# In vitro differentiation of skin-derived precursors into sensory neurons

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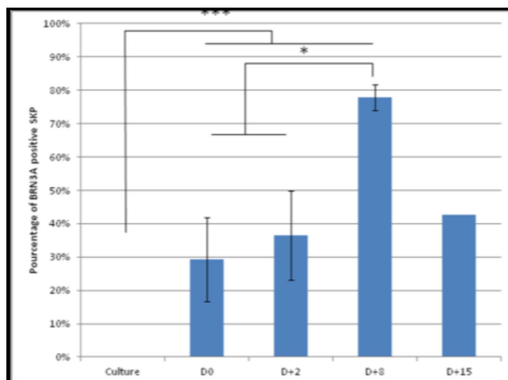
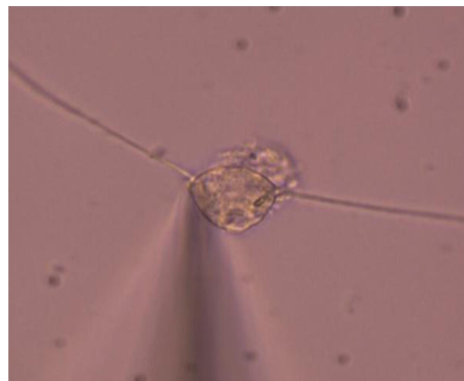
**SUMMARY :**

The culture of animal's dorsal root ganglia sensory neuron is an *in vitro* model commonly used for the study of neurogenic inflammation, pain or pruritus. However, ethical problems may appear and projection of the results into humans is very difficult. In the last decade, studies have highlighted the ability for iPS (induced pluripotent stem cells) or ES (embryonic stem cells) to differentiate into cells with sensory neuron characteristics. In most of these studies, these neurons are obtained in two main steps. From ES or iPS, cells are induced into cells expressing markers of neuronal precursors and the neural crest. Then in a second step, the induction of Wnt and / or BMP pathways allows differentiation into sensory neurons. Our work aimed at determining if it was possible to directly differentiate stem cells derived from the neural crest into sensory neurons. For this, we used the SKP (skin-derived precursors), which are derived from the neural crest and extracted from the skin tissue. SKPs were extracted by enzymatic and mechanical dissociation of small pieces of abdominal skin sample. These cells were grown and maintained in neurosphere in a DMEM/F12 medium containing FGF2 and EGF. After few weeks, the cells adhered spontaneously and were used for the differentiation experiment. To induce the Wnt pathway in SKP, we chose the CHIR99201 that activates the Wnt pathway by inhibiting glycogen synthase kinase 3 beta. For induction of BMP pathway we added BMP4. We obtained 1) confirmation by PCR that our SKP cells expressed markers of neural crest and precursors (p75NTR, SOX9, AP2, PAX3) and 2) after differentiation, evidence that they acquired a sensory neuron phenotype. Part of the cells also acquired a bipolar neuronal morphology. In qPCR, the Brn3a (marker sensory neurons) expression was increased by 7 after 20 days with CHIR99201 and 8 days of BMP4 compared to undifferentiated cells. At the same time, 100% of cells expressed the neuronal marker neurofilaments and p75NTR in immunochemistry. 78 and 75% of cells expressed Brn3a and peripherin (a peripheral neuronal marker), respectively. The presence of the TRPV1 channel was also evidenced by immunochemistry and PCR. Altogether, these results demonstrate that we can obtain cells with a sensory neuron phenotype from SKP. A functional study is underway.

**ILLUSTRATION :**



**Differentiated SKP into bipolar cell attached with a micropatch clamp**

**KEYWORDS :**

SKP Sensory Neurons Skin Differentiation Model

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# Development of 3D micropatterned intestinal crypts to study intestinal stem cell fate and proliferation

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**SUMMARY :**

The behavior of mammalian cells in a tissue is influenced by the three-dimensional microenvironment and involves a dynamic interplay between biochemical and mechanical signals. At present time, most *in vitro* studies are restricted to two-dimension culture systems, which do not match the physiological growth conditions of cells. The development of three-dimension models like spheroids or organoids has already shown their relevance to modelize specific tissues *in vitro* but these models still fail in recapitulating the specificity of *in vivo* 3D microenvironments, notably tissue architecture, stiffness and spatial distribution.
In this context, we are developing new 3D models to grow and study intestinal stem cells and their progenies, with controlled physicochemical properties (topography, stiffness, porosity…) using photopolymerizable hydrogels. These hydrogels are processed by 3D printing using a stereolithography approach to create artificial scaffolds on which cells are seeded and/or directly printed in the matrix. We performed extensive testing in culture and selected a PEG-DA (PolyEthylene Glycol DiAcrylate)/acrylic acid mix that can be supplemented with biological matrices such as collagen, fibronectin or laminin. Using this material, we succeeded in 3D printing microenvironment matching the dimensions of mouse intestinal crypts/villi. This system is first being tested with colorectal cancer cells and then will be optimized with sorted intestinal stem cells.
Finally we ambition to progressively add complexity to this system by tuning the microenvironment niche (fibroblasts, immune cells…), growth factors gradients and mimicking biomechanical forces (peristaltism and shear stress) using microfluidics.
These intestinal crypt/villi scaffolds may a complementary approach to organoids culture system in order to study intestinal stem cells and their progenies *in vitro*. This 3D model, by allowing guided self-organization and controlled differentiation, may allow the reconstitution of natural cellular heterogeneity and 3D spatial distribution of the intestinal epithelium.

**ILLUSTRATION :**

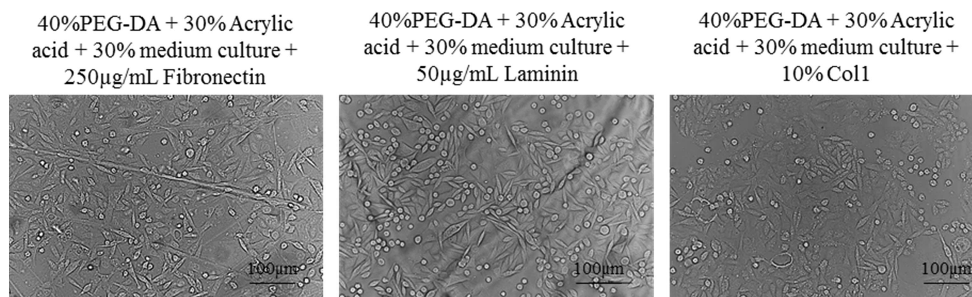


Fig. 1: Development of biocompatible photosensitive hydrogel to grow colorectal cancer cells

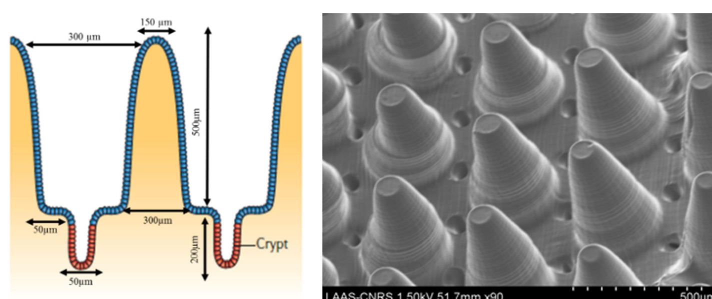


Fig. 2: Printing of PEG-DA/Acrylic acid mix matching dimensions of mouse intestinal crypt/villi

**KEYWORDS :**
3D Bioprinting intestinal stem cell tissue engineering

# Use of skin biopsies for assessing delivery of actives from topical film

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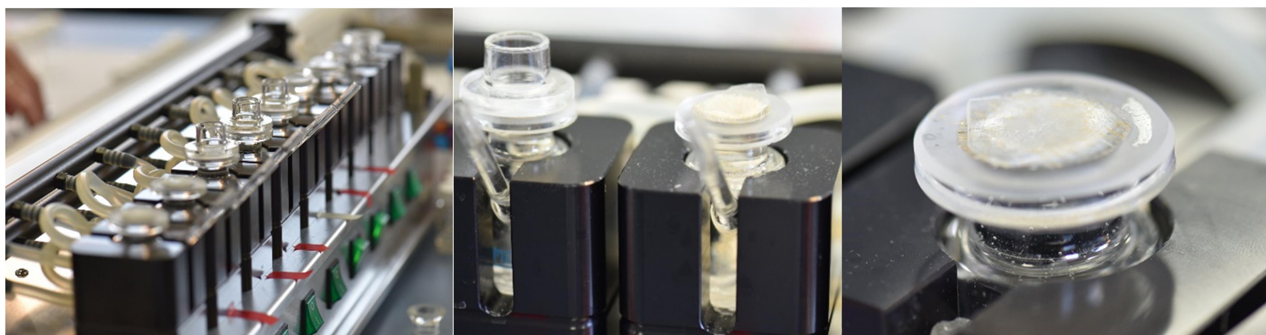
## SUMMARY :

In this work, we present our investigations on the transference of actives from polymeric film (typically used for face masks and eye patches) to skin by means of percutaneous absorption test with porcine skin biopsies at physiological temperature. With this method, it is possible to quantify the amount of active that is present in every skin layer (surface, *stratum corneum*, epidermis, and dermis) by extraction with selective solvents and further analysis, typically by HPLC. Furthermore, the active which has permeated until the hypodermis level (blood stream) is also quantified by analyzing the fluid in the receptor chamber. The residual active which has not been transferred to skin is found by chromatographic analysis of the application polymer. The amount of active found in the epidermis (without the *stratum corneum*), dermis and receptor fluid is considered to have penetrated into the skin; the amount found in the *stratum corneum* is not considered as penetrated because of its lost by desquamation.
By carrying out testing at different incubation times, the kinetic pattern is obtained, thus allowing a certain prediction of in vivo results and the possibility of screening different candidates for both the polymer and the impregnating liquid, and also rationalize the application conditions for the polymeric mask.

## ILLUSTRATION :



Franz cell setting for a polymeric film on a porcine skin biopsy

## KEYWORDS :

Topical delivery, Percutaneous absorption, Skin biopsy, Polymeric film, Cosmetics, Dermatology

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# Beating mammalian heart grafts on the chorioallantoic membrane: an alternative model to animal testing.

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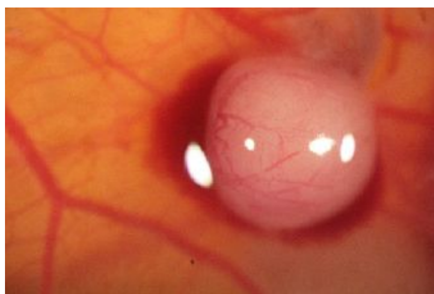
## SUMMARY:

There is currently no method available to study ex vivo cardiac function over an extended time period. Due to its extensive vasculature, the chorioallantoic membrane (CAM) is an excellent model to support the growth of a variety of tissues as well as to study various biological processes such as angiogenesis. Here we present a method to graft dissociated cardiomyocytes or pieces of avian (allograft) or mammalian (xenograft) hearts fragments on the CAM.

Ex ovo preparations were prepared at x days post fertilization. Isolated cardiomyocytes were grafted at day 7 post fertilization or cardiac tissue grafts of mammalian or avian origin were grafted at 11-13 days post fertilization on the CAM. Grafted tissue or cells were vascularized by the CAM as determined by intra-vital microscopy techniques such as echography or echo-Doppler analysis. The grafts recuperated functionality as evidenced by beating 2-3 days post engraftment. Stimulation by epinephrine applied either locally on the graft or by an I.V. injection significantly enhanced the beat rate frequency of the grafted tissues. Grafts were viable from 5-10 days post engfraftment.

Our results demonstrate that cardiac grafts find a complex and supportive environment (ex ovo) to recover functional properties, likely due to the development of a rich vasculature network. This work provides a novel method to prolong ex vivo studies of cardiac function.

## ILLUSTRATION :



KEYWORDS :

cardiac tissue xenograft, chorioallantoic membrane, beating heart, shell-less, functional cell, animal testing alternatives, mammalian

## REFERENCES:

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# Tardigrades (water bears): an emerging model animal to dry storage desiccation-sensitive cells

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**SUMMARY:**

We present an emerging and alternative model organism for bio-medical research: tardigrade (water bear). Tardigrades are microscopic aquatic animals (body length < 1 mm) that survive a remarkable array of stresses including, freezing and high temperatures, irradiation, exposure to the vacuum of outer space, and desiccation (Rebecchi et al., 2007).
Water is essential for life, but tardigrades have the ability to survive complete desiccation (losing 97% of body water) by entering in a state of reversible suspension of the metabolism called anhydrobiosis (Fig. 1). Today it is known that the ability of tardigrades to survive desiccation involves a complex array of mechanisms working at structural, physiological, and molecular/biochemical levels (Guidetti et al., 2011). In particular, the formation of a tun body-shape, the accumulation of compatible solutes (e.g. trehalose), the activation of antioxidant enzymes, and the synthesis and accumulation of unique tardigrade proteins. Among proteins there are: i. a DNA –associating protein (Dsup), which suppresses X-ray induced DNA damage and improves radiotolerance (Hashimoto et al., 2016); ii. specific intrinsically disordered proteins (TDPs) which are able to increase desiccation tolerance when expressed in heterologous systems (Bootbhy et al., 2017).
The next challenge will be the ability to induce or engineer complete desiccation tolerance in cells/tissues of desiccation sensitive organisms, using xeroprotectants detected in tardigrades.

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**ILLUSTRATION :**

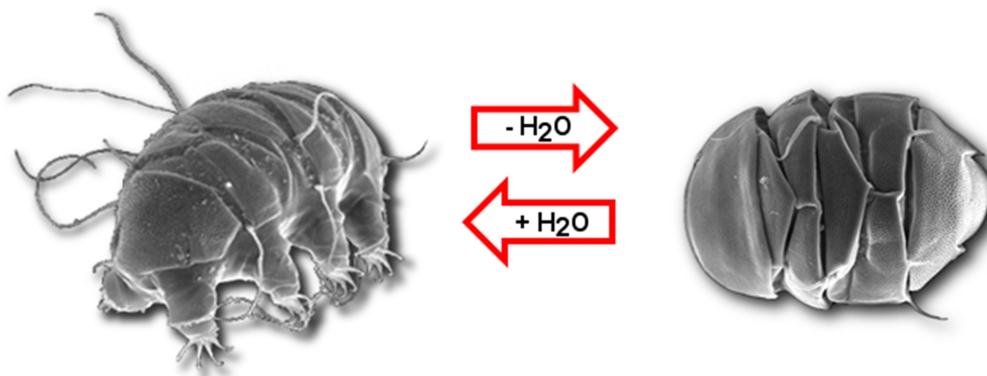


**Figure 1.** Two physiological states of a tardigrade: animal with active metabolism (left), animal in a desiccated state (right).

**KEY WORDS:**
Tardigrades, desiccation tolerance, anhydrobiosis, bio-protectans, xeroprotectans, sensitive desiccation organisms

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