

## The Benzoin-Based Complex: A New Frontier for Skin Repair

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The skin acts as a protective barrier for the human body and is constantly being remodeled in order to retain maximum protection. When the skin suffers a severe wound, the body reacts by overproducing matrix metalloproteinases (MMPs) and inflammatory cytokines [1]. Matrix metalloproteinase-9 (MMP-9) prolongs the degradation taking place at the site of the wound and inhibits skin recovery [2]; whereas fibronectin, collagen and elastin are proteins that promote wound repair and skin elasticity [3]. Furthermore, severe trauma to the skin is exacerbated by oxidative stress in which free radicals cause further damage to the site of the injury.

Benzoin is synthesized from benzaldehyde, which has been claimed by folklore to have beneficial effects on the skin. However, this has never been scientifically proven. [4]. Olive fruit extract, which contains the powerful antioxidant hydroxytyrosol, is known to prevent the damage caused by oxidative stress and free radicals induced by a severe wound to the skin [5]. In this study, the ability of benzoin and olive fruit extract to improve the treatment of wounds was investigated. It was hypothesized that the combination of benzoin and olive fruit extract would decrease the levels of MMP-9 in human keratinocytes and increase the levels of fibronectin, collagen, and elastin in dermal fibroblasts in a dose-dependent manner.

Human keratinocytes (HaCaT) from the epidermis and dermal fibroblasts from the dermis were used in this study. Benzoin was applied to cells in concentrations of 0 $\mu$ M, 0.5 $\mu$ M and 0.05 $\mu$ M, diluted in DMSO. The olive fruit extract was standardized to contain 25% hydroxytyrosol, and diluted in distilled water to 0%, 0.001%, 0.002%.

An MTS assay showed that all concentrations of benzoin and olive fruit extract used were not cytotoxic. Furthermore, the combination of benzoin and olive fruit extract induced statistically significant proliferation in the HaCaT cell line ( $p < 0.05$ ). A DPPH antioxidant assay showed that olive fruit extract exhibited antioxidant ability by reducing 2,2-diphenyl-1-picrylhydrazyl (DPPH) in a dose-dependent manner across all concentrations.

Results from enzyme-linked immunosorbent assays (ELISA) showed that MMP-9 levels in HaCaT cells decreased as the concentrations of benzoin and olive fruit extract increased, both independently and in combination ( $p < 0.05$ ). Benzoin and olive fruit extract, when combined, caused a slight increase of fibronectin in HaCaTs, and a significant increase of collagen and elastin in dermal fibroblasts ( $p < 0.05$ ).

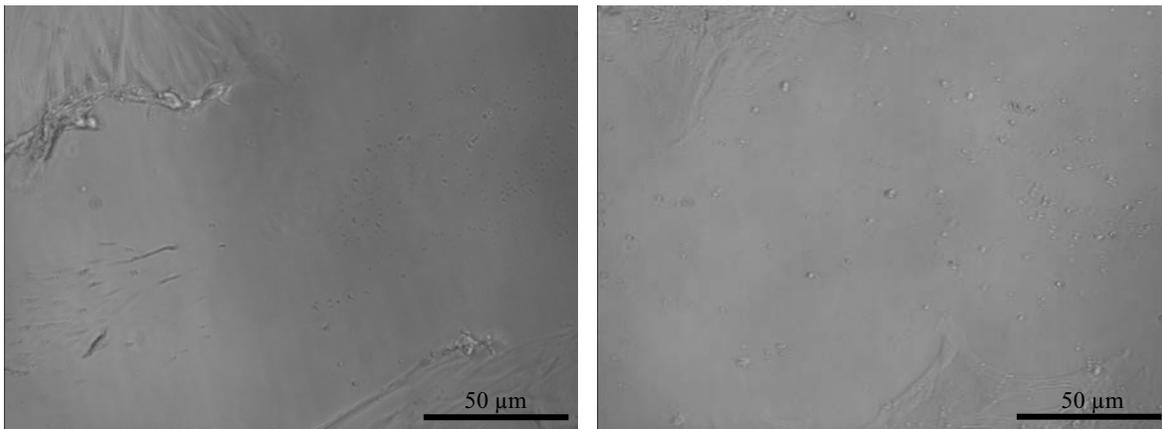
A scratch assay was also performed to qualitatively observe the wound healing abilities of the treatment. After scratching the plated dermal fibroblasts to simulate a wound, the proliferation and migration of the cells were observed using phase contrast microscopy. The assay demonstrated that

benzoin and olive fruit extract caused faster recovery (coverage of cells across the scratched region) compared to the control (Figures 1 and 2).

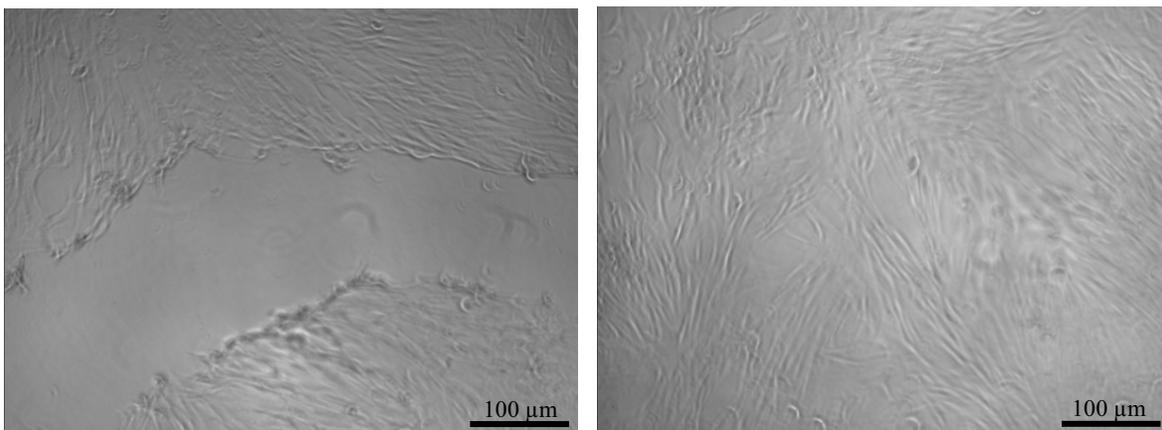
These results suggest that benzoin and olive fruit extract could mediate degradation of the skin after severe trauma by down-regulating MMP-9, promoting the production of fibronectin, collagen and elastin, and improving the migration of cells to cover the wound. Future tests on *in vivo* models, as well as improved delivery method for the treatment will be studied.

#### References:

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**Figure 1:** Scratch assay. Dermal fibroblasts (control) at time zero (left) and after six hours (right).



**Figure 2:** Scratch assay. Dermal fibroblasts treated with 0.5µM benzoin and .001% olive fruit extract at time zero (left) and after six hours (right).