Bioéconomie & Santé

Ressources naturelles au service de la santé humaine

Session:
Le biosourcé appliqué aux biocomposites et aux matériaux biocompatibles

Biomatériaux pour la santé et actifs naturels: biofonctionnalisation et régulation de la réponse biologique

Emmanuel Pauthe

CERGY PARIS UNIVERSITÉ

ERRMECe
équipe de recherche sur les relations matrice extracellulaires-cellules

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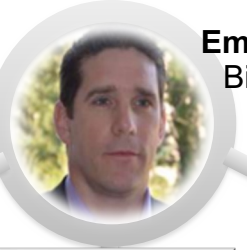
Diversity & Complementarity

Emmanuel Pauthe
Biochemist



Violeta Rodriguez
Pharmacist

Adeline Gand
Enzymologist

Damien Seyer
Microbiologist

Michel Boissiere
Chemist, biophysist

Carla Palomina
Cell biologist

Agnes Mihajlovski
Molecular biologist

Ph.D. students

Maxime Gobin

Phuong A. Dang
Sept 2018

Rosa Calderon

Solene Rota
Oct 2018

Audrey Deraine

Anamar Miranda
Sept 2019

Proactive and biomimetic
Biomaterial

Biological env. Cellular and Tissular

biological response

- Reparation & subtitution
- Biofunctinality
- Delivery

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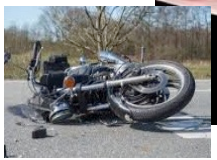
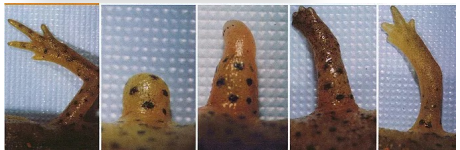
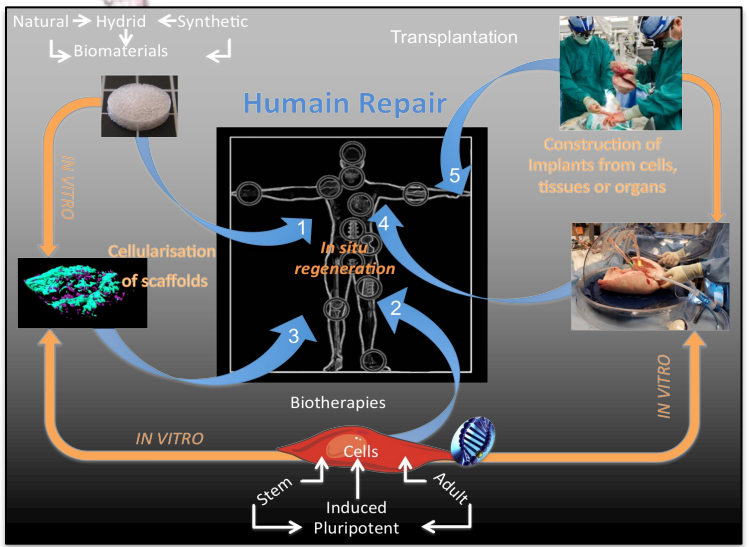
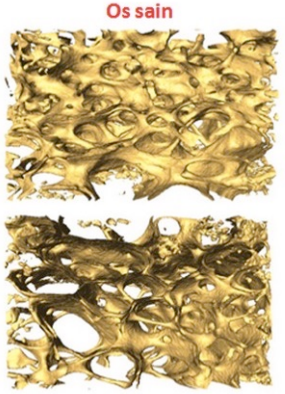


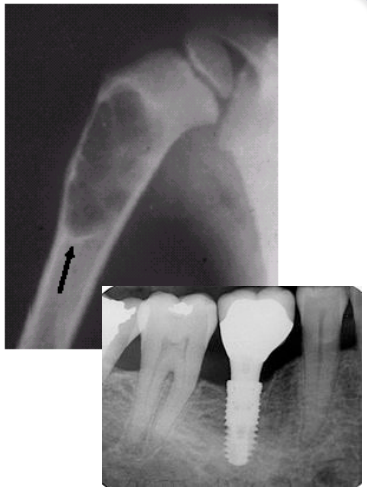




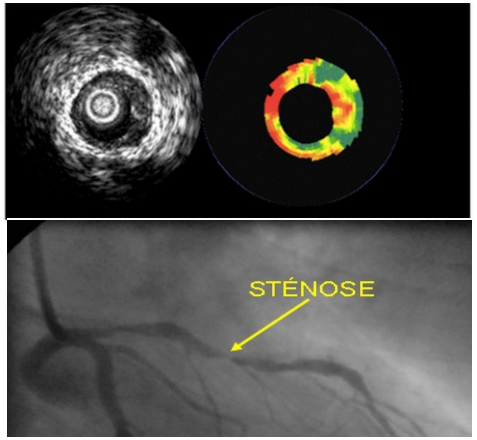
Various physiological situations

Mineralized tissues

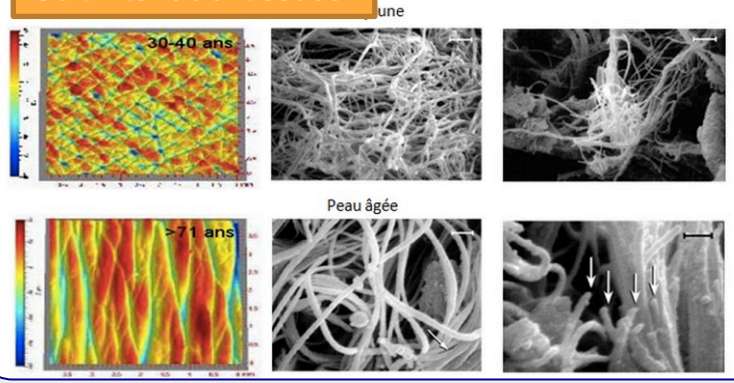




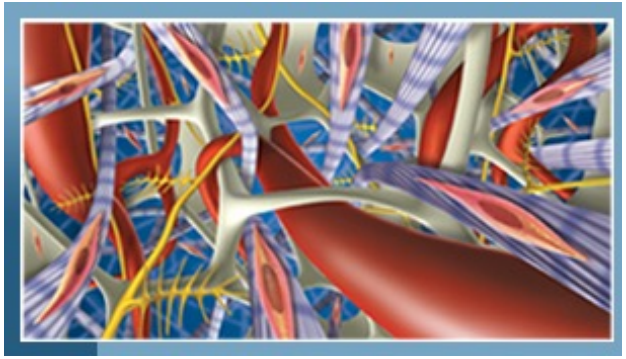
Soft deep tissues



Soft interfacial tissues



New exigence / new definition…..



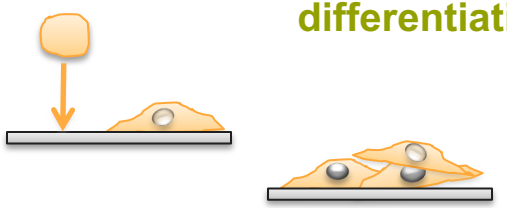
Accommodation of the bioactivity:
updating of the original definition of biocompatibility

*"**ability of a biomaterial to perform its desired function** with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but **generating the most appropriate beneficial cellular or tissue response to that specific situation**, and optimizing the clinically relevant performance of that therapy" Williams 2008*

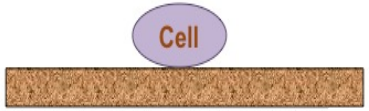
New challenges: "Biocontrol", concept of proactivity

Biomaterials: control of the cells & vice-versa

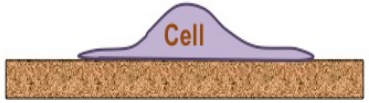
→ cell adhesion,
proliferation,
differentiation…..

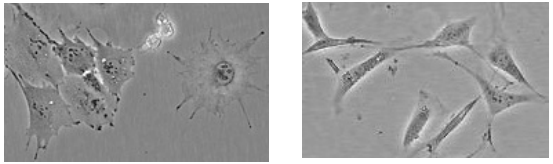


Original paradigm: Inert biomaterial

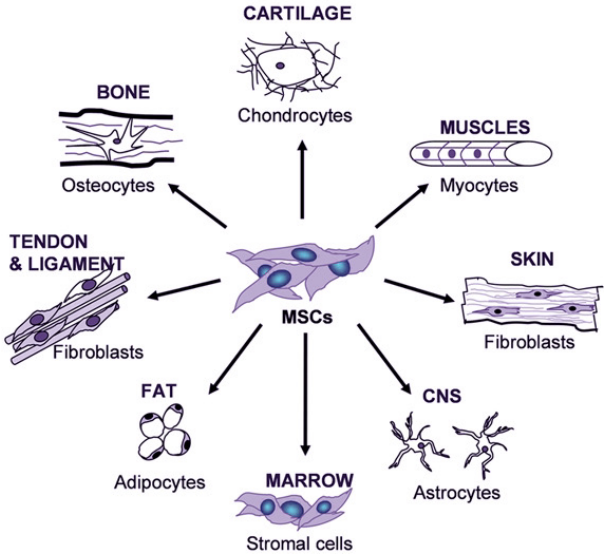


Current paradigm: Interactive biomaterial, via interface



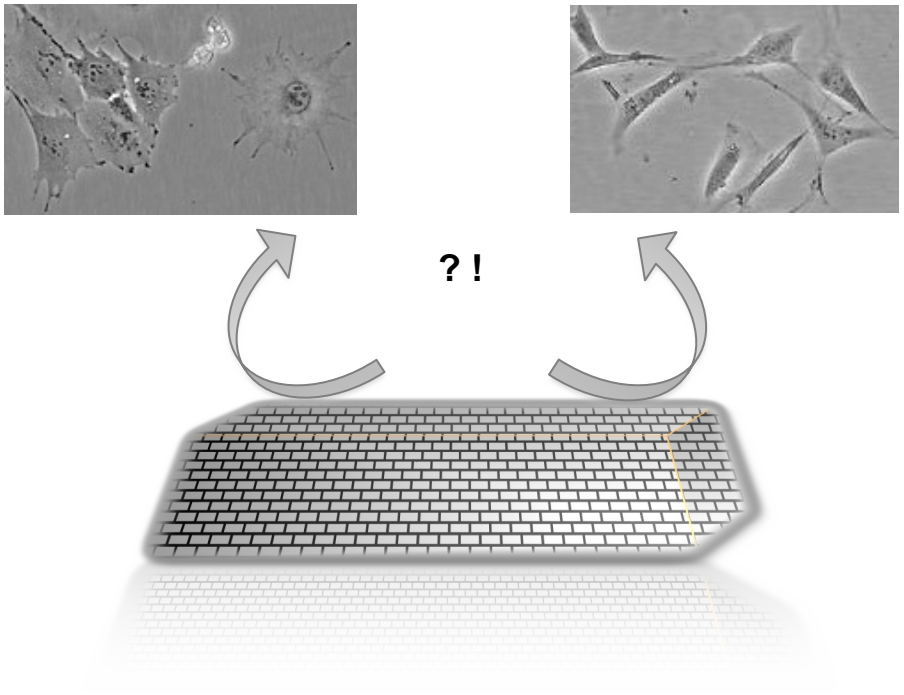


Direct, influence



Cells-biomaterials dialogue

How ?



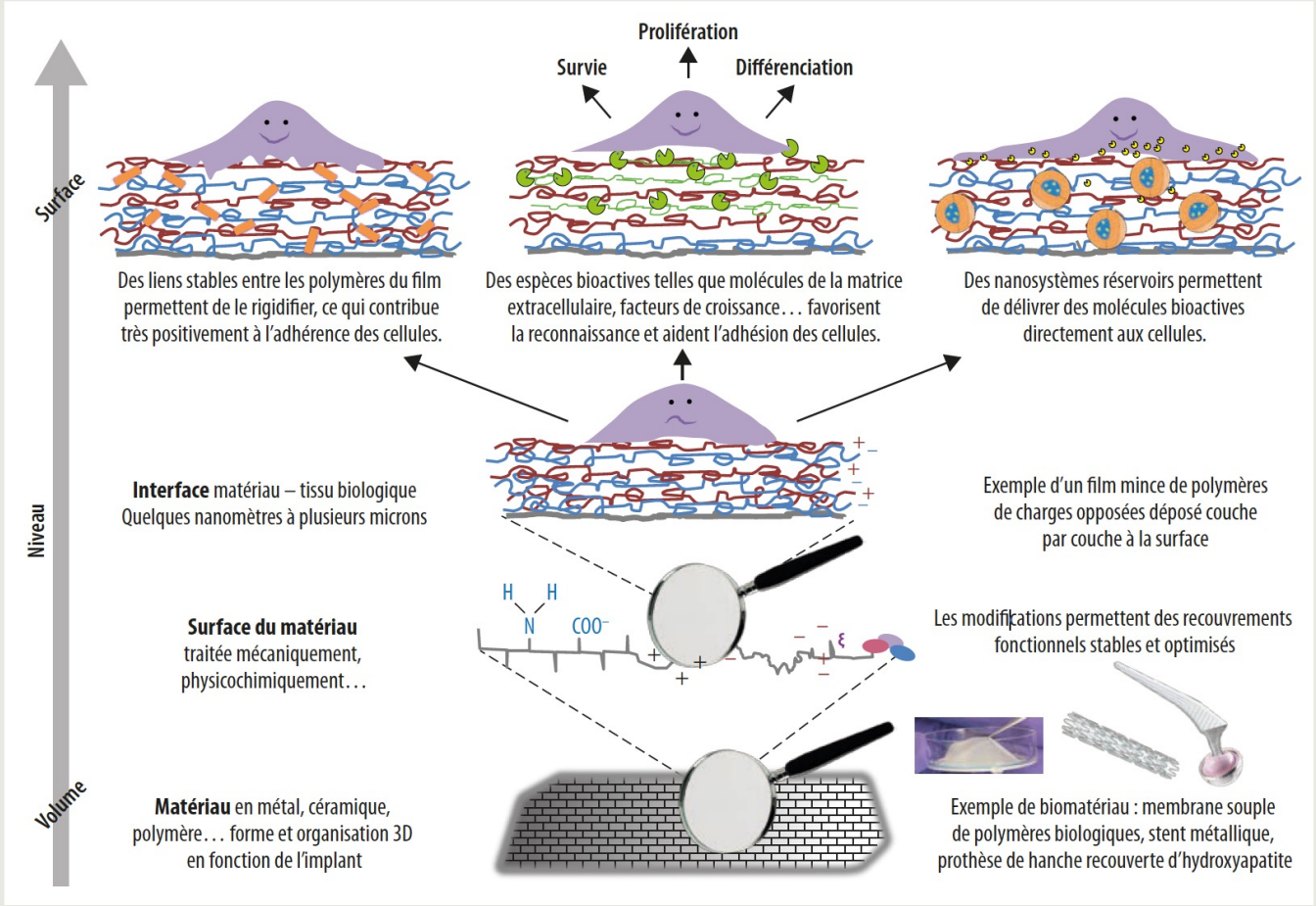


Figure 1. Stratégies de modifications chimiques, physiques et biologiques de l'interface matériau-tissu.

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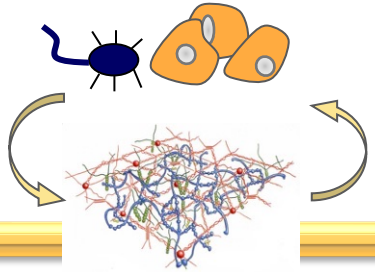

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Molecular and supramolecular approaches

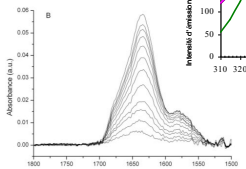
- Structure and organisation of matrix proteins
- Structural and conformationnal dynamics
- Organisation in solution, at interfaces and in gelified states

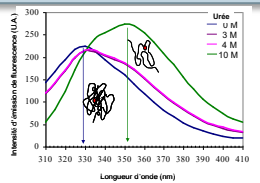
Proactive and biomimetic Biomaterial

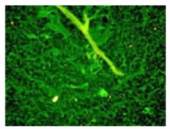
interface

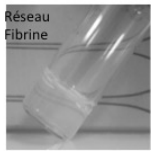
Biological environment

Cellular and Tis









Toward an optimized biological response

- Reparation & subtitution
- Biofunctionality
- Delivery

Biomaterial for Health

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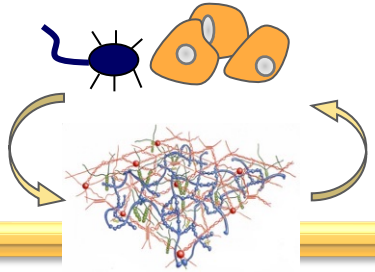
sur les relations matrice
extracellulaires-cellules



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Molecules naturelles

- Algues
- Fruits,
- Plantes, racines…..

Proactive and biomimetic Biomaterial

interface

Biological environment

Cellular and Tis

Toward an optimized biological response

- Reparation & subtitution
- Biofunctionality
- Delivery

Development of a curcumin loaded-NLCs hydrogel system for topical applications

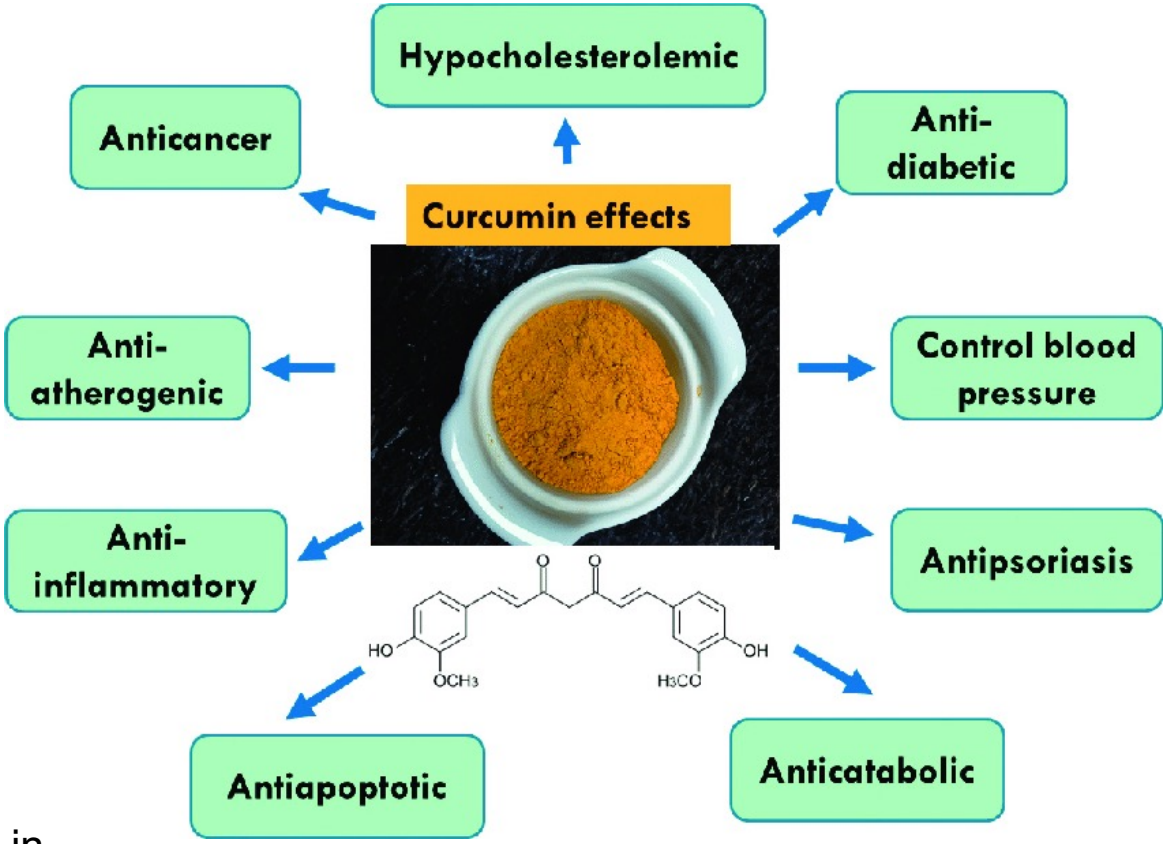
R. Calderon-Jacinto
V. Rodriguez-Ruiz, P. Matricardi, G. Pavon-Djavid, V. Gueguen, E. Pauthe

NLCs HYDROGEL: ALLOWING THE TOPICAL APPLICATION OF CURCUMIN

Prevents oxidative stress during wound-healing

By directly neutralising ROS (O_2^- , H_2O_2, HOCl)

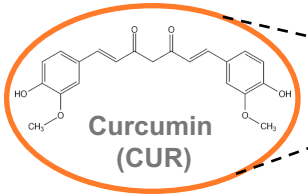
By activating cytoprotective pathways in dermal cells



Development of a curcumin loaded-NLCs hydrogel system for topical applications

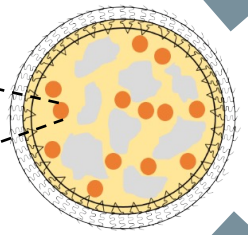
- Antioxidant
- Cytoprotective towards cells

Prevents oxidative stress during wound-healing

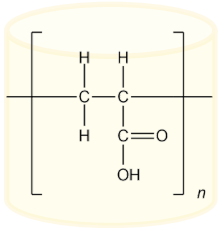


Problems for topical application

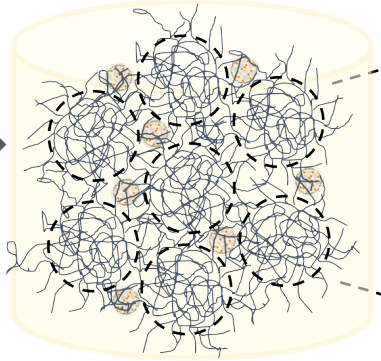
- ↓ Water solubility
- ↓ Stability: heat and light



CUR-NLCs



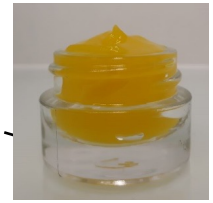
Hydrogel



Human **fibroblasts** (BJ)

Human **keratinocytes** (HEKn)

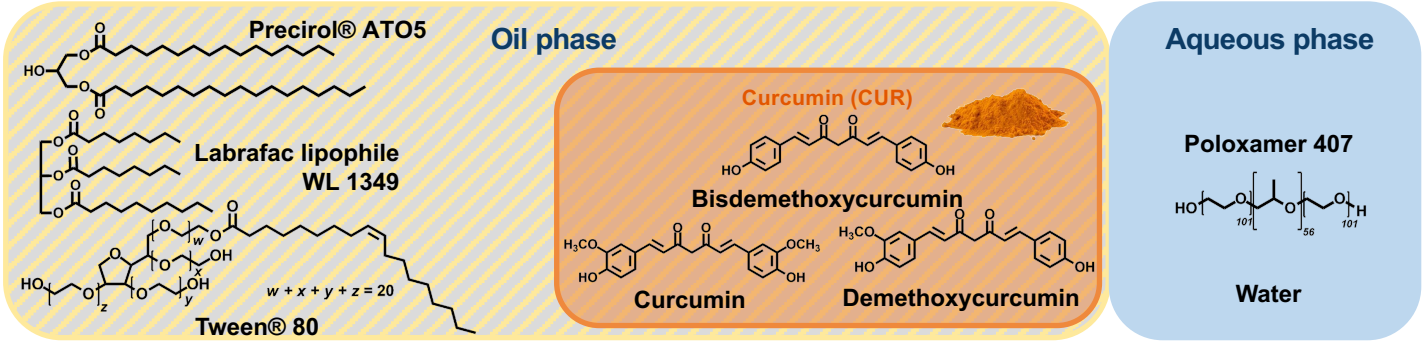
Hydrogel
+
CUR-NLCs



PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION OF THE NLCs@Curcumin

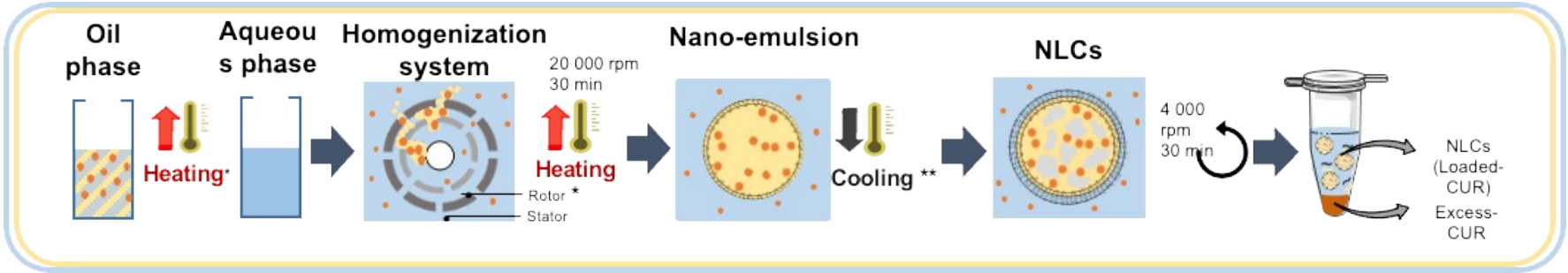
Components of NLCs:

lipids (solid and liquid), co-surfactant (yellow and grey stripped box)
CUR composed of three curcuminoids (orange box)
aqueous phase containing the surfactant (blue box)



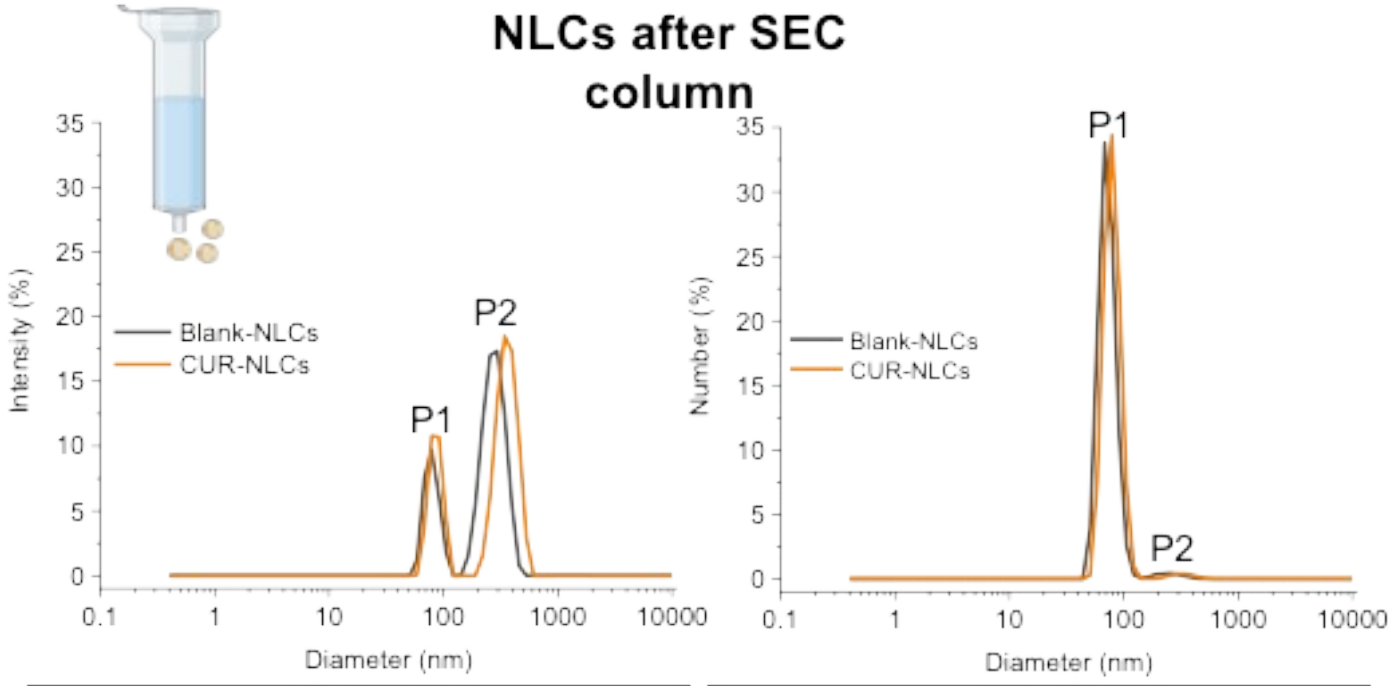
NLCs preparation by Hot Homogenization

Method at heating temperature of 70°C and separation of non loaded CUR (Excess-CUR) by centrifugation

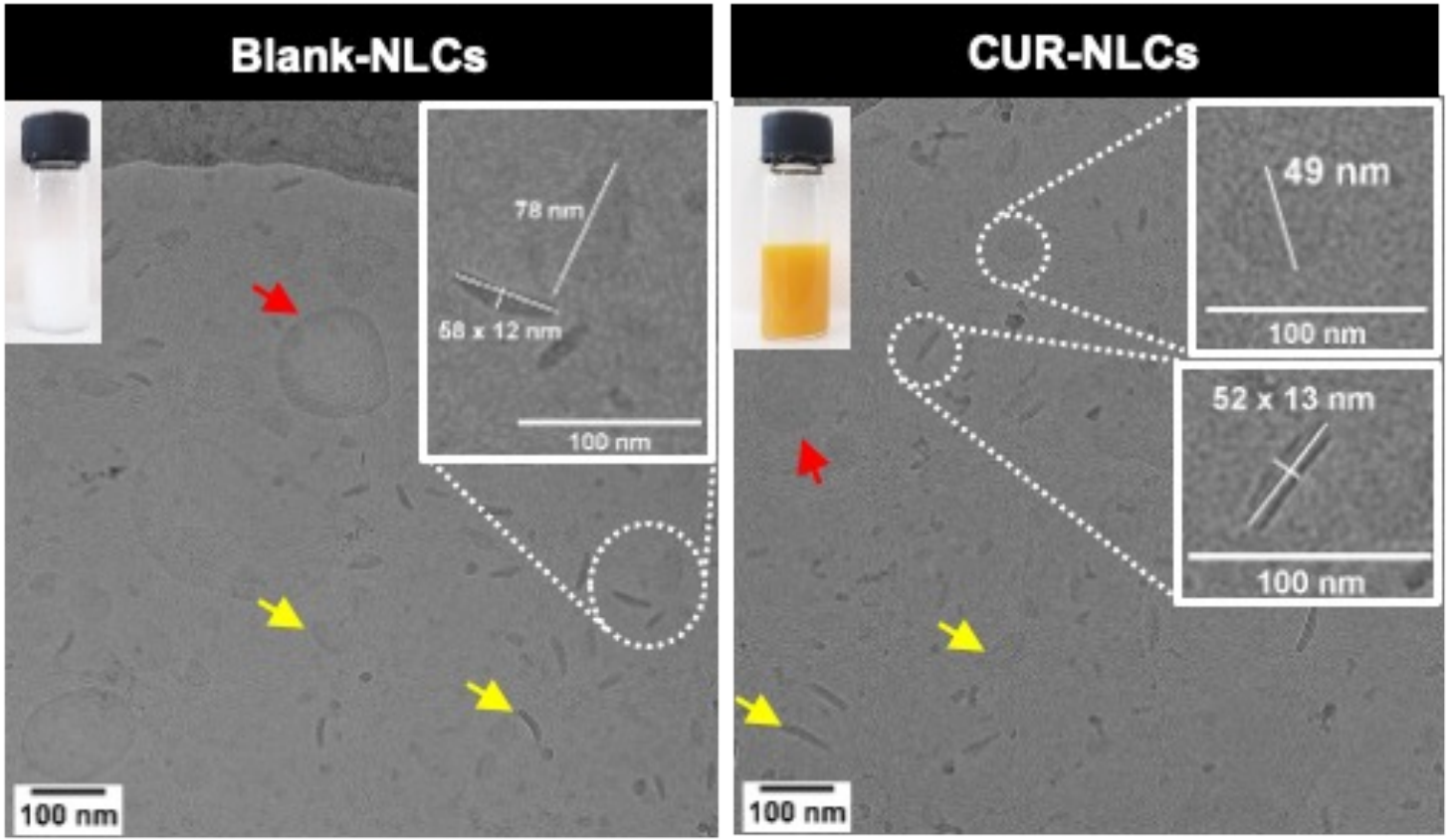


Physicochemical and Morphological Characterization of Blank-NLCs and CUR-NLCs suspensions

Particle size analysis in % intensity and in % number



Visual aspect and Cryo-TEM images of samples ions



.

Yellow arrows show circular platelet shape or rod shape of small size NLCs population (~ 75 nm, P1).

Red arrow shows view of large size NLCs population **(~** 300 nm, P2).

Size, Charge

~ 0.8 g CUR/L
EE ~ 85 %

Room temperature

Size and ZP

CUR-NLCs

299 +- 32 nm
(~3 %)
72 +- 8nm
(~97 %)

ZP ~ -10 mV

Blank-NLCs

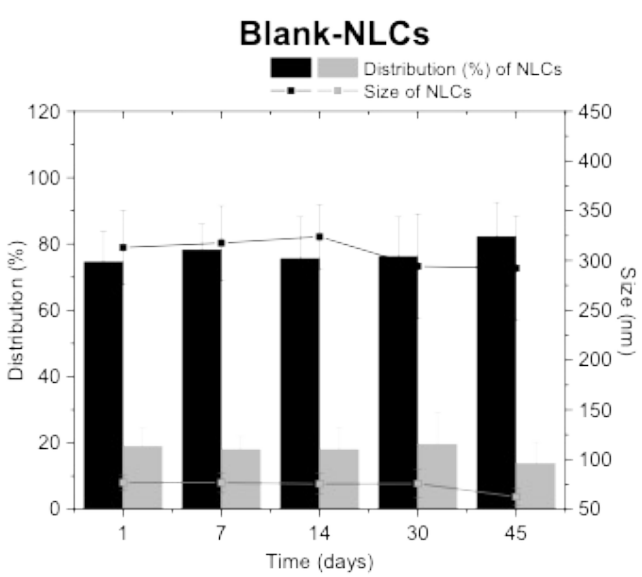
317 +- 33 nm
(~5 %)
80 +- 7nm
(~95 %)

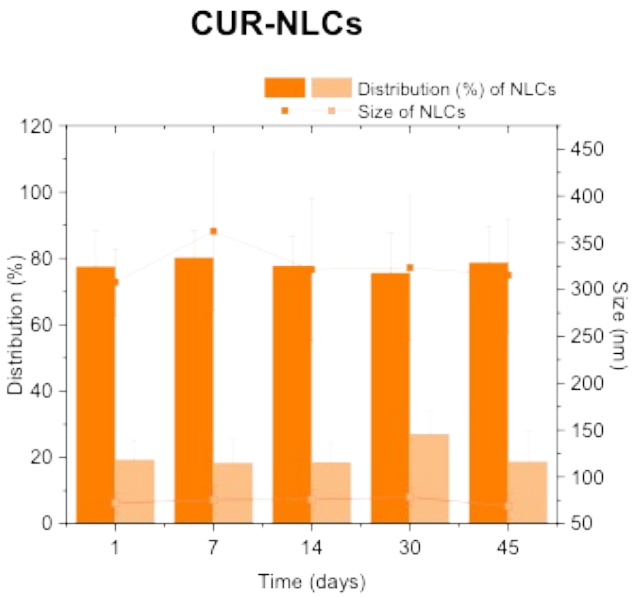
- **La taille des objets**
- **La répartition entre les 2 populations**
- **La charge**

ne sont pas influencés par la présence de CUR

Stability

Blank-NLCs & CUR-NLCs at 1, 7, 14, 30 and 45 days after preparation.



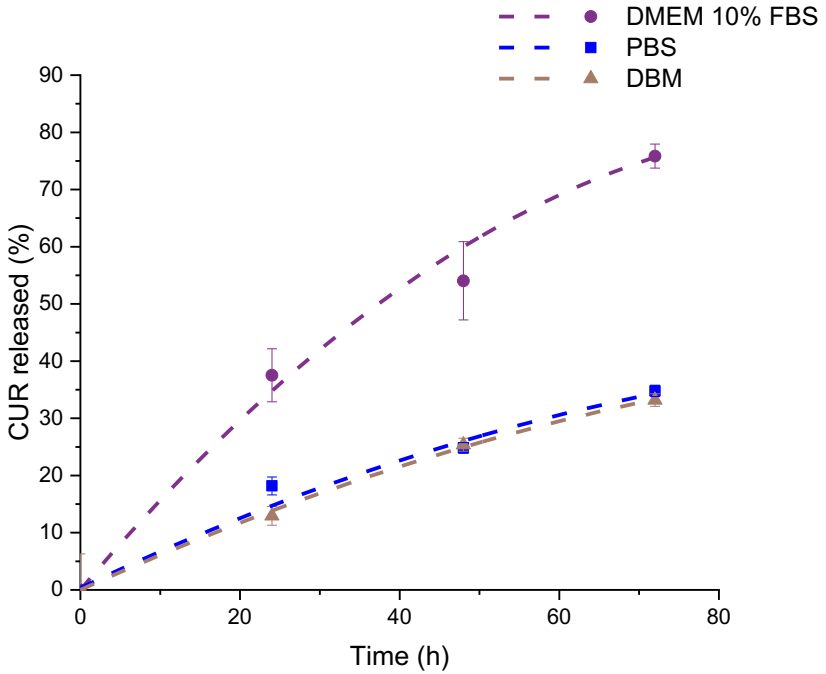


Les particules sont stables dans le temps et peuvent être stockées

Bar graphs indicate the distribution in % intensity of NLCs while line and dot graphs indicate the size of each NLCs population in terms of their diameter

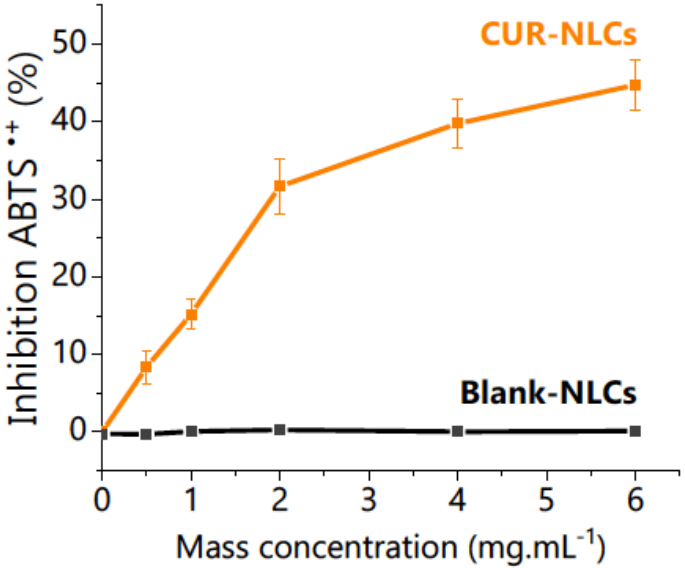
CUR delivery & properties

CUR release from NLCs in different biological medium



Le milieu de culture cellulaire favorise le relargage de la curcumine

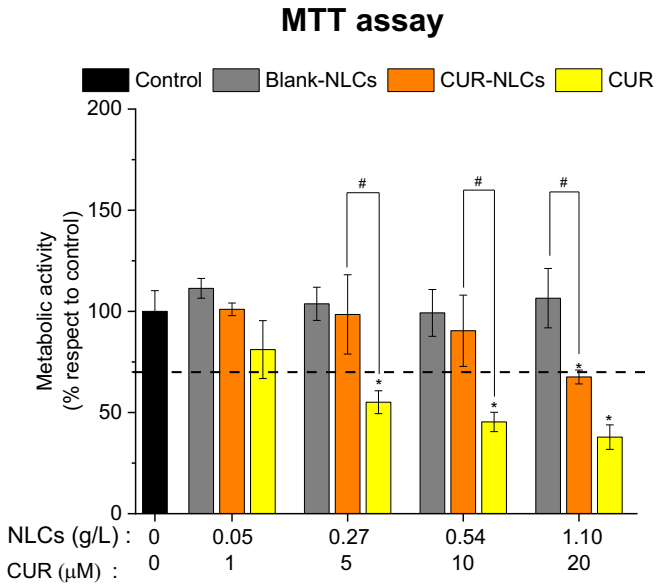
Antioxidant activity

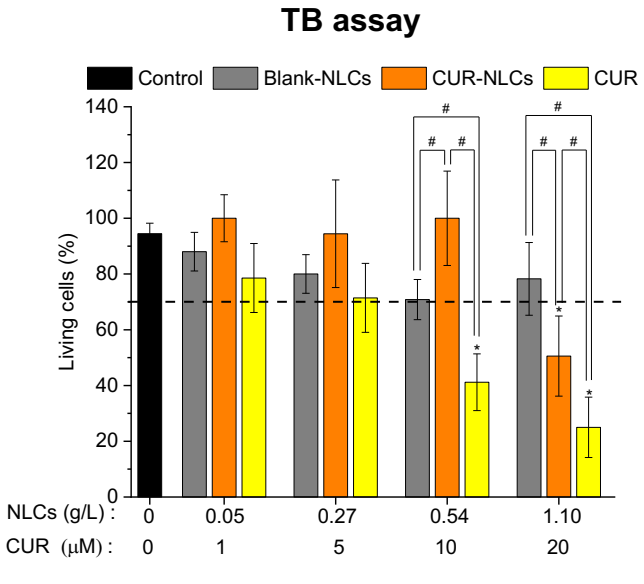


Les propriétés antioxydantes de la curcumine sont préservées

Cell viability assays

BJ Fibroblasts in basal conditions





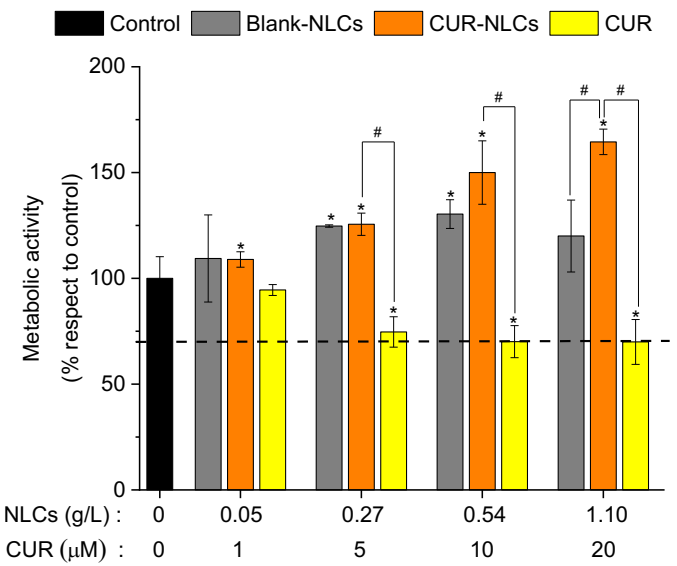
L'encapsulation de la CUR diminue sa cytotoxicité

Possibilité de monter jusqu'à 10 μM

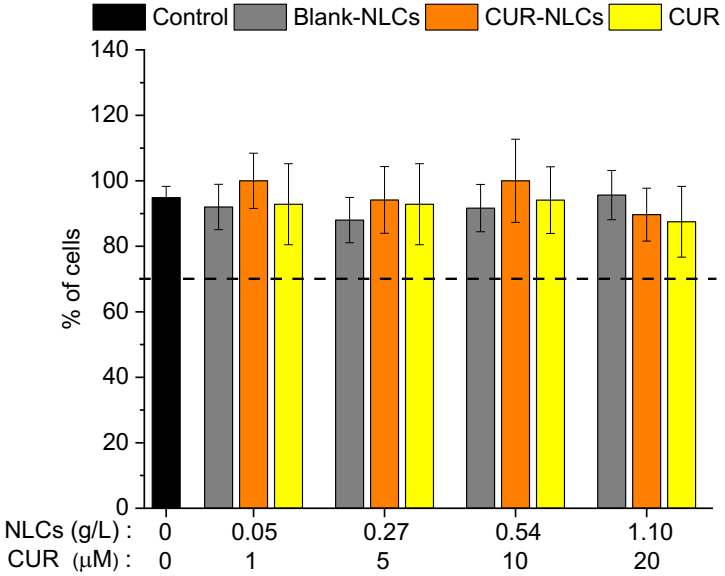
Cell viability assays

HEKn in basal conditions

MTT assay



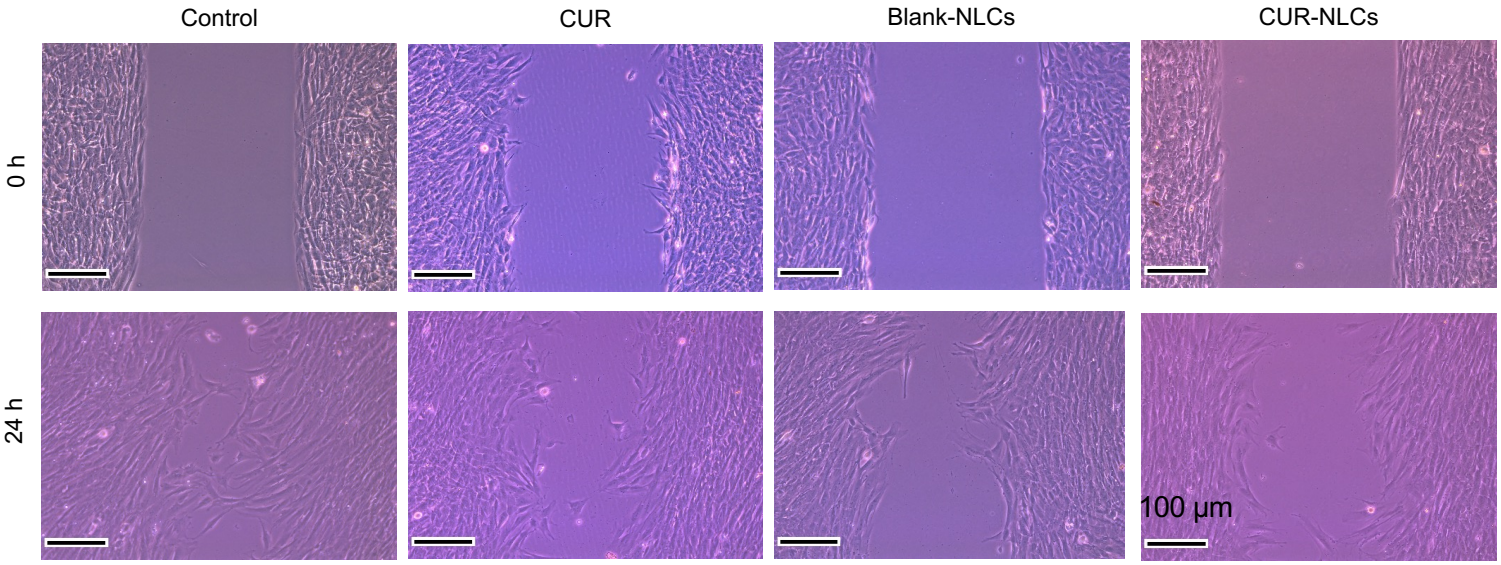
TB assay



Kératynocites sont plus résistants à la CUR libre

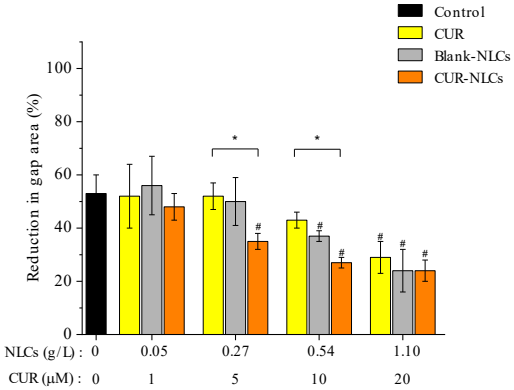
NLCs semblent favoriser l'activité des Kératinocytes

Cell migration/proliferation studies



BJ in

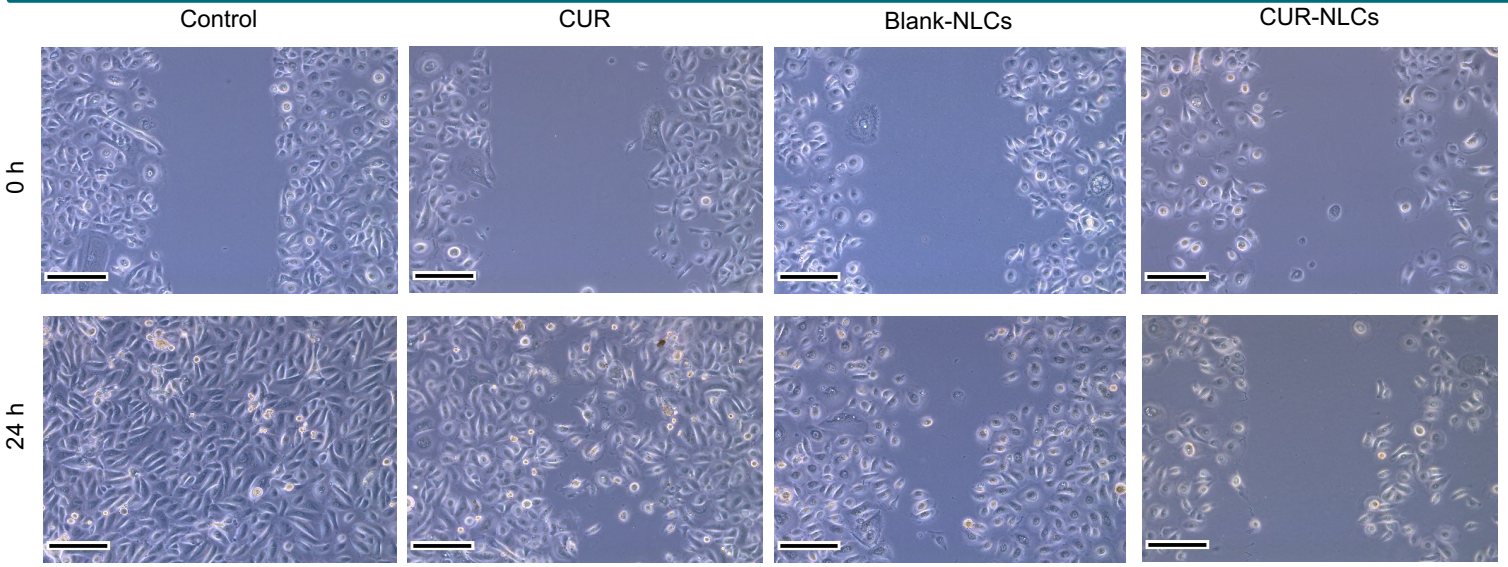
- basal conditions
- CUR (5 µM)
- Blank-NLCs (0.27 g/L)
- CUR-NLCs (0.27 g/L containing 5 µM of Loaded CUR)..



Phase contrast images of the gap at 0h and after 24h of treatment for BJ Fibroblasts. Control group was treated with DMEM 10%FBS

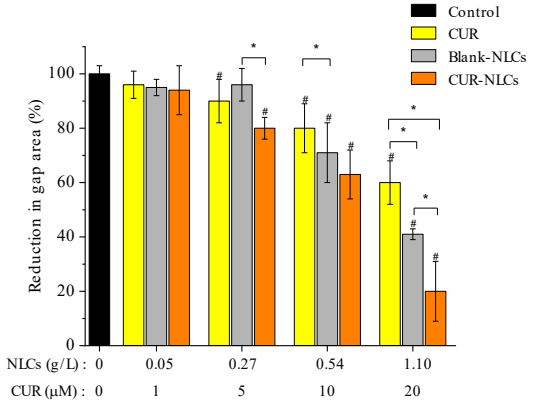
Diminution migration et prolifération des fibroblastes en fonction de la quantité de NLC et de NLC@CU

Cell migration/proliferation studies



HEK in

- **basal conditions**
- CUR (5 µM)
- Blank-NLCs (0.27 g/L)
- CUR-NLCs (0.27 g/L containing 5 µM of Loaded CUR)..

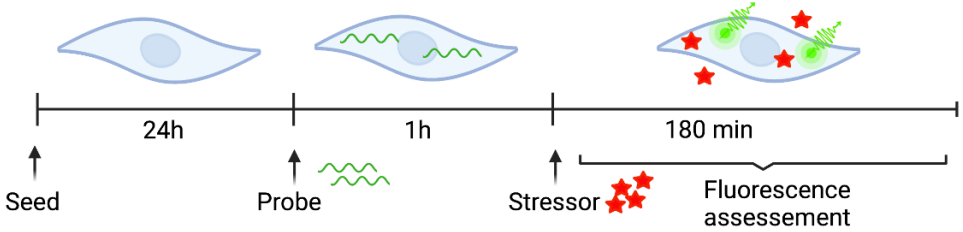


Phase contrast images of the gap at 0h and after 24h of treatment for Keratinocytes Control group was treated with DMEM 10%FBS

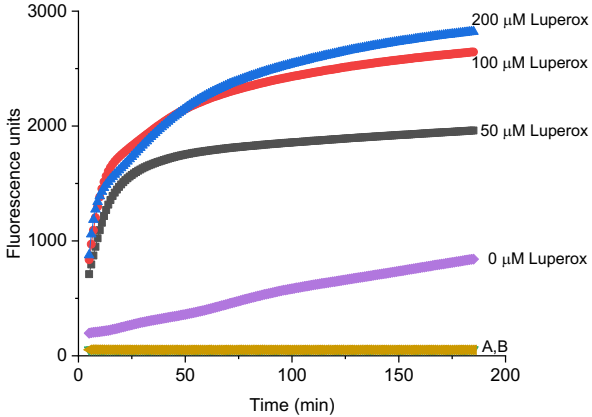
La CUR, libre, mais aussi et surtout encapsulée dans les NLCs perturbe la migration cellulaire

Intérêt / conditions physiopathologiques critiques tel que psoriasis, dermatites, plaies aigues….

Oxidative stress induction and evaluation on BJ Fibroblasts

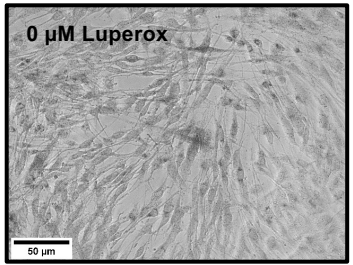


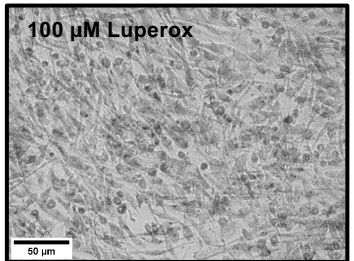
In vitro stress model for the induction and the evaluation of oxidative stress



Evaluation of oxidative stress induced by different concentrations of the stressor (Luperox)

Phase contrast images of BJ Fibroblasts after 1h treatment with 0 µM or 100 µM of stressor and subsequently incubation with MTT for 2h30

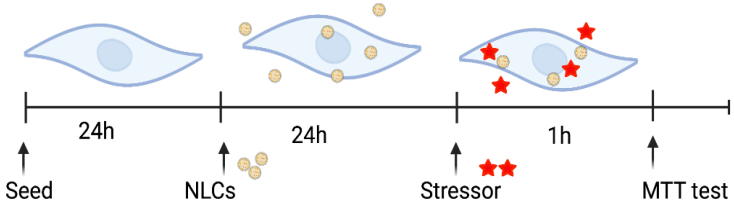




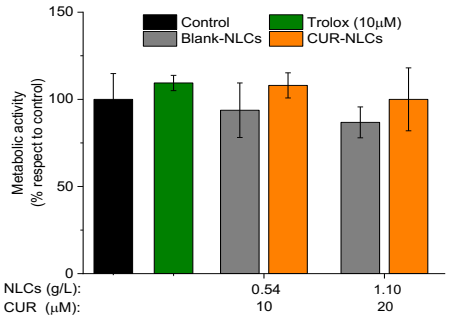
Cellules « bien stressés »:

- **produisent fortement espèces réactives de l'oxygène (ROS)**
- **activité métabolique conservée, tapis cellulaire**

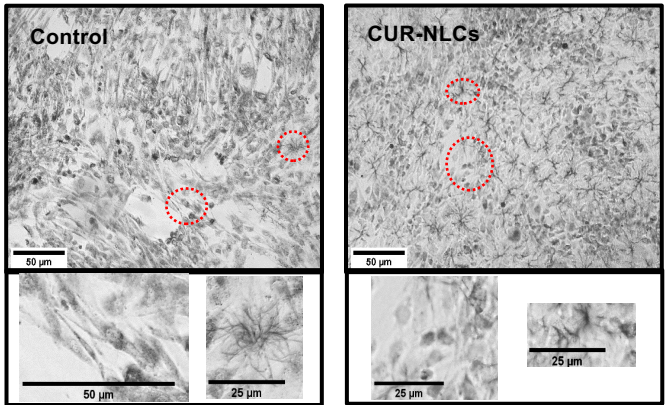
Effect of NLCs on BJ Fibroblasts under oxidative stress



In vitro model for the evaluation of the effect of NLCs on BJ Fibroblasts metabolic activity after undergoing oxidative stress for 1h



Impact of Blank-NLCs and CUR-NLCs on the metabolic activity of BJ Fibroblasts undergoing oxidative stress; Trolox 10 μM was used as an antioxidant standard

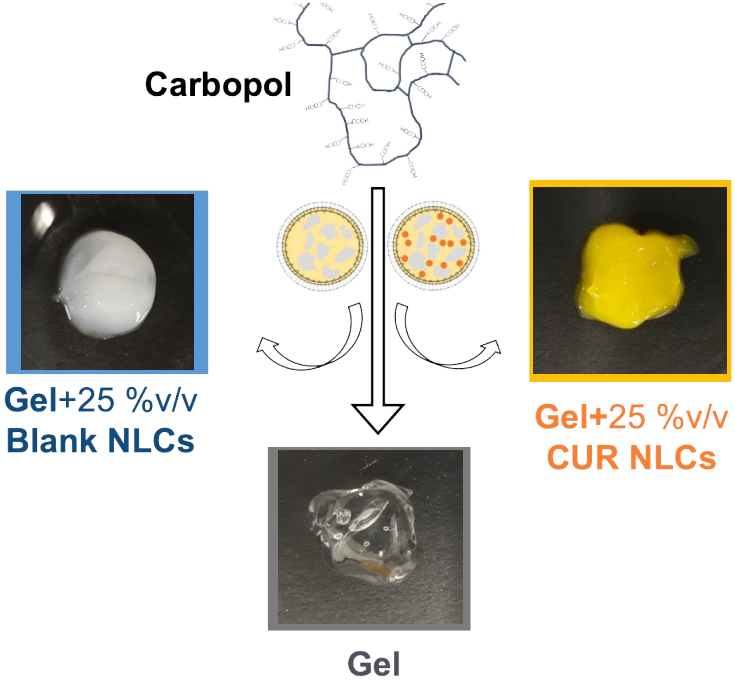


Phase contrast images of BJ Fibroblasts after 24h treatment with DMEM 10% FBS control *vs* 1.10 g/L of CUR-NLCs 20 μM of CUR

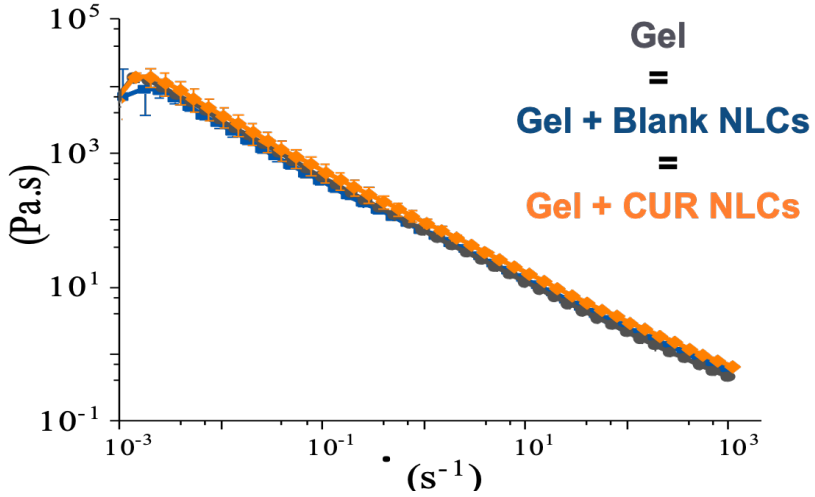
exposition to 100 μM of stressor for 1h and subsequently incubation with MTT for 2h30.

extended morphology of fibroblasts as well as the formation of formazan crystals can be distinguished, evidencing metabolically active cells

Gel preparation and properties

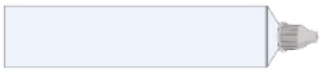


Shear-thinning behaviour



Le comportement pseudo-élastique du gel n'est pas modifié par la présence des particulers +/- CUR

Rheological characterization, at 32°C (skin temperature), in conditions mimmicking a topical application



Formulation in tube

High shear

Squezeed from tube

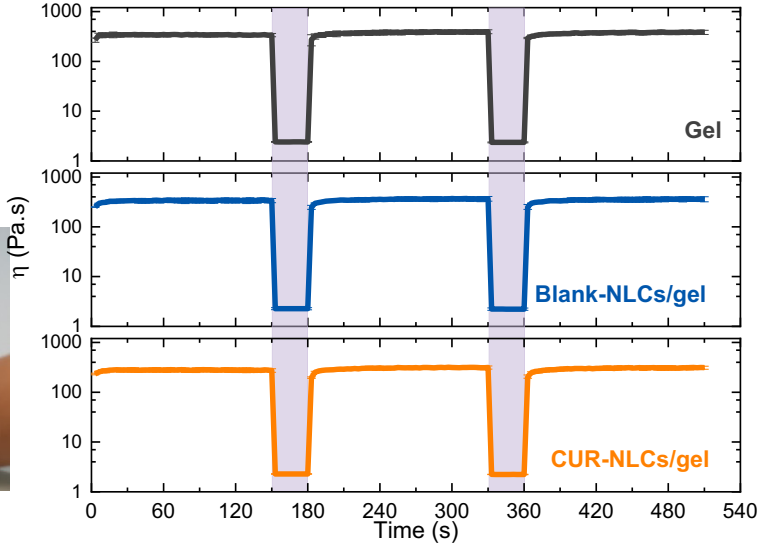
Laying on skin

High shear

Spreaded

Remaining on skin

Time-dependent flow behaviour



Composite gels rapidly recover its structure after undergoing high shear periods of time.

CONCLUSIONS AND PERSPECTIVES

CUR-NLCs

Significantly **increased apparent water solubility** of CUR.

Preserved antioxidant properties of CUR.

Show **a suitable cell response in dermal cells and controlled** CUR release.

CUR-NLCs/hydrogel

Rheological behavior **compatible with a topical application**

Seems to be a **suitable formulation for the delivery of curcumin** to the skin.

Works in progress to investigate the capacity of the hydrogel to release the NLCs

Anti**B**iofilm **C**ompress:
Développement d'une compresse à activité antibiofilm sur une base d'Algostéril

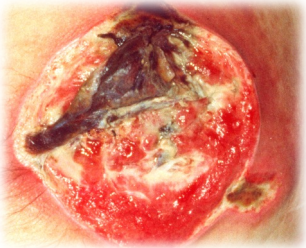
M. Gobin
D. Seyer, A. Gand, R. Proust, L. Duciel, S. Lack, C. Descourtils, E. Pauthe

ERRMECe
équipe de recherche sur les relations matrice extracellulaire -cellules

LABORATOIRES BROTHIER

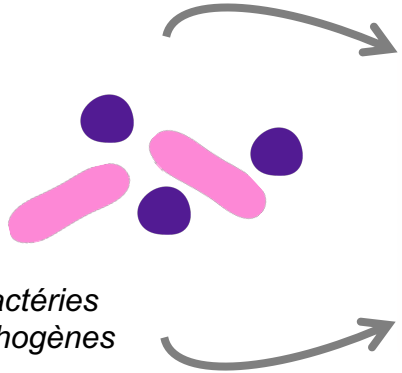
Plaies et cicatrisation

Plaie fibrineuse



Processus normal de cicatrisation

Réparation de la peau

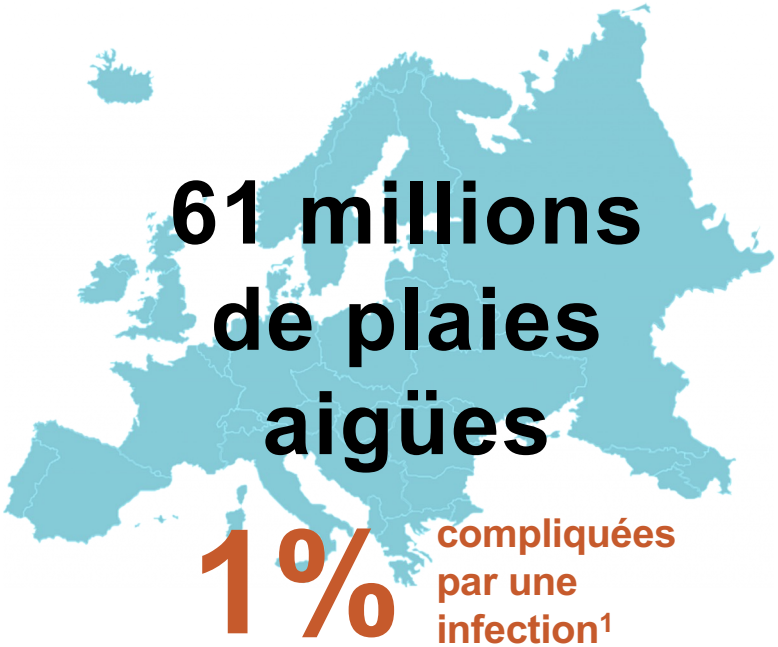


Bactéries pathogènes

Inflammation prolongée
Retard de cicatrisation
Persistance des plaies

Infection clinique pertinente
10^5UFC/g ou /cm^2 de biopsie de plaie

Plaies et cicatrisation, quelques chiffres



61 millions de plaies aigües

1% compliquées par une infection[1]

1 à 3%

2,5 millions

avec plaies[2]

= plaies chroniques

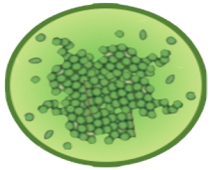
jusqu'à **90%** avec *Biofilm*

contre 6% des plaies aigües[3,4]

[1]Edmiston CE, *et al*. A narrative review of microbial biofilm in postoperative surgical site infections: Clinical presentation and treatment. *J Wound Care* 2016
[2]Meaume S, *et al*. Workload and prevalence of open wounds in the community: French Vulnus initiative. *J Wound Care* 2012
[3]Wolcott RD, *et al*. Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. *Wound Repair Regen* 2016
[4]James GA, *et al*. Biofilms in chronic wounds. *Wound Repair Regen* 2008

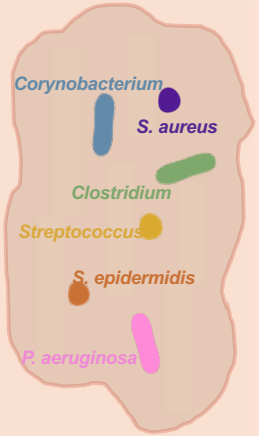
Le biofilm, redoutable mode de vie des bactéries

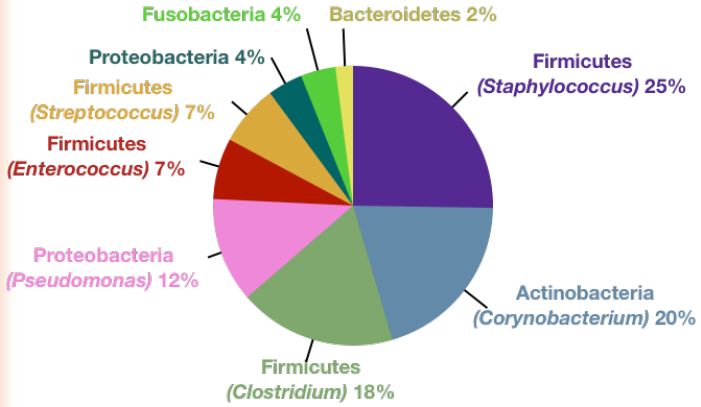
99%
des bactéries[5]



Biofilm

Définition : Communauté bactérienne située à une interface et engluée dans une matrice d'exopolysaccharides et de protéines apportant protection et nouvelles propriétés





***Quorum sensing* = communication bactérienne**

Diffusion ↘ **Virulence** ↗

Diversité phénotypique

S. aureus ● G+

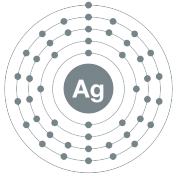
P. aeruginosa ▬ G-

→ **Tolérance accrue aux antibiotiques**
(x10 à x1000)[6]

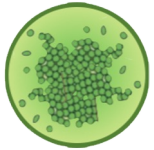
→ **Bactéries prédominantes dans les plaies**
Représentent une urgence élevée par l'OMS[7]

[5]Garrett TR, *et al*. Bacterial adhesion and biofilms on surfaces. *Prog Nat Sci* 2008
[6]Flemming HC, *et al*. Biofilms: An emergent form of bacterial life. *Nat Rev Microbiol* 2016
[7]Govindaraj Vaithinathan A, *et al*. WHO global priority pathogens list on antibiotic resistance: an urgent need for action to integrate One Health data. *Perspect Public Health* 2018

Traitement des biofilms dans les plaies



Tolérance
Résistance[8]



Résistance
Cytotoxicité[9]

Effet antibiofilm modéré[10]
(3,5log de réduction)

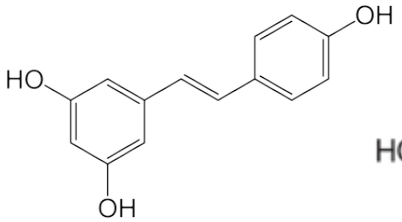
Effet antibiofilm fort
5log de réduction
biomasse bactérienne

Besoin de trouver une solution plus efficace face à l'installation des biofilms dans les plaies

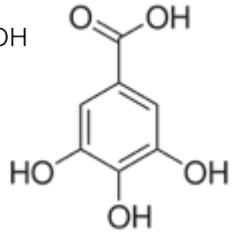
[8]Sanchez-Vizuete P, *et al*. Pathogens protection against the action of disinfectants in multispecies biofilms. *Front. Microbiol* 2015
[9]Murphy OS, *et al*. Advances in Wound Healing: A Review of Current Wound Healing Products. *Plast. Surg* 2012
[10]Brackman G, *et al*. Biofilm inhibitory and eradicating activity of wound care products against Staphylococcus aureus and Staphylococcus epidermidis biofilms in an in vitro chronic wound model. *J. Appl. Microbiol* 2013

Molécules contre les biofilms

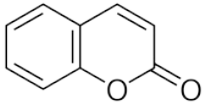
Formation du biofilm : molécules anti-adhérence



Resveratrol

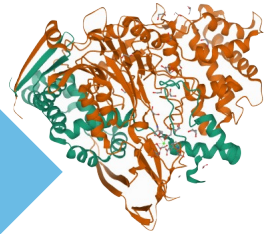


Acide gallique

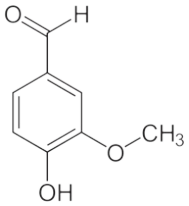


Coumarin

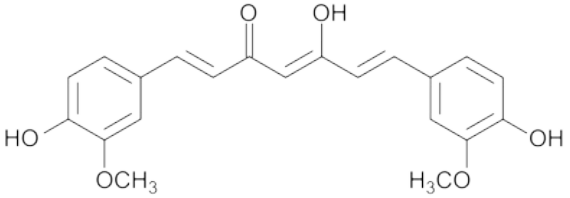
Inhibition du *Quorum sensing* *(communication bactérienne)*



Acylase

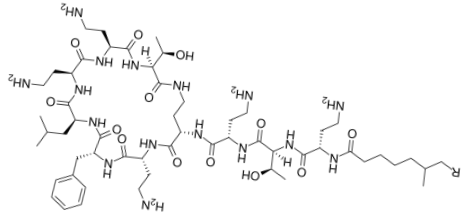


Vanilline

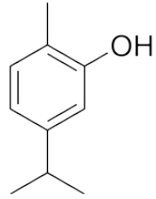


Curcumine

Éradication bactérienne



Polymyxine B



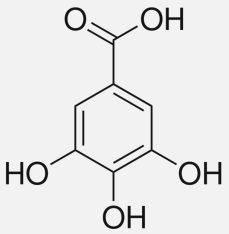
Carvacrol

Des actifs d'origine naturel contre les biofilms

- Les Laboratoires Brothier, produits

ORIGINE 100% VEGETALE

Acide Gallique G

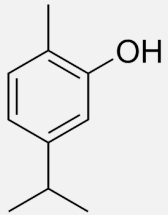


Noix de galle

Effet anti-adhérence des bactéries

Planctonique *Biofilm*

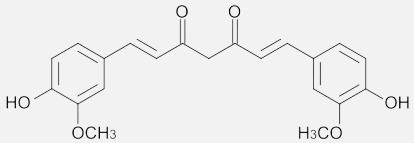
Carvacrol K



Origan

Déstabilisation de la membrane bactérienne

Curcumine Q



Curcuma

Brothier et la cicatrisation des plaies

LABORATOIRES BROTHIER

ORIGINE 100% VEGETALE

Valorisation alginate → **Produits pour l'hémostase et la cicatrisation**

Laminaria hyperborea

Transformation
Textilisation

Algostéril

11-14

- **Drainage et Absorption**
- **Piégeage des bactéries dans les fibres**
- **Pro-cicatrisantes**
- **Limite le risque infectieux**

➔ **Ø sur les biofilms**

Collaboration
Projet ABC
ANTIBIOFILM COMPRESS

ERRMECe
équipe de recherche sur les relations matrice extracellulaire –cellules

THÈSE Cifre Conventions

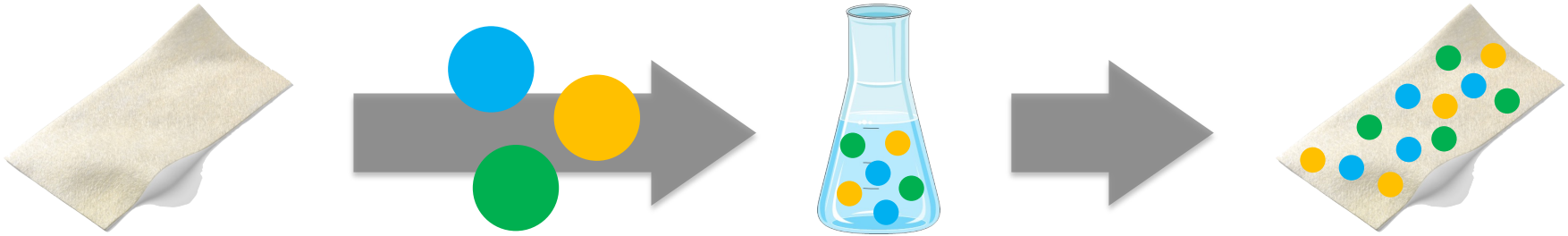
[11]André J, *et al.* Intérêt de la mèche d'alginate de calcium et de la mèche imprégnée de polyvidoneiodée dans le traitement local du sinus pilonidal abcédé. *Revue de l'ADPHSO* 1997
[12]Lalau JD, *et al.* Efficacy and tolerance of calcium alginate versus vaseline gauze dressings in the treatment of diabetic foot lesions. *Diabetes Metab* 2002
[13]Costagliola M, *et al.* Algosteril° dressing (calcium alginate) versus Flammazine° (silver sulphadiazine) in the treatment of second-degree burns. *Fourth Australian Wound Management Association Conference* 2002
[14]Servant J, *et al.* Algosteril® range (calcium alginate rope/dressing/powder) versus Tulle gras Lumière®Naseline®(vaseline gauze) in the treatment of lesions due to Verneuil's disease. *Fourth Australian Wound Management Association Conference* 2002

Stratégie

DÉVELOPPEMENT DE COMPRESSES D'ALGINATE DE CALCIUM À ACTIVITÉ ANTIBIOFILM

→ **Prévenir la formation de biofilm**
(plaies à haut risque infectieux)

→ **Détruire le biofilm mature**
(plaies infectées)

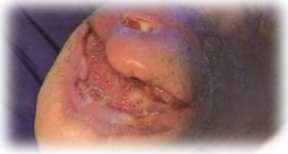


Objectifs du projet ABC

Contexte :

- Les bactéries sont majoritairement retrouvées sous forme de biofilm
- Le biofilm permet aux bactéries d'être jusqu'à 1000 fois plus résistantes aux antibiotiques / antiseptiques
- Le biofilm est une cause de chronicité des plaies

Ajout d'actifs dans une compresse ALGOSTERIL pour prévenir/lutter contre les biofilms dans la plaie



Plaies à haut risque infectieux

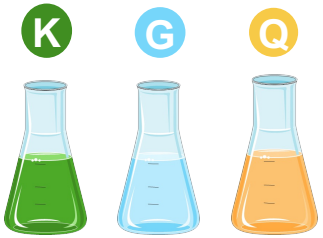
⇨ *Prévenir la formation de biofilm*

Plaies infectées

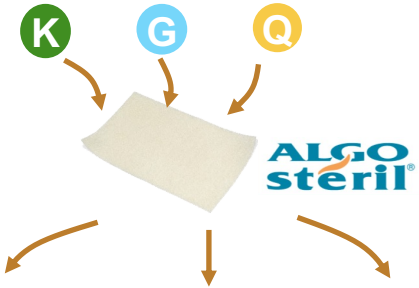
⇨ *Détruire le biofilm mature*

Méthode d'évaluation de l'efficacité antibiofilm

1. Solution d'actifs

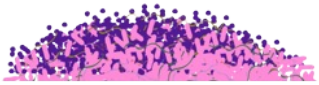


2. Actifs en solution ou Compresses imbibées d'actifs



Choix des bactéries ciblées

- Bactéries les plus retrouvées dans les plaies :
  Staphylococcus aureus
  Pseudomonas aeruginosa



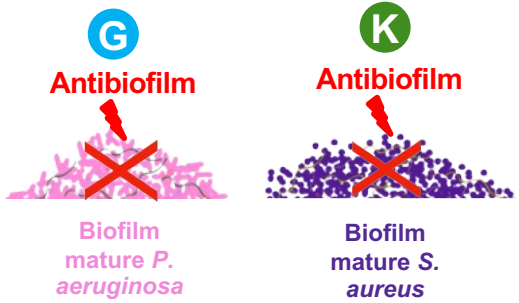
✓ **ANTIBIOFILM = -5 log**

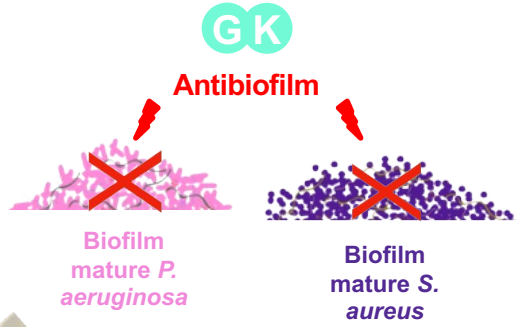
- Test sur modèle de biofilm mature *in vitro* :

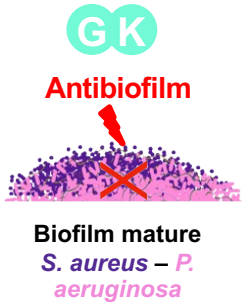
S. aureus

P. aeruginosa

Conclusions

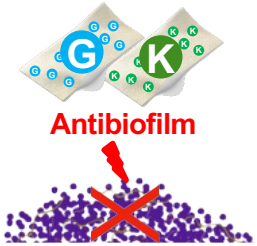


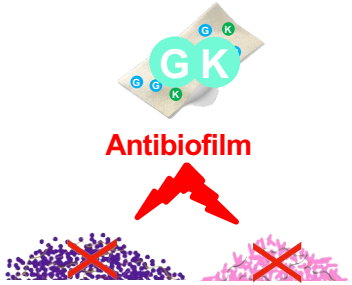




Prototypes

Compresses imprégnées





Produits finis

Compresses séchées

- **Quantité d'actifs suffisantes pour un effet antibiofilm**
- **Instabilité de G et K au stockage dans les compresses**

inpi BREVET DÉPOSÉ INSTITUT NATIONAL DE LA PROPRIÉTÉ INDUSTRIELLE

ASSOCIATION DU CARVACROL AVEC L'ACIDE GALLIQUE ET/OU LA CURCUMINE

Gobin M, Gand A, Seyer D, Lack S, Pauthe E, Proust R (n° FR2007652)

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