Constructs combining a resorbable scaffold and cultured fibroblasts and keratinocytes
**Requirements to develop a skin substitute**

- **Presence of an epidermal sheet**
  - To protect from outside aggressions

- **Use a dermal-epidermal complex**
  - To facilitate skin wound healing
  - To reinforce mechanical properties

- **Try to avoid animal products**
  - To limit the risk of immune reaction
  - Degradation of dermal scaffold independent from cellular or enzymatic activities
Solution using poly($\alpha$- hydroxy acid)s

- Why use Poly($\alpha$- hydroxy acid)s?
  - Various hydrolytic degradation times (3-48 months)
  - Various mechanical properties
  - Polymers already used in several biomedical applications

- Objective

Conception of a dermal scaffold made from bioresorbable and biocompatible artificial polymers

Seeding then culturing of human fibroblasts + keratinocytes

Skin substitute
Specifications

- **Biocompatibility**
  - Cytocompatibility: assessment of human skin cell adhesion and proliferation
  - Innocuousness (Cytotoxicity or Immunogenicity)

- **Biofunctionality**
  - Possibility of processing (dermal scaffold)
  - Mechanical properties (adapted to surgical handling)
  - Degradation properties (adapted to wound healing time)
  - Sterilization
**Assessment of cytocompatibility by MTT test**

**Adhesion of keratinocytes**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Polystyrene</th>
<th>PLA37.5 GA25</th>
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<tbody>
<tr>
<td>15</td>
<td>0.15</td>
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<td>30</td>
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**Proliferation of keratinocytes**

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- Keratinocytes adhere to PLAGA support
- After 6 days, keratinocytes do not proliferate on PLAGA support
- Glycolic acid inhibits the keratinocytes proliferation from 210 µg/ml

**PLA_{37.5 \ GA_{25}}**

Composed of 37.5 % of L-lactic acid, 37.5 % of D-lactic acid and 25 % of glycolic acid
**PLA$_{50}$**

Composed of 50% of L-lactic acid and 50% of D-lactic acid

- **Assessment of cytocompatibility by MTT test**

**Adhesion of keratinocytes**

<table>
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**Proliferation of keratinocytes**

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- Keratinocytes adhere to PLA$_{50}$ support
- Keratinocytes proliferation is delayed then reaches PS values
- Fibroblasts proliferation on PLA is quite identical to FBH proliferation on PS
Epidermal sheet culture

- We obtained a monocellular layer after 10 days’ culture
- The polymer films appeared somewhat brittle
- Surgical manipulation seems to be really difficult

Chemical modifications to obtain a polymer which presents better mechanical properties
**PLA<sub>50</sub>-PEG-PLA<sub>50</sub>**

- **Introduction of PEG (hydrophilic polymer)**
  - Improvement of mechanical properties
  - Decrease of the hydrolytic degradation time
  - Increase of surface contact angle

- **PEG does not undergo hydrolytic degradation but PEG can be eliminated by kidney filtration**

- **PEG can cause a decrease of cell adhesion and proliferation**
**PLA$_{50}$-PEG-PLA$_{50}$**

- Assessment of cytocompatibility by MTT test
  - We synthetized 4 batches of PLA$_{50}$-PEG-PLA$_{50}$
    - Batch 1 (LA/EG=0.5), B2 (R=1), B3 (R=1.5) et B4 (R=2)

**Proliferation of keratinocytes**

**Proliferation of fibroblasts**

- PEG influences negatively the cell proliferation (fibroblasts)
- Results obtained with batch 4 are not significantly different from those obtained with PLA$_{50}$
PLA<sub>50</sub>-PEG-PLA<sub>50</sub>

**Scaffold processing**

Necessity to produce a scaffold with interconnected pores

- To allow the circulation of biological liquids
- To promote the fibroblast colonization

*Processing using the “salt-leaching technique” (ammonium bicarbonate)*

- Air-contacting surface
- Glass-contacting surface

- Pores present on two sides
- Interconnected network

ESEM: Environmental Scanning Electronic Microscopy
**PLA_{50}-PEG-PLA_{50}**

- Study of angiogenesis *in vitro* from an aortic ring segment

Aortic ring included in collagen close to polymer scaffold

Circle of vascular cell proliferation for polymer scaffold is larger than the control

Vascular cells grew on polymer scaffold surface

Scar Meeting 2006

Friday 31^{th} March
**PLA_{50}-PEG-PLA_{50}**

- **Skin substitute making**

  Dermal layer composed of polymer scaffold colonized by dermal fibroblasts + pores filled with type 4 collagen (0.1 % m/mt)

  Epidermal layer obtained after culture of keratinocytes during 21 days

- Well-differentiated epidermis

- Porous scaffold colonized by human fibroblasts
**Conclusion**

- **PLA$_{50}$-PEG-PLA$_{50}$**
  - Skin cytocompatibility from a molar ratio LA/EG $> 2$
  - Possibility to make a scaffold with an interconnected network of pores
  - Culture support for vascular cells
  - Possibility to produce a dermal-epidermal complex *in vitro*

- **In prospect (*in vivo*)**
  - Degradation study of polymer scaffold
  - Angiogenesis study
  - Engraftment on SCID mice
Thanks to

- Centre de Recherche sur les Biopolymères Artificiels
- Laboratoire de Dermatologie Moléculaire (JP Moles)
- Dr Luc TEOT (Plastic and Reconstructive Surgery)
- Laboratoire Rein et Hypertension (D Casellas)