

## Spheroid cell culture of human skin-derived precursors

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### Organisation

**Name of the organisation** Vrije Universiteit Brussel (VUB)

**Department** Pharmaceutical and Pharmacological Sciences

**Specific Research Group or Service** In Vitro Toxicology and Dermato-Cosmetology

**Country** Belgium

**Geographical Area** Brussels Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research, Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	human skin stem cells

## DESCRIPTION

### Method keywords

tree-dimensional spheroid culture  
hepatic differentiation

### Scientific area keywords

spheroids  
skin stem cells  
differentiation

### Method description

For the generation of the human skin-derived precursors (hSKP) spheroids, ultra-low attachment 96-well plates, cell-repellent (Greiner) are used. hSKP cultured on tissue culture plates are detached with trypple (5 minutes) and collected in a tube. After centrifugation, hSKP are counted at the Burker chamber and seeded at 2,000 cells per well. The 96-well plates are placed on an orbital shaker, shaking at 40 rpm overnight in a humidified incubator. The outer wells are filled with PBS to reduce evaporation of cell culture medium. The day after, hSKP aggregates of approximately 200-250 µm diameter size are formed. Culture medium is refreshed twice in a day every 3 days to allow

complete medium replenishment.

### **Lab equipment**

Cell culture laboratory;  
Laminar air flow;  
Ultra-low attachment 96-well plates;  
Shaker.

### **Method status**

Still in development

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

Emulation of 3D cell organization, simple method, applicable to many cell types.  
Spheroid formation has been shown to enhance anti-inflammatory effects, augment tissue regenerative and reparative effects, facilitate differentiation potentials of multiple lineages, improve stemness properties of adult stem cells.

### **Challenges**

Spheroid handling and transfer step (e.g. spheroids may be easily lost by aspiration with pipette during refreshment of cell culture medium).  
Presence of a necrotic core inside the sphere that could lead to poor cell viability in long-term culture.

### **Future & Other applications**

Since spheroid generated from mesenchymal stem cells exert strong anti-inflammatory and immuno-modulatory effects, they could be employed in regenerative medicine and autoimmune diseases.

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **References**

Cesarz Z, Tamama K (2016) Spheroid Culture of Mesenchymal Stem Cells. Stem Cells Int 2016:. doi: 10.1155/2016/9176357  
Tsai A-C, Liu Y, Yuan X, Ma T (2015) Compaction, Fusion, and Functional Activation of Three-Dimensional Human Mesenchymal Stem Cell Aggregate. Tissue Eng Part A 21:1705–1719. doi: 10.1089/ten.tea.2014.0314

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