

**Regenerative Potential of Cultured Gingival fibroblast-
Mesenchymal Stem Cells in Treatment of Periodontal
Intrabony Defects (Randomized Clinical and
Biochemical Trial)**

Protocol of thesis

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Introduction:

Fibroblasts are biologically dynamic and morphologically heterogeneous and are the most plentiful connective tissue cells, with different structures depending on their location and activity. The main function of fibroblasts is to keep the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix. Fibroblasts may act as a novel clue for in situ tissue repair and contribute to cellular mechanisms of mesenchymal stem cell-like features under normal or pathological conditions (Haniffa et al 2007).

Nyman et al.,1989. were claimed that GTR procedure aims at the reconstruction of a periodontal ligament (PDL) with proper oriented and organized collagen fibers inserted in newly formed cementum and newly regenerated alveolar bone. Guided tissue membranes are employed in the hope of excluding epithelium and gingival connective tissue from the root surface due to that they interfere with periodontal regeneration and provide mechanical support for clot formation.

Melcher hypothesis were experimentally established and histologically verified by Karen et al. They have shown that periodontal regeneration occurs when gingival epithelial cells or fibroblasts are excluded from the wound space and periodontal ligament cells are allowed to migrate and populate the wound space. On the other side of research, other studies were reported that gingival connective tissue cells may contribute to the regenerative process. (Lallier et al 2005,(Bartold PM and Narayanan AS.2006).

A later study by *Mitrano et al.2010* showed that gingival tissue cells fulfill the minimal criteria proposed by the International Society for Cellular Therapy to be defined as mesenchymal stem cells. Human gingival tissue-derived mesenchymal stem cells GMSCs are easy to isolate and proliferate faster than BMSCs without any use of growth factors. They have a constant morphology , they maintain a normal karyotype and telomerase activity in long-term cultures and are not tumorigenic(Tomar et al.,2010,Tang et al., 2011) . GMSCs exhibit

strong suppressive effects on the proliferation and activation of human peripheral blood mononuclear cells (PBMC)(Zhang et al., 2009)

MSC are currently defined as plastic adherent, multipotential fibroblast-like cells expressing CD73, CD105 and negative for the hematopoietic markers CD14, CD34 and CD45(Dominici M et al., 2006) but that properties and markers are also shared by fibroblasts. Osteoblastic, chondrogenic, adipogenic differentiation from fibroblasts has also been described (Haniffa MA et al., 2000). Fibroblasts have also the immunosuppressive properties. In fact, it had been comprehensively demonstrated that fibroblasts from various tissue sites inhibit mitogen and allo-antigen stimulated T-cell proliferation (Sarkhosh et al., 2003) and IFN γ production in exactly the same vein as more recent reports using MSC (Klyushnenkova EN et al., 1998).

A novel perforated collagen membrane as a sensory system that could enable participation of gingival fibroblasts and gingival stem cells in GTR procedures (Gamal et al., 2013). That approved in a clinical study that the utilization of a perforated membrane (PM) improved clinical outcomes significantly more than those observed with the use of occlusive membranes. It has likewise been suggested that growth and differentiation factors from cells in the gingival connective tissue could pass through the membrane perforations and augment regeneration. Gamal et al. reported that PM use was associated with significantly higher GCF levels of BMP-2, PDGF-BB and VEGF compared to occlusive membrane (OM) treated sites. They hypothesized that; blood clot occluded membrane perforations could allow for more physiologic growth factor release compared to occlusive membranes (Gamal et al., 2014,2015)

Since cellular responses mainly dependent on specific interactions with extracellular matrix components(ECM), growth factors, or cell surface receptors, ECM act as a constitutive part of stem cell niche, ECM components are key players of the niche instructive power(R. Peerani, P.W. Zandstra.2010) These extracellular macromolecules, by their assembly and three-dimensional arrangement, supply a microenvironment in which the signals deriving from cell-ECM interaction, as well as soluble and ECM-bound factors, are integrated

in a functional manner to allow the maintenance of stem cell homeostasis (M.F. Pera, P.P.L. Tam, 2010).

We hypothesized that if the gingival fibroblasts translocated away from its extracellular matrix components into the periodontal defect to be subject to its same mechanical and biologic media, it could behave the same way as the MSCs. This experimental study was performed in order to evaluate clinically and radiographically regenerative potentials of cultured GMSCs and gingival fibroblasts into intrabony periodontal defects in human.

Aim of the study:

Primary outcome:

Clinical and radiographic evaluation of the regenerative potentials of cultured gingival fibroblasts and GMSCs carried by tri calcium phosphate into intrabony periodontal defects in human.

Secondary outcome:

Biochemical analysis during the healing of intrabony periodontal defect .

Subject and methods:

Twenty patients diagnosed as a moderate to severe chronic periodontitis will be participate in this study, they will be selected from the outpatient clinic, Department of Oral Medicine and Periodontology, Ain Shams University.

All patients will be fully informed about the aim and purpose of this study. An informed consent will be signed by each patient.

I-Patient Selection:**A) Inclusion Criteria:**

Patients were selected according to these criteria.

- 1- Age range from 25 to 50 years.
- 2- Presence of at least two bilateral interproximal osseous defects estimated from radiographic evaluation and transgingival bone sounding ≥ 3 mm of two or three osseous walls.
- 3- Probing Depth ≥ 5 mm after initial therapy.
- 4- Attachment loss ≥ 5 mm.
- 5- Thick gingival biotype more than 1 mm with enough width of attached gingiva.

B) Exclusion Criteria:

- 1- Patients with systemic disease or compromised immune illness regarding Cornell medical index.
- 2- Smoker's patients.
- 3- Pregnant and lactating females.
- 4- Patients with regular use of medications that could compromise wound healing.
- 5- Uncooperative patients (low compliance).

II-Patients grouping:

Group 1:

Ten periodontal defects will receive tri calcium phosphate alone.

Group 2:

The other ten periodontal defects will receive gingival MSCs - fibroblast culture in tri calcium phosphate as a scaffold.

III- Clinical and Radiographic Assessments:

For the selected sites, the following clinical parameters will be assessed preoperative (baseline), 3, and 6 months after the surgical procedure using the same William's graduated periodontal probe.

- 1- Plaque index (PI) *Löe 1967.*
- 2- Gingival sulcus bleeding index (SBI) *Muhlemann, 1963.*
- 3- Probing depth (PD) *Glavind and Loe, 1967.*
- 4- Clinical attachment level (CAL) *Glavind and Loe, 1967.*
- 5- Gingival recession: considered the distance of the gingival margin from the cemento-enamel junction if there were recession preoperative, while if there is no recession the gingival margin above the cemento-enamel junction is considered preoperatively and postoperatively.

Radiographic assessment:

- 1- Assessed at baseline, 3, 6 and 9 months postoperatively using indirect digital radiography (Digora)♦.
- 2- Each radiograph was standardized using a paralleling device with an acrylic bite block that was fixed by the opposite arch by the indentations of the opposing teeth.

♦ Digora Soredex USA www.soredex.com

The radiographic parameters were measured on 2.7 version of Digora software:

- 1- Intrabony defect height.
- 2- Defect surface area.
- 3- Bone density

IV- the gingival crevicular PDGF-BB levels at 1,3, 7, 14,and 21 day:

The measurement of platelet derived growth factor level in the gingival crevicular fluid at different interval will detect the regenerative process in periodontal defect.

V-Surgical protocol:

A) Surgical procedures:

- gingival biopsy will be taken from retromolar area , the tissue will be kept in a-MEM medium in sterile container. And so it will be cultured for 14 days in biochemical department in Cairo University.
- Biopsy medium is consists of Dulbecco's modified Eagle's medium (DMEM) plus penicillin and streptomycin.
- The samples will be cut into small pieces displaced on glass slides and placed in culture plate with basic medium (DMEM+ fetal bovine serum + penicillin and streptomycin)(*Alireza M et al., 2014*).
- It will be maintained in an incubator at 37°C and humidified air (5% CO₂, 95% air). From days 14 to 21, gingival fibroblast cell cultures will reach 80% confluence; the cells will be transferred to tissue culture flasks using a solution of trypsin and EDTA.
- After centrifugation, cells will be resuspended with serum supplemented medium and incubated in 50 cm²culture flask (Falcon).
- After that the minimally invasive technique will be made at the site of the defect and the graft material will be applied.

-All patients will take antibiotics for 1 week (3 · 500mg amoxicillin*/day), (3.250mg metronidazole**/ day) and an anti-inflammatory (3.alphintern***/ day).

-Patients will instruct to rinse twice daily with a 0.12% chlorhexidine digluconate[▫] mouth rinse and to avoid mechanical plaque removal at the site of surgery for 15 to 30 days.-Periodontal dressing was removed after 1 week.

-Sutures will removed 10 day following surgery.

-Patients will instruct not to brush for 2 weeks and not to floss the surgical area for 4 weeks.

- After 15 days patient were instructed to use Bass technique for tooth brushing.

VI-Postoperative surgical evaluation and assessment:

- Weekly recall appointments will schedule during the first 6 weeks after surgery and once per month.
- The follow up period included general and oral examination, plaque and calculus removal when necessary, at the surgical site careful subgingival debridement will carry out with hand instruments.
- Both clinical and radiographic parameters will be recorded at 3and 6 months postoperative.

*The following rule in the unit of Biochemistry and Molecular Biology will be applied to maintain cells culture:

- ✓ Hood preparation
- ✓ Sterile handling .All biological contaminated tissue culture plates, flask, and other non sharps will be placed in non – sharp biohazard waste container. However, an effort to minimize entry /exit from the hood will be made to minimize disturbances in laminar flow at the entrance, which may create the potential to waft in contaminants. Removal of all these biohazards will be under supervision of researcher.
- ✓ Cleaning up

* Amoxil MUP Egypt
 ** Flagyl Sanofi Aventis Egypt
 *** Alphintern Amoun Egypt
 ▫Antiseptol Kahira Pharm Egypt

- ✓ Cell feeding , To enable cells to grow and divide in dish, they will be fed by the nutrients provided by the media. Old media will be replaced with fresh media every two or three days for most animal cell lines
- ✓ Cell splitting ,Cell growing in dish begin to get crowded and then stop growing. This crowded state is called (confluence) and to maintain cells growth, confluent cultures will be split and reseeded into new culture dishes at a lower density.

VII- Statistical analysis:

- All data will be subjected to statistical analysis.
- Description of quantitative variable will be in the form of mean and standard deviation (SD).
- Qualitative variable will be described as number and percentage.
- Comparison between quantitative variable will be carried out using student -t test when normally distributed, and Mannwhitney test for abnormal distribution.
- Categorical data will be compared using Chi-square test.
- Significant value will be defined as P values <0.05 .

This research will be reviewed by the research ethics committee faculty of dentistry ain shams university

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