Functionality of umbilical cord stem cells secretome in human fibroblast proliferation in vitro

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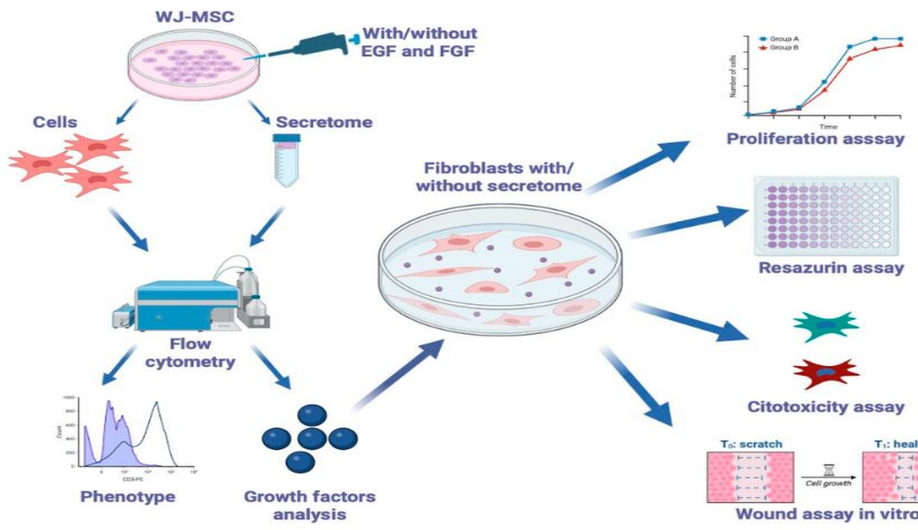
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Introduccion

In regenerative medicine, there is interest in using acellular therapy based on the secretome of mesenchymal stem cells (MSC) to promote wound healing. Wharton's jelly cells (WJ-MSCs) are a readily available source. Their secretion has been optimized when stimulated with FGF and EGF to induce proliferation and prevent senescence. Therefore, evaluating the effect on proliferation and wound closure of human fibroblasts in vitro with different concentrations of the secretome of WJ-MSCs stimulated with growth factors is necessary to identify the most efficient work concentration.

Materials and methods



Results

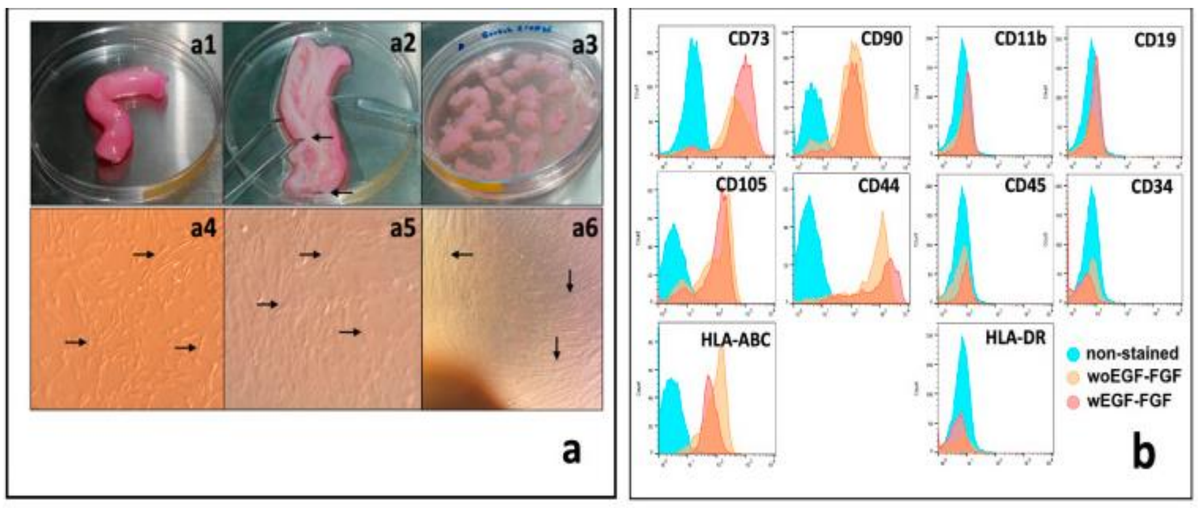


Figure 1 Isolation and characterization of human WJ-MSCs. (a) Processing of the umbilical cord and culture of WJ-MSCs. (b) Flow cytometry phenotype analysis of WJ-MSCs. Histograms show the expression of typical positive and negative markers of MSCS in cultured cells at passage 2, stimulated or unstimulated with EGF and FGF (ns). Magnification of (a4–a6) = 40×.

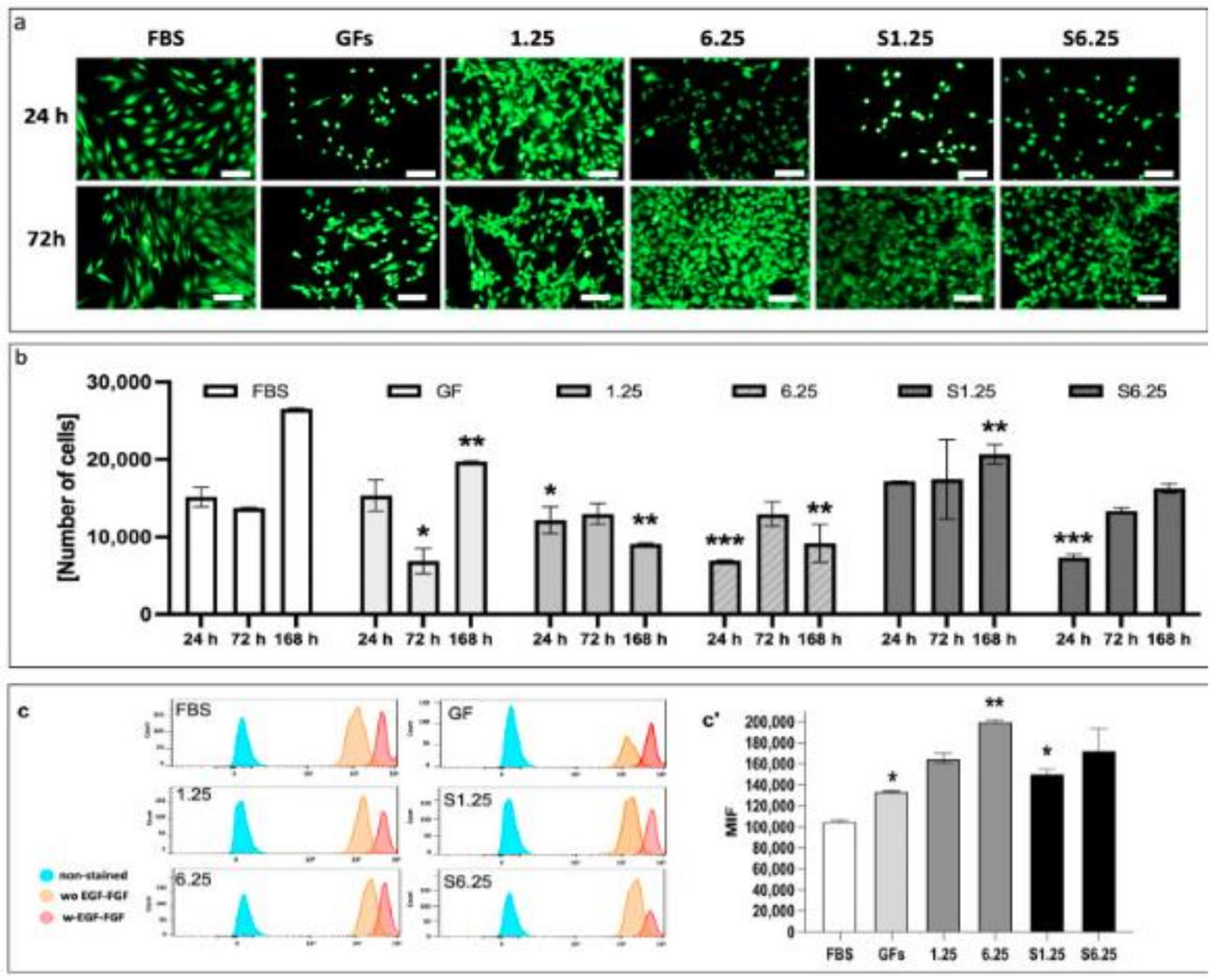


Figure 3 Analysis of cytotoxicity, metabolic activity, and proliferation in human dermal fibroblasts cultured with the secretome of WJ-MSCs. (a) Live and dead assay. (b) Prestoblue colorimetric assay. Metabolic activity (* $p < 0.03$, ** $p < 0.01$, and *** $p < 0.008$). (c) Histograms of CFSE in human fibroblasts with different concentrations of WJ-MSC secretome obtained from cells stimulated and unstimulated with FGF-EGF. (c') MIF graphic of CFSE.

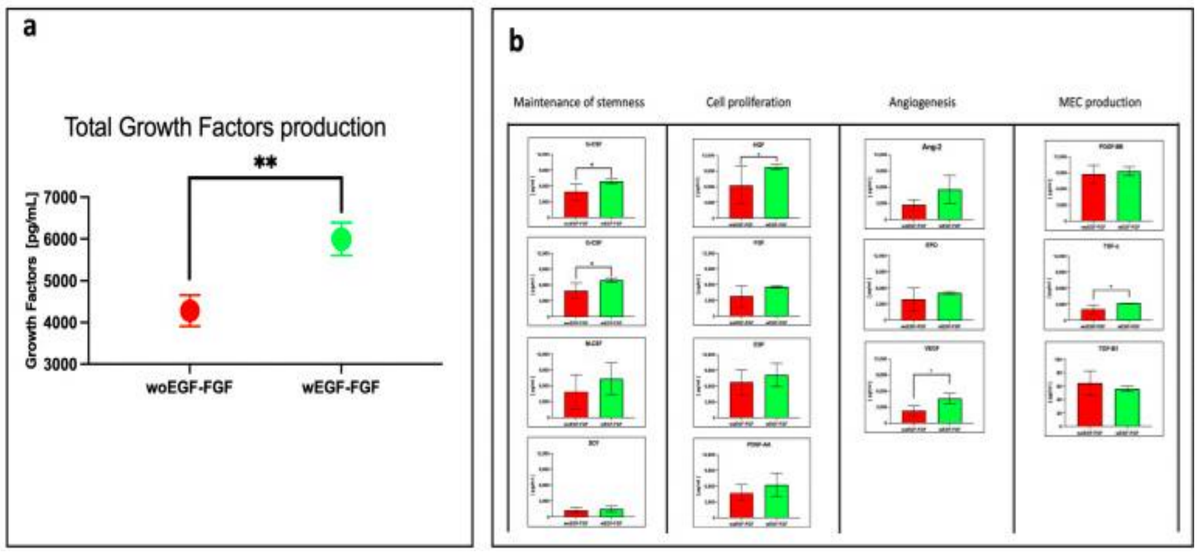


Figure 2 Determination of some growth factors produced by non-stimulated and stimulated WJ-MSCs. (**a**) Comparison of total growth factors production by WJ-MSCs. WJ-MSCs cultured in the presence of EGF-FGF stimulation showed a significantly greater total production of growth factors than unstimulated ones (** $p < 0.01$). (**b**) Comparison of each of the 14 Growth Factors produced by WJ-MSCs. (* $p < 0.05$). n = 3 independent experiments, each performed in triplicate.

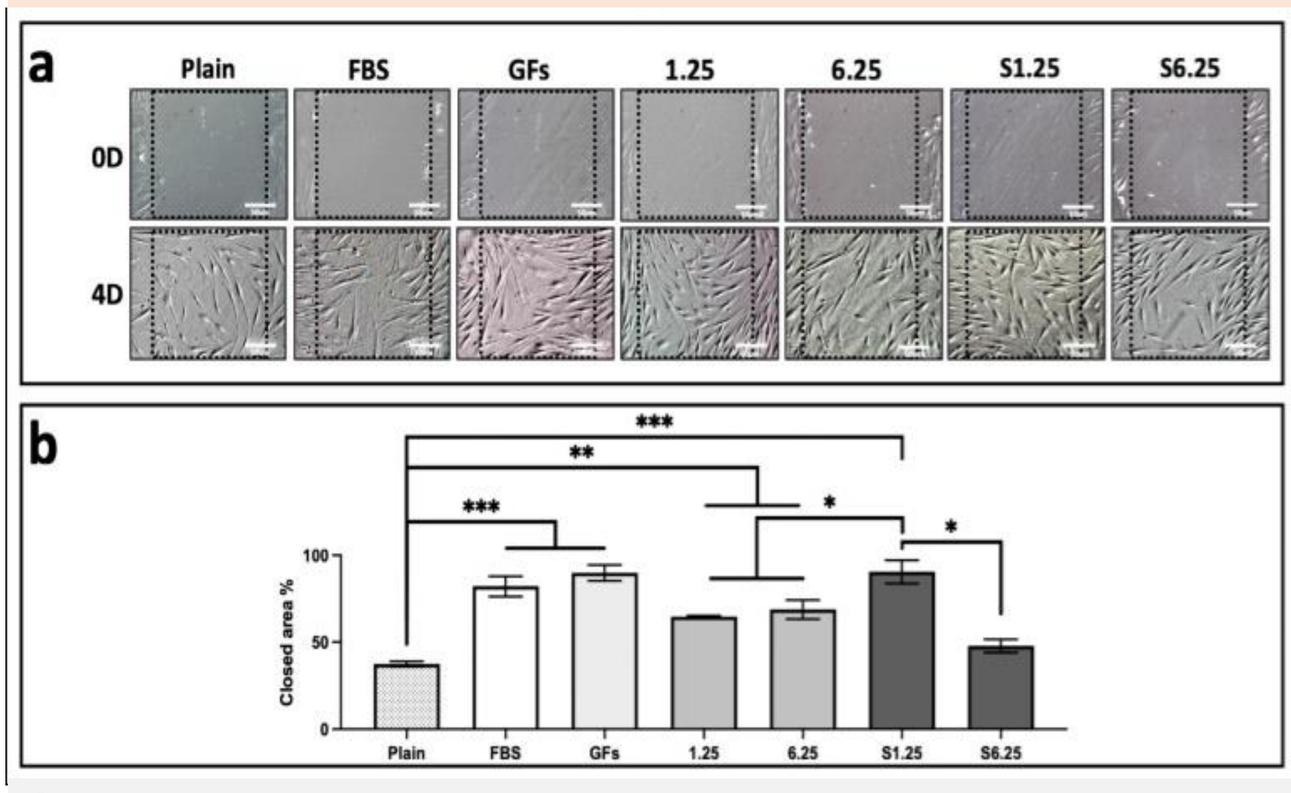


Figure 4 Scratch wound assay on fibroblast cultured with WJ-MSC secretome. (**a**) DIC photomicrographs of fibroblast cultures at day 0 and day 4 after scratching. (**b**) Quantification of scratch area occupied by fibroblasts expressed as a percentage, (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

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