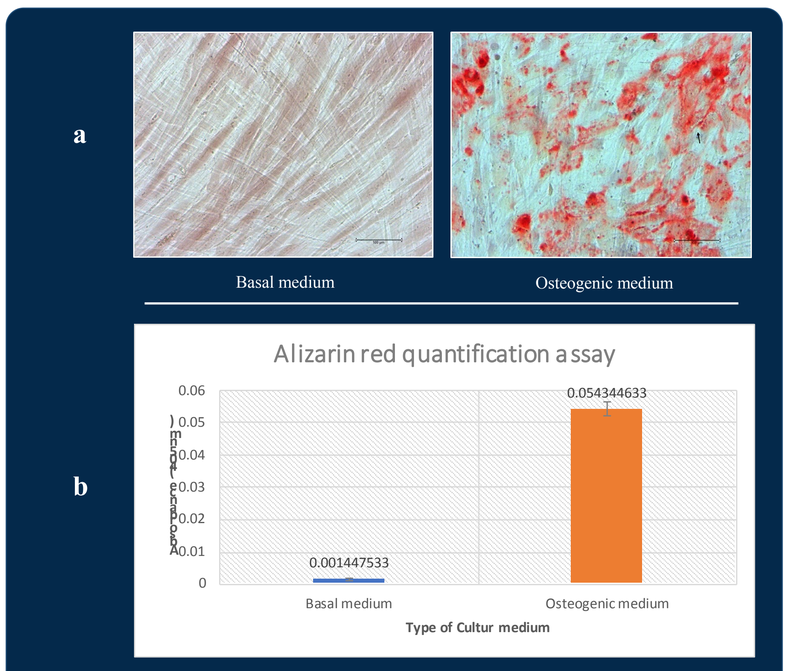

Figure 4: Comparison of calcium deposition level of hPDLCs cultured in basal and osteogenic media for 21 Days. a. Alizarin red stain (black arrows). b. Alizarin red quantification assay.

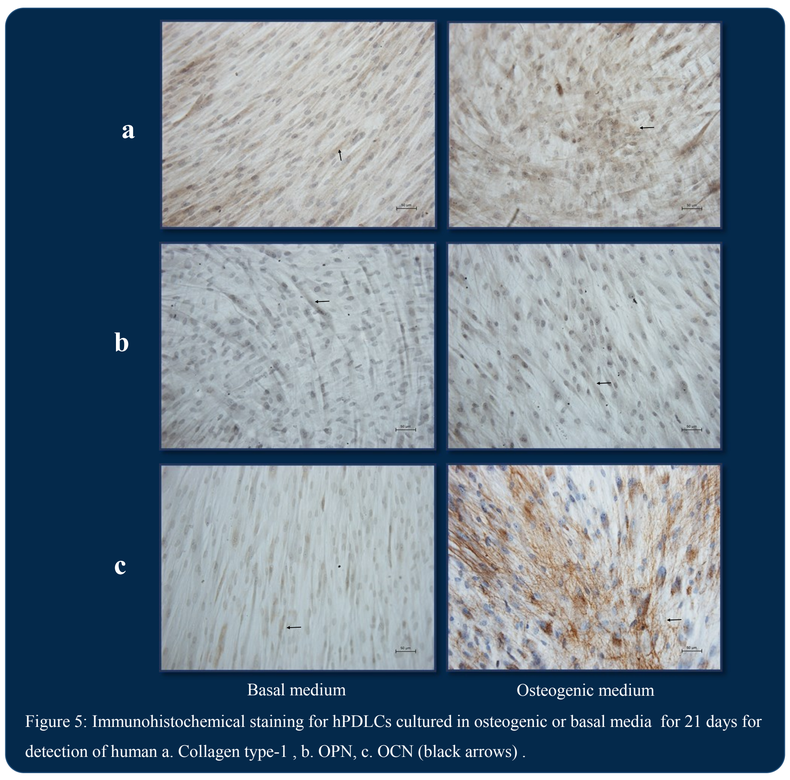

Figure 5: Immunohistochemical staining for hPDLCs cultured in osteogenic or basal media for 21 days for detection of human a. Collagen type-1 , b. OPN, c. OCN (black arrows) .

Biological behaviour of 3D seeded HPDLCs both in-vitro and in-vivo:

Seeding HPDLCs cells on silk scaffold prove the ability of those cells to attach and proliferate on a 3D scaffold (figure 6-a). Also, cells were capable of forming the extracellular collagenous matrix, which is both seen in in-vitro and in-vivo models, Figures (6-b and 6-c).

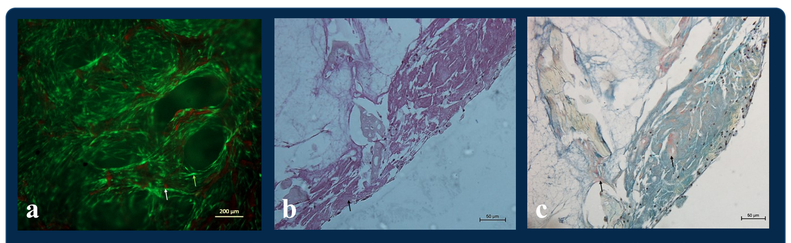

Figure 6: The Cellular activity of HPDLCs seeded on 3D silk scaffold. a. HPDLCs labelled with Calcein-Ethidium homodimer fluorescent live-dead stain (green and white arrows) after four weeks of seeding in-vitro. b. Cellular growth on silk scaffold implanted in peritoneal space of nude mice for seven weeks. Sections stained with Van Gieson's stain. c. Alcian blue/ picrosirius red stained section demonstrating extracellular collagen matrix formation (black arrows).

CONCLUSIONS

Human periodontal ligament cells that isolated from extracted teeth can proliferate and differentiate into osteoblast-like cells when grown in particular culturing conditions, also, they could enhance bone-like tissue formation, both in-vitro and in-vivo.

CONFLICT OF INTEREST STATEMENT

The authors whose names are listed above certify that they have no financial or non-financial conflict of interest in the subject matter or materials discussed in this project.

ACKNOWLEDGEMENT

F Al-Dabbagh is sponsored by a scholarship grant that is offered by the higher Committee of Education Development in Iraq.

Contact us