Técnicas de manipulación/preservación para tejidos blandos: Descontaminación, **descelularización**, liofilización

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Técnicas de manipulación/preservación para tejidos blandos: Descontaminación, **descelularización**, liofilización

- introducción
- métodos y verificación
- aplicaciones
- preservación

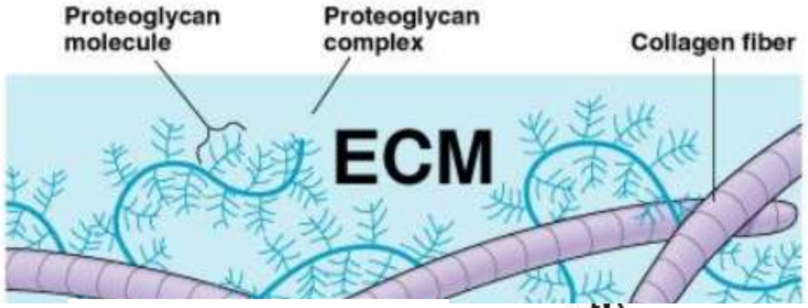
Descelularización. Introducción

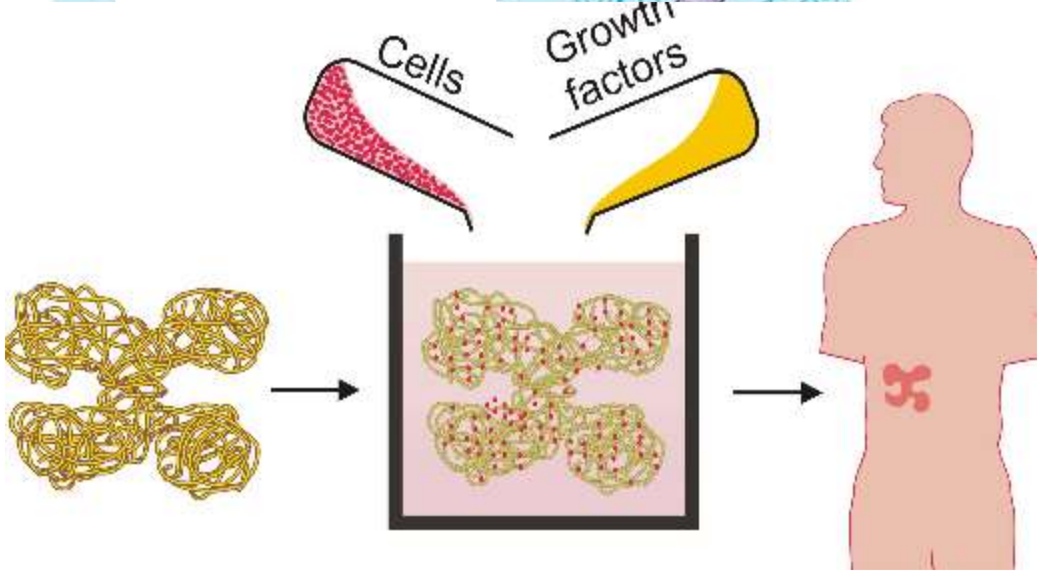
Qué es:
_eliminación del componente celular
_mantenimiento de la matriz extracelular

Tejido para uso terapéutico:
_seguridad
_eficacia
_mantenimiento de las características del tejido

Aplicaciones:
_Ingeniería de tejidos

_Regeneración/reparación/sustitución



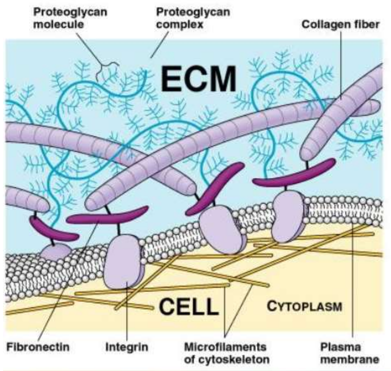


Descelularización. Métodos

Tratamientos mecánicos:

Temperatura
Agitación
Electroporación
Sonicación

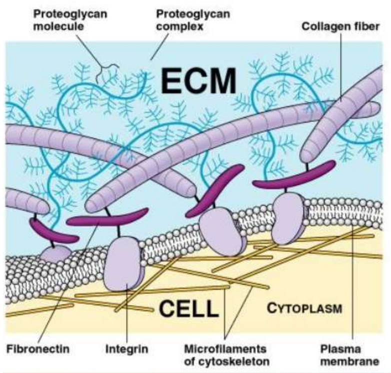
ciclos congelación / descongelación

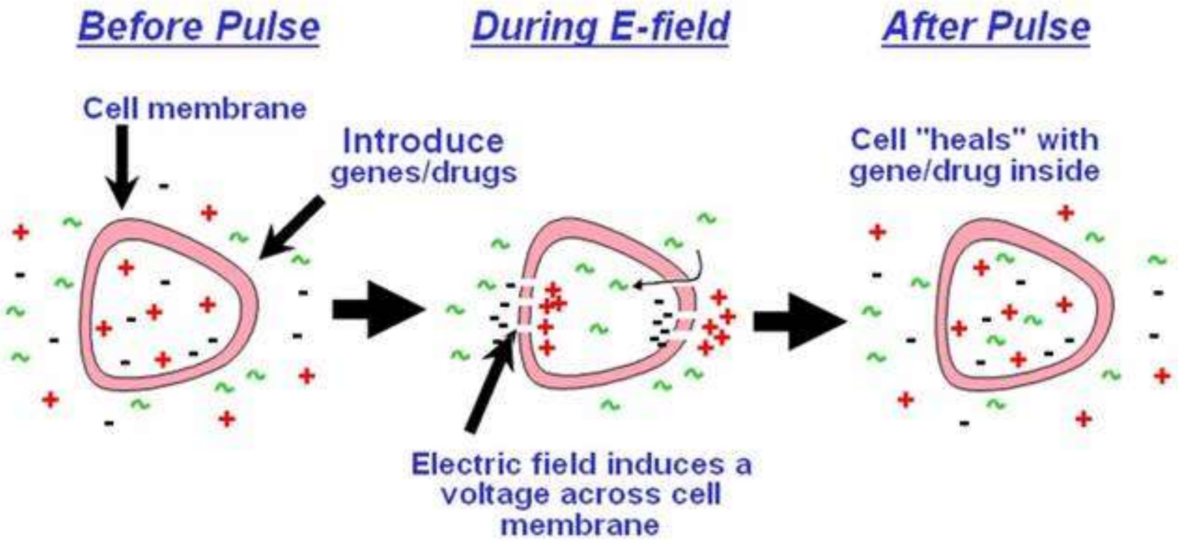


Descelularización. Métodos

Tratamientos mecánicos:

Temperatura — ciclos congelación / descongelación
Agitación
Electroporación — campo eléctrico
Sonicación

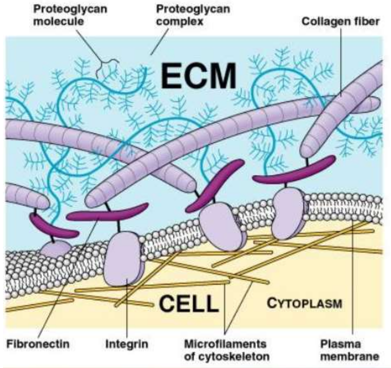


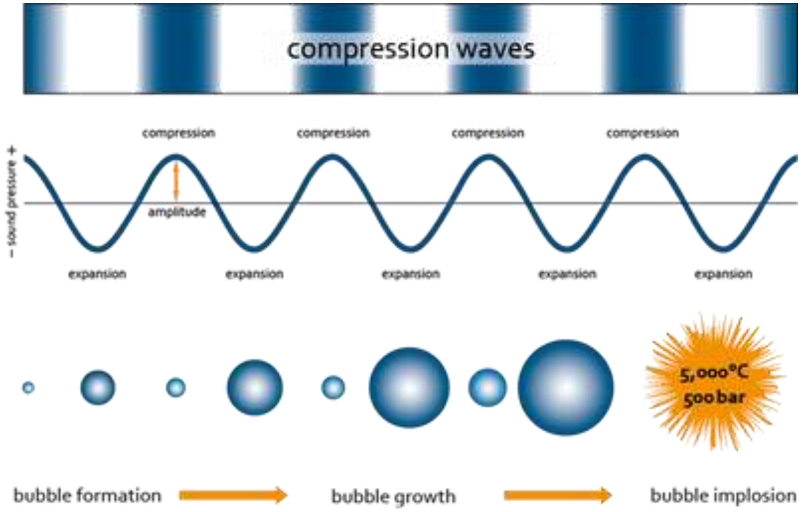


Descelularización. Métodos

Tratamientos mecánicos:

Temperatura	ciclos congelación / descongelación
Agitación	
Electroporación	campo eléctrico
Sonicación	ultrasonidos





Descelularización. Métodos

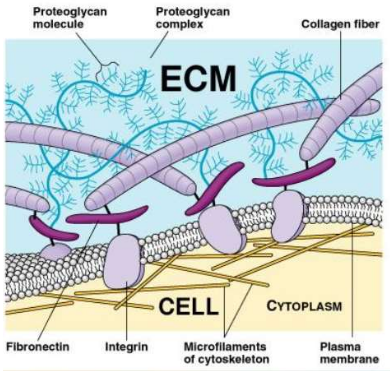
Tratamientos químicos:

Soluciones hipo e hiper tónicas
_H_2O destilada, tris buffer, EDTA;
_cloruro sódico, sacarosa, cloruro de potasio

Ácidos y bases
_peracético, desoxicólico, acético;
_hidróxido de amonio, sódico, cálcico

Disolventes orgánicos
_etanol, acetona

Detergentes



Descelularización. Métodos

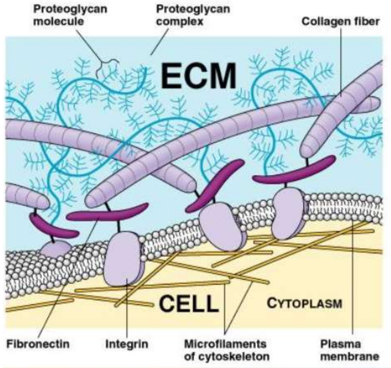
Tratamientos químicos:

Soluciones hipo e hiper tónicas

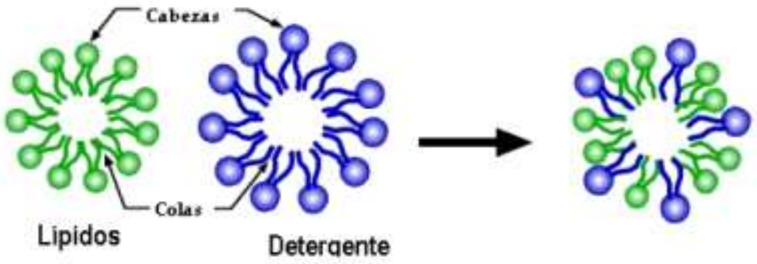
Ácidos y bases

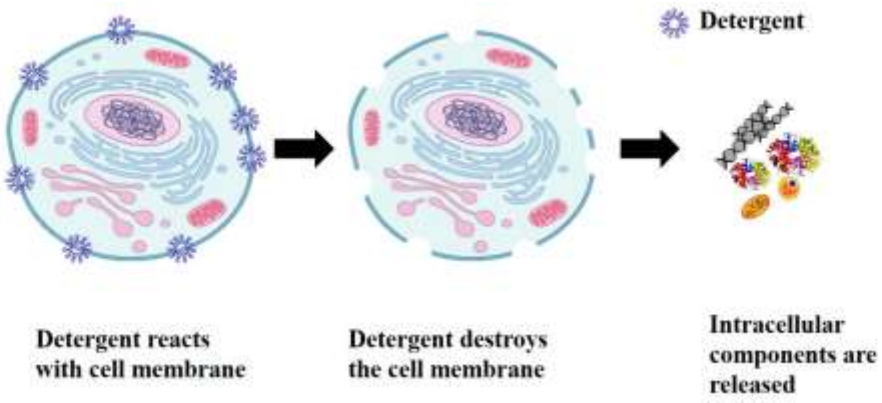
Disolventes orgánicos

Detergentes



Surfactante: **Sur**face **act**ive **agent**





Descelularización. Métodos

Tratamientos químicos:

Soluciones hipo e hiper tónicas

Ácidos y bases

Disolventes orgánicos

Detergentes: iónicos
no iónicos
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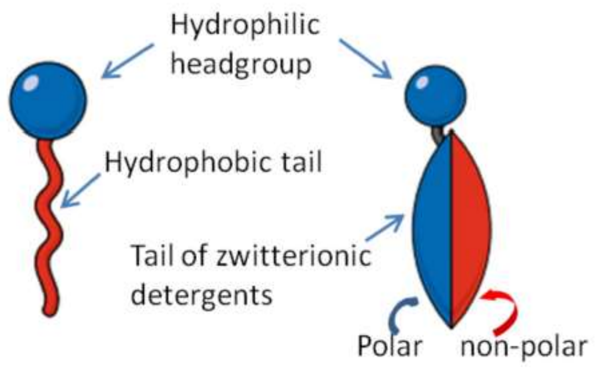
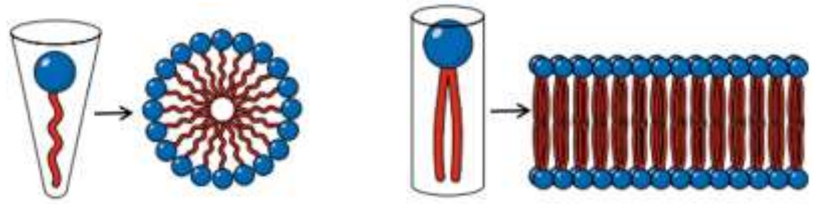
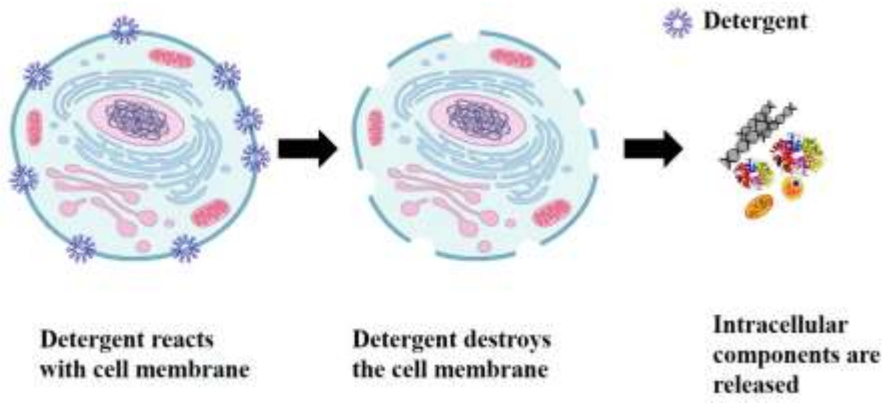
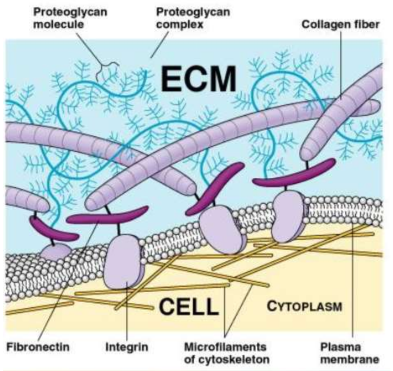




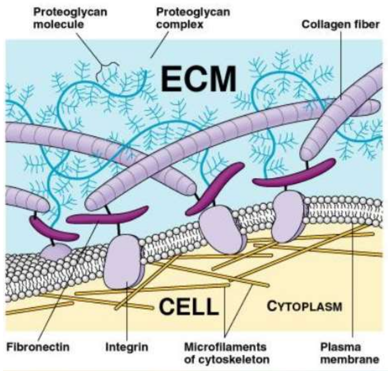




Descelularización. Métodos

Tratamientos biológicos: Enzimas _proteasas
_nucleasas

A la hora de diseñar un protocolo de descelularización se combinarán todos los métodos, teniendo en cuenta las características del tejido y la aplicación final



dispositivo donde tiene lugar una reacción

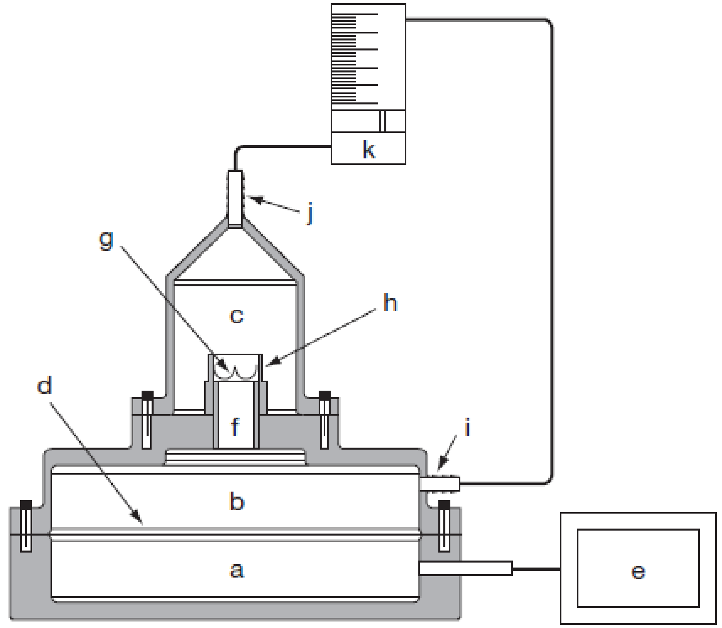
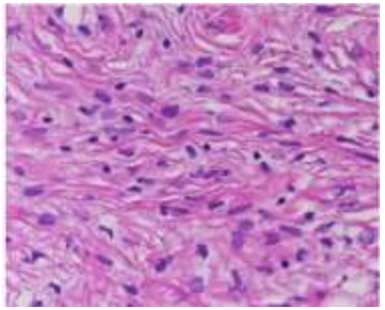
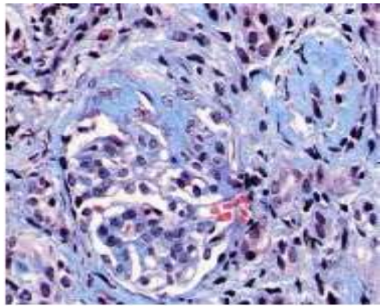


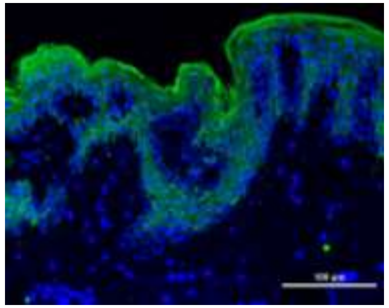
Figure 5 The two-chamber bioreactor design. The pulsatile flow chamber (bioreactor) consists of two principal chambers: the air chamber (a) at the bottom level and the cell media fluid chamber (b,c) at the upper level, separated by a thin silicone diaphragm (d; 0.5 mm). The air chamber is connected to a respirator pump (e; dual phase). The fluid chamber (total volume: 500 ml) is divided into two compartments: the lower compartment (b) is connected via a tube (f) to the smaller top compartment (c; valve perfusion chamber). The tissue-engineered heart valve (g) is fixed to a removable silicone tube (h) by suturing. The media flow is directed from the bottom compartment (b) via the connecting tube (f) to the mounted tissue-engineered heart valve in the perfusion chamber. The upper compartment is connected via a valved inlet (i) and outlet (j) tubing to a separate media reservoir (k; total volume: 500 ml).

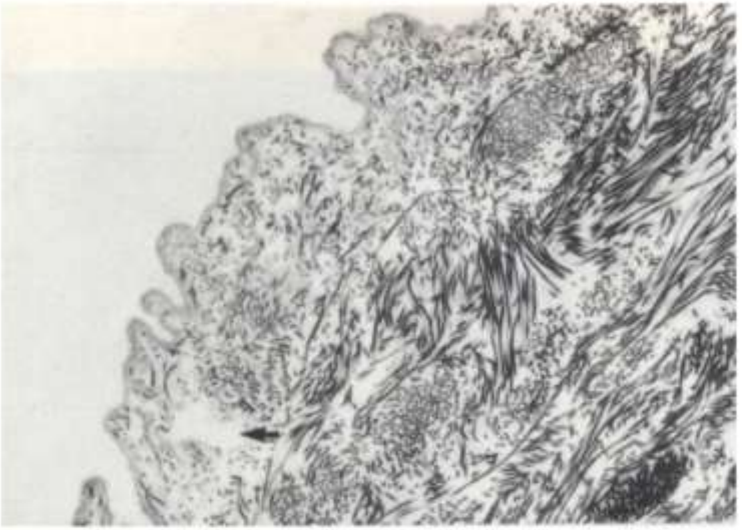
Descelularización. Verificación

Técnicas cualitativas:
microscopía


Hematoxilina-eosina


Tricrómico de Masson


DAPI


SEM

Descelularización. Verificación

Técnicas cuantitativas:
espectrofotometría, cromatografía

Cuantificación proteína total
Cuantificación proteína estructural
Cuantificación proteína específica

Cuantificación DNA residual

Especificación tejido descelularizado

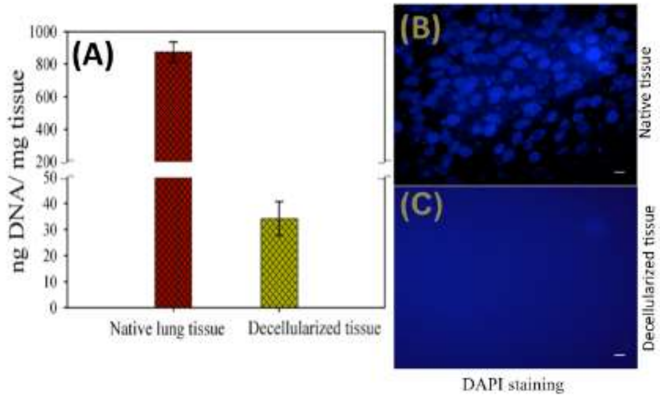


Figure 1. (A) DNA content of native and decellularized tissue indicating significant decrease in the DNA after decellularization, and (B) DAPI staining showing positive nuclear staining in the native tissue indicating the presence of cells, and (C) negative nuclear staining in the decellularized tissue indicating absence of cells. Sacle bars: 50 μm.

Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011;32(12):3233-43.

Descelularización. Verificación

Técnicas cuantitativas:
espectrofotometría, cromatografía

Cuantificación proteína total
Cuantificación proteína estructural
Cuantificación proteína específica

Cuantificación DNA residual

Especificación tejido descelularizado

Evaluación de las propiedades biomecánicas

Residuos de reactivos en el tejido: cromatografía
citotoxicidad

Descelularización. Aplicaciones. Órganos

- Reactores: dispositivo donde tiene lugar una reacción
- Perfusión: paso de los reactivos a través de un sistema circulatorio

Figure 2

Reported perfusion protocols for decellularization of heart, liver, and lung. Abbreviations: DMEM, Dulbecco's Modified Eagle's Medium; EDTA, ethylene diamine tetraacetic acid; FBS, fetal bovine serum; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate.

Badylak SF et al. Whole-organ tissue engineering: Decellularization and Recellularization of Three-Dimensional Matrix Scaffolds. Ann. Rev. Biomed. Eng. 2011;13:27-53.

Descelularización. Aplicaciones. Órganos

nature medicine

2008
SDS1%

Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart

Harald C Ott[1], Thomas S Matthiesen[2], Saik-Kia Goh[2], Lauren D Black[3], Stefan M Kren[2], Theoden I Netoff[3] & Doris A Taylor[2,4]

Arch Bronconeumol. 2018;54(1):31–38

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE Bronconeumología

www.archbronconeumol.org

Review article

Bioengineered Lungs: A Challenge and An Opportunity

Ramon Farré[a,b,c,*], Jordi Otero[a,b], Isaac Almendros[a,b,c], Daniel Navajas[a,b,d]

[a] Unitat Biofísica i Bioenginyeria, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Spain
[b] CIBER de Enfermedades Respiratorias, Madrid, Spain
[c] Institut Investigacions Biomèdiques August Pi Sunyer, Barcelona, Spain
[d] Institut de Bioenginyeria de Catalunya, The Barcelona Institute of Science and Technology, Barcelona, Spain

Transplant International

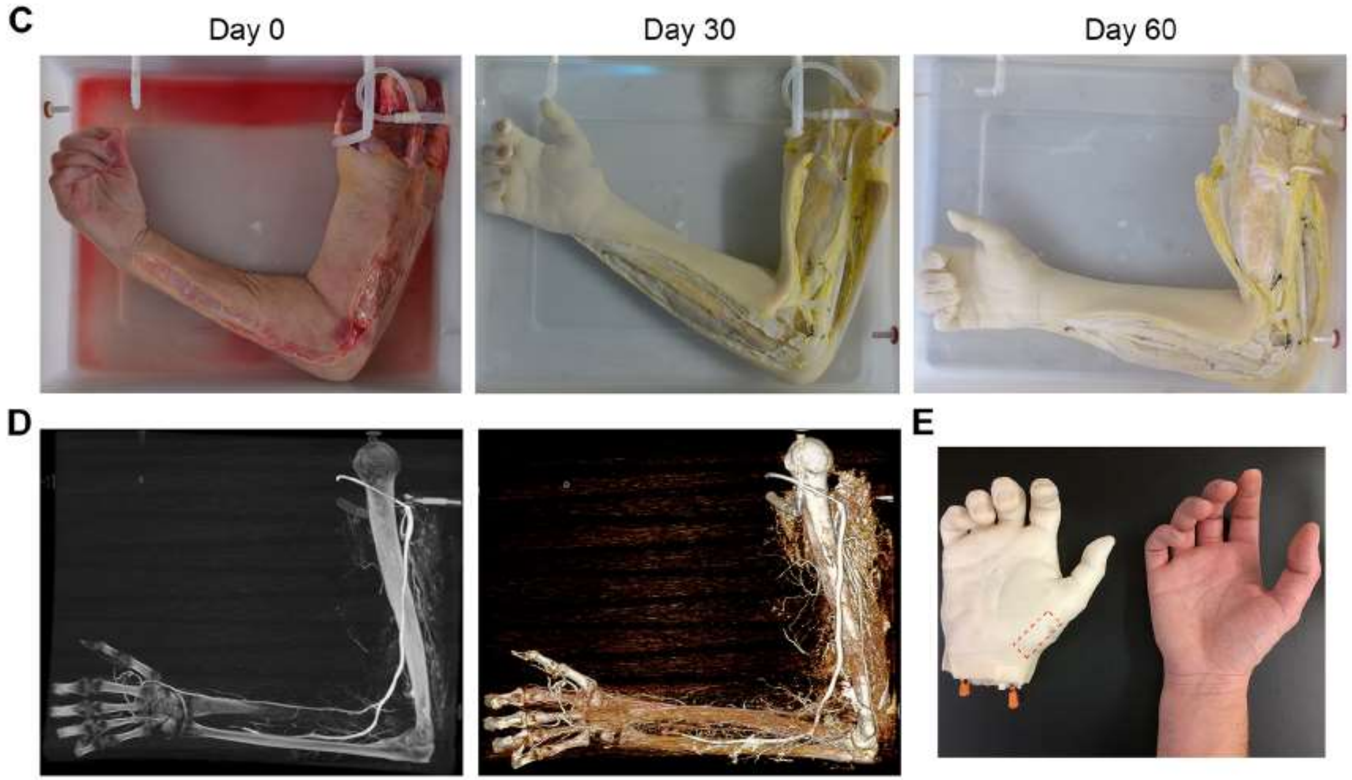
REVIEW

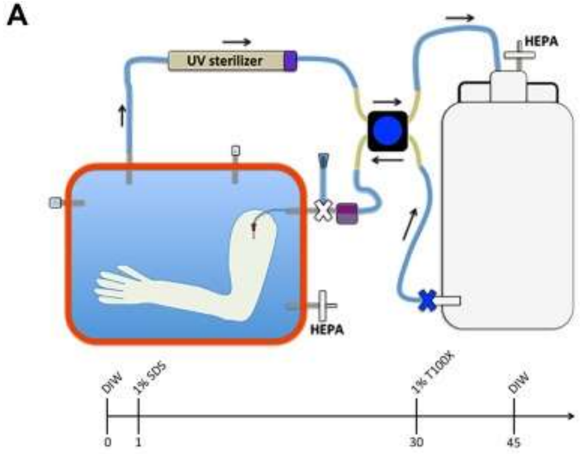
Strategies based on organ decellularization and recellularization

Karl H. Hillebrandt, Hannah Everwien, Nils Haep, Eriselda Keshi, Johann Pratschke & Igor M. Sauer

2019

Descelularización. Aplicaciones. Órganos





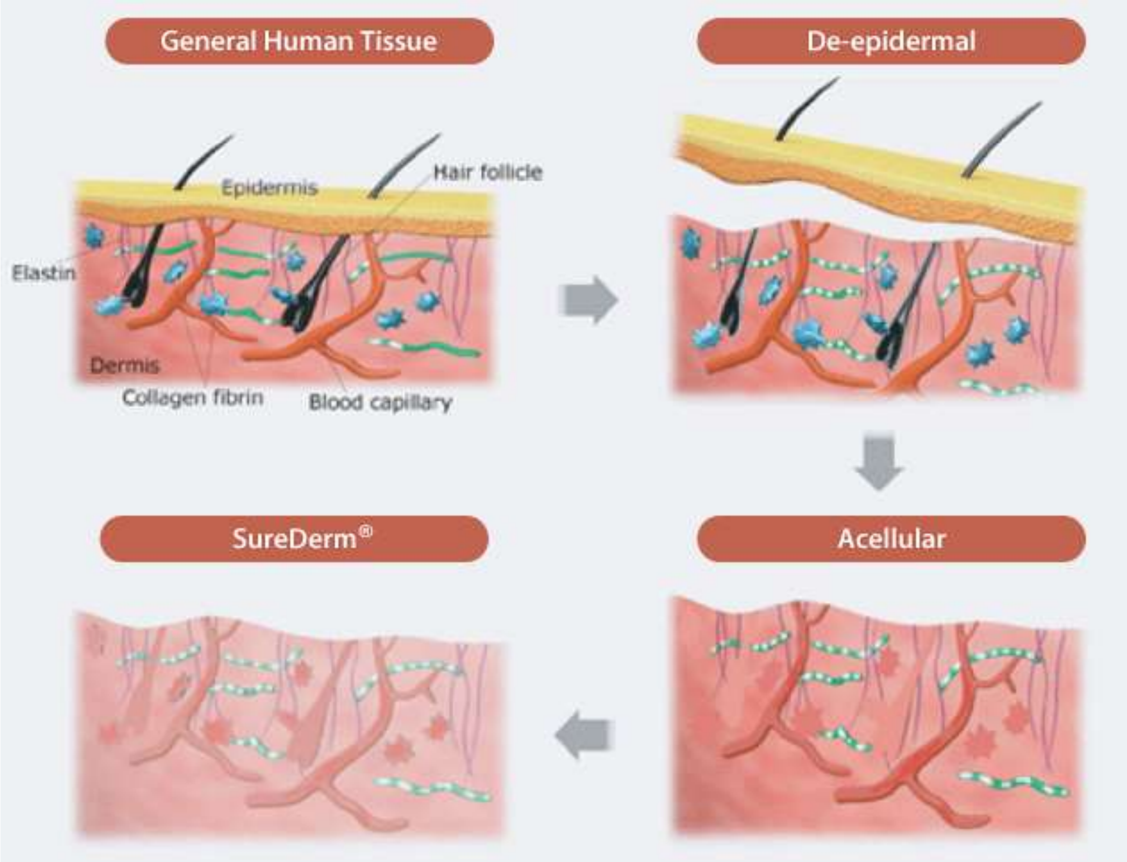
RESEARCH ARTICLE

Perfusion decellularization of a human limb: A novel platform for composite tissue engineering and reconstructive surgery

Mattia Francesco Maria Gerli[1,2¤], Jacques Paul Guyette[1,2], Daniele Evangelista-Leite[1], Brian Burns Ghoshhajra[2,3,4], Harald Christian Ott[1,2,5,6]*

2018

Descelularización. Aplicaciones. Tejido cutáneo



BUTTERWORTH HEINEMANN

Burns Vol. 21, No. 4, pp. 243–248, 1995

0305-4179/95 $10.00 + 0.00

Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns

D. J. Wainwright

Division of Plastic Surgery, University of Texas Health Science Center at Houston and Hermann Hospital Burn Unit, Houston, Texas, USA

ELSEVIER

Burns Vol. 22, No. 3, pp. 182–190, 1996

0305–4179/96 $15.00 + 0.00

0305-4179(95)00123-9

Dispase/detergent treated dermal matrix as a dermal substitute

Y. Takami[1,2], T. Matsuda[2], M. Yoshitake[2], M. Hanumadass[2] and R. J. Walter[3]

[1]Department of Plastic Surgery, Sapporo Medical College, Sapporo, Japan, [2]Burn Unit, Cook County Hospital, Chicago and [3]Departments of Surgery, Cook County Hospital and Rush Presbyterian, St Luke's Medical Center, Chicago, Illinois, USA

Descelularización. Aplicaciones. Tejido cutáneo

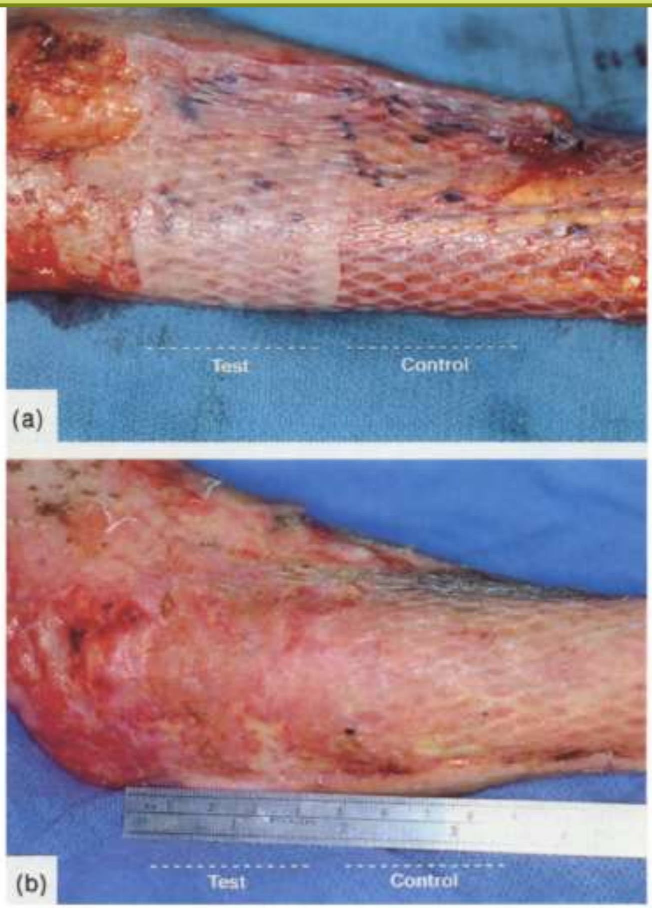
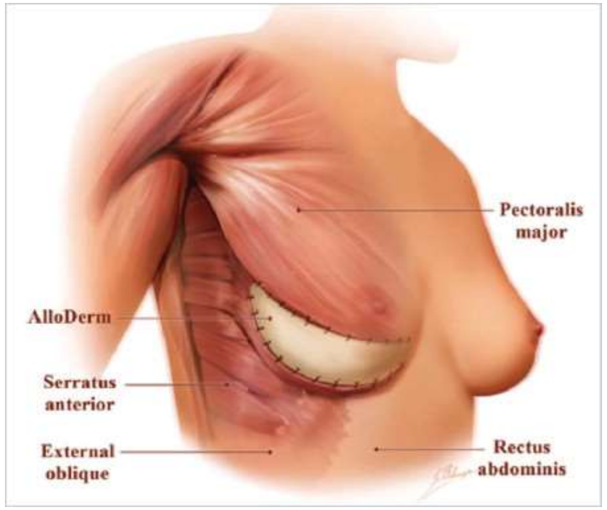


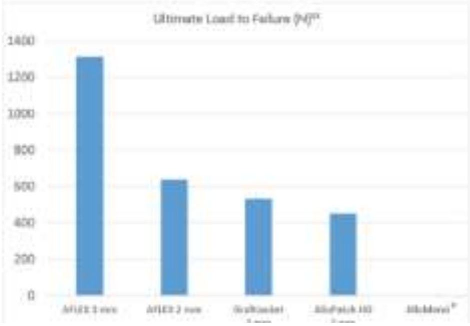
Figure 2. **a**, Simultaneous application of an acellular allograft dermal matrix (Test) and a 3 : 1 STSG (Control) in a full-thickness burn wound described in *Case 1*. **b**, At day 16 postsurgery, both the test and the control sites had fully epithelialized.

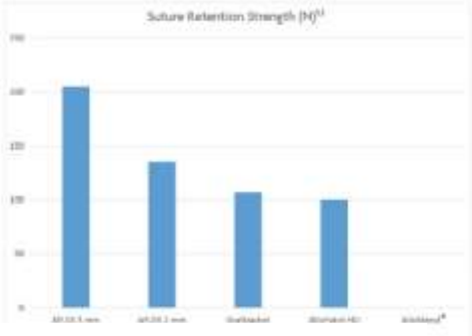
Figura 2: Superficies radiculares expuestas antes de injertar con AlloDerm

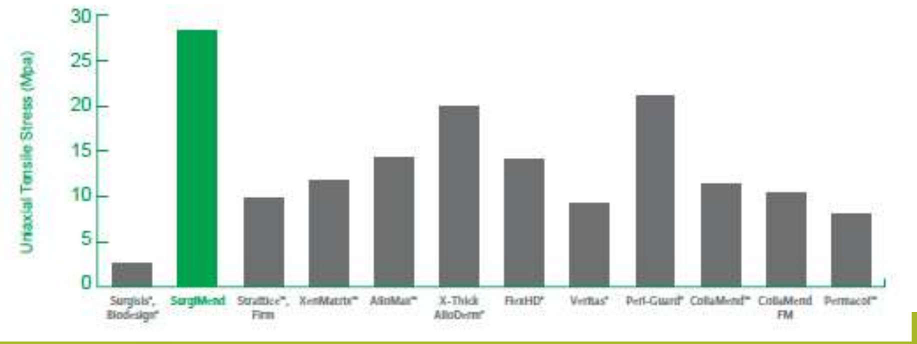
Figura 3: Las superficies radiculares cubiertas y protegidas tras el injerto con AlloDerm



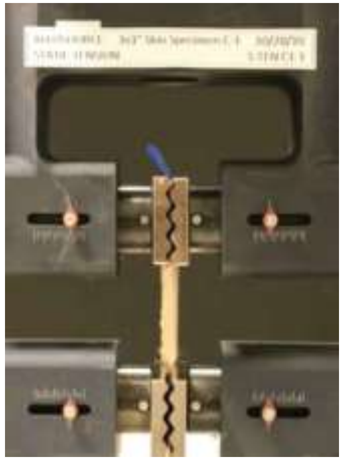
Descelularización. Aplicaciones. Tejido cutáneo

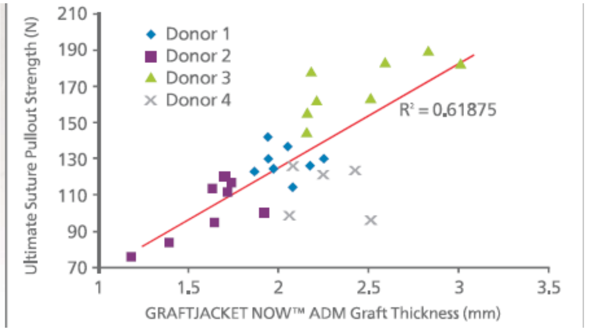




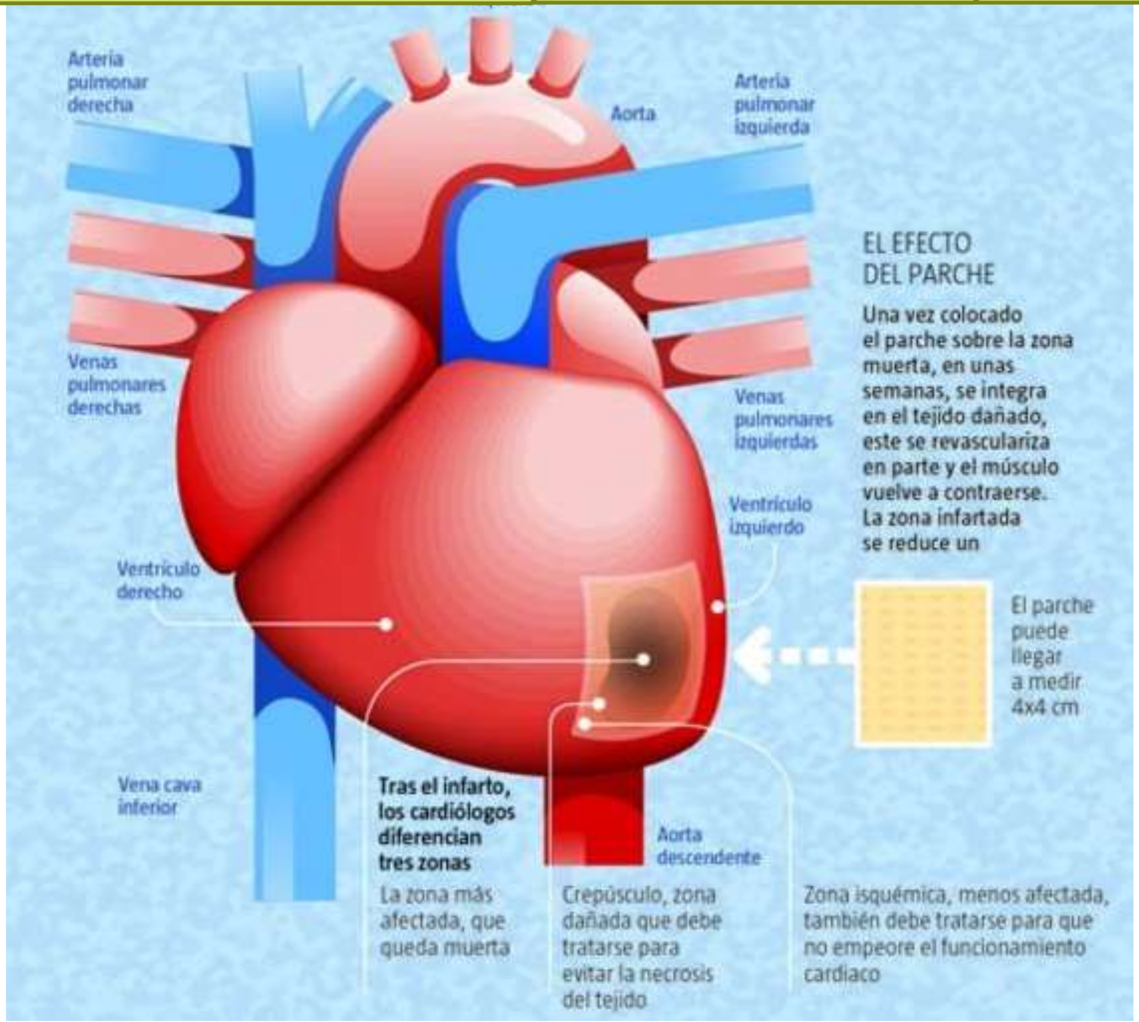


Definición de las propiedades del tejido





Descelularización. Aplicaciones. Pericardio



Descelularización. Aplicaciones. Válvulas cardiacas

European Journal of Cardio-Thoracic Surgery 50 (2016) 281–290
doi:10.1093/ejcts/ezw050 Advance Access publication 24 March 2016

ORIGINAL ARTICLE

Cite this article as: Sarikouch S, Horke A, Tudorache I, Beerbaum P, Westhoff-Bleck M, Boethig D *et al.* Decellularized fresh homografts for pulmonary valve replacement: a decade of clinical experience. Eur J Cardiothorac Surg 2016;50:281–90.

Decellularized fresh homografts for pulmonary valve replacement: a decade of clinical experience†

Samir Sarikouch[a,*], Alexander Horke[a,*], Igor Tudorache[a], Philipp Beerbaum[b], Mechthild Westhoff-Bleck[c], Dietmar Boethig[a,b], Oleg Repin[d], Liviu Maniuc[d], Anatol Ciubotaru[d], Axel Haverich[a] and Serghei Cebotari[a]

[a] Department for Cardiothoracic, Transplant, and Vascular Surgery, Hannover Medical School, Hannover, Germany
[b] Department for Pediatric Cardiology and Pediatric Intensive Care, Hannover Medical School, Hannover, Germany
[c] Department for Cardiology and Angiology, Hannover Medical School, Hannover, Germany
[d] Cardiac Surgery Center, State Medical and Pharmaceutical University, Chisinau, Moldova

* Corresponding author. Hannover Medical School, Department for Cardiothoracic, Transplant, and Vascular Surgery, (Director: Prof. Dr. med. A. Haverich), Carl-Neuberg-Str. 1, 30625 Hannover, Germany. Tel: +49-051-15325567; fax: +49-051-153218502; e-mail: sarikouch.samir@mh-hannover.de (S. Sarikouch).

Received 22 September 2015; received in revised form 7 December 2015; accepted 19 December 2015

Abstract

OBJECTIVES: Decellularized homografts have shown auspicious early results when used for pulmonary valve replacement (PVR) in congenital heart disease. The first clinical application in children was performed in 2002, initially using pre-seeding with endogenous progenitor cells. Since 2005, only non-seeded, fresh decellularized allografts have been implanted after spontaneous recellularization was observed by several groups.

METHODS: A matched comparison of decellularized fresh pulmonary homografts (DPHs) implanted for PVR with cryopreserved pulmonary homografts (CHs) and bovine jugular vein conduits (BJVs) was conducted. Patients' age at implantation, the type of congenital malformation, number of previous cardiac operations and number of previous PVRs were considered for matching purposes, using an updated contemporary registry of right ventricular outflow tract conduits (2300 included conduits, >12 000 patient-years).

RESULTS: A total of 131 DPHs were implanted for PVR in the period from January 2005 to September 2015. Of the 131, 38 were implanted within prospective trials on DPH from October 2014 onwards and were therefore not analysed within this study. A total of 93 DPH patients (58 males, 35 females) formed the study cohort and were matched to 93 CH and 93 BJV patients. The mean age at DPH implantation was

Descelularización. Aplicaciones. Válvulas cardiacas

European Journal of Cardio-Thoracic Surgery 50 (2016) 89–97
doi:10.1093/ejcts/ezw013 Advance Access publication 18 February 2016

ORIGINAL ARTICLE

Cite this article as: Tudorache I, Horke A, Cebotari S, Sarikouch S, Boethig D, Breymann T *et al*. Decellularized aortic homografts for aortic valve and aorta ascendens replacement. Eur J Cardiothorac Surg 2016;50:89–97.

Decellularized aortic homografts for aortic valve and aorta ascendens replacement[†]

Igor Tudorache[a,†,*], Alexander Horke[a,†], Serghei Cebotari[a], Samir Sarikouch[a], Dietmar Boethig[a], Thomas Breymann[a], Philipp Beerbaum[a], Harald Bertram[a], Mechthild Westhoff-Bleck[a], Karolina Theodoridis[a], Dmitry Bobylev[a], Eduard Cheptanaru[b], Anatol Ciubotaru[a,b] and Axel Haverich[a]

[a] Department of Cardiac-, Thoracic-, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany
[b] State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

* Corresponding author. Department of Cardiac-, Thoracic-, Transplantation and Vascular Surgery, Hannover Medical School, Carl-Neuberg Str. 1, 30625 Hannover, Germany. Tel: +49-5115322125; fax: +49-5115325404; e-mail: tudorache.igor@mh-hannover.de (I. Tudorache).

Received 23 September 2015; received in revised form 8 December 2015; accepted 11 December 2015

Abstract

OBJECTIVES: The choice of valve prosthesis for aortic valve replacement (AVR) in young patients is challenging. Decellularized pulmonary homografts (DPHs) have shown excellent results in pulmonary position. Here, we report our early clinical results using decellularized aortic valve homografts (DAHs) for AVR in children and mainly young adults.

METHODS: This prospective observational study included all 69 patients (44 males) operated from February 2008 to September 2015, with a mean age of 19.7 ± 14.6 years (range 0.2–65.3 years). In 18 patients, a long DAH was used for simultaneous replacement of a dilated ascending aorta as an extended aortic root replacement (EARR). Four patients received simultaneous pulmonary valve replacement with DPH.

Descelularización. Aplicaciones. Nervio

NEUROSURGICAL FOCUS

Neurosurg Focus 42 (3):E4, 2017

Optimizing decellularization techniques to create a new nerve allograft: an in vitro study using rodent nerve segments

Caroline A. Hundepool, PhD,[1,2] Tim H. J. Nijhuis, MD, PhD,[2] Dimitra Kotsougiani, MD,[1] Patricia F. Friedrich, AAS,[1] Allen T. Bishop, MD,[1] and Alexander Y. Shin, MD[1]

[1]Department of Orthopedic Surgery, Microvascular Research Laboratory, Mayo Clinic, Rochester, Minnesota; and [2]Department of Plastic, Reconstructive and Hand Surgery, Erasmus Medical Center, Rotterdam, The Netherlands

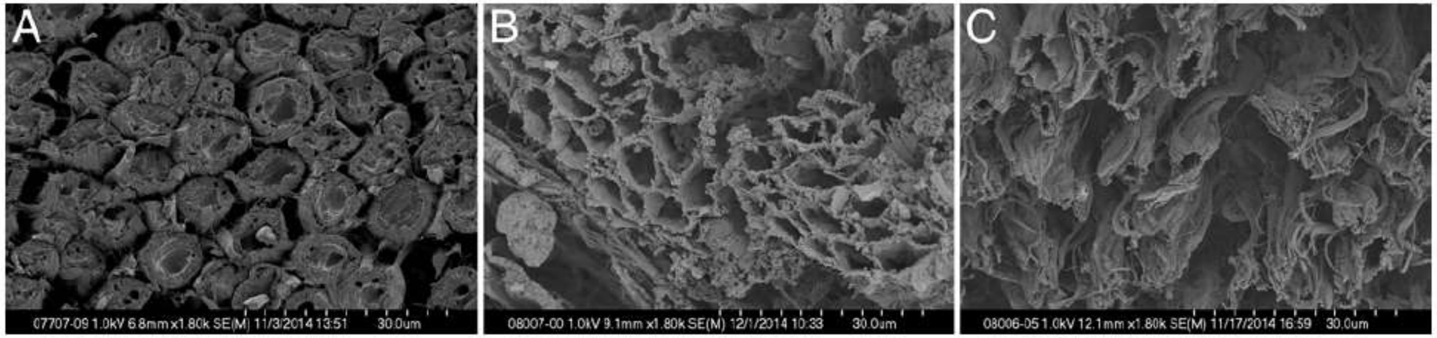


FIG. 6. Scanning electron microscopic images of the nerve graft. The images show native nerve: Group V (**A**); cold-preserved nerve allograft: Group II (**B**); and frozen nerve allograft: Group III (**C**).

Descelularización. Preservación final del tejido

Temperatura ambiente: glicerolización
liofilización
irradiación

refrigerado, congelado, criopreservado??

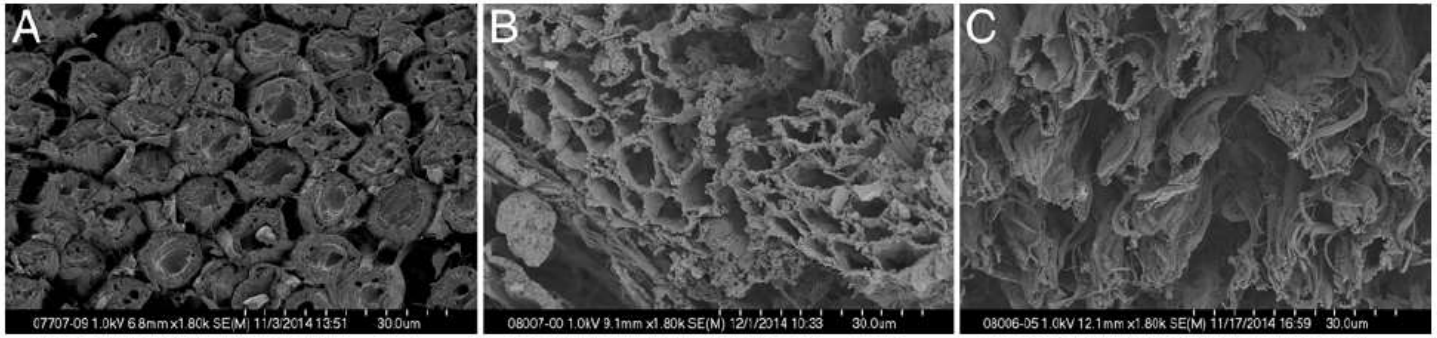


FIG. 6. Scanning electron microscopic images of the nerve graft. The images show native nerve: Group V (**A**); cold-preserved nerve allograft: Group II (**B**); and frozen nerve allograft: Group III (**C**).

Muchas gracias

mlperez@bst.cat