IN VITRO EFFECTS OF NATURAL GLYCOSAMINOGLYCANS ON HUMAN PRIMARY FIBROBLASTS

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INTRODUCCIÓN

Glycosaminoglycans (GAGs) are one of the main components of the dermal extracellular matrix (ECM), and their composition, sulfation degree and molecular weight determine skin physiological and biochemical properties, as well as the degree of skin hydration.

Skin aging is characterized by a progressive deterioration of its functional properties, which is closely related to impaired synthesis, structure and function of GAGs. We have recently developed the ingredient Wharton Gel Complex® (WGC®), which derives from Wharton's jelly of the umbilical cord of animal origin.

WGC® consists of a unique blend of natural GAGs and growth factors that mimics the composition of a functional ECM, achieving a filler effect, while acting on the ECM components, promoting a dermal redensification. WGC® has shown significative chemotactic properties, inducing the migration of fibroblasts to it.

OBJECTIVE

To demonstrate the effects of WGC® on parameters involved in skin ageing and dermis deterioration, specifically evaluating its effects on the physiology of fibroblasts.

MATERIALS AND METHODS

Human primary fibroblasts were cultured for up to 72h in the presence or absence of 10% WGC® or a synthetic comparator (SC). Cell viability and proliferation were evaluated by MTT (3-[4,5-dimethylthiazolyl-2]-2,5 diphenyl tetrazolium bromide) assay and live/dead staining followed by fluorescence microscopy. Cell morphology changes and cell migration were observed by phase contrast microscopy. Finally, the ability of WGC® to induce the synthesis of ECM proteins was investigated by immunocytochemistry, or RT-PCR.

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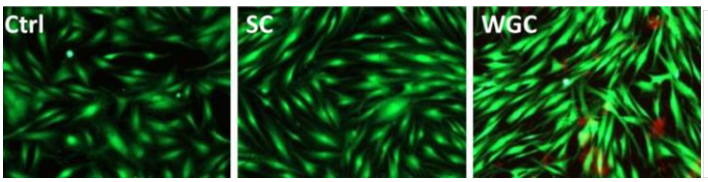

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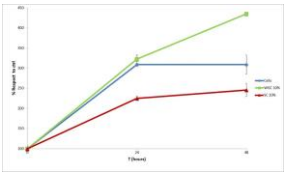


Figure 1a: Fluorescence optical microscopy with Live/dead staining (Green: living cells; Red: dead cells), after 72h, showing an increase in the number of fibroblasts *vs* control and SC. The increase of dead cells is due to an over confluence of the plate.
Figure 1b: MTT cell proliferation assay. WGC® increases cell proliferation by 150% compared to control cultures, and 100% compared to SC.

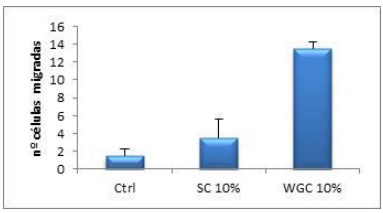


Figure 2: Transwell migration assay showing the number of fibroblasts that have migrated through the *transwell* plates to the compartment where control, SC or WGC® culture media were added. WGC® has a significant chemotactic activity compared to control and SC media.

Figure 3: Fluorescence optical microscopy after 7 days (Green: collagen I; Red: fibronectin; Blue: Hoechst nuclear counterstaining). WGC® induces an increase in the levels of both collagen I and fibronectin compared to control cultures and, specially, compared to SC.

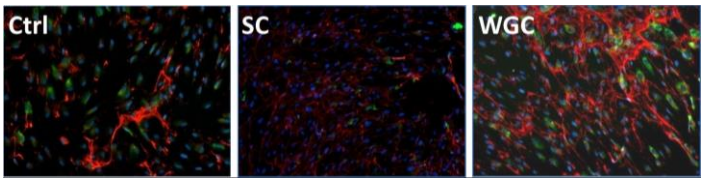
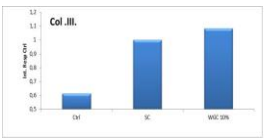
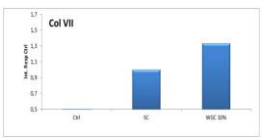
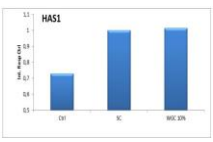
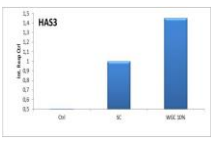


Figure 4: Gene expression in fibroblasts by RT-PCR. The semi-quantitative analysis of gene expression of collagen III and VII, and hyaluronic synthases 1 and 3 showed the ability of the ingredient to induce the synthesis of these molecules, significantly more than control medium or the synthetic comparator.









CONCLUSIONS

WGC® has shown to increase human primary fibroblasts proliferation while preserving their viability and morphology. Furthermore, WGC® promotes fibroblast migration, exhibiting a high chemotactic capacity. The analysis of ECM components proved the ability of WGC® to increase the levels of collagen I and fibronectin fibers, as well as the synthesis of collagen III and VII, and hyaluronic synthases 1 and 3.

Thus WGC® is an effective ingredient with regenerative properties which could help to improve firmness of the skin. Further investigation in human beings will be necessary to confirm the clinical efficacy of WGC®.

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