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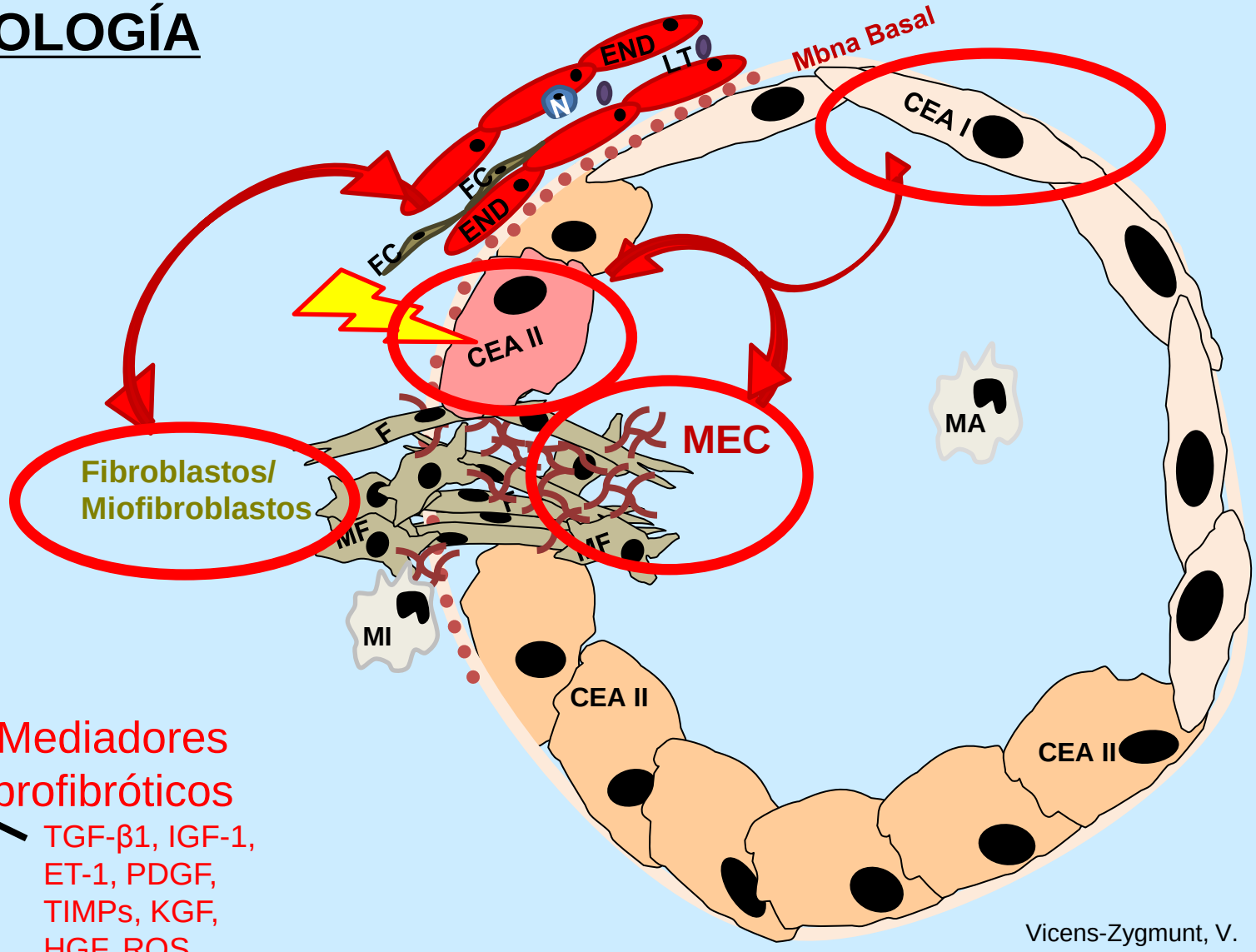
Bellvitge Hospital

CÉLULA-MATRIZ EXTRACELULAR EN FPI

Vanesa Vicens Zygmunt

FISIOPATOLOGÍA



Vicens-Zygmunt, V.

Mediadores antifibróticos
PGE2, MMPs

Mediadores profibróticos
TGF-β1, IGF-1, ET-1, PDGF, TIMPs, KGF, HGF, ROS, Th1/Th2

El microambiente pro-fibrótico de la MEC contribuiría a los cambios fenotípicos y de función celular --- perpetuación del proceso.

OBJETIVOS

Caracterizar el perfil proteico de la MEC en pulmones de pacientes con FPI.

Desarrollar una matriz 3D *in vitro* que mimetice las condiciones biomecánicas del pulmón FPI, que permita el cultivo celular en su interior.

CARACTERIZACIÓN PROTEICA DE LA MEC EN FPI

7 normal
17 FPI

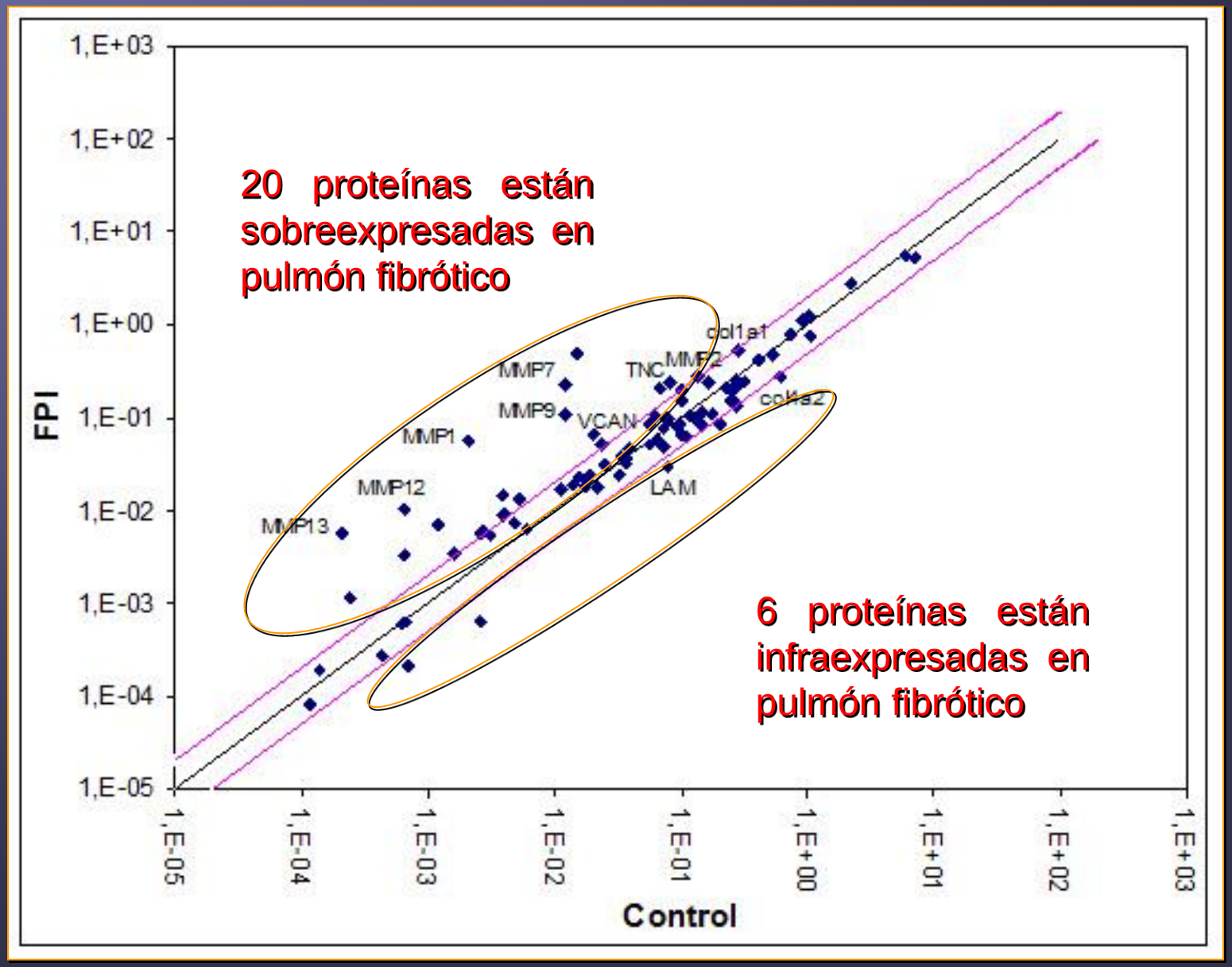
RNA → cDNA (*Transcript Reversa*) → **MICROARRAYS MEC (84 prots)** → Expresión génica

qPCR → Expresión génica

WB → Confirmación de proteïnas

IHQ → Localización de proteïnas

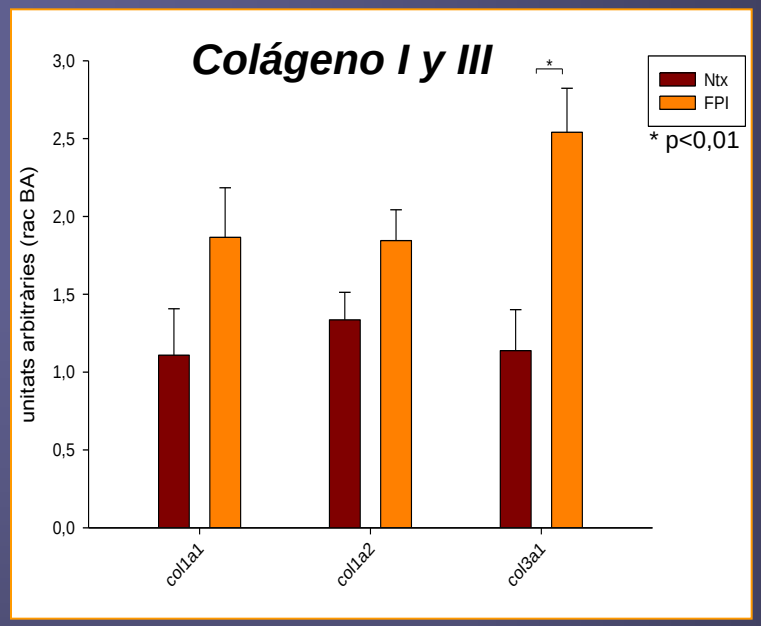
DNA-MICROARRAYS



Estany S, et al. BMC Pulm Med. 2014;14:120

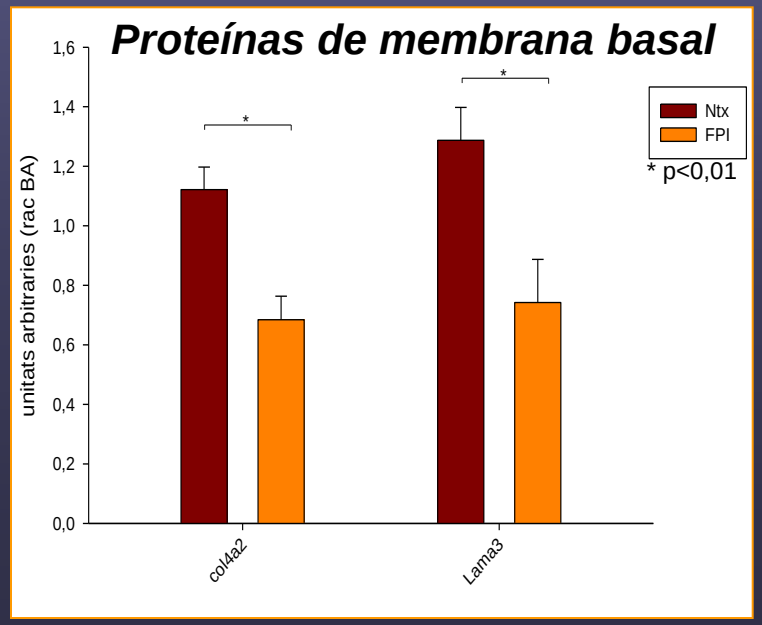
Rt-PCR

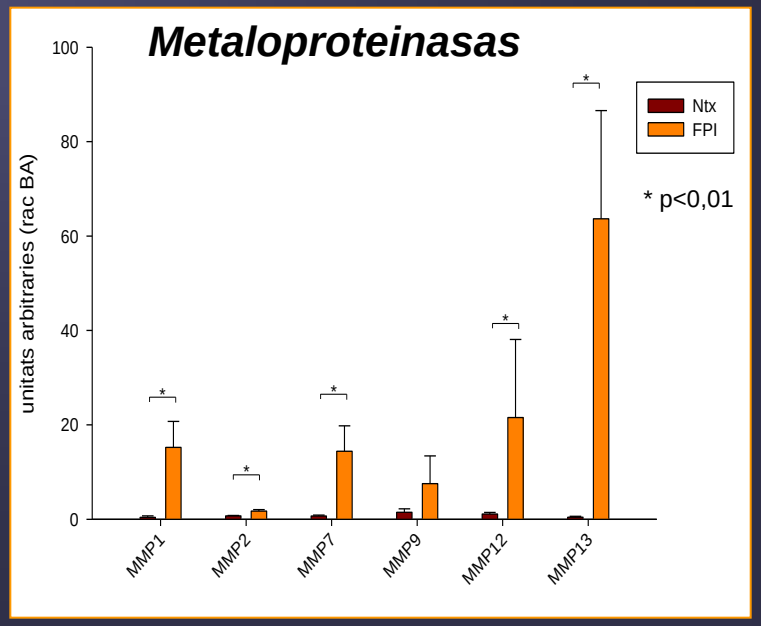
Confieren resistencia al estiramiento



Anclaje celular a la membrana basal

Responsables de la degradación de la MEC, de la mbna basal y del remodelado tisular.



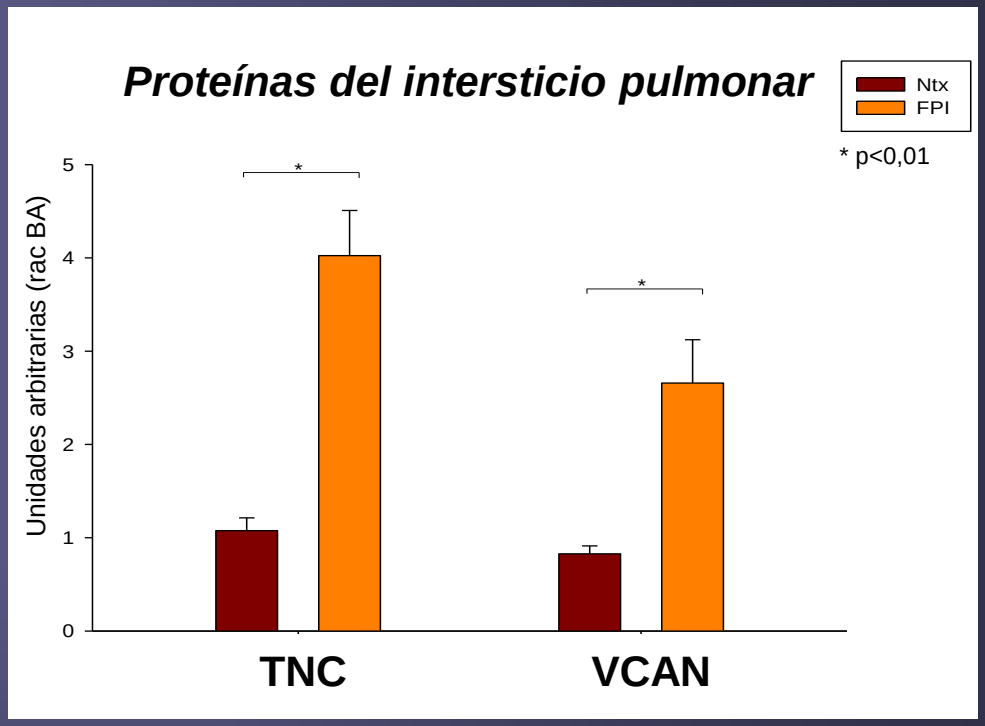


Estany S, et al. BMC Pulm Med. 2014;14:120

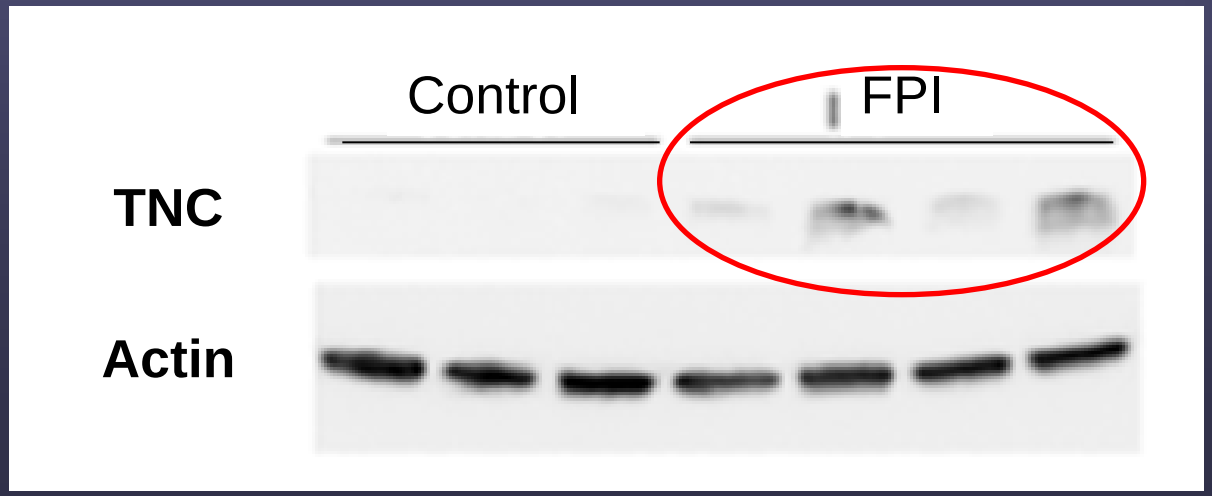
RESULTADOS

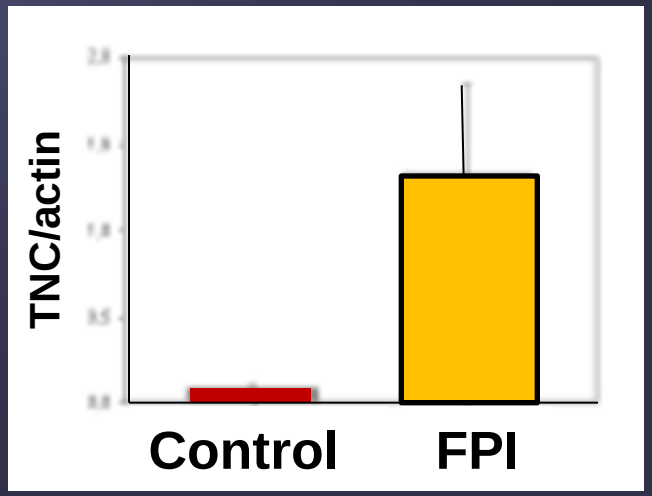
Rt-PCR

Favorecen la diferenciación celular, adhesión y migración.

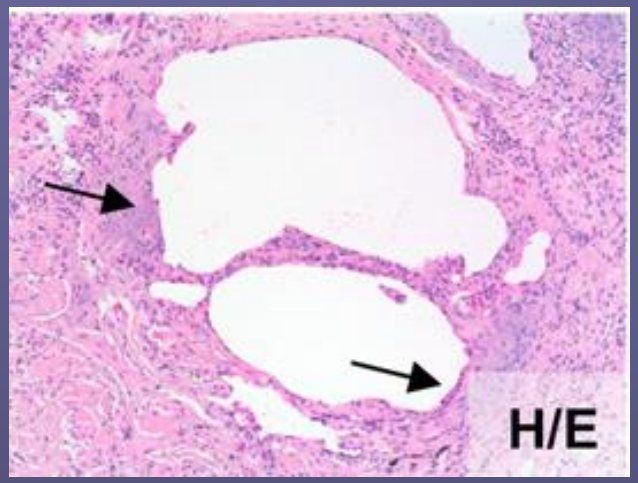


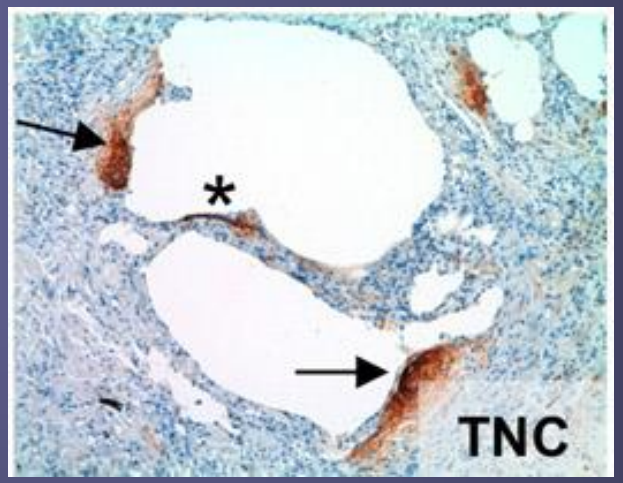
WB

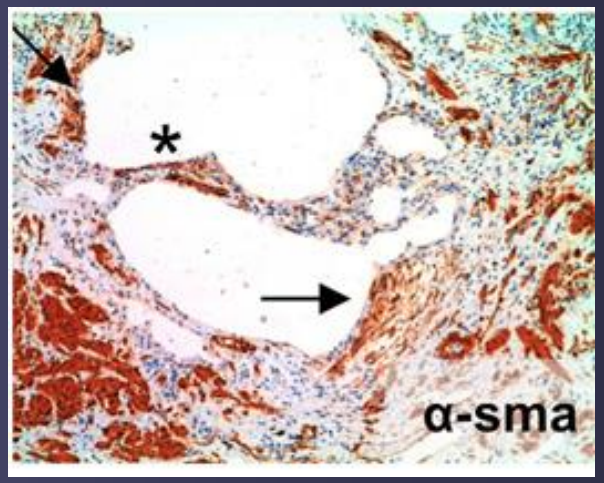


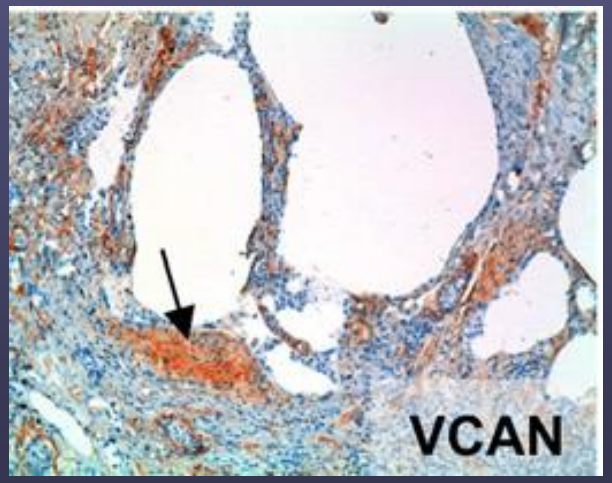


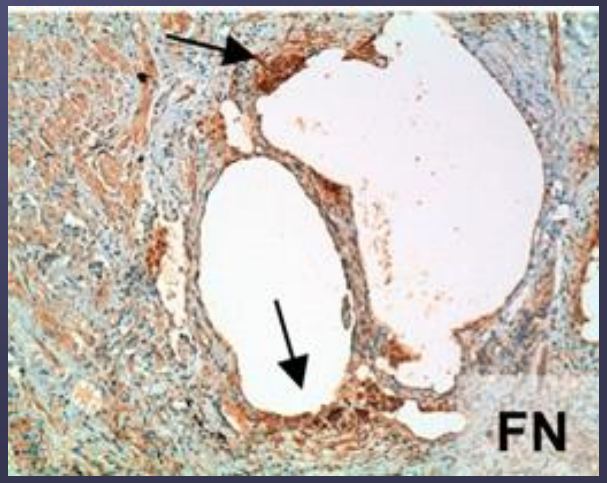
Inmunohistoquímica



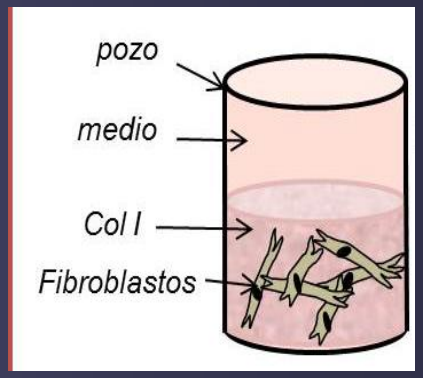






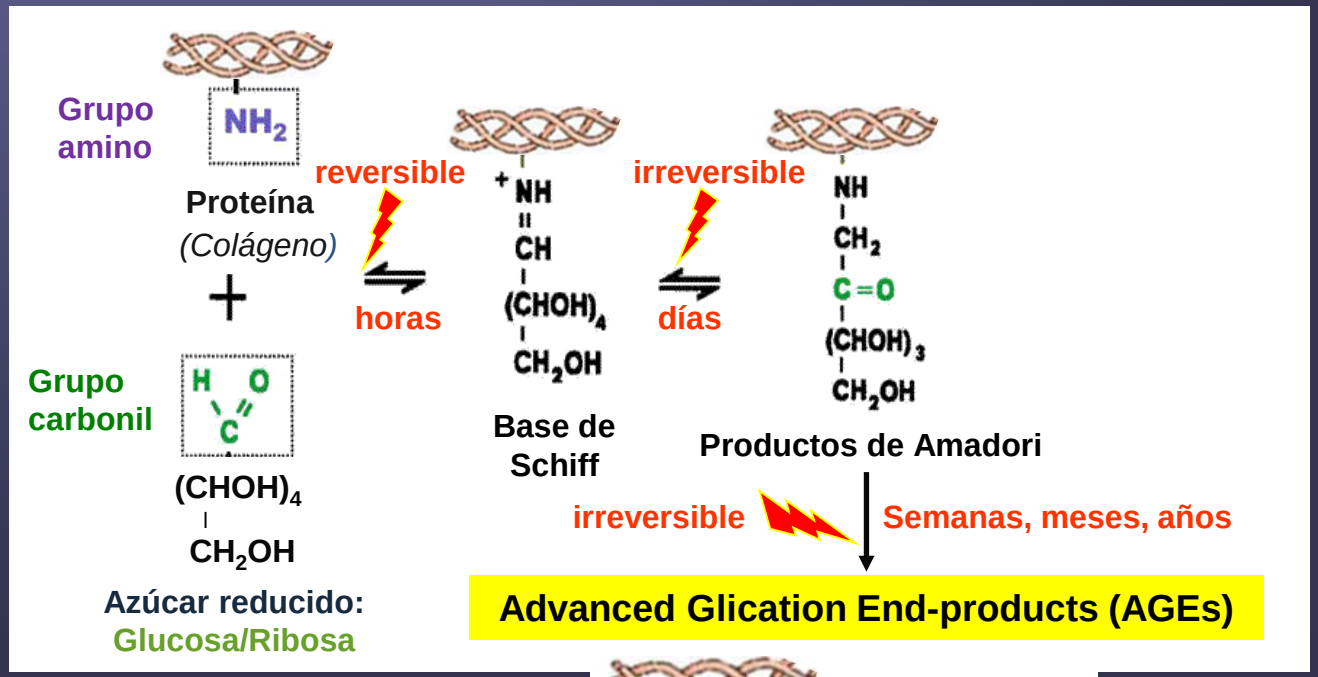


DESARROLLO DE UNA MEC 3D



COLÁGENO TIPO I con FIBROBLASTOS en su INTERIOR

GLICACIÓN NO-ENZIMÁTICA



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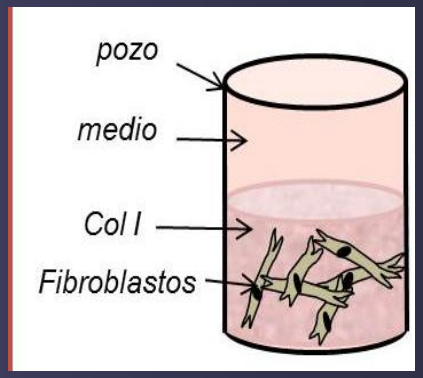
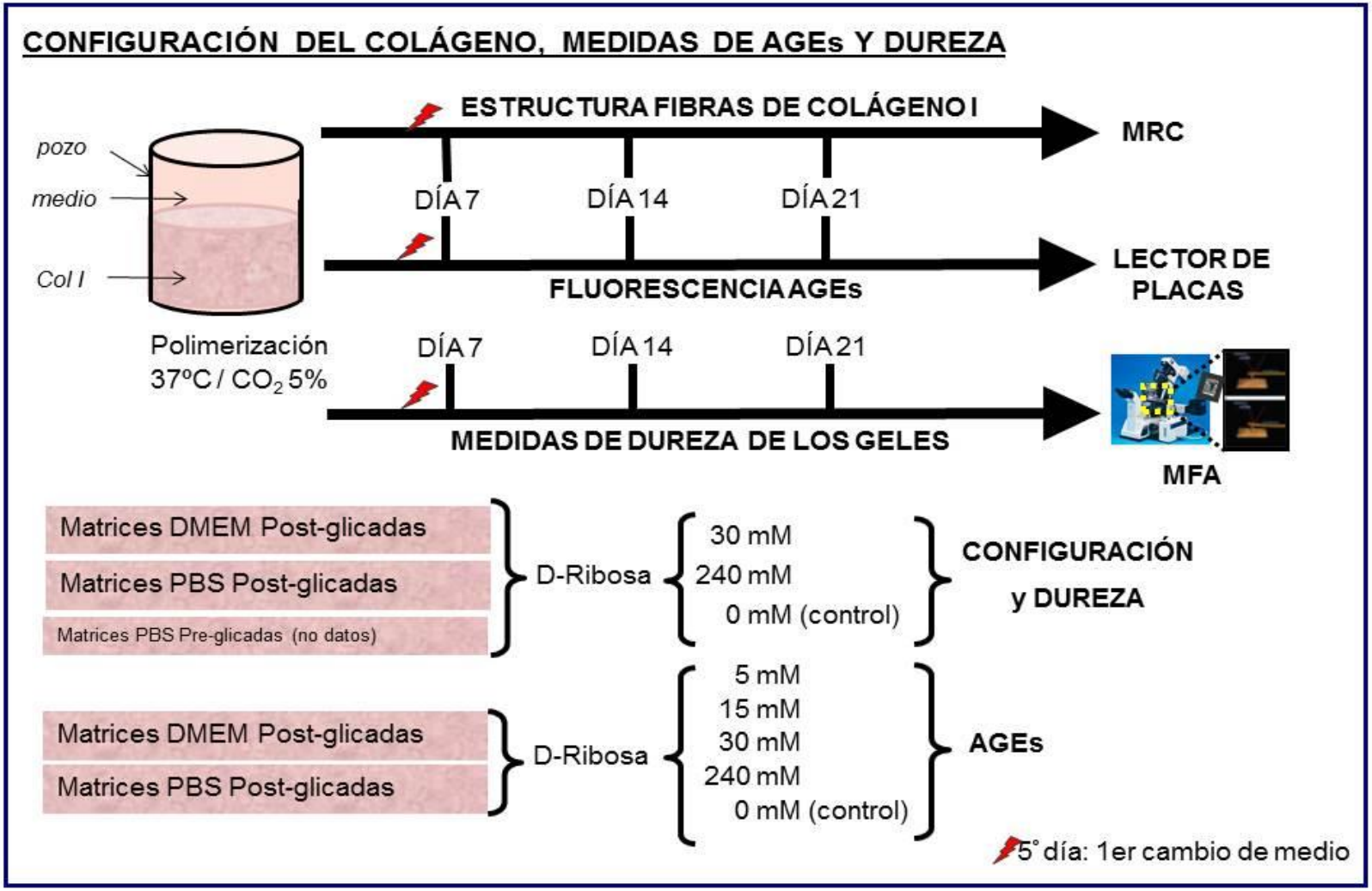


COLÁGENO TIPO I con

FIBROBLASTOS en su INTERIOR

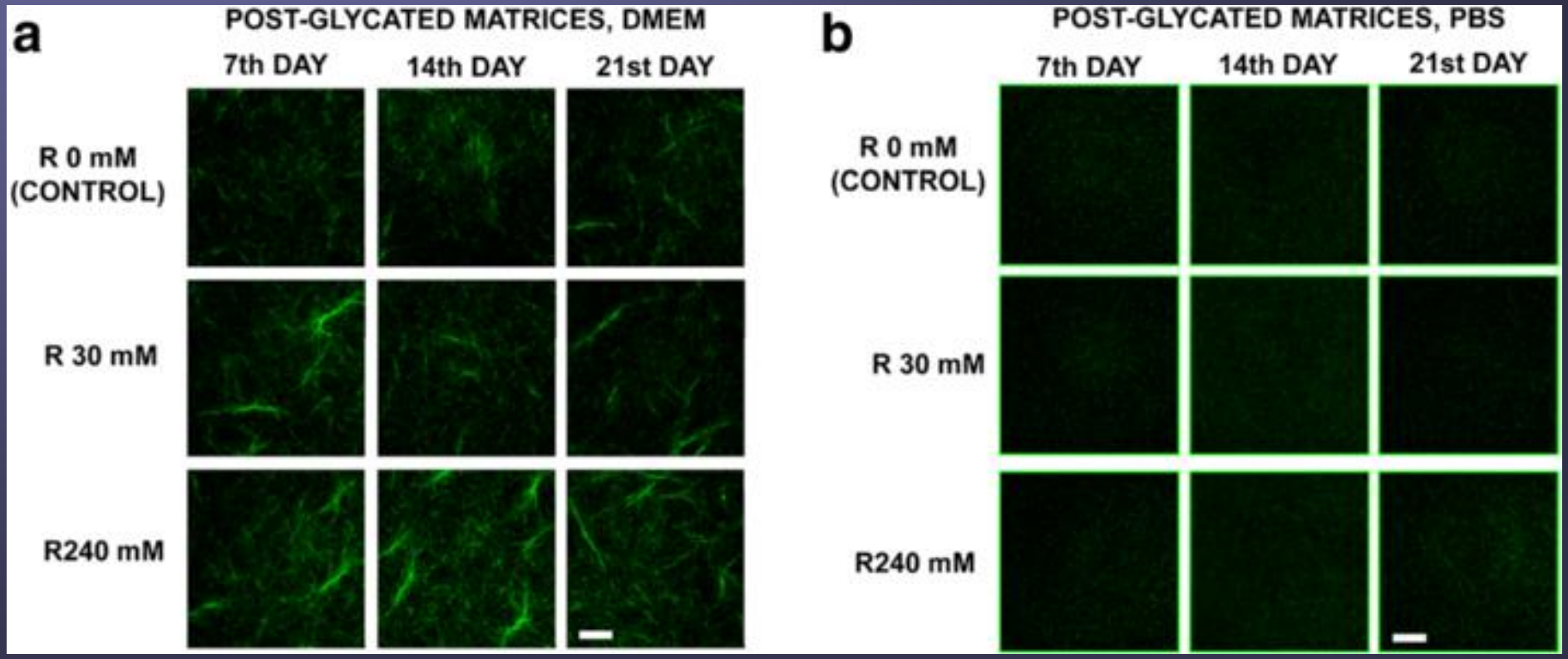
GLICACIÓN NO-ENZIMÁTICA

PROPIEDADES FÍSICO-QUÍMICAS DE LAS MEC 3D



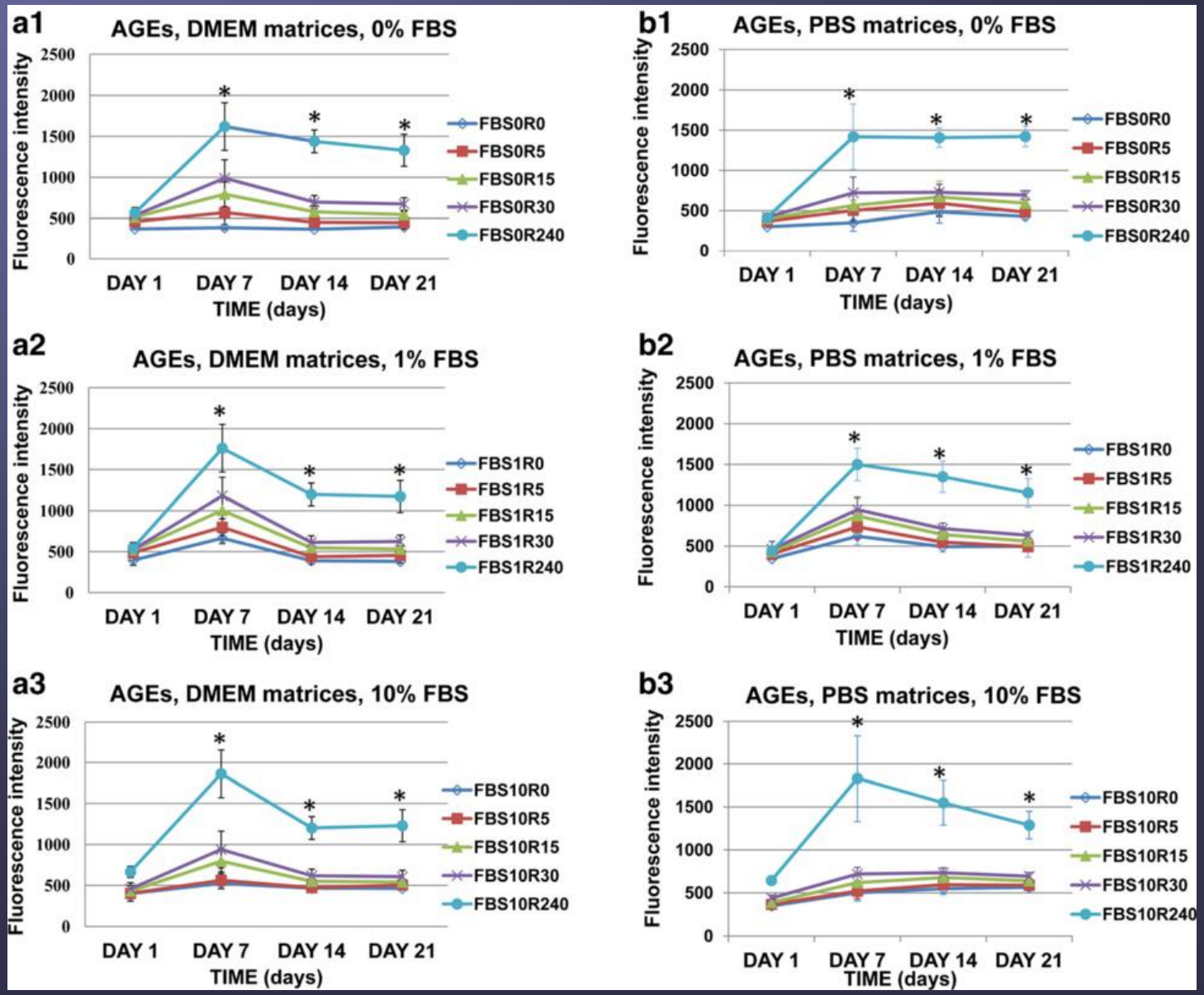
PROPIEDADES FÍSICO-QUÍMICAS DE LAS MEC 3D

Estructuras de las fibras de Colágeno I



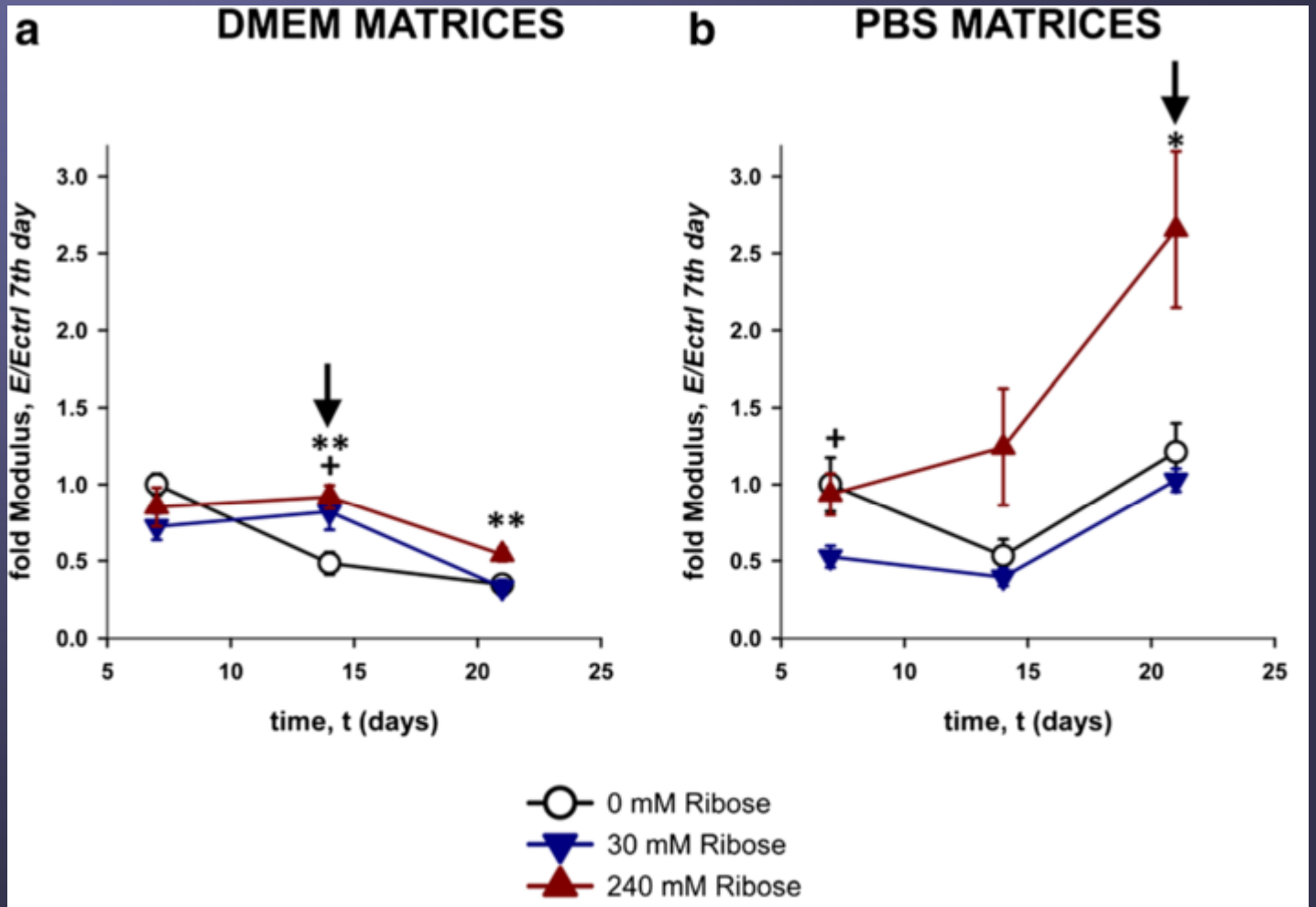
PROPIEDADES FÍSICO-QUÍMICAS DE LAS MEC 3D

Formación de AGEs



PROPIEDADES FÍSICO-QUÍMICAS DE LAS MEC 3D

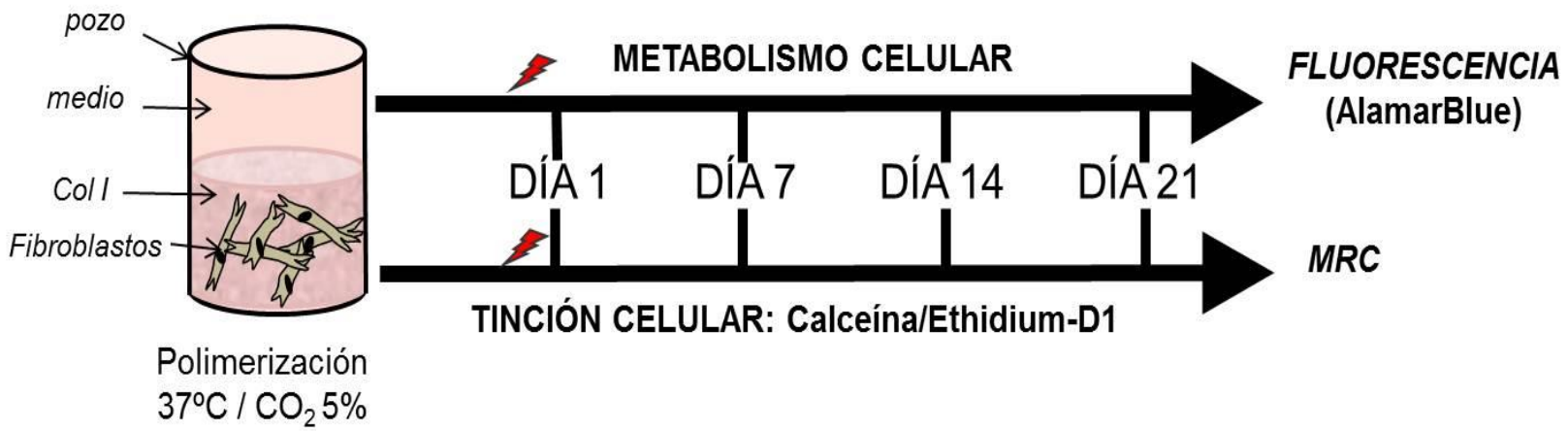
Dureza



NOTE: E/Ectrl 7th day: Elastic modulus (E) normalized to E control (Ectrl) at the 7th day.

VIABILIDAD CELULAR DENTRO DE LAS MEC 3D

VIABILIDAD FIBROBLASTOS



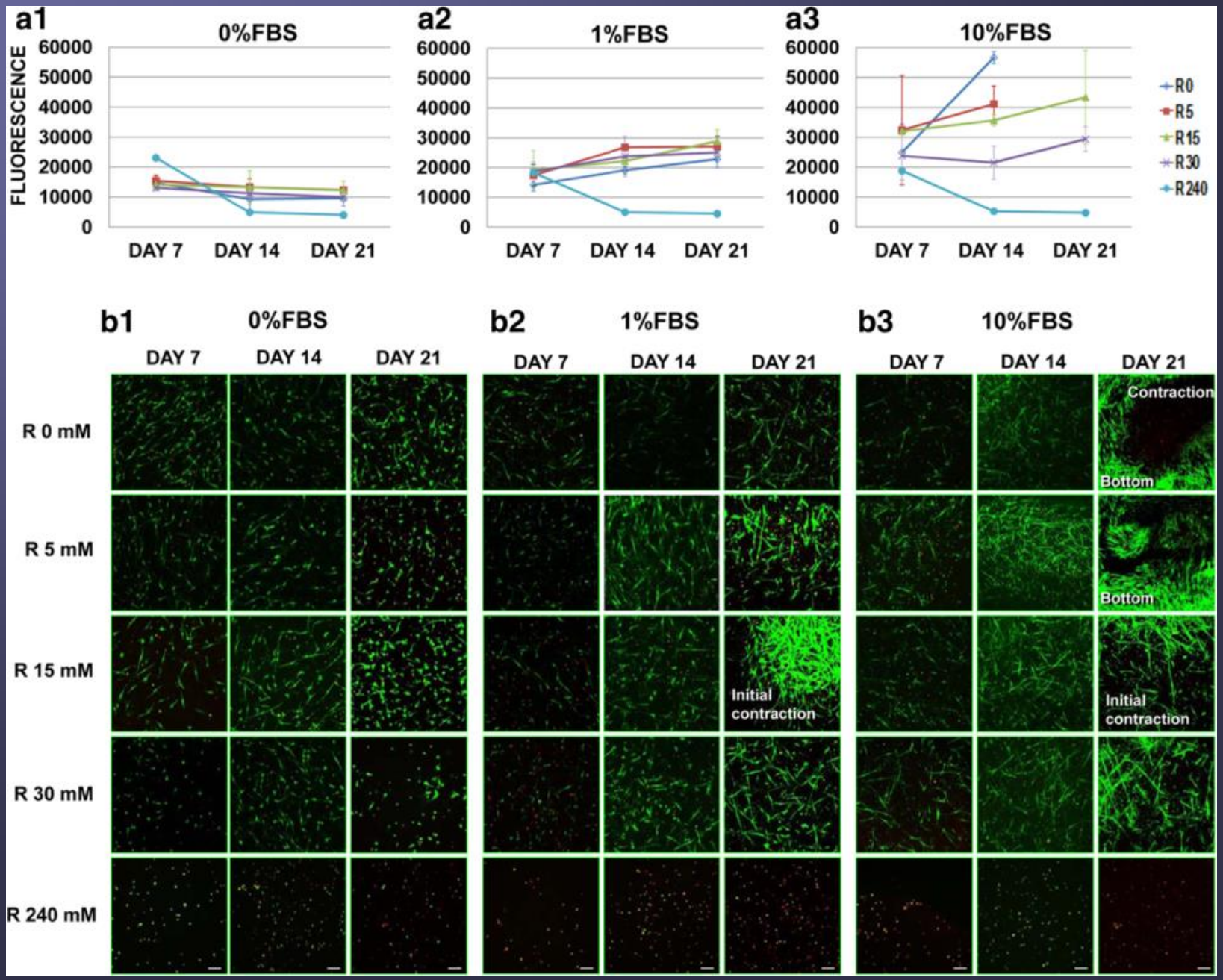
Matrices DMEM Post-glicadas
Matrices PBS Post-glicadas

D-Ribosa: 5 mM, 15mM, 30 mM, 240 mM, 0 mM (control)

FBS: 0%, 1%, 10%

5ª día: 1er cambio de medio

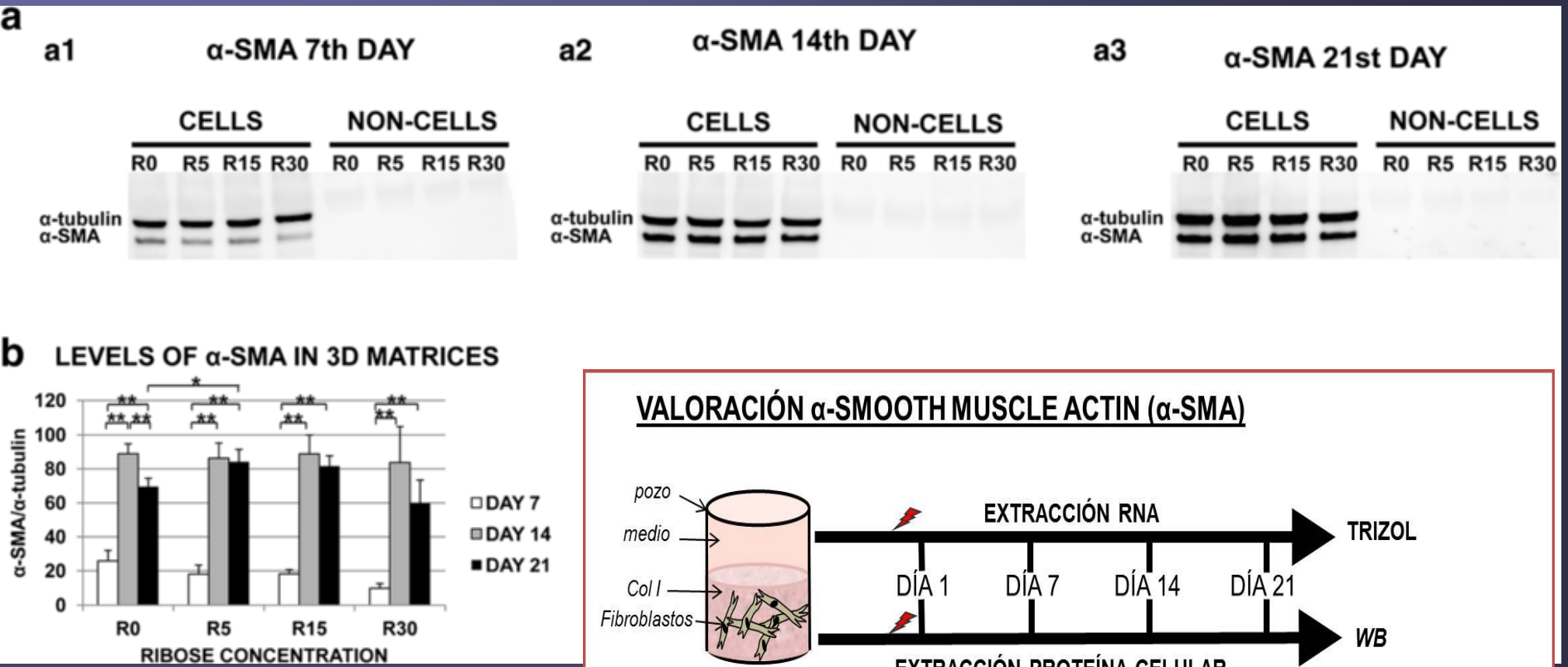
VIABILIDAD CELULAR DENTRO DE LAS MEC 3D



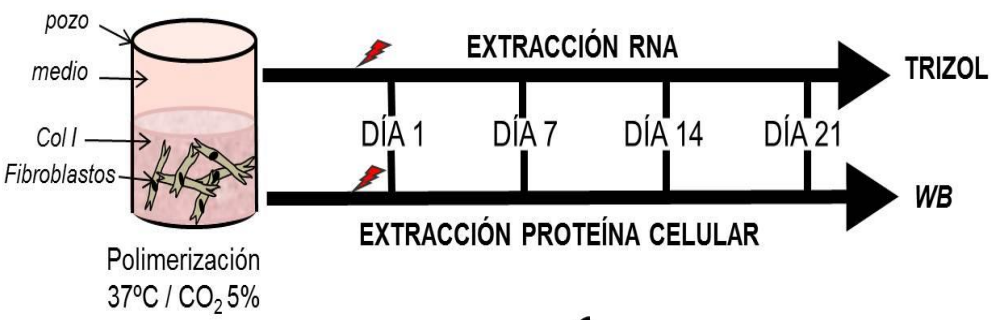
VIABILIDAD CELULAR DENTRO DE LAS MEC 3D

video

CAMBIO EN EL FENOTIPO CELULAR



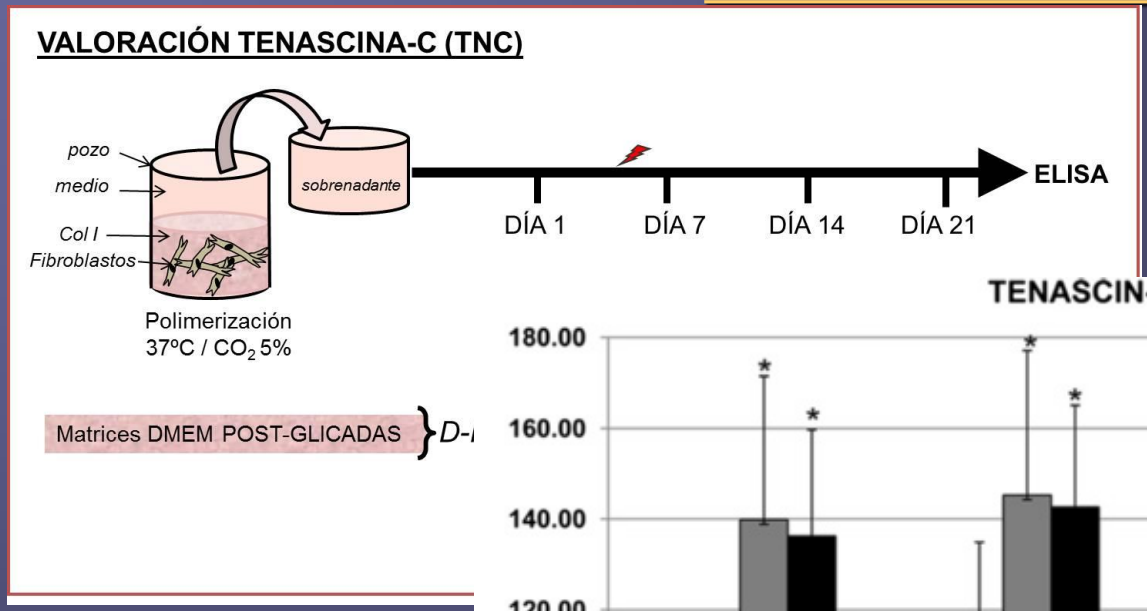
VALORACIÓN α-SMOOTH MUSCLE ACTIN (α-SMA)

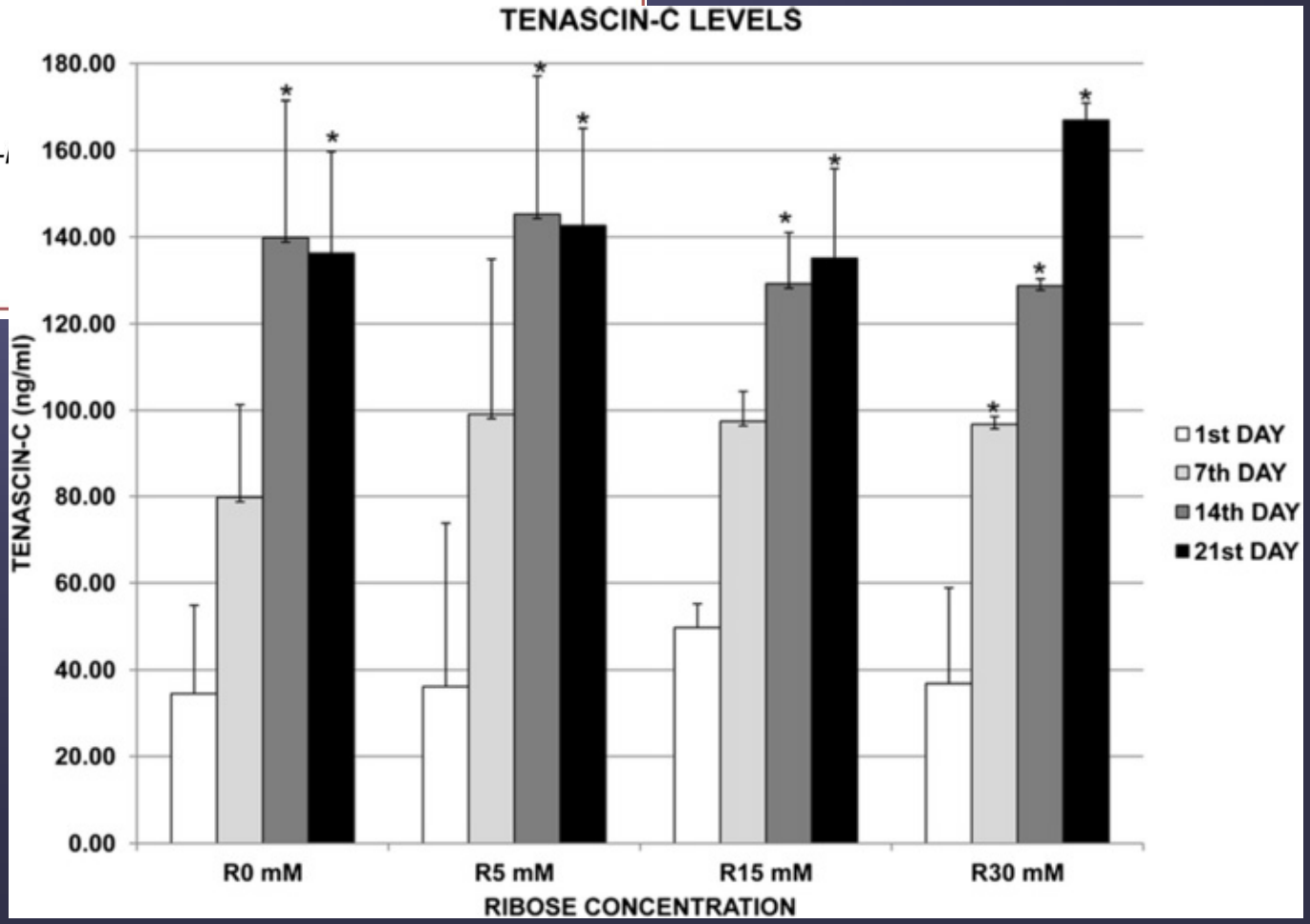


Matrices DMEM POST-GLICADAS } *D-Ribosa* { 5 mM, 15mM, 30 mM, 0 mM (control) → FBS 10%

5ª día: 1er cambio de medio

PRODUCCIÓN DE PROTEÍNAS DE MATRIZ (TNC) EN CULTIVO 3D





MUCHAS GRACIAS!

Equipo ufip

Estany S, Vicens-Zygmunt V, Llatjós R, Montes A, Penin R, Escobar I, Xaubet A, Santos S, Manresa F, Dorca J, Molina-Molina M. ***Lung fibrotic tenascin-C upregulation is associated with other extracellular matrix proteins and induced by TGFβ1.*** **BMC Pulm Med. 2014;14:120.**

Vicens-Zygmunt V, Estany S, Colom A, Montes-Worboys A, Machahua C, Sanabria AJ, Llatjos R, Escobar I, Manresa F, Dorca J, Molina-Molina M. ***Fibroblast viability and phenotypic changes within glycated stiffened three-dimensional collagen matrices.*** **Respir Res. 2015;16:82.**

Proyecto financiado por: ISCIII-FIS, SEPAR, SOCAP, FUCAP