A new image analysis-based approach to evaluate cytoxicity on 3D models

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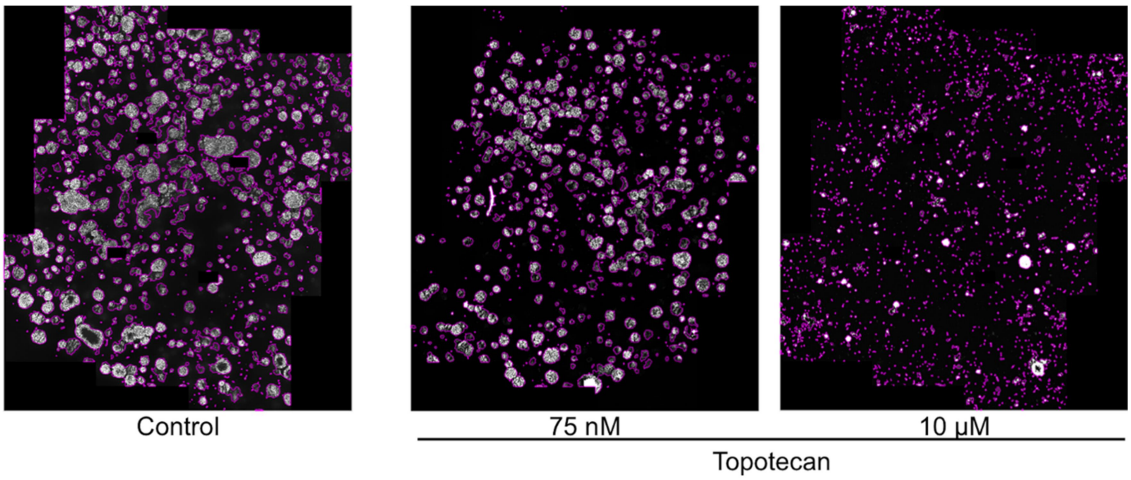
SUMMARY :

Pharmacological evaluation of anticancer drugs using 3D pre-clinical in vitro model provides invaluable information for the prediction of in vivo activity. The work reported here presents an efficient and robust imaging and analysis process to assess with quantitative parameters the efficacy of a cytotoxic drug. To illustrate our service offer, we present the quantitative evaluation of dose-dependent Topotecan cytotoxicity on HCT116 colon adenocarcinoma tumor cells multispheres grown in 3D.

Our workflow involves 3D microscopy with structured illumination, image processing and feature extraction performed with custom analysis tools. Our procedure allows automatic detection of spheres in a large volume of matrix in 96-wells plate. The resulting quantification of morphometric parameters such as cluster's size distribution shows variation correlated with Topotecan concentration.

This procedure allows by using quantitative image analysis to evaluate and quantify the cytotoxic activity of anticancer drugs on 3D multicellular models, and can be applied to multi-parametric analysis to address customers' specific needs.

ILLUSTRATION :



KEYWORDS :

Imaging	Pharmacological evaluation	Image Analysis
Multi-spheroid	Pre-clinical study	3D model

REFERENCES

GOMES and al. Evaluation by quantitative image analysis of anticancer drug activity on multicellular spheroids grown in 3D matrices. ONCOLOGY LETTERS 12: 4371-4376, 2016.

Re-innervated human skin explant: optimized model for study pruritus

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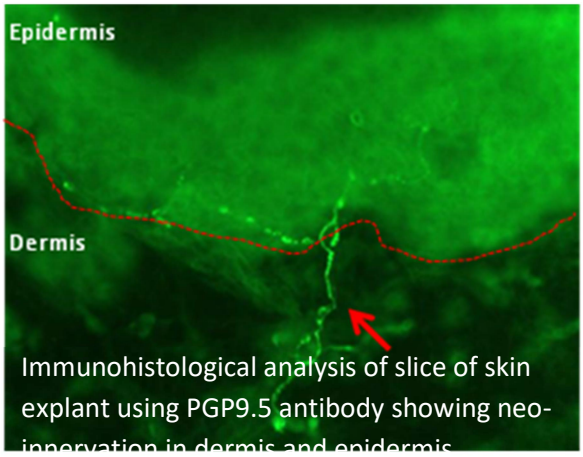
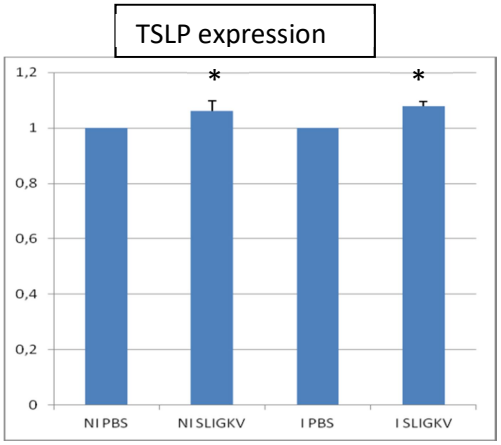
SUMMARY :

In order to study pruritus, we adapted a previously published skin explant model re-innervated with sensory neurons. This model is based on a co-culture between a human skin explant (dermis-epidermis) and sensory neurons of rats from dorsal root ganglia. In this adapted model, after several days of co-culture in transwell, we were able to confirm the presence of nerve fibers in the epidermis. Thus, we have showed that innervation (I) decreased the apoptosis of keratinocytes compared to skin explant cultured without neurons (NI), and maintained the thickness of the epidermis in culture compared to normal skin. For the study of neurogenic inflammation and non-histaminergic pruritus, we showed by IHC that the major actors (PAR-2, TSLP, TSLPr, TRPA1, IL31, IL31r) were present in neurons and epidermis. The functionality of the model was confirmed by the release of TSLP in the supernatant after topical application of PAR-2 agonist (SLIGKV-NH_2). In conclusion, we have optimized a model for studying itch to replace the use of living animals.

ILLUSTRATION :



Immunohistological analysis of slice of skin explant using PGP9.5 antibody showing neo-innervation in dermis and epidermis



KEYWORDS :

Skin | Innervation | Organotypic | Explant
Ex vivo

REFERENCES

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In vitro differentiation of skin-derived precursors into sensory neurons

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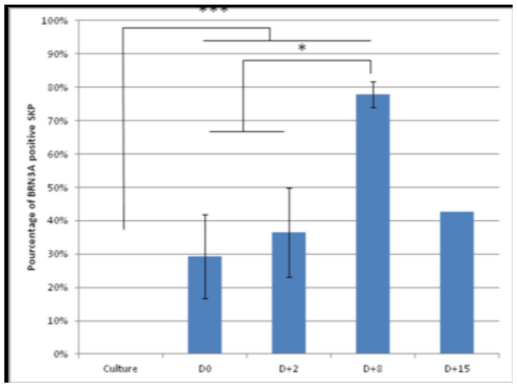
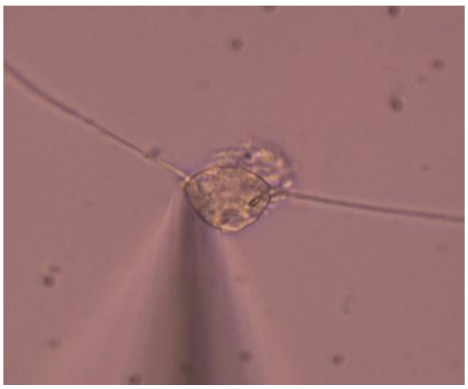
SUMMARY :

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

REFERENCES

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Lebonvallet N, Boulais N, Le Gall C et al. Exp Dermatol 2012 :21 :195-200

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 :

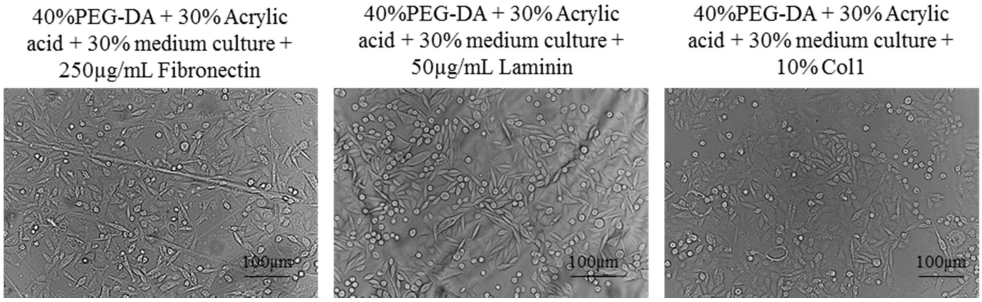

Fig. 1: Development of biocompatible photosensitive hydrogel to grow colorectal cancer cells

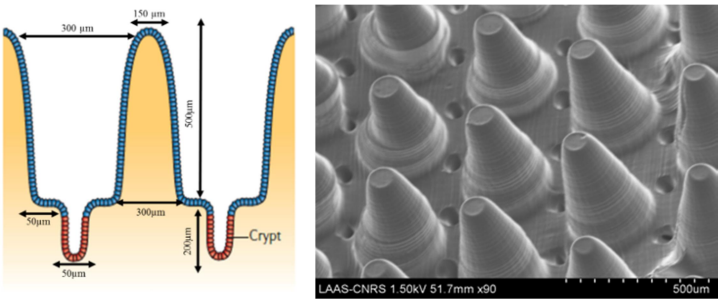

Fig. 2: Printing of PEG-DA/Acrylic acid mix matching dimensions of mouse intestinal crypt/villi

KEYWORDS :
3D Bioprinting intestinal stem cell tissue engineering

Modelling catecholaminergic polymorphic ventricular tachycardia using patient-specific iPSC-derived engineered heart tissues

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2:University Medical Center Hamburg-Eppendorf, Department for Experimental Pharmacology and Toxicology, Building N30 Martinistraße 52 20246 Hamburg, Germany,

SUMMARY

Background and objectives :

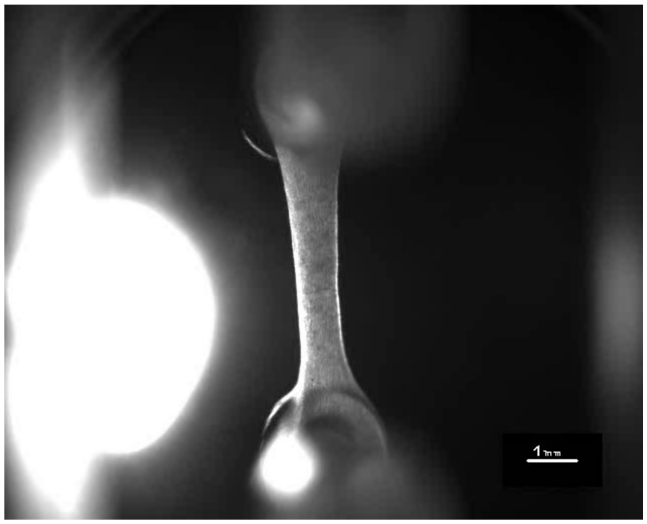
Induced Pluripotent Stem Cells were proven to efficiently model Mendelian diseases *in vitro*. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a disease involving a mutation in the ryanodine receptor 2 (RyR2), provoking life threatening arrhythmias dependent on β-adrenergic stimulation. The disease was already successfully modelled in patient-specific iPSC-derived cardiomyocytes, however studies performed in single cells ignore the complexity of cell/cell interaction. In this study, we used fibrin matrix based engineered heart tissue (EHT) to provide multicellular complexity level to patient-specific iPSC-derived cardiomyocytes and to confirm a model for CPVT phenotype.

Methods:
iPSCs were cultured and differentiated into cardiomyocytes thanks to Embryoid Body (EB) formation and small molecules, both mimicking early embryonic stages. EHTs were then casted between two silicone posts where the polymerisation is quickly achieved by mixing together thrombin and fibrinogen. The embedded cardiomyocytes will then progressively build cell/cell contact in two weeks and contract coherently within the entire EHT, allowing tissue contractility measurements.
Those contractions were analysed by a video-optical recording linked to a custom-made software with a figure recognition capability. The force was then calculated depending on the movement, each individual contraction was identified by the program and average parameters such as force, frequency, relaxation time or irregularity indicators were analysed.

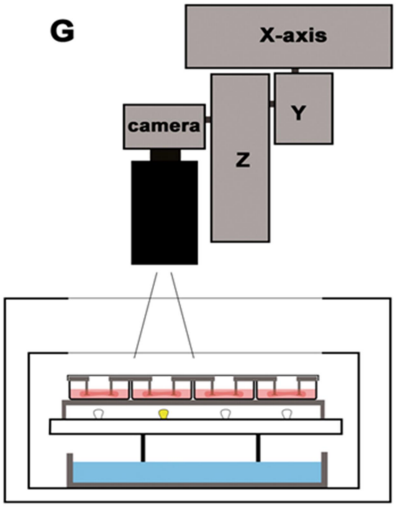
Results :
CPVT EHTs displayed a longer relaxation time than the healthy controls. This longer relaxation time could however not be rescued by a RyR2 stabilising drug and arrhythmias could not be observed despite isoprenaline stimulation.
In a second experiment, CPVT EHTs measured overnight in high calcium displayed an irregular beating behaviour after more than 6 hours recording. This behaviour was specific to the disease-specific EHTs. Irregularity could have been caused by a calcium overload of the sarcoplasmic reticulum leading to an increase of the RyR2 opening probability. However, stabilising the RYR2 could not rescue the phenotype, therefore casting doubts that the EHTs successfully model the CPVT disease.

Conclusion and perspectives:
We have previously shown that EHTs were a good model to include the complexity of cell/cell interaction of a heart tissue. In this study, we found that disease-specific EHTs had a significantly longer relaxation time than the controls, suggesting a disease-specific phenotype. However, this longer relaxation time could not be rescued by RyR2 stabilisers. Furthermore, even if isoprenaline induction successfully displayed the expected increase of frequency of our model, it did not display the typical disease-specific isoprenaline-induced arrhythmias. The system is well adapted for long automated measurements which allowed us to unmask arrhythmic behaviour in high calcium containing tyrode. However, these findings could not be further confirmed as the attempt to rescue the arrhythmic phenotype with a RyR2 stabiliser was not successful. In conclusion, the prolongation of $T2_{80\%}$ should be further confirmed by using other control cell lines, and patients with a more severe CPVT conditions should be used to further test the EHT model.

ILLUSTRATION :



Engineered Heart Tissue (EHT)



Automated recording incubation chamber

KEYWORDS :

iPSC	Tissue Engineering	Disease modelling	Cardiomyocyte
Differentiation	Contraction recording	CPVT	Stem cells

REFERENCES

Letuffe-Brenière, D., 2016. *Modelling catecholaminergic polymorphic ventricular tachycardia with patient-specific iPSC-derived engineered heart tissues*. Universitätsklinikum Hamburg-Eppendorf (UKE). Available at: http://ediss.sub.uni-hamburg.de/volltexte/2016/7798/pdf/Dissertation.pdf.

Percutaneous penetration of the DEET through damaged skin

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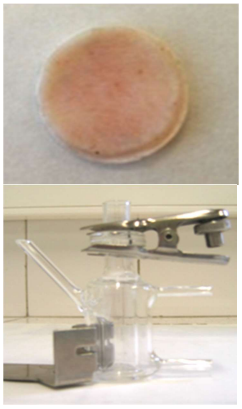
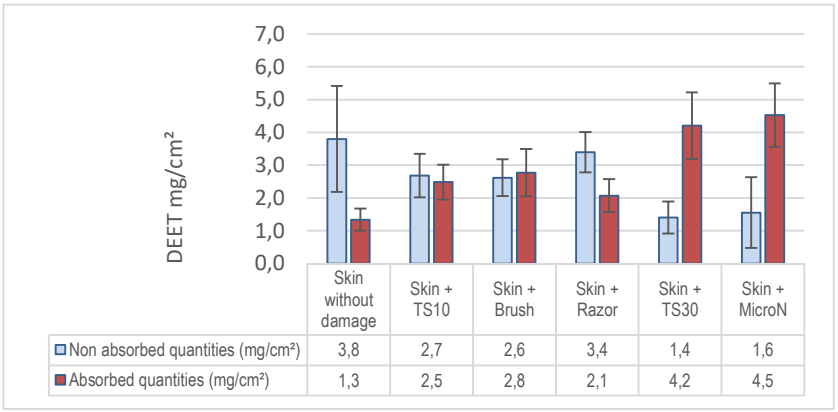
SUMMARY :

Introduced for public use in 1957, N,N-diethyl-m-toluamide (DEET) is an common topical insect repellents. This compound is currently used in many formulations proposed to consumer. Reported adverse side effects after using DEET product appear to be the result of a significant absorption through human skin. Mosquito bites can create a bump that is typically swollen, sore, or itchy. Some injuries were observed by scraping or grazing the skin, and percutaneous absorption of chemicals may seriously increase when the skin is damaged. The aim of this study was to mimic *in vivo* conditions of topical repellent applied to skin with the SC impaired due to various mechanical reasons. Skins were mechanically damaged by 10 and 30 sequential tape strips as model systems[1], and with razor, rotated-brush and finally with microneedles. These studies were conducted according to the OECD (2004)[2], as well as the SCCS (2010)[3] guidelines for studies on in vitro dermal absorption with biopsies of porcine skins and static Franz cells.

All mechanical damage to the skin increases the systemic availability of DEET. The results with 10 tape strips can be compared to the damage obtained by means of a razor and a rotated-brush. In this case, the absorption is multiplied by twice. In addition, to obtain an increase in gravity, 30 tape strips were used and compared to damages caused by microneedles. In this case, we showed a strong extension in deeper skin layers with a large increase in systemic concentration of the repellent.

ILLUSTRATION :





	Skin without damage	Skin + TS10	Skin + Brush	Skin + Razor	Skin + TS30	Skin + MicroN
Non absorbed quantities (mg/cm²)	3,8	2,7	2,6	3,4	1,4	1,6
Absorbed quantities (mg/cm²)	1,3	2,5	2,8	2,1	4,2	4,5

Quantification of DEET penetration in the different compartments of skin (n=4-6)

KEYWORDS :

In vitro Skin absorption Franz cells Repellent DEET

REFERENCES :

H. Dabboue, N. Builles, É. Frouin, D. Scott, J. Ramos and G. Marti-Mestres. Assessing the Impact of Mechanical Damage on Full-Thickness Porcine and Human Skin Using an In Vitro Approach. BioMed Research International. (2015) ID 434623, 10 pp

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Studies supported by ANSM French Health Agency: ThroughSkin Project.

Skin barrier disruptions in damaged human skin have no effect on dermal penetration and systemic distribution of Titanium dioxyde nanoparticles

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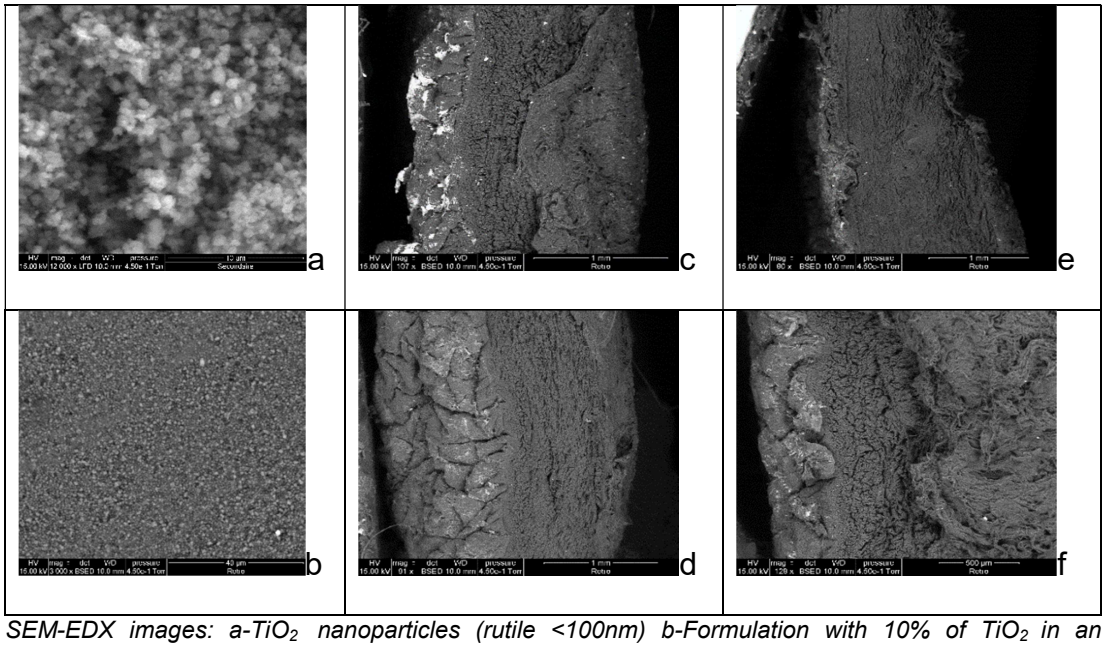
Summary :

Titanium dioxide (TiO_2) nanoparticles has become a frequently used physical UV filter in cosmetic formulations. And, penetration of nanoparticles TiO_2 into human skin seems to be possible because of the particle size under of 100 nm. But, the small particle size results in a high surface activity of the primary particles and causes a formation of agglomerates in the formulation which also limited the transdermic penetration.[1]

The aim of this study was to investigate the *ex vivo* penetration behaviour of the physical UV filter into human skin intact and damaged[2] by 30 Tape-stripping, brush razor and also microneedles. Furthermore, a stable sunscreen formulation with different NP TiO_2 rutile form (<100nm) and anatase form (15 nm) was manufactured in an emulsion.

According to our experiments, we compared the skin penetration characteristics and systemic distribution of Titanium dioxide-NP at 10% in a cosmetic formulation in healthy human skin versus different damaged skin by SEM-EDX analysis and completed by AAS. In conclusion, Titanium dioxide (TiO_2) nanoparticles seem not to cross the normal or injured human skin.

Illustrations :



SEM-EDX images: a-TiO_2 nanoparticles (rutile <100nm) b-Formulation with 10% of TiO_2 in an emulsion ; Biopsies human skin after 24 hours application of the NP TiO_2 formulation damaged before by : c- 30 Tape-stripping, d- Brush, e-Razor, and f-Microneedles

Keys Words :

Ex vivo – Human skin – Titanium dioxyde nanoparticles – Franz cells – Scanning Electron Microscopy/Energy Dispersive X-Ray Analysis (SEM/EDX) – Atomic Absorption Spectrometry (AAS)

References :

1-C. Bennat, C.C. Müller-Goymann, Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. Int. J. Cosmet. Sci., 22 (4) (2000), pp. 271-283

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The authors have no conflicts to disclose. The ANSM (project ThroughSKIN) is thanked for financial support.

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

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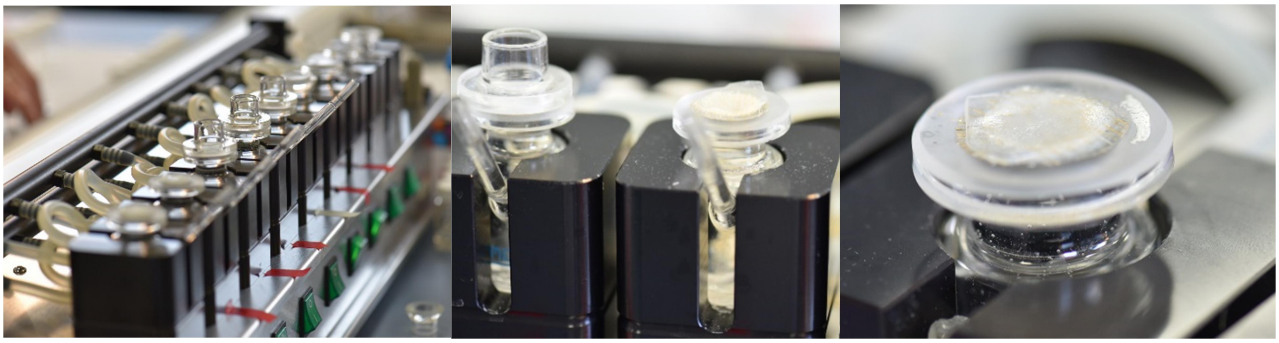
SUMMARY :

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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Screening natural LDHA inhibitors from wild mushrooms

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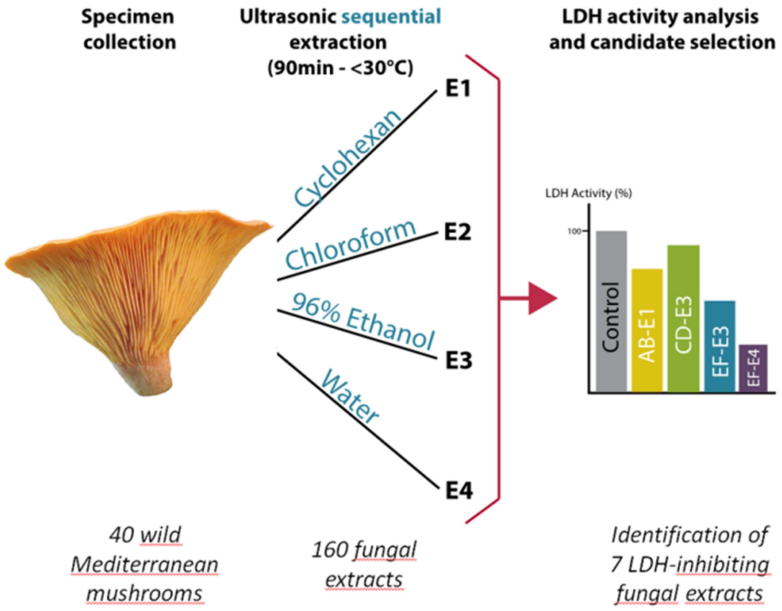
SUMMARY :

Human lactic acid dehydrogenase (LDH) is a tetrameric enzyme composed of two subunits, LDHA and LDHB. Although LDHB is ubiquitously expressed, LDHA expression is predominantly found in skeletal muscle and other highly glycolytic tissues. LDH catalyzes the reversible transformation of pyruvate into lactate in anaerobic conditions, coupled with NADH oxidation into NAD+. LDH thus regulates the final step of anaerobic glycolysis. It is now a well-known fact that most malignancies rely on a high rate of glycolysis to produce the energy necessary for tumor growth and progression [1]. Numerous studies have demonstrated that natural substances can be considered as strong therapeutic resources in the fight against cancer [2]. We sought to identify natural LDH inhibitors within fungi. 160 fungal extracts were obtained by ultrasonic sequential extractions, using 4 different solvents, of 40 Mediterranean wild mushrooms. We then screened the 160 fungal extracts for their potential LDH-inhibitory properties. Through this method, we have identified 7 fungal extracts that harbor LDH inhibitory properties. Because these active extracts originate from different extraction solvents, we suspect that the LDH inhibitory properties of these fungal extracts are exerted by different types of molecules. Further bioguided purification will carried out to identify bioactive compounds. Our findings thus point to the interest of investigating the potential application of these mushroom-derived substances in therapeutic strategies.

ILLUSTRATION :



KEYWORDS :

LDH mushroom cancer activity
natural products

REFERENCES :

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Juan Bautista CHAPE y GUISADO and artificial emulsions, precursor of current *in-vitro* studies

Paloma RUIZ VEGA

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SUMMARY :

Goals : JB.CHAPE y GUISADO was a translator and analyst at the Journal of the Royal Academy of Medicine and Surgery of Cadiz, author of a work on artificial emulsions which had been published in French by A. Baudrimont in the Journal de Pharmacie et des Sciences Accessoires de Paris, corresponding to the n ° 1 of 1830.

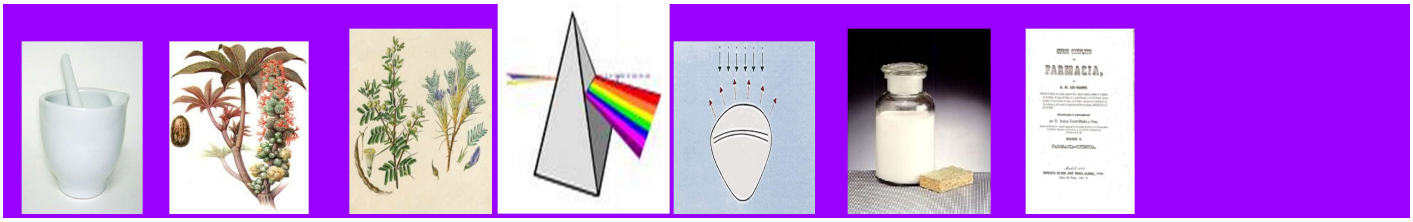
Results : JB.CHAPE indicates that all forms or treaties of pharmacy published at the time recommended for the preparation of artificial emulsions the use of gum or water to which oil was added in small proportions, which gave a White emulsion, not very stable at rest and separating into two parts, one transparent and the other white opaque to the upper phase.

Discussion: JB.CHAPE notes that there is a better method, known to a certain number of pharmacists (although not published), which consists in putting oil and gum in powder in a mortar, and agitating while adding a certain amount Amount of water.

However, although very viscous, these emulsions tend to overflow from the mortar, indicating that they have not been well prepared. On the other hand, when they have been well prepared, they adhere to the pestle and during the agitation, a slight crackling is perceived. The emulsions thus obtained are of a white color which is all the more intense as they reach perfection.

Conclusion: JB. CHAPE, after having translated the work of Monsieur BAUDRIMONT on artificial emulsions, notes that this method of preparation is certainly not unknown to some Spanish Pharmacy professors, but wishing that it is generalized in view of its good results, confirms by his experiments On lochs and mixtures enclosed in oils and resins, that it deserves to be known to all.

ILLUSTRATION:



Mortar | *Ricinus communis* | *Acacia Senegal* | *Refraction* | *Reflexion* | *Emulsion* | *LE CANU,Tomol*

KEYWORDS:

Artificial emulsions | Refraction | Mixtures
R. Acad. Med. Surg. Cadiz | J. Pharm. Sci. Acces. Paris

REFERENCES:

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Bi-directional passage through a fragment of skin grafted onto a chorioallantoic membrane.

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2 : INSERM U1058 –Pathogenèse et contrôle des infections chroniques - 60 rue de Navacelles– CS 34493 – 34394 Montpellier cedex 5.

SUMMARY

Punch biopsies of human skin were grafted on chorioallantoic membrane (CAM) of a fertilized chicken egg. The shell-less model allows a continuous observation of the grafts throughout the experiment. The vascularization of the grafted tissue was observed within 48 hours.

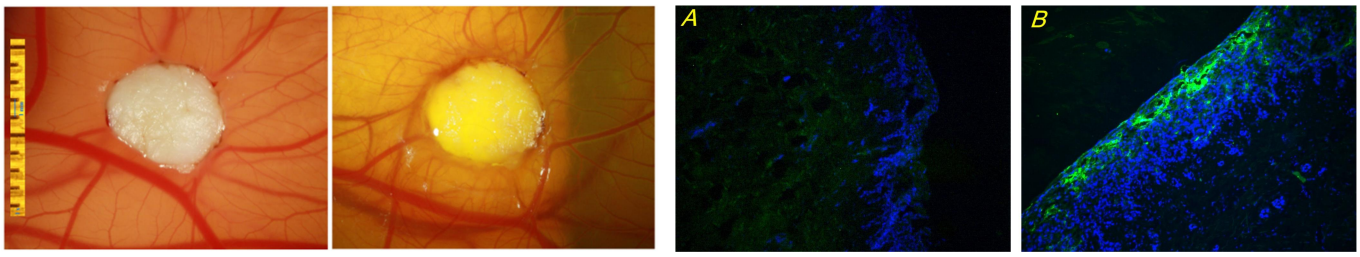
We aim at demonstrating that such a model can be used to evaluate the effect of drugs on normal or pathological skin.

Firstly we showed that when 150 kDa FITC-dextran is injected into the CAM vessels, the rapid passage of the dye from the blood to the vascularized graft can be observed in the epidermis whereas no dye was seen in the non-vascularized grafts. Reciprocally, some molecules such as nicotine applied on a skin fragment can be recovered into the blood as demonstrated by HPLC analysis.

Secondly, we were able to graft 3D carcinoma-like skin (CLS) on the CAM and showed that the graft vascularized. Both normal skin and CLS were treated with 255 µM vitamin D3 analog by a single intravenous injection. After 2 days, immuno-histochemical analysis of the cytokeratin 10, a differentiation marker, was performed on frozen sections. While CK10 was not detected in vehicle-treated CLS, its expression was observed in Vit-D3-treated graft as well as in all normal skins.

Faced with the exclusion of animals use for the development of cosmetic products (European Commission 1223/2009), the CAM appears to be a model of choice for grafting skin pieces and i) To evaluate the positive effects of new drugs devoted to treat pathological skin affections by a systemic route, or, ii). to demonstrate that new cosmetic compounds applied on the skin does not reached the vessels even for long term goal experiment.

ILLUSTRATION :



native skin **FITC-dextran** **untreated** **treated**

KEYWORDS :

xenografting	treatment	carcinoma like skin	oncology
chorioallantoïc membrane	skin	cutaneaous passage	FITC-dextran

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

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SUMMARY:

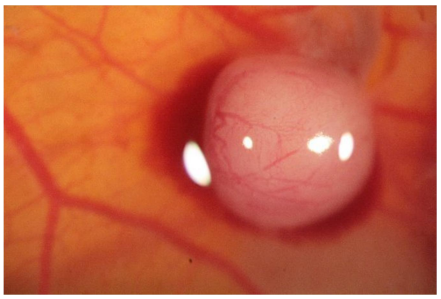
There is currently no method available to study ex vivo cardiac function over an extended time period. Due to its extensive vasculature, the chorioallantoic membrane (CAM) is an excellent model to support the growth of a variety of tissues as well as to study various biological processes such as angiogenesis.

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 4 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 engraftment.

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 :



Beating heart

Echo Doppler

KEYWORDS :

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

REFERENCES:

Patent : Nirdé Ph., Richard S. Dépôt FR 155 5364, 12 juin 2015. Procédé de greffe de cellule cardiaque sur la membrane chorioallantoïde d'œuf fécondé ».
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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.

Research supported with the grant "Fondo di Ateneo per la Ricerca -Progetti di ricerca di dipartimento (FAR) 2015 (Dr. 267/2016, P.n. 8176)" of the University of Modena and Reggio Emilia to LR.

ILLUSTRATION :

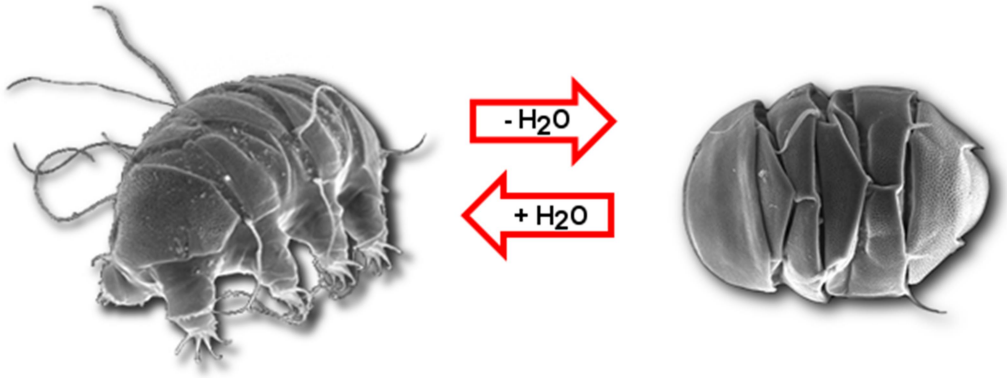

Figure 1. Two physiological states of a tardigrade: animal with active metabolism (left), animal in a desiccated state (right).

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

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Integrated model for assessment of significant biological mechanisms in pharmacology, aging and toxicity studies: *Caenorhabditis elegans*.

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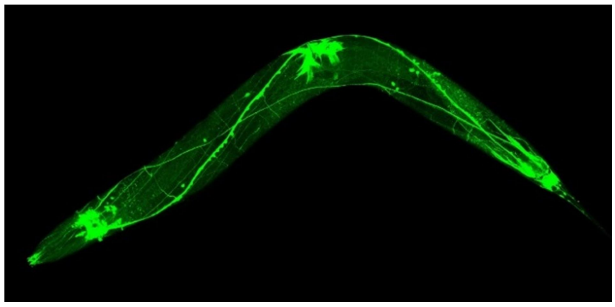
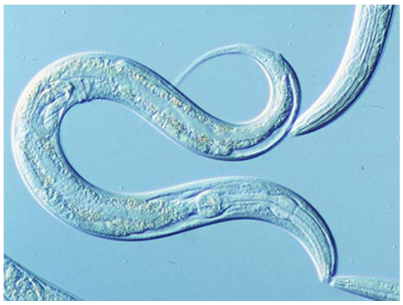
SUMMARY :

Caenorhabditis elegans is a non-parasitic free living bacterivorous nematode. This model organism gives several advantages. It is a small organism composed of 959 cells at the adult stage. Adult hermaphrodite is 1 mm length and 60 µm diameter. The developmental life cycle of this worm span only 3 days at 20°C. *C. elegans* is a well-studied organism and allows quantitative observations. This nematode is a suitable laboratory model that allows physiological impact evaluation upon exposure to exogenous molecules.
We have developed new molecular and genetic tolls that allow us to look at the aging process control in relation with various treatments.
For example, Chicoric acid can act on muscle mitochondria homeostasis as well as on metabolism linked with type 2 diabetes. We have started to analyze *C. elegans* aging impact of Chicoric acid and found an unexpected positive effect even at unexpected very low (micromolar) concentrations.
We have also tested some gas in term of toxicity, development, fertility and mutagenicity for an industrial toxicity assessment.
Our quantitative *C. elegans* technology can include liquid, gas, soluble or insoluble drug screenings on identified or unidentified molecular targets (or pathways) as well as monitoring of mitochondrial, metabolism or aging impact as well.

ILLUSTRATION :





KEYWORDS :
Nematoda *Caenorhabditis elegans* Model Organism Toxicity studies
Aging

REFERENCES

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Preve C, Maladen R, Lahaye G, Penelon T, **Richaud M**, and **Galas S**. CIRED, 24th International Conference on Electricity Distribution, Glasgow, 12-15 June 2017. Hazard study of MV Switchgear with SF6 alternative gas in electrical room. Paper 0385.

The impact of water composition in aging field using *Caenorhabditis elegans* model.

Myriam RICHAUD[1], Maëlys GARNIER[2], Hinda DABBOUE[b], Simon GALAS[1] and Gilberte MARTI-MESTRES[2],

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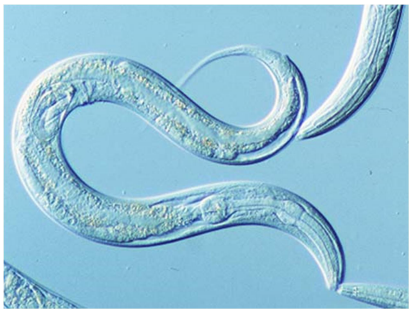
SUMMARY :

Caenorhabditis elegans is a non-parasitic free living bacterivorous nematode. This model organism (Maupas, N2 var Bristol) was primarily found in terrestrial soils but also lives in fresh water systems. *C. elegans* has several advantages. This organism is well studied and very easy to cultivate. It is small and composed of 959 cells at the adult stage. Adult hermaphrodite is 1 mm length and 60 µm diameter. The developmental life cycle of this worm span only 3 days at 20°C. *C. elegans* allows quantitative observations.
Water has always been known to be a key element of life. Our body is composed of water at 60 to 70 percent. Thermal waters have been used in therapeutics since the Greeks. All these elements show the importance of water for humans.
Water composition can be very different. We can distinguish very mineralized waters or little mineralized waters depending on their composition.
In this study, we used the model organism *C. elegans* to evaluate the impact of several waters (*Hepar*, *Volvic* and *Contrex*) on aging. Numerous mineralized waters are used to formulate cosmetics and these results could be an important information for selecting the type of water for this field specially for anti-aging formulations.

ILLUSTRATION :



KEYWORDS :
Water *Caenorhabditis elegans* Model Organism Aging

REFERENCES

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The tardigrade *Hypsibius dujardini*, a new model for studying resistance mechanisms.

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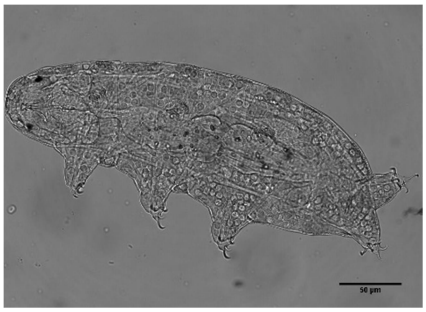
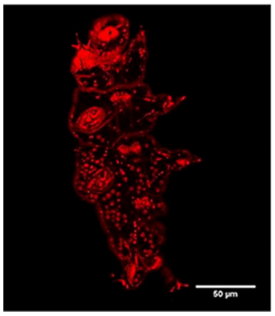
c- Centre de Recherche en Biologie cellulaire de Montpellier - UMR 5237 - CNRS - Université de Montpellier, France

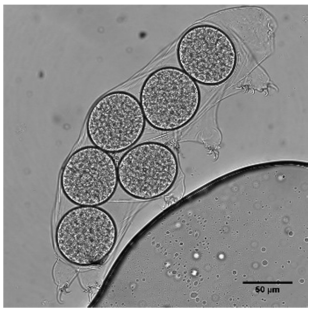
SUMMARY :

Tardigrades are minute metazoan animals from approx. 0.1-1.2 mm in size that inhabit diverse habitats such freshwater, marine or water films of terrestrial moss and lichens. Tardigrades species hold their own phylum that stands in between the phylum Arthropoda and Nematoda. They can cope with harshest environments.
The species *Hypsibius dujardini* for example hold exceptional characteristics that enable it to withstand high pressure, space vacuum or radiation levels that are generally fatal for living organisms. This tardigrade is able to resist but also to repair biological damage he suffered with any impact on subsequent life span expectancy.
H. dujardini can adopt a resistance state called anhydrobiosis.
Beside this stage, physiological and biochemical modifications have been described. However, lots of data about the molecular and physiological mechanisms that can be accounted for the Tardigrade anhydrobiotic stage resistance are still missing. To date, any detectable metabolic activity of anhydrobiotic Tardigade species have been uncovered.
We have adapted new techniques for living Tardigrade to uncover mitochondria adaptation and control during metabolic activity shutdown of the anydrobiotic state.
In a second part, we assessed if *H. dujardini* can accumulates protein carbonylation during either anhydrobiotic “tun” state or the living stage.

ILLUSTRATION :







KEYWORDS :

Tardigrada	*Hypsibius dujardini*	Model Organism	Resistance
Anhydrobiosis	Metabolism	Protein carbonylation	

REFERENCES

Author index