



Dermal stem cells – our next target

PhytoCellTec™ Argan

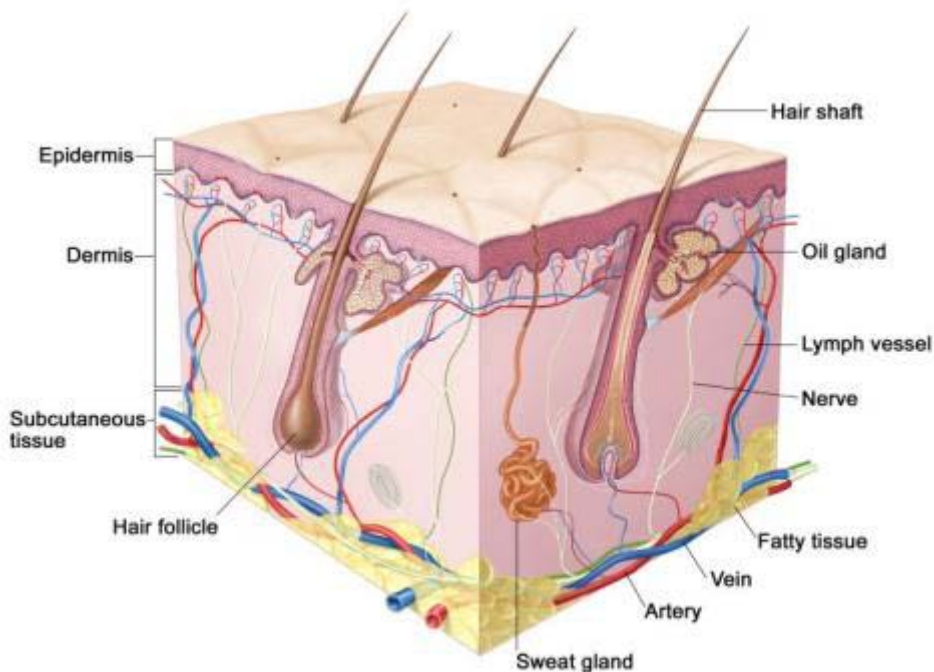
Vitalizing dermal stem cells for
fundamental anti-aging effectiveness

DermaI Stem Cells – Our Next Target

“The Second Generation of Stem Cell Cosmetics”

- Adult stem cells guarantee a continuous regeneration of skin tissue.
- But as we age, the skin loses its elasticity and firmness and forms wrinkles → the regenerative potential of the stem cells does not last forever.
- In 2008, Mibelle Biochemistry was the first company that developed a cosmetic active based on plant stem cells to protect **epidermal skin stem** cells (PhytoCellTec™ Malus Domestica).
- In 2011, Mibelle Biochemistry is the first company to launch an ingredient designed to delay the depletion of the **dermal stem cells**; again a path breaking innovation.

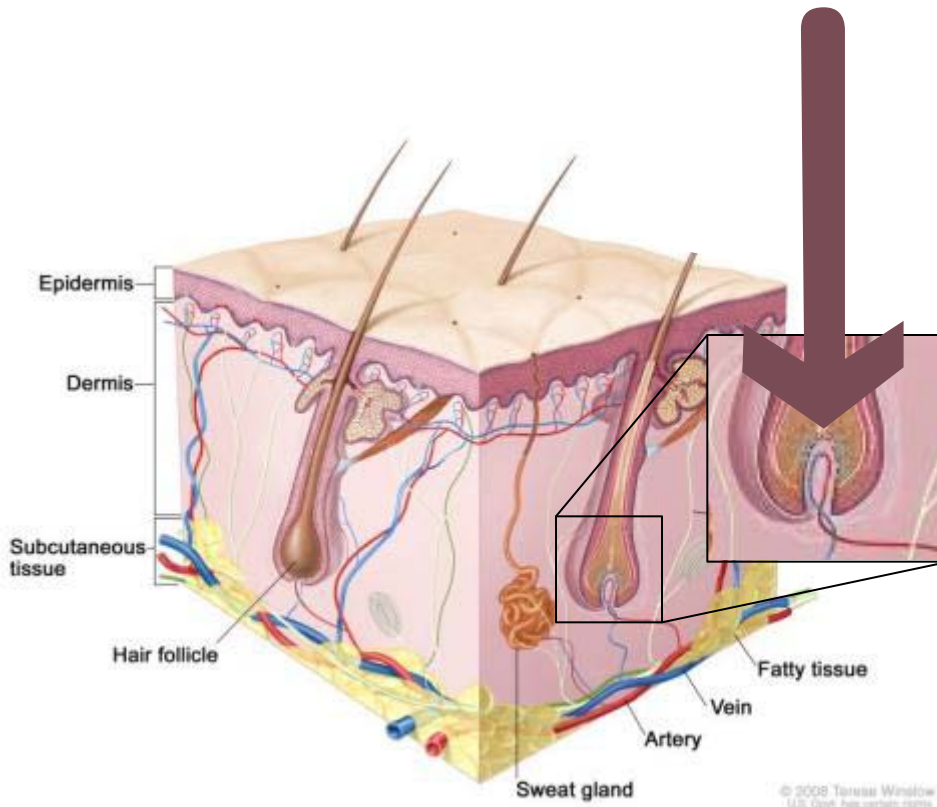
Where are the Dermal Stem Cells?



- **Epidermis:** epidermal stem cells in the basal layer continuously regenerate the epidermal layer
- **Dermis:** dermal stem cells confer firmness and elasticity to the skin and are involved in wound healing processes

But: the stem cell reservoir in the dermis remained a secret for a long time

New Stem Cell Niche Discovered!



End of 2009 a research group from the University of Toronto (Freda Miller et al., Hospital for Sick Children, Toronto, CA) discovered that the **dermal papilla is a niche for dermal progenitor/stem cells.**

Dermal papilla cells were found to:

- express the stem cell marker gene Sox2
- grow in 3D spheres
- induce the formation of hair follicles
- differentiate into fibroblasts

Dermal Papilla Stem Cells and their Role in Skin



sphere formation



wound healing

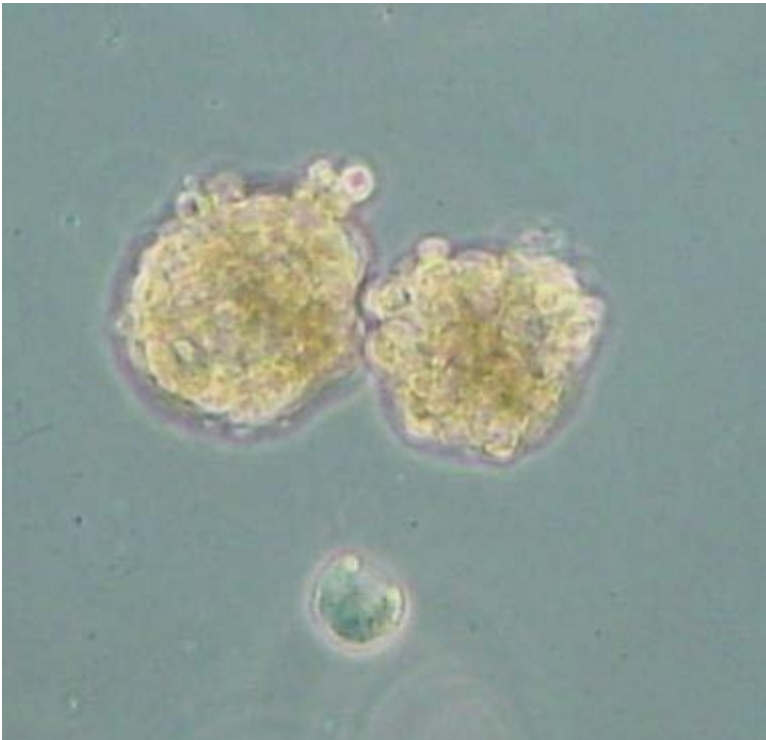


differentiation into inter-follicular fibroblasts



self renewal

Characterization of “Dermal Stem Cells”



Sphere-forming properties

Human dermal papilla cells grow in three dimensional spheres.

Sox2 expression

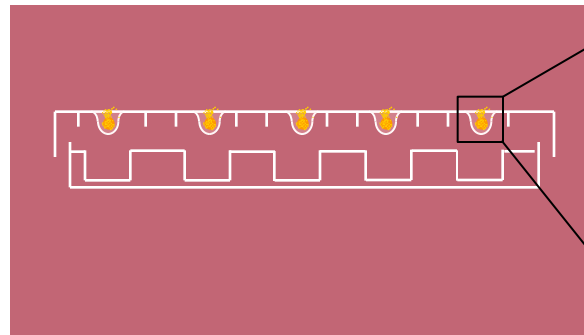
The expression of the specific Sox2 marker gene is required for maintaining the pluripotency in stem cells.

1. Establishment of a Cell Line with Human Dermal Papilla Cells

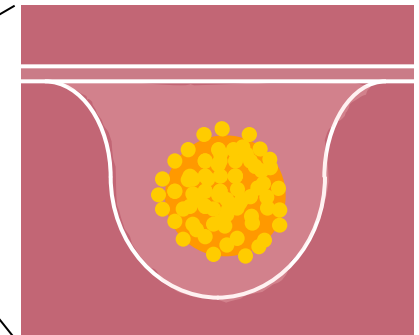


Isolation of dermal papilla cells from a hair bulb

Passaging

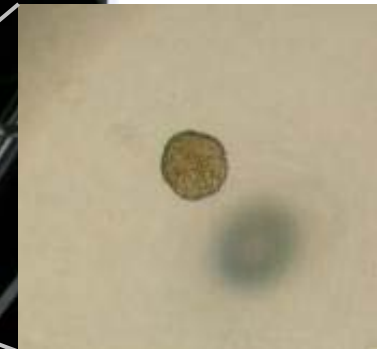
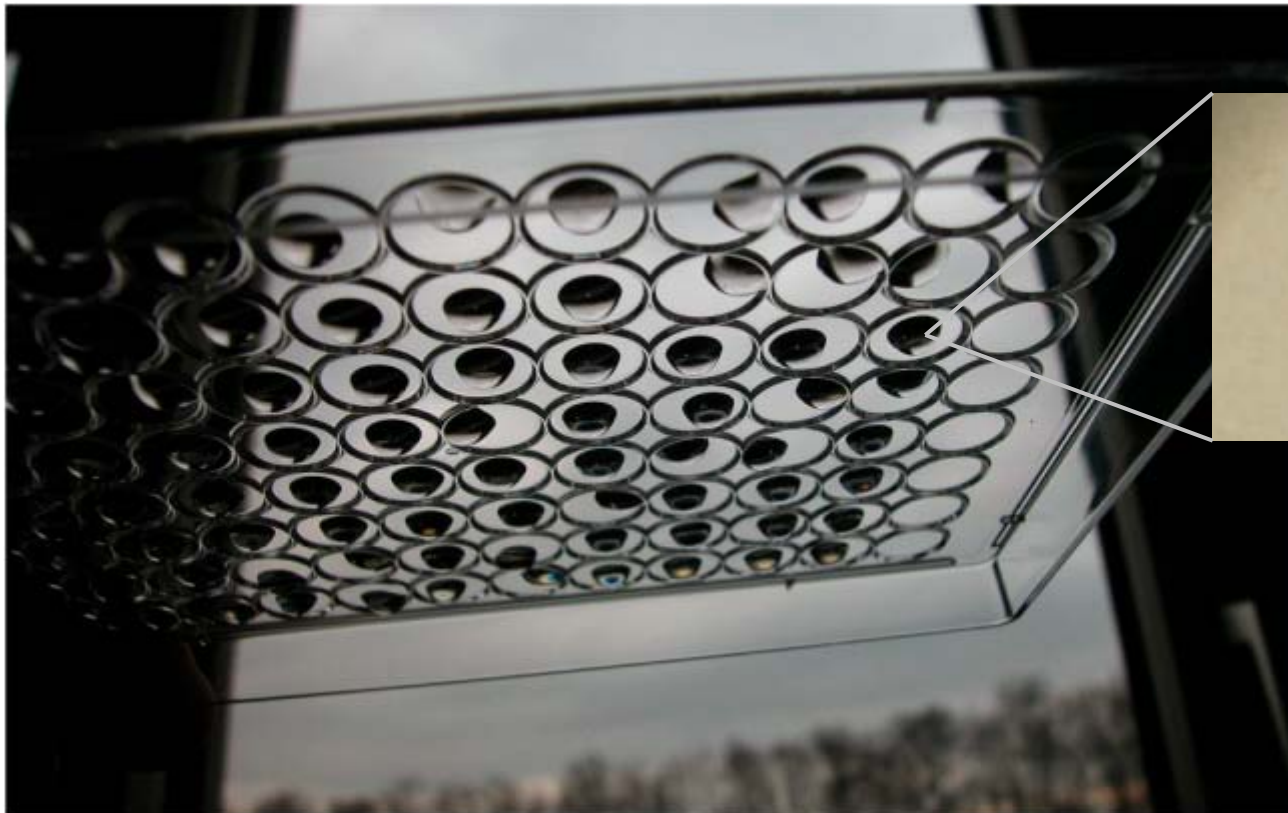
Hanging drop culture in 96 well plate
 → Formation of primary spheres



Sphere of dermal papilla cells
 (ca. 3000 cells/ 10 µl drop)

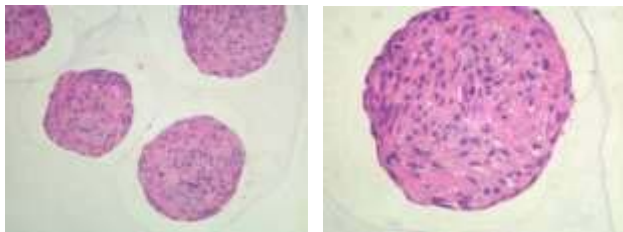
Hanging drops after Higgins et al., 2003

Hanging Drop Cell Cultures



Hanging drop sphere
after Higgins et al.
(phase contrast after
24h)

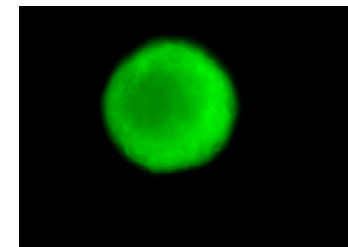
Staining of Spheres



Histological analysis of primary spheres



Nuclear staining

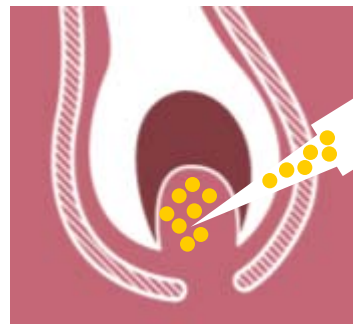


Sox2 staining

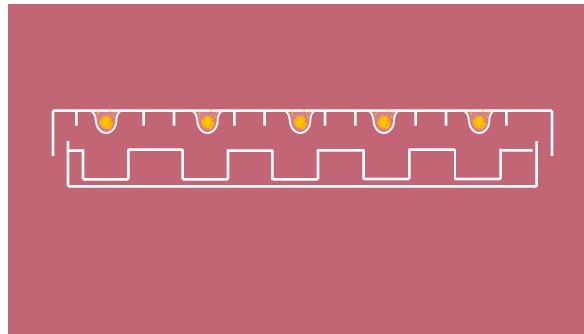


Real dermal stem cells!

2. Preparation of Secondary Spheres

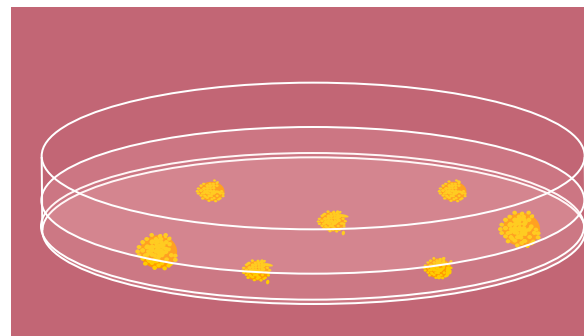


Passaging



Hanging drop culture in 96 well plate
→ Formation of primary spheres

Spheres are trypsinised and solubilised



→ Spontaneous formation of secondary spheres (10'000 cells/0.5 ml)

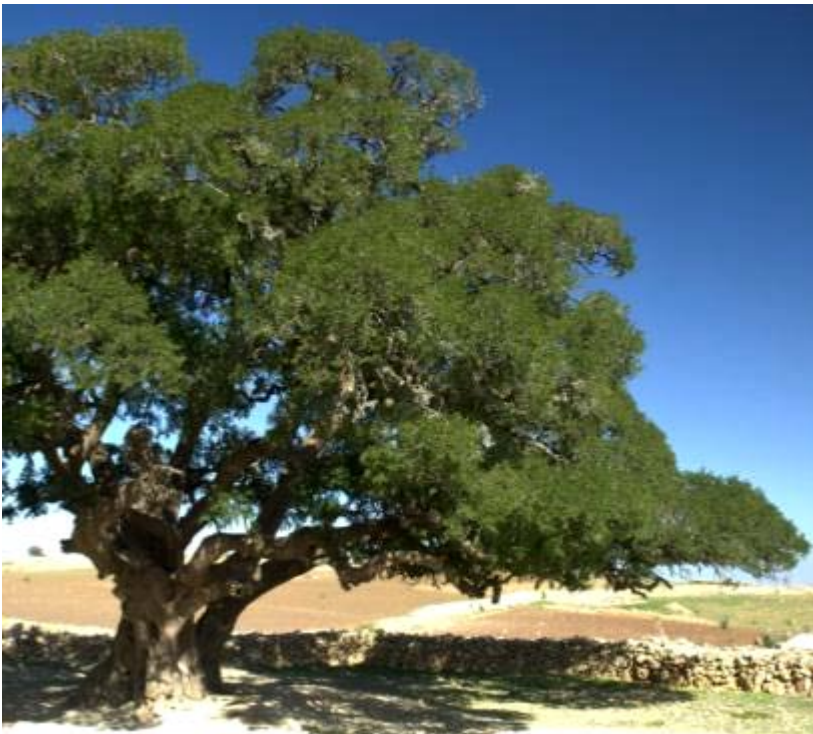


Test system for novel cosmetic actives!

Innovation: Next Generation of Stem Cell Cosmetics

1. The identification of dermal stem cells opens the door to the next generation of stem cell cosmetics: Protection and vitalization of human dermal stem cells for a deep-seated rejuvenation of the skin, resulting in restoration of firmness and wrinkle reduction.
 2. We have established a specific test system: cell cultures of human dermal stem cells that.
 3. Screening for a cosmetic active that has the potential to enhance the vitality of dermal stem cells.
- Plant stem cells obtained with our PhytoCellTec™ technology from a very resistant and rare plant – the argan tree.

PhytoCellTec™ Argan a New Active from – the Ancient Berber Tree



- Argan (*Argania spinosa*) grows to 8-10 metres high, and lives to 150–200 years.
- Belongs to the oldest tree species of the world (sapotaceae) – its age is 80 Mio years (when the dinosaurs still roamed the earth)!
- Argan trees grow only in the southwestern part of Morocco in a small area covering only 8'000 km².

Argan – Adapted to Extreme Drought



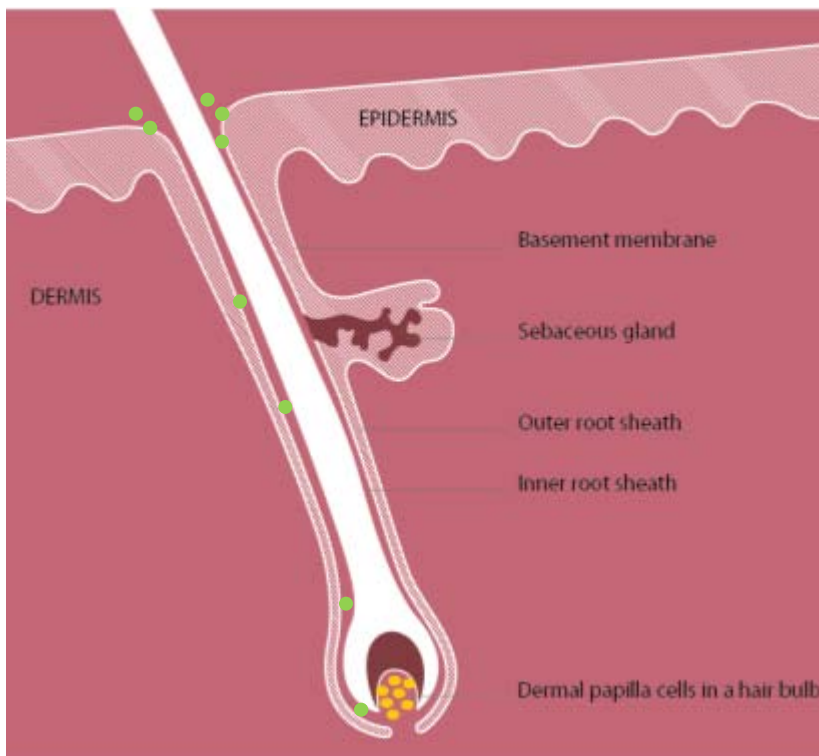
- Argan trees are able to resist extreme droughts and extremely high temperatures $> 50^{\circ}\text{C}$.
- Roots can grow up to 30 metres long
- During prolonged drought, the trees dry out and remain in a state of dormancy for several years. When the humidity level rises, it suddenly comes back to life.
- Argan trees have become a rare and endangered species.

Our 4 Steps of Plant Cell Cultures to Produce PhytoCellTec™ Argan

1. Wounding argan **sprouts** to induce the formation of a callus tissue
 2. Growing callus cells in liquid cultures
 3. Disrupture of the cells, extracting the oil- and water-soluble ingredients with liposomal bilayers
 4. Spraying on a powder based on Isomalt
- Sustainable technology for rare and endangered plant species like the argan
(only a small part of the plant is needed)



Penetration into the Dermis



In PhytoCellTec™ Argan the oil- and water-soluble ingredients are vectorized in liposomes

Hair follicles represent a highly relevant and efficient penetration pathway and reservoir for topically applied substances

→ dermal papilla, where the dermal stem cell pool is localized, can be reached over follicular penetration

Evaluation of the Activity of PhytoCellTec™ Argan on Dermal Stem Cells

1. Effect of **PhytoCellTec™ Argan** on Sox2 expression

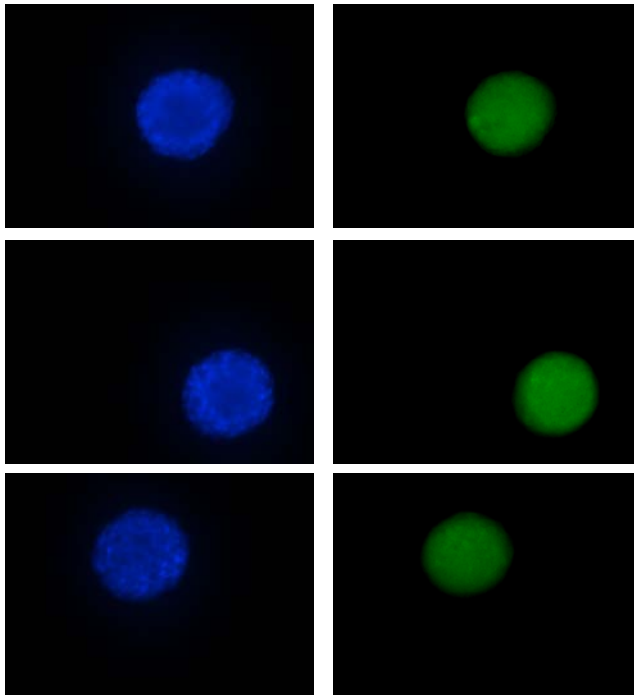
(Sox2 is a marker for the “stemness” of a stem cell, the better the vitality of a stem cell, the stronger Sox2 is expressed)

2. Effect of **PhytoCellTec™ Argan** on sphere number

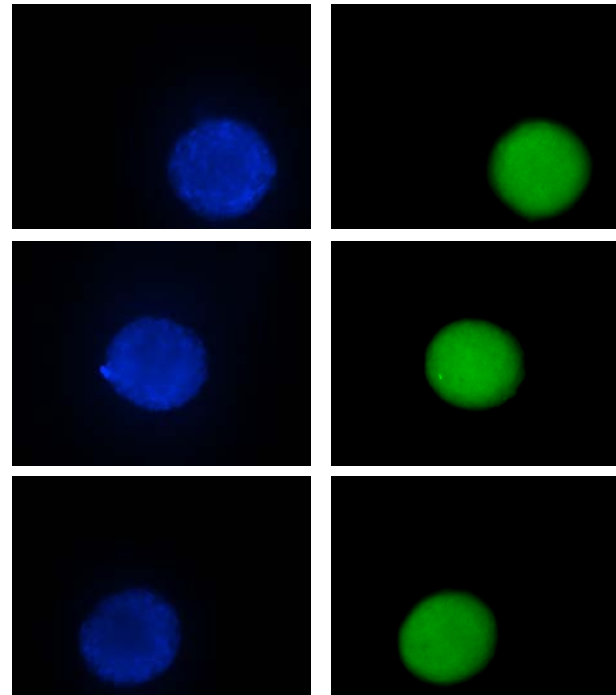
→ Sphere Forming Efficiency

1. Effect of PhytoCellTec™ Argan on Sox2 Expression

Control



+ PhytoCellTec™ Argan

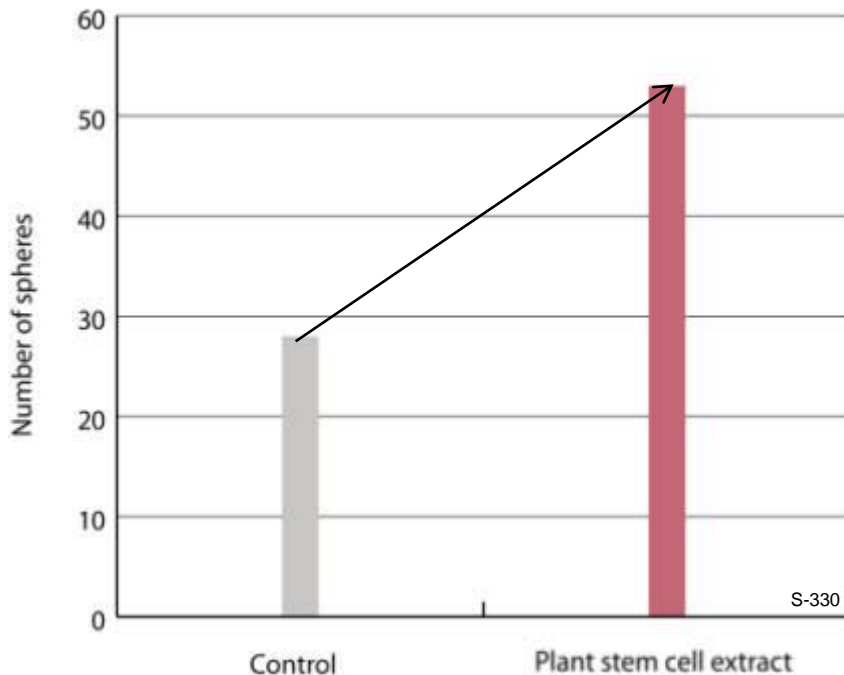


DP passage 9,
16 days



Sox2 expression is enhanced
“stemness” of the dermal stem cells is improved

2. Effect of PhytoCellTec™ Argan on Sphere Forming Efficiency (SFE)



Sphere Formation Efficiency SFE

- Secondary spheres of human dermal stem cells
- Microscopic counting of sphere number after 3 week

➔ Increase of **sphere number with PhytoCellTec™ Argan**

Claim Ideas with PhytoCellTec™ Argan



- Dermal rejuvenation of the skin
- Vitalizes dermal stem cells, which are responsible for collagen and elastin production
- Accelerates skin's natural repair process
- Combats chronological aging
- Fights wrinkles and loss of firmness