## Biomolecular and ultrastructural investigation on fibroblasts treated with an aqueous formulation of equine collagen type I

## Indagine biomolecolare ed ultrastrutturale su fibroblasti trattati con una formulazione acquosa di collagene equino di tipo l

**Objectives:** equine collagen type I is known to mimic the effects of the tissue collagen, helping to accelerate the healing process of chronic wounds, having documented effects on fibroblast proliferation, tissue regeneration and collagen biosynthesis stimulation. So, it is used in dermatology as well as plastic and vascular surgery for wound healing and skin ulcer therapy from at least thirty years. The aim of the present work was to evaluate, in vitro, the biomolecular and ultrastructural effects of heterologous collagen type I cell on 3T3 mouse fibroblasts.

**Materials and methods:** treatments of the cell cultures with different concentrations of collagen at different time intervals have been evaluated on cell viability and proliferation as well on the expression of proteins involved in the synthesis of collagen fibers i.e. HSP-47 (heat shock protein 47) and P4H-A1, known to stabilize the collagen triple helix under physiological conditions and recognized as a "rate-limiting enzyme" in collagen production. The expression of  $\alpha$ -SMA, a known marker of myofibroblast differentiation, have been also analyzed. The collagen 1 and 3 neosynthesis has been investigated by immunostaining assay. To verify the potential ultrastructural changes induced by treatment, fibroblasts were analyzed by SEM to verify the phenomenon of microvescicle shedding from plasma membrane. Of note, the cells undergo dramatic changes both in cell shape and in cell surface extensions, becoming highly convoluted with numerous filopodia and microvescicles budding from the plasma membrane.

**Results and conclusions:** overall, our results firstly indicated that the treatment with equine collagen aqueous solution provides favorable conditions for stimulating collagen processing in vitro and the process of microvescicle schedding which in turn may be causally related with its effects of tissue regeneration and biorejuvenation.

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