

**Protection and preventive activity of AC-11 after UV irradiation  
and anti ageing activity on human skin explants maintained in  
survival**

**Study 08E1576**

**Tested Products:**

- AC-11 at 10µg/ml
- AC-11 at 25µg/ml

**Study report delivered by BIO-EC, the 05 August 2008  
Study director: M. P. Gasser**

***Study 08E1576 according to the quotation D08-77-2***

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**AIM OF THE STUDY**

The goal of this study is to evaluate the **preventive (and curative) DNA protection activity** of cosmetic products on human skin explants maintained in survival.

An anti-ageing activity was followed on non-irradiated batches.

After a pre-treatment of 48h with products, explants receive UVB irradiations.

Different stainings permit to visualize modifications:

- Masson's trichrom staining to visualize general morphology and Sun Burn Cells.
- Thymin dimmers immunostaining to visualize DNA damages.

Non-irradiated batches were treated during 9 days to evaluate anti-ageing activity.

**METHODS**

**1. Tested product**

Cat's Claw extract; AC-11 at 10µg/ml tested at 10 µg/ml (P1) and 25 µg/ml (P2).

**2. Explants preparation**

On an abdominal plasty, 51 explants were prepared and put in survival in BEM (BIO-EC's Explants Medium).

They were distributed in 17 batches as following:

Batches	Nb explants	Treatments	UVB mJ/cm <sup>2</sup>	Sampling
T D0	3	None	0	D0
T	6	None	0	D4, D11
TUV1	3	None	50	D4
TUV2	3	None	100	D4
P1	6	Treatment with active 10µg/ml	0	D4, D11
P1UV1	3	Preventive treatment 48H with active 10µg/ml	50	D4
P1UV1C	3	Preventive treatment 48H with active 10µg/ml + treatment 24H post irradiation	50	D4
P1UV2	3	Preventive treatment 48H with active 10µg/ml	100	D4
P1UV2C	3	Preventive treatment 48H with active 10µg/ml + Treatment 24H post irradiation	100	D4
P2	6	Treatment with active 25 µg/ml	0	D4, D11
P2UV1	3	Preventive treatment 48H with active 25 µg/ml	50	D4
P2UV1C	3	Preventive treatment 48H with active 25 µg/ml + Treatment 24H post irradiation	50	D4
P2UV2	3	Preventive treatment 48H with active 25 µg/ml	100	D4
P2UV2C	3	Preventive treatment 48H with active 25 µg/ml + Treatment 24H post irradiation	100	D4

### **3. Treatment**

On day 0, explants were placed in 2 ml of culture medium. Treatment is performed by incorporation of products in the medium. Concentration of the products was calculated in function of oral dosage. Control batch doesn't receive treatment. Treatment was renewed at day 1. On day 2, treatment was realized 3 hours before UV irradiation.

Treatment will be renewed after UV irradiation on **P1**, P1UV1, P1UV1C, P1UV2, P1UV2C, **P2**, P2UV1, P2UV1C, P2UV2 and P2UV2C explants batches.

Three explants of P1 and P2 batches receive the same treatment during 10 days.

### **4. Irradiation**

On day 2, culture medium was substituted by HBSS (Hank's Balanced Saline Solution).

Batches TUV1, TUV2, P1UV1, P1UV1C, P1UV2, P1UV2C, P2UV1, P2UV1C P2UV2 and P2UV2C receive UVB irradiations.

Explants were removed in survival in BEM culture medium.

Non irradiated batches don't receive any UV and are placed in a dark place during the irradiation time.

### **5. Sampling**

On day 4 (24h after irradiation), explants were taken off. On each sampling time, explants were cut in two parts, one half was frozen at  $-80^{\circ}\text{C}$  for different immunostainings, the other half was fixed in formol solution for the general morphology staining.

On day 10, three explants of T, P1 and P2 batches were taken off and treated by the same method.

### **6. General morphology and SBC counting:**

The morphological study of epidermal and dermal structures was realized on sections stained according to Masson's trichrom staining.

Sun burn cells are counted along epidermis (mean by cm of epidermis).

### **7. Thymin dimers Immunostaining**

Immunostaining of thymin dimers was performed on paraffin sections with anti-thymin dimers (clone KTM53 Kamiya Biomedical Company).

Lighting cells will be counted along epidermis (mean by cm of epidermis) by image analysis.

**Following parameters for anti ageing activity:****8. Epidermal thickness**

Epidermal thickness was measured on non-irradiated explants: T0, T D11, P1 D11 and P2 D11 or 12 explants.

**9. Ki67 immunostaining**

Immunostaining of cells in mitosis was performed on frozen sections with polyclonal anti-Ki 67 (Novo Castra) revealed in DAB.

Positive cells were counted on epidermis and the average will be expressed in cells/cm. This immunostaining was realized on: T0, T D4, P1 D4 and P2 D4 batches (12 explants).

**10. Collagen I immunostaining**

The immunostaining was realized on frozen sections with an anti-collagen-I, polyclonal (from SBA) and revealed by FITC (in green). The nucleuses were post stained with propidium iodide.

This staining was realized on explants of batches: T0, T J11, P1 D11 and P2 D11, or 12 explants.

**11. Collagen III immunostaining**

The immunostaining was realized on frozen sections with an anti-collagen-III, polyclonal revealed by VIP (in violet). The nucleuses were post stained with Masson hemalun. Immunostaining was realized on T0, T D11, P1 D11 and P2 D11 batches or 12 explants.

**12. Results :**

Microscopic observation will be realized by Leica DMLB optic microscope. Photos will be taken by Sony camera tri CCD with an IM 1000 Leica system.

Results will be presented in a report illustrated with representative's photos.

## RESULTS

### A-General morphology

#### Observations on day 0 (T0):

The stratum corneum is moderately thick, slightly laminated, clearly keratinized on its surface and on its base. The epidermis presents 5 to 6 cellular layers with a good morphology. The relief of the dermal-epidermal junction is very clear. In the papillary dermis, collagen appears as thick fibres forming a moderately dense network. It is well cellularized.

#### Observations on day 4:

##### Untreated explants: T

The stratum corneum is moderately thick, very slightly laminated, very moderately keratinized on its surface and very clearly at its base. The epidermis presents 4 to 5 cellular layers with a good morphology. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a dense network. It is well cellularized.

##### Explants treated with product 1: P1

The stratum corneum is moderately thick, very slightly laminated, moderately keratinized on its surface and very clearly on its base. The epidermis presents 4 to 5 cellular layers with a good morphology. Spongiosis is moderate in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a dense network. It is well cellularized.

##### Explants treated with product 2: P2

The stratum corneum is moderately thick, very slightly laminated, moderately keratinized on its surface and clearly at its base. The parakeratosis is clear. The epidermis presents 4 to 5 layers with a good morphology. Spongiosis is moderate in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents thick collagen fibres showing a dense network. It is well cellularized.

##### Untreated and irradiated explants at 50mJ/cm<sup>2</sup>: T UV1

The stratum corneum is moderately thick, very slightly laminated, slightly keratinized on its surface and clearly at its base. The parakeratosis is moderate. The epidermis presents 5 to 6 cellular layers with a good morphology. Spongiosis is slight in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a dense network. It is well cellularized.

**Treated explants with the product 1 and irradiated at 50mJ/cm<sup>2</sup>: P1 UV1**

The stratum corneum is moderately thick, very slightly laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is slight. The epidermis presents 5 to 6 cellular layers with a good morphology. Spongiosis is moderate in the basal and supra basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a dense network. It is well cellularized.

**Treated explants with the product 2 and irradiated at 50mJ/cm<sup>2</sup>: P2 UV1**

The stratum corneum is moderately thick, very moderately laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is slight. The epidermis presents 5 to 6 layers with a good morphology. Spongiosis is slight in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents thick collagen fibres showing a dense network. It is well cellularized.

**Treated explants with the product 1 and irradiated at 50mJ/cm<sup>2</sup>: P1 UV1C**

The stratum corneum is moderately thick, very moderately laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is slight. The epidermis presents 5 to 6 layers with a good morphology. Spongiosis is clear in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents thick collagen fibres showing a dense network. It is well cellularized.

**Treated explants with the product 2 and irradiated at 50mJ/cm<sup>2</sup>: P2 UV1C**

The stratum corneum is moderately thick, very moderately laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is slight. The epidermis presents 5 to 6 layers with a good morphology. Spongiosis is moderate in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents thick collagen fibres showing a dense network. It is well cellularized.

**Untreated and irradiated explants at 100 mJ/cm<sup>2</sup>: T UV2**

The stratum corneum is moderately thick, very slightly laminated, moderately keratinized on its surface and clearly at its base. Parakeratosis is moderate. The epidermis presents 5 to 6 cellular layers with a good morphology. Spongiosis is moderate in the basal layer. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a dense network. It is well cellularized.

**Treated explants with the product 1 and irradiated at 100 mJ/cm<sup>2</sup>: P1 UV2**

The stratum corneum is moderately thick, very slightly laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is clear. The epidermis presents 5 to 6 cellular layers with a good morphology. Spongiosis is slight on the basal layer. The relief of the dermal-epidermal junction is moderate. The papillary dermis presents moderately thick collagen fibres showing a dense network. It is well cellularized.

**Treated explants with the product 2 and irradiated at 100 mJ/cm<sup>2</sup>: P2 UV2**

The stratum corneum is moderately thick, very slightly laminated, moderately keratinized on its surface and clearly at its base. The parakeratosis is slight. The epidermis presents 5 to 6 cellular layers with a good morphology. Spongiosis is moderate in the basal and supra basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents thick collagen fibres showing a very dense network. It is well cellularized.

**Treated explants with the product 1 and irradiated at 100 mJ/cm<sup>2</sup>: P1 UV2C**

The stratum corneum is moderately thick, moderately laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is moderate. The epidermis presents 5 to 6 cellular layers slightly altered. These alterations are characterized by a clear spongiosis in the basal and supra basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a moderately dense network. It is well cellularized.

**Treated explants with the product 2 and irradiated at 100 mJ/cm<sup>2</sup>: P2 UV2C**

The stratum corneum is moderately thick, moderately laminated, moderately keratinized on its surface and clearly on its base. The parakeratosis is moderate. The epidermis presents 5 to 6 cellular layers slightly altered. These alterations are characterized by a clear spongiosis in the basal and supra basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a moderately dense network. It is well cellularized.



**Observations on day 11:****Untreated explants: T**

The stratum corneum is thick, moderately laminated, slightly keratinized on its surface and very clearly at its base. Parakeratosis is moderate. The epidermis presents 4 to 5 cellular layers with a quite good morphology. Spongiosis is very clear in the basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a moderately dense network. It is well cellularized.

**Explants treated with product 1: P1**

The stratum corneum is moderately thick, very moderately laminated, slightly keratinized on its surface and clearly on its base. The parakeratosis is strong. The epidermis presents 4 to 5 cellular layers with a quite good morphology. Spongiosis is clear in the basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a moderately dense network. It is well cellularized.

**Explants treated with product 2: P2**

The stratum corneum is moderately thick, very moderately laminated, slightly keratinized on its surface and clearly on its base. The parakeratosis is strong. The epidermis presents 4 to 5 cellular layers with a quite good morphology. Spongiosis is very clear with a moderate acantholysis in the basal layers. The relief of the dermal-epidermal junction is clear. The papillary dermis presents moderately thick collagen fibres showing a moderately dense network. It is well cellularized.

**B- SBC counting**

Batch	Nb Cell +	Lenght (cm)	Nb Cell+ / cm	Mean	Standart deviation
D0	0	0,89	0	<b>0,0</b>	0,0
	0	0,97	0		
	0	0,94	0		
D	1	0,9	1	<b>0,7</b>	0,5
	0	0,94	0		
	1	0,95	1		
P1	0	0,95	0	<b>0,0</b>	0,0
	0	1,05	0		
	0	1,07	0		
P2	1	0,94	1	<b>0,4</b>	0,5
	0	1,22	0		
	0	1,13	0		
T UV1	1	0,96	1	<b>1,2</b>	0,4
	2	1,14	2		
	1	1,06	1		
P1 UV1	1	0,94	1	<b>1,4</b>	0,5
	1	0,98	1		
	2	0,92	2		
P2 UV1	0	1,01	0	<b>0,9</b>	0,7
	1	1,07	1		
	2	1,12	2		
P1 UV1 C	0	1,15	0	<b>1,0</b>	0,8
	2	1,00	2		
	1	1,08	1		
P2 UV1 C	0	0,98	0	<b>0,3</b>	0,4
	0	1,04	0		
	1	1,10	1		
T UV2	3	1,02	3	<b>2,6</b>	0,4
	3	1,09	3		
	2	0,97	2		
P1 UV2	0	1,05	0	<b>0,7</b>	0,5
	1	1,15	1		
	1	0,91	1		
P2 UV2	2	1,09	2	<b>1,6</b>	0,4
	2	1,08	2		
	1	0,94	1		
P1 UV2 C	0	1,09	0	<b>0,0</b>	0,0
	0	1,10	0		
	0	1,08	0		
P2 UV2 C	0	1,01	0	<b>0,6</b>	0,5
	1	1,07	1		
	1	0,99	1		



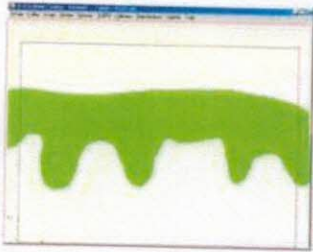
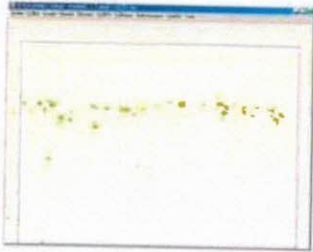
The low UV doses used in this study are too low to induce a significant number of Sunburn cells



**C- Thymin dimers Immunostaining**

The thymin dimers expression was analyzed by Image analysis. For each explants 6 microscopic fields were analyzed.

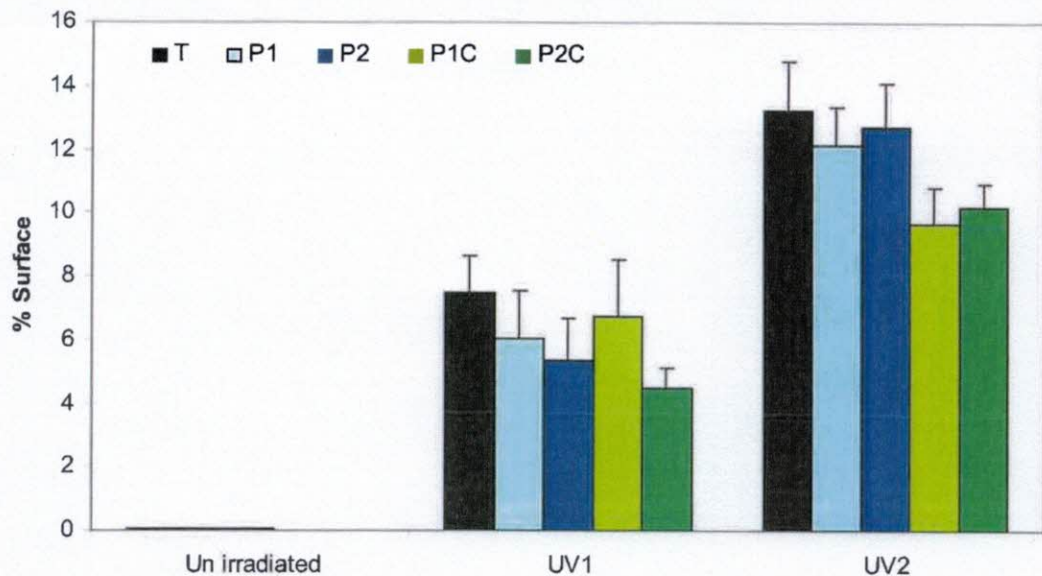
LEICA QWIN software, routine: « 08E1576 Surf et int Dimers Thy.Q5R »

1. **Digitized image (obj x40)**  
Thymine dimers Immunostaining  
Revelation by VIP 
2. **Detection of immunostaining by intensity level selection**  
binary mask 0 
3. **Determination of the interested zone,**  
Binary mask 2 
4. **Selection of the immunostaining in the interested zone**  
Binary mask 3=2+0 
5. **Surfaces measuring**  
Measuring of binary 3 mask  
Measuring of binary 2 mask
6. **Results**  
Exportation of results to Excel

**% of surface of thymine dimers in the epidermis**

% of surface	Un irradiated		UV1: 50 mJ/cm <sup>2</sup>		UV2: 100 mJ/cm <sup>2</sup>	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
T (Day 0)	0,02	0,01				
T	<b>0,04</b>	0,03	<b>7,49</b>	1,12	<b>13,22</b>	1,56
P1	<b>0,07</b>	0,01	<b>6,05</b>	1,48	<b>12,16</b>	1,18
P2	<b>0,07</b>	0,00	<b>5,37</b>	1,29	<b>12,71</b>	1,39
P1C			<b>6,73</b>	1,80	<b>9,65</b>	1,16
P2C			<b>4,49</b>	0,64	<b>10,21</b>	0,74

**Thymine dimers UV induced in the epidermis**



The irradiation UV1 (50 mJ/cm<sup>2</sup>) induces a significant increase of 17573% of the expression of thymine dimers, compared to the untreated and unirradiated batch.

- The 3 days pretreatment with the tested product at 10µg/ml (P1UV1) induces a non significant decrease of 19%, compared to the untreated and irradiated batch
- The 3 days pretreatment with the tested product at 25µg/ml (P2UV1) induces a non significant decrease of 28%, compared to the untreated and irradiated batch
- The 3 days pretreatment completed with a 1 day curative treatment with the tested product at 10µg/ml (P1UV1C) induces a non significant decrease of 10%, compared to the untreated and irradiated batch

- The 3 days pretreatment completed with a 1 day curative treatment with the tested product at 25µg/ml (**P2UV1C**) induces **a significant decrease of 40%**, compared to the untreated and irradiated batch

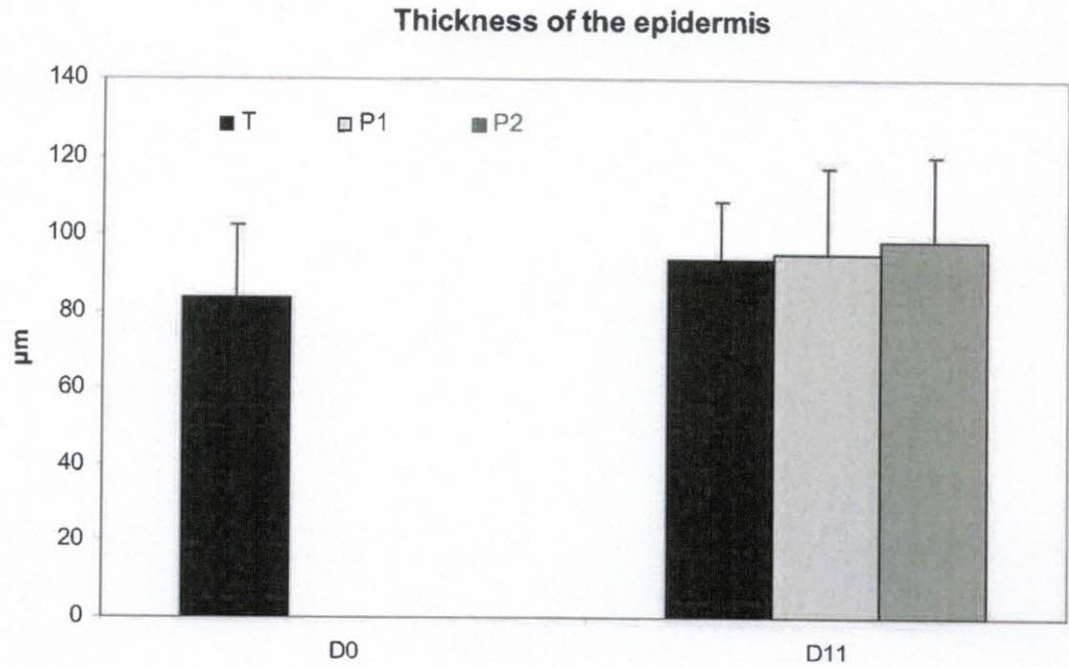
The irradiation UV2 (100 mJ/cm<sup>2</sup>) induces a significant increase of 31083% of the expression of thymine dimers, compared to the untreated and unirradiated batch.

- The 3 days pretreatment with the tested product at 10µg/ml (P1UV2) induces a non significant decrease of 8%, compared to the untreated and irradiated batch
- The 3 days pretreatment with the tested product at 25µg/ml (P2UV2) induces a non significant decrease of 4%, compared to the untreated and irradiated batch
- The 3 days pretreatment completed with a 1 day curative treatment with the tested product at 10µg/ml (P1UV2C) induces a non significant decrease of 27%, compared to the untreated and irradiated batch
- The 3 days pretreatment completed with a 1 day curative treatment with the tested product at 25µg/ml (P2UV2C) induces a significant decrease of 23%, compared to the untreated and irradiated batch

**D- Epidermis thickness**

Epidermal thickness was measured on non-irradiated explants: T0, T D11, P1 D11 and P2 D11 or 12 explants.

<b>Thickness of the epidermis(µm)</b>				
	<b>T0</b>	<b>TJ11</b>	<b>P1J11</b>	<b>P2J11</b>
	93	104	117	77
	61	104	143	74
	72	94	112	84
	113	91	72	84
	89	75	89	110
	88	88	84	103
	114	92	84	82
	74	74	65	113
	79	91	94	148
	55	127	93	111
	52	113	87	110
	62	91	82	98
	111	111	118	55
	93	72	98	59
	65	77	85	83
	89	87	106	137
	127	96	93	124
	80	103	81	98
	87		70	101
	84		76	91
	92		50	95
	52		120	
	61		112	
	87		84	
	76			
	92			
	102			
<b>Ave</b>	<b>83,80</b>	<b>94,00</b>	<b>95,30</b>	<b>98,60</b>
<b>SD</b>	<b>18,71</b>	<b>14,52</b>	<b>21,88</b>	<b>21,74</b>



The 11 days treatment with the tested product at 10µg/ml (P1) induces a non significant decrease of 1 % of the thickness of the epidermis.

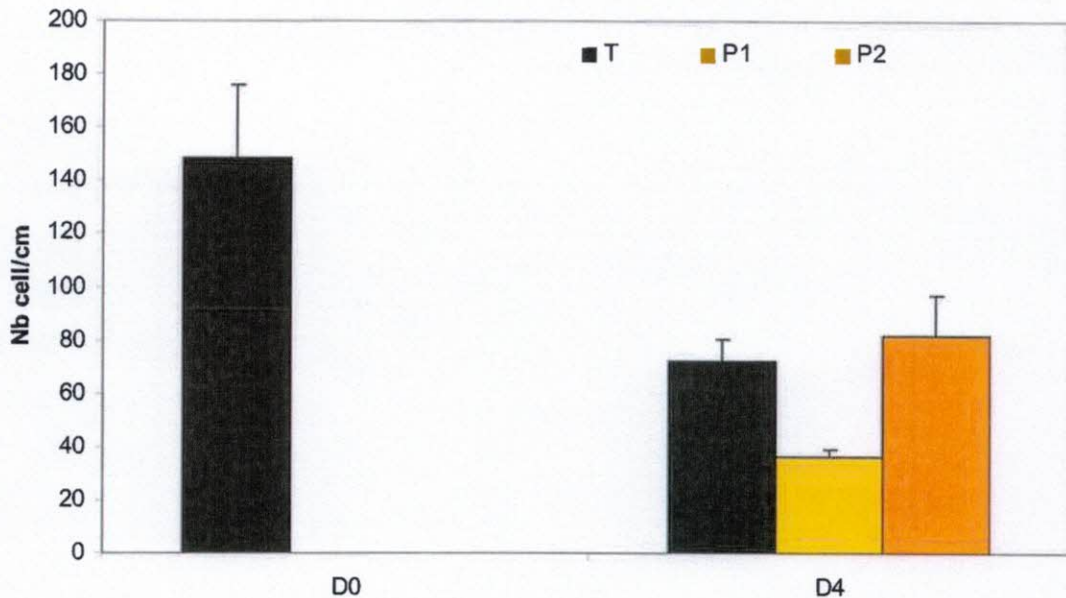
The 11 days treatment with the tested product at 25µg/ml (P2) induces a non significant increase of 5 % of the thickness of the epidermis.

**E- Ki67 immunostaining:**

This immunostaining will be realized on: T0, T D3, P1 D3 and P2 D3 batches (12 explants).

Batch	Nb Cell +	Lenght (cm)	Nb Cell+ / cm	Mean	Standart deviation
T D0	133	0,89	149	<b>148,2</b>	27,6
	176	0,97	181		
	107	0,94	114		
TD4	66	0,9	73	<b>72,1</b>	8,6
	77	0,94	82		
	58	0,95	61		
P1D4	33	0,95	35	<b>36,4</b>	2,7
	36	1,05	34		
	43	1,07	40		
P2D4	59	0,6	98	<b>81,9</b>	14,9
	71	1,14	62		
	96	1,13	85		

**Ki67 positives cells in the epidermis**



The 4 days treatment with the tested product at 10µg/ml (P1) induces a significant decrease of 50 % of the number of Ki67 positive cells.

The 4 days treatment with the tested product at 25µg/ml (P2) induces a non significant increase of 14 % of the number of Ki67 positive cells.



### ***F- Collagen I immunostaining***

The immunostaining will be realized on frozen sections with an anti-collagen-I, polyclonal (from SBA) and revealed by fluorescence. The nucleus will be post stained with propidium iodide. This staining will be realized on explants of batches: T0, T J10, P1 D10 and P2 D10, or 12 explants.

#### **Observations on day 0 (T0):**

##### **Untreated explants: T**

The staining is very slight, slightly dense with thin fibres in the papillary dermis.

#### **Observations on day 11:**

##### **Untreated explants: T**

The staining is moderate, moderately dense with thin fibres in the papillary dermis.



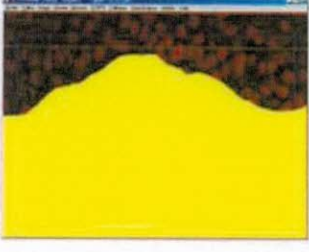

##### **Explants treated with product 1: P1**

The staining is very moderate, slightly dense with thin fibres in the papillary dermis.

##### **Explants treated with product 2: P2**

The staining is quite clear, moderately dense with thick fibres in the papillary dermis.

For each batches, 9 microscopic field have been analyzed with the LEICA QWIN, software using the following process: « 08E1576 Surf et Int Coll I.Q5R »

1. **digitized image (obj X40)**  
Immunostaining of collagen I revealed in FITC
 
2. **Detection of immunostaining by intensity level selection**  
binary mask 0
 
3. **Determination of the interested zone,**  
Binary mask 2
 
4. **Selection of the immunostaining in the interested zone**  
Binary mask 3=2+0
 
5. **Surfaces measuring**

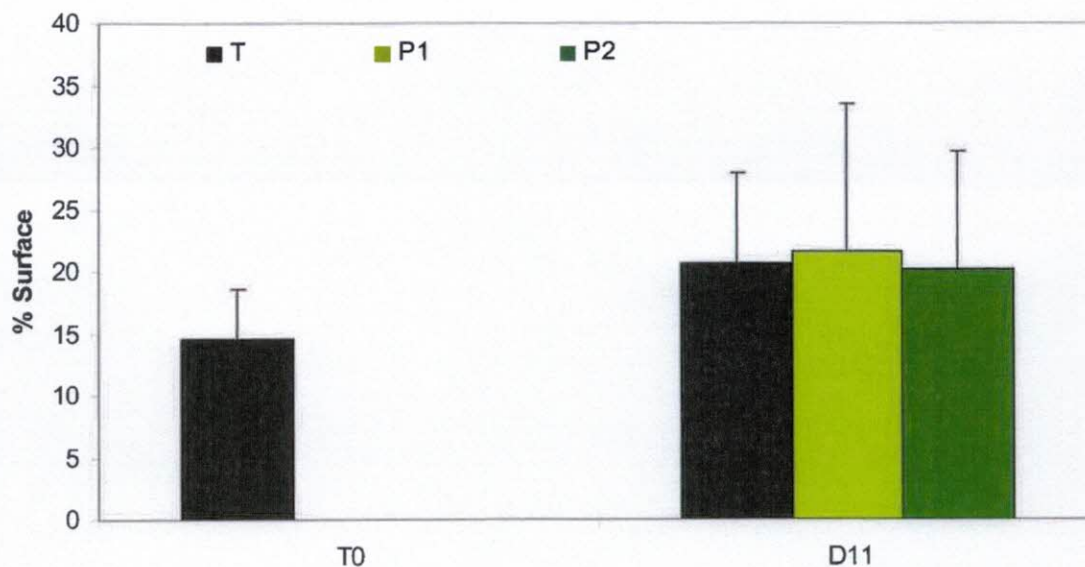
Measuring of binary 3 mask  
Measuring of binary 2 mask
6. **Results**

Exportation of results to Excel

Percentage of surface of collagen I in the papillary dermis.

% Surface	Day 0		Day 11	
	Average	SD	Average	SD
T	<b>14,5</b>	4,0	<b>20,6</b>	7,3
P1			<b>21,7</b>	11,8
P2			<b>20,2</b>	9,5

**Percentage of surface of collagène I in the papillary dermis**



The 11 days treatment with the tested product at 10µg/ml (P1) induces a non significant increase of 5 % of the percentage of collagen I in the epidermis.

The 11 days treatment with the tested product at 25µg/ml (P2) induces a non significant decrease of 2 % of the percentage of collagen I in the epidermis.

### **G- Collagen III immunostaining**

The immunostaining will be realized on frozen sections with an anti-collagen-III, polyclonal revealed by DAB. The nucleus will be post stained with Masson hemalun. Immunostaining will be realized on T0, T D11, P1 D11 and P2 D11 batches or 12 explants.

#### **Observations on day 0 (T0):**

##### **Untreated explants: T**

The staining is very slight, slightly filamentous in all the papillary dermis.

#### **Observations on day 11:**

##### **Untreated explants: T**

The staining is very moderate, slightly filamentous in all the papillary dermis.

##### **Explants treated with product 1: P1**

The staining is very moderate, moderately filamentous in all the papillary dermis.

##### **Explants treated with product 2: P2**

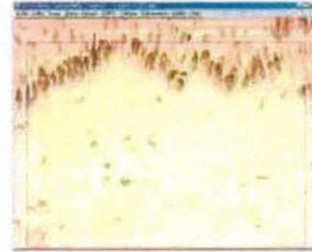
The staining is quite clear, clearly filamentous in all the papillary dermis.

### Collagen III immunostaining image analysis

For each batches, 9 microscopic field have been analyzed with the LEICA QWIN, software using the following process: « 08E1576 Surf et Int Coll III.Q5R »

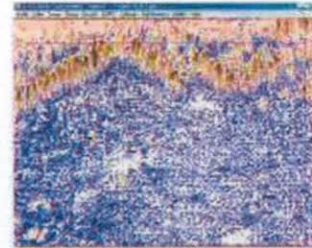
1. **digitized image (obj X40)**

Exemple of immunostaining of collagen III revealed in DAB

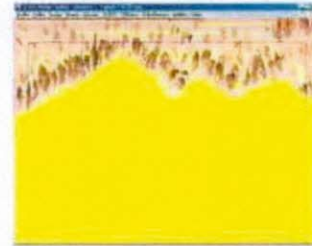


2. **Detection of immunostaining by intensity level selection**

binary mask 0

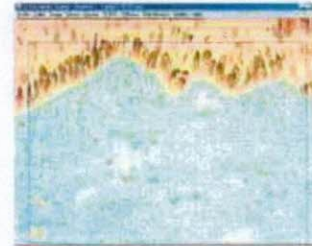


3. **Determination of the interested zone, Binary mask 2**



4. **Selection of the immunostaining in the interested zone**

Binary mask 3=2+0



5. **Surfaces measuring**

Measuring of binary 3 mask  
Measuring of binary 2 mask

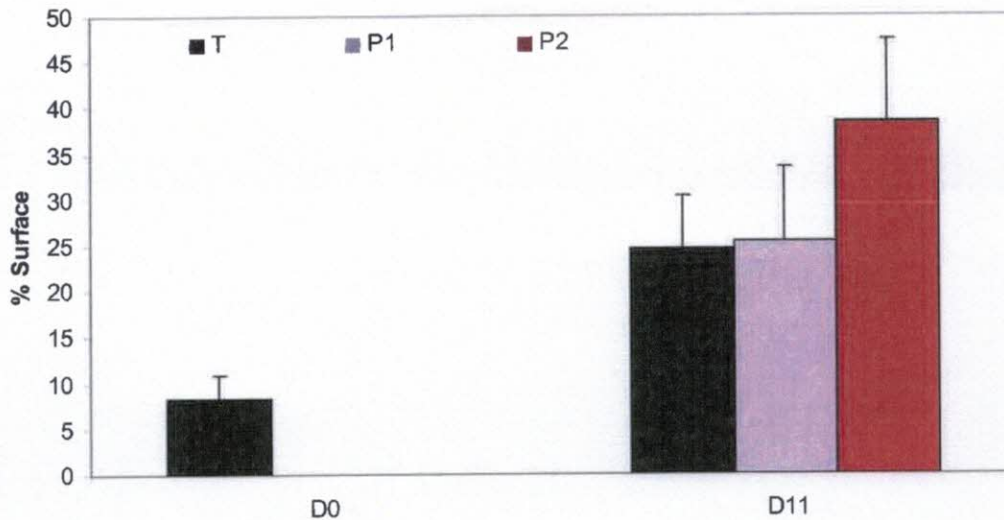
6. **Results**

Exportation of results to Excel

Percentage of surface of collagen III in the papillary dermis.

% Surface	Day 0		Day 11	
	Average	SD	Average	SD
T	<b>8,2</b>	2,6	<b>24,6</b>	5,8
P1			<b>25,4</b>	8,1
P2			<b>38,5</b>	8,9

Percentage of surface of collagène I in the papillary dermis



The 11 days treatment with the tested product at 10µg/ml (P1) induces a non significant increase of 3 % of the percentage of collagen III in the epidermis.

The 11 days treatment with the tested product at 25µg/ml (P2) induces a **significant increase of 56 %** of the percentage of collagen III in the epidermis.

## **DISCUSSION**

### **Preventive (and curative) DNA protection activity**

#### **General morphology:**

##### **On day 0**

The stratum corneum is moderately thick, slightly laminated, clearly keratinized on its surface and on its base. The epidermis presents 5 to 6 cellular layers with a good morphology. The relief of the dermal-epidermal junction is very clear. In the papillary dermis, collagen appears as thick fibres forming a moderately dense network. It is well cellularized.

##### **On day 4**

On the untreated explants, the epidermal structure is closed to that observed on day 0. The papillary dermis presents moderately thick collagen fibres showing a moderately dense network.

Whatever the treated batch, no significant alterations have been observed in the epidermal and dermal structure.

#### **Anti ageing activity:**

#### **General morphology:**

##### **On day 0**

The stratum corneum is moderately thick, slightly laminated, clearly keratinized on its surface and on its base. The epidermis presents 5 to 6 cellular layers with a good morphology. The relief of the dermal-epidermal junction is very clear. In the papillary dermis, collagen appears as thick fibres forming a moderately dense network. It is well cellularized.

##### **On day 11**

On the untreated explants, the epidermal structure is moderately altered with a moderate parakeratosis and a very clear spongiosis in the basal layer.

On treated explants with AC-11 at 10µg/ml, P1, the general morphology is closed to that observed on the untreated ones.

On treated explants with AC-11 at 25µg/ml, P2, the general morphology is slightly more thick than that observed on explants with P1.

#### **SBC counting:**

##### **On day 0**

##### **On day 11**

#### **Thymin dimers Immunostaining**

##### **On day 0**

**On day 11**

**Ki67 immunostaining**

**On day 0**

**On day 11**

**Collagen I immunostaining:**

**On day 0**

Collagen I is very slight, slightly dense with thin fibres in the papillary dermis.

**On day 11**

On untreated explants, collagen I is slightly increased compared to that observed on the untreated ones.

Collagen I is not increased on explants treated with AC-11 at 10µg/ml, P1, compared to the untreated ones.

Collagen I is moderately increased on explants treated with AC-11 at 25µg/ml, P2, with a more filamentous structure.

**Collagen III immunostaining:**

**On day 0**

Collagen III is very slight, slightly filamentous in all the papillary dermis.

**On day 11**

On untreated explants, collagen III is slightly increased compared to that at D0.

Collagen I is slightly increased on explants treated with AC-11 at 10µg/ml, P1 compared to the untreated ones.

Collagen I is clearly increased on explants treated with AC-11 at 25µg/ml, P2, with a more filamentous structure.



## **Anti ageing activity**

**According to these operating conditions after 11 days survival:**

**AC-11 at 10µg/ml induces:**

**No epidermal stimulation**

**No collagen I over expression**

**No collagen III over expression**

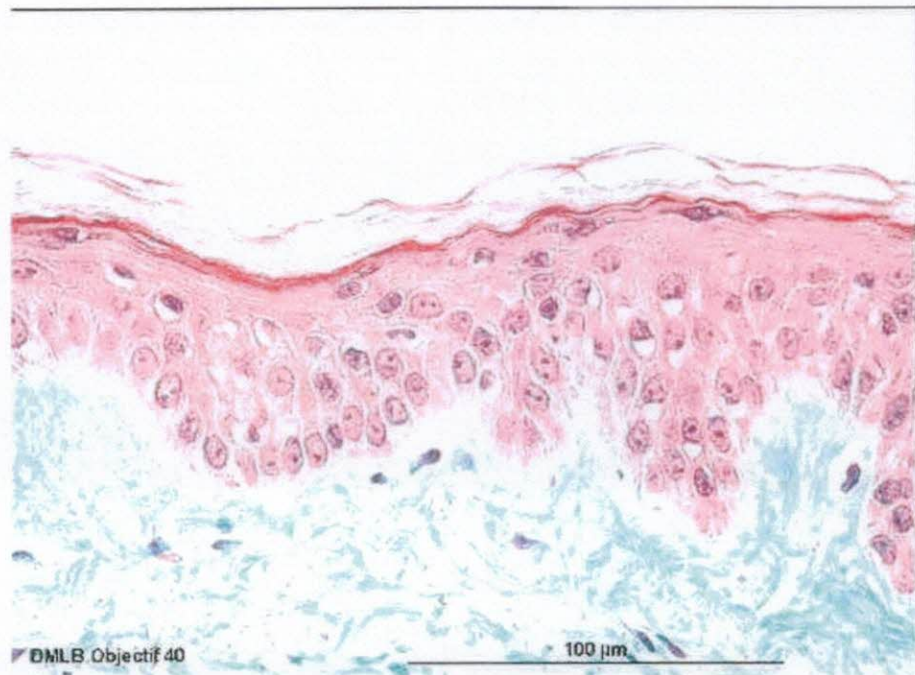
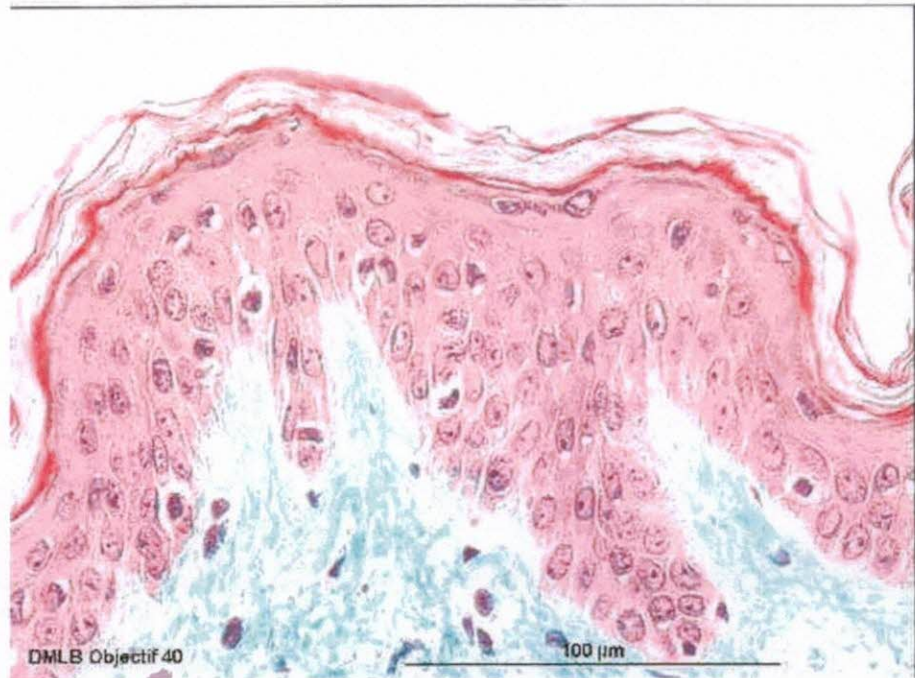
**AC-11 at 25µg/ml induces:**

**A slight epidermal stimulation**

**A slight collagen I over expression**

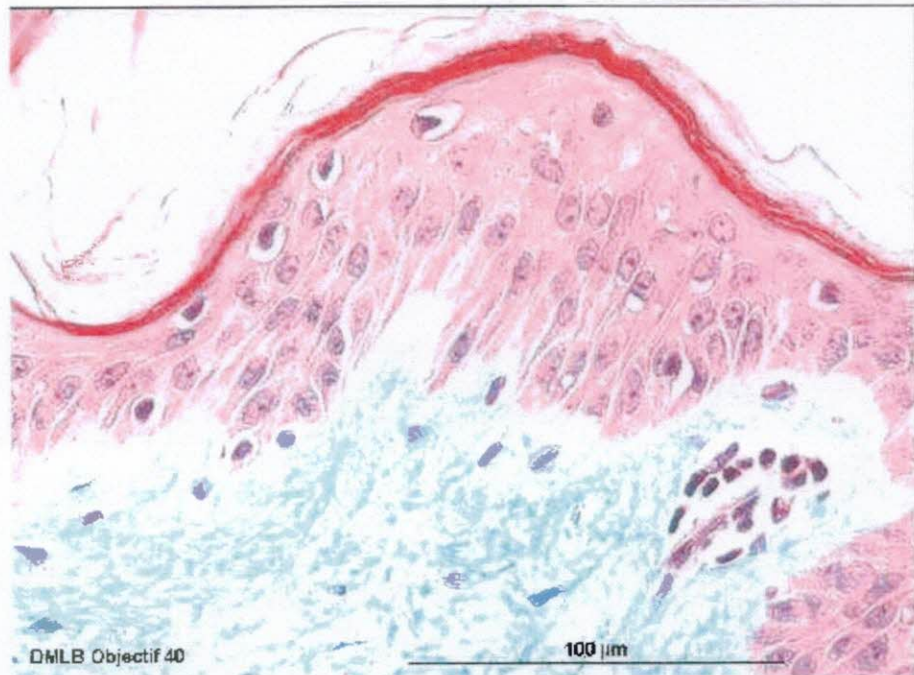
**A moderate collagen III over expression**

### General morphology



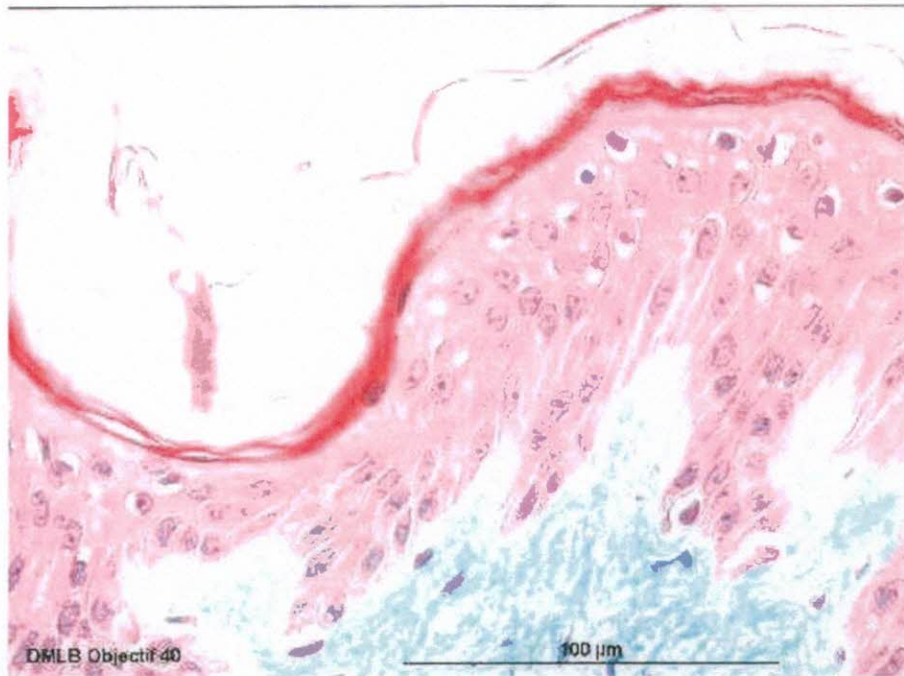
**Blank explant on D0**

### General morphology



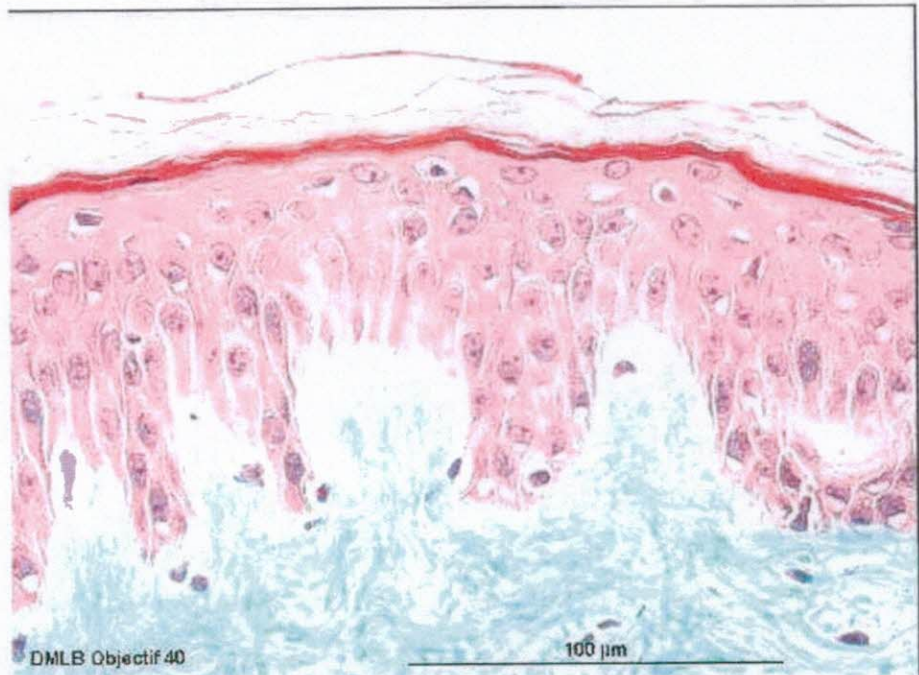
**Untreated explant on D4 (T)**

### General morphology



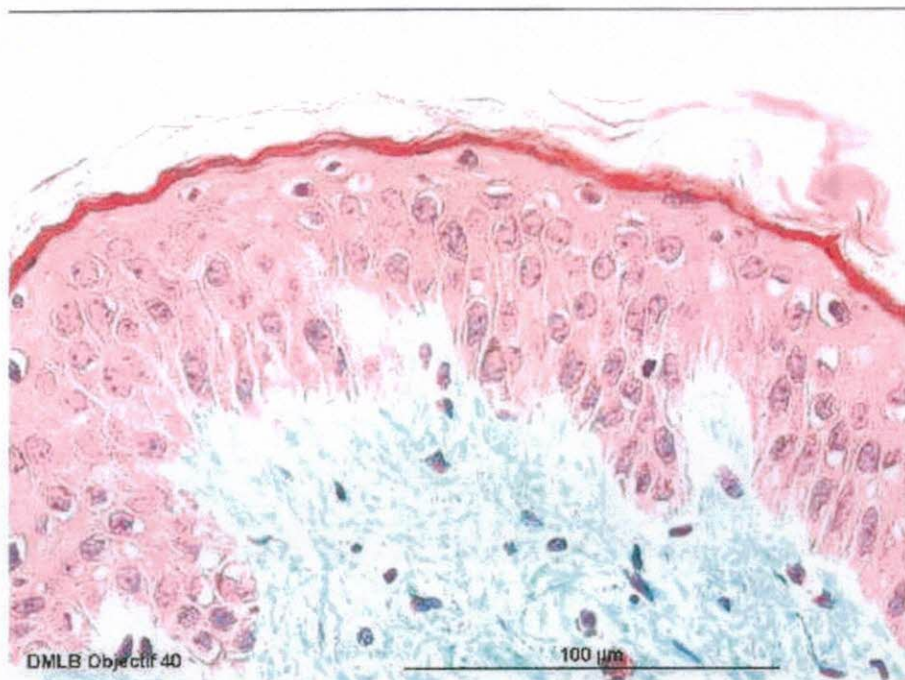
**Explant treated with product 1 on D4 (P1)**

### General morphology



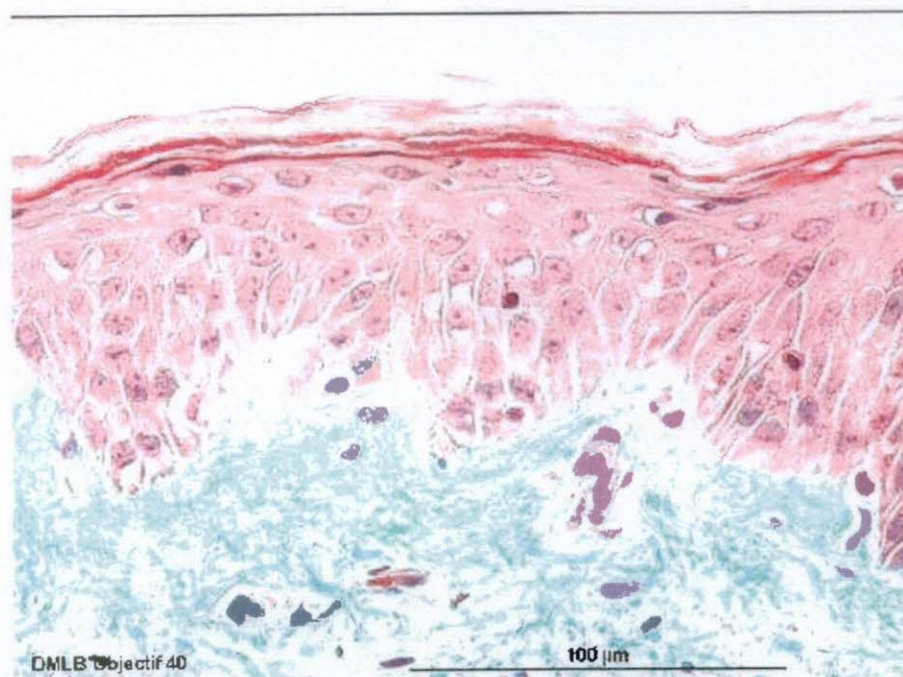
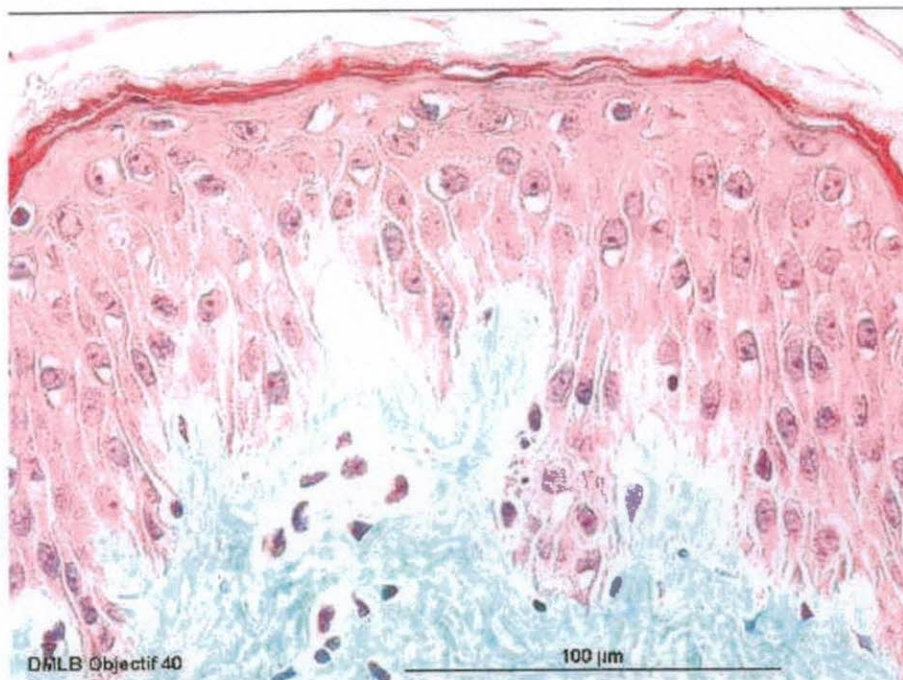
**Explant treated with product 2 on D4 (P2)**

### General morphology



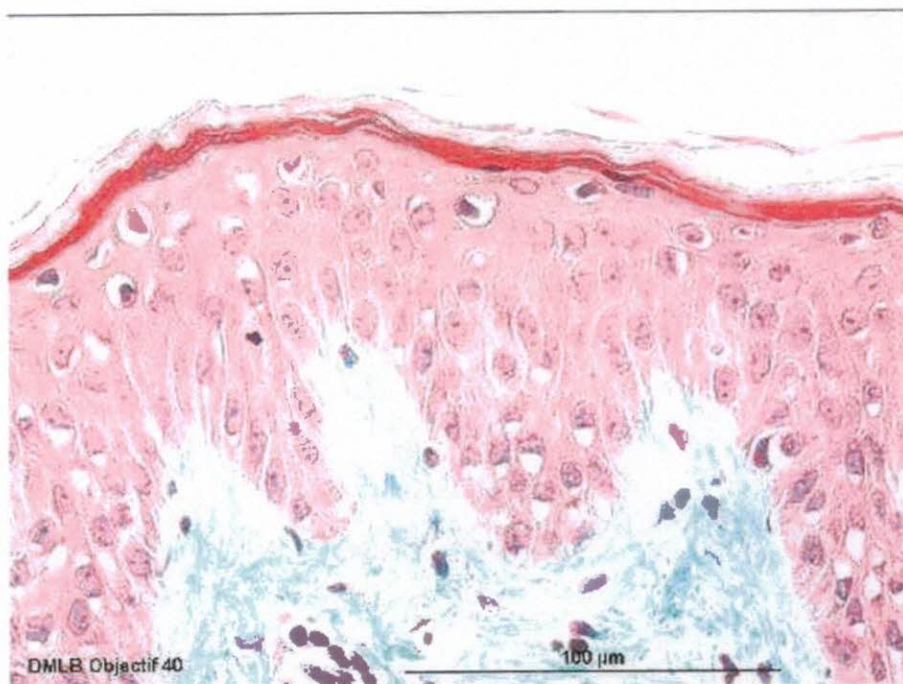
**Untreated explant and irradiated at 50mJ on D4 (TUV1)**

### General morphology



**Treated explant with product 1 and irradiated at 50mJ on D4 (P1UV1)**

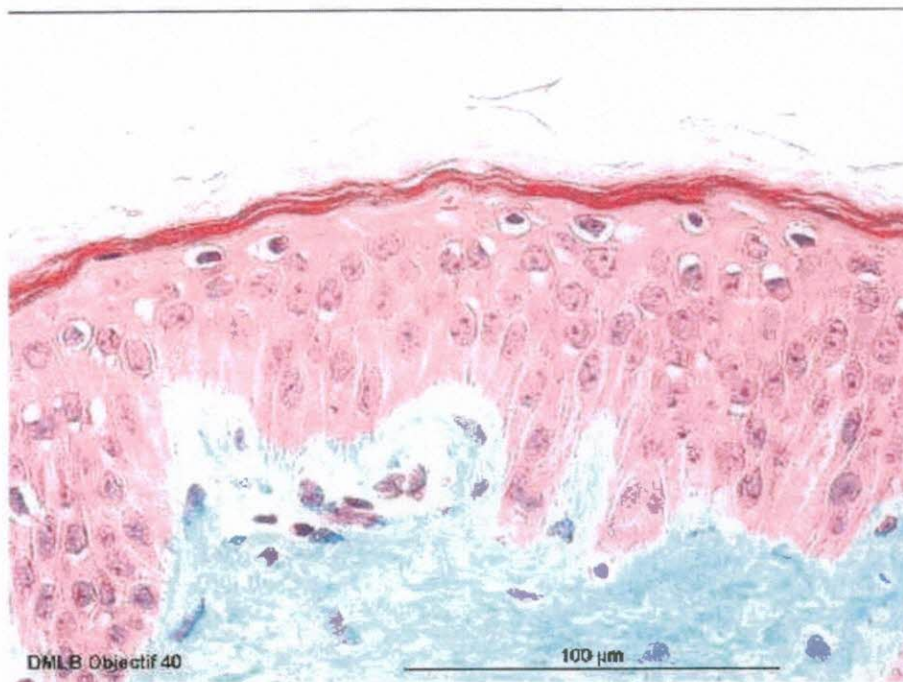
### General morphology



**Treated explant with product 2 and irradiated at 50mJ on D4 (P2UV1)**

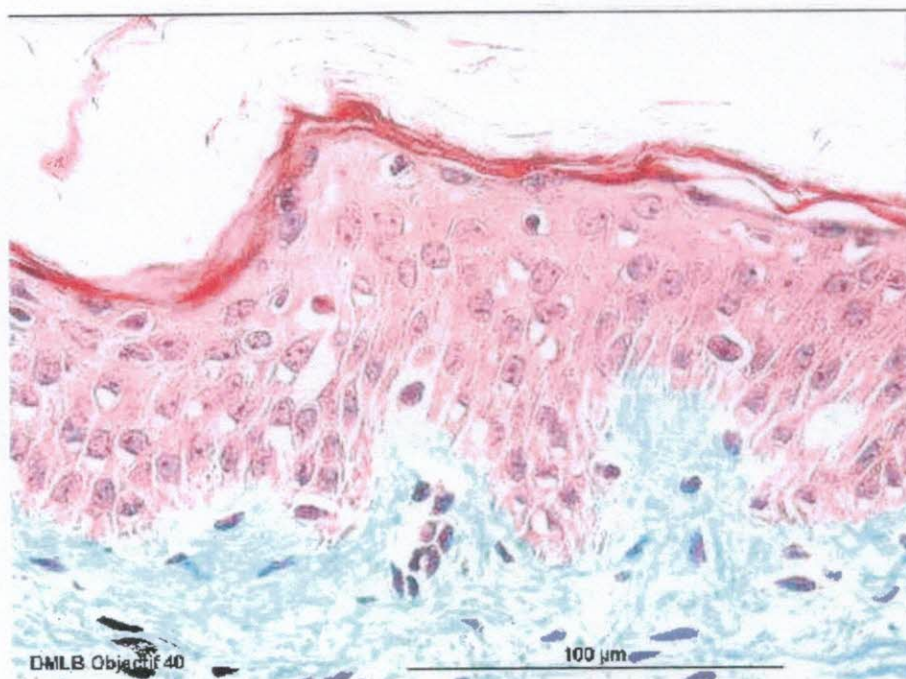


### General morphology



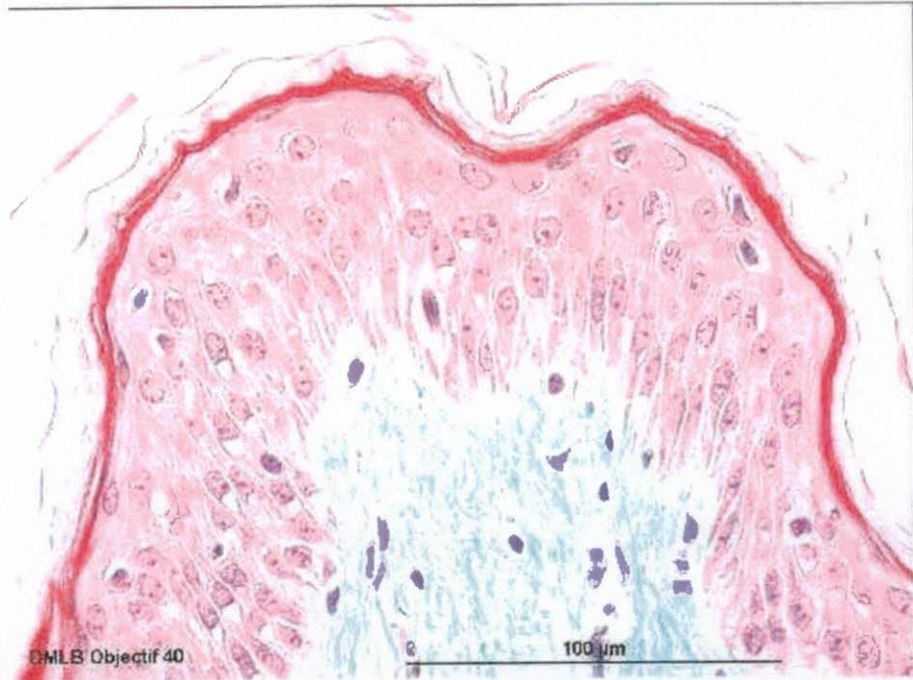
**Treated explant with product 1 and irradiated at 50mJ on D4 (P1UV1C)**

### General morphology



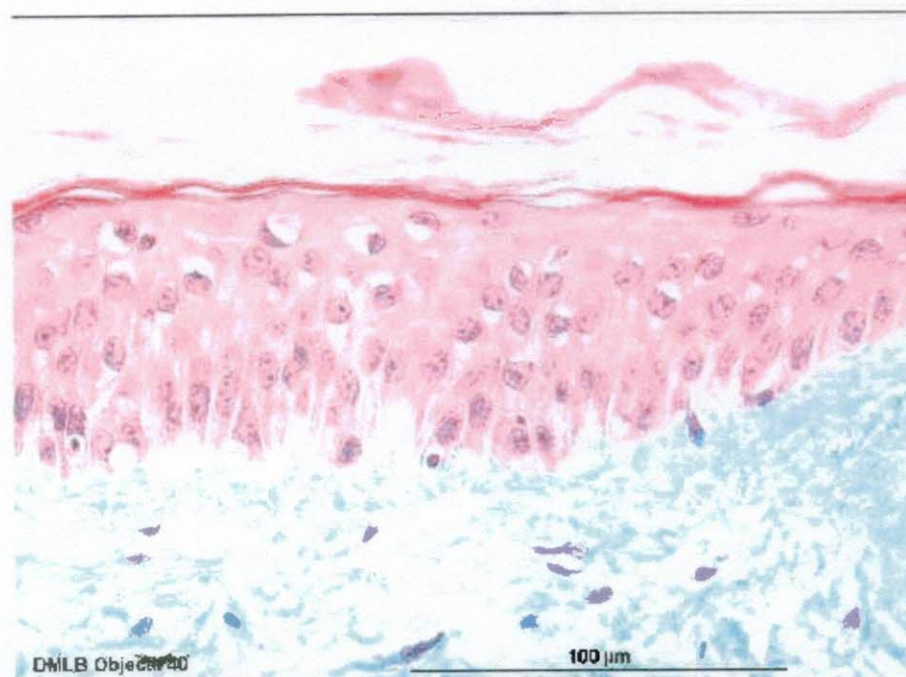
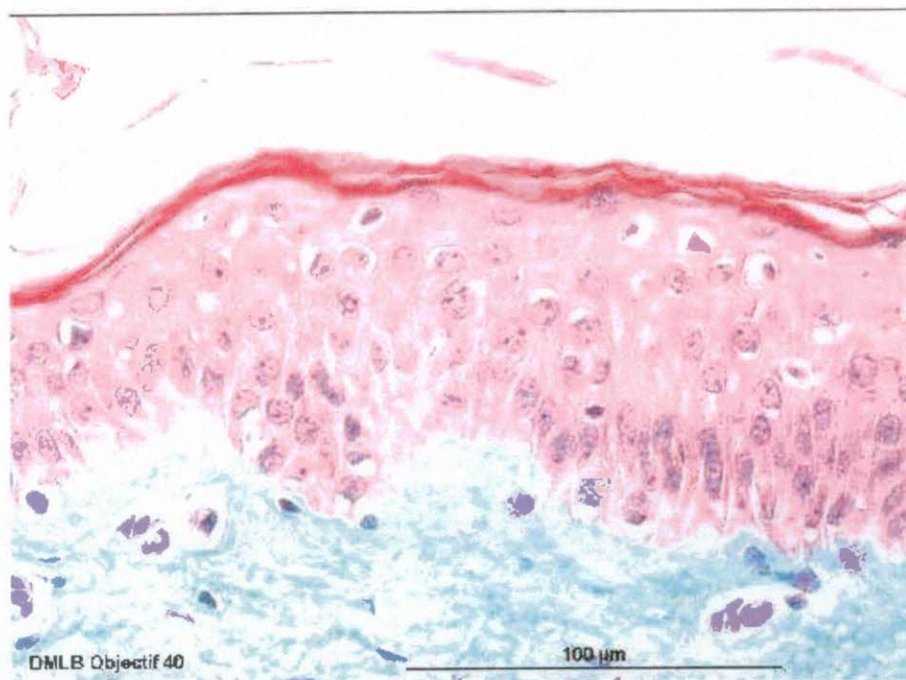
**Treated explant with product 2 and irradiated at 50mJ on D4 (P2UV1C)**

### General morphology



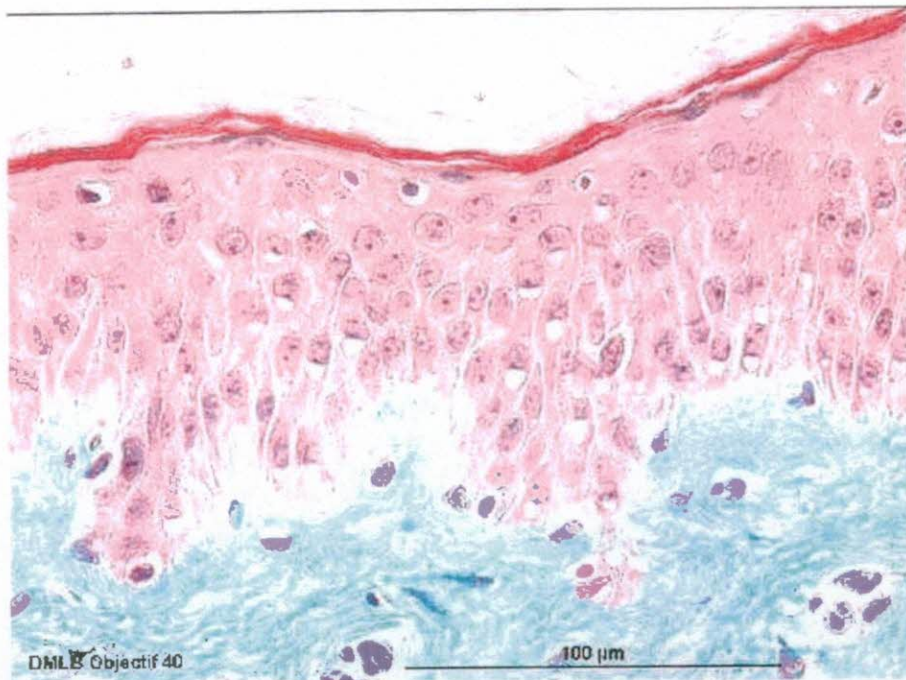
**Untreated explant and irradiated at 100mJ on D4 (TUV2)**

### General morphology



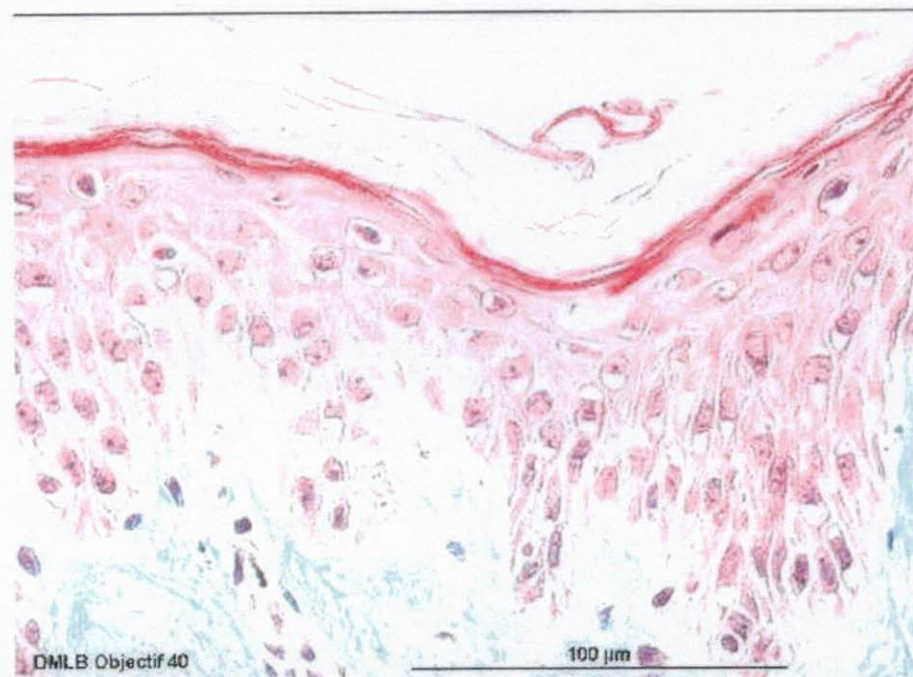
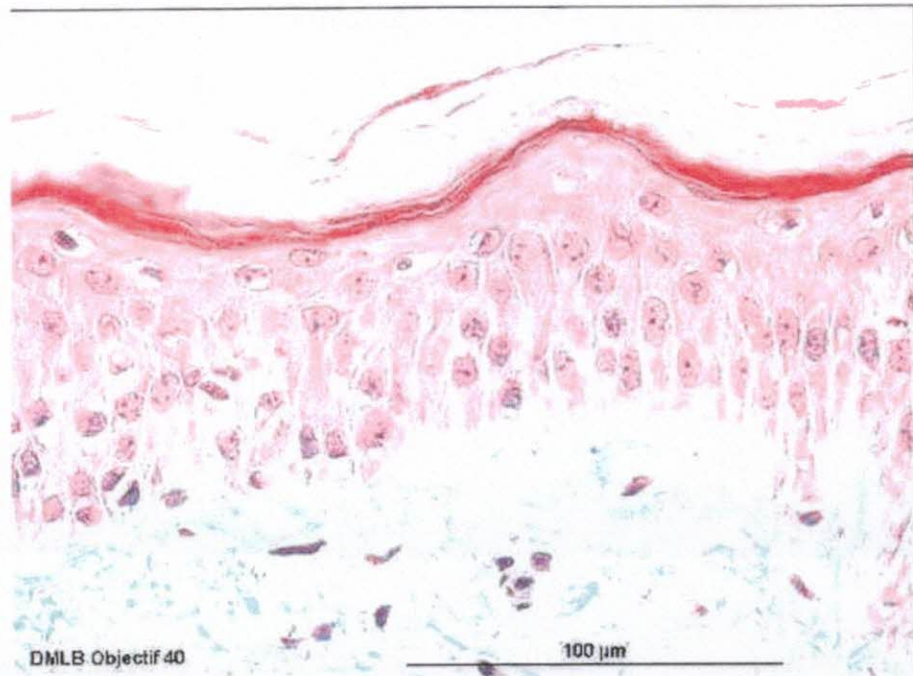
**Treated explant with product P1 and irradiated at 100mJ on D4 (P1UV2)**

### General morphology



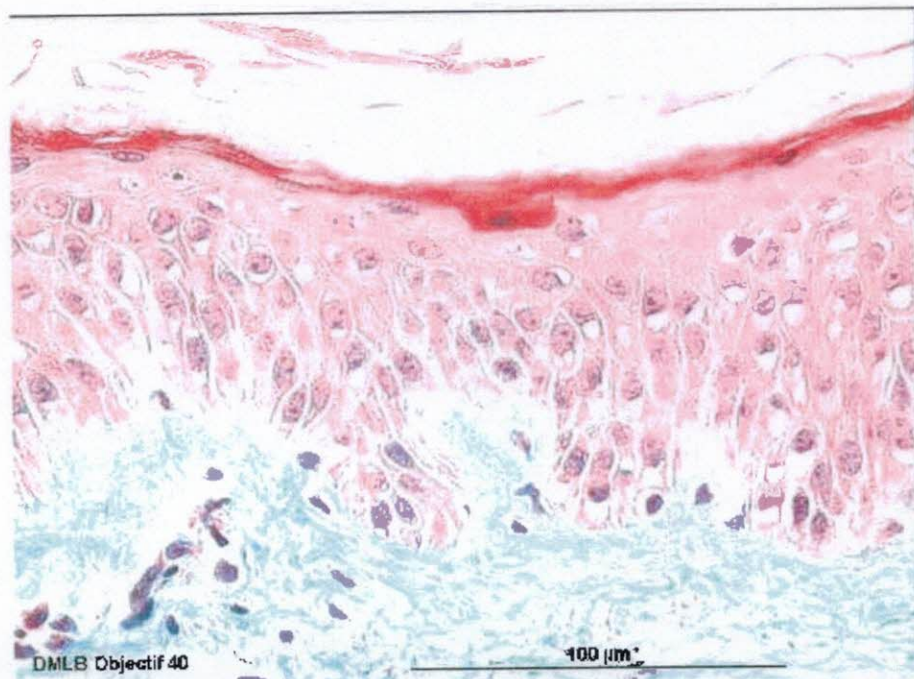
**Treated explant with product P2 and irradiated at 100mJ on D4 (P2UV2)**

### General morphology



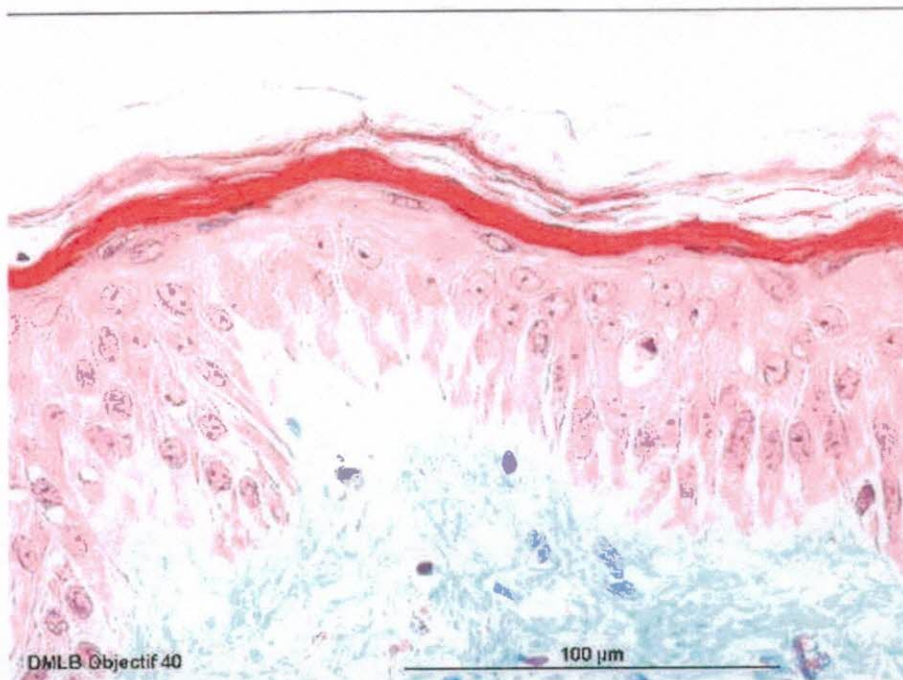
**Treated explant with product P1 and irradiated at 100mJ on D4 (P1UV2C)**

### General morphology



**Treated explant with product P2 and irradiated at 100mJ on D4 (P2UV2C)**

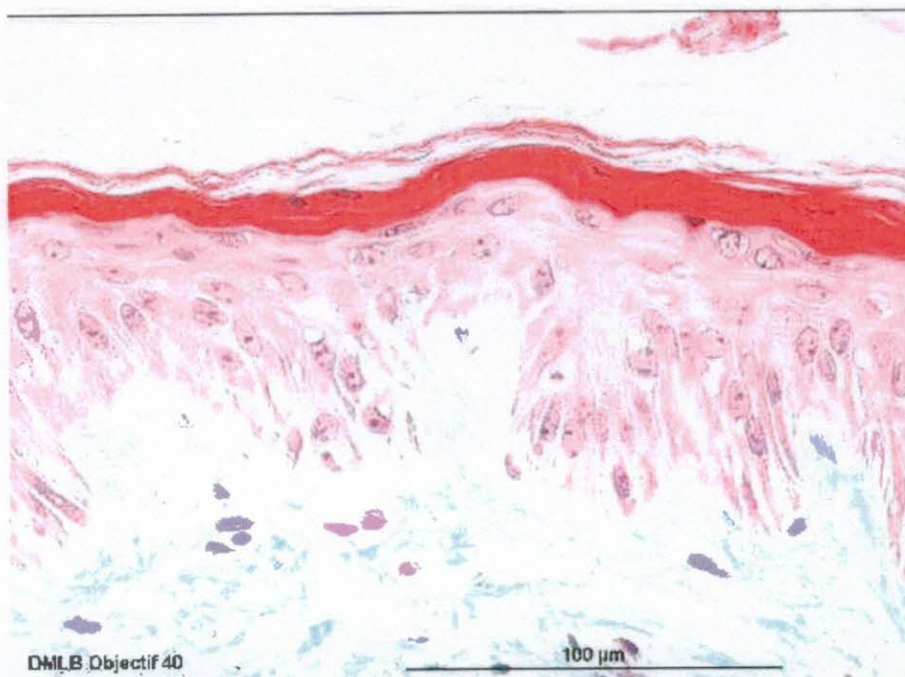
### General morphology



Untreated explant on D11 (T)

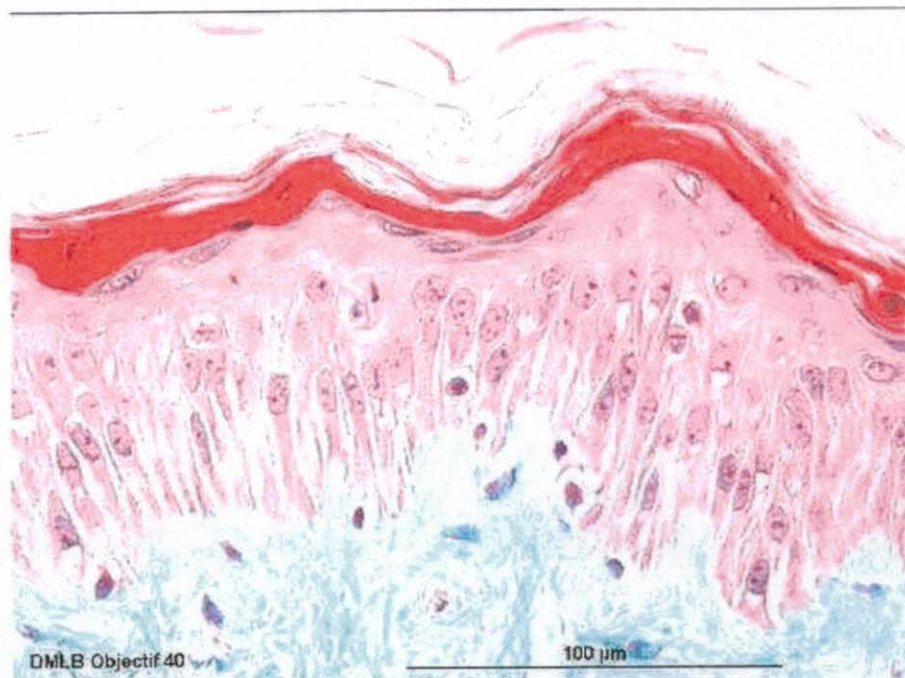


### General morphology

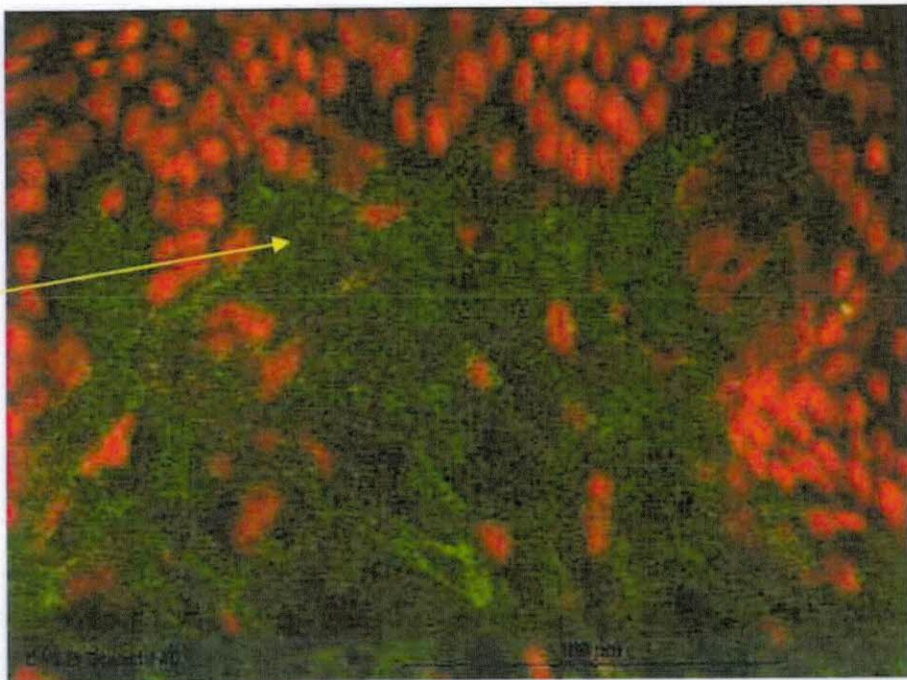
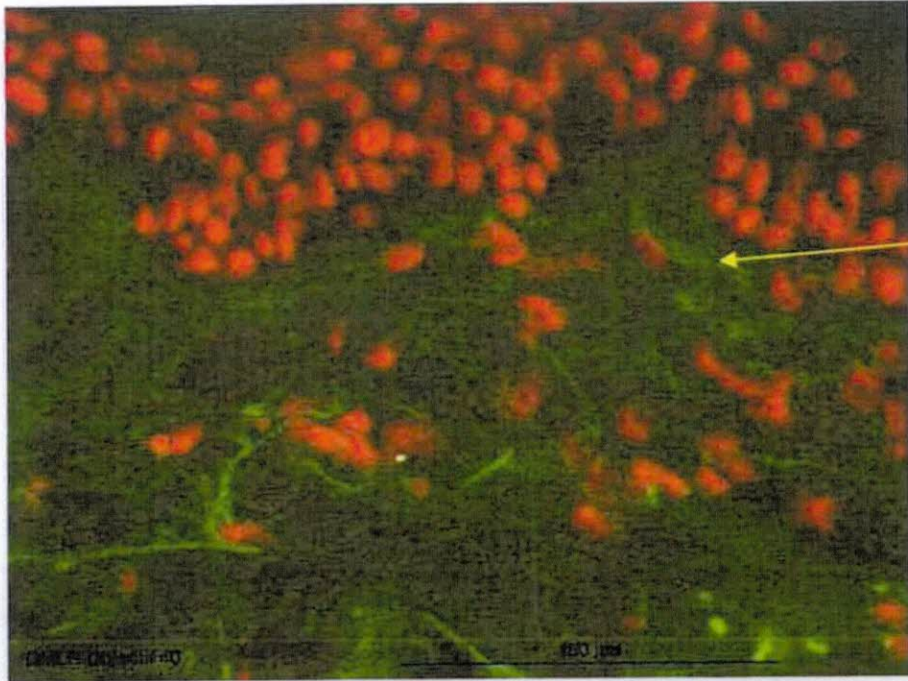


**Treated explant with product P1 on D11 (P1)**

### General morphology

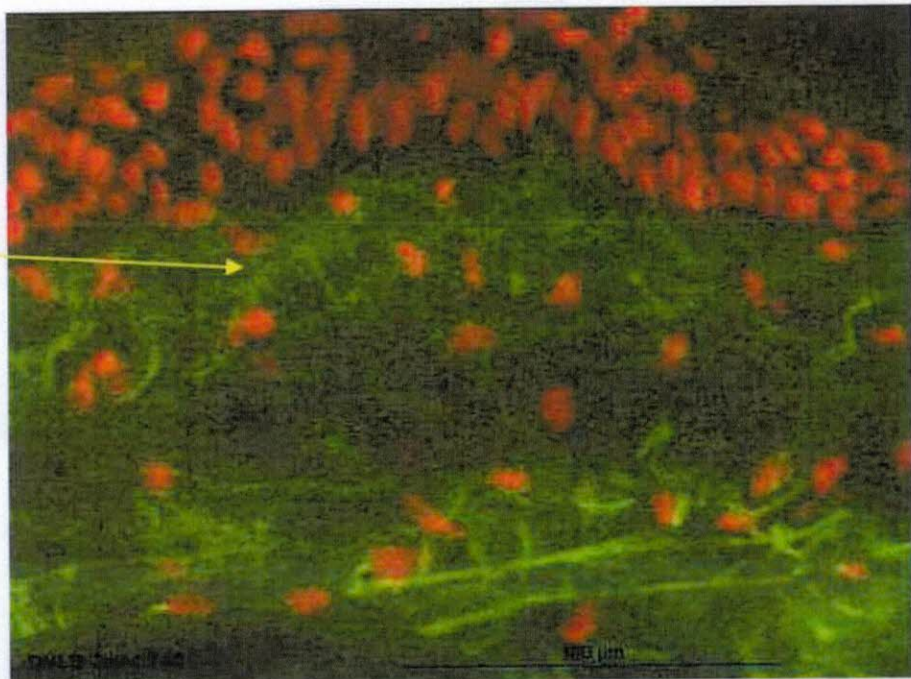
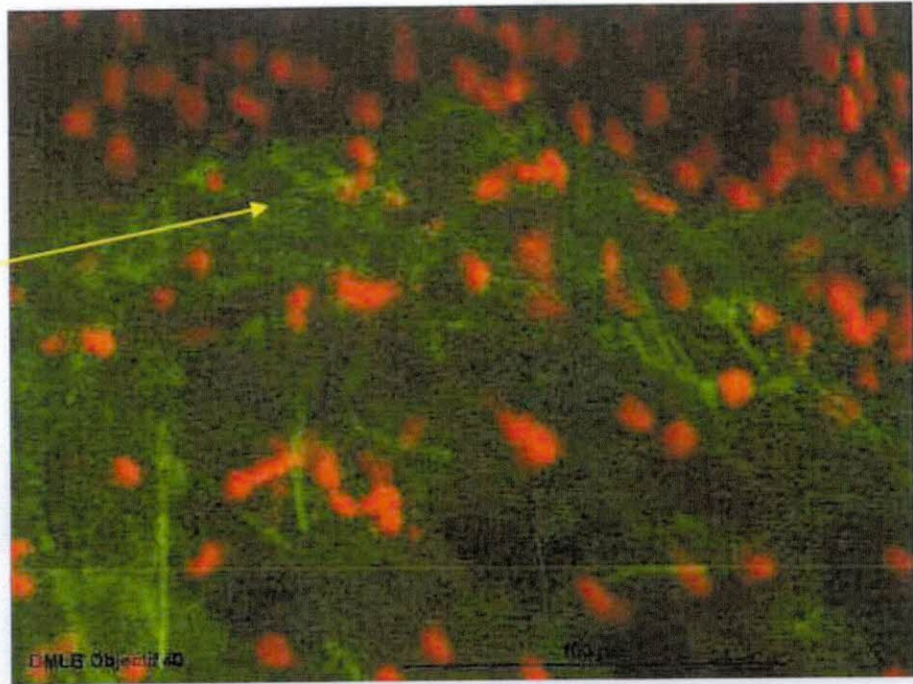


**Treated explant with product P2 on D11 (P2)**



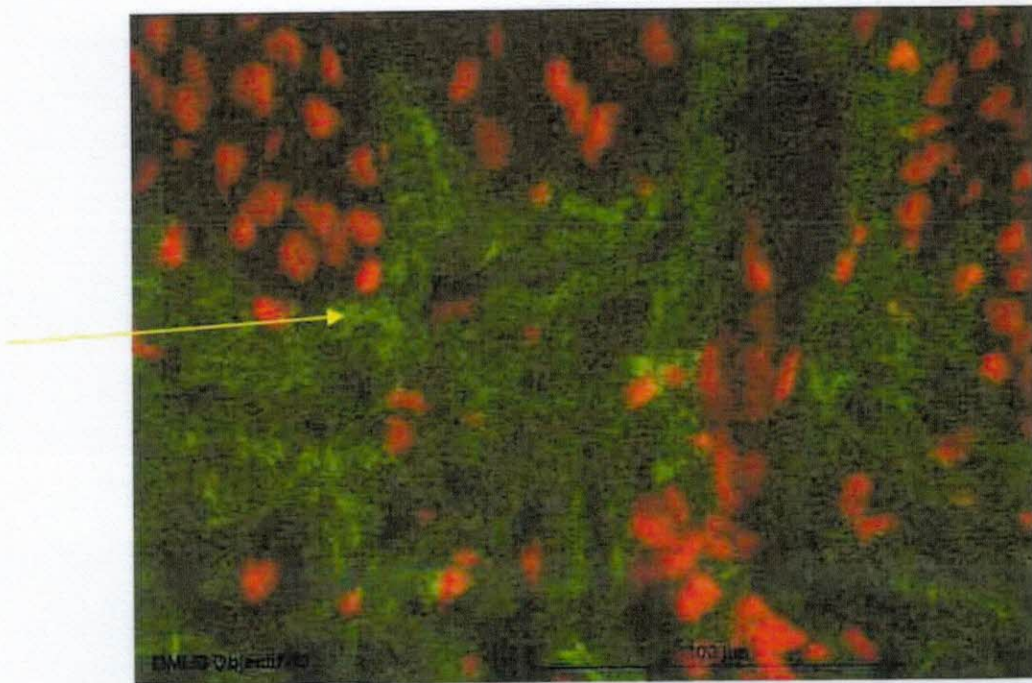
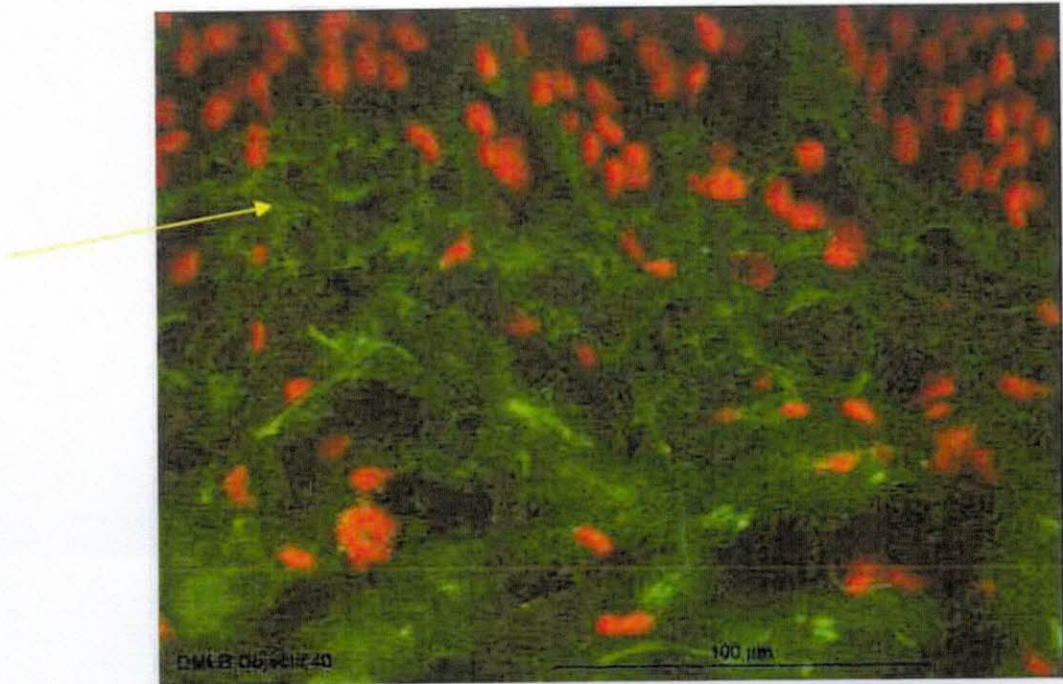
**Untreated explant on D0 (T)**

### Collagen I



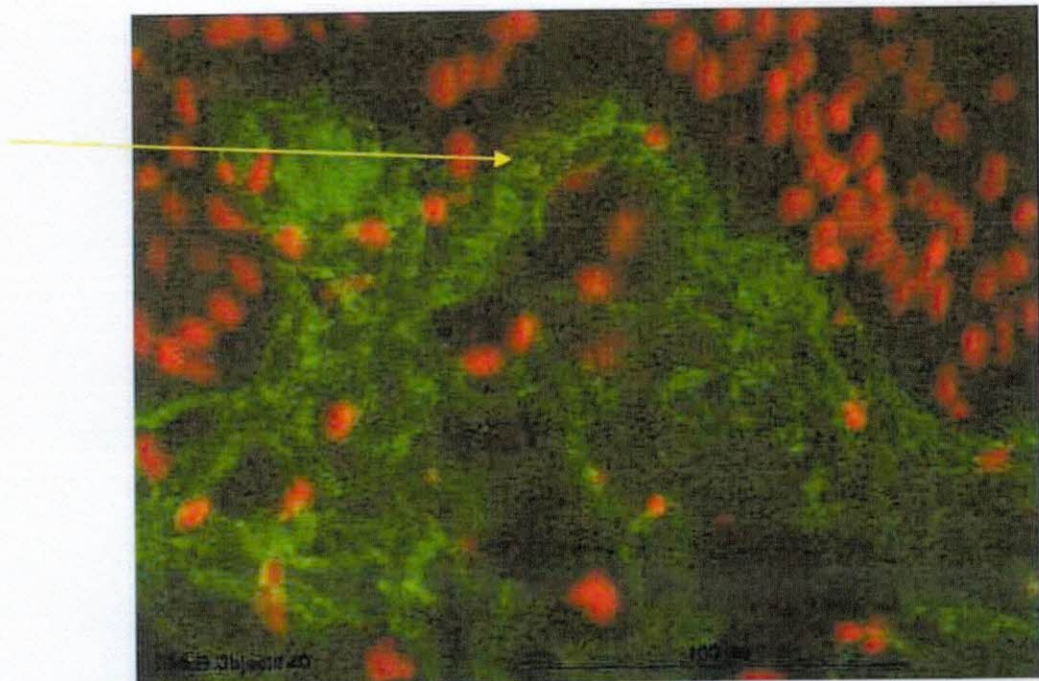
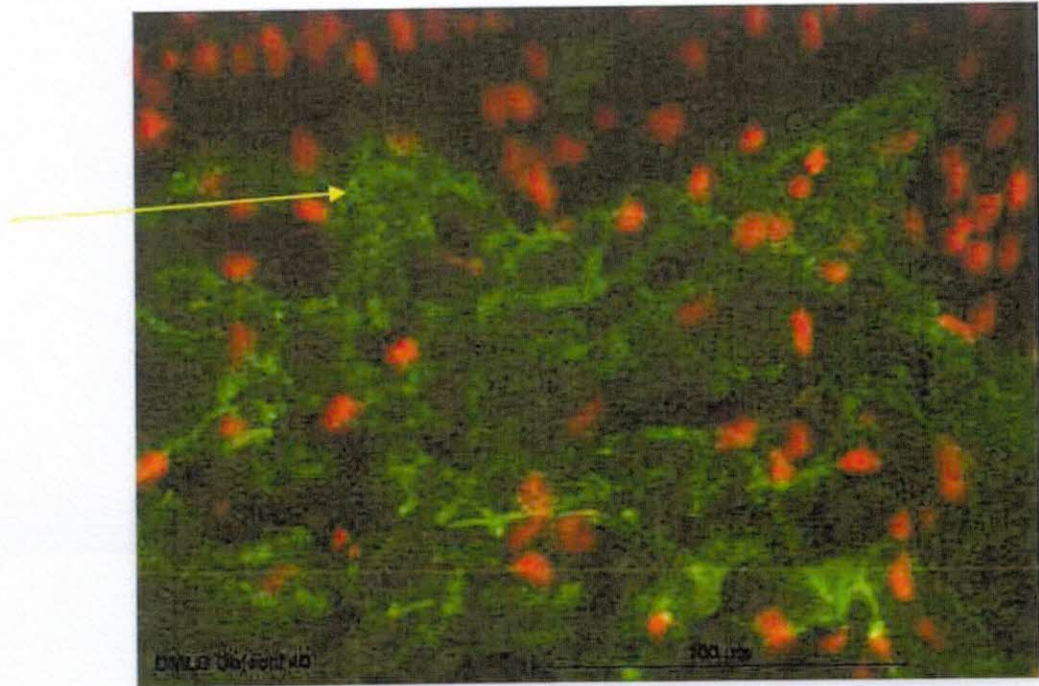
**Untreated explant on D11 (T)**

Collagen I



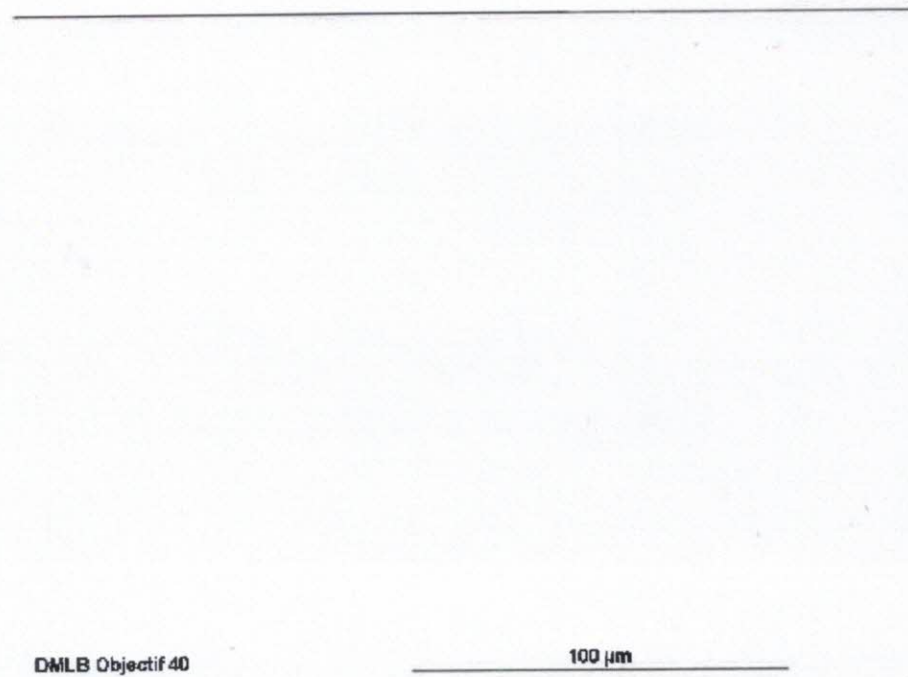
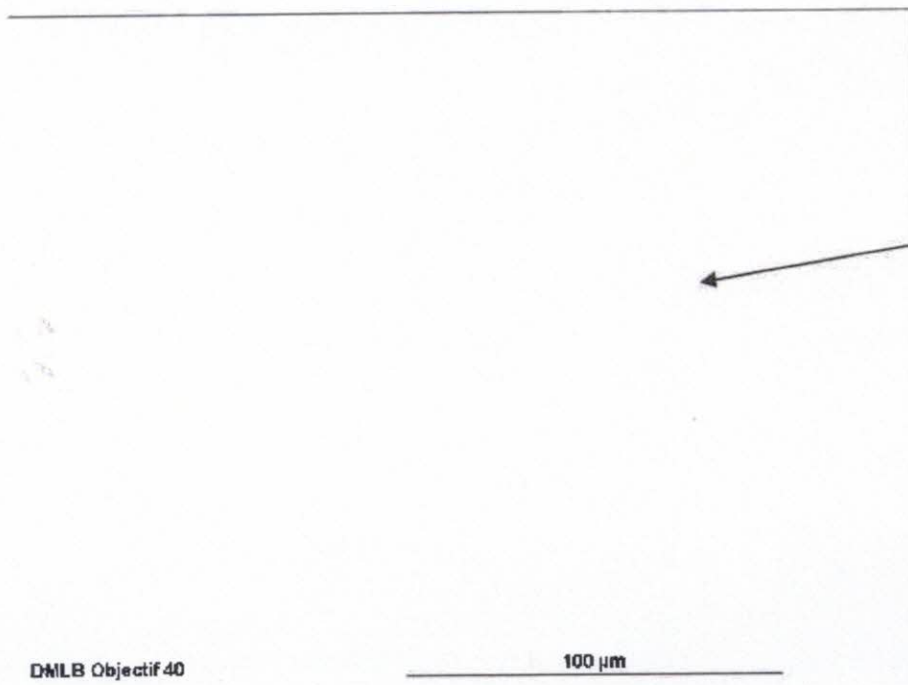
**Treated explant with product P1 on D11 (P1)**

### Collagen I



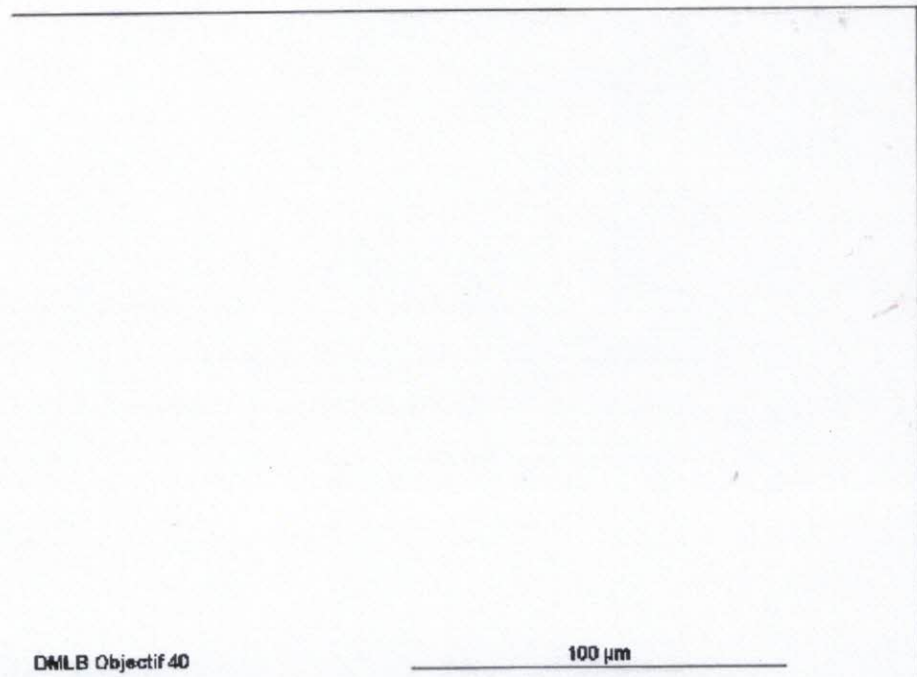
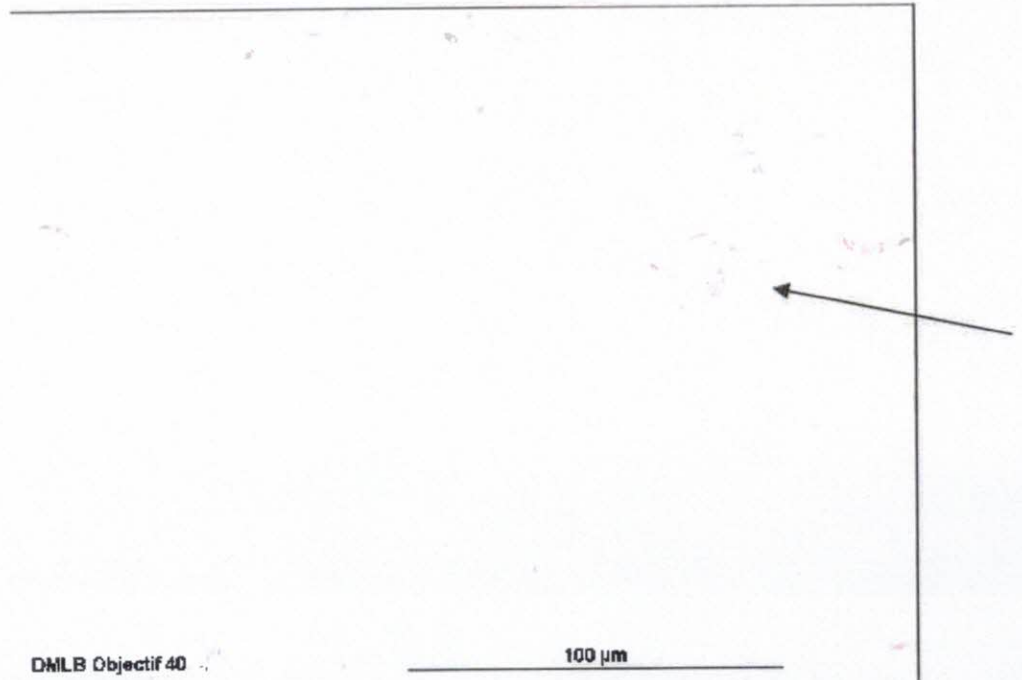
**Treated explant with product P2 on D11 (P2)**

### Collagen III



**Untreated explant on D0 (T)**

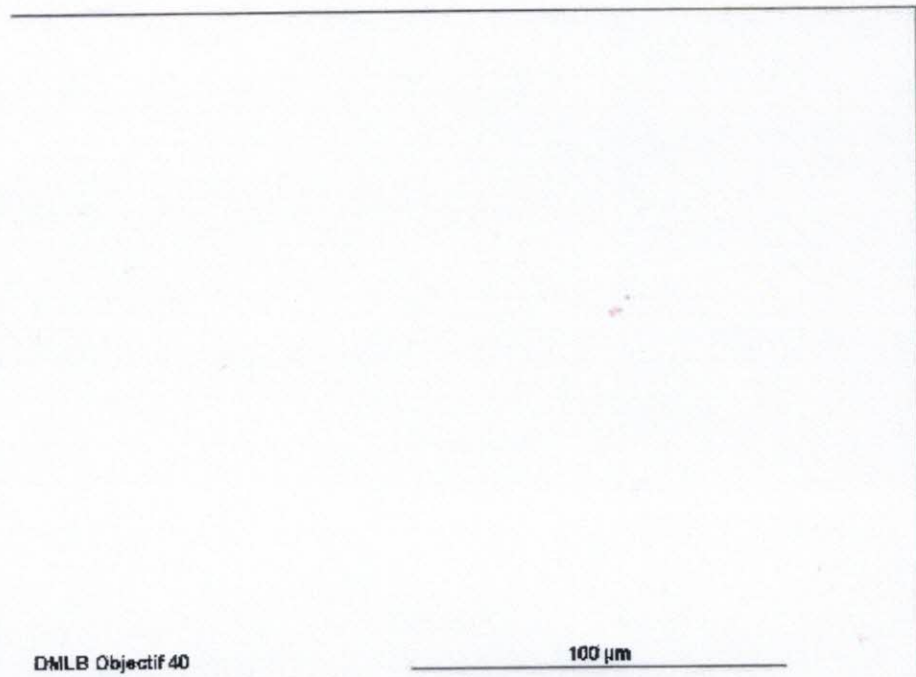
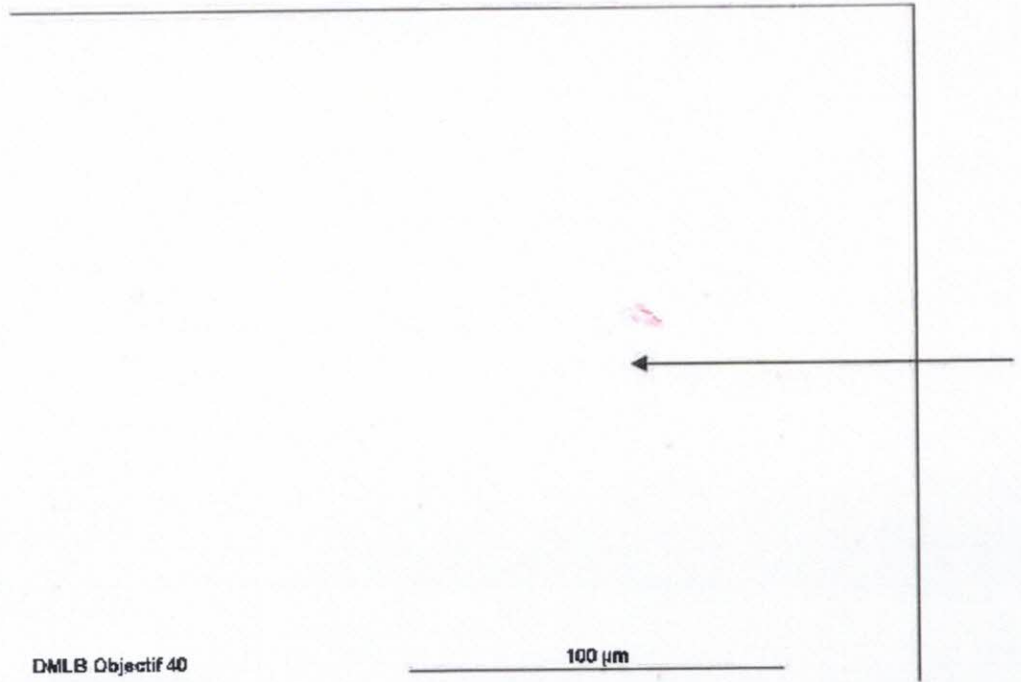
### Collagen III



Untreated explant on D11 (T)

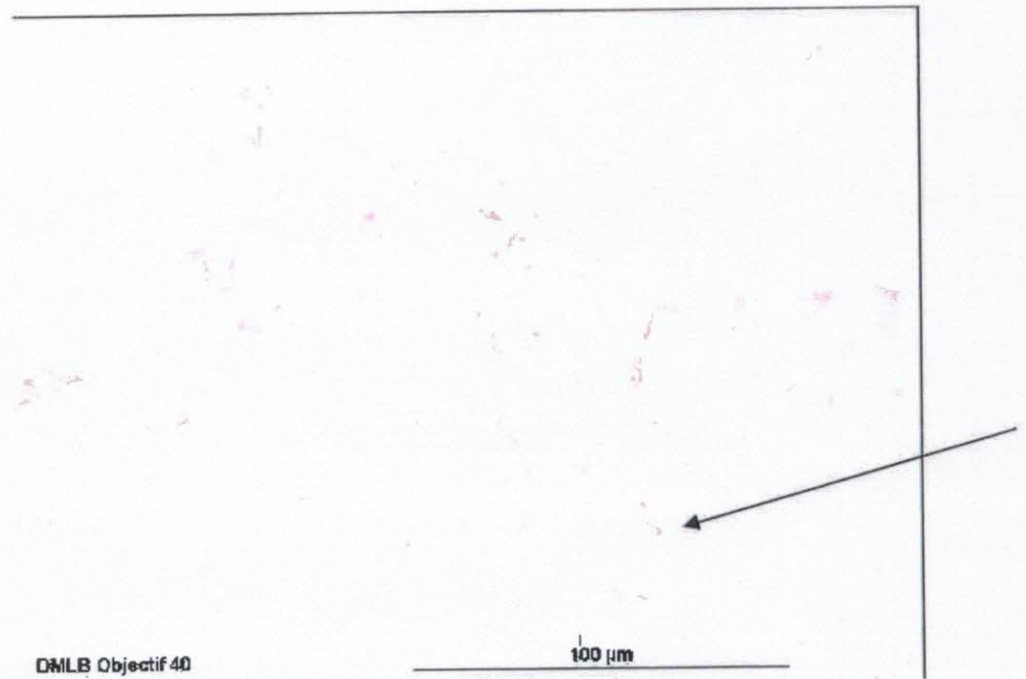
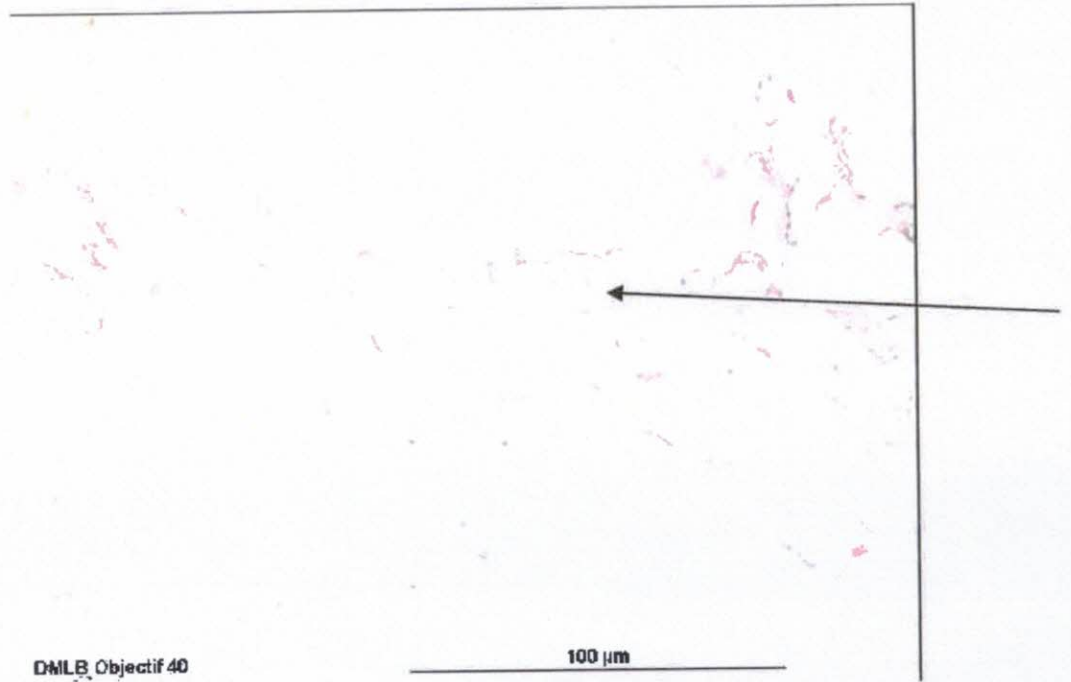


### Collagen III



**Treated explant with product P1 on D11 (P1)**

### Collagen III



**Treated explant with product P2 on D11 (P2)**