

# 3D Organoids from primary melanoma cell lines and from iPSc-derived neural crest stem cells

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## Organisation

**Name of the organisation** VIB - KU Leuven

**Department** Department of Brain and Disease Research

**Specific Research Group or Service** Lab of Computational Biology

**Country** Belgium

**Geographical Area** Flemish Region

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	Human iPSC; Primary melanoma cells

## DESCRIPTION

### Method keywords

3D culture

organoid

single-cell

ECM  
hydrogel  
PEG

### **Scientific area keywords**

melanoma  
enhancer  
single-cell RNA seq  
single-cell ATAC seq

### **Method description**

We propose to generate three-dimensional tumoroids from the primary melanoma cell lines, as well as 3D organoids from the iPSc-derived neural crest stem cells. We will use the AggreWell system (STEMCELL Technologies) to generate uniform, size-controlled three-dimensional spheroids. After 5 days in the AggreWell plate, the spheroids are moved to a PEG-based artificial ECM hydrogel (Gjorevski et al.; Nature Protocols 2017). The organoids can be cultured for weeks in these PEG-droplets. At different time points during organoid culture, organoids will be used for immunostaining and/or for single-cell sequencing. We will dissociate the PEG gel to obtain single cells by use of the cell-dissociation enzyme TrypLE.

### **Lab equipment**

Biosafety cabinet ;  
Cell incubator CO2-connected ;  
Centrifuge for plates.

### **Method status**

Published in peer reviewed journal

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

### **Advantages**

3D organoids mimic tissue architecture heterogenous cell culture to study cellular differentiation enhancer testing.

## Challenges

Not every cell type/tissue can be studied.

## Modifications

Different cell types are studied to form organoids.

## Future & Other applications

Drug application: concentration and activity can be tested.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

Gjorevski N & Lutolf MP. Synthesis and characterization of well-defined hydrogel matrices and their application to intestinal stem cell and organoid culture. Nature Protocols 12 (11); 2263-2274 (2017)

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