School of Dentistry

FACULTY OF MEDICINE AND HEALTH

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Periodontal regeneration capacity of human periodontal ligament-derived stromal cells

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INTRODUCTION

Restoring periodontal defect is still one of the clinical challenges; this is due to the complex structure and diversity of cell types in this unique joint. The advancement in tissue engineering and cell therapy, make it possible to recruit these approaches to overcome this challenge. This study aims to investigate the capacity of human periodontal ligament stromal cells (HPDLSCs) to differentiation into the main periodontal cell types and enhance the regeneration process.

METHODOLOGY

All cells were isolated from human healthy molar teeth extracted for orthodontic purposes. Periodontal tissues were scraped from the middle part of these teeth and cultured in αMEM media supplemented with 10% FBS, Penicillin-Streptomycin and L-Glutamine.

In order to characterise and biologically evaluate isolated HPDLCs cells, several tests were performed, including:

1. **Colony-forming-unit fibroblast (CFU-F) assay:** Cells isolated from three different donors were cultured in 35mm plastic dishes at a cell density of 100 cells/dish. After ten days of culture, cells were fixed and stained with 0.5% crystal violet. Colonies of more than 50 cells only counted.
2. **Examining the expression of Mesenchymal stem cells and Haematopoietic markers:** at passage two, HPDLCs were labelled with fluorescent mesenchymal stem cells markers (Cd29, CD73 and STRO-1) as well as haematopoietic cells antibodies including CD34 and CD45. Labelled cells then examined for their expression levels of these marker using flow cytometry.
3. **Inducing Multilineage differentiation:** Special types of media used to induce the differentiation of HPDLCs from three donors into adipogenic, chondrogenic and osteogenic cues in addition to fibrogenic lineage. Specific histology and immunohistology stains, as well as biochemical assays were used to confirm the phenotyping of each type.
4. **Evaluation of cellular activity both in-vitro and in-vivo:**

   To assess cells biological activities, HPDLCs were seeded on 3D Bombyx mori silk fibroin scaffolds in vitro for five weeks and analysed using histology, immunohistochemistry and biochemical assays to confirm proliferation and differentiation processes. Afterwards, the samples were implanted into the peritoneal space of nude mice using diffusion chambers for a further seven weeks. All samples were then retrieved from animal models and processed for histology.

RESULTS

Colony-forming-unit fibroblast assay:

Results CFU-f assay demonstrated the ability of HPDLCs subpopulation to adhere to the vessels plastic surface and proliferate, forming colonies of more than 50 cells, see figure (1-a). However, the number of colonies formed is different between the various donors (figure1-b).

Figure 1-a: CFU-F assay for HPDLCs obtained from three donors. Violet circles represent the colonies after ten days of culture, with each of > 50 cells.

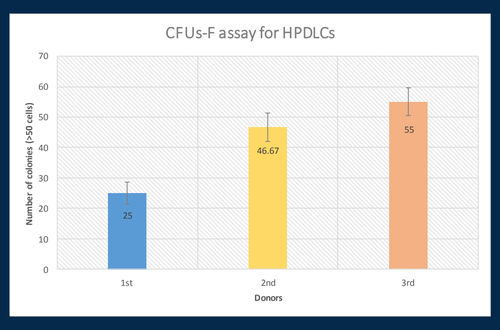

Figure 1-b: Histogram demonstrates the number of HPDLCs colonies formed after ten days of culture.

Mesenchymal stem cells and Haematopoietic markers expression levels:

Human periodontal ligament cells expressed high levels of CD29 and CD73 markers. However, STRO-1 stem cells marker demonstrated in a small fragment of this population. On the other hand, CD34 and CD45 also expressed in the isolated cells but with at lower levels, figure (2).

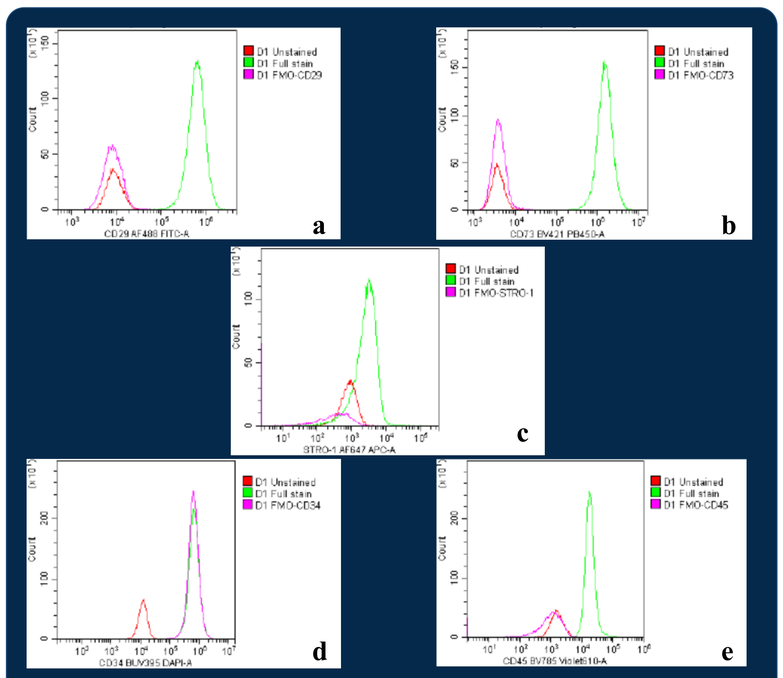

Figure 2: Expression levels of mesenchymal stem cells' markers CD29, CD73 and STRO-1 (a,b and c) and Hematopoietic cells' markers CD34 and CD45 (d and e) of the isolated HPDLCs.

Multilineage differentiation of HPDLCs:

After culturing for 21 days in adipogenic and chondrogenic media, a subpopulation of HPDLCs demonstrated morphological transformation into adipogenic and chondrogenic cells. Oils red stain showed the formation of lipid droplets inside the adipocyte cells (figure 3-a). Furthermore, the cells, where chondrogenic differentiation was induced, showed the formation of collagen type III, figure (3-b).

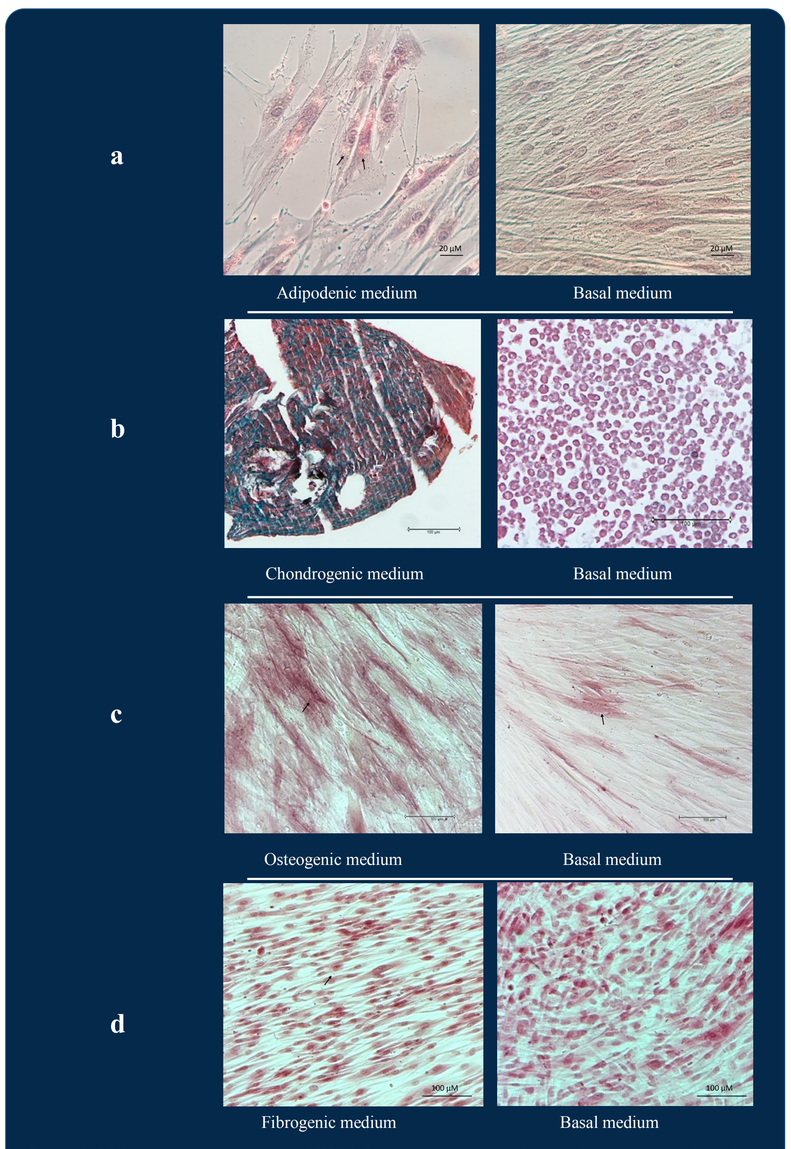

Figure 3: Multilineage differentiation of HPDLCs. a. adipogenic morphological changes as well as lipid droplet (black arrows) formation that detected with Oil red stain, b. collagen formation after culturing HPDLCs in chondrogenic media for 21 Day (black arrow refer to collagen type III) where sections stained withAlcian blue - Picrosirius red; c. ALP stain (black arrows) refer to induced osteogenic differentiation of HPDLCs, d. fibroblast-like cells (black arrow)and their orientation after staining with Van Gieson's stain.

ALP stain and assay results revealed the osteogenic differentiation capacity of HPDLCs after being cultured in Osteogenic medium for 14 days in comparison to the same type of cells grown in the basal medium. Also, evidence of extracellular mineral deposition became evident after 21 days of culture in differentiation medium (Figure 4-b). Immunohistochemistry staining of monolayer cells culture demonstrates the expression of Osteocalcin (OCN) and Osteopontin (OPN) proteins at a higher level than in basal media group (Figures 5-a and 5-b).

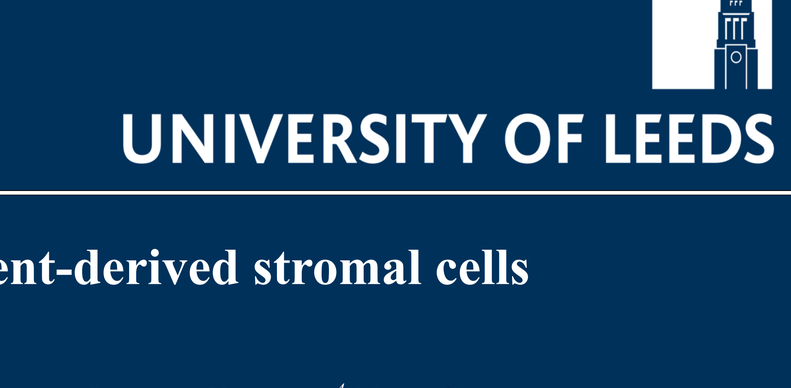

Figure 4: Osteogenic differentiation of HPDLCs a. Non-specific ALP assay to compare the ALP levels of HPDLCs cultured in basal and osteogenic media. b. Extracellular mineral deposition (black arrows) after 21 days of inducing the osteogenic differentiation.

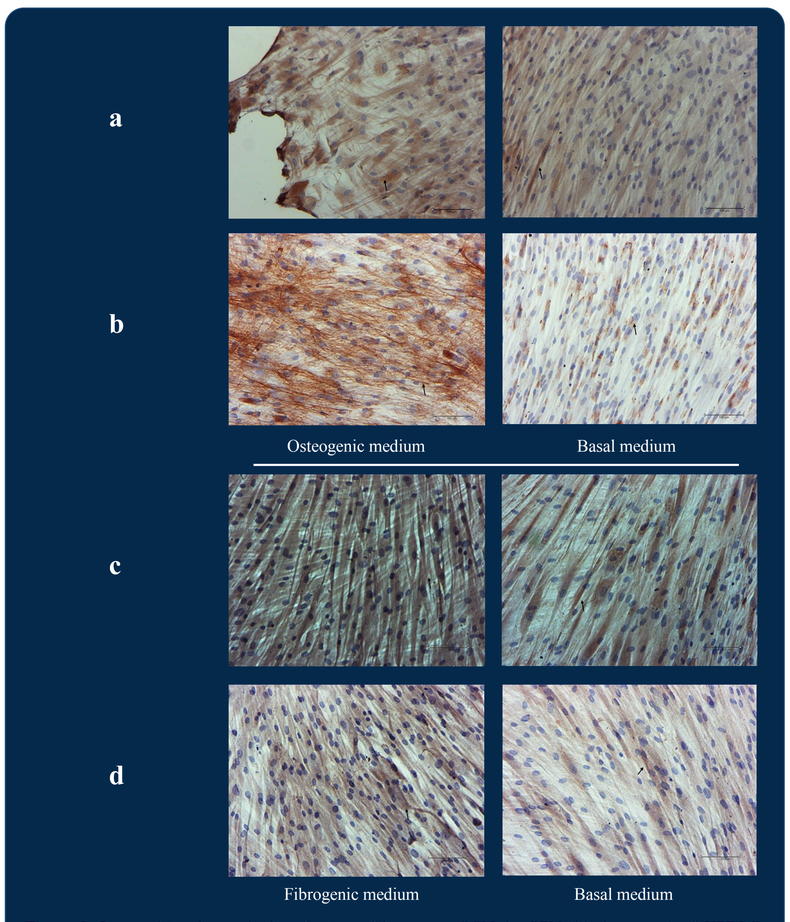

Figure 5: Immunohistochemical identification of human a. OCN, b. OPN (black arrows) of HPDLCs cultured in osteogenic media for 21 days. c. Collagen type 1, d. Vimentin (black arrows) expression in cells with induced fibrogenic differentiation.

After 14 days of culturing in fibroblast differentiation medium, the cells became more oriented and paralleled to each other, with spindle-like morphology more apparent, figure (3-d); with an abundance formation of collagen type 1 and vimentin. (Figures 5-c and 5-d)

HPDLCs biological behaviour 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).

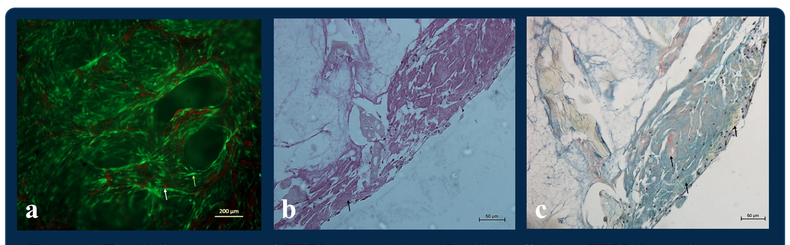

Figure 6: The Cellular activity of HPDLCs seeded on 3D silk scaffold. a. HPDLCs labelled with CMFDA/Ethidium homodimer fluorescent live-dead stain (green and white arrows) after four weeks of seeding in-vitro. b. Cellular growth on silk scaffold implanted in peritoneal space of nude mice for seven weeks.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

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CONCLUSIONS

In conclusion, this study showed that HPDLCs contain the essential progenitor stromal cells, at variable ratio between different donors. Moreover, Those cells have the capacity to differentiate into the main periodontal cells including the osteoblast and fibroblast cell, which could potentially contribute in enhancing the periodontal regeneration process.