Self- and Graft-Supported Human Gingival Fibroblasts Derived Cell Sheets As A Potential Source of Periodontal Regeneration: In Vitro Evaluation

Ghada G A.GamalElDin (g.ag.ghada@gmail.com)
Periodontology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt

Ahmed Y Gamal
Oral Medicine, Periodontology and Diagnosis Department, Ain Shams University, Cairo, Egypt
https://orcid.org/0000-0002-7511-4083

Sara El Moshy
Oral Biology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt https://orcid.org/0000-0002-2860-8523

Karim M. Fawzy El-Sayed
Oral Medicine and Periodontology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt
https://orcid.org/0000-0002-6261-3609

Method Article

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Abstract

Cellular deficiency in periodontal and peri-implant diseases represents the major challenge in treatment outcomes. Cell sheet therapy is an approach by which cell-to-cell communication through endogenous matrix secretion offers a good modification to increase the reliability of traditional cell therapy. In this study, sandwiching the rapidly resorbable scaffold with gingival fibroblasts to produce cell sheet supported graft material will be tried to produce a sheet with adequate thickness and stiffness for more predictable clinical outcomes in soft tissue augmentation. The present study will be performed to investigate the physicochemical characteristics of the produced cell sheets with the primary outcome, which is cell sheet thickness, and secondary outcomes, which are the number of viable cells, laminin and fibronectin production, resistance to mechanical forces, and cell morphology following gingival fibroblast expansion with or without rapidly resorbable construct.

Introduction

Reagents

Equipment

Procedure

The protocol was reviewed and accepted by the Research Ethics Committee, Faculty of Dentistry, Cairo University.

A) Samples, Intervention and Outcomes

1. **Cell Isolation and Culture:**

Create the gingival fibroblast-derived cell sheets in the stem cell center, Faculty of Dentistry, Cairo University, as described by Diar-Bakirly and El-Bialy (2021). Briefly:

I) Obtain gingival tissue from patients undergoing gingivectomy or gingivoplasty, after assigning the informed consent.

II) Cut the gingival tissues into small pieces, plate over tissue culture dishes, and incubate at 37°C in a humidified incubator with 5% CO_2.

III) Expand the cells to passages until the number of cells is sufficient for experimentation.

2. Characterization of Gingival Fibroblasts:

Examine the morphological characterization of gingival fibroblast using an inverted microscope. (Gamal et al., 2017).

3. Intervention for Each Group, Cell Sheet Fabrication:

After passages of expansion, seed cells on culture dishes and culture in a complete medium containing ascorbic acid (Wei et al., 2012) with and without rapidly resorbable scaffold. Harvest and evaluate autologous gingival fibroblasts-derived cell sheets.

4. Examine physicochemical properties of the produced cell sheets:

Primary Outcome:

Cell Sheet Thickness (Histomorphometric Evaluation).

Secondary Outcomes:

1- Number of Cells and Cell Survival Rate using Trypan Blue Staining.

2- Laminin and Fibronectin Production using Enzyme-Linked Immunosorbent Assay (ELISA).

3-Resistance to Mechanical Forces using Universal Testing Machine.

4- Cell Morphology (Scanning Electron Microscope (SEM) Evaluation).

5. Test data statistically after checking its normality by a blinded statistician.

B) Assignment to Intervention

1- Sequence Generation

Cell culture dishes will be randomly assigned to intervention and control groups.

Random allocation sequence will be generated using computer-assisted randomization.

2- Allocation Concealment

The generated random allocation sequence will be sealed using numbered opaque sealed envelopes. Opaque envelopes will be numbered and each envelope will receive the group number according to the generated random allocation sequence, then will be adequately sealed to the time of applying the procedures of cell sheet formation.

3- Implementation

Sequence generation and allocation concealment will be performed by a person not involved in the study.

C) Blinding

Outcome assessors and statistician will be blinded and will not be informed about the samples assignment to which study group.

References

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