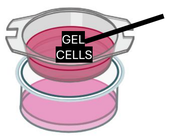
Establishing suitable *in vitro* methodologies for assessing cytocompatibility and functionality of injectable polysaccharide hydrogels with dermal cells

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The aim was to systematically compare the cytocompatibility (direct vs. indirect) and functional performance of water-soluble cellulose-based polysaccharide (CPS) and polysaccharide-polyethylene glycol (CPS-PEG) hydrogels by evaluating their growth factor (GF) release kinetics and the subsequent biological effect of the released GF on seeded skin cells.

The methodology employed a comprehensive approach to assess CPS and CPS-PEG hydrogels using Human Dermal Fibroblasts (HDFn) and HaCaT Keratinocytes. This assessment began with cytocompatibility tests, where cells were evaluated via direct contact with the gels and through indirect seeding using culture inserts to test leachable components. GF release kinetics were determined by mixing AF647-labeled GF within the gels, monitoring the fluorescence released. Finally, the bioactivity of the released GF was confirmed functionally using a scratch assay, where GF-loaded gels allowed the released factor to stimulate the migration and wound closure of the underlying cell monolayer. Moreover qPCR were performed to observe the expression of some genes.

(I.) Gels applied in a direct (topical) way



Cells seeded directly on an insert, gel (70 µl) added 2 days post-seeding

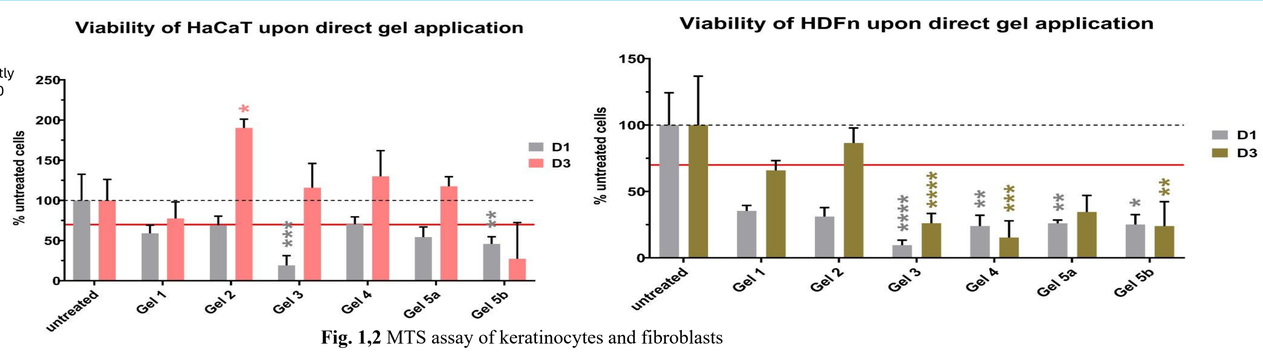


Fig. 1,2 MTS assay of keratinocytes and fibroblasts

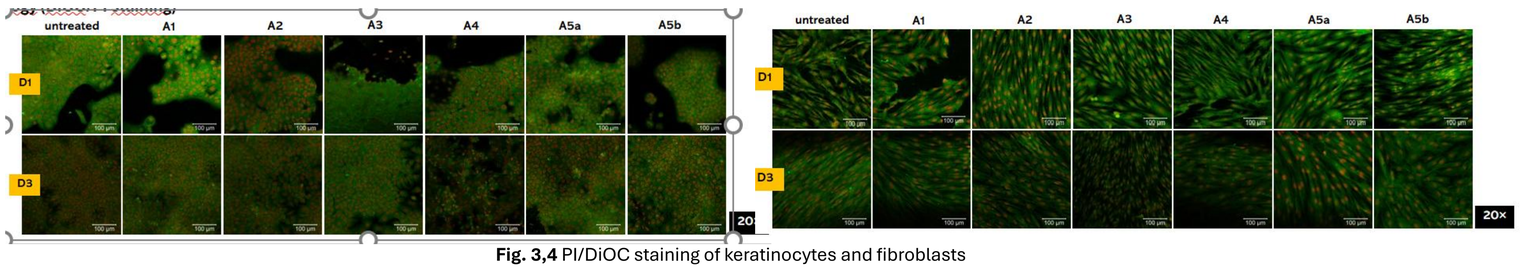
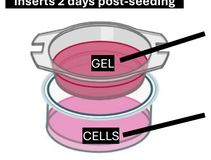


Fig. 3,4 PI/DiOC staining of keratinocytes and fibroblasts

Results: Direct seeding of cells to the scaffolds is not suitable due to lack of cell adhesion and a significant decrease in their metabolic activity.

(II.) Cells seeded on TCP, gels applied in hanging inserts 2 days post-seeding



(2) Gels added to inserts 2 days post-seeding

(1) Cells seeded in wells

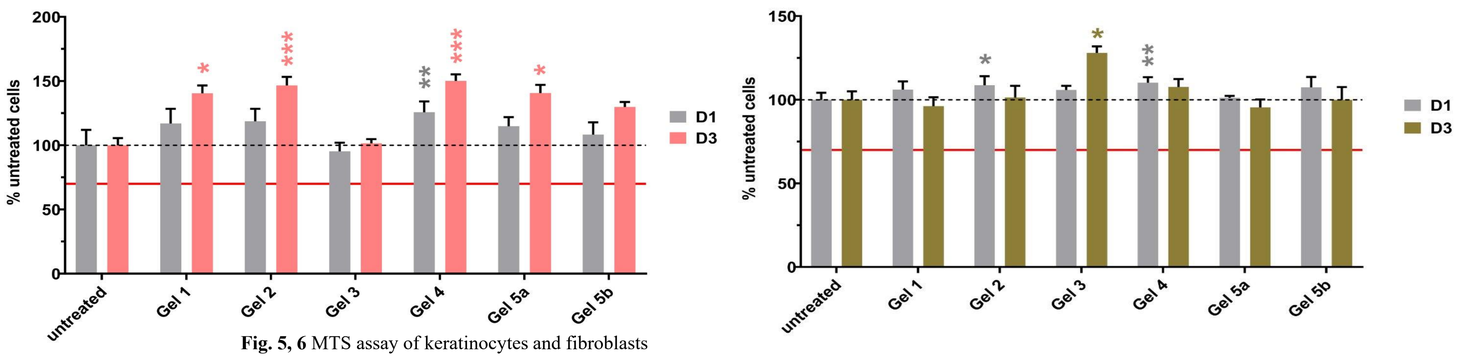


Fig. 5, 6 MTS assay of keratinocytes and fibroblasts

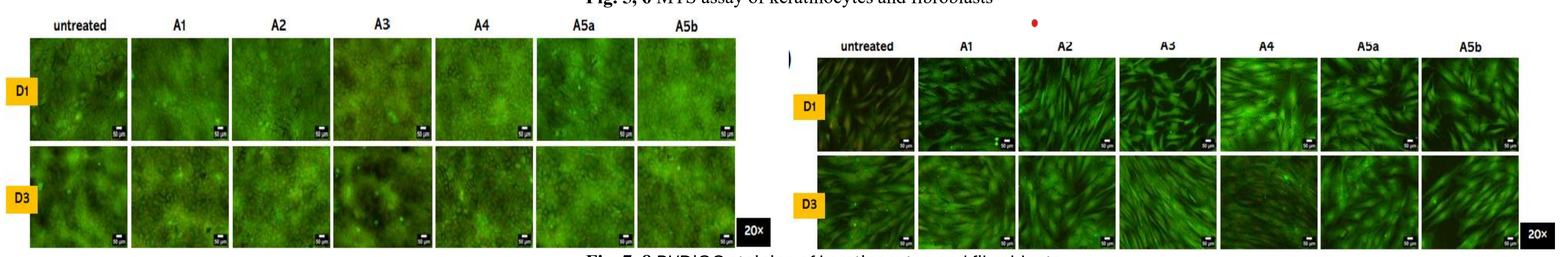


Fig. 7, 8 PI/DiOC staining of keratinocytes and fibroblasts

Results: Indirect application supports cell metabolic activity, thus enabling the testing of various growth factors added to the gels.

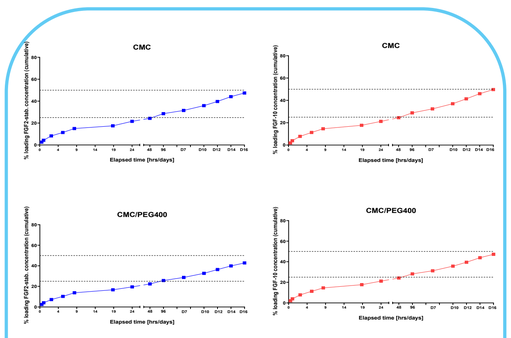


Fig 9,10 Release of FGF-2 stab from gels

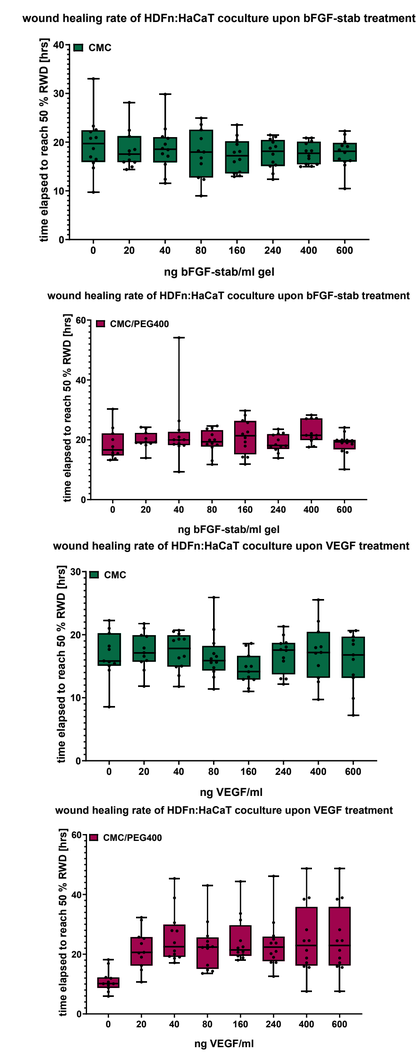
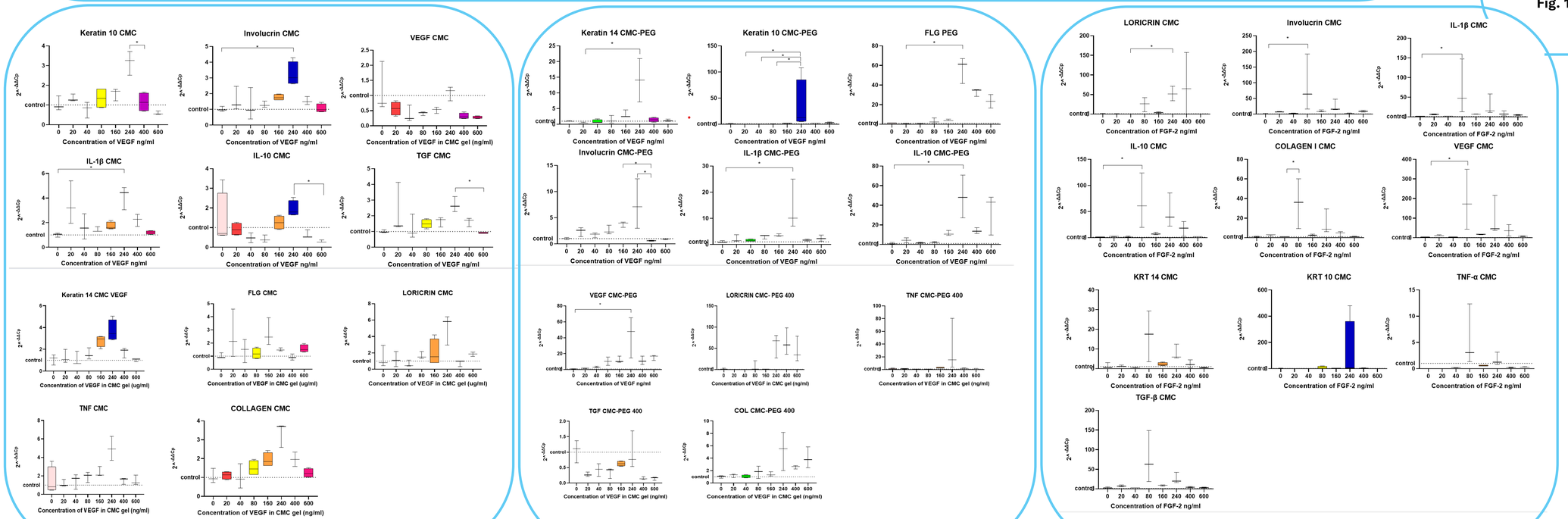


Fig. 11-14 Scratch test



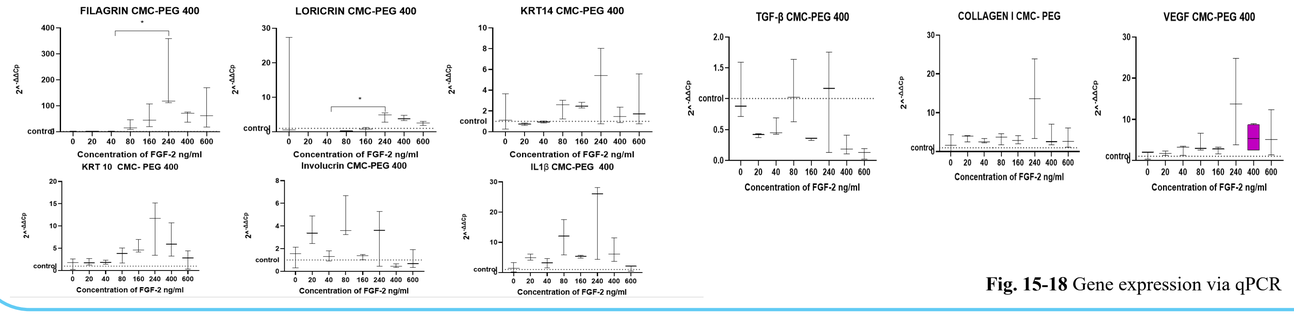


Fig. 15-18 Gene expression via qPCR

Results: While the scratch test showed no statistically significant differences, varying the concentrations of the selected growth factors led to significant differences in the expression of certain genes.

The study concludes that indirect seeding is superior in vitro method for CPS and CPS-PEG hydrogels, effectively circumventing the reagent interference issues seen in direct metabolic assays. This approach successfully validated the hydrogels' therapeutic potential by linking the quantifiable release kinetics of labeled GF to a positive bioactive effect on expression of some genes of interest.

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