

## Effects of Resolvin-E1 on Primary Human Gingival-Fibroblast in Bioactive Hydrogels

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**Objective:** Gingival recession is defined as migration of the gingival margin apical to the cemento-enamel-junction (CEJ). Due to this condition, root exposure can lead to sensitivity, root caries, and an unaesthetic appearance. Resolvin E1's (RvE1) are anti-inflammatory and pro-resolving lipid mediators that exhibit significant therapeutic potential in gingival healing. They can stimulate the proliferation of human gingival fibroblasts (hGFs) and promote tissue regeneration, rendering them as a minimally invasive approach to healing periodontal disease. The primary objective of this analytical study was to quantify the viability of hGFs supported in bioactive and highly tunable 3D hydrogels treated with and without RvE1.

**Experimental Methods:** The experimental design consisted of fresh 100 ng/mL RvE1 and vehicle control treatments every two days. A live/dead assay (Calcein AM/Ethidium Homodimer III and Hoechst) was performed, and time-matched pucks were fixed with 4% paraformaldehyde per the following timepoints (days): 2, 5, 8, 11, and 14 for downstream immunocytochemistry to determine proliferation (Ki67), phenotype (vimentin), and extracellular matrix deposition (collagen-1). Cells were imaged using an A1R/MP Confocal Microscope, and the Imaris software was used to quantify fluorescence micrographs to determine cell viability. Through the spot detection feature object counts were obtained and two filters were created to represent the colocalized regions between live/dead cells (green/red) and live cells/nuclei (green/blue). By identifying cells that exhibited signals from two different markers, cell viability was assessed more accurately.

**Results:** The analysis showed that treatment with RvE1 notably increased viability of hGFs (Calcein AM<sup>+</sup>) beginning at Day 11 (\*\* p<0.01) when compared to the vehicle control group.

**Conclusions:** Our hydrogel system supports long-term interrogation of primary derived hGFs. In terms of clinical applications, due to its unique ability to lower inflammation and promote tissue regeneration, RvE1 treatment could potentially serve as a non-invasive treatment option for periodontitis.

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