

# ADULT HUMAN EPIDERMAL STEM CELLS RECRUITMENT BY AN OLIGOALGINATE SOLUTION



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## INTRODUCTION

- After injury to the skin, healing processes are activated under the control of a molecule called Endothelial Growth Factor (EGF), which stimulates the division of adult stem cells, still called epidermal stem cells (ESC), at the healing site in order to rebuild the skin. ESC are all the more effective for rebuilding of the skin because of their high division potential and their colony forming efficiency (CFE).
- With age and the accumulation of skin injuries, ESC are less numerous, they progressively lose their capacities to renewal, cannot repair epidermal damages or replace dead cells...

## OBJECTIVE

- Assess the effect of an Oligoalginate solution (OS) on Human Epidermal Stem Cells (HESC) to protect epidermal stem cells and maintain their renewal capacities without overstimulation.

## RESULTS

### ■ Characterization of HESC

Cells at the periphery of obtained colony are characterized by a high expression of  $\beta$ 1-integrin and no expression of MHC class I  $\beta$ 2-microglobulin.

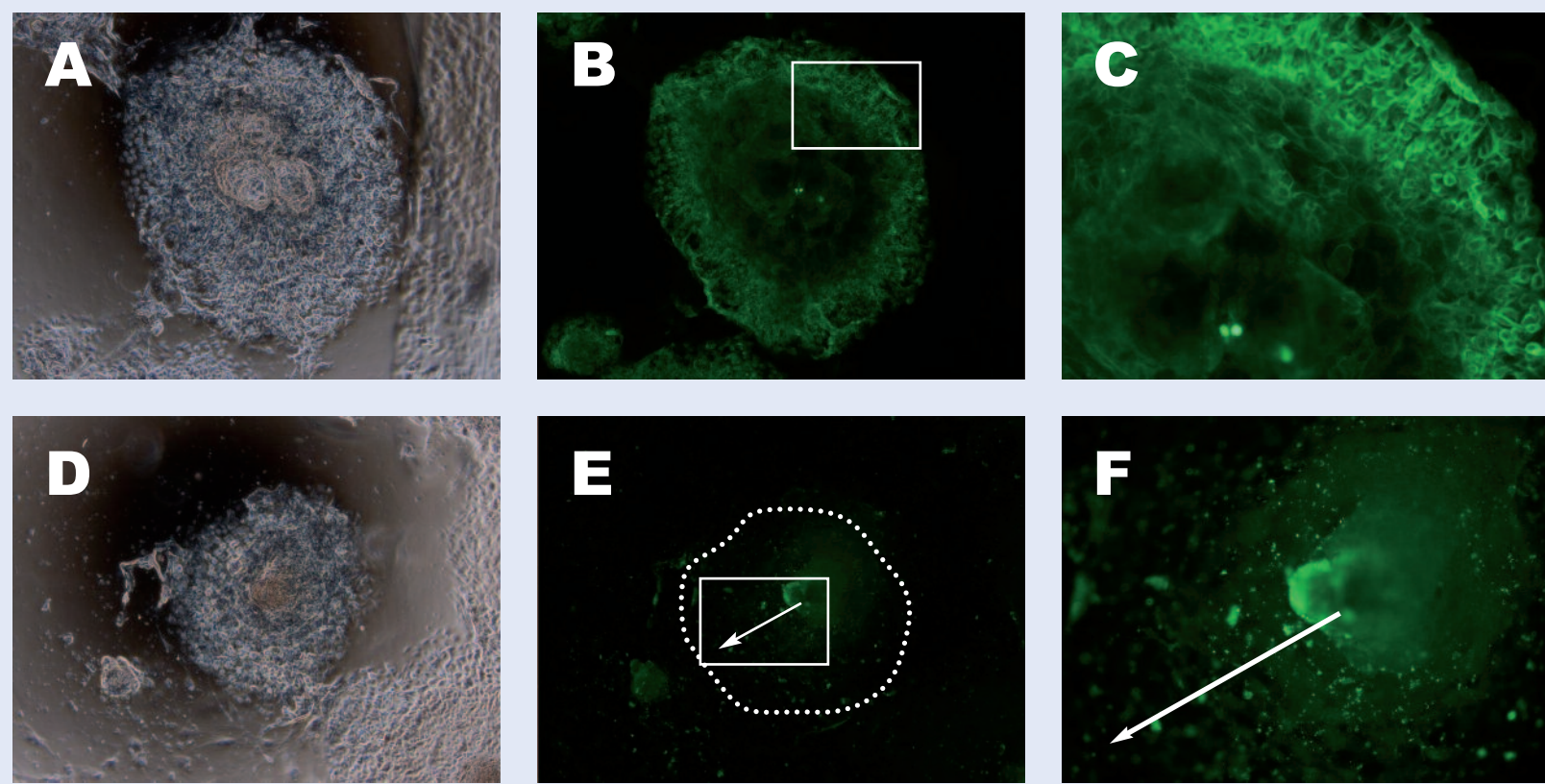


Figure 1: Photography of a colony of Human keratinocytes obtained after culture of one Epidermal Stem Cell, for 8 days, observed in phase contrast (A, D) and location of  $\beta$ 1-integrin (B, C) and  $\beta$ 2-microglobulin (E, F) in this colony observed at different magnification (objective x4 for figures A, B, D, E and objective x10 for figure C and F). C is a detail of B; F is a detail of E. The dotted white line delineates the contour of the colony on figure E. The white arrow in figures E and F represents the direction of keratinocytes migration.

### ■ No effect of OS on HESC phenotype

The treatment of HESC by OS, for 8 days, does not modify the phenotype of HESC.

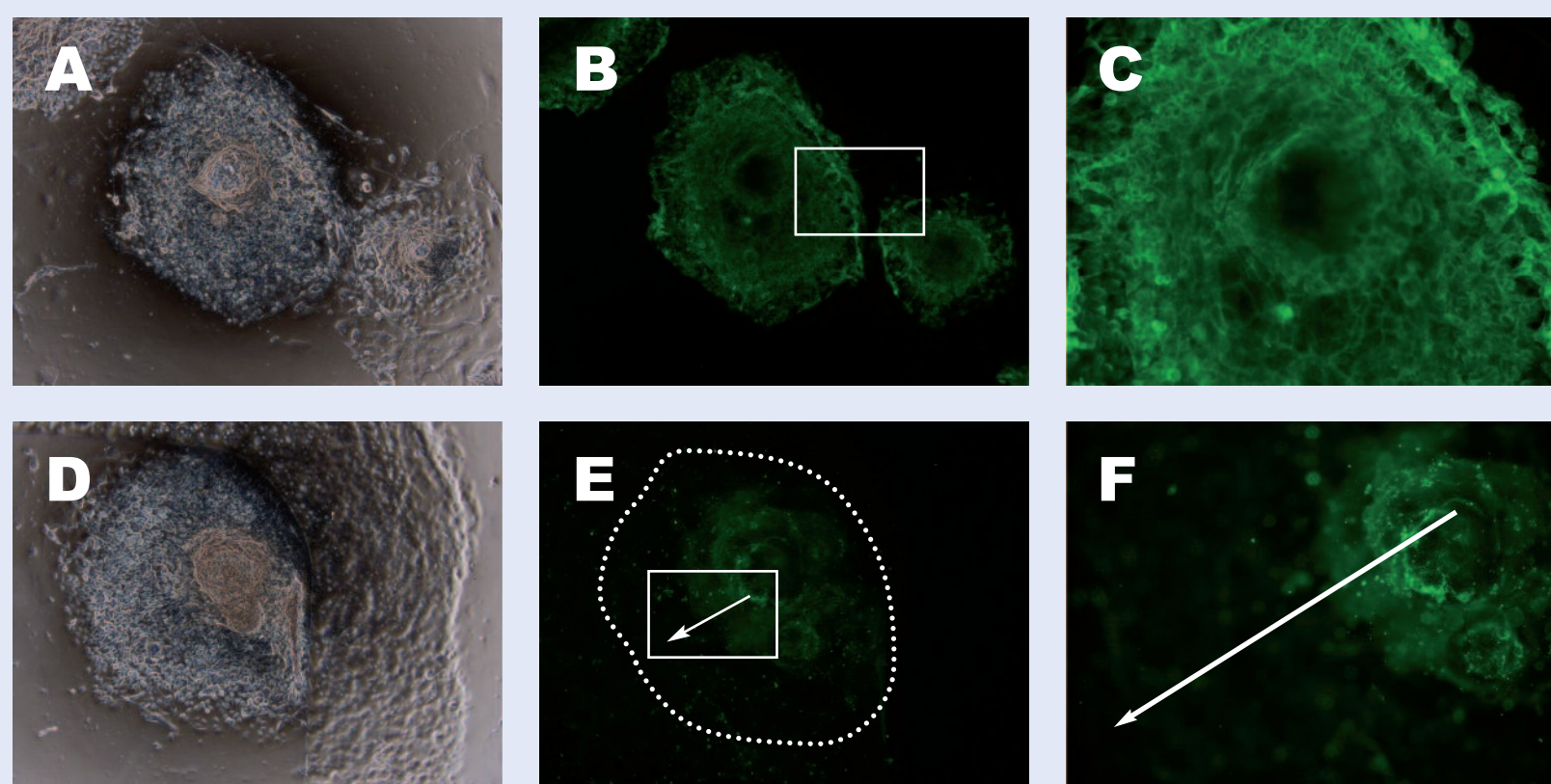


Figure 2 : Photography of a colony of Human keratinocytes treated by OS at 1% for 8 days, observed in phase contrast (A, D) and location of  $\beta$ 1-integrin (B, C) and  $\beta$ 2-microglobulin (E, F) in this colony observed on the same conditions than figure 1.

## CONCLUSION

- OS may optimize binding of EGF to HESC. This therefore leads to a capitalization of its properties: there are an increasing number of much larger colonies leading to a much more rapid and effective regeneration of the epidermis.
- The benefits of this action could be used for wound healing stimulation but also for the preservation of epidermal stem cells in aged skin

**THE OLIGOALGINATE SOLUTION (OS) IMPROVES THE ACTIVITY OF ADULT HUMAN EPIDERMAL STEM CELLS WITHOUT AFFECTING THEIR PHENOTYPE.**

## MATERIAL AND METHODS

- **Product:**  
Oligoalginate solution (OS) containing 5% of an oligosaccharide produced by enzymatic digestion of polysaccharides purified from the brown alga *Laminaria digitata* and hydrolyzed using a specific enzyme (alginate lyase). It is produced by Codif International.
- **Cells:**  
HESC are human keratinocytes with the highest division capacity isolated from skin plastic surgery, cultured on 3T3 feeder cells. A unique HESC produces a big colony of cells in only 8 days.
- **Methods:**  
Culture of HESC for 8, 10 or 12 days on a fibroblast-based matrix in keratinocytes medium with or without 0.1%, 0.5 % or 1% of OS. Number of cells evaluated by optical density; Rhodamine coloration for image analysis of the area of colonies and percentage of big colonies; immunostaining (Anti- $\beta$ 1-integrin and anti- $\beta$ 2-microglobulin; Tebu Bio).

### ■ Effect of OS on the division capacity of HESC

OS optimizes the division of HESC from 0.1%. At 1%, OS increased by +57% the renewal capacity of HESC.

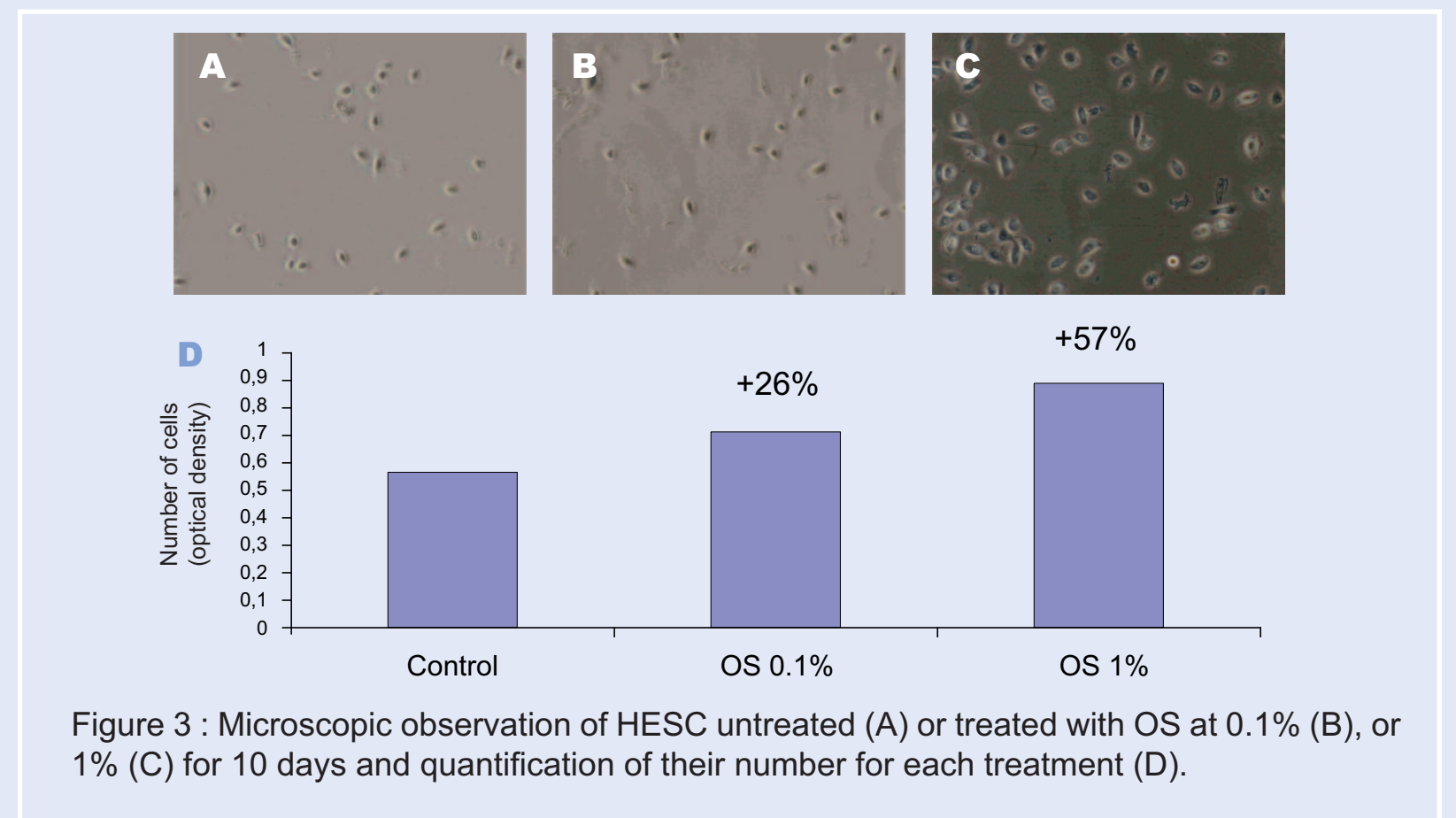


Figure 3 : Microscopic observation of HESC untreated (A) or treated with OS at 0.1% (B), or 1% (C) for 10 days and quantification of their number for each treatment (D).

### ■ Cumulative effect of OS on the division and migration of HESC

- As EGF does, OS promoted HESC division (increase in cell number) and their migration (increase the diameter of colonies).The effect of OS could be explained by the EGF-like effect.
  - In the cellular model used, OS acted as a co-factor stimulating the division of HESC and / or a factor promoting the migration of HESC
- OS also stimulated the percentage of big colonies of HESC.

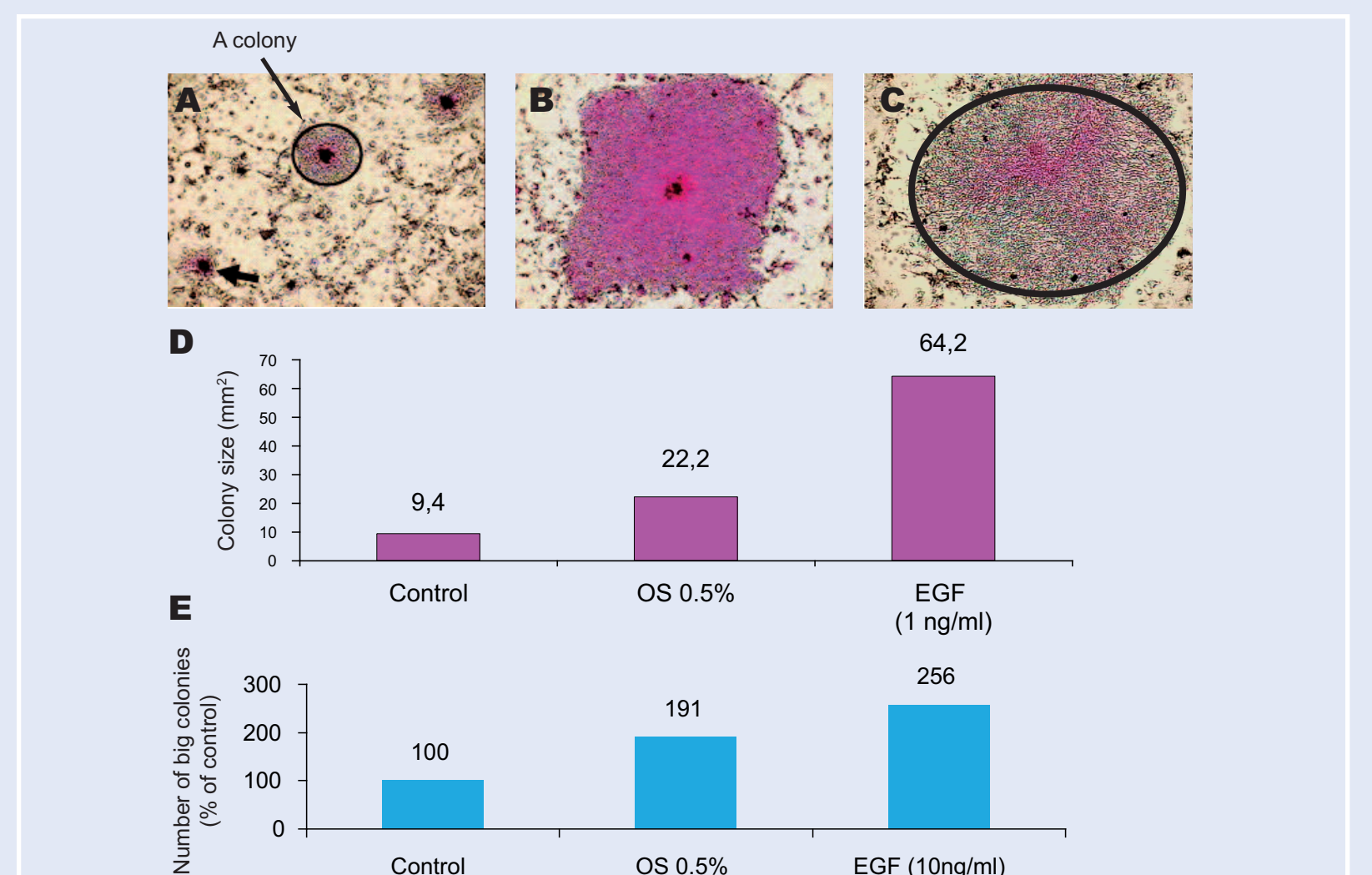


Figure 4 : Microscopic observation of the colonies of HESC non treated (A) and treated with OS at 0.5% (B) or EGF at 1ng/ml (C) for 12 days and quantification of the total surface of the colonies (D) and of the percentage of big colonies (E).