



# HUMAN ORGANOTYPIC SKIN EXPLANT CULTURE (hOSEC): *an alternative method for tropical diseases and cosmetics.*



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*University of São Paulo (USP)*

*Marco Andrey Cipriani Frade 2018*



## CONFLICT OF INTEREST



**Narcissus**  
Pesquisa Clínica e Biotecnologia



**SUPERA**

Parque de Inovação e Tecnologia de Ribeirão Preto

# hOSEC

## human Organotypic Skin Explant Culture

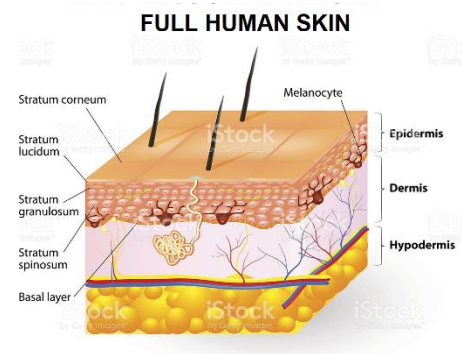


✓ An alternative method for animal use



✓ Skin pieces from plastic surgeries

✓ A full 3D model with melanocytes, keratinocytes, Langerhans cells, fibroblasts, glycosaminoglycans, collagen and a natural dermal-epidermal junction



Frade et al., 2015

Frade et al., 2015; Xu et al., 2012; Lebonvallet et al., 2010; Jacobs et al., 2000, 2006

Marco Frade 2018



# hOSEC

## human Organotypic Skin Explant Culture

### INVESTIGATION

347

#### Prolonged viability of human organotypic skin explant in culture method (hOSEC)\*

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DOI: <http://dx.doi.org/10.1590/abd1806-4841.20153645>

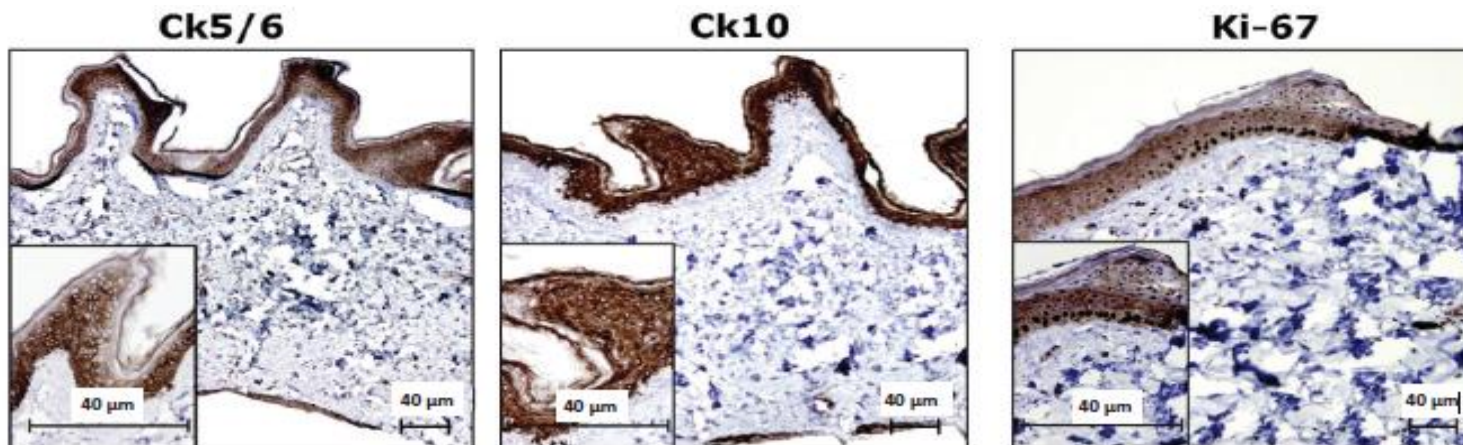
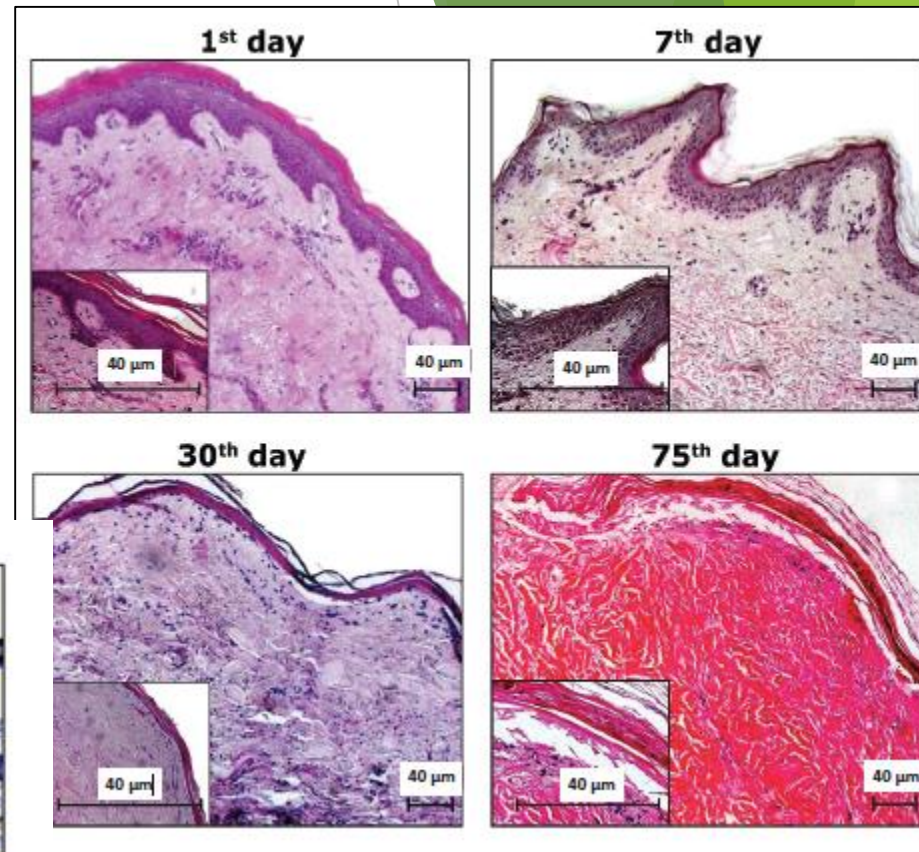


Fig. 2. Immunohistochemical staining of skin on culture for 75 days for Ck5/6, Ck10 and Ki-67 proving the viability of hOSEC method by proliferative capacity of keratinocytes (magnification: 100x and 400x) [10].



This is our first paper proving the human skin viability for 75 days in culture

# hOSEC MODEL APLICATIONS:

✓ for Tropical Disease Studies

**LEPROSY**

**LEISHMANIASIS**

**ZIKA**

**MICROORGANISM CULTURE  
DRUG TESTS  
SKIN INTERACTIONS and  
IMMUNOLOGICAL RESPONSE**



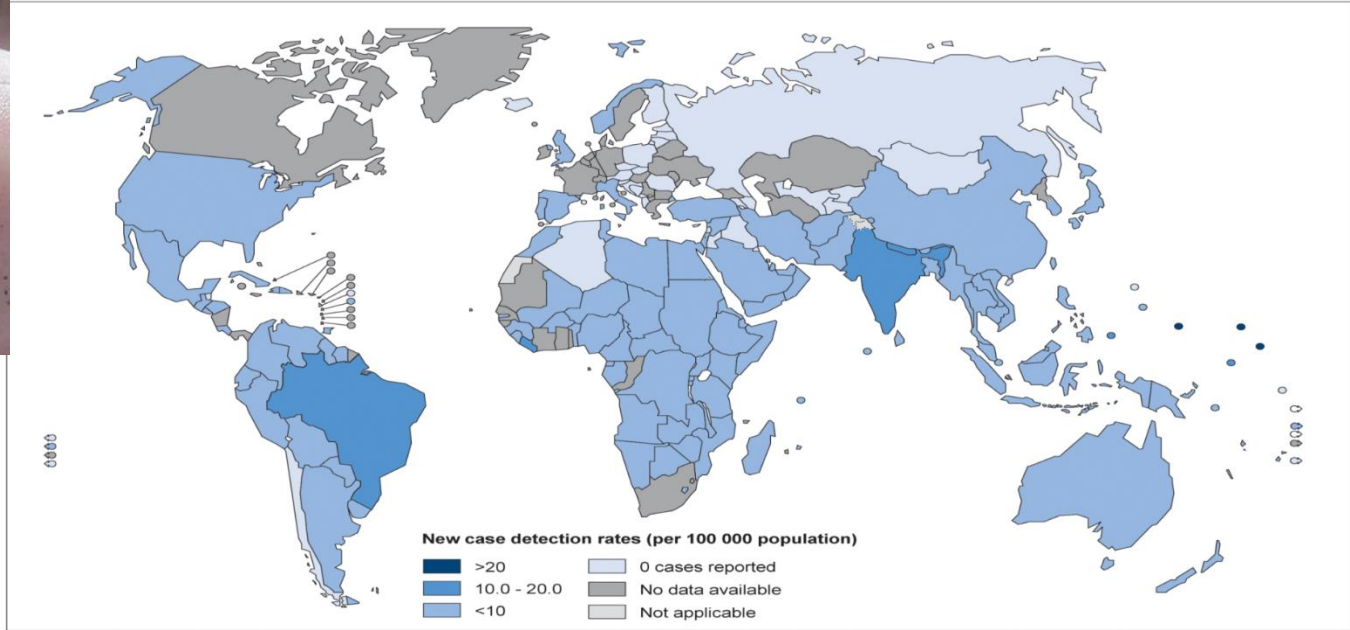
✓ FOR COMESTIC TEST

**TOPICAL PRODUCTS**

**SAFETY AND EFFICACY TESTS  
CORROSIVITY AND  
PERMEATION**

Marco Frade 2018

# LEPROSY - *Mycobacterium leprae*



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Data Source: World Health Organization  
Map Production: Control of Neglected  
Tropical Diseases (NTD)  
World Health Organization



Leprosy is still one important epidemiological problem in the world mainly because incapacities  
BR~30,000 new cases a year

# LEPROSY - *Mycobacterium leprae*

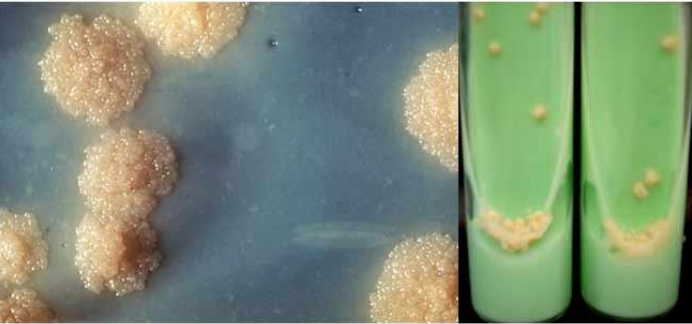


Fig: Cultural Characteristics of *Mycobacterium tuberculosis*

➤ *Mycobacterium tuberculosis*  
In vitro cultured in Lowenstein-Jensen medium  
Growth in 3 weeks - 60 days

Multiply in 12-18 hours

➤ **M. LEPRAE NOT ABLE BE CULTIVATE IN VITRO!!**

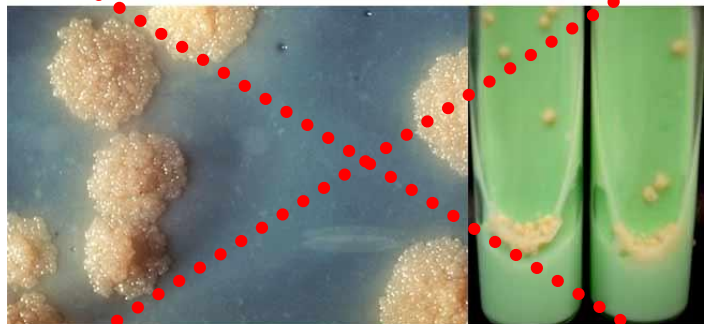
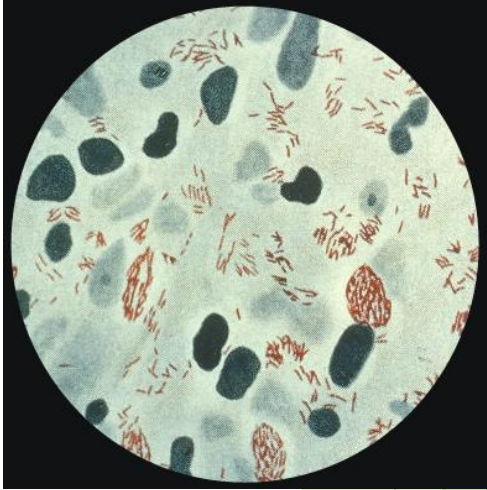


Fig: Cultural Characteristics of *Mycobacterium tuberculosis*



*Mycobacterium leprae*

Multiply in **2-3 weeks** in humans



delay

Diagnostic

Drug study

Research

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Frade, MAC 2018



***MAINTAINING THE VIABILITY OF Mycobacterium  
leprae IN EX-VIVO MODEL OF HUMAN SKIN “HOSEC”  
(Human Organotypic Skin Explant Culture)***



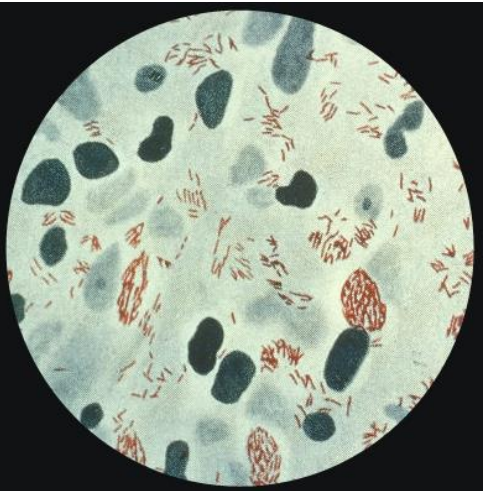
Natália Ap. de Paula  
npbiomed@yahoo.com.br

PhD. student – Program of Cellular and Molecular Biology- FMRP  
Adviser: PhD Marco Andrey Cipriani Frade



# hOSEC MODEL: for Leprosy

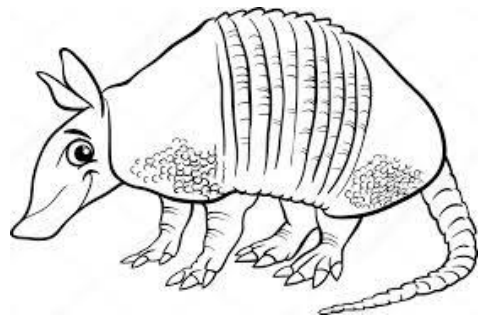
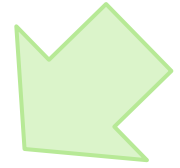
✓ In vivo inoculation



*Mycobacterium leprae*



Foot pad of the nude mice  
(Shepard, 1960)



Tatus  
(Kirchheimer, W. F. e Storrs, E. E, 1971).



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PhD student  
Biocell Program - USP

6 months to grow bacilli  
Until 12 months for drug  
resistance experiments

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# hOSEC MODEL: for Leprosy

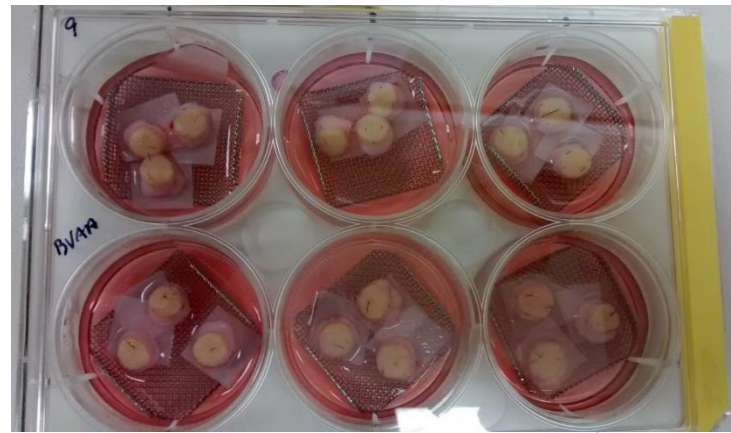
The bacilli are then inoculated into the skin fragment



Bacilli are obtained from nude mice of the ILSL-Bauru-SP



10<sup>4</sup> bacilli/ fragmento  
25uL



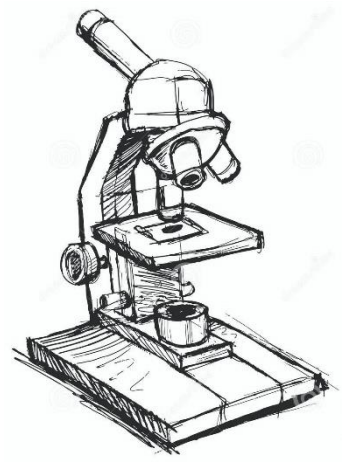
Control: *M. leprae* autoclaved and saline

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# hOSEC MODEL: for Leprosy

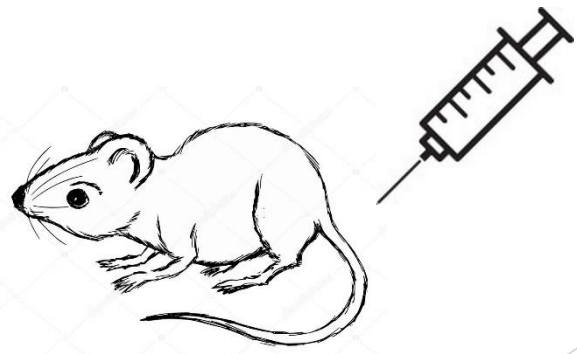
Analysis are done at 4, 7, 14, 28, 60 days after the inoculate to assess viability and growth of the bacillus.



Microscopy techniques  
Fite Faraco protocol  
Histopathology,  
Immunohistochemistry



PCR  
RNA e DNA



Inoculation *in vivo*



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PhD student  
Biocell Program - USP



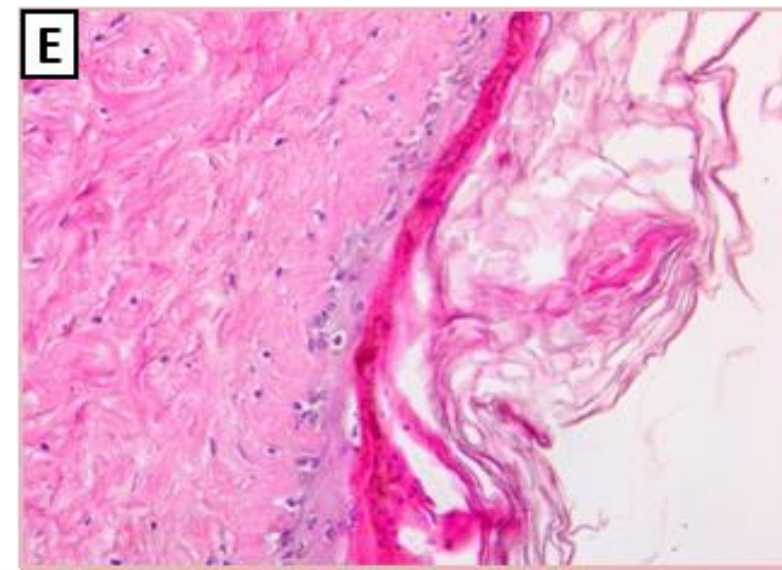
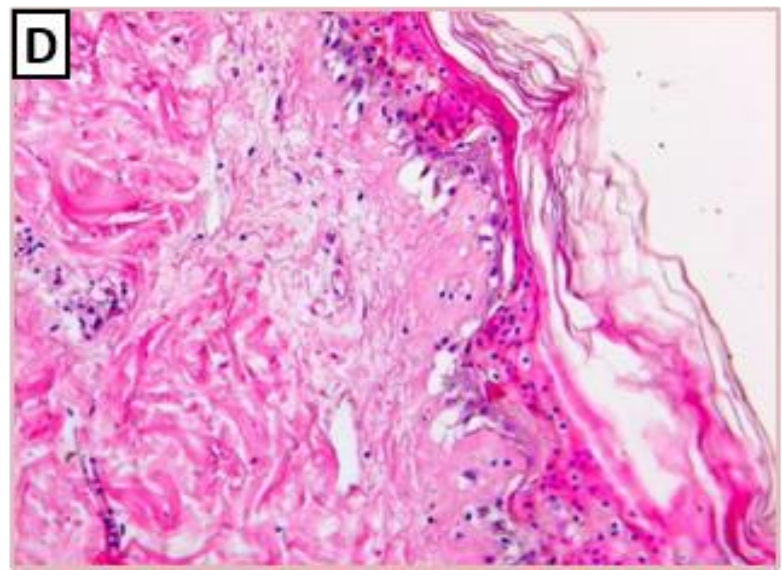
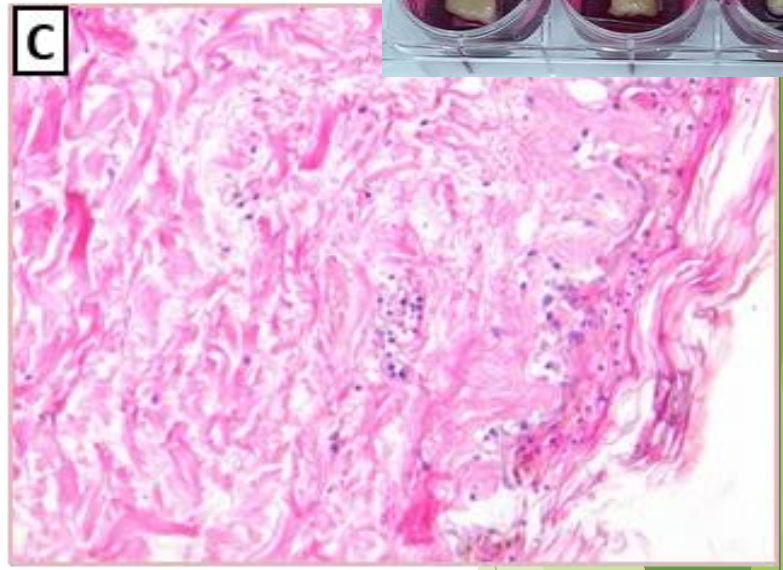
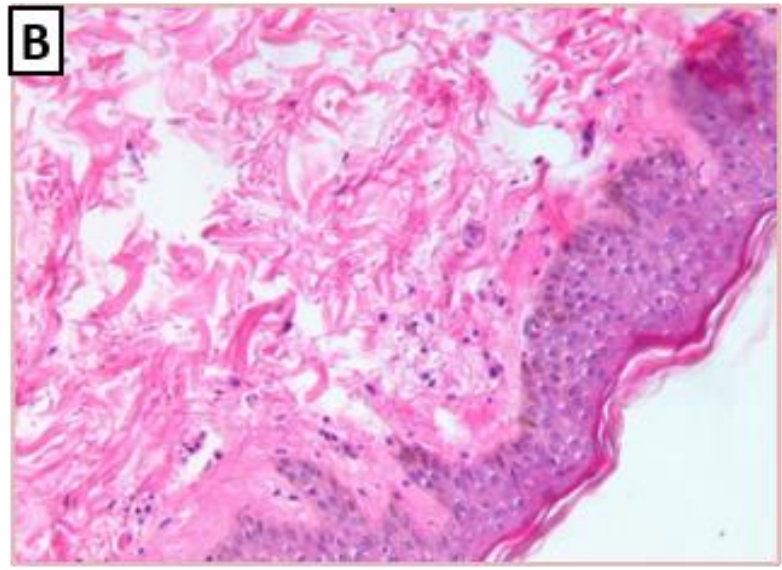
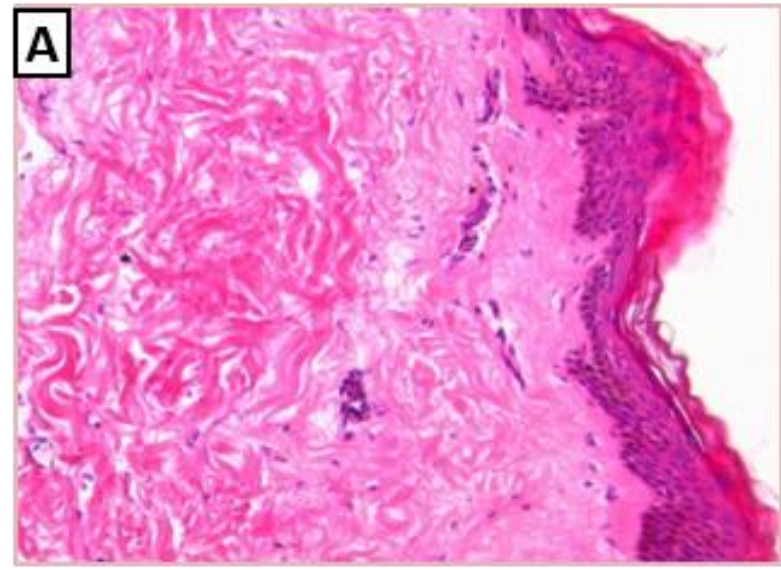
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# hOSEC MODEL: for Leprosy

H&E stain →

(A) D0 (B) D7 (C) D14 (D) D28 (E) D60

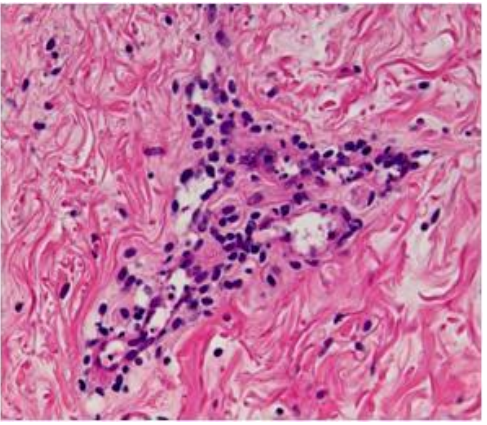
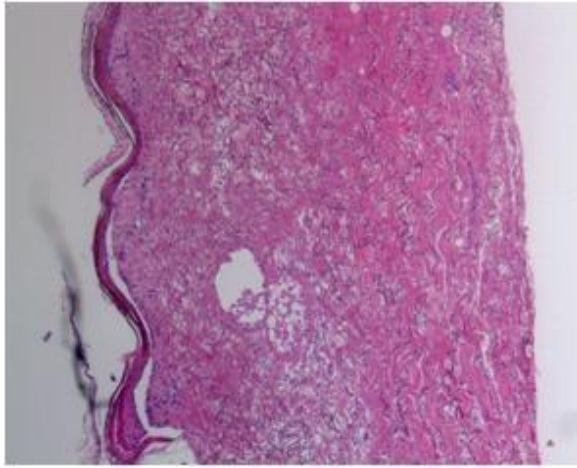


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Frade, MAC 2018*

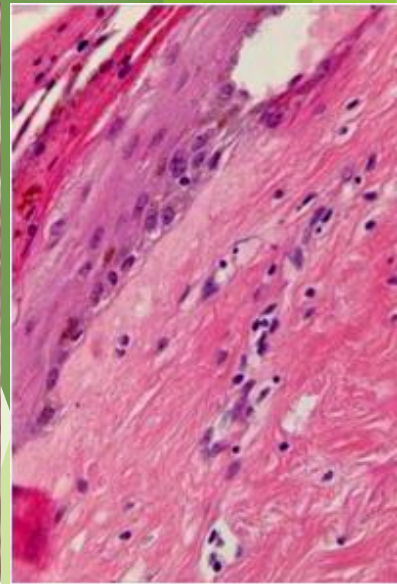
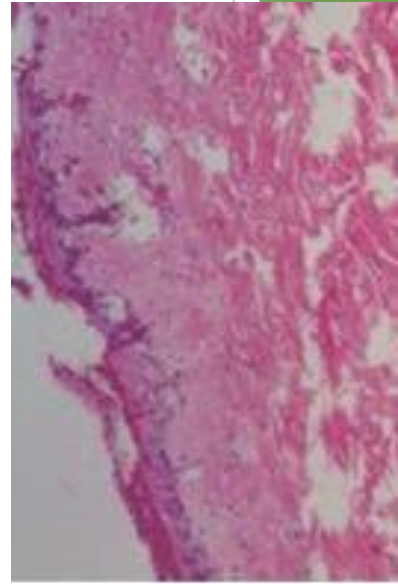
# hOSEC MODEL: for Leprosy

Control

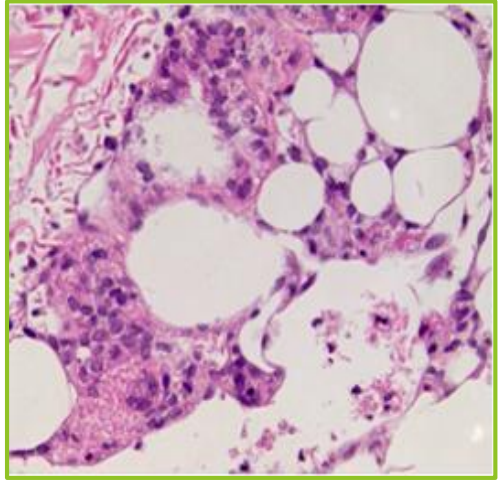
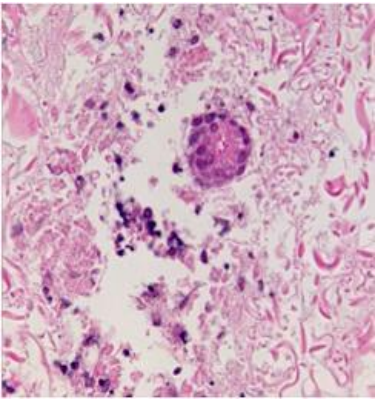
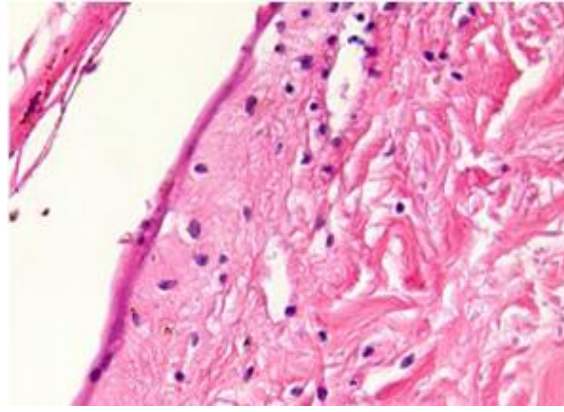
HE - after D28 (28 days in culture)



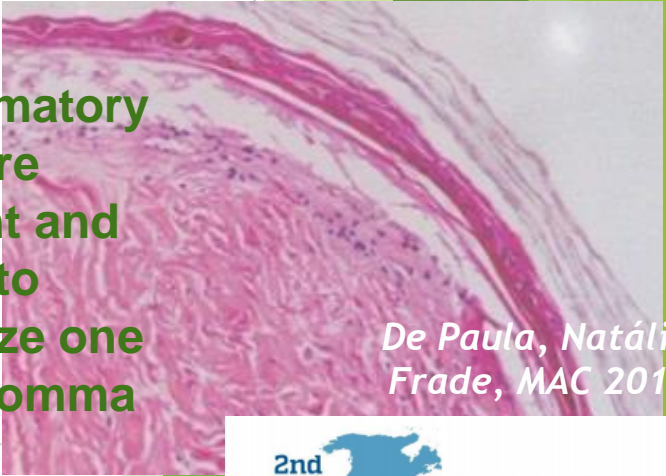
**M. Leprae pieces**  
Thin epidermis, less collagen fiber and inflammatory cells trying to organize one granuloma, not observed in control ones



HE - after D60 (60 days in culture)



**Inflammatory cells are present and trying to organize one granuloma**



*De Paula, Natália Frade, MAC 2018*

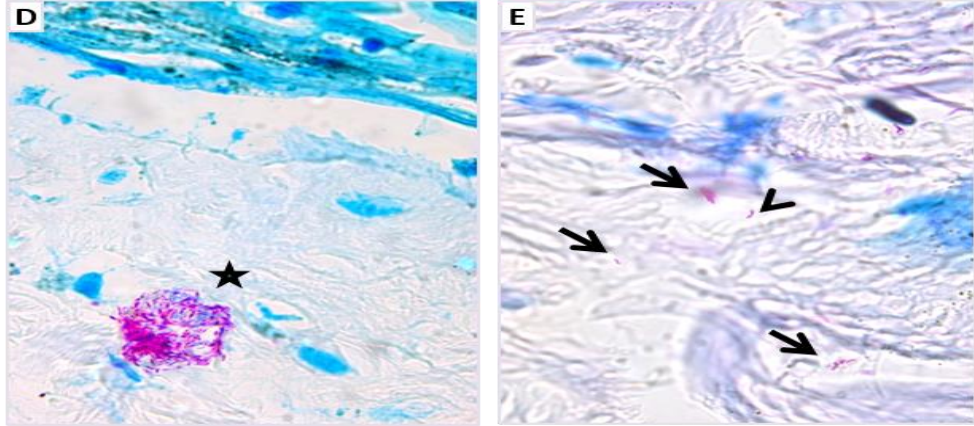
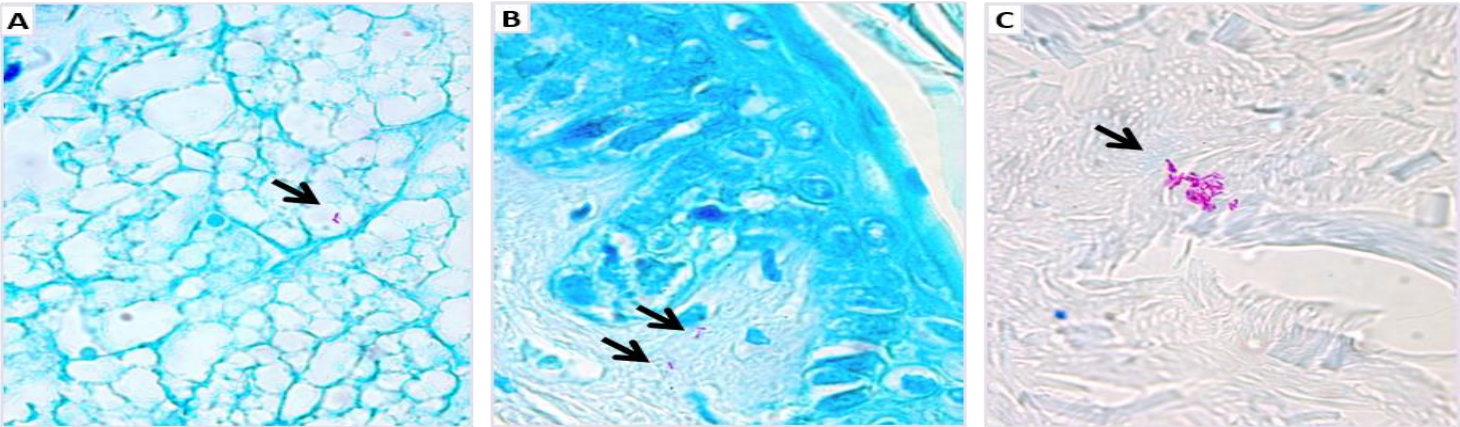
**✓ *M. leprae* seems to induce tissue morphological modifications of hOSEC model**

# hOSEC MODEL: for Leprosy

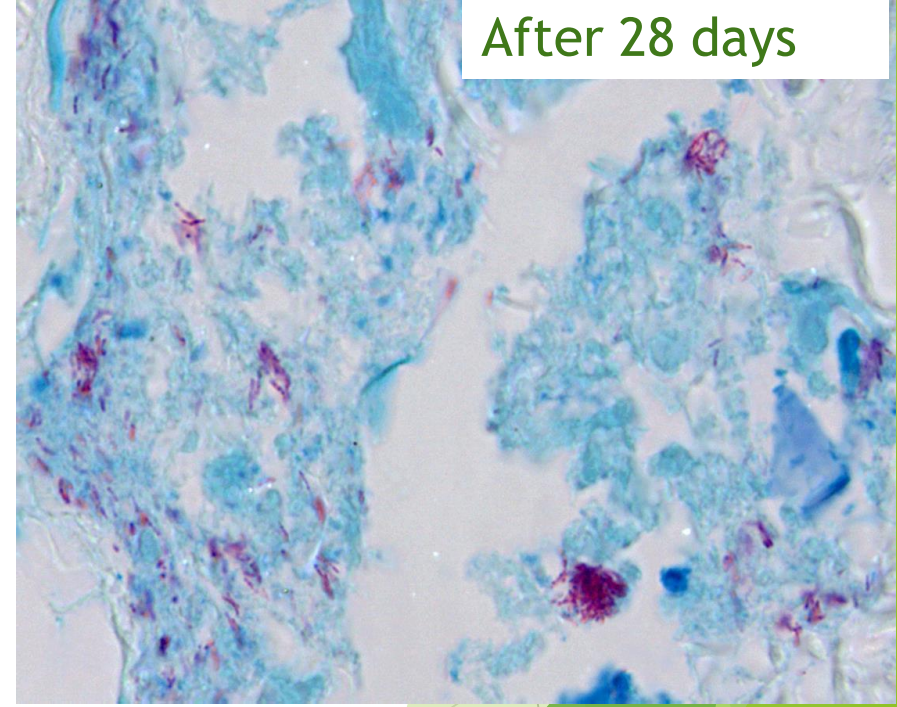
## Morphology of *M. Leprae*

Fite Faraco Stain →

(A) D0 (B) D7 (C) D14 (D) D28 (E) D60



Integral bacillus (arrow), fragment bacillus (arrow head) and globia (star).



D28 - many bacilli were observed in the Fite-Faraco staining, where vast majority (more of 90%) were integral bacilli.

D60 - many bacilli became fragmented, however 64 % integrate bacilli were SEEN

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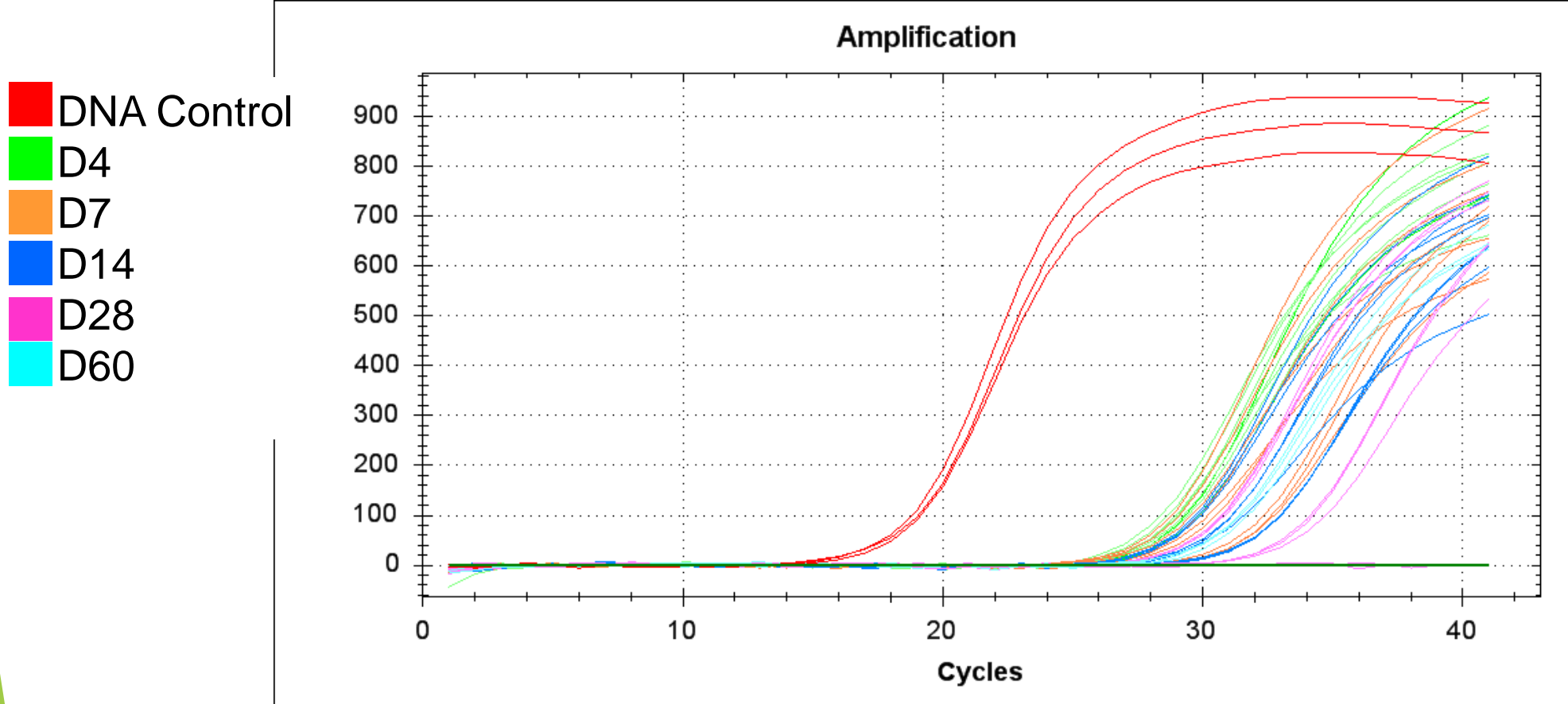


# hOSEC MODEL: for Leprosy

## Viability of *M. Leprae*



Amplification curve of RT-PCR with primers 16sRNA, specific to bacillus



For the quantification of the number of bacilli, we are using PCR with DNA

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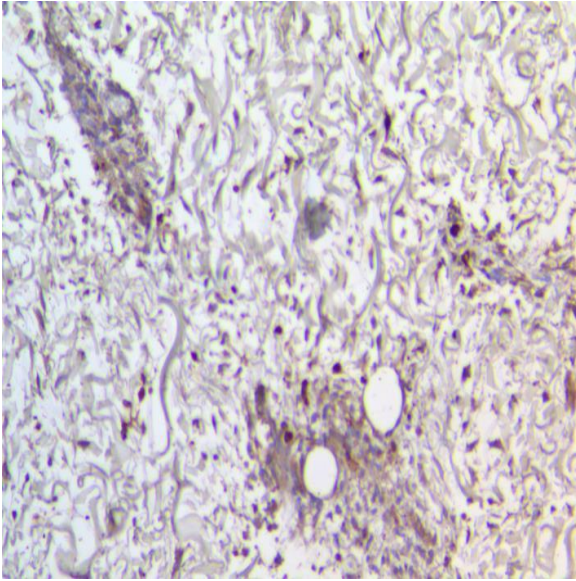


# hOSEC MODEL: for Leprosy

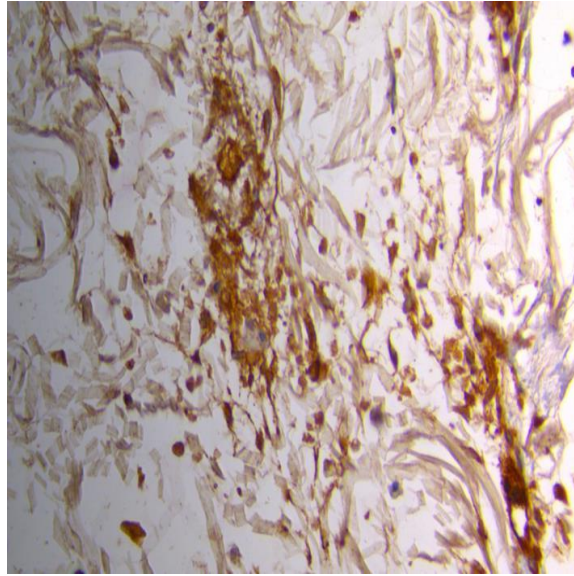
IMMUNOHISTOCHEMISTRY - D60

*M. Leprae* and immunology

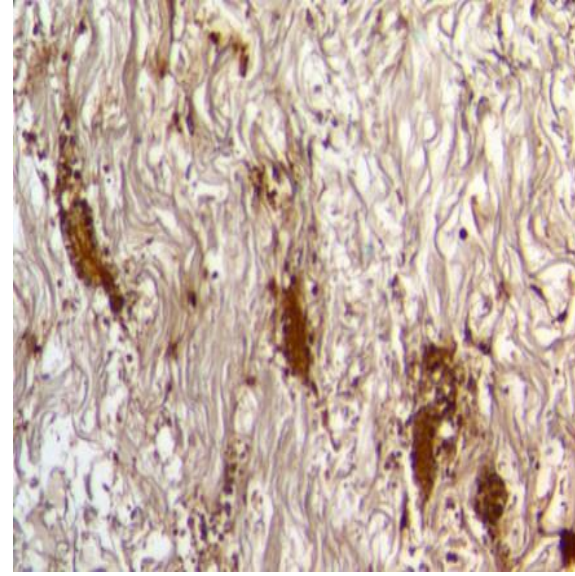
CD-68



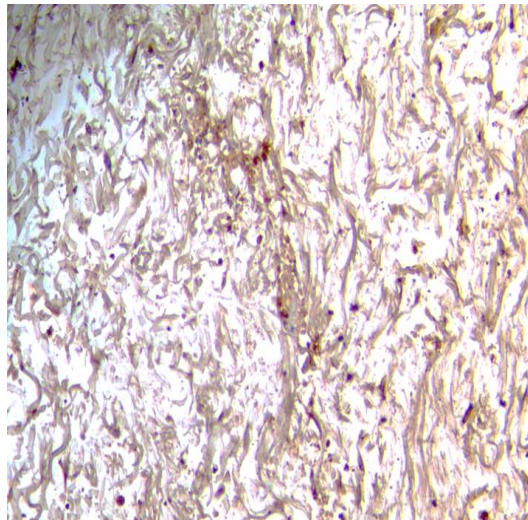
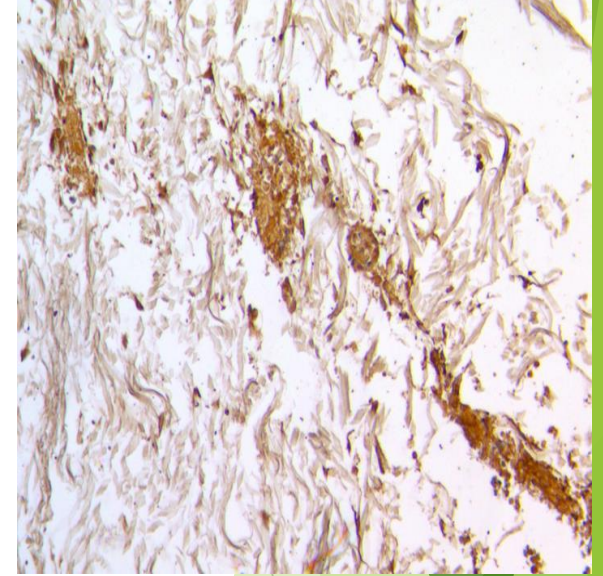
IFN $\gamma$



IL-12



iNOS



✓ *M. leprae* seems to induce the innate immune response around bacillar area in hOSEC model

Anti-MLSA-LAM  
specific antibody for ML

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Frade, MAC 2018



# hOSEC MODEL: for Leprosy

- ✓ SO, THE *M. LEPRAE* MAINTAINED VIABLE IN THE EX-VIVO SKIN CULTURE AND SEEMS BE ABLE TO MULTIPLY AFTER 28 AND 60 DAYS IN CULTURE
- ✓ THIS IS THE FIRST ARTIFICIAL CULTURE ASSAY TO KEEP *M. LEPRAE* FOR SO LONG TIME
- ✓ IMPORTANT STEP TO IMPROVE THE STUDIES ABOUT:
  - ✓ *M. LEPRAE* MICROBIOLOGY
  - ✓ SKIN INTERACTIONS AND IMMUNOLOGY
  - ✓ DRUG RESISTANCE TEST FOR CLINICAL APPLICATION



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Frade, MAC 2018

# hOSEC MODEL



Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

Procedia Engineering 110 (2015) 67 – 73

Procedia  
Engineering

[www.elsevier.com/locate/procedia](http://www.elsevier.com/locate/procedia)

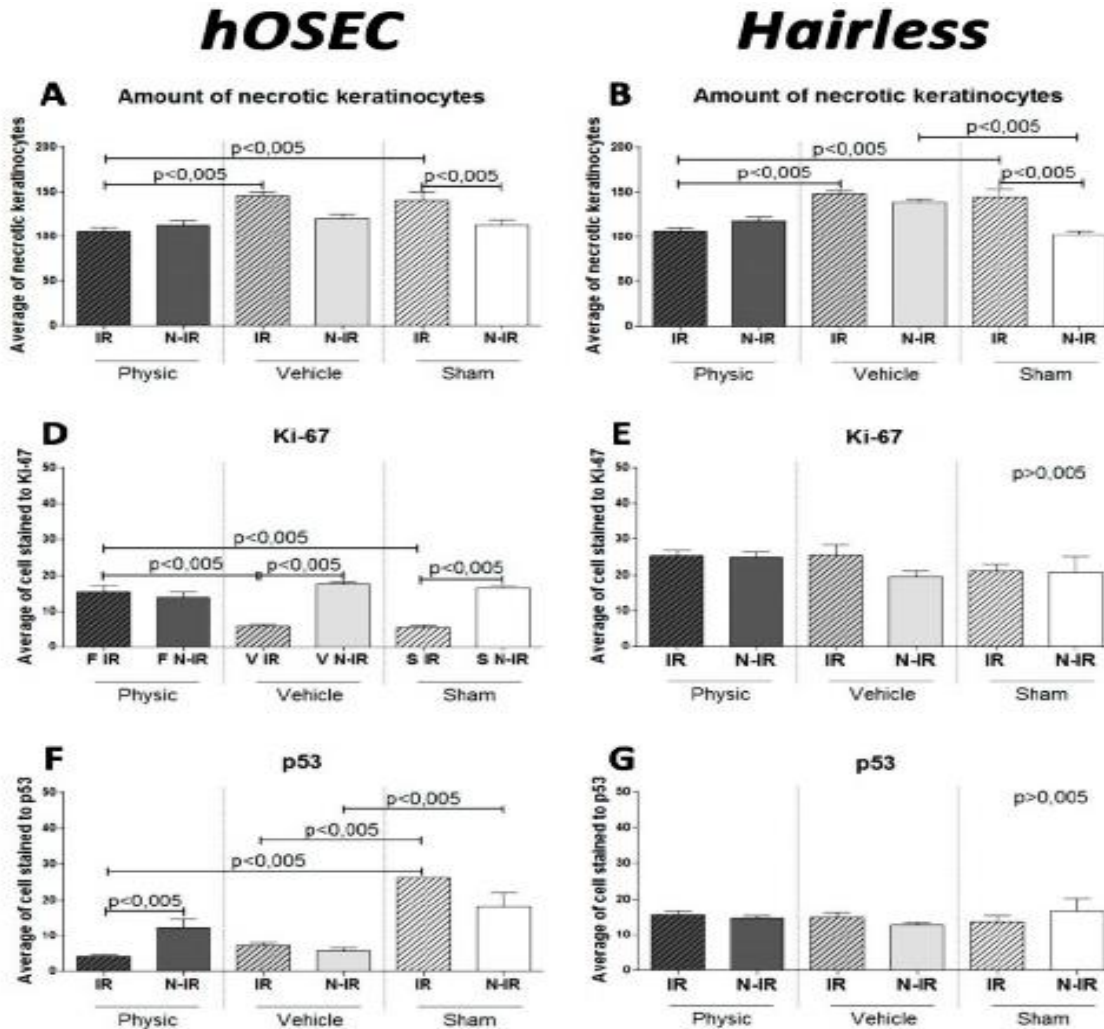


✓ FOR COMESTIC TEST

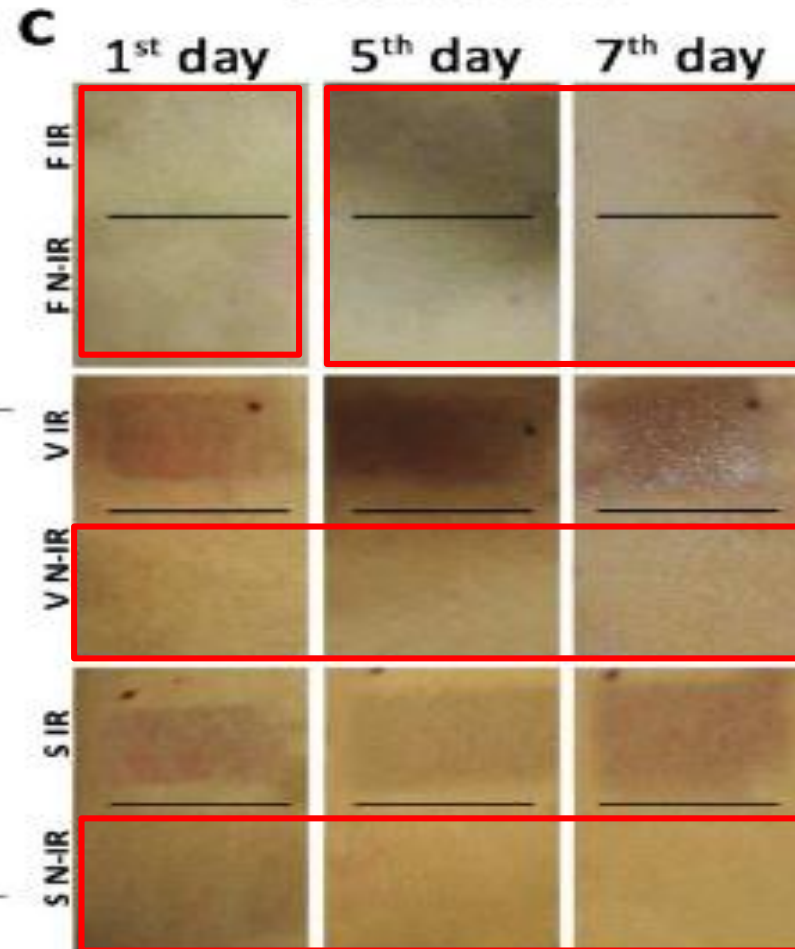
4th International Conference on Tissue Engineering, ICTE2015

Ex vivo model of human skin (hOSEC) as alternative to animal use for cosmetic tests

Andrade TA<sup>a\*</sup>, Aguiar AF<sup>a</sup>, Guedes FA<sup>a</sup>, Leite MN<sup>a</sup>, Caetano GF<sup>b</sup>, Coelho EB<sup>c</sup>, Das PK<sup>d</sup>, Frade MA<sup>b</sup>



# Human



Similar results were obtained comparing hOSEC model with animal model and also human clinical model according necrotic KC, Ki67 and p53 cells. EFFICACY/SAFETY TEST FOR UV PROTECTION

Frade, MAC 2018



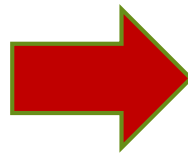
# hOSEC MODEL

✓ FOR CORROSIVITY TEST

➤ Safety test for reagentes and also finished products



hOSEC



Cell Viability

TTC



Figueiredo S,  
Frade, MAC 2018

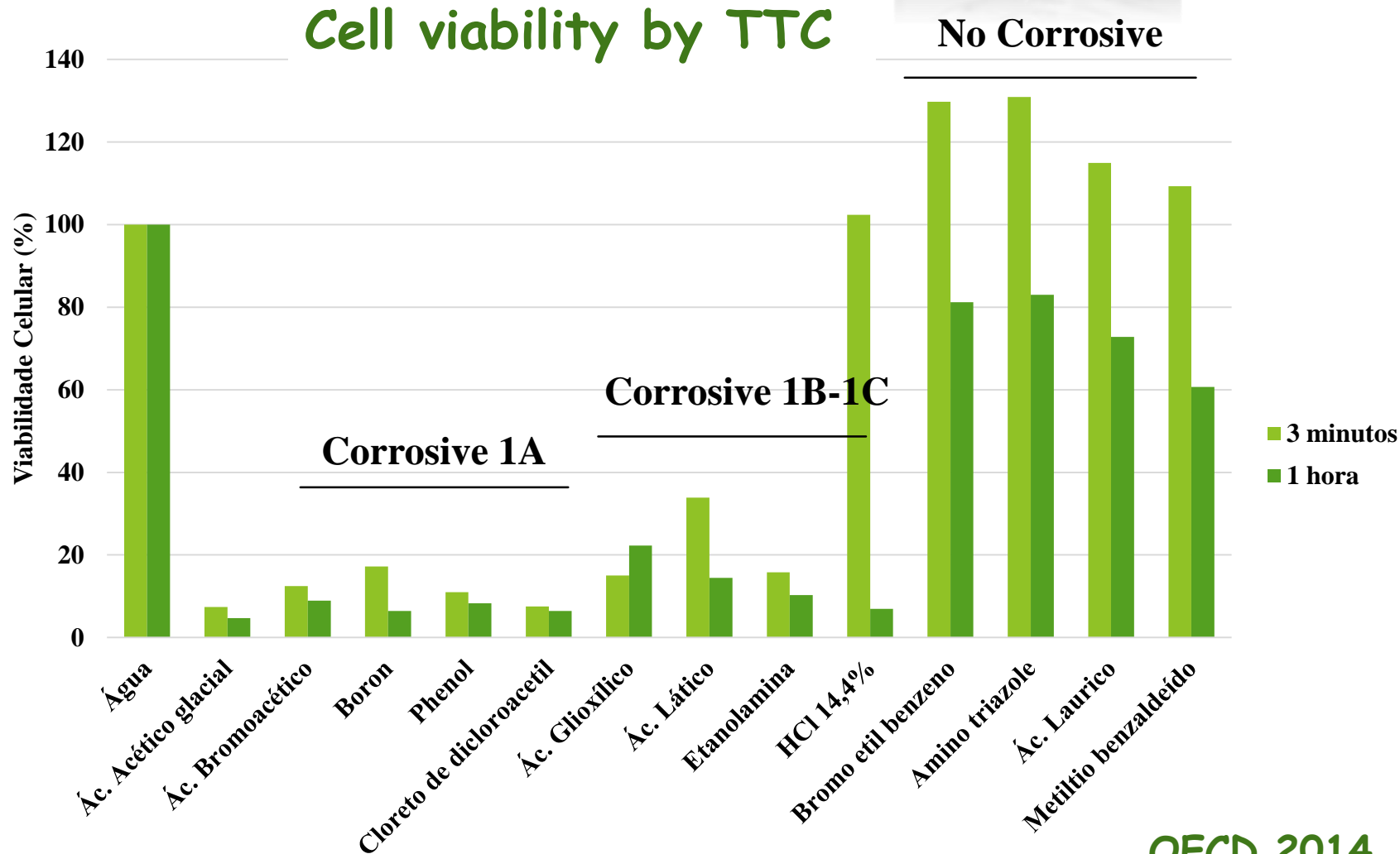


# hOSEC MODEL

✓ FOR CORROSIVITY TEST



No Corrosive



2,3,5-  
TRIPHENYL-TETRAZOLI  
UM CHLORIDE (TTC)  
Cell viability test with no  
interference with  
chemical painel

Figueiredo S, Leite, MN  
Frade, MAC 2018



OECD 2014

<http://www.oecd.org/env/ehs/testing/The%20Short%20Time%20Exposure%20In%20Vitro%20Test%20Method.pdf>

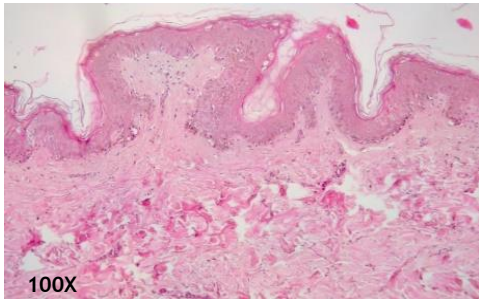


# hOSEC MODEL

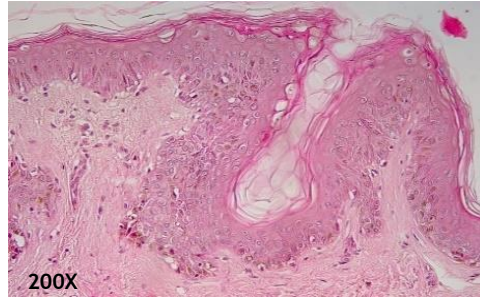
✓ FOR SUN EXPOSING TEST



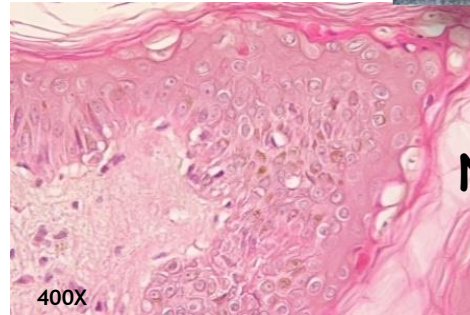
Pele Normal D0



100X

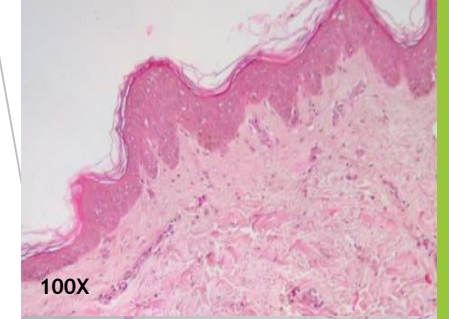


200X

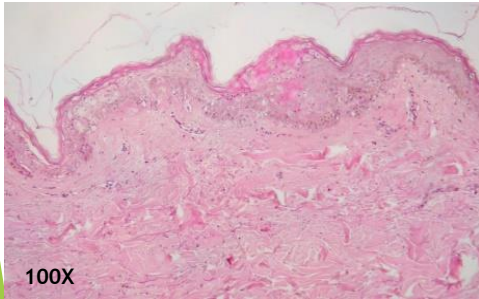


400X

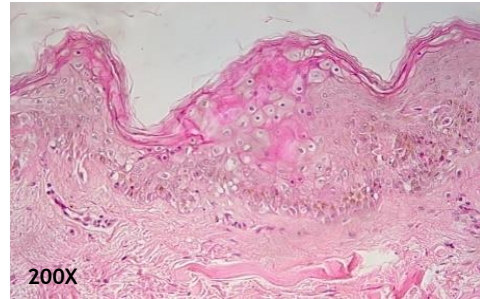
No IR SKIN-10 / D7



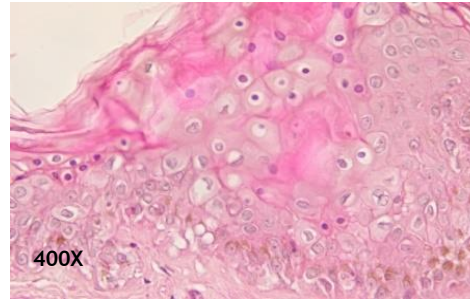
100X



100X

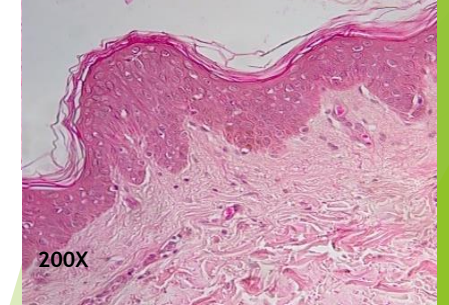


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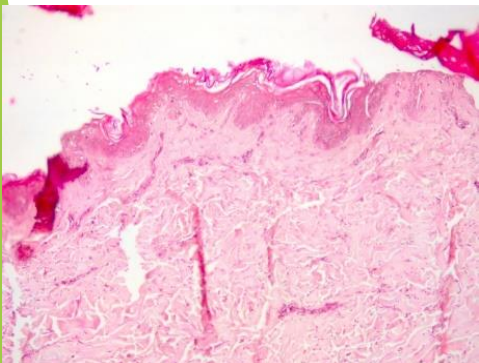


400X

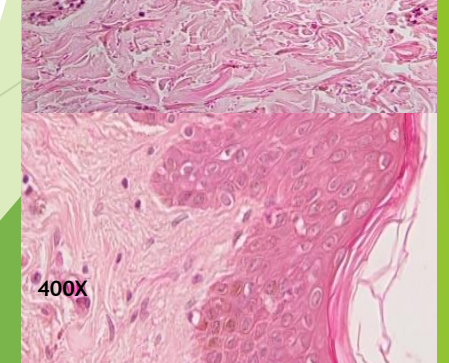
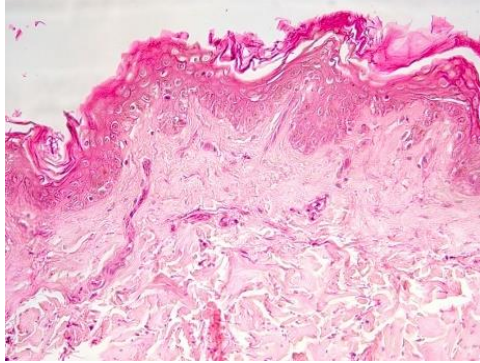
IR SKIN-10 / D7



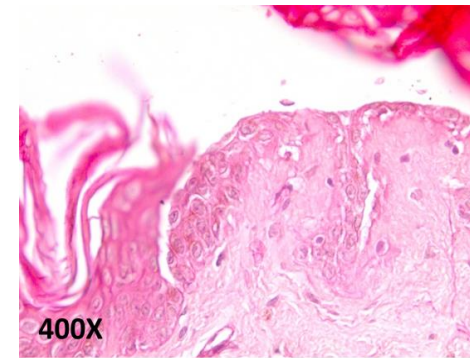
200X



IR SKIN -20 / D7



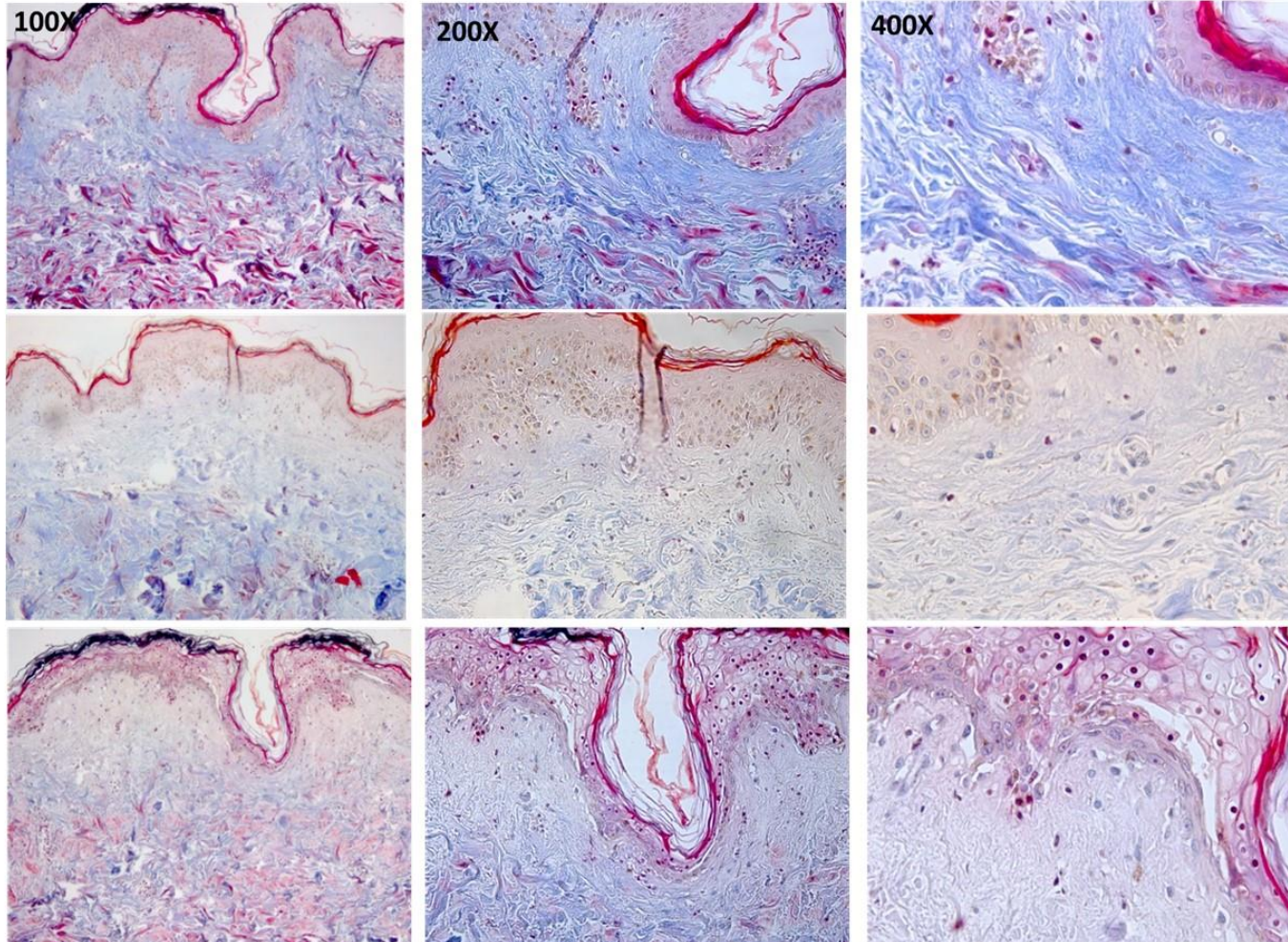
400X



400X

# hOSEC MODEL

✓ FOR SUN EXPOSURE TEST



No IR SKIN-10 / D7

IR SKIN-10 / D7

IR SKIN -20 / D7

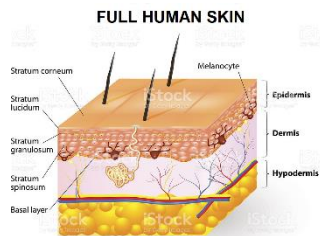
To analyze the collagen modification by UV exposure for 7 days using 2 doses for 10 and 20 minutes

Leite, MN., Figueiredo S, De Paula, NA; Frade, Fonseca MJ, Campos, P. MAC 2018

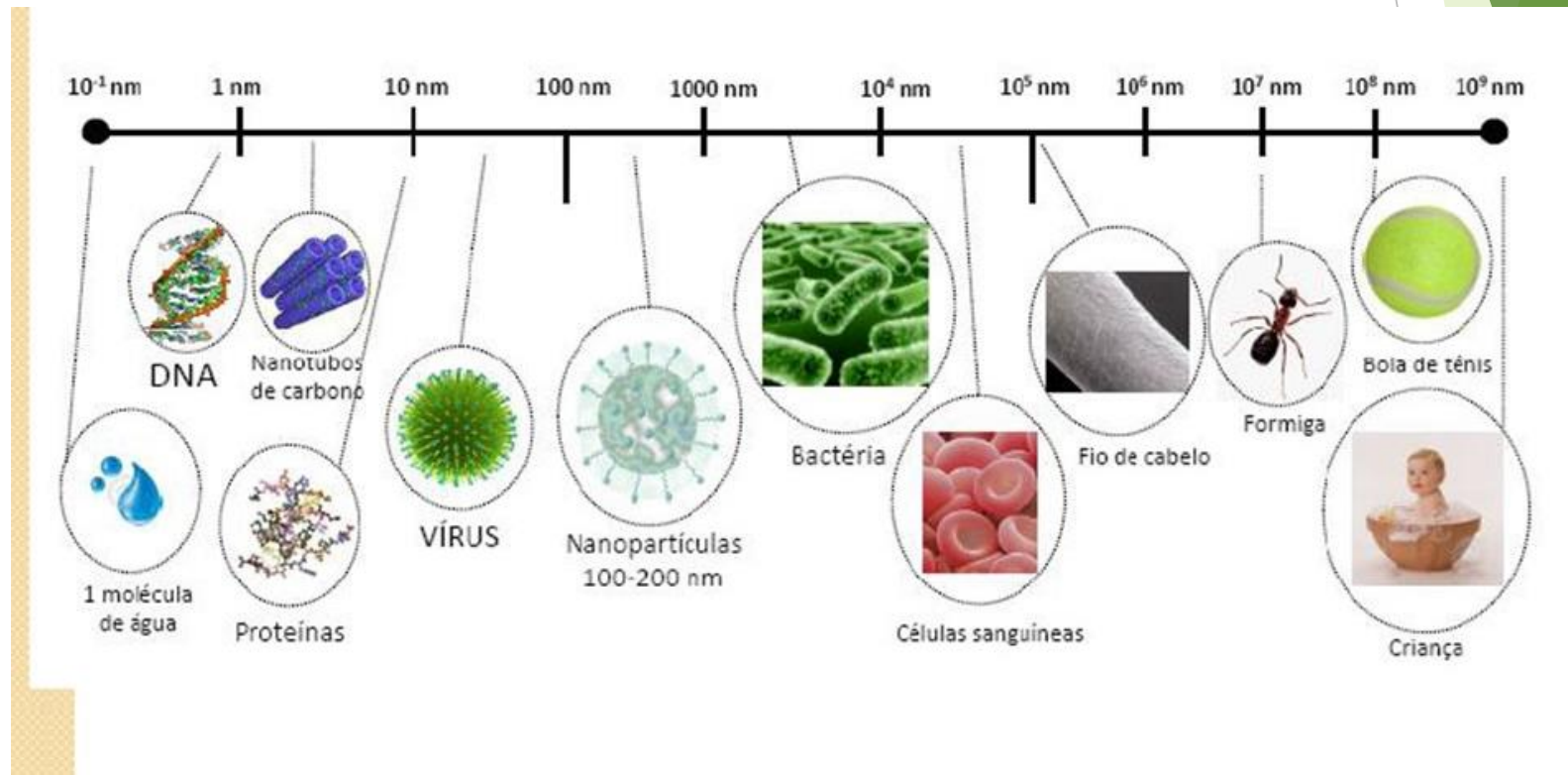


# hOSEC MODEL

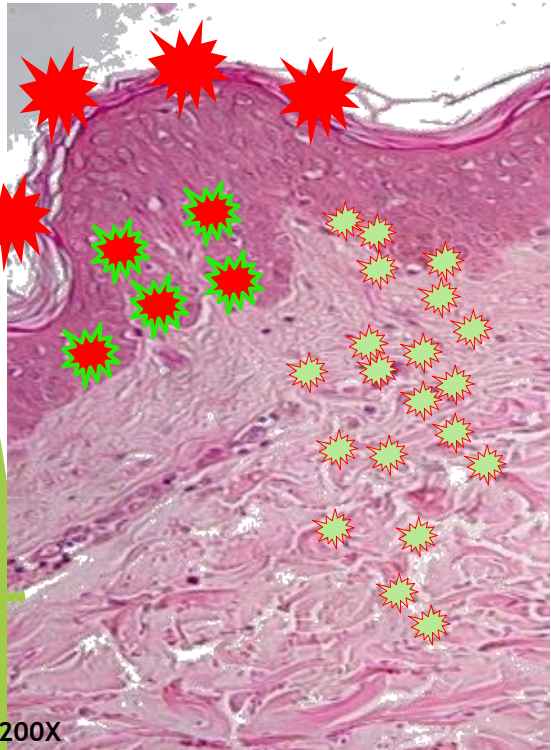
✓ FOR EFFICACY AND SAFETY TESTS



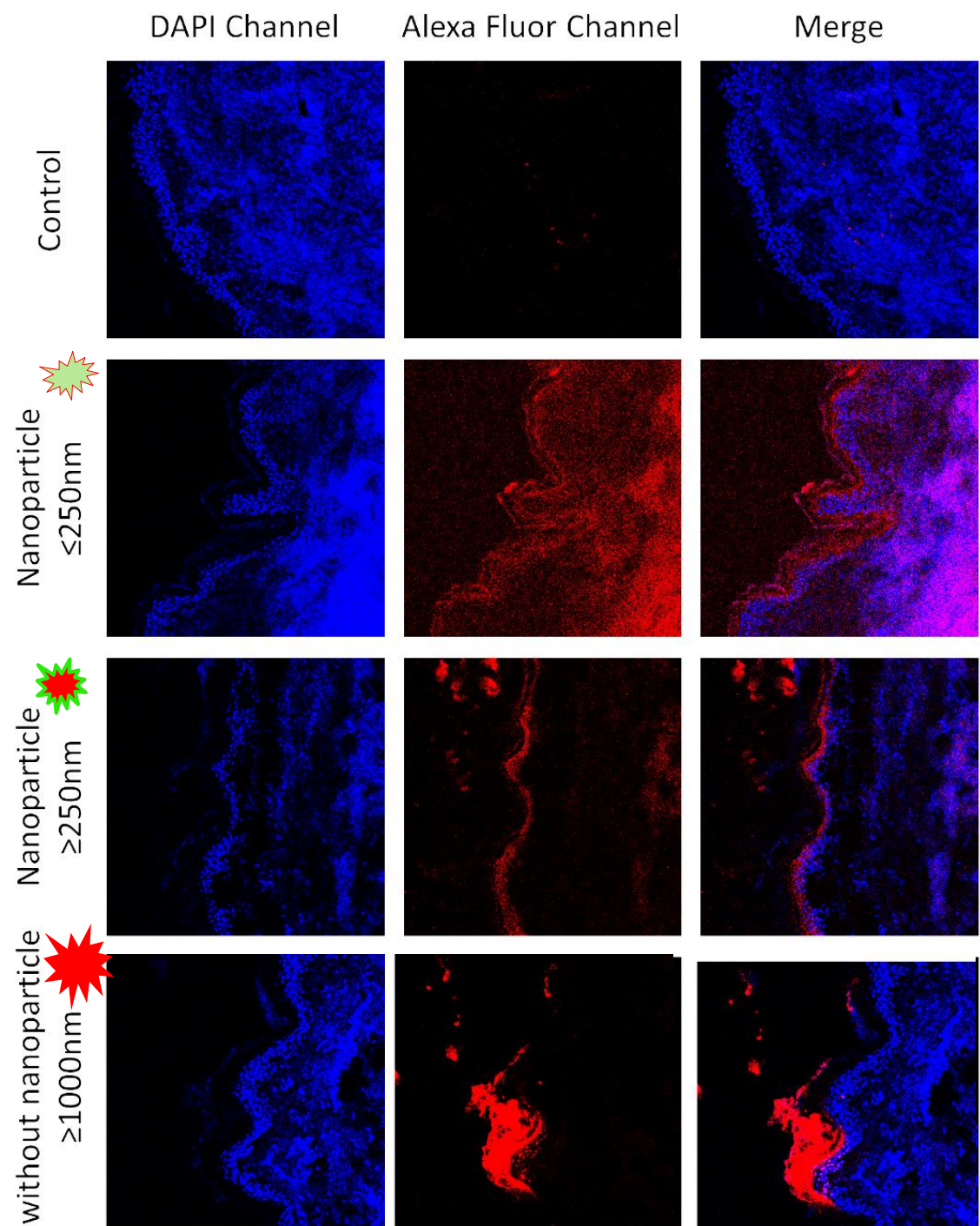
## NANOMETRIC SCALE



# What is your asset target in the skin?



200X



**Ex: antitumor topical asset**  
**Nanoparticle applied for 7 days**

\*to clear the skin's autofluorescence

The nanoparticle was able to penetrate the cutaneous tissue in all layers

The nanoparticle was able to penetrate only the stratum corneum and the superficial epidermis

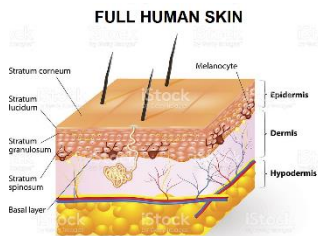
Retained in the stratum corneum

**Juliana dos Santos Rosa**  
M.Sc student  
Prof. Bentley, MVB.





# hOSEC MODEL



In conclusion, the hOSEC model is one interesting alternative model for animals experiments:

- To study tropical disease and propose a nice results for important clinical questions as in leprosy;
- to test topical products on the skin, applied such as the use of the final consumer for a considerable time;

Using a complete skin considering the dermoepidermal natural junction, immune cells, nerve and vascular fragments

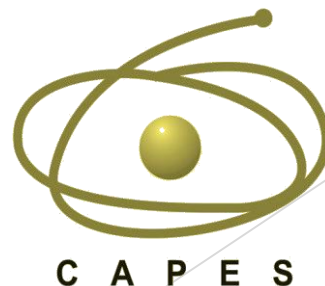
The disadvantage is dependence of human skin sources  
Difficulty: Regulatory standards

# Acknowledgement



**SUPERA**

Parque de Inovação e Tecnologia de Ribeirão Preto



Wound Healing and  
Leprosy Group

*Thiago Andrade  
Guilherme Caetano  
Marcel Nani Leite  
Natália de Paula  
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# Financial Support



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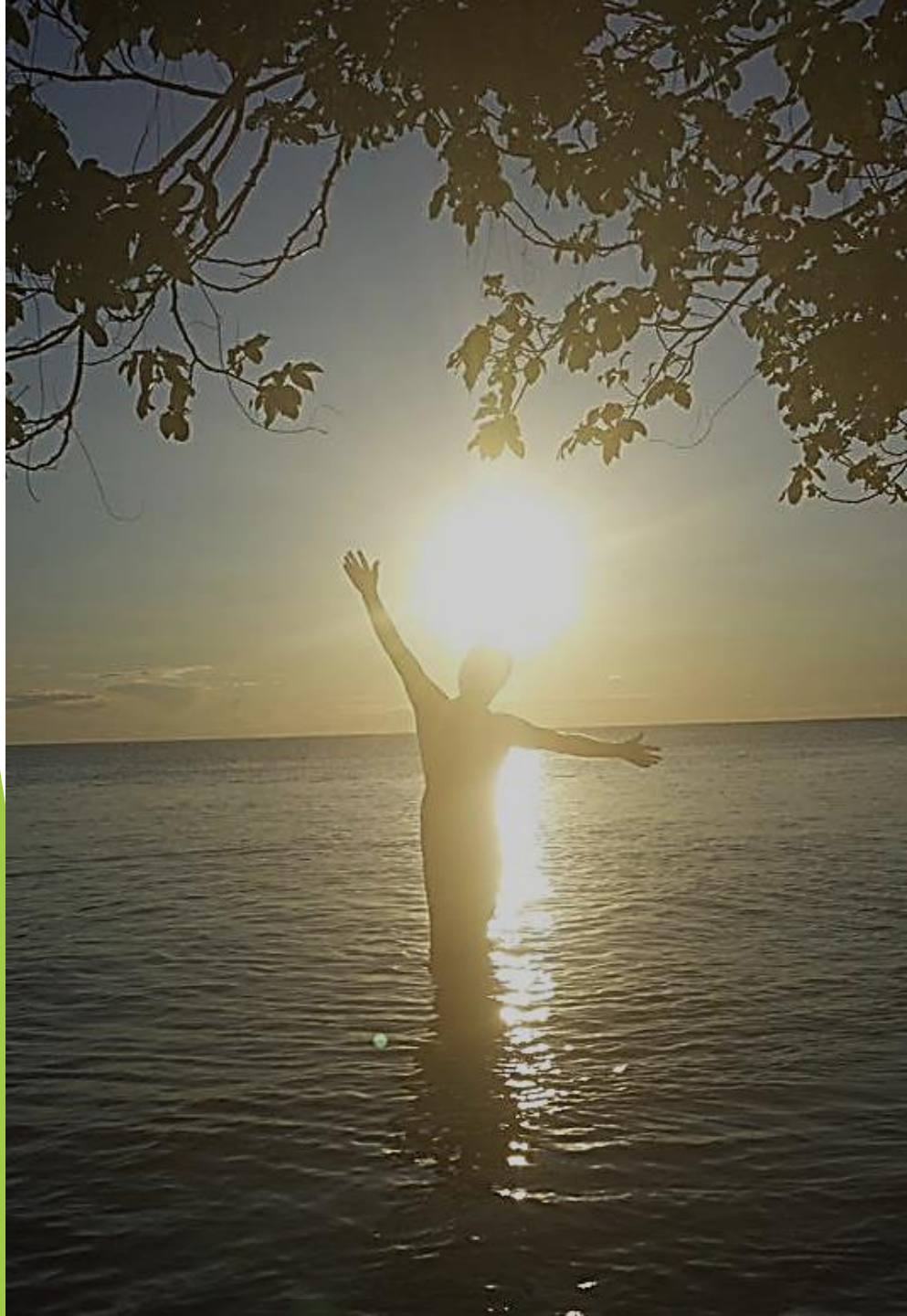


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Marco Frade 2018

2nd  
**PAN-AMERICAN**  
Conference for Alternative Methods  
August 23-24, 2018  
Rio de Janeiro



Muito obrigado!

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