

**XXII EEBA**  
European Eye Bank Association  
Annual Meeting



## Techniques for *ex vivo* expansion of epithelial cells

Ricardo P. Casaroli-Marano

Instituto Clínic de Oftalmología – Hospital Clínic de Barcelona  
Departamento de Cirugía y Especialidades Quirúrgicas – UB  
Transplant Services Foundation (TSF) – Corporación Sanitaria Clínic  
Instituto de Investigaciones Biomédicas August Pi i Sunyer - IDIBAPS

 UNIVERSITAT DE BARCELONA


European Eye Bank Association – Annual Meeting 2010

**CLÍNIC**  
BARCELONA  
Hospital Universitari


**XXII EEBA**  
European Eye Bank Association  
Annual Meeting

Introduction

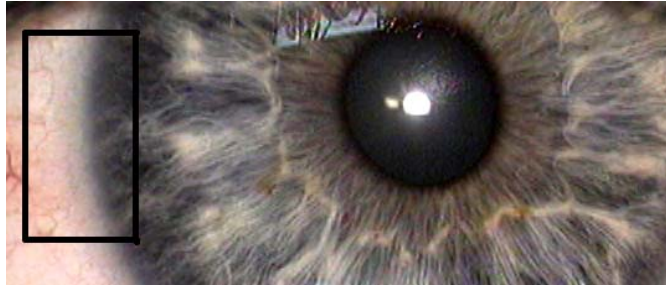
epithelium



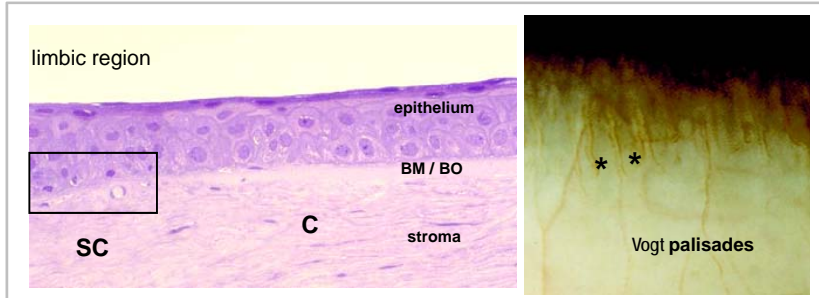
stroma



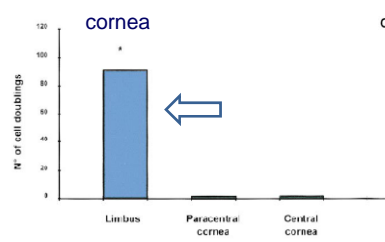
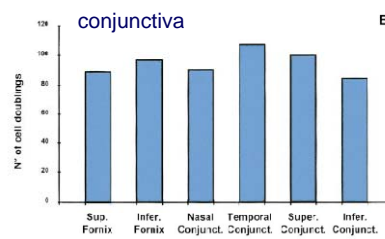
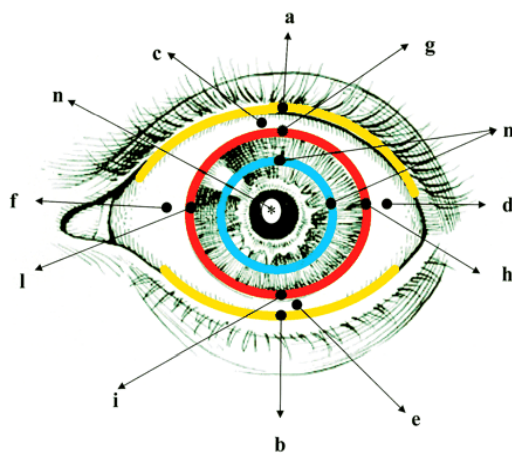
endothelium



- sclerocorneal limbus
- limbus
- limbic region

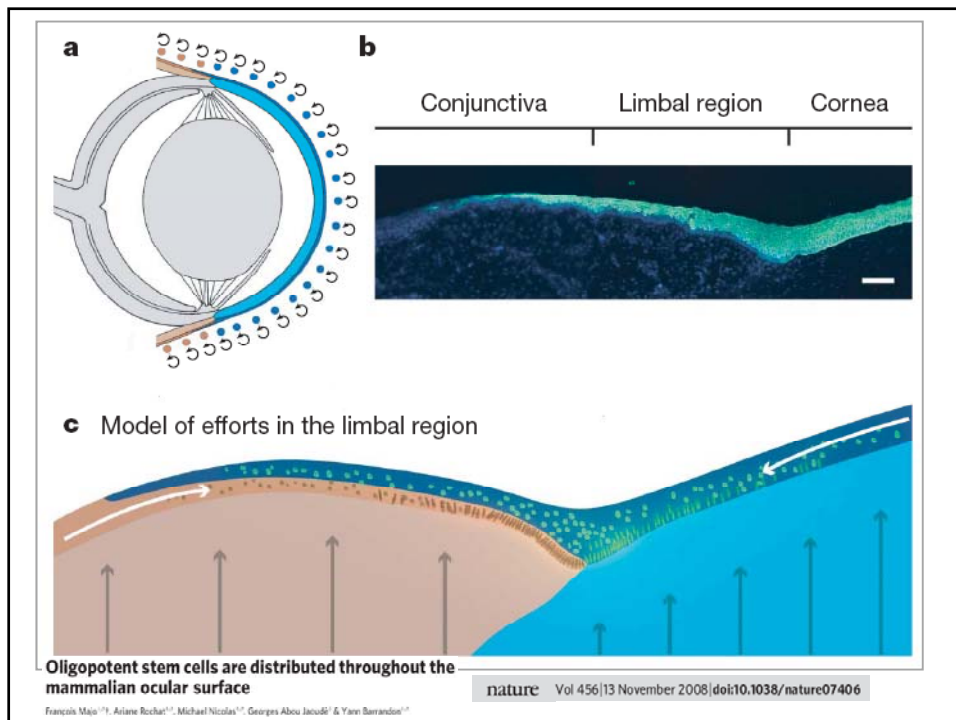
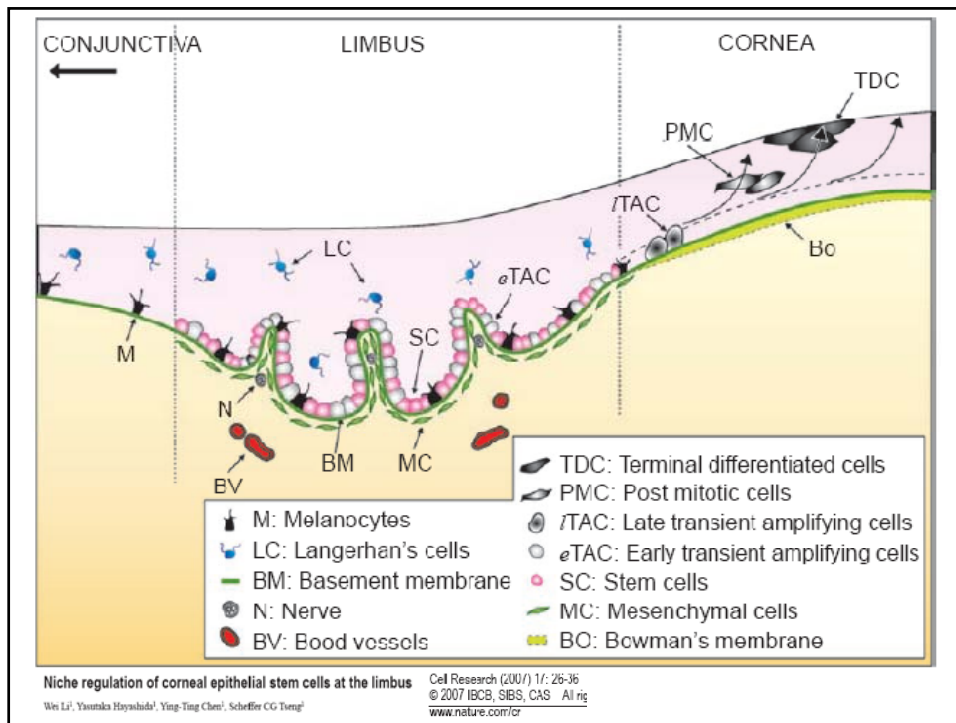


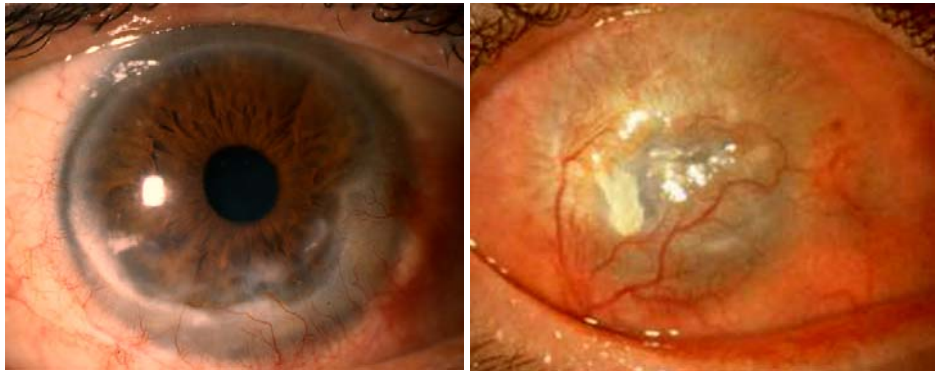
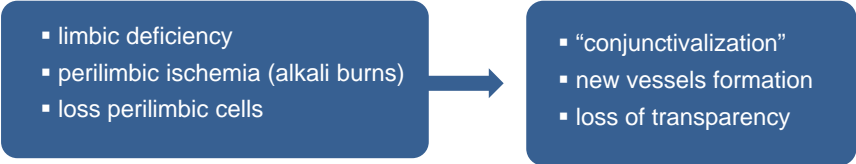
Ocular Surface: clonal characteristics and growth potential



Location and Clonal Analysis of Stem Cells and Their Differentiated Progeny in the Human Ocular Surface

Graziella Pellegrini,<sup>1</sup> Osvaldo Golubano,<sup>2</sup> Patrizia Paterna,<sup>3</sup> Alessandro Lambiase,<sup>1,4</sup> Stefano Borini,<sup>4</sup> Paolo Rama,<sup>2</sup> and Michele De Luca<sup>1</sup>  
The Journal of Cell Biology, Volume 145, Number 4, May 17, 1999 769-782





*Analysis of Indication for Treatment with Cultured Limbal or Oral Mucosal Epithelium*

Indication for Treatment with Cultured Limbal Epithelium	No. of Patients Treated	Percentage of Total
<b>Group I - Established LESC deficiency</b>		
Alkali/chemical or thermal burn	122	63.4
Stevens-Johnson syndrome	24	12.4
Ocular cicatricial pemphigoid/ Pseudopemphigoid	8	4.1
Retreatment of previously failed grafts	4	1.5
Ectodermal dysplasia or other congenital connective tissue disorder	3	1.5
Rosacea blepharoconjunctivitis/ phlyctenular disease	2	1.0
Vernal keratoconjunctivitis	2	1.0
Neurotrophic ulcer	1	0.5
Aniridia	1	0.5
Other	4	2.1
<b>Group II - Surgical excision limbal pathology:</b>		
Recurrent Pterygia	16	8.2
Pseudopterygium	4	2.1
CIN/premalignant/malignant conjunctival disease	3	1.5
<b>Total</b>	<b>194</b>	<b>100</b>

*Outcomes by Disease*

Treatment of LESC Deficiency with Cultured Limbal Epithelium	Primary Pathology	Treated (n)	Successful (n)	Success Rate (%)
Treatment of LESC Deficiency with Cultured Limbal Epithelium	Alkali/chemical or thermal burn (chronic phase)	122	94	77
	Stevens-Johnson	24	16	67
	Ocular cicatricial pemphigoid/ Pseudopemphigoid	8	6	75
	Retreatment of previously failed cultured epithelial grafts	4	4	100
	Ectodermal dysplasia or other congenital connective tissue disorder	3	2	67
	Rosacea blepharoconjunctivitis/ phlyctenular disease	2	2	100
	Vernal keratoconjunctivitis	2	2	100
	Neurotrophic ulcer	1	1	100
	Aniridia	1	1	100

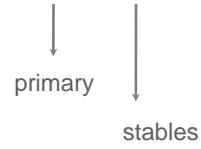
**Transplantation of Ex Vivo Cultured Limbal Epithelial Stem Cells: A Review of Techniques and Clinical Results**

Alex J. Shorn, MSc, MRCOphth,<sup>1,2</sup> Genevieve A. Secker, BSc,<sup>1</sup> Maria B. Nistor, PhD,<sup>3</sup> G. Anil Limb, PhD,<sup>3</sup> Prong T. Khan, PhD, FRCOphth,<sup>1,2</sup> Stephen J. Tuft, MD, FRCOphth,<sup>1,2</sup> and Julie T. Daniels, PhD<sup>1</sup>

*Methods for cell and tissue cultures*



**1. Cell cultures**

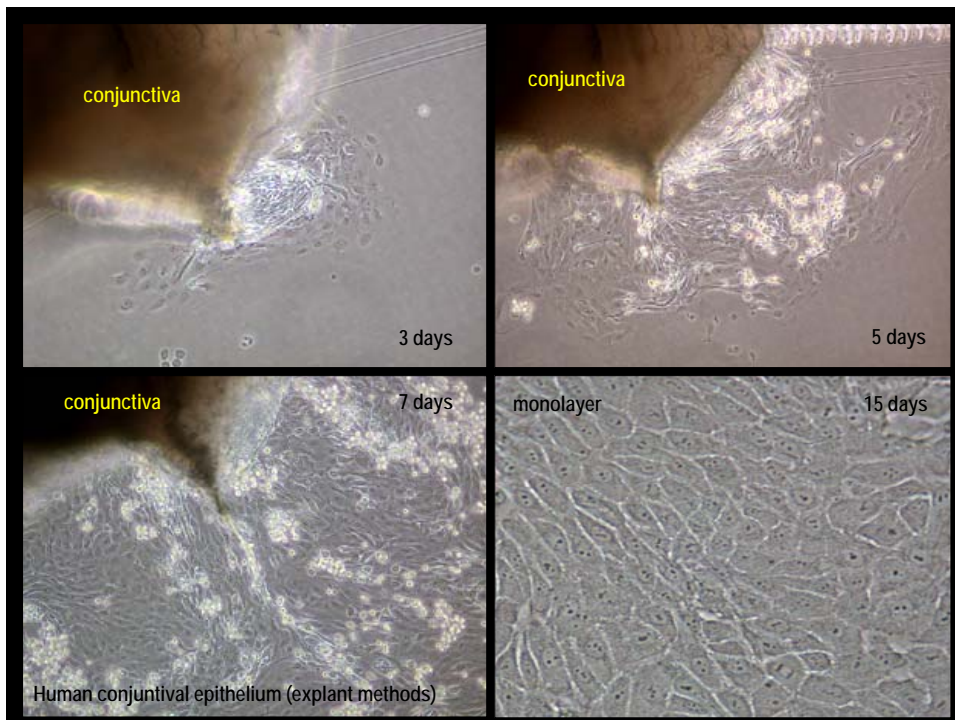


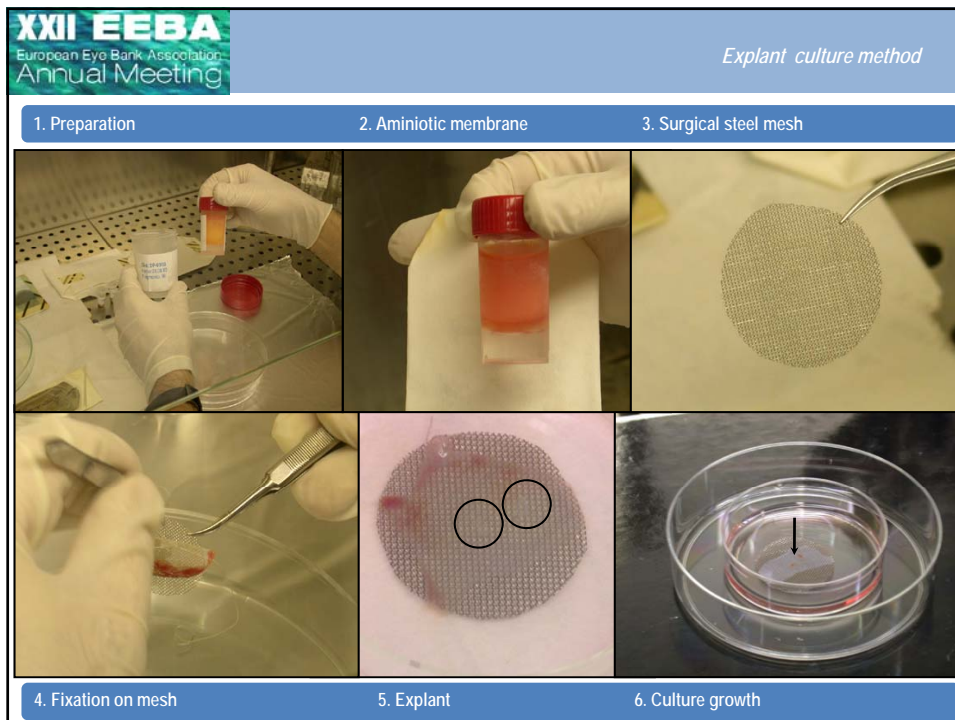
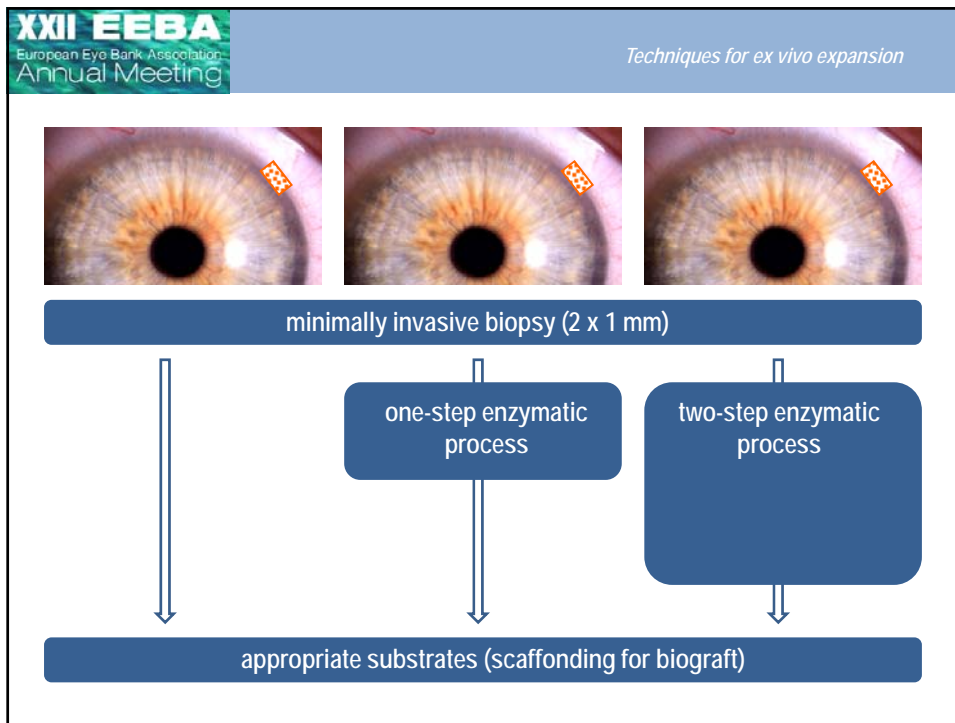
**2. Explant cultures**

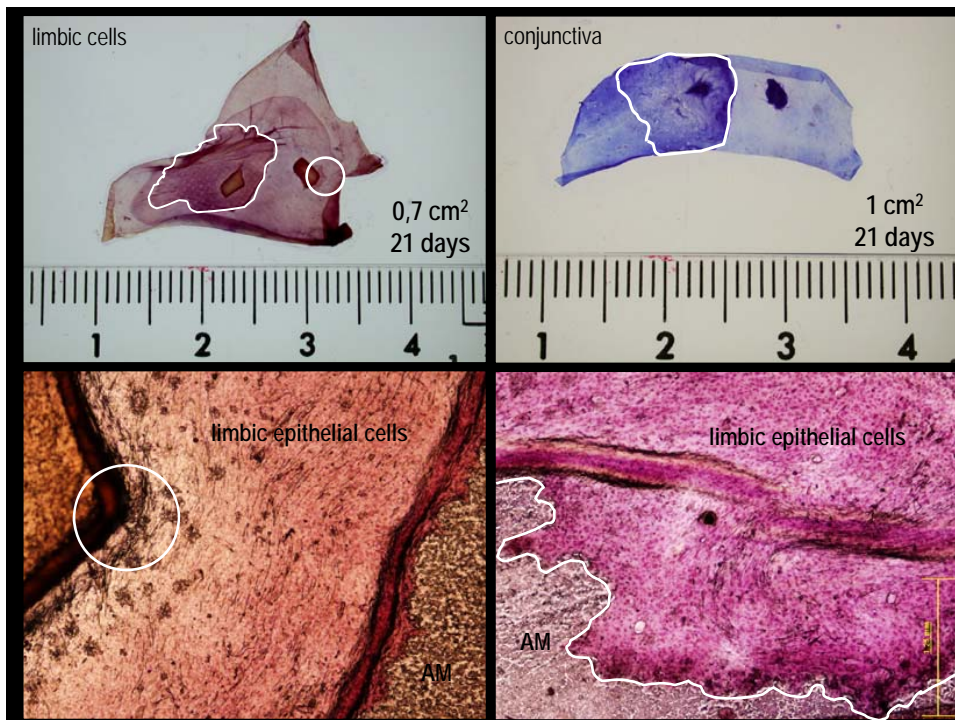
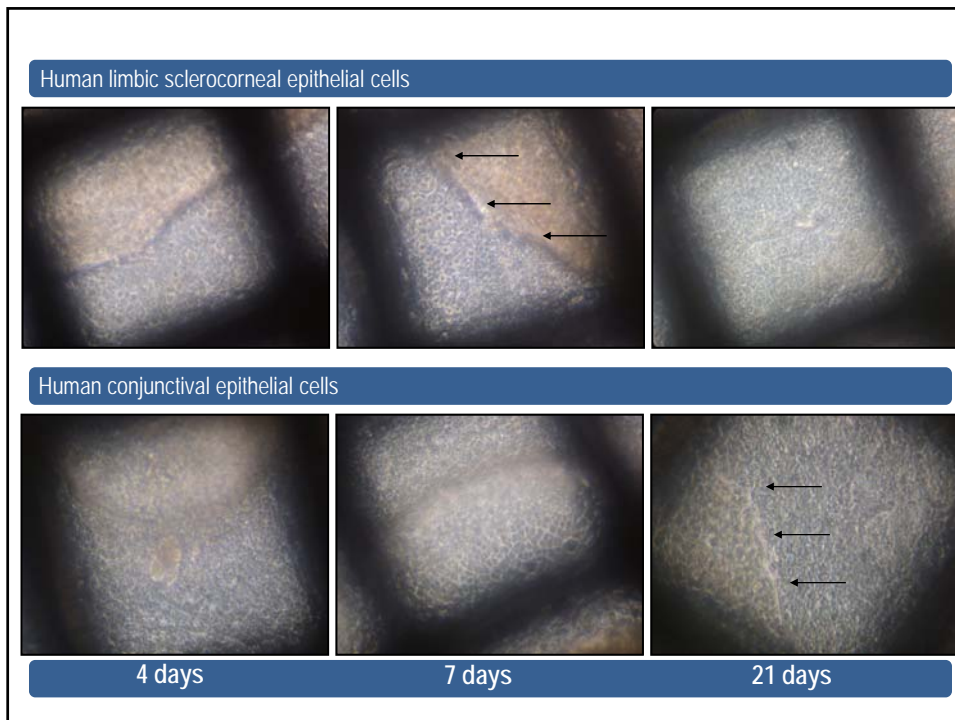
for tissues

**3. Organotypic cultures**

for complex structures







**XXI EEBA**  
European Eye Bank Association  
Annual Meeting

*Explant culture method*

Well of tissue culture plate  
Plastic culture insert  
Amniotic membrane attached to bottom of culture insert  
Limbal epithelial cell outgrowth from limbal biopsy  
Limbal biopsy attached to basement membrane side of amniotic membrane

Well of tissue culture plate  
Plastic culture insert  
Amniotic membrane attached to bottom of culture insert and suspended above feeder layer  
Limbal epithelial cell outgrowth from limbal biopsy  
Growth arrested 3T3 fibroblast feeder layer  
Limbal biopsy attached to basement membrane side of amniotic membrane

Transplantation of Ex Vivo Cultured Limbal Epithelial Stem Cells: A Review of Techniques and Clinical Results  
Alex J. Shortt, MSc, MRCOphth,<sup>1,2</sup> Graziella A. Sedore, BSc,<sup>2</sup> Maria D. Notara, PhD,<sup>2</sup> G. Astrid Limb, PhD,<sup>3</sup> Peng T. Khaw, PhD, FRCOphth,<sup>1,2</sup> Stephen J. Tuohi, MD, FRCOphth,<sup>1,2</sup> and Julie T. Daniels, PhD<sup>2</sup>

SURVEY OF OPHTHALMOLOGY VOLUME 52 • NUMBER 2 • SEPTEMBER-OCTOBER 2007

*Transwell device system*

**XXI EEBA**  
European Eye Bank Association  
Annual Meeting

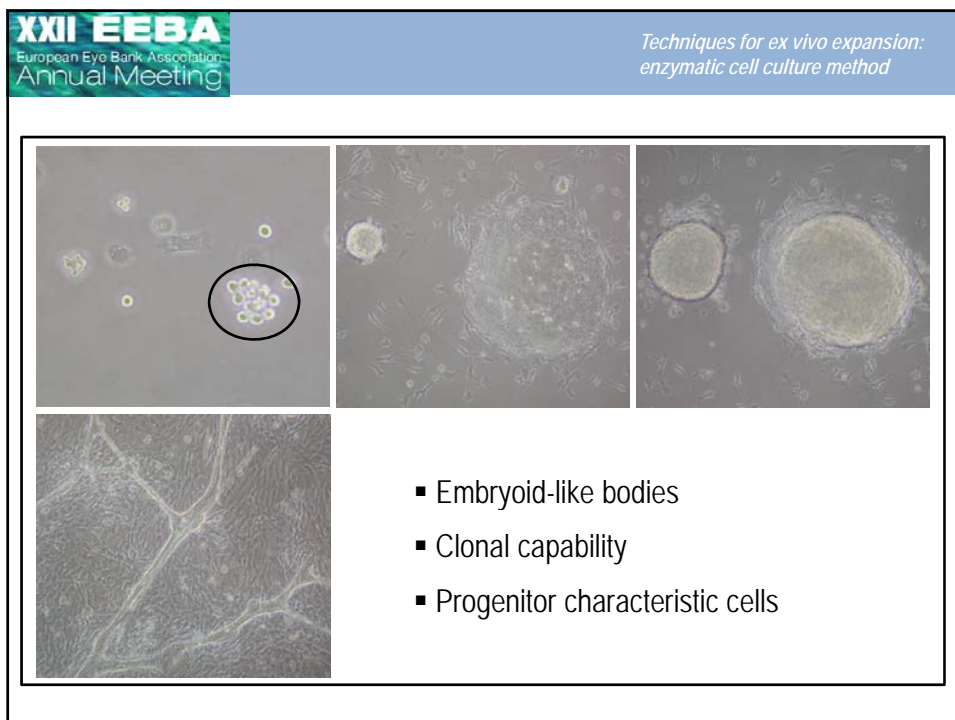
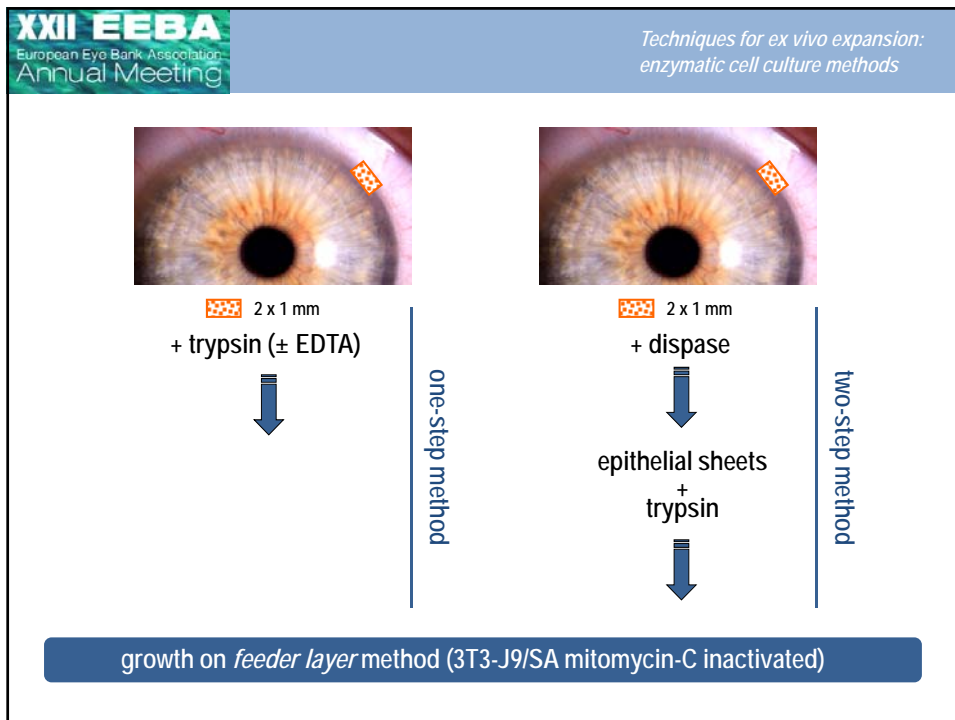
*Techniques for ex vivo expansion*

minimally invasive biopsy (2 x 1 mm)

one-step enzymatic process

two-step enzymatic process

appropriate substrates (scaffolding for biograft)



Explant culture method	
ADVANTAGES	DRAWBACKS
<ul style="list-style-type: none"> <li>✓ Simple methodology</li> <li>✓ Optimal cost-effectiveness correlation</li> <li>✓ Simple infrastructure</li> </ul>	<ul style="list-style-type: none"> <li>✓ Culture "contamination" (fibroblasts, antigen presenting cells, etc.)</li> </ul>

Enzymatic cell culture method	
ADVANTAGES	DRAWBACKS
<ul style="list-style-type: none"> <li>✓ Selected cell population</li> <li>✓ Optimal cost-effectiveness correlation</li> <li>✓ High efficiency</li> </ul>	<ul style="list-style-type: none"> <li>✓ Several steps</li> <li>✓ Additional tissue handling</li> <li>✓ Methodologically complex</li> <li>✓ Complex infrastructure</li> <li>✓ Learning curve process</li> </ul>



Appropriate conditions to handling cell and tissues for human transplant purpose

### Requirements



#### 1. Culture media

adequate nutritional intake

#### 2. Culture conditions

proper handling      optimize cultures

#### 3. Culture protection

product      operator      environment



Laminar flow

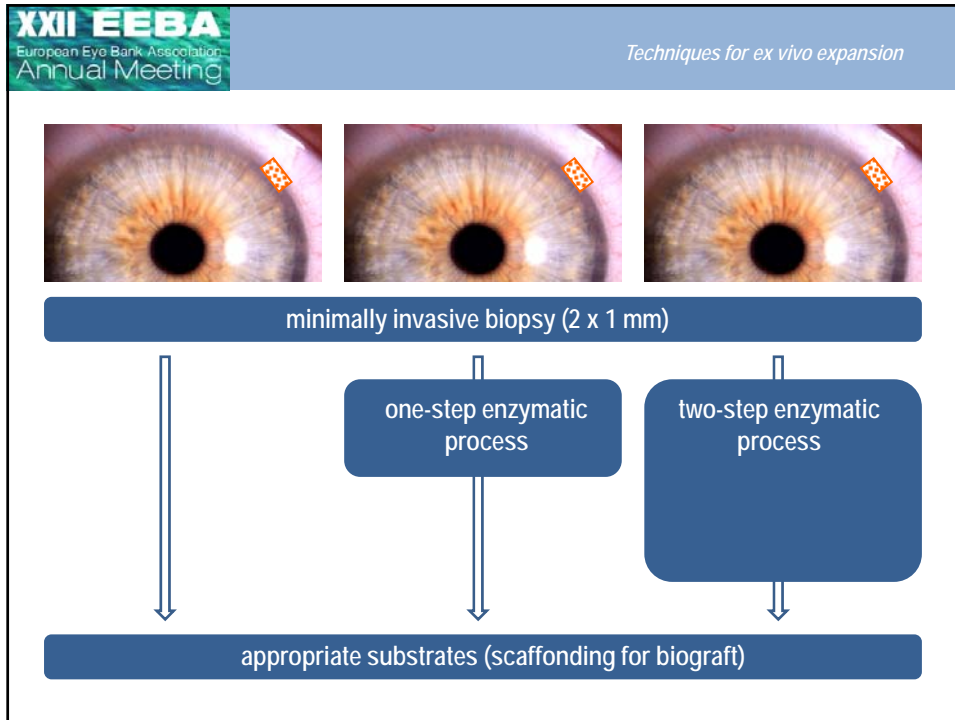


Culture media and supplements



Sterile handling conditions





**XXI EEBA**  
European Eye Bank Association  
Annual Meeting

*Techniques for ex vivo expansion: substrates*

appropriate substrates (scaffonding for biograft)

Aminiotic membrane	Fibrin gel	Contact lenses	PRP / PRGF
			

- Collagen shields
- Paraffin gauze
- Polymer membranes (biocompatibles)