In Vitro Evaluation of the Human Gingival Fibroblasts - GMSCs Dynamics Through Perforated Guided Tissue Membranes: Cell Proliferation, Migration and Membrane Stiffness Assay

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Dedication

This work is dedicated to my dear
Parents and my lovely son Anas

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Acknowledgement

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- α-MEM: alpha modified minimal essential medium.
- ALP: Alkaline phosphatase.
- BMP: Bone morphogenetic protein
- bFGF: basic fibroblast growth factor.
- CAL: Clinical attachment level.
- CD: Cluster of differentiation.
- CFU-F: Colony-forming unit-fibroblast.
- CT: Connective tissue.
- DMEM: Dulbecco's modified Eagle's medium.
- DNA: Deoxy ribonucleic acid.
- ECM: Extracellular matrix.
- EGF: Epidermal growth factor.
- e-PTFE: Expanded polytetrafkuoroethylene.
- FBS: Fetal bovine serum.
- FGF: Fibroblast growth factor.
- GBR: Guided bone regeneration.
- GCF: Gingival crevicular fluid.
- GMSCs: Gingival mesenchymal stem cells.
- GTR: Guided tissue regeneration.
- IL: Interleukin
- MPM: Modified perforated membranes.
- MMP: Matrix metalloproteinase

- MSCs: Mesenchymal stem cells.
- MTT: Methyl thiazolyl-diphenyl-tetrazolium bromide.
- NADH: Nicotinamide adenine dinucleotide.
- NADPH: Nicotinamide adenine dinucleotide phosphate.
- NK cells: Natural killer cells.
- OCN: Osteocalcin.
- OM: Occlusive membranes.
- PBS: Phosphate buffered saline.
- PDGF: Platelet derived growth factor.
- PDL: Periodontal ligament.
- PTFE: Polytetrafluoroethylene.
- SD: Standard deviation.
- SDF-1: Stromal cell derived factor-1.
- SEM: Scanning electron microscope.
- TGF: Transforming growth factor.
- TIMP: Tissue inhibitor of metallo-proteinase.
- TNF: Tumor necrosis factor.
- TSP: Thrombospondin.
- VEGF: vascular endothelial growth factor.

Introduction and Review Of Literature

Introduction and Review of Literature

Chronic periodontitis is an inflammatory disease that causes gradual destruction of the tooth-supporting tissues including periodontal ligaments, cementum and alveolar bone [(1)]. Regenerative periodontal therapy aims at restoration of the original pattern and function of the tooth supporting apparatus. However, it is still a challenging task with unpredictable outcome in most situations. Guided tissue regeneration (GTR) is considered the most well documented procedure for periodontal regeneration especially for intrabony defects and class II furcation defects [(2)].

The concept of GTR was introduced in the mid-1980s by Gottlow *et al.* as they observed through an animal study that the placement of a membrane over denuded root surfaces favored new attachment formation compared to control roots treated by open flap debridement [(3)]. The rationale behind GTR is that using a physical barrier over the denuded root surfaces prevents migration of gingival epithelium and connective tissue and offers selective guidance for cells originating from periodontal ligament and alveolar bone to repopulate the wound area and proliferate to reform the lost tissue compartments [(2-4)].

Scantlebury described five essential design criteria for membranes used for guided tissue regeneration. These criteria include biocompatibility, space-provision, cell-occlusiveness, tissue integration and clinical manageability [(5)]. Barrier membranes are classified according to their degradation rate into nonresorbable and bioabsorbable categories. Currently, the most commonly used nonresorbable material is the expanded polytetrafluoroethylene (ePTFE) membrane. Examples of bioabsorbable membranes include collagen, polylactic acid, polyurethane, polyglactin, acellular dermal matrix, dura mater, periosteum, and chitosan [(2)].

Comparative studies have shown that resorbable collagen membranes have similar clinical effectiveness as non-resorbable membranes. When compared to open flap debridement, collagen barriers have shown better probing depth reduction, clinical attachment level (CAL) gain, and defect fill [(6).] Collagen membranes have the advantages of easy manipulation, and weak immunogenicity. Collagen also shows a hemostatic function, as it can aggregate platelets, facilitating early clot formation and wound stabilization. In addition, collagen possesses a chemotactic function for gingival and periodontal ligament fibroblasts, thus promoting cell migration, proliferation and extracellular matrix maturation to enhance wound healing. Taken together, these properties make it a successful material for regeneration [(6, 7)].

The periodontal defect represents a challenging wound as it is composed soft tissue components (gingival epithelium, gingival connective tissue and periodontal ligament), and the mineralized tissues (cementum, dentin, and alveolar bone). The structural and functional differences of these tissue compartments affect the healing events of periodontal wounds. Periodontal wound healing is also challenged by the presence of two different wound margins; the rigid, nonvascular usually contaminated root surface on one side, and the rapidly proliferating gingival connective tissue and epithelium of the gingival flap on the other side [(8)].

In an effort to enhance the regenerative capacity of the remaining periodontal structures, recently, Gamal and Iacono introduced a novel perforated collagen membrane, which showed improved clinical outcomes when it was compared with occlusive membranes. They claimed that during GTR procedure, periosteal elevation and the placement of an occlusive membrane may limit the regeneration by depriving the wound area of regenerative elements derived from periosteum and gingiva, especially gingival fibroblasts and gingival mesenchymal stem cells (GMSCs) [(9)].

Gamal *et al.* suggested that membrane macropores could allow the migration of gingival stem cells to the defect to share in the healing process. Small perforations and wide inter-pore

distance were suggested to preserve membrane rigidity, providing a membrane that prevents soft tissue invasion and at the same time allows for the passage of cells and mediators through fibrin clot-occluded perforations. They also claimed that mechanical interlocking of fibrin strands from the blood clot with membrane perforations could offer additional stabilization of the flap [(10)].

The optimal perforation diameter that favors maximum cell migration into the defect area and at the same time acts as an occlusive barrier for gingival epithelium and its associated gingival extracellular matrix component is not yet identified. In this study we evaluated 3 different perforation diameters in order to test gingival fibroblasts and their associated GMSC locomotion and activity through resorbable collagen membrane.

Periodontal wound healing:

One of the main determinants of the outcome of the healing process is the phenotype of the cells repopulating the healing site. Animal studies of periodontal wound healing showed that cells from bone, periodontal ligament possess a repopulation response following wounding. Immediately after reconstructive surgery, increased proliferative activity is detected in the most coronal parts of bone and periodontal ligament. This activity continues adjacent to the forming junctional epithelium for 15 days [(11)].