
leti
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TECHNOLOGY
RESEARCH
INSTITUTE
TECHNOLOGIES
FOR BIOLOGY
AND HEALTH
2019
SCIENTIFIC
REPORT

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TECHNOLOGY
RESEARCH
INSTITUTE

COMMITTED TO INNOVATION, CEA-Leti CREATES DIFFERENTIATING SOLUTIONS WITH ITS PARTNERS.

CEA-Leti is a research institute of CEA Tech and a recognized global leader in miniaturization technologies.

CEA-Leti's teams are focused on developing secure solutions that will enable future information and communication technologies, health and wellness approaches, clean and safe energy production and recovery, sustainable transport, space exploration and cybersecurity.

For 50 years, the institute has built long term relationships with its industrial partners, tailoring innovative and differentiating solutions to their needs. Its entrepreneurship programs have sparked the creation of 65 start-ups.

CEA-Leti and its industrial partners work together through bilateral projects, joint laboratories and collaborative research programs, as illustrated in this report.

CEA-Leti maintains an excellent scientific level by working with the best research teams worldwide, establishing partnerships with major research technology organizations and academic institutions. CEA-Leti is also a member of the French Carnot Institutes network*.

*Carnot Institutes network: French network of 39 institutes serving innovation in industry.

DE LA RECHERCHE À L'INDUSTRIE
cea

CEA Tech is the technology research branch of the French Alternative Energies and Atomic Energy Commission (CEA), a key player in research, development and innovation in defense & security, nuclear energy, technological research for industry and fundamental physical and life sciences.

www.cea.fr/english

CEA-Leti at a glance

800
publications per year

ISO 9001
certified since 2000

114
European projects

Founded in
1967

Based in
France (Grenoble)
with offices in the
US (San Francisco) and
Japan (Tokyo)

300
industrial partners

1,850
researchers

3,100
patents in portfolio

10,000
sq. meters cleanroom
100-200-300 mm wafers

65
startups created

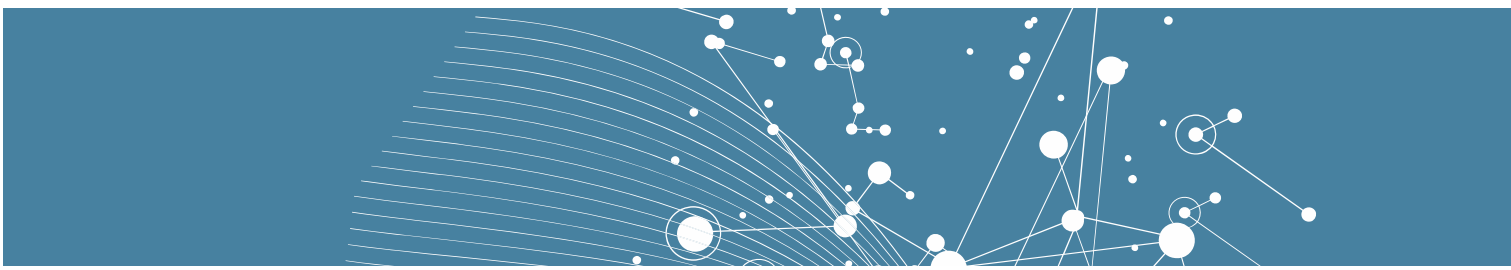
TECHNOLOGIES FOR BIOLOGY AND HEALTH

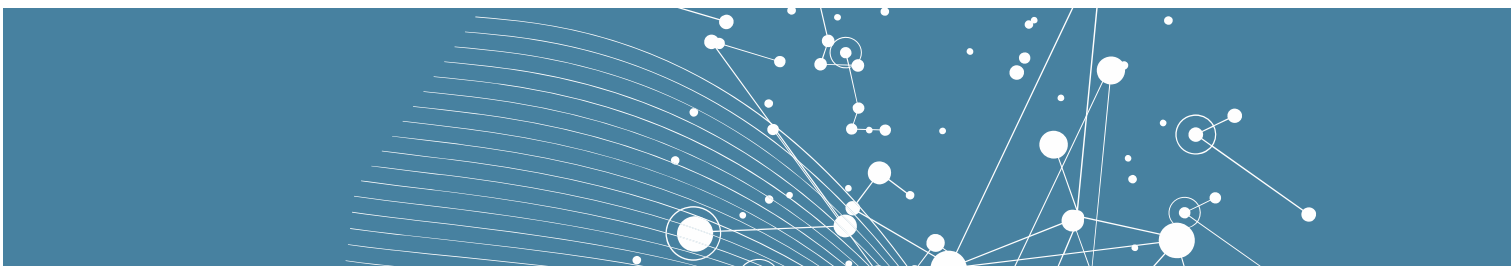
Core R&D competencies of technologies for **Biology and Health Division** are the development, design, integration and qualification of micro- and nanotechnologies in application to health (in the braod sense as defined by WHO) and life sciences the. These include sensors and actuators, imaging technologies, microfluidics, chemistry, biochemistry and electrochemistry, biology and instrumentation, including mechanics, software, information processing and electronics.

Our teams have acquired expertise in developing product prototypes with a system-development perspective.

Our facilities include cleanrooms dedicated to biochip packaging (230 m^2) , and to surface functionalization, (bio)probes grafting and (bio)reagent loading (100 m^2), biological laboratories with L2 rooms dedicated to bacteria, cells and human samples handling and biological characterization equipment such as PCR, cell microscopy and FACS (100 m^2). We also have laboratories for synthetic chemistry, electrochemistry and characterization (430 m^2) and a microfluidic platform dedicated to technologies and system validation (300 m^2). These facilities are complemented with technical platforms dedicated to optics instrumentation and to electronic and integration.

With **Clinatec**, we placed our state-of-the-art technology and biology laboratories under one roof with a fully equipped preclinical facility hosting small and large animals and an integrated cutting-edge clinical platform operated by Grenoble University Hospital. This unit is optimal for conducting the first human medical-device clinical trials for safety and efficacy studies, as well as for hosting clinician partners for the duration of their clinical research projects.





TECHNOLOGIES FOR BIOLOGY AND HEALTH

FOREWORD

Patrick Chaton,
Head of Microtechnology for Biology and Healthcare division

Prof. Alim Louis Benabid,
Chairman of the Clinatec Board

Prof. Stephan Chabardes,
Clinatec Clinical sector Director

Health is commonly associated to the medical status of a person. However, such a definition clearly shortens the reality of health that covers not only medical aspects but also prevention aspects associated to food, environment, wellness and security, since these factors may critically alter human life. Such a global view of health, reported as One-Health approach, is particularly pregnant nowadays with ecological concerns coupled to population ageing and demography evolution. Leti Health is clearly involved in the One-Health strategy through its two constitutive divisions, DTBS (Microtechnologies for Biology and Health Division) and Clinatec (Technology and Medical Research Center) to address the overall field of health from the design / maturation of new technologies to preclinical studies, and even to clinical trials dedicated to innovative medical device evaluation, including implantable device assessment.

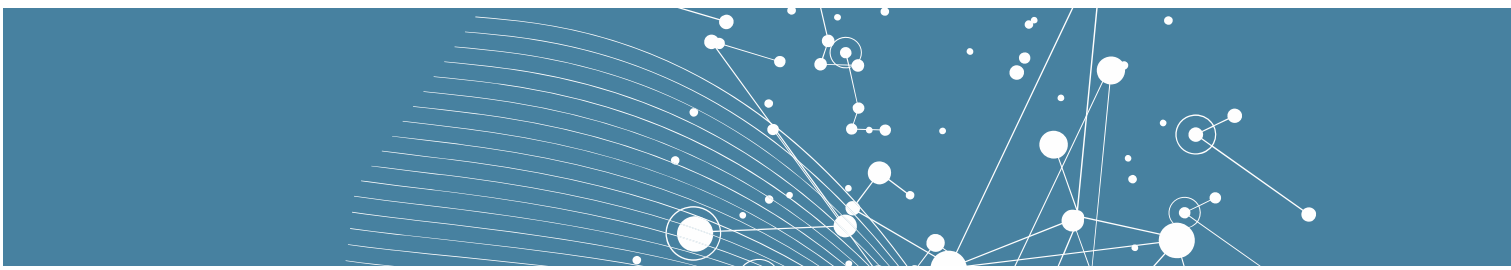
To address the one-health challenge, DTBS was reorganized in 2019 within two R&D sectors that cover the scientific and technologic aspects of five Investigation axes (diagnostics, monitoring, drug delivery, environment-exposome, pharma-bioproduction). This redefinition of the DTBS strategic contours comes into perfect resonance with the two collaborative actions initiated in 2018 dedicated to industrial maturation of innovative medical devices (Hub4Aim) and to the deployment of connected medical devices developed with our industrial partners (E-Meuse Santé).

2019 has also seen the quadrennial evaluation of Leti Health by the HCERES state organization. HCERES report has pointed out the remarkable interactions of Leti Health with the socio-economical actors of health and the quality and the coherence of its research project around medical devices.

In a clinical research point of view, 2019 was a great year for Leti-Health with the publication of two clinical trials in a prestigious journal, The Lancet. These trials relate to two of our emblematic developments: the artificial pancreas (The Lancet Digital Health) and the brain-computer interface (The Lancet Neurology).

Regarding technological research, 2019 has also seen the recognition of Leti-Health knowhow in microfluidics with the obtaining of the "InnovationTeam Best Practices" award decreed by the "Club de Paris des Directeur de l'Innovation. This trophy awards our collaborative research with CEA fundamental research teams (DRF-IRIG) for the design of an integrated blood sample preparation tools dedicated to proteomics. In addition, 2019 has seen the acceptance of an emblematic clinical trial by the French agency ANSM that will allow evaluating an innovative neuroprotective approach for Parkinson's disease, based on intracranial near-infrared illumination.

In summary, 2019 was for us a great year with the concretization of emblematic researches on e-health and BCI.

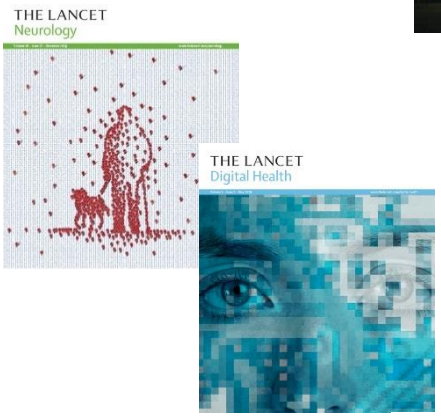


KEY FIGURES

144 Researchers

41 Post-docs and short-term contracts

30 PhDs and apprentices



59 Book chapters and journal articles

55 Conferences and workshops

31 Patents filled

388 Patents in portfolio

9 Start-ups created

230 m² Cleanroom for surface chemistry and biochip packaging

100 m² Biological laboratory (L1 and L2 facilities)

430 m² Chemistry laboratory

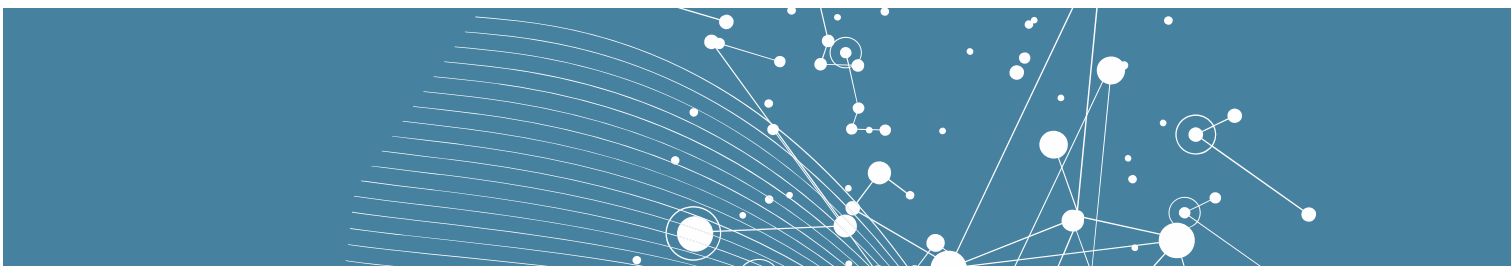
230 m² Microfluidic laboratory

6 Rooms for patients and monitoring technologies

A fully equipped surgery room with Intraoperative MRI

Multimodal investigation capabilities

MEG, SPECT-CT, gait analysis



SCIENTIFIC ACTIVITY

Publications

59 Book chapters and journal articles. These include Lancet Neurology, Lancet Digital Health, Nature Acta Diabetologica, Analytical Chemistry, Material sciences and engineering C, Journal of Biomedical Optics, Biomedical Optics Express, Physical Review Letters, PLoS One, Cellulose, Cancer, Journal of Carbohydrates Plymers…
36 Conferences and workshops
Main papers are:

"**An exoskeleton controlled by an epidural wireless brain–machine interface in a tetraplegic patient: a proof-of-concept demonstration**", A. L.Benabid, T. Costecalde, A. Eliseyev, G. Charvet; A. Verney; S.I Karakas; M. Foerster; A. Lambert; B. Morinière; N. Abroug; M-C.Schaeffer, A. Moly; F. Sauter-Starace, D. Ratel, C. Moro, N. Torres-Martinez, L. Langar; M. Oddoux, M. Polosan, S. Pezzani; V. Auboiroux, T. Aksenova, C. Mestais; S. Chabardes,
The Lancet Neurology, 18, (2019) 1112-1122
Doi: 0.1016/S1474-4422(19)30321-7

"**Closed-loop insulin delivery in adults with type 1 diabetes in real-life conditions: a 12-week multicentre, open-label randomised controlled crossover trial**", P-Y.Benhamou, S. Franc, Y. Reznik, C. Thivolet, P. Schaepelynck, E. Renard, B. Guerci, L. Chaillous, C. Lukas-Croisier, N. Jeandidier, H. Hanaire, S. Borot, M. Doron, P. Jallon, I. Xhaard, V. Melki, L. Meyer, B. Delemer, M. Guillouche, L. Schoumacker-Ley ,A. Farret, D. Raccah, S. Lablanche, M. Joubert, A. Penfornis, G. Charpentier, on behalf of the DIABELOOP WP7 Trial Investigators
The Lancet Digital Health, 1 (2019) e17
Doi: 10.1016/S2589-7500(19)30003-2

Experts

2 Research Directors
3 International Expert
10 Senior Experts
14 Experts
13 owning the HDR.

Participation in normalization groups

Convenior (Nicolas Verplanck) of the European (CEN/TC332/WG7 and International ISO/TC48/WG3 regulation groups for the normalization of microfluidic systems.

International Collaborations

MIT (USA)
Politecnico di Milano, University of Pisa (Italy)
Helmoltz Association, Franhaufer, Charité Berlin (Germany)
University of Twente, UMC Ultrecht (Netherland)
Tyndall (Ireland)
University of Birmingham (United Kingdom)
SINTEF (Norway)
University of Aalto (Sweden)
CIDETEC, CSIC (Spain)
University of Sidney (Australia)
CSEM, EMPA (Switzerland)
Nanomedicine European Platform (Europe)

01 BIOMATERIALS & DRUG DELIVERY

- Treatment for resistant bacteria
- Biobased antimicrobial dressings
- Cancer photodynamic therapy
- Electrospun hyaluronic acid nanofibers

Co-delivery of free vancomycin and transcription Factor decoy-nanostructured lipid carriers can Enhance inhibition of methicillin resistant Staphylococcus A+ureus (MRSA)

RESEARCH TOPIC:
Bacterial resistance, Vancomycin, Cationic nanostructured lipid-carriers, Chitosan nanoparticles, Transcription factor decoys

AUTHORS:
A. Hibbitts, A. Lucía, I. Serrano-Sevilla, L. De Matteis, M. McArthur, J. M. de la Fuente, J. A. Aínsa, F. Navarro

Bacterial resistance to antibiotics is widely regarded as a major public health concern with last resort MRSA treatments like vancomycin now encountering resistant strains. TFDs (Transcription Factor Decoys) are oligonucleotide copies of the DNA-binding sites for transcription factors. They inhibit transcription of many genes in bacteria. TFDs present as a potential method for inhibiting microbial growth without encountering typical resistance mechanisms. However, the efficient protection and delivery of the TFDs inside the bacterial cells is a critical step for the success of this technology. Therefore, in our study, specific TFDs against S. aureus were complexed with two different types of nanocarriers: cationic nanostructured lipid carriers (cNLCs) and chitosan-based nanoparticles (CS-NCs). Co-delivery of cNLC-TFD with vancomycin reduced the MIC of vancomycin by over 50% in MSSA and resulted in significant decreases in viability compared with vancomycin alone in MRSA cultures. To our knowledge, this is the first attempt at a combined antibiotic/oligonucleotide-TFD approach to combatting MRSA and, as such, highlights a new avenue of MRSA treatment combining traditional small molecules drugs and bacterial gene inhibition.

SCIENTIFIC COLLABORATIONS: Universidad de Zaragoza (Spain), CIBER (Madrid, Spain), University East Anglia (Norwich, UK)
This work was undertaken as part of the NAREB European research network supported by the EU FP7 (grant agreement 604237)

Context and Challenges

Antimicrobial resistance to conventional antibiotics is an increasingly serious threat to global public health that requires urgent action. Typically, antimicrobial-resistant infections carry higher incidents of mortality and present a considerable economic burden of over 20 billion dollars per year in the US alone. Of the multitude of microorganisms currently presenting antimicrobial resistant strains, Staphylococcus aureus represents a particularly serious challenge to healthcare professionals. S. aureus is a versatile Gram-positive human pathogen that is commonly found in the respiratory tract, open wounds and the urinary tract among others. Furthermore, the World Health Organisation (WHO) published recently that there is a high priority for developing novel antimicrobials against MRSA [1]. Although vancomycin has been used for over 40 years, it remains the standard treatment for infections caused by MRSA with the obvious dangers of this over-reliance now becoming apparent. Specifically, reports describing clinical failures of vancomycin treatment due to the emergence of S. aureus with reduced vancomycin susceptibility have now been published [2]. To address the growing threat of antibiotic resistance, effective alternatives to the use of antibiotics are in high demand [16](16). Recent research among the authors has focused on the development of transcription factor decoys (TFDs) as a new avenue of inhibiting bacterial replication. TFDs are short length oligonucleotides (10-80 base pairs) carrying the conventional binding sequence of a bacterial essential transcription factor [3].

Like other nucleic acid-based technologies, the successful delivery and protection of the cargo prior to reaching the inside of the bacteria is an essential step in order to achieve an efficient therapeutic effect. Therefore, this study investigated the suitability of two different nanoparticle systems to deliver TFDs to both methicillin susceptible S. aureus (MSSA) and MRSA. The nanocarriers investigated were developed in-house and consisted of a cationic nanostructured lipid carrier (cNLC) and chitosan-based nanocarrier (CS-NCs).

Main Results

Initial experiments were undertaken using the MSSA reference strain CECT794. Formulations displaying anti-microbial effects were brought forward for testing in CECT5190, which is the methicillin resistant strain commonly used for drug susceptibility assays. cNLC-TFD nanocomplexes did not demonstrate any antibacterial activity in MSSA strains by themselves. However, the presence of cNLC-TFD nanocomplexes in combination with vancomycin resulted in a 50% decrease in the MIC. Following this, the cNLC-TFD nanocomplexes were assayed to determine if the boosting effect when co-administered with vancomycin was also possible in MRSA. It was found that while there was not total inhibition of the MRSA growth, there were significant decreases in viability observed when the samples were treated with cNLC-TFD nanocomplexes in the presence of vancomycin (fig 1) [4]. Furthermore, no cellular toxicity or hemolytic activity were observed by using cNLC-TFD nanocomplexes.

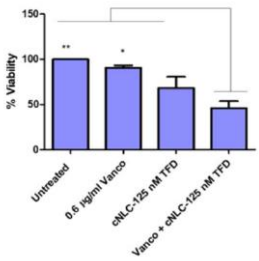


Figure 1: Synergy assay demonstrating enhanced antimicrobial effect against MRSA strain CECT 5190

Perspectives

This approach has remained unreported but highlights a new possibility in overcoming resistance mechanisms in S. aureus. Optimizations are being carried out in order to further improve their combined efficacy against MRSA. It is hoped that in-depth screening in biofilm models and *in vivo* infection studies may occur. This paves the way of new therapeutic strategies to fight against antimicrobial resistance.

RELATED PUBLICATIONS:
[1] https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
[2] Melo-Cristino J, Resina C, Manuel V, Lito L, Ramirez M. First case of infection with vancomycin-resistant Staphylococcus aureus in Europe. The Lancet. 2013;382(9888):205.
[3] Marín-Menéndez A, et al. Antimicrobial Nanoplexes meet Model Bacterial Membranes: the key role of Cardiolipin. Scientific reports. 2017;7:41242.
[4] A. Hibbitts, A. Lucía, I. Serrano-Sevilla, L. De Matteis, M. McArthur, J. M. de la Fuente, J. A. Aínsa, F. Navarro. Co-Delivery of Free Vancomycin and Transcription Factor Decoy-Nanostructured Lipid Carriers Can Enhance Inhibition of Methicillin Resistant Staphylococcus aureus (MRSA) PLoS ONE Vol14, Issue 9, 2019

Towards biobased antimicrobial Dressings based on functionalized Nanocellulose using supercritical CO_2

RESEARCH TOPIC:

Nanocellulose, supercritical CO_2, antimicrobial, functionalization, cryogel, aerogel, green chemistry, biomaterials

AUTHORS:

G. Nonglaton, C. Darpentigny, P. Marcoux, F. Ricoul, M. Menneteau

In this work, we present an innovative strategy for functionalizing nanocellulose-based materials using supercritical CO_2. This green chemistry strategy has been applied here to the development of new antimicrobial materials. Several antibacterial agents have been used. On one side, ciprofloxacin, a synthetic antibiotic and thymol, a natural molecule from essential oil and used for its antiseptic, antibacterial and antifungal properties have been impregnated for diffusion inhibition. On the other side, an aminosilane was covalently grafted to the structures for contact killing. The cytotoxicity and the antimicrobial properties of the materials were assessed. The results are very promising for the design of antimicrobial biobased and biocompatible medical devices using supercritical conditions.

SCIENTIFIC COLLABORATIONS: Bruno JEAN (CERMAV-CNRS), Julien BRAS (LGP2-PAGORA)

Context and Challenges

In a context where the demand for innovative medical devices is increasing and where the environmental question is becoming a major concern, the objective of this project was to prepare antimicrobial dressings by following as much as possible the principles of "green chemistry". For this, nanocelluloses were selected as biosourced and biocompatible basic bricks for the design of porous architectures. Their functionalization by antimicrobial agents was then undertaken in supercritical CO_2 medium (scCO2) used as an alternative to current organic solvents by taking advantage of its specific features such as high diffusivity, easy removal of solvent and residual reagents and compatibility with fragile materials.

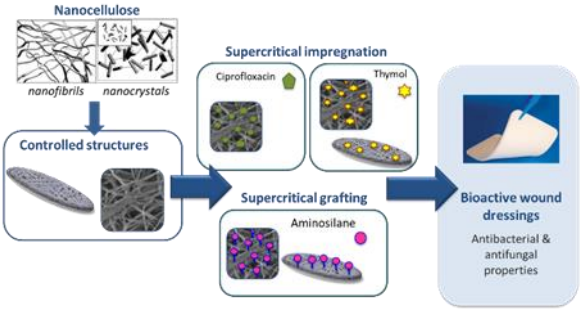


Fig. 1: Synopsis of preparation and functionalization of antimicrobial nanocellulose materials

Main Results

Thus, 2D and 3D structures, nanopapers, cryogels and aerogels, having varied properties in terms of porosity, morphology and specific surface were prepared from cellulose nanofibrils (CNFs) and nanocrystals (CNCs) using different techniques such as lyophilization or critical point drying. [1, 2] First, in order to introduce antibacterial functionality, cryogels with varied surface chemistries were impregnated in scCO2 with a synthetic antibiotic, ciprofloxacin. Impregnated cryogels exhibited antibacterial activity against both Gram-negative and Gram-positive bacterial strains (*Fig. 1*). Then, four materials of increasing specific surface area, all prepared from CNFs, were impregnated with an essential oil molecule, thymol (*Fig. 2A*) . Results show a direct relationship between of the amount of impregnated molecules and the specific surface that leads in the case of cryo- and aerogels to good antimicrobial properties against two types of bacteria and yeast. [3] The second functionalization strategy focused on the covalent grafting of an antimicrobial amino silane in scCO2 to increase the duration of effectiveness of the antimicrobial effect (*Fig. 2B*). Surface analysis methods (X-ray photoelectron spectroscopy, contact angle andsurface zeta potential analysis) have confirmed the success of the grafting. The cytotoxicity was studied by investigating the cell viability in contact and no or low toxicity was revealed. Antibacterial properties on contact have been confirmed. [4]

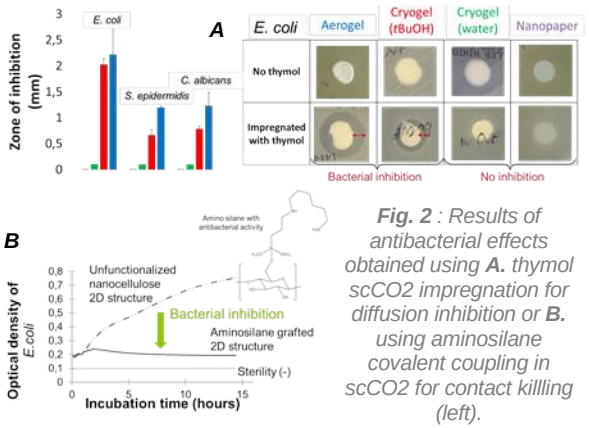


*Fig. 2 : Results of antibacterial effects obtained using **A.** thymol scCO2 impregnation for diffusion inhibition or **B.** using aminosilane covalent coupling in scCO2 for contact killling (left).*

Perspectives

These newly designed materials are very promising for the design of antimicrobial biobased and biocompatible medical devices using supercritical conditions. Perspectives for topical administration and wound dressing design could be considered. Furthermore, these innovative and versatile functionalization methods could be applied to different bio-based materials.

RELATED PUBLICATIONS:

[1] Darpentigny, C.; Molina-Boisseau, S.; Nonglaton, G.; Bras, J.; Jean, B., Ice-templated freeze-dried cryogels from tunicate cellulose nanocrystals with high specific surface area and anisotropic morphological and mechanical properties. *Cellulose* **2020**, *27* (1), 233-247. https://doi.org/10.1007/s10570-019-02772-8

[2] Darpentigny, C.; Nonglaton, G.; Bras, J.; Jean, B., Highly absorbent cellulose nanofibrils aerogels prepared by supercritical drying. *Carbohydr. Polym.* **2020**, *229*, 115560. https://doi.org/10.1016/j.carbpol.2019.115560

[3] Darpentigny, C.; Marcoux, P. R.; Menneteau, M.; Michel, B.; Ricoul, F.; Jean, B.; Bras, J.; Nonglaton, G., Antimicrobial Cellulose Nanofibril Porous Materials Obtained by Supercritical Impregnation of Thymol. *ACS Appl. Bio Mater.* **2020**, *3* (5), 2965-2975. https://doi.org/10.1021/acsabm.0c00033

[4] Darpentigny, C.; Sillard, C.; Menneteau, M.; Martinez, E.; Marcoux, P. R.; Bras, J.; Jean, B.; Nonglaton, G., Antibacterial cellulose nanopapers via aminosilane grafting in supercritical carbon dioxide, *submitted to ACS Appl. Bio Mater*.

Verteporfin-loaded nanostructured lipid carriers improve ovarian cancer photodynamic therapy *in vitro* and *in vivo*

RESEARCH TOPIC:
Photo Dynamic Therapy (PDT). Drug Delivery System. Nanovectorization. Lipid Nanoparticles.

AUTHORS:
T. Michy, T. Massias, C. Bernard, L. Vanwonterghem, M. Henry, M. Guidetti, G. Royal, JL. Coll, I. Texier, V. Josserand, A. Hurbin

Advanced ovarian cancer is the most lethal gynecological cancer, with a high rate of chemoresistance and relapse. Photodynamic therapy offers new prospects for ovarian cancer treatment, but current photosensitizers lack tumor specificity, resulting in low efficacy and significant side-effects. In the present work, the clinically approved photosensitizer verteporfin was encapsulated within nanostructured lipid carriers (NLC) for targeted photodynamic therapy of ovarian cancer. Encapsulation prolonged blood lifetime of verteporfin and slowed down drug uptake in tumor cells, but importantly considerably decreased drug dark toxicity. Overall, verteporfin-NLC based photodynamic therapy was both safe and efficient to decrease tumor growth.

SCIENTIFIC COLLABORATIONS: Institute for Advanced Biosciences, INSERM U1209, CNRS UMR5309. Centre Hospitalier Universitaire Grenoble Alpes. CNRS UMR5250, Département de Chimie Moléculaire, Université Grenoble Alpes.

Context and Challenges

Ovarian cancer accounts for about 4% of worldwide cancer incidence and is the most lethal gynecological cancer [1]. Seventy five percent of ovarian cancer cases are detected in late stages, and the 5-year survival rate of patients in advanced-stages is barely 30% [2]. Due to its non-symptomatic advancement, high metastases rate, and its resistance to chemotherapy, treatment of ovarian cancer constitutes a clinical challenge. Photodynamic therapy (PDT) offers new prospects for ovarian cancer treatment, but current photosensitizers lack tumor specificity, resulting in low efficacy and significant side-effects. In the present work, the clinically approved photosensitizer verteporfin was encapsulated within nanostructured lipid carriers (NLC) for targeted photodynamic therapy of ovarian cancer.

Main Results

Verteporfin encapsulation yield in NLC was quantitative (> 95%) and yielded as expected neutral nanoparticles of 47.9 ± 1.0 nm (polydispersity index < 0.2) [3]. NLC-verteporfin displayed similar absorption and emission profiles compared to free verteporfin in culture medium, showing that verteporfin molecules were non aggregated inside the NLC lipid core.

Cellular uptake and phototoxicity of free verteporfin and NLC-verteporfin were studied *in vitro* in human ovarian cancer cell lines cultured in 2D and 3D-spheroids. The cellular uptake of NLC-verteporfin was found to be slower (maximum 24h after incubation) than for free verteporfin (maximum 2h after incubation) in three different ovarian cancer cell lines. Both free verteporfin and NLC-verteporfin induced high phototoxicity in ovarian cancer cells *in vitro*. Increasing concentrations of free verteporfin or NLC-verteporfin exposed to NIR light significantly reduced the viability of SKOV3 and OVCAR3 cells cultured in monolayers and in spheroids, whereas they had no toxic effect on their proliferation in the dark.

Biodistribution and photodynamic therapy of free and NLC-loaded verteporfin were then evaluated *in vivo* in mice. *In vivo* biodistribution and pharmacokinetic studies evidenced a long circulation time of NLC associated with efficient tumor uptake (*Fig. 1A*). Administration of 2 mg.kg^{-1} free verteporfin induced severe phototoxic adverse effects leading to the death of 5 out of 8 mice. In contrast, laser light exposure of tumors after intravenous administration of NLC-verteporfin (8 mg.kg^{-1}) significantly inhibited tumor growth without visible toxicity (*Fig. 1 B*).

NLC-verteporfin thus led to efficient photosensitizer vectorization to the tumor site and protection from side-effects, providing promising therapeutic prospects for cancer PDT.

Perspectives

In this first study, verteporfin dose and latent time between verteporfin-NLC injection and photo-treatment appeared as key parameters to obtain full benefit of this innovative ovarian cancer treatment. These parameters and others, such as the number of therapeutic sessions, will be further investigated for treatment optimization, paving the way towards clinical trials.

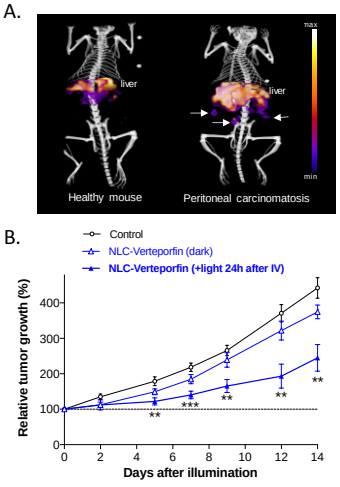


Fig. 1 :

***A**. PET/Fluorescence images evidence uptake of NLC-verteporfin in peritoneal carcinomatosis nodules and*

***B.** NLC-verteporfin in combination with light significantly inhibit tumor growth*

RELATED PUBLICATIONS:
[1] Torre, L.A.; Islami, F.; Siegel, R.L.; Ward, E.M.; Jemal, A. Global Cancer in Women: Burden and Trends. Cancer Epidemiol. Biomarkers Prev. 2017, 26, 444–457, 10.1158/1055-9965.EPI-16-0858,
[2] Narod, S. Can advanced-stage ovarian cancer be cured? Nat. Rev. Clin. Oncol. 2016, 13, 255–261,10.1038/nrclinonc.2015.224,
[3] T. Michy, T. Massias, C. Bernard, L. Vanwonterghem, M. Henry, M. Guidetti, G. Royal, J.L. Coll, I. Texier, V. Josserand, A. Hurbin, Verteporfin-Loaded Lipid Nanoparticles Improve Ovarian Cancer Photodynamic Therapy In Vitro and In Vivo, Cancer 2019, 11, 1760, 10.339/cancers11111760

Electrospinning in water and in situ crosslinking Of hyaluronic acid/cyclodextrin nanofibers: Towards wound dressing with controlled Drug release

RESEARCH TOPIC:
Nanofibers, hyaluronic acid, electrospinning, wound dressing, cyclodextrin

AUTHORS:
M. Seon-Lutz, A- C. Couffin, S. Vignoud, G.Schlatter, A. Hebraud

Hyaluronic acid (HA) is a polysaccharide for which biocompatibility and bioactivity properties make it a very interesting compound as wound dressings. In this work, biocompatible insoluble HA-based nanofibrous dressings were designed by electrospinning in pure water in order to overcome any toxicity issues. To this purpose, poly(vinyl alcohol) and hydroxypropyl-β-cyclodextrin were added to form uniform nanofibrous scaffolds. An in situ crosslinking process of the scaffolds is also investigated to ensure the stability of the fibrous structure during the use of the dressing. For opening the scope of wound application, pathways of functionalization of these materials have been envisaged. For this purpose, the direct impregnation of naproxen, a model drug with anti-inflammatory properties, has been performed into the scaffolds either in aqueous solution or under supercritical CO_2.

SCIENTIFIC COLLABORATIONS: ICPEES, Strasbourg University

Context and Challenges

Hyaluronic acid (HA) is widely investigated due to its high potential for wound dressing applications. The fabrication of biomimetic HA-based scaffolds by electrospinning, a process allowing the formation of polymeric nanofibers, is thus extensively studied. However, HA is often dissolved in toxic organic solvents to allow the efficient production of electrospun nanofibers. Indeed, although HA is soluble in water, its ionic nature leads to high viscosity of aqueous HA solutions without insuring enough chain entanglements necessary for a stable electrospinning [1].
The goal of this work was to prepare biocompatible insoluble HA-based nanofibers using only water as solvent during the electrospinning process.

Main Results

To electrospun HA in aqueous solutions, poly(vinyl alcohol) (PVA) was added as a carrier polymer and it was found that the addition of hydroxypropyl-βcyclodextrin (HPβCD) stabilized the process of electrospinning and led to the efficient formation of uniform nanofibrous scaffolds [2]. As illustrated in *Fig. 1*, the resulting membranes are composed of uniform nanofibers with an average diameter of 150 ± 30 nm. An in situ crosslinking process of these scaffolds is also realized, by adding the coupling of EDC with NHS, known to form ester bonds between carboxylic acid groups and hydroxyl groups, insuring a whole fabrication process of insoluble membranes without any toxicity. Furthermore, the beneficial presence of HPβCD in the nanofibers, known for its encapsulation capabilities [3], paves the way for wound dressing applications with controlled drug encapsulation-release properties. As a proof of concept, naproxen, a non-steroidal anti-inflammatory drug was chosen as a model drug. Naproxen was impregnated into the scaffolds either in aqueous solution or under supercritical CO_2. Drug was released from the functional nanofibrous scaffolds over several days, with a maximum of release during the first 24 h without losing the fibrous structure. Thus, the scaffolds are ideal candidates to deliver a drug directly to the wound, with a fast enough kinetics to counter the inflammation and avoid acute pain.

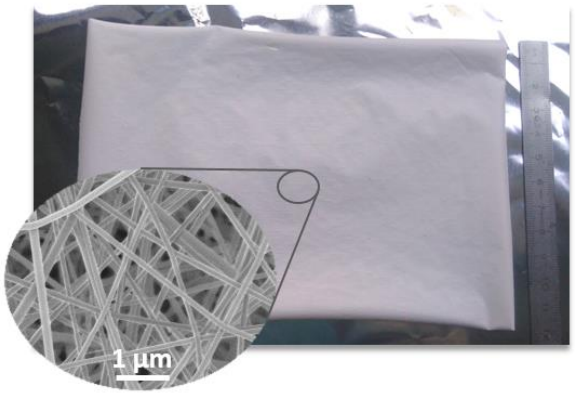


***Fig. 1**: HA-PVA-HPβCD nanofibrous membrane formed by electrospinning*

Perspectives

This study proposes a simple approach to form stable HA-based nanofibrous scaffolds embedding HPβCD using water as the only solvent, enabling the development of safe functional wound dressings. Thanks to possible host-guest supramolecular interactions between HPβCD and a wide variety of molecules, scaffolds with drug encapsulation and release properties can be envisaged for multiple drugs and diverse biomedical applications.
Furthermore, following this study, it has also been demonstrated that it was possible to incorporate, within the nanofibers, lipid nanoparticles in which drugs can be encapsulated and delivered to the wound site [3].

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[4] Séon-Lutz M. (2019) *Elaboration et caratérisation de matériaux nanofibreux fonctionnels à base d'acide hyaluronique et de nanoparticules lipidiques pour des applications à usage biomédical*, Thèse de doctorat, http://www.theses.fr/2019STRAE026


Graphique
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Temps en heure
Insuline (U/h)
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02 DIGITAL MEDICAL DEVICES

- Characterization of daily glycemic variability
- Physical activity and glucose prediction
- Closed-loop insulin delivery

Characterization of daily glycemic Variability in subjects with type 1 Diabetes using a mixture of metrics

RESEARCH TOPIC:
Diabetes, multivariate statistics, glycemic variability

AUTHORS:
F Zheng, M Jalbert, F Forbes, S Bonnet, A Wojtusciszyn, S Lablanche, P-Y Benhamou

Glycemic variability is a key component in glycemic control in patients with type-1 diabetes (T1D). Continuous Glucose Monitoring (CGM) systems allow quantifying accurately the intra- and inter-day variations of interstitial glycemia. The papers aims at using modelization techniques taken from multivariate statistics and machine learning to propose a new score that combines in a statistical way the different metrics of glycemia variability taken from the literature. An approach based on a reference population modeling is described, the score is simply the log-likelihood of an observation according to the reference model. The reference population dataset is built from data collected in the TRIMECO clinical trial (NCT01148680) where patients have been transplanted with pancreatic islets and thus restore their glycemic control after islet transplantation.

SCIENTIFIC COLLABORATIONS: INRIA, CHU Grenoble-Alpes, CHU Montpellier

Context and Challenges

In the literature, different metrics have been proposed to evaluate the glycemic control of a T1D patient. Some metrics are based on simple statistics from the CGM signal (e.g. variability coefficient), on the extrema amplitudes (e.g. MAGE: mean amplitude glycemic excursion) and some are purely heuristic. So far, no consensus has been reached among physicians as to which metrics to use in practice. However, doctors need quantitative tools to assess the diabetes liability of a T1D patient in order to propose an islet transplantation procedure. This work is an attempt to provide them such tool.

Main Results

In collaboration with Pr. P-Y Benhamou, CHU Grenoble, we have used the TRIMECO islet transplantation clinical trial to build a reference model [1]. In this study, data are collected before transplant and 6 and 12 months after [2]. For each patient, since we know their beta-score (medical composite score), we are able to isolate data that correspond to a (back to) normal glycemic control. These data are used to build a reference model using multivariate statistics.
More precisely, we model the distribution of classical daily glycemic variability metrics using a mixture of multivariate Student distribution [1]. The EM algorithm is used to estimate the model parameters. Using this reference model, we can then compute the likelihood of an observation, as presented in the framework depicted in *Fig. 1*.

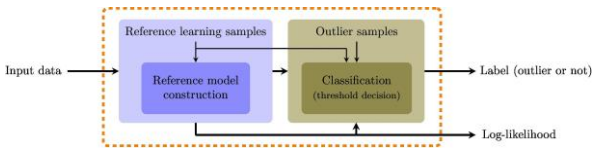

***Fig. 1**: Framework used to compute the likelihood of an observation*

If the likelihood is high, the day will be quantified as stable and below a learned threshold, it will be labelled as instable.
The proposed characterization framework integrates multiple standard metrics and provides a comprehensive daily glucose variability (GV) index, based on which, long-term variability evaluations and investigations on the implicit link between variability and beta-score can be carried out (*Fig. 2*). Evaluation, in a daily GV classification task, shows that the proposed method is highly concordant to the experience of diabetologists.

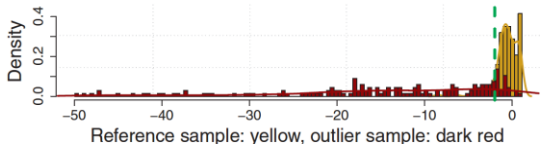

***Fig. 2**: Distribution of the Log likelihood for Inliers (days qualified as stable coming from the reference sample) and Outliers (days qualified as instable) Threshold is also learnt from data.*

Perspectives

A multivariate statistical model is proposed to characterize the daily glucose variability of subjects with T1D. The model has the advantage to provide a single variability score that gathers the information power of a number of canonical scores, too partial to be used individually. A reliable decision rule to classify daily variability measurements into stable or unstable is also provided.
This new metric can for instance be used to assess the gain in glycemic control between T1D patients using open-loop treatment and T1D patients using a closed-loop artificial pancreas system. The proposed framework can also accept new metrics and new reference samples.

RELATED PUBLICATIONS:
[1] Fei Zheng, Manon Jalbert, Florence Forbes, Stéphane Bonnet, Anne Wojtusciszyn, Sandrine Lablanche, and Pierre-Yves Benhamou. Diabetes Technology & Therapeutics. Apr 2020. 301-313. http://doi.org/10.1089/dia.2019.0250
[2] Jalbert, M., Zheng, F., Wojtusciszyn, A. et al. Glycemic variability indices can be used to diagnose islet transplantation success in type 1 diabetic patients. Acta Diabetol 57, 335-345 (2020), https://doi.org/10.1007/s00592-019-01425-3.

ARX model for interstitial glucose Prediction during and after Physical activities

RESEARCH TOPIC:
Closed-Loop, Diabetes, Control.

AUTHORS:
H Romero-Ugalde, M Garnotel, M Doron, P Jallon, G. Charpentier, S Franc, E Huneker, C Simon, S Bonnet

Since 2015, CEA-Leti and Diabeloop SA team together to build a hybrid closed-loop artificial pancreas. Such system is primarily intended to people with type 1 diabetes that do not secret insulin. A therapeutic solution consists thus in a device that continuously monitors interstitial glucose and automatically delivers personalized insulin doses to maintain blood sugar in a normal range. One core ingredient of the artificial pancreas is the capacity to forecast glycaemia in the next coming hours based on insulin pump history and past interstitial glucose values. The article [1] aims to improve such glycaemia forecasting by taking into account other factors like energy expenditure and ingested carbohydrates that will impact the future glycaemia.

SCIENTIFIC COLLABORATIONS: Centre de Recherche en Nutriton Humaine (CRNH) Rhône-Alpes, DIABELOOP SA

Context and Challenges

The context of the article deals with diabetes therapeutics and more precisely artificial pancreas. Since 2016, three commercial solutions have been regulatory approved worldwide with systems from MedTronic (640G), Diabeloop (DBLG1, CE approval 2018) and recently Tandem (control-IQ).

One challenge to build a fully automatized artificial pancreas is to seamlessly account for physical activity so that the person does not have to warn the system that a physical exercise will start. Moreover it is difficult for a person to precisely quantify the intensity of the physical activity.

Physical activity is known to lower blood glucose so that insulin infusion should be decreased or even halted when a physical exercise is ongoing. Accelerometers and heart rate monitor can be used to precisely estimate energy expenditure and this information can be used in the glycaemia prediction.

Main Results

The article [1] presents the first autoregressive with exogenous input (ARX) model that uses energy expenditure, carbohydrates on board, and insulin on board as input to predict interstitial glucose (IG). The proposed model may be used for predicting IG even during physical activity (PA). The system has been patented in [3]

Two different databases were used to estimate and validate the proposed ARX model. In each database, CGM data, insulin pump history, accelerometer data, heart rate data and meal annotations (quantity of ingested carbohydrates) are fused by the model to predict IG between 30 and 120 minute ahead. An illustration is shown in *Fig. 1*.

The accelerometer and heart rate data are first transformed into energy expenditure (EE) using the approach described in [2]. Such transformation has been validated using labeled data from a previous experiment in collaboration with CRNH, Lyon (Prof. C. Simon). A vigorous EE will lower the blood glucose ahead and thus EE is a good predictor in the ARX model.

Furthermore, Insulin on Board (IoB) and Carbohydrates on Board (CoB) are computed using standard pharmaco-kinetic (PK) models. These quantities, if non zero, will also affect the future glycaemia and, thus, are good regressor variables in the ARX model.

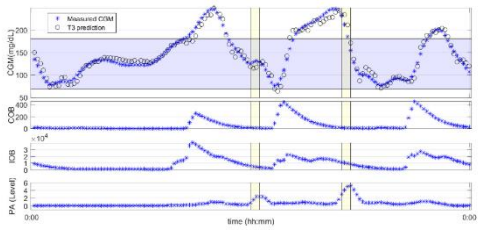

***Fig. 1** : Interstitial glucose (IG) prediction 30 min ahead by a patient specific ARX model using as inputs EE, CoB and IoB. Every 5 min, the ARX model uses these inputs to predict IG 30 min ahead.*

A population-based model, obtained from a first database composed of 14 type 1 diabetes (T1D) patients, achieved a root-mean-square error (RMSE) of 16.7 ± 15.6 mg/dL, on IG prediction (30-min ahead) at the end of a PA, on a second database (15 T1D patients). Patient-specific ARX models, obtained on the second database, improved prediction accuracy (RMSE = 7.8 ± 4.5 mg/dL), outperforming the results found in the literature.

Perspectives

A perspective of this work is the physical implementation of such models in an artificial pancreas with onboard accelerometer data processing and model personalization. These developments will allow fully automated artifical pancreas with a minimum user interaction. It could also be beneficial for young adults or teens that have a somewhat higher physical activity level.

RELATED PUBLICATIONS:
[1] H Romero-Ugalde, M Garnotel, M Doron, P Jallon, G Charpentier, S Franc, E Huneker, C Simon, S Bonnet (2019) ARX model for interstitial glucose prediction during and after physical activities, Control Engineering Practice, Control Engineering Practice · Volume 90, September 2019, Pages 321-330.
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[3] H Romero-Ugalde, S Bonnet, M Doron (2018) Système de prédiction de la glycémie d'un patient. FR3079130A1, Mar. 2018

Closed-loop insulin delivery in adults with type 1 Diabetes in real-life conditions: a 12-week Multicentre, open-label randomised Controlled crossover trial

RESEARCH TOPIC:
Automated Insulin Delivey, Closed-Loop, Diabetes, Type 1 Diabetes, Control.

AUTHORS:
P-Y. Benhamou, M. Doron, P.Jallon, et al., on behalf of the DIABELOOP WP7 Trial Investigators

Development of a hybrid closed-loop system for the Automated Insulin Delivery for People living With Type 1 Diabetes started in 2012 with a collaboration between CERITD and CEA-LETI. Since 2015, a common laboratory with Diabeloop SA, CERITD and CEA-LETI furthers develops the prototypes and improvements in the commercial product. Closed-loop insulin delivery systems are expected to become a standard treatment for patients with type 1diabetes. In the paper, we aimed to assess whether the Diabeloop Generation 1 (DBLG1) hybrid closed-loop artificial pancreas system improved glucose control compared with sensor-assisted pump therapy.

SCIENTIFIC COLLABORATIONS: CERITD, DIABELOOP SA, Centre de Recherche en Nutrition Humaine (CRNH) Rhône-Alpes, 12 Centres hospitalo-universitaires français (CHSF, CHUGA, Besançon, Caen, Lyon, Marseille, Montpellier, Nancy, Nantes, Reims, Strasbourg, Toulouse

Context and Challenges
In France, 200 000 patients live with Type1 diabetes, a chronic lifelong condition, which leads to a daily burden of the treatment and comorbidities. In this multicentre, open-label, randomised, crossover trial, we recruited adults (aged ≥18 years) with at least a 2 year history of type 1 diabetes, who had been treated with external insulin pump therapy for at least 6 months, had glycated haemoglobin (HbA1c) of 10% or less. After a 2-week run-in period, patients were randomly assigned (1:1) with a web-based system in randomly permuted blocks of two, to receive insulin via the hybrid closed-loop system (DBLG1; using a machine-learning-based algorithm) or sensor-assisted pump therapy over 12 weeks of free living, followed by an 8-week washout period and then the other intervention for 12 weeks. The primary outcome was the proportion of time that the sensor glucose concentration was within the target range (3·9–10·0 mmol/L) during the 12 week study period. Efficacy analyses were done in the modified intention-to-treat population [1-4].

Main Results
Between March 3, 2017, and June 19, 2017, 71 patients were screened, and 68 eligible patients were randomly assigned to the DBLG1 group (n=33) or the sensor-assisted pump therapy group (n=35), of whom five dropped out in the washout period (n=1 pregnancy; n=4 withdrew consent). 63 patients completed both 12 week treatment periods and were included in the modified intention-to-treat analysis. The proportion of time that the glucose concentration was within the target range (*Fig. 1*) was significantly higher in the DBLG1 group (68·5% [SD 9·4] than the sensor-assisted pump group (59·4% [10·2]; mean difference 9·2% [95% CI 6·4 to 11·9]; p<0·0001). Five severe hypoglycemic episodes occurred in the DBLG1 group and three episodes occurred in the sensor-assisted pump therapy group, which were associated with hardware malfunctions or human error.

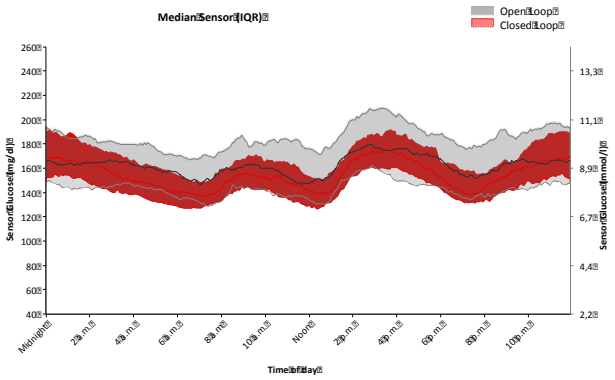
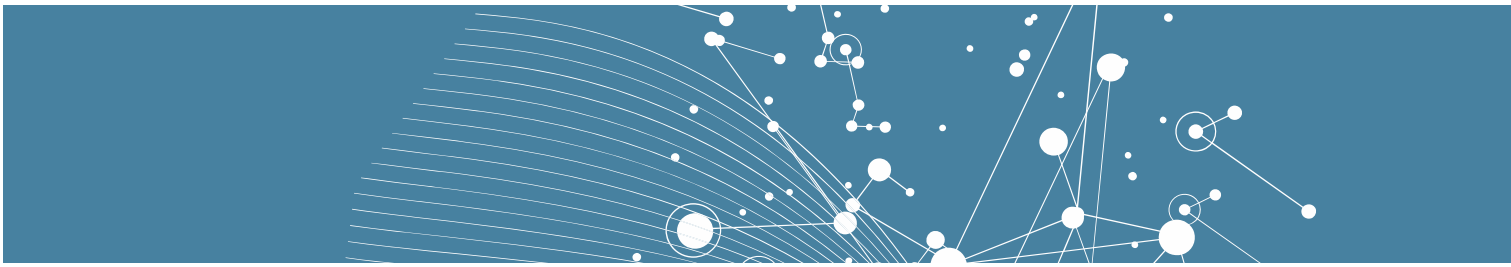


***Fig. 1**: Median (IQR) sensor glucose concentrations during closed-loop and control periods for the 24 h duration over the study period. In red: closed-loop and in grey: control period.*

Perspectives
The DBLG1 system improves glucose control compared with sensor-assisted insulin pumps. This finding supports the use of closed-loop technology combined with appropriate health care organisation in adults with type 1 diabetes.

RELATED PUBLICATIONS:
[1] Benamou P-Y. et al, " Closed-loop insulin delivery in adults with type 1 diabetes in real-life conditions: a 12-week multicentre, open-label randomised controlled crossover trial", The Lancet - Digital Health, 1, e17, 2019.
H. Hanaire, S. Franc S. Borot, A. Penfornis, P.-Y. Benhamou, P. Schaepelynck, E. Renard, B. Guerci, N. Jeandidier, C. Simon P. Hannaert, I. Xhaard, M. Doron, E. Huneker, G. Charpentier, Y. Reznik, "Efficacy of the Diabeloop closed-loop system to improve glycaemic control in patients with type 1 diabetes exposed to gastronomic dinners or to sustained physical exercise", Diabetes Obes Metab., 1–11, 2019.
[2] R. Blanc ; H. M. Romero Ugalde ; P.-Y. Benhamou ; G. Charpentier ; S. Franc ; E. Huneker ; E. Villeneuve ; M. Doron, "Modeling the variability of insulin sensitivity for people with Type 1 Diabetes based on clinical data from an artificial pancreas study", 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC 2019), 23/07/2019 - 27/07/2019, Berlin, Allemagne, 2019.
[3] Benhamou P. Y., E. Huneker, S. Franc, M. Doron, and G. Charpentier on behalf of the Diabeloop Consortium, "Customization of home closed-loop insulin delivery in adult patients with type 1 diabetes, assisted with structured remote monitoring: the pilot WP7 Diabeloop study", Acta Diabetologica, 55, 549-556, 2018.



03 NON-CONVENTIONAL MICROSCOPY

- Quantitative phase imaging of adherent cells
- Platelet counting
- Large field of view phase and fluorescence
- Compact phase and fluorescence microscope

Quantitative phase imaging of Adherent mammalian cells: A comparative study

RESEARCH TOPIC:
Quantitative cell Imaging

AUTHORS:
C. Allier, L. Hervé, O. Mandula, P. Blandin, Y. Usson, J. Savatier, S. Monneret, S. Morales

Quantitative phase imaging (QPI) methods have several advantages when monitoring adherent mammalian cell cultures. Because of low photo-toxicity and no need for staining, we can follow cells in a minimally invasive way over a long period. The ability to measure the optical path difference in a quantitative manner allows the measurement of the cell dry mass, an important metric for studying the growth kinetics of mammalian cells. Here, we compare for the first time cell measurements obtained with three different techniques: digital holographic microscopy, lens-free microscopy and quadriwave lateral sheering interferometry. We report a linear relationship between optical volume density values measured with these different techniques and estimate the precisions of this measurement for the different individual instruments.

SCIENTIFIC COLLABORATIONS: TIMC-IMAG, Uni. Grenoble Alpes, Institut Fresnel, Marseille, France

Context and Challenges

In this article [1], we present a comparison study of three different QPI techniques [2]. We used three commercially available QPI instruments: digital holographic microscopy (DHM) [3], quadriwave lateral sheering interferometry (LSI) [4] and lens-free microscopy (LFM) [5]. To ensure consistency, the measurements with the different techniques were performed on the same day, in the same room and on the same set of cells. Each instrument was operated by a separated experienced team using this particular instrument on daily basis. The present study is not an intrinsic comparison of different optical methods, since the instruments are based on different principles. The magnification, coherence of the source, detector type etc. cannot be matched. Nevertheless, this study brings together and compares for the first time the characteristics of different QPI methods, when measuring the exact same set of adherent mammalian cells. In the first place, this study is intended for biologists to help in experimental design using QPI. The choice of an optimal QPI method will depend on each particular experiment.

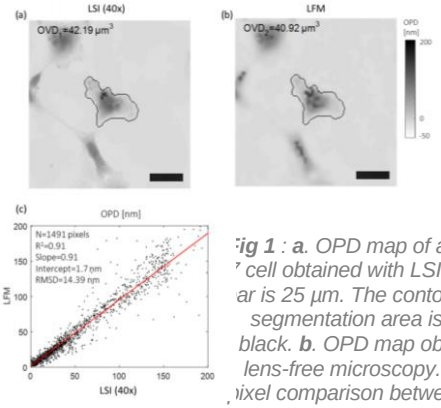


Fig 1 : a. OPD map of a fixed COS-7 cell obtained with LSI(40×). Scale bar is 25 µm. The contour of the cell segmentation area is shown in black. b. OPD map obtained with lens-free microscopy. c. Pixel to pixel comparison between the OPD maps (within segmented cell area).

Main Results

The comparison between the three different QPI methods shows a good agreement when measuring cell optical path difference (OPD) and optical volume difference (OVD) values. We estimated precision of the OVD measurements for different modalities from statistical analysis of pair-wise comparison measurements. The precision was in the range of 1 to 4 µm³ depending on the modality for cell OVD values ranging between 10 and 100 µm³ (*Fig. 1*). These precision values were achieved with finely adjusted parameters of the baseline subtraction and automatic segmentation algorithms (*Fig. 2*).

Perspectives

Importantly, the measurements obtained with lens-free microscopy correlate linearly with the measurements obtained with DHM and LSI. It implies that the lens-free microscopy setup developed at CEA Leti can thus be considered as a quantitative phase imaging technique for the measurements of adherent cells. We believe that the reported characteristics of the lens-free microscopy setup will help to design and interpret biological experiments with cellular cultures involving this technique.

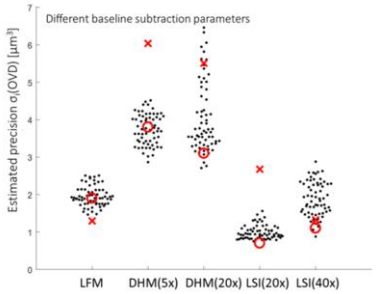


Fig. 2 : Estimations of the instrument precision. The different point correspond to different parameters used in the baseline subtraction algorithm. The red circles correspond to the results obtained with threshold values of 10 nm for LFM, 12.5 nm for DHM and 5 nm for LSI.

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High accuracy platelet counting Using lensfree imaging

RESEARCH TOPIC:
Microscopy, In-line holography, Lensfree, Hematology

AUTHORS:
D. Isèbe, E. Gremion, A. Daynès, B. Thouy, N. Rongeat, S. Bressieux, A. Ali-Cherif, V. Rebuffel, P. Joly, S. Morales, O. Cioni, P. Blandin

Flow cytometry is the main technology used in hematology analyzers. However, this technology requires bulky and complex hardware systems. Lens-free imaging is an emerging microscopy technique based on a simple and compact in-line holography setup. This technique enables to image a large field-of-view (≈30mm²) leading to statistical counting (>10 000 cells) in a single-shot acquisition consistent with performances required in hematology. We report high accuracy platelet count in 54 platelet-rich plasma samples. This accuracy can be achieved through a wise choice of the illumination spectral properties and an optimized algorithmic chain dedicated to small pure phase objects.

SCIENTIFIC COLLABORATIONS: HORIBA ABX SAS (Montpellier, France)

Context and Challenges

Complete blood count (CBC) is the first prescribed test in the world. This test includes platelet (PLT) counting. PLT counting could also be performed in platelet-rich plasma (PRP), that is investigated and used in the field of treatment of joint pathologies. The hematology laboratory analyzers used, based on flow cytometry, are bulky and quite complex systems.

Lensfree imaging is now a very widespread imaging modality [1-4], that reveals phase and absorption contrasts without staining requirement. The raw holograms acquired are "numerically" focused, phase and modulus maps of the field in the sample plane are reconstructed. Lensfree imaging can be implemented with a magnification factor close to 1, and then the field of view is large (few tens of mm²). The resolution is limited by the pixel size of the detector (between 1.2 µm and 4 µm for current CMOS). Consequently, it is relevant for blood cell population's study: it provides access to a significant number of objects (between a few hundreds and several tens of thousands), which enables to accede representative statistical information in a single-shot acquisition.

Whereas RBC and WBC are extensively studied in optical imaging, few results are reported on platelets: they are indeed smaller (typically between 2 and 4 µm greatest diameter) and exhibit pure phase contrast. Especially, to our knowledge, no statistical representative PLT counting has been reported with lensfree imaging. In this article [5], we present high precision platelet counting using lensfree imaging in PRP.

Main Results

Since PLT are small and possess low contrasts, to detect them we use a state-of-the art iterative reconstruction algorithm from a single image based on gradient L1-norm minimization, developed in our team. In the reconstructed map in a single plane, we do not detect all the cells present in the 100 µm chamber height. We have demonstrated that a multiplane detection is necessary.

We have also tested different light sources to study their influence on the platelet detection and counting. The "classical" light source consists of a RGB LED associated with a 150 µm pinhole, to ensure a sufficient spatial coherence to perform in-line holography. The blue and red LED emit light with peak wavelength of 450, 640 nm respectively and spectral bandwidth Δλ of approximatively 20 nm. Moreover, 405 and 650 nm laser diodes are also tested. The power supply intensity of these laser diodes is set below the laser threshold to adjust the spectral bandwidth of the emission. With these sources, no pinhole is required. Qualtitatively and quantitatively, we have demonstrated that the source with the shortest wavelength and the highest temporal coherence (laser diode at 405 nm with a spectral bandwidth of 4,6 nm) gives the best results (*Fig. 1*).

Perspectives

Future works will be oriented on whole blood, to demonstrate the potential of this method for in vitro diagnostic in hematology.

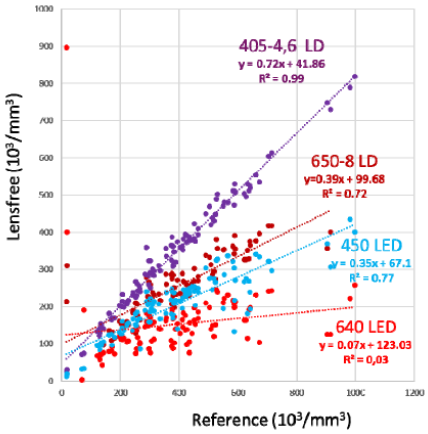

Fig. 1 : Correlations lensfree versus reference lab instrument (ABX Pentra 120®, HORIBA Medical, France) on 54 PRP for different illumination sources. 405-4.6 LD = 405 nm laser diode with Δλ = 4.6 nm; 650-8 LD = 650 nm laser diode with Δλ = 8.0 nm; 450 LED = 450 nm LED, 640 LED = 640 nm LED.

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[3] Liu et al., "A Microfluidic Cytometer for Complete Blood Count With a 3.2-Megapixel, 1.1- µm-Pitch Super- Resolution Image Sensor in 65-nm BSI CMOS", IEEE Transactions On Biomedical Circuits and System 11, 4, 794 (2017).
[4] Cornelis et al., "Fast and robust Fourier domain-based classification for on-chip lens-free flow cytometry", Optics Express 26, 11, 14329 (2018)
[5] D. Isèbe, et al., "High accuracy platelet counting using lensfree imaging", SPIE Proceedings Volume 11075, Novel Biophotonics Techniques and Applications V; 110750O (2019)

Large field-of-view phase and fluorescence mesoscope with microscopic resolution

RESEARCH TOPIC:
Microscopy, Fluorescence, Phase, In-line holography

AUTHORS:
I. de Kernier, A. Ali-Cherif, N. Rongeat, O. Cioni, S. Morales, J. Savatier, S. Monneret and P. Blandin

Phase and fluorescence are complementary contrasts that are commonly used in biology. However, the coupling of these two modalities is traditionally limited to high magnification and complex imaging systems. For statistical studies of biological populations, a large field-of-view is required. We describe a 30 mm^2 field-of-view dual-modality mesoscope with a 4-µm resolution. The potential of the system to address biological questions is illustrated on white blood cell numeration in whole blood and multiwavelength imaging of the human osteosarcoma (U2-OS) cells.

SCIENTIFIC COLLABORATIONS: Institut Fresnel, Aix-Marseille Université, Horiba ABX SAS (Montpellier France)

Context and Challenges

Microscopy systems continuously strive to provide superior image quality through higher resolution and signal-to-noise ratio (SNR), resulting in great imaging performances but with bulky, ultraspecific, and expensive systems. Furthermore, these systems tend to sacrifice the field-of-view (FOV) for the sake of resolution. Recently, some research teams have loosened the requirements for image quality and have started to develop miniaturized and affordable systems [1]. Unfortunately, these imaging systems are usually unimodal. Phase imaging [2] offers a label-free contrast to image unstained cells that would have a low contrast in brightfield microscopy. Phase contrast can be used to study cell morphology, cell–cell interactions,. Fluorescence provides a complementary contrast to phase when observing biological objects [3]. It allows obtaining specificity and has become a reference technique in biological microscopy. Specific cells, structures in a given cell, or specific functions of a cell can be highlighted. Combining phase and fluorescence contrasts has been demonstrated on systems imaging at diffraction- limited resolutions and beyond. In this article [4], we describe a wide FOV phase and fluorescence imaging system. To our knowledge, this is the first report of coupling fluorescence imaging and phase imaging on an ultrawide FOV with a micrometric resolution.

Main Results

We developed an experimental setup to perform phase and fluorescence imaging over a 30-mm^2 FOV without the need for postregistration of images. The system has a unique detection pathway and two separate illumination modules. The detection pathway included a consumer single lens reflex (SLR) camera lens and an industrial CMOS sensor. Semicoherent brightfield illumination was achieved by using a low-cost LED, and fluorescence excitation used either a LED or a laser diode. The phase map of the sample could be numerically retrieved from a single out-of-focus image. The multimodal resolution was found to be 3 to 4 µm. The FOV of our 1× magnification system allowed performing statistical studies of cells (*Fig. 1*).

Perspectives

The system described in this work was developed for cell population analysis. Our aim is to propose a single-shot imaging alternative to flow cytometry for specific applications in hematology. We believe in this case that phase contrast and diffraction flow cytometry can provide similar information, whereas fluorescence imaging gives indications comparable to fluorescence flow cytometry. However, spatial resolution brings additional information that cannot be obtained by conventional flow cytometry, such as cell morphology, spatial distribution, or the intracellular location of biomolecules.

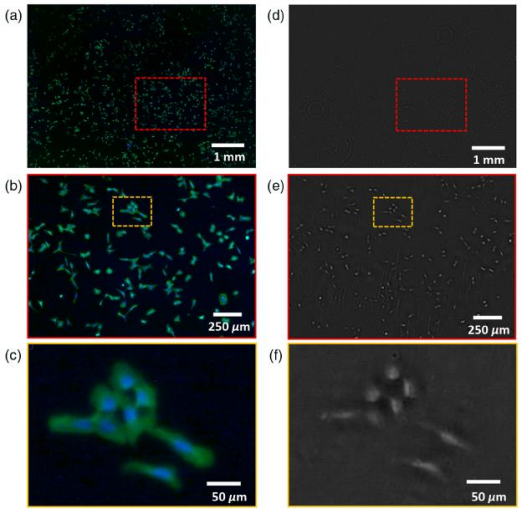

Fig. 1 : *(a)-(c) Wide-field bicolor fluorescence data of U-2 OS cells The blue fluorescence reveals the DNA in the nucleus stained with Hoechst 33342 and the actin filaments are green because of Alexa Fluor 488 phalloidin. (d)–(f) Corresponding phase images obtained from holographic reconstruction of a single-defocus image. The red boxes in (a) and (d) correspond to the zoomed images in (b) and (e), respectively. The yellow boxes in (b) and (e) correspond to the zoomed images in (c) and (f), respectively*

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[1] D. N. Breslauer et al., “Mobile phone based clinical microscopy for global health applications,” PLoS One 4, e6320 (2009)
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Surprisingly simple and compact microscope For time-lapse phase and fluorescence Imaging based on chromatic aberration

RESEARCH TOPIC:
Phase and fluorescence microscopy of live cell cultures

AUTHORS:
O. Mandula, J-P. Kleman, F. Lacroix, C. Allier, D. Fiole, L.Hervé, P. Blandin, D. C. Kraemer, and S. Morales

We designed a particularly simple, compact and robust microscope for phase and fluorescent imaging. The phase-contrast image is reconstructed from a single, approximately 100 µm defocused image with an algorithm based on a constrained optimization of Fresnel diffraction model. Fluorescence image is recorded in-focus. No mechanical movement of neither sample nor objective or any other part of the system is needed to change between the phase-contrast and fluorescence modality. The change of focus between phase (out-of-focus) and fluorescence (in-focus) imaging is achieved with chromatic aberration specifically enhanced by the optical design of our system. Our microscope is sufficiently compact (10x10x10 cm^3) to fit into a standard biological incubator. The simple and robust design reduces the vibration and the drift of the sample. The absence of motorized components makes the system robust and resistant to the humid conditions inside the biological incubator.

Context and Challenges

Simple and compact microscopes present a significant advantage in biological research: compactness allows the system to be used directly in a standard incubator, while simplicity makes the microscope stable and robust [1].
Phase contrast and fluorescence microscopy are of particular interest in biomedical research. Phase imaging is a minimally invasive technique for the observation of non-absorbing, unstained specimens. The contrast in phase images reveals local changes of a sample's optical properties and allows observation of morphological changes. On the other hand, fluorescence can be used for highlighting specific structures, events or cell sub-populations. In other words, the phase-contrast image provides an overall context while fluorescence gives specificity.

Main Results

We used our microscope for the observation of HeLa cell culture marked with Hoechst 33342 and a sub-population of cells expressing GFP tubulin or mCherry. Fluorescence and defocus images were recorded every 10 min over 40 hours directly in the incubator with the full field of view is of 3 mm2 containing ~103 of individual cells.
Snapshots of the central region of interest (ROI) in 5-hours intervals are shown in *Fig. 1a*. The cells were dividing up to confluence at t=40 hours. The phase images *Fig. 1b* show details of the individual cells. Nucleus with nucleoli and details of thin and dynamic lamellipodia can be observed. Red arrows point to the cells in division. Due to the large optical thickness of the mitotic cells the algorithm encounters phase-wrapping problems. Phase unwrapping will be addressed in future work.
The resolution of the system was determined from the in-focus images of the absorption USAF resolution target. We can resolve down to the 5th element of group 8 with 1.23 µm line width. The reconstruction of a 100 µm defocused image of our custom-made phase resolution target (silica slide with 300 nm thick engraving of the USAF resolution target) shows that at least the 2nd element of group 8 with line width 1.74 µm can be resolved.

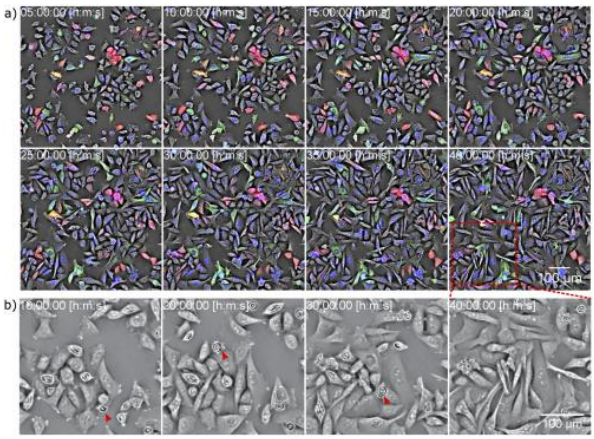

***Fig. 2 : a**. ROI of the phase and multi-color fluorescence of triple stained HeLa cells observed over 40 hours directly in the incubator, **b**. Details of Individual cells*

Perspectives

Currently, we are using the system for studying of cell cycle of cultures with FUCCI system. FUCCI (fluorescence ubiquitination cell-cycle indicator), is a genetically encoded, two-colour (red and green), indicator of the progression through the cell cycle: the cells in G1 phase show red fluorescence nuclei while the cells in S, G2 and M phase display green fluorescence within the nuclei. We use phase images for segmentation and tracking of the individual cells, which allows us to determine the level of fluorescence in each cell in the green and red fluorescence channel. We compare the obtained statistics with the data from flow cytometer acquired at the end of the observation. We show that we can produce a statistically relevant time-resolved measurement of a cell population while keeping access to the individual cells.

RELATED PUBLICATIONS:
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DELL

04 INNOVATIVE THERAPIES

- BCI clinical trial
- Hypothermia for glioblastoma treatment
- Laser Cooling for biomedical applications
- Collagen/lipidot cryogel for wound healing

The first long-term proof-of-concept of a four Limb exoskeleton succesfully driven by a brain Computer interface by a tetraplegic patient to Compensate his motor deficits

RESEARCH TOPIC:
Brain Computer Interface, Tetraplegia, Implantable Medical Device, Long-Term Application

AUTHORS:
A.L. Benbabid, T. Costecalde, S. Karakas, C. Larzabal, G. Charvet, et al.

Brain Computer Interfaces have been studied for several decades in several applications and multiple methods. The project developed at Clinatec has been designed to compensate the motor deficits of tetraplegic patients by controlling a full body exoskeleton using a Brain Computer Interface (BCI). For 32 months, we have achieved a successful high-dimensional control in multiple limbs. This chronic application is a real step forward to consider the use of such neuroprostheses at home to improve patients' quality of life.

SCIENTIFIC COLLABORATIONS : CHU Grenoble-Alpes, CEA-LIST

Context and Challenges
Approximately 20% of traumatic cervical spinal cord injuries result in tetraplegia. We investigated the possibility for these patients to control an exoskeleton using their brain signals. Some necessary challenges were reached to achieve this goal such as the developments of semi-invasive implants (WIMAGINE® [1]) as well as a four-limb neuroprosthetic exoskeleton. Two patients were currently trained to pilot this neuroprosthesis.

Main Results
For 32 months, a patient has succesfully controlled a four-limbs exoskeleton to precisely reach some targets with his upper limbs or activate a walking cycle using only his brain activity. These results were published in *The Lancet Neurology* [2], *Table 1* presents the most important results of this study.

***Table 1** : most important results from the study published in the Lancet Neurology*

	DoF	Number of experiments	Calibration duration (min)	Brain-machine interface control duration (min)	Number of targets		Hit (%)		Ratio	
TARGET 2D_LH	2	17	7·7 ± 1·3	9·1 ± 2·1	20·1 ± 8·4		80 ± 15·5		2·8 ± 1·4	
TARGET 2D_RH	2	19	9·5 ± 2	7·7 ± 3·6	16·8 ± 6		82·2 ± 12		3·3 ± 1·5	
EMM 3D_LH	3	7	20·5 ± 4·2	17·2 ± 4·4	31·3 ± 9·5		56·9 ± 15·3		6·8 ± 4·1	
EMM 3D_RH	3	11	22·6 ± 2·2	12·2 ± 6·9	23·2 ± 9·5		52·5 ± 11		6·6 ± 3·6	
EMM 2D_2H	4	7	27·6 ± 2·3	12·5 ± 2·2	32·3 ± 7·1		69·6 ± 6·1		3·8 ± 1·5	
EMM 3D_2H	6	6	38·6 ± 5·8	29·4 ± 8·3	39·7 ± 10·9		57·2 ± 9·5		6·3 ± 3·2	
EMM 3D_2H PRONO	8	11	No recalibration	29·2 ± 5·2	3D_2H 38·7 ± 10	PRONO 26·8 ± 9·5	3D_2H 64 ± 5·1	PRONO 89·7 ± 5·2	3D_2H 5·2 ± 1·4	PRONO 5 ± 1·5
EMY 3D_LH	3	2	25·4 ± 7·7	11·4 ± 2·4	30·5 ± 0·5		68·9 ± 1·1		5·7 ± 2·4	
EMY 3D_RH	3	1	33·9	10·1	26		61·5		6·1 ± 2·5	
EMY 2D_2H	4	1	41·1	15·9	37		83·8		8·4 ± 4·7	
EMY 3D_2H	6	1	37·6	22·4	42		71·4		5·3 ± 1·4	
EMY 3D_2H PRONO	8	5	No recalibration	22·3 ± 7·7	3D_2H 21·4 ± 5·5	PRONO 21·8 ± 3·3	3D_2H 70·9 ± 11·6	PRONO 99·2 ± 1·8	3D_2H 9·8 ± 3·5	PRONO 4 ± 1

This publication highlighted three major advances.
Firstly, the WIMAGINE® implants developed by the CEA allowed long-term and chronic human sudies using ElectroCorticoGram (ECoG) signals. It showed a long-lasting tolerance, and no side effects, nor signal degradation.
Secondly, brain activities were decoded in a self-paced manner online and in real-time, without recalibration during several months [2 and 3].
Thirdly, the patient increased progressively his high-dimensional control of multiple limbs. He started from walking tasks to 8D bimanual tasks from two upper limbs (3D from each arm and prono-supination of the wrist). Recently, he could control the four limbs of the exoskeleton. *Fig. 1*, issued from the publication in *The Lancet Neurology,* illustrates this progression in the number of Degree of Freedom controlled.

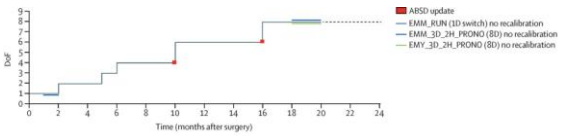

***Fig. 1 :** Progression in the patient control of the four limb of the exoskeleton expressed in the degree of freedom controlled*

Additionally, we explored the possibility to control by BCI a wheelchair and the proof-of-concept of this control was successfully demonstrated. Concerning the function of prehension, we have started developping a prosthetic hand to be used in the last experiments with the patient.

Perspectives
The experiments are still underway, the final aim is to show that a tetraplegic patient is able to control all the functions of the exoskeleton by his brain activities. We have designed a complex experiment, the patient has to walk to a table, activate one arm to reach a target in a 3D space, catch this target with the hand prosthesis, keep it, walk again and finally put the target on an another table.
The confirmation of all these results is in progress with a new patient who started the study since 6 Months ago.
The aim now is to push all this method to be deployed at home, outside of the laboratory to demonstrate that the quality of life of tetraplegic patients could be improve by the combination of BCI and exoskeleton to achieve some everyday gestures.

RELATED PUBLICATIONS:
[1] "WIMAGINE: Wireless 64-Channel ECoG Recording Implant for Long Term Clinical Applications," *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, 23(1), 10-21. C. Mestais, G. Charvet, F. Sauter-Starace, M. Foerster, D. Ratel, and AL. Benabid, (2015).
[2] "An exoskeleton controlled by an epidural wireless brain–machine interface in a tetraplegic patient: a proof-of-concept demonstration," *The Lancet Neurology*, 2019, Alim-Louis Benabid et al.
[3] "Recursive exponentially weighted n-way partial least squares regression with recursive-validation of hyper-parameters in brain-computer interface applications," *Scientific Reports*, 2017.
Andrey Eliseyev, Vincent Auboiroux, Thomas Costecalde, Lilia Langar, Guillaume Charvet, Corinne Mestais, Tetiana Aksenova, Alim-Louis Benabid.

Moderate hypothermia inhibits both Proliferation and migration of human Glioblastoma cells

RESEARCH TOPIC:
Brain tumor (glioblastoma), Therapeutic hypothermia, Cell culture

AUTHORS:
C. Fulbert, C. Gaude, E. Sulpice, S. Chabardès and D. Ratel

Therapeutic hypothermia is a promising approach in various medical applications. We studied its potential in the treatment of glioblastoma, an aggressive brain tumor with poor survival. To achieve this, we performed *in vitro* experiments on human glioblastoma cell lines and investigated the ability of hypothermia to prevent cancer cell growth. Results demonstrated a significant reduction of glioblastoma cell migration and a total inhibition of proliferation by moderate hypothermia. These results support a therapeutic role for hypothermia as an adjuvant therapy for treating glioblastoma, and open the way to preclinical studies in order to evaluate anti-tumor effects of hypothermia *in vivo*.

Context and Challenges

Glioblastoma is a particularly aggressive brain tumor, treated by surgical resection with combined radiotherapy and chemotherapy [1]. However, tumor recurs in the resection margins after surgical removal in almost all patients because of the activation of residual glioblastoma cells, resulting in a median survival of only 15 months. Hence, there is a crucial need for developing novel therapies that improve survival outcome. To handle this issue, we suggest therapeutic hypothermia, an interesting treatment due to its neuroprotective effects and good clinical tolerance. We propose hypothermia as an adjuvant treatment, to place residual tumor cells in a dormant state by inhibiting their proliferation and migration, with the aim of preventing tumor recurrence [2].

Main Results

We performed *in vitro* experiments and evaluated the impact of different temperatures on four human glioblastoma cell lines. Results were similar for all cell lines, demonstrating a consistent effect of hypothermia and a high sensitivity of cells to this treatment. First, we demonstrated that hypothermia reduces glioblastoma cell proliferation in a dose-dependent manner, with proliferation arrest below 28°C. We further investigated the impact of moderate hypothermia at 28°C on cell proliferation and showed that proliferation arrest was long lasting, up to at least 30 days (*Fig. 1*).

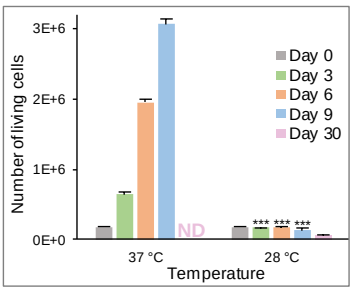

***Fig. 1** : Influence of moderate hypothermia on glioblastoma cell proliferation over time in U251 cell line. Bar graphs represent adherent living cells, ND not determinable due to confluenece, ***p < 0.001 versus control at 37°C.*

Then, we investigated the impact of hypothermia on glioblastoma cell migration (both oriented and non-oriented), a key process involved in tumor growth. We demonstrated a strong inhibition for both of them, with notably a 91% reduction of non-oriented migration in U251 cell line (Figure 2).

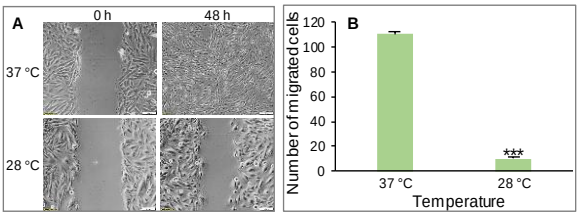

***Fig. 2** : Influence of moderate hypothermia on glioblastoma cell non-oriented migration in U251 cell line. **A**. Follow-up of lesion recolonization over time (scale bar, 100 µm). **B**. Quantification of migration test after DAPI staining. Bar graphs represent migrating cells In 100 µm length wound line, ***p < 0.001 versus control at 37°C.*

Finally, we showed that hypothermia not only alters glioblastoma cell growth but also intrinsic mechanisms like cell morphology and cell cycle. Indeed, hypothermia modifies cell adhesion properties and deteriorates cell membranes. Moderate hypothermia also alters cell cycle distribution by inducing cell cycle arrest. In fact, we observed an accumulation of cells in the division phase (G2/M), which could explain proliferation arrest.

Perspectives

Our results demonstrate that moderate hypothermia could be a promising adjuvant therapy for glioblastoma treatment, as it strongly inhibits both cell proliferation and migration. After further *in vitro* experiments, preclinical studies are required in order to evaluate the therapeutic potential of hypothermia *in vivo*. If these results show the same inhibitory effects, moderate hypothermia applied at the resection margins could delay tumor recurrence, in combination with current treatments.

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[1] R. Stupp et al., "Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival…", Lancet Oncol., 10, 459-466, 2009.
[2] C. Fulbert et al., "Moderate hypothermia inhibits both proliferation and migration of human glioblastoma cells", J. Neurooncol., 144, 489-499, 2019.

Laser cooling of solids: Towards biomedical application

RESEARCH TOPIC:
Laser cooling of solids, Optical refrigeration, Anti-Stokes fluorescence, Intractable focal epilepsies, Active implantable medical devices (AIMD)

AUTHORS:
Quentin Mermillod, Johan Cazals, Alain Glière, Mathieu Dupoy, Nicolas Aubert, Stephan Chabardès

Focal cooling is a promising alternative therapy for intractable focal epilepsies, avoiding the irreversible neuronal damages induced by resection surgery. However, due to thermal conduction losses, local cooling of a deep brain region remains a challenging objective for thermoelectric or fluidic technologies. Here, we investigated the viability of an optical micro-cooler based on anti-Stokes refrigeration of ytterbium doped YLF crystals, taking into account the medical constraints for implantable device [1]. We realized significant cooling under atmospheric pressure and developed a solution drastically reducing the harmful fluorescence heating of brain-like liquids below 2 K, thus demonstrating the relevance of this technology for biomedical applications.

Context and Challenges

The technological challenge is to design a localized cooling system that can be long-term implanted in the brain, allowing rapid and effective inhibition of the epileptic foci [1]. This system has to meet all the meeting all the Active Implanted Medical Device (AIMD) constraints gathered in the ISO14708-1 standard. The energy consumption, compatible with the use of implantable batteries is also a crucial Issue. In such a context, our long-term objective is to develop a micro-cooler based on laser cooling technology that will be then integrated into an AIMD prototype compatible with preclinical studies.

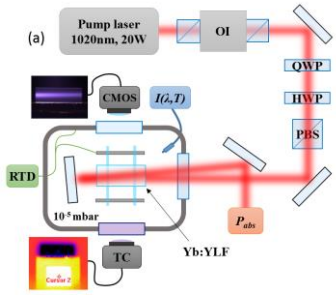

***Fig. 1**: Schematic of the experimental set-up*

Main Results

The laser-cooling set-up is outlined in *Fig. 1*. A fiber laser generates high energy photons (20 W) in resonance with the E4/E5 transition (1020 nm) with a beam diameter of 1 mm. An optical isolator (OI) protects the laser from reinjections. A quarter-wave plate (QWP) is used to slightly correct the beam ellipticity. A half-wave plate (HWP), combined with a polarizing beam splitter (PBS) allows to control the polarization axis orientation and the excitation power. The laser beam is then directed to a pressure-controlled chamber containing a 10% wt. Yb^{3+}:YLF crystal. This crystal is placed on two bare optical fibers to limit thermal conduction losses The beam travels back and forth through the crystal, and the transmitted light is collected in a thermal power sensor [2].

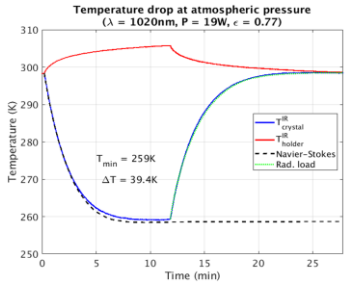

***Fig. 2**: Temperature dynamics of a 10% wt. Yb3+:YLF crystal (blue) and the holder (red) under atmospheric pressure.*

In dry nitrogen, the natural convection limits the temperature drop to ΔT ~ 40 K. To our best knowledge this result is the best ever reported at atmospheric pressure.
We realized opto-thermal simulation of a realistic cooling probe with the size of a DBS probe in 310 K water representative of the human brain environment. This simulation study demonstrates the *ability* of this technology to cool thermal load composed of liquids, but also points out the harmful heating due to fluorescence absorption [3]. Thus, we study a solution based on dichroic filtering to drastically reduce this drawback and to enhance by 70% the cooling efficiency.

Perspectives

In this paper, we report the first possible biomedical application of optical refrigeration. We demonstrate the possibility to cool a volume of a mm3 below 300 K in a deep brain region inaccessible so far by other cooling technologies. This result is an important achievement towards the realization of a fully implantable device able to treat medically refractory epilepsy. In addition, we propose a solution based on dichroic filtering to drastically reduce the harmful heating induced by the fluorescence absorption and to enhance by 70% the cooling efficiency. Beside of that, these results pave the way towards new medical approaches using optical cooling for cryosurgery or cryotherapy in deep brain region.

RELATED PUBLICATIONS:
[[1] Q.Mermillod, J. Cazals, A.Glière, M. Dupoy, N. Aubert, S. Chabardès, "Laser cooling of solids: towards biomedical applications," Proc. SPIE 10936, Photonic Heat Engines: Science and Applications, 109360M (1 March 2019); doi: 10.1117/12.2507828
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[3] Kern, M., Jeske, J., Lau, D. W. M., Greentree, A. D., Jelezko, F., and Twamley, J., \Optical cryocooling of diamond," Physical Review B 95, 235306 (June 2017).

Preparation and characterization of Hybrid collagen/nanostructured lipid Carriers cryostructurates for wound Healing applications

RESEARCH TOPIC:
Drug Delivery System. Collagen. Lipid Nanoparticles. Wound healing. Curcumin. SEM. TEM. AFM.

AUTHORS:
V. Laghezza Masci, A.R. Taddei, T. Courant, O. Tezgel, F. Navarro, F. Giorgi, D. Mariolle, A.M. Fausto, I. Texier

Curcumin-loaded collagen cryogels have been designed for wound healing applications. Curcumin displays strong antioxidant, antiseptic, and anti-inflammatory properties, while collagen is acknowledged for promoting cell adhesion, migration and differentiation. However, when curcumin was loaded directly onto collagen hydrogels, it formed large molecular aggregates and clogged the matrix pores. A double encapsulation strategy was therefore developed by loading curcumin into nanostructured lipid carriers (NLC), and embedding these particles inside collagen scaffolds. The resulting collagen/NLC cryogels were thoroughly structurally characterized by a large range of techniques. They displayed an optimal fibrous structure with ≈ 100 µm average pore size for sustaining cell migration.

SCIENTIFIC COLLABORATIONS: Department for Innovation in Biological, Agrifood and Forestry Systems (DIBAF) & Section of Electron Microscopy, Tuscia University, Italy.

Context and Challenges

Collagen is one of the best biomaterial to be employed as a prosthetic medical device for wound healing, thanks to its relative abundance in bodily proteins, its structural role as a main component of the extracellular matrix, its low immunogenicity and high stability [1]. Several therapeutic strategies have been recently proposed to complement these scaffolding features with antimicrobial or wound-healing promoting properties. Curcumin exhibits some antimicrobial, antioxidant, antiseptic and anti-inflammatory properties, besides expressing a potential MMP inhibiting activity, and has recently aroused much interest for wound healing applications [2]. New collagen/curcumin cryogels were therefore designed with the intent of preserving all major hallmarks of collagen matrix for wound healing, i.e. high microporosity, convenient swelling ratio, good mechanical properties and favorable conditions for cell interaction. Since direct curcumin loading in the collagen matrix occluded the cryogel pores, the drug was encapsulated into nanostructured lipid carriers (NLC) embedded in the cryogel to preserve all the properties of the collagen scaffold.

Main Results

Hybrid collagen/nanoparticles (NLC) materials were prepared by a freeze-drying process. NLC were homogeneously distributed amidst collagen fibers (fluorescence microscopy, SEM observation). Collagen was structurally unaltered by the presence of NLC up to 10% lipid/collagen w/w, as demonstrated by mechanical (swelling, compression), thermal (DSC, TGA) and structural (SEM, TEM, AFM) analysis. In particular, materials displayed macropores of about 100 µm and fiber helical structure was maintained (*Fig.1 A*) [3].
Materials soaked in saline buffer yielded hydrogels releasing about 20% to 30% of their NLC content within 24h. Prolonged release of drug-loaded NLC from the scaffolds was achieved: 25 days were necessary to release all NLC [3].
When exposed to NIH 3T3 fibroblasts or keratinocytes, the hybrid collagen/NLC hydrogels provided a satisfactory scaffold for cell interaction as early as 4 h after seeding with no cytotoxic counter effect (*Fig. 1B*). Seven days after starting cell incubation, collagen/NLC scaffolds still presented fiber structure, contrary to pure collagen for which fibers were digested into smaller fibrils [3].
These positive features make the collagen/lipid cryogels a promising scaffold to promote wound healing.

Perspectives

The hybrid collagen/NLC biomaterials have demonstrated *in vitro* their potential as scaffolds able to both promote tissue regeneration (collagen and lipids) and deliver on long term therapeutic drugs such as curcumin [3] and siRNA [4]. In a context of increased economic and social burdens of non-healing chronic wounds, these new materials could present an innovative therapeutic option.

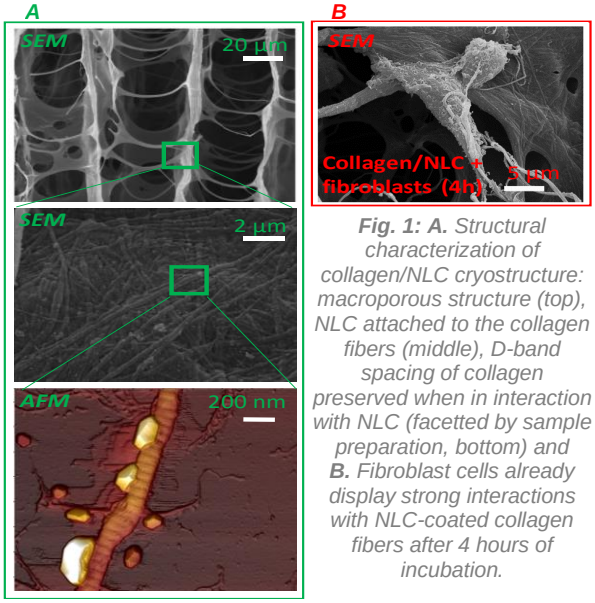

Fig. 1: A. *Structural characterization of collagen/NLC cryostructure: macroporous structure (top), NLC attached to the collagen fibers (middle), D-band spacing of collagen preserved when in interaction with NLC (facetted by sample preparation, bottom) and* ***B.*** *Fibroblast cells already display strong interactions with NLC-coated collagen fibers after 4 hours of incubation.*

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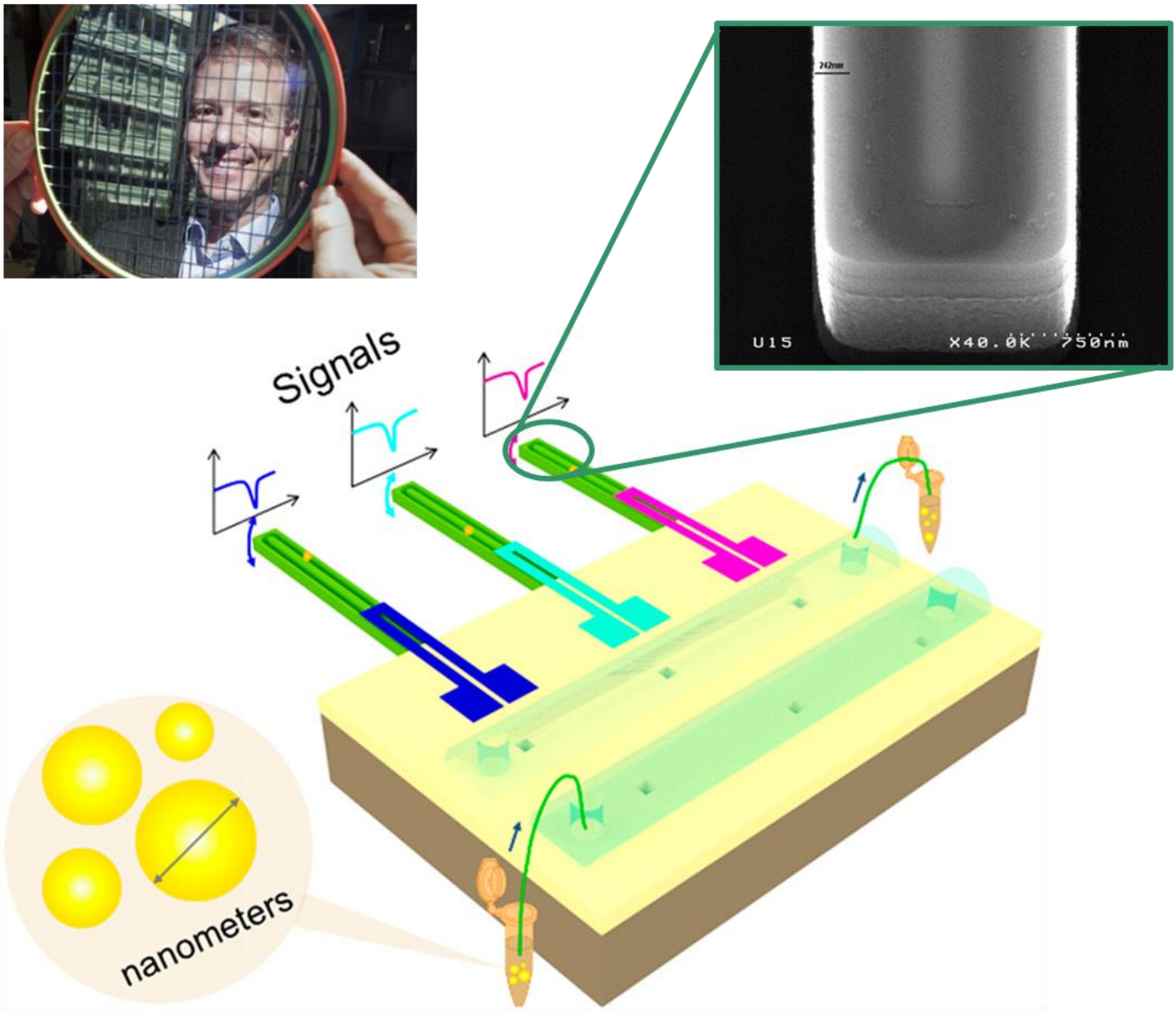
[2] D. Kumar, M. Kumar, C. Saravan, S.K. Singh, Curcumin: a potential candidate for matrix metalloproteinase inhibitors, Expert Opin. Ther. Targets 2012, 16(10) 959-972,
[3] V. Laghezza Masci, A.R. Taddei, D. Joud, T. Courant, F. Navarro, F. Giorgi, G. Gambellini, D. Mariolle, A.F. Fausto, I. Texier, Characterization of collagen/lipid nanoparticle cryostructurates for wound healing applications, Macromol. Biosci. 2019, 19(5), e1800446.
[4] O. Tezgel, N. DiStasio, V. Laghezza-Masci, A.R. Taddei, A. Szarpak-Jankowska, R. Auzély-Velty, F. P. Navarro, I. Texier, Collagen Scaffold-Mediated Delivery of NLC/siRNA as Wound Healing Materials, J. Drug Delivery Sci.Technol. 2020, 55, 101421.



DOI: (10.1021/acssensors.0c00394)

05 SENSORS & ACTUATORS FOR MEDICAL DEVICES

- Suspended microchannel resonator array
- Bipolar electrochemistry at micropores
- Porous silicon membranes for biofuel cells
- Real-time sweat excretion monitoring
- Implantable parylene based multielectrode array

Rapid and high-precision sizing of single particles using parallel suspended microchannel resonator arrays and deconvolution

RESEARCH TOPIC:
Suspended microchannel resonator (SMR), SMR array, Single particle sizing, Microfluidic device.

AUTHORS:
M. A. Stockslager, S. Olcum, S. M. Knudsen, R. J. Kimmerling, N. Cermak, K. R. Payer, V.t Agache, S. R. Manalis

Suspended microchannel resonators (SMRs) are microfluidic devices that directly measure particle mass by detecting a shift in resonance frequency as particles flow through a resonating microcantilever. While these devices offer high precision for sizing particles by mass, throughput is fundamentally limited by the small dimensions of the resonator. Here, we introduce two complementary technical advancements that vastly increase the throughput of SMRs: i. deconvolution-based approach for extracting mass measurements from resonance frequency data throughput increases from 120 to 2000 particles/min) and ii. design and operation of new devices containing up to 16 SMRs connected fluidically in parallel and operated simultaneously on the same chip (throughput increases to 6800 particles/min without degrading precision). Finally, we estimate that future systems designed to combine both of these techniques could increase throughput by nearly 200-fold (up to 24 000 particles/min)

SCIENTIFIC COLLABORATIONS: MIT (Boston, USA)

Context and Challenges

SMRs are microfluidic devices based on vibrating cantilevers on which a microchannel is micromachined. They allow the sizing of individual particles, circulating in the microchannel, such as cells, through direct measurement of their buoyant mass. However, to date, their flow-rate is limited to tens of particles per minute, restricting their use to applications requiring highly precise measurements of relatively small numbers of objects. The technological challenge of the presented work [1] deals with increasing the throughput of SMRs to provide faster screening of individual cells. Such an improvement in the SMR working function can be addressed owing to two independent strategies, concomitantly implemented, and relying to data analysis and devices evolution through SMRs parallelization respectively.

Main Results

This work represents a first demonstration in SMR parallelization for single particles or cells mass measurement at high throughput. To address such increase in measurement throughput, the first advancement deals with a model-based deconvolution algorithm of the SNR frequency response owing to piezoresistors measurements. The typical signal shape related to the flow of a particle to the tip exhibits 3 minima in frequency (2 having the same amplitude) as exemplified on Fig1.A for cantilevers actuated in their second vibration mode. According to the transit time of the particle within the cantilever and to the measurement bandwidth of the system, such a signal can be distorted. The deconvolution operation consists on the postprocessing de-blurring of the signal (*Fig. 2B*). The second advancement is a microfluidic device containing 16 SMRs connected fluidically in parallel and operated simultaneously (*Fig. 1C*). Using techniques described previously [2] shifts in the resonance frequency of each cantilever can be tracked independently, and frequency-multiplexing allows each cantilever to be continuously driven at resonance using a single actuation channel and single detection channel. The precision of the parallel SMR arrays was assayed by measuring suspensions of monodisperse polystyrene beads, obtaining coefficients of variation up to ~4 times lower than a commercial Coulter counter configured for a similar size range. Finally, using both complementary advancement we were able to size 6800 particles per minute without degrading the precision of mass measurement.

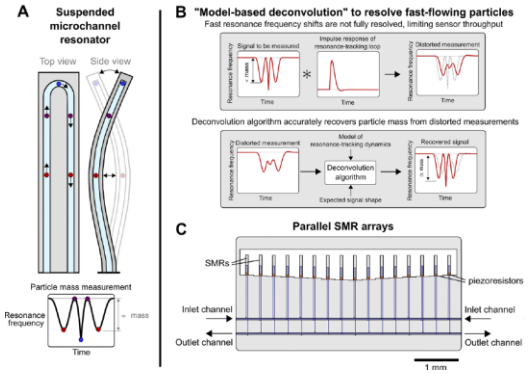


*Fig. 1. : **A**. Schematic of the SMR and of its operation in the second vibration mode resulting in three local minima in resonance frequency as a particle flows to the tip of the cantilever and back. **B**. A model-based deconvolution algorithm increases the maximum particle speed for which accurate mass measurements can be obtained. **C**. Schematic of parallel SMR array devices, which contain 16 SMRs connected fluidically in parallel and operated simultaneously to further increase throughput.*

Perspectives

We envision that increased throughput will extend the range of applications for which mass-based particle sizing can be employed. While mass has traditionally been less commonly used than volume for characterizing particle size, in some applications it provides unique advantages, such as the ability to distinguish particles of the same size but different densities Otherwise, such parallelized systems will be employed for characterizing particles of nanometric size such as exosomes.

RELATED PUBLICATIONS:
[[1] M. A. Stockslager, S. Olcum, S. M. Knudsen, R. J. Kimmerling, N. Cermak, K. R. Payer, V.t Agache, S. R. Manalis, " Rapid and high-precision sizing of single particles using parallel suspended microchannel resonator arrays and deconvolution " Rev. Sci. Instrum. 90, 085004, 2019.
[2] N. Cermak, S. Olcum, F. F. Delgado, S. C. Wasserman, K. R. Payer, M. A. Murakami, S. M. Knudsen, R. J. Kimmerling, M. M. Stevens, Y. Kikuchi, A. Sandikci, M. Ogawa, V. Agache, F. Baléras, D. M. Weinstock, and S. R. Manalis, "High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays", Nat. Biotechnol. 34, 1052, 2016.

Enhanced bipolar electrochemistry at solid-State micropores: demonstration by wireless Electrochemiluminescence imaging

RESEARCH TOPIC:

Bipolar electrochemistry, Planar micropore, Microfluidic channel, Electrochemiluminescence.

AUTHORS:

A. Ismail, S. Voci, P. Pham, L. Leroy, A. Maziz, L. Descamps, A. Kuhn, P. Mailley, T. Livache, A. Buhot, T. Leichlé, A. Bouchet-Spinelli, N. Sojic

Bipolar electrochemistry (BPE) is a powerful method based on the wireless polarization of a conductive object that induces the asymmetric electroactivity at its two extremities. Micrometric and nanometric objects are extremely difficult to address by BPE due to the very high potentials required (tens of kV). Herein, the synergetic actions of BPE and of planar micropores integrated in a microfluidic device lead to the spatial confinement of the potential drop at the level of the solid-state micropore, and thus to a locally enhanced polarization of a bipolar electrode. Electrochemiluminescence (ECL) is emitted in half of the electroactive micropore and reveals the asymmetric polarization in this spatial restriction. Micrometric deoxidized silicon electrodes located in the micropore are polarized at a very low potential (7 V), which is more than 2 orders of magnitude lower compared to the classic bipolar configurations.

SCIENTIFIC COLLABORATIONS: CEA DRF-IRIG , ISM CNRS-Université de Bordeaux, LAAS CNRS- Université de Toulouse

Context and Challenges

BPE simultaneously promotes oxidation and reduction reactions at both sides of the same conductive objects placed in solution, hence the term "bipolar" electrode that reflects this electrochemical dichotomy [1]. In the most common BPE approach, a conductive object is immersed in an electrolyte solution and positioned between two feeder electrodes, which are not in physical contact with it. However, BPE faces severe limitation when implemented to micro and nanometric objects due to the high potential drops required. Controlling the spatial distribution of the potential drop and its localization may allow manipulating the BPE behavior [2]. The aim of this work [3] is to explore this original concept by using micropores and ECL as field focusing and reading tools respectively.

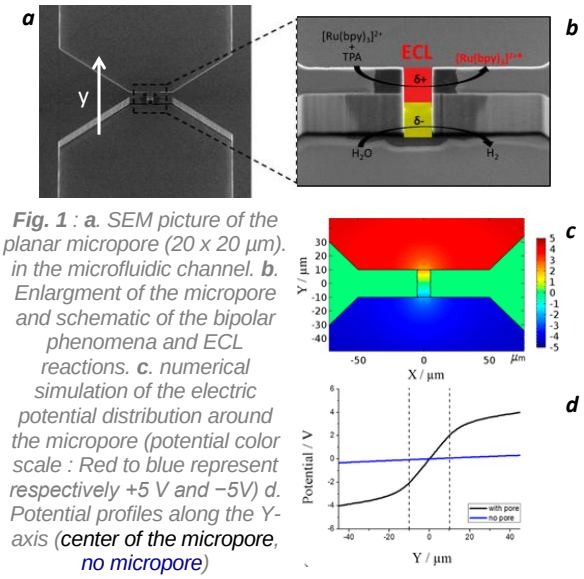


***Fig. 1** : **a**. SEM picture of the planar micropore (20 x 20 µm). in the microfluidic channel. **b**. Enlargment of the micropore and schematic of the bipolar phenomena and ECL reactions. **c**. numerical simulation of the electric potential distribution around the micropore (potential color scale : Red to blue represent respectively +5 V and −5V) d. Potential profiles along the Y-axis (center of the micropore, no micropore)*

Main Results

To demonstrate the concept of focused field aided BPE, microfluidic channel and planar micropore were fabricated in a p-type silicon using a top-down micromachining. A dense thermal silica layer was grown at the surface of the silicon and gold feeding electrodes were deposited in each compartment of the microfluidic channel. To mimic a conductive micro-object, the silica layer was etched in the micropore area (*Fig. 1 a-b*). Thereby ECL reaction can take place in the anodic side of the micropore whereas water hydrolysis represents the cathodic reaction (*Fig1 b*). Numerical simulation highlighted the effectiveness of the potential drop within the pore (*Fig. 1 c-d*) and a voltage of 7 V was applied between the feeder electrodes to generate the right potential drop at the edges of the micropore. Fig.2 illustrates the existence of an ECL behavior that is co-localized to the micropore site at half of the silicon electrode as expected. This represents the first in situ experimental demonstration of BPE at a micropore and a first proof of concept of contactless ECL generation at a micropore.

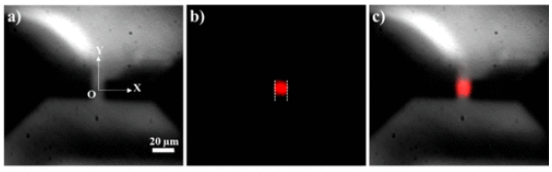


***Fig. 2**: **a**. Photoluminescence, **b**. ECL and **c**. overlay of both luminescence images of the same region of interest around the micropore.*

Perspectives

The presented approach offers exciting perspectives for BPE of micro/nano-objects, such as dynamic BPE with objects passing through the pores or wireless ECL-emitting micropores. Such single wireless ECL micropores pave the way for the development of original analytical applications such as cell capturing coupled with ECL detection.

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[2] A. Bouchet-Spinelli, P. Mailley, et al., Polarization induced electrofunctionalization of pore walls: a contactless technology", Biosensors, 9, 121, 2019.

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*

Chemical functionalization of porous silicon membranes for the development of implantable reactors

RESEARCH TOPIC:
Functionalization, Silane PEG, Porous silicon, Membrane, Implantable reactors, Glucose biofuel cell

AUTHORS:
G. Nonglaton, G. Costa, F. Gaillard, M-L. Cosnier, P. Cinquin, D. K. Martin, C. Gondran

The natural biodegradability of porous silicon (pSi) in physiological media limits its wider usage for implantable systems like glucose biofuel cell (GBFC). In this study, we report the stabilization of pSi membranes by chemical surface oxidation followed by a PEGylation process. These surface modifications stabilized the pSi to allow a long period of immersion in PBS, while leaving the pSi surface sufficiently hydrophilic for good filtration and diffusion of several biomolecules of different sizes without any blockade of the pSi structure. After 2 months immersion in PBS, the pSi membrane continued to operate, but with a reduced glucose diffusion coefficient. The chemical stabilization of pSi membranes provided almost 1 week stable and functional biomolecule transport in blood plasma and opens the possibility for its short-term implantation as a diffusion membrane in biocompatible systems.

SCIENTIFIC COLLABORATIONS: TIMC-IMAG, UGA, DCM, UGA

Context and Challenges

Glucose biofuel cell (GBFC) is a power supply implantable device that can convert the chemical energy of glucose and oxygen from the extracellular fluid (ECF) directly into electric energy by using enzymes as the catalyst. GBFC generally consists in two bioelectrodes, within which redox reaction occurs, mechanically confined using a semi-permeable membrane such as dialysis membrane. The purpose of the membrane is to prevent the diffusion of the enzymes while letting small molecules like glucose and O_2 from the outside flow into the device. A future generation of GBFC could be packaged between two silicon chips within nanoporous membranes made of porous silicon (*Fig. 1*).

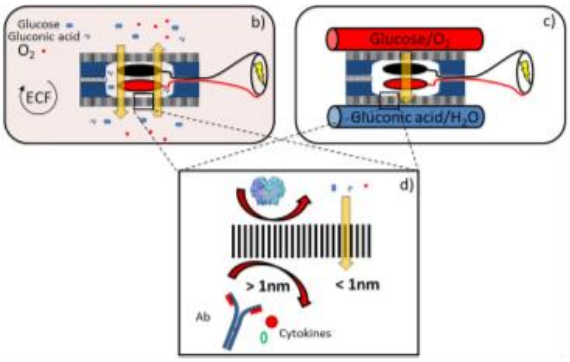

***Fig. 1** : Schematic of the pSi GBFC concept*

pSi is widely used in biomedical applications due its biocompatibility and many other attractive physical and chemical properties. Nonetheless, the major hurdle for an implantable pSi membrane is to overcome its natural degradation in physiological solutions

Main Results

In the present research, we investigate the means to stabilize pSi for its use in physiological conditions. We report the stabilization of pSi) embranes by chemical surface oxidation using RCA1 and RCA2 protocols standard cleaning procedures of silicon wafers, which was followed by a PEGylation process using a silane-PEG 2-[Methoxy-(polyethyleneoxy)propyl] trichlorosilane. [1] These surface modifications stabilized the pSi to allow a long period of immersion in PBS, while leaving the pSi surface sufficiently hydrophilic for good filtration and diffusion of several biomolecules of different sizes without any blockade of the pSi structure. The pore sizes of the pSi membranes (thickness around 70 µm) were between 5 and 20 nm. The diffusion coefficient for fluorescein through the membrane was 2×10^{-10} $cm^2.s^{-1}$, and for glucose was 2.2×10^{-9} $cm^2.s^{-1}$. The pSi membrane maintained that level of glucose diffusion for one month of immersion in PBS. After 2 months immersion in PBS the pSi membrane continued to operate, but with a reduced glucose diffusion coefficient. The chemical stabilization of pSi membranes provided almost 1-week stable and functional biomolecule transport in blood plasma.

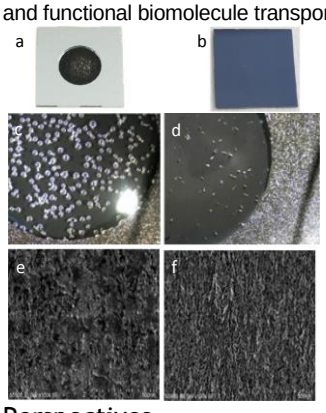

***Fig. 2**: Different views of a pSi membrane back (**a**) and front sides (**b**). Hydrogen bubbles formation in presence of PBS onto an unstabilized pSi membrane (**c**) and absence of bubbles for a stablized pSi (**d**). SEM cross sectional view of the membrane for a collapsed structure of non-silanized pSi after 3 days in PBS (**e**), and silanized at 1% after 2 months in PBS (**f**).*

Perspectives

The stabilization of pSi membranes in physiological media is very promising for packaging inside implantable devices. An example of potential usage of pSi that is stabilized against degradation deals with the packaging of a glucose biofuel cell.

RELATED PUBLICATIONS:
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Developing a new device for continuously Recording, in vivo, the excretion rate of Sweat (perspiration) in humans

RESEARCH TOPIC:
antiperspirant, continuous recording, electronic sensor, sweat excretions

AUTHORS:
E. Caberlotto, C. Guillou, L. Colomb, Charlie Barla, S. Salah, M. Vicic, F. Revol-Cavalier, V. Rat, S. Filipe

A textile sensor (20 cm^2) including electrodes is fixed onto the armpits. A microcontroller is used to permanently record changes in the conductance between two electrodes during exposure of subjects to different sweat-inducing conditions and allow to assess the efficacy of applied aluminum hydrochloride (ACH)-based roll-ons at two concentrations (5% and 15%). In vivo, results show that casual physical exercise leads to sweat excretions much higher than in warm environment (37 or 45°C). Only, an exposure to a 50°C environment induced comparable sweat excretion. Decreased sweat excretions were recorded following applications of ACH,with a dose effect.

SCIENTIFIC COLLABORATIONS: L'Oréal Research and Innovation (Chevilly-Larue, France)

Context and Challenges

The human skin, apart from its specific and well-established protective characteristics (barrier to chemicals, microbes, water, UVs) and its sensorial or aesthetical components, plays an important role in the physiology of the whole body. The constant maintenance of an internal temperature (37°C) is a vital need that is ensured in a very large part by skin when the organism is submitted to intense physical activity or very warm external conditions [1]. Hence, these conditions lead the human eccrine sweat glands to rapidly deliver sweat onto the skin surface that rapidly evaporates

Assessing the efficacy of antiperspirant agents has been, for long, a difficult challenge with regard to technical approaches (different signals recorded), and the strong influence of various personal hygiene habits in frequency and various products used. To evaluate the perspiration rate of people, the gold standard procedure consist in measuring the flux of sweat by pre-and post-weightings of cotton pads fixed onto the armpits for a 20-minute session under a 38°C ambient temperature. This approach remains static since restricted to a 20-minute collection. In order to obtain dynamic recordings of sweat excretion, a new electronic sweat patch was developed by LETI to enable real time measurement of perspiration rate for long durations [2].

Results

The sweat sensor, adapted to the armpit geometry, comprises 16 electrodes patterned on a flexible polyimide sheet (Kapton®). Nine small apertures (Ø = 2 mm) are homogeneously shaped within the flexible plastic material to allow water evaporation to occur, The sensor is then laminated with a non-woven absorbent pad (4 cm by 5 cm) that is secured under the armpit with non-occlusive adhesive tape to ensure close contact between the sensor pad and the armpit skin.

The patch is connected to an electronic board through a multiplexer that connects successively electrodes by pairs among the 16 electrodes available. The electronic records the conductivity of the fabrics between the selected electrode pairs. The increased conductance of the medium in contact with a pair of neighboring electrodes progressively induced by the sweat flow rate is converted by the electronic card and is further expressed as changes in millivolts (mV) that are compared to a stable DC current (1.25 V) obtained with dry textile

A total of 114 healthy Caucasian men and women have tested the aforementioned sweat patch in different thermal conditions. Static perspiration generated by different exposure to a thermal stress (sauna) at different temperatures (37, 40, 45, and 50°C) was compared to dynamic perspiration generated by physical exercise (walking at 4 km/h for 10 minutes, recovery/rest for 5 minutes, walking at 6 km/h for 10 minutes, and recovery/rest for 5 minuntes). Physical exercises appears strikingly stronger sweat inducer than exposure to sauna conditions, up to 50°C.

The effectiveness of the dose of antiperspirant (ACH) deposited onto armpits is measured with the sweat patch. Two roll-ons containing two different concentrations of ACH (5% and 15%) in a same vehicle were applied to both armpits leading to dose effect observation. ACH deposited onto armpits reduces the amount of sweat exuded during physical activity (*Fig.1A*) by 25%. Applying ACH15% is more efficient than ACH 5% (*Fig. 1 B*). The starting time of sweat exudation is also elongated when ACH 15% (*Fig. 1A*).

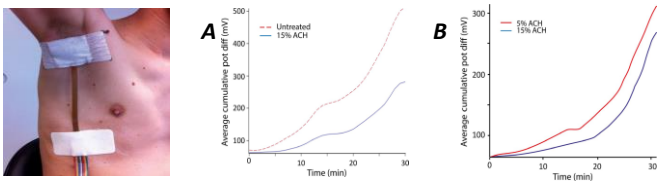

***Fig. 1** : Picture of the sweat patch fixed under the armpit and kinetics of average sweat excretion during physical exercise **A**. Comparison between untreated and antiperspirant-deposited (15 % ACH) armpits, **B** Effect of the ACH antiperspirant contain (5% or 15%) on perspiration kinetic.*

Perspectives

The sweat patch presented here allows the real-time monitoring of the dynamic aspects of sweat excretion. It appears applicable to a wide range of human activities or states including physical activities, working condition, mental stress or exposure. The sweat patch will be further improved by using a wireless connection (smartphones included) to allow real-life measurements.

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[1] Wilke K, Martin A, Terstegen L, et al, "A short history of sweat gland biology", Int J Cosmet Sci., 29,169-179, 2007.
[2] Caberlotto E, Guillou C, Colomb L,et al. Developing a new device for continuously recording, in vivo, the excretion rate of sweat (perspiration) in humans. Skin Res Technol. 2019;25:489–498. https://doi.org/10.1111/art.12677

Reliability of parylene-based multi-electrode Arrays chronically implanted in adult rat brains, And evidence of electrical stimulation on Contact impedance

RESEARCH TOPIC:

Micro Electrode Array, Long-term implantation, Electrical Stimulation, Biocompatibility

AUTHORS:

F. Sauter-Starace, Napoleon Torres-Martinez, S. Maubert

The goal of this study was to evaluate the long-term behavior of the surface electrode through electrochemical characterization and follow up of implanted parylene/platinum microelectrodes. To this aim, we designed and manufactured specific planar electrodes for cortical implantation for a rat model. This work was included in the INTENSE® project, one of the goals of which was to prove the feasibility of selective neural recording or stimulation with cuff electrodes around the vagus nerve. After a 12-week implantation on a rat model, we can report that these microelectrodes are suitable for in-vivo use. We here demonstrated the biocompatibility of the electrodes (materials and manufacturing process). After the three months implantation, we characterized limited tissue reaction beneath the electrodes and showed an increase and a stabilization of their impedance. Interestingly, the follow up of the electrochemical impedance combined with electrical stimulation highlighted a drop of the impedance up to 60% @1kHz after ten minutes of mild electrical stimulation at 110Hz.

SCIENTIFIC COLLABORATIONS: Livanova, MXM, INRA and INRIA

Context and Challenges

In the framework of INTENSE, a PSPC project, industrials and academic players collaborated to develop an implantable device to record and stimulate the vagus nerve for cardiac impairment and obesity. Apart the development of electronic and software, we developed microelectrode arrays and had to demonstrate their biocompatibility and ability to record or stimulate selectively [1] the electrophysiological activity of the vagus nerve.

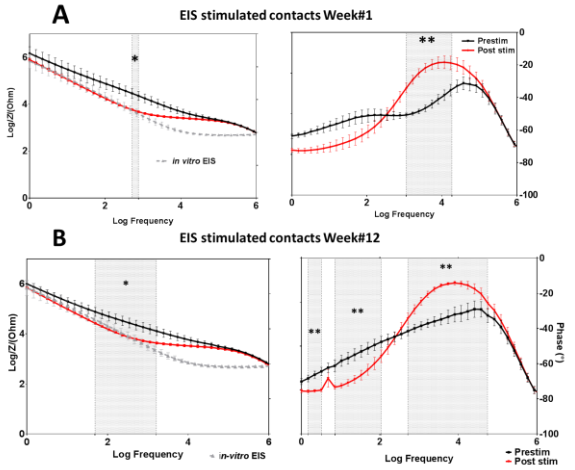

*Fig. 1: Evolution of impedance (EIS) of stimulated electrodes pre and post stimulation. (**A**) EIS of all stimulated electrodes at week#1 post implantation; (**B**) EIS of all stimulated electrodes at week#12 post implantation. In grey, the contact impedance in PBS medium.*

Main Results

The first outcome of this in-vivo study is the reliability and good local tolerance of the microelectrode array developed by CEA Leti. From the follow-up of the impedance, we found as expected a monotonous increase of impedance, which stabilized after 4 weeks of implantation (*Fig. 1*). This type of change in impedance in-vivo is explained by thickening of the dura and growth of a fibrotic encapsulation of the electrodes, increasing the impedance of the recording with a serial resistance. More surprising this study highlighted impedance drops (60%) immediately after electrical stimulation. Weiland [2] and Wang[3] also reported an impedance drop after electrical stimulation, using penetrating electrodes but here the effect is longer than previously reported. We attribute this difference to different stimulation parameters and different tissue sheaths.

Perspectives

This article [4] illustrated that CEA Leti was able to design, manufacture and characterize long-term stable microelectrode array based on parylene/platinum technological stack. CEA Leti will benefit of this kind of publications (see also [5] for platinum with carbon nanotubes and boron-doped diamond), which demonstrate its ability in academic or Industrial framework to manufacture electrodes or to assess their long-term biocompatibility.

RELATED PUBLICATIONS:

[1] Bonnet, S., Rubeck, C., Agache, V., Bourgerette, A., Fuchs, O., Gharbi, S, et al, "Selective ENG recordings using a multi-contact cuff electrode". Proceeding of the 6th International IEEE/EMBS Conference on Neural Engineering (NER), 923-926, 2013.
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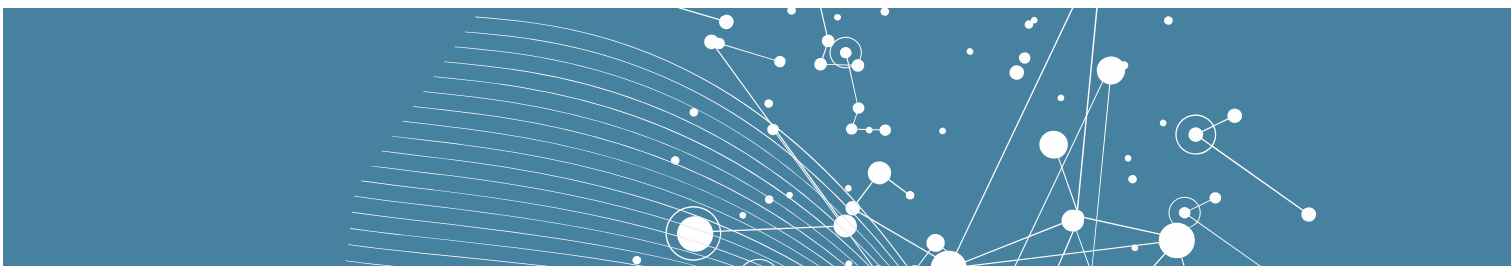
[5] Torres-Martinez, N., Cretallaz, C., Ratel, D., Mailley, P., Gaude, C., Costecalde, T., et al., "Evaluation of chronically implanted subdural boron doped diamond/CNT recording electrodes in miniature swine brain", Bioelectrochemistry, 129, 79-89, 2019.




CLINATEC

06 HDR & PHD DEGREES AWARDED

HDR

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- Jean-Maxime ROUX
- Stéphane BONNET

PHD

- Clémentine DARPENTIGNY
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- Thibault KRAMER
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- David ORIVE-MIGUEL

Dr. Cécile MORO (Senior Expert)

INTEREST OF PRECLINICAL MODELS TO EVALUATE INNOVATIVE MEDICAL DEVICES

Ecole doctorale Ingénierie pour la Santé, la Cognition et l'Environnement
Université Grenoble Alpes (France)

The development and testing of preclinical and medical devices dedicated to health is essential to innovative therapies. Since ten years, the innovative treatments based on photobiomodulation in human health is expanded, and its use for Parkinsons' disease has been my main project since 2011. My HDR manuscript exposes my initial training and current research topics, and I will consider research projects that I wish to lead in the next years.

Dr. Jean-Maxime ROUX (Senior Expert)

CONTRIBUTIONS TO ELECTRO-FLUIDO-DYNAMICS (EFD) FOR THE DEVELOPMENT OF BIOLOGICAL ANALYSIS DEVICES

Ecole doctorale Ingénierie - Matériaux, Mécanique, Environnement, Energétique, Procédés, Production
Université Grenoble Alpes (France)

The dissertation, submitted with a view to obtaining the Habilitation à Diriger des Recherches diploma, presents the contributions to Electro-Fluido-Dynamics (EFD) carried out at CEA/DTBS for the development of biological analysis devices. This dissertation is subdivided into three parts which follow the works carried out within three scientific subfields related to EFD and on which biological considerations were gradually being introduced:

1 / Deformation of interfaces to control the movement of microdroplets

The detachment of microdroplets under the effect of an electric field is the result of a complex deformation process. The latest work carried out by our team proposes a detachment model based on a description of the fluid/fluid interface under the combined action of gravity and the imposed electric field. Numerical results are compared with analytical results obtained in the context of small deformations and experimental results. Development axes are identified in order to better describe the case of large deformations.

2 / Electrostatic precipitation for the collection of microorganisms

The second part of the work described here, focused on electric discharges, is approached through two parts: one devoted to electrostatic precipitation and the other to the effects of discharges on microorganisms. This part will begin with a presentation of the reasons that led to the study of electrostatic precipitation for the collection of microorganisms and will continue with a presentation of work in the field, in particular the bioaerosol collectors developed.

3 / Effect of discharges on microorganisms

Since electrostatic precipitation is based on the use of an electrical discharge, its use for the collection of airborne pathogens naturally leads to questions about the effects that electrical discharges can cause on microorganisms. The presentation that was made follows two studies carried out successively in the laboratory:

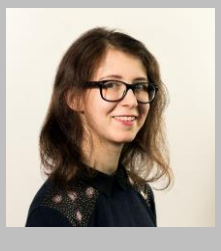
- to preserve viable and cultivable microorganisms for later analysis by culture;
- to extract the intracellular content of the microorganisms such as their DNA for identification by an analytical method based on its amplification. This second application route is also of interest for the extraction of bioproduct compounds such as lipids synthesized by microalgae.

Dr. Stéphane BONNET (Senior Expert)

EXPERIMENTAL AND THEORETICAL CONTRIBUTIONS IN BIOMEDICAL ENGINEERING

Ecole Doctorale Ingénierie pour la Santé, la Cognition et l'Environnement
Université Grenoble Alpes (France)

My HDR presentation includes all the contributions made for the last ten years in the field of motion capture and electrical measurement (passive measurements in EEG (electroencephalography) and active measurements in bioimpedance).
The dissertation details the contributions in signal processing in various fields Biomedical engineering with in particular the introduction of Riemannian geometry for the processing of multivariate EEG signals for the brain-machine interfaces. The dissertation is a synthesis of research work that combines methodology, practical aspects and technological developments.



Clémentine DARPENTIGNY

EMBEDDED BROADBAND MEASUREMENT OF ELECTRICAL IMPEDANCE : APPLICATION TO BATTERY

Ecole Doctorale Ingénierie - Matériaux, Mécanique, Environnement, Energétique, Procédés, Production
Université Grenoble Alpes (France)

In a context where the need for innovative medical devices is increasing and the environmental issue is becoming a major concern, the aim of the thesis was to prepare antimicrobial wound dressings using the greenest possible way. For this purpose, nanocelluloses have been chosen as bio-based and biocompatible building blocks for the design of porous architectures and their functionalization with antimicrobial agents was then undertaken in supercritical CO2 medium (CO2sc) used as an alternative to organic solvents and by taking advantage of its specificities such as high diffusivity, easy removal of solvent and residual reagents and compatibility with fragile materials. Thus, 2D and 3D structures, nanopapers, cryogels and aerogels, exhibiting various physicochemical properties were prepared from cellulose nanofibrils (CNFs) and nanocrystals (CNCs). In order to introduce antibacterial functionality, porous materials, prepared from nanocellulose, were impregnated in scCO2 with a natural oil, Thymol. Results show a direct relationship between the amount of impregnated molecules and the specific surface that leads in the case of cryo- and aerogels to good antimicrobial properties against two types of bacteria and yeast. In a second strategy, covalent grafting of CNFs structures in scCO2 was investigated with a novel antibacterial aminosilane. Surface analysis characterizations methods confirmed the successful grafting on nanopapers. The contact active properties of grafted nanopapers and cryogels were assessed. These results are very promising for the design of antimicrobial biobased and biocompatible medical devices using supercritical conditions.

Morgane SEON-LUTZ

DEVELOPMENT AND CHARACTERIZATION OF FUNCTIONAL NANOFIBERS BASED ON HYALURONIC ACID AND LIPID NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

École Doctorale Physique et Chimie-Physique
Université de Strasbourg (France)

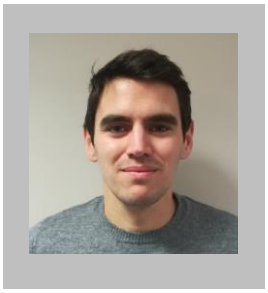
Hyaluronic acid (HA) is a polysaccharide for which biocompatibility and bioactivity properties make it a very interesting compound as wound dressings. In this work, biocompatible insoluble HA-based nanofibrous dressings were designed by electrospinning in pure water in order to overcome any toxicity issues. To this purpose, poly(vinyl alcohol) and hydroxypropyl-β-cyclodextrin were added to form uniform nanofibrous scaffolds. An in situ crosslinking process of the scaffolds is also investigated to ensure the stability of the fibrous structure during the use of the dressing. For opening the scope of wound application, various pathways of functionalization of these materials have been envisaged. The first one was the direct impregnation of naproxen, a model drug with anti-inflammatory properties, into the scaffolds either in aqueous solution or under supercritical CO2. The second way is based on the incorporation, within nanofibers, of lipid nanoparticles in which drugs can be encapsulated and then delivered to its wound sites.

Isaure LE CARDINAL DE KERNIER

CYTOMETRY BY WIDE FIELD PHASE AND FLUORESCENCE IMAGING. APPLICATIONS IN HEMATOLOGY

Ecole doctorale Physique et Sciences de la Matière
Aix-Marseille Université (France)

Blood cell population analyses allow detecting a wide scope of clinical disorders, ranging from anemias to malaria. A very large number of cells ought to be considered so as to ensure the statistical significance of the result, and in turn, yield a reliable diagnosis. Currently, hematology analyses are based on flow cytometry techniques and performed in specialized laboratories. In these devices, high throughput is obtained at the expense of the information content of each acquisition. To reduce the time-to-result, and to minimize the complexity and cost of the systems dedicated to analyzing cell populations, the current need is to reduce the number of acquisitions and optimize the information content. This thesis focuses on single-shot image cytometry as an alternative to flow-based cytometry. It aims at obtaining a set-up based on optical contrasts for the study of large cell populations while preserving the ability to resolve individual cells. We investigate a multi-scale and multi-modal approach to detect, characterize, and classify blood cells. To evaluate the feasibility and clinical relevance of the method, we developed two proof-of-concept set-ups, respectively called the mesoscope and the miniscope. The mesoscope, based on optical developments, combines phase contrast with fluorescence. The complementarity of morphological features and the expression of specific fluorophores enables us to accurately classify blood cells, and for example assess Plasmodium falciparum parasitemia in whole blood samples. The results are benchmarked to reference techniques. However, to address the need for point of care analyses, the system should be miniaturized. Hence, we designed the miniscope, a chip-based bimodal imager.



Thibault KRAMER

DEVELOPMENT OF A MICROVASCULAR NETWORK ON CHIP FOR TISSUE RECONSTRUCTION

École Doctorale Chimie et Sciences du Vivant
Université Grenoble-Alpes (France)

Tissue engineering aims to develop functional tissues or organs in vitro to provide drug testing platforms or transplantable tissues and to improve treatments provided to patients. However, physiological tissue structures developed to date do not integrate perfusion vascular network. In vivo, the vascular network supplies body cells with oxygen and nutrients and removes cell waste and carbon dioxide. It also has a major role in maintaining organ homeostasis. Blood capillaries are hollow vessels whose walls are only composed of a layer of endothelial cells. The blood capillary network is dense and perfuse all tissues. Due to the limited oxygen diffusion inside tissues, each cell is located at most 200µm away from a capillary. During my thesis, thick tissue micro-vascularization was developed using an innovative method consisting on growing a network of capillaries through the pores of a stacked-assembly of spherical tissue micro-units within a µfluidic chamber. Tissue micro-units are composed of extracellular-matrix biopolymers and cells from the tissue of interest. A layer of endothelial cells is developed on the surface of these microspheres. The stacking of tissue microspheres creates a porous structure in which nutrient medium is perfused. Flow control within such a structure allows the application of physical stimuli influencing the self-assembly of endothelial cells into capillaries. During this thesis, a system dedicated to tissue microspheres biomanuacturing from natural biopolymers was developed. The structure formed by the stacks of spheres was studied and the flow within such environments were characterized. A bioreactor-like perfusion system was built. A thick tissue structure was formed within this system and the growing of the vascular network was promoted and demonstrated. The developed technique is promising for the design of a range of tissues and for organ-on-chip or tissue engineering devices.

Roxane CROUÏGNEAU

NEW STANDARDIZED AND FUNCTIONALIZED MICROCAPSULES : APPLICATIONS TO TYPE 1 DIABETES CELL THERAPY

Ecole doctorale Ingénierie pour la Santé, la Cognition et l'Environnement
Université Grenoble-Alpes (France)

Cell therapy with allogenic pancreatic islets is a treatment used for type 1 diabetic patients for whom the usual insulin treatments does not work anymore. However, this therapy requires administration of an immunosuppressive treatment that leads to many side effects and complications. The implantation of microencapsulated islets that become stealth to the host's immune system consists in an alternative to immunosuppressive treatments. This technique presents very promising results in several clinical trials. The FUTURE Caps project, in which my thesis is done, tryes to tackle some of the remaining challenges : the properties of the encapsulation polymers by using chemically modified alginates, that increase the mechanical stability of the capsules ; and the control in size and shape of the capsules, that enables to have an optimal diffusion barrier, by using a microfluidics flow focusing (MFFD) droplet generation system, developed at the CEA. The physicochemical properties of the different alginates were first characterized, and theoretical rheological models that define their behavior were determined. Then, the parameters involved in the formation and gelation of the droplets were studied. Following these studies, monodisperse capsules (variation coefficients under 5 %) were obtained in microfluidics with the different alginates (that have very diverse properties), and these latter were characterized in terms of size, shape and permeability. Eventually, neo-natal pig, and human pancreatic islets were encapsulated in the alginates studied. The encapsulated islets viability was measured in vitro, and they have also been implanted in mice, in order to check for biotolerability and viability in vivo after 15 days.

David ORIVE-MIGUEL

TIME-RESOLVED MULTISPECTRAL OPTICAL TOMOGRAPHY FOR RECONSTRUCTION OF DEPTH-RESOLVED CHANGES IN OXY- AND DEOXYHEMOGLOBIN

Ecole Doctorale Electronique Electrotechnique Automatique Traitement du Signal
Université Grenoble Alpes (France)

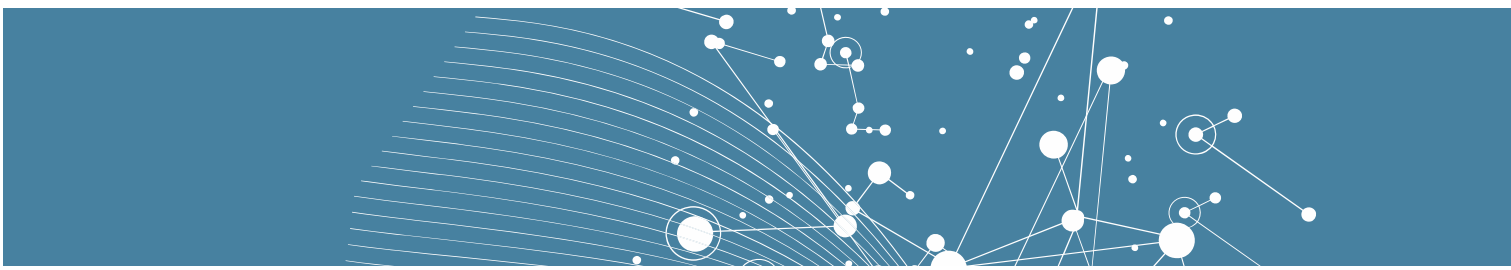
Noninvasive and continuous monitoring of patients are key features in the future of medical imaging. Biophotonics is a field that is attracting a lot of interest because its technology is intrinsically noninvasive and potentially miniaturizable and wearable. Regarding the imaging of human tissue using photonics, it has been proven that near-infrared diffuse optical tomography (DOT) permits to probe noninvasively and in depth the human tissue by reconstructing parameters of the composition of biological tissues such as the concentrations of oxygenated and deoxygenated hemoglobin in the blood. In this thesis, I describe the novel improvements I developed in the field of time-resolved DOT algorithms. First, I introduce a novel method to compute datatypes for tomographic reconstruction of time-resolved measurements. The results show that with this new approach the noise of datatypes are decorrelated and resolution in depth of reconstructions is improved significantly for inclusions deeper than 2.5 centimeters. After, I describe two different approaches to perform total variation regularization for DOT reconstruction on irregular meshes. The knowledge developed in previous parts was applied to in-vivo experiments on human subjects. In collaboration with Politecnico di Milano, I tested a time-resolved tomographic system with two probes of three source fibers and four silicon photomultiplier detectors each. Arm occlusion experiments were performed to validate the technology. After, I did motor cortex activation experiments on three different subjects. The results show that it is possible to monitor with one-second resolution the motor cortex activation and that spatial and in depth information can also be retrieved. Finally, I introduce the reader to the effort that is being done at BitMap network to push the standardization of diffuse optics field. I describe the work I did to build an open dataset with the measurements performed at twenty-eight instruments from eight different European institutions using three validated European protocols.

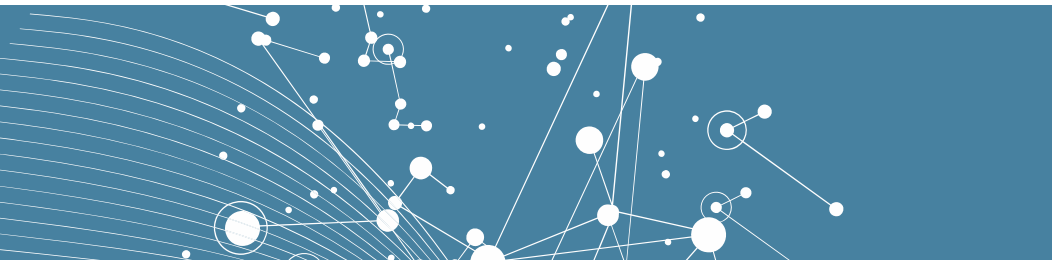
Raphaël BOUVET

NANOENCAPSULATION OF DRUGS TO TREAT SKIN ULCERATIONS. A PROOF OF CONCEPT

Ecole doctorale Ingénierie pour la santé la Cognition et l'Environnement
Université Grenoble Alpes (France)

Wound healing is a multifactorial process. In diabetes, there is a change in the processes involved in healing. Indeed, chronic hyperglycemia in diabetic patients modifies tissue function at the endothelial level and at the cutaneous level. Chronic ulcers in diabetic patients are a major public health problem with limited efficacy of current therapy. They represent a major factor in the deterioration of quality of life with a significant risk of lower limb amputation .In this thesis, we evaluated the potential of encapsulation of active ingredients in lipid nanoparticles for local delivery. Lipid nanoparticles are galenic forms that stabilize the active pharmaceutical ingredients and improve skin delivery. The active pharmaceutical ingredients used in this thesis will be vasodilators. They can increase wound healing by their effect on vascular function, but also on the processes of inflammation, proliferation and tissue remodelling. We were able to validate the effect of encapsulation in lipid nanoparticles to improve the stability of formulations of different vasodilator active ingredients. In the healing of diabetic wounds, we have observed an improvement in healing when administering prostacyclin analogues. Additional studies are being carried out to describe the mechanisms used to improve healing by these formulations.





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