

ADULT HUMAN DERMAL STEM CELLS PROTECTION AND ACTIVATION BY A LAMINARIA DIGITATA EXTRACT



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INTRODUCTION

- Cells renewal is possible thanks to stem cells.
- Mesenchymal stem cells (MSCs) are multipotent stem cells with fibroblast morphotype that can differentiate into a variety of cell types: adipocytes, osteoblasts, chondrocytes.
- With age, stress and external aggressions, MSCs become older, lose their vitality and capacity to divide, so that skin renewal becomes slower and wrinkles appear and deepen.

OBJECTIVE

Prove that a *Laminaria digitata* extract (LDE) rejuvenates dermal adult stem cells

- to reinitialize their capacity to divide,
- to rejuvenate fibroblasts.

RESULTS

■ No effect of LDE on HMSC phenotype: Stem cells stay stem cells

HMSC treated 24 hours by LDE 1% or 2.5% expressed the same pattern of expression than untreated control cells: CD13+, CD29+, CD31-, CD34-, CD45-, CD73+, CD90+, CD105+ and CD166+.

Table 1 : Results of the flow cytometry analysis (% of cells expressing this phenotype in comparison with the total number of cells)

Antibody		% stained cells		
		Untreated cells (Control)	LDE 1%	LDE 2.5%
X	CD13	90	86	84
X	CD29	92	87	85
X	CD31	0.3	0.1	0.2
X	CD34	0.6	0.5	0.04
X	CD45	0.8	1.5	1.2
X	CD73	94	90	89
X	CD90	90	85	82
X	CD105	90	86	84
X	CD166	84	83	74

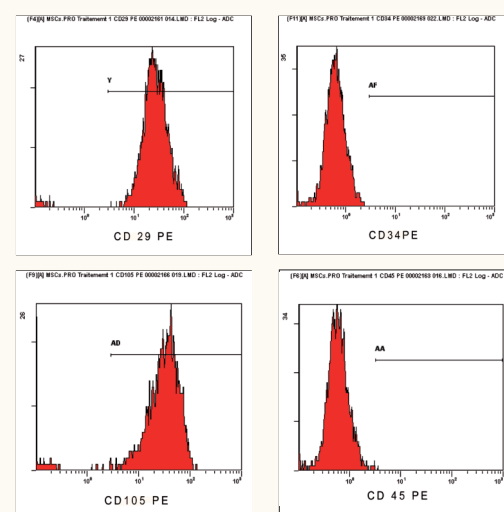


Figure 1: Histograms of flow cytometry for CD29, CD34, CD105 and CD45 markers of 1% LDE treated HMSC.

■ Revitalising effect of LDE on Human Mesenchymal Stem Cells (HMSC)

In bad conditions (Fig 2A), the addition of 1% LDE stimulated the growth of HMSC after 24 and 48 hours of culture. In optimal conditions (Fig 2B), the LDE, tested at 1%, stimulated the growth of HMSC after 48 hours of culture

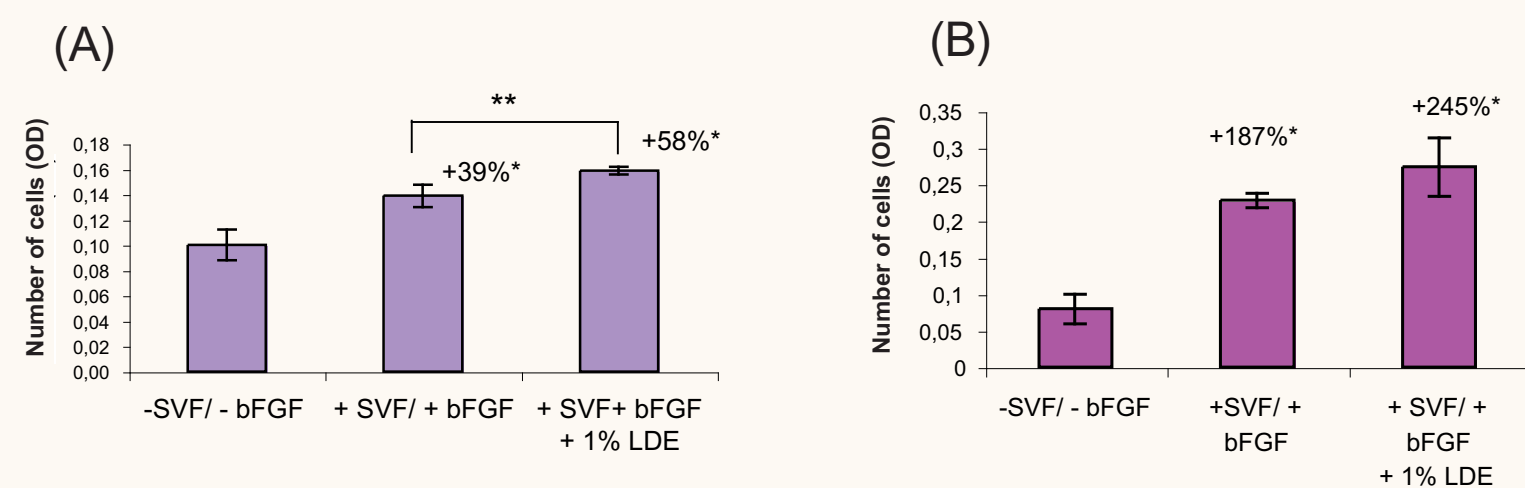


Figure 2 : Effect of the LDE on the viability of HMSC after 24 hours (A) and 48 hours (B) in bad conditions (A) and good conditions (B). *p<0.05 Comparison to control without serum and b-FGF; **p<0.05 Comparison to control with low serum and low b-FGF.

■ Protective effect of 1% LDE on HMSC exposed to UVB

In non optimal conditions (Fig 3A), the UVB radiation decreased the viability of HMSC after 72 h of culture but LDE, tested at 1% and 5%, stimulated the growth of HMSC exposed to UVB as well as for unirradiated cells. The stimulating effect seems to be higher for stressed cells.

In optimal conditions (Fig 3B), the LDE, tested at 5%, stimulated the growth of HMSC exposed to UVB, even more than the unirradiated cells. The extract adapted its protective effect in function of the intensity of stress.

MATERIAL AND METHODS

• **Product:** *Laminaria digitata* extract (LDE) is obtained by leaching the freeze-dried alga, followed by sterilizing microfiltration and reverse osmosis to concentrate the active molecules. It is produced by Codif International.

• **Cells:** Human Mesenchymal Stem Cells (HMSC) isolated from the human bone marrow of a 42 years old male, by centrifugation. They are characterized by a stem cells specific phenotype (CD13+, CD29+, CD73+, CD90+, CD105+, CD166+, CD31-, CD34-, CD45- staining with flow cytometry) and by collagen-1 production.

• **Method:** 24H and 48H culture of HMSC in 2 conditions:
 - Good conditions "Young skin": 10% FCS + 1 ng/ml bFGF
 - Bad conditions "Old skin": 2% FCS + 0.2 ng/ml bFGF
 (FCS = Foetal Calf serum; bFGF = basic Fibroblast Growth Factor)

Cell ageing process is simulated by UVB radiation. HMSC cultures were then incubated for 24 h before and after UVB radiation (10 to 30 mJ/cm²). Cellular vitality was measured by MTT method.

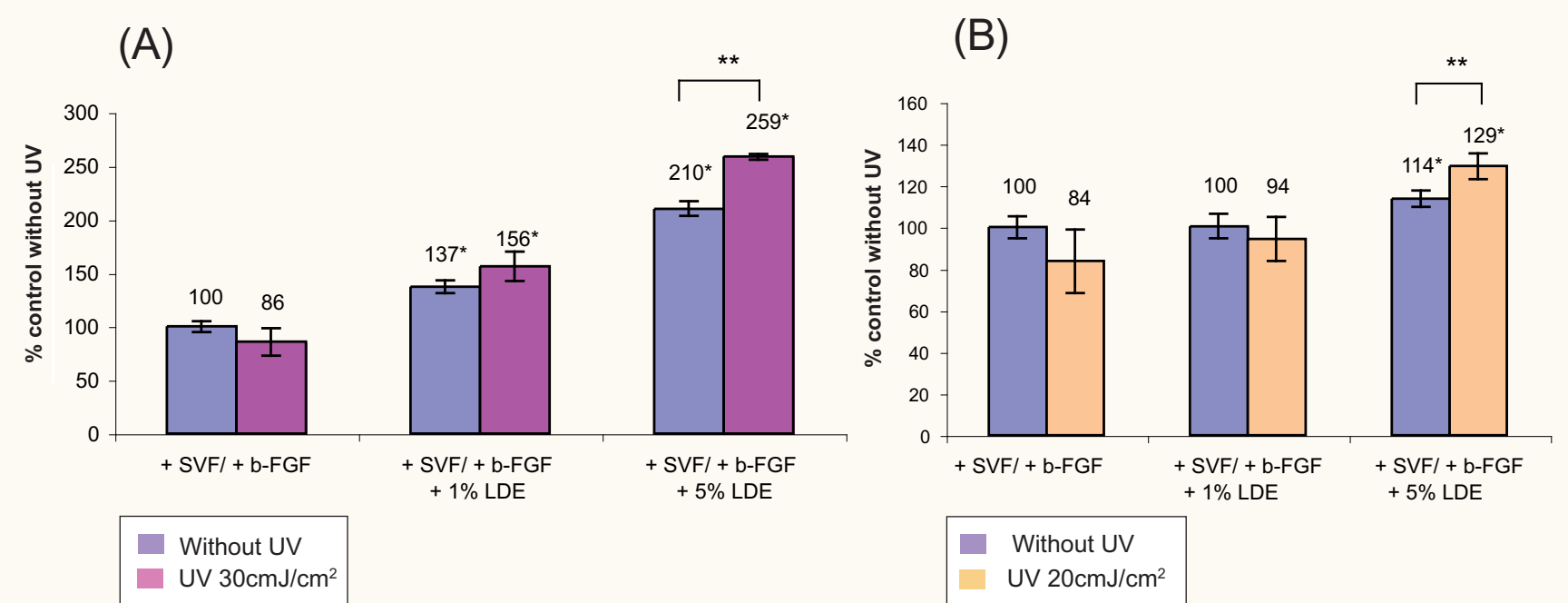


Figure 3 : Effect of the LDE on the viability of HMSC cultured in bad (A) or good (B) conditions and unirradiated or exposed to UV (20-30 mJ/cm²). *p<0.05 (Comparison to the control 2% SVF/ 0.2ng/ml b-FGF); **p<0.05 (Comparison between the same condition +/- UV)

■ Reinitialization of the division capacities of HMSC exposed to UVB by 1% LDE

The LDE decreased the UV-induced cellular changes and stimulated HMSC growth.

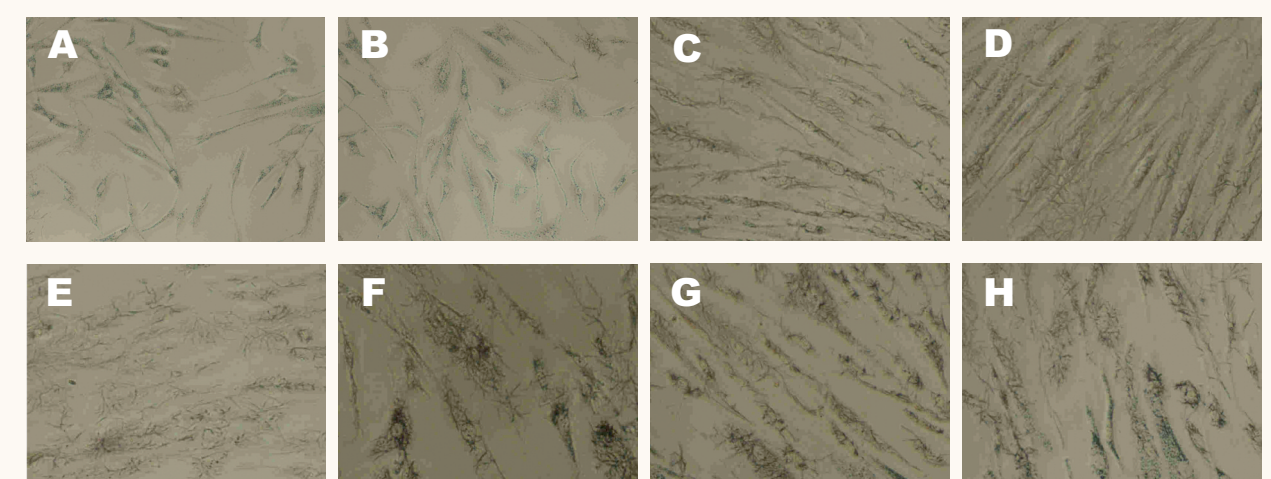


Figure 4 : Microscopic observation (during MTT test) of HMSC cultured in bad (A, B, C, D) and good (E, F, G, H) conditions without UV rays (A, E) or exposed to UVB (10mJ/cm²) without the LDE (B, F) and with the LDE at 1% (C, G) and 5% (D, H)

■ Rejuvenating effect on the crow's feet wrinkles

- 15 volunteers aged from 40 to 55 years; 2 applications per day for 28 days on the face.
- After 28 days of twice-daily use of 1% LDE, 80% of volunteers observed a significant reduction in the main wrinkle of -6.2% on average and up to -15.6%. This reduction in the depth of wrinkles was accompanied by a smoothing effect, with a reduction in mean roughness of up to -29.6%.



Figure 5 : Photographs of crow's foot wrinkles of a volunteer before (D0) and after 28 days of treatment (D28) with a cream containing 1% LDE

CONCLUSION

- By its revitalizing action on adult stem cells, 1% LDE restores their capacity for division without affecting their phenotype. Ageing skins recover similar levels of adult stem cells as young skins.
- From 1%, LDE has a significant anti-ageing effect on stem cells from young and old skin. This anti-ageing effect is more visible on older skins as cell ageing weakens the internal defense systems.

THE LAMINARIA DIGITATA EXTRACT (LDE) REJUVENATES AND PROTECTS "ADULT STEM CELLS" IN THE DERMIS, BY PROLONGING TISSUE YOUTH AND RESPECTING SKIN ETHICS.