ESTUDIO DE GMI.
ESTUDIO PROTEÓMICO COMPARATIVO ENTRE LOS IMPLANTES DENTALES DE GMI Y DE STRAUMANN

PIMA Grup de Polímers i Materials Avançats | Universitat Jaume I | Universidad del País Vasco - Euskal Herriko Unibertsitatea | CIC bioGUNE Bilbao

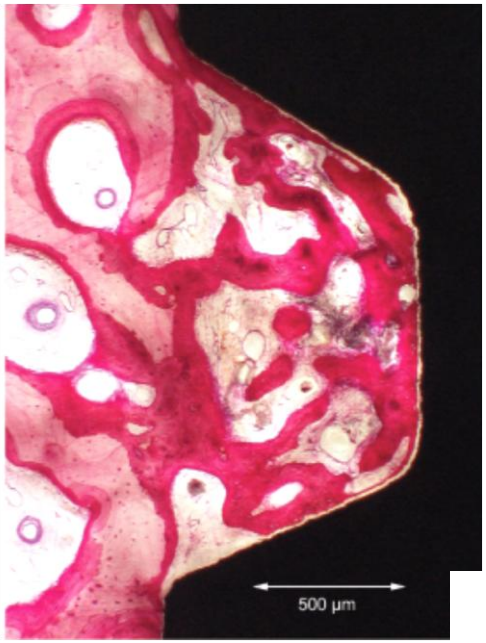
Hipótesis. Estudio proteómico de la primera capa de proteínas depositada

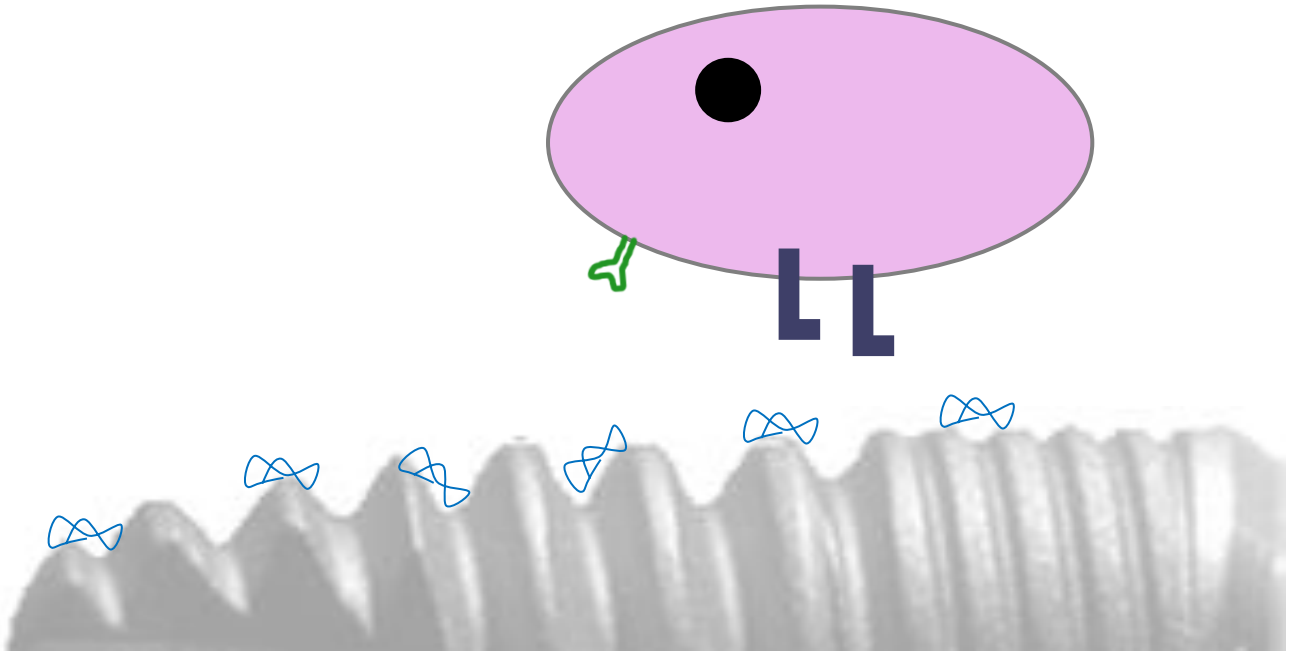
Después de la implantación: el biomaterial entra en contacto con la sangre

(en segundos)

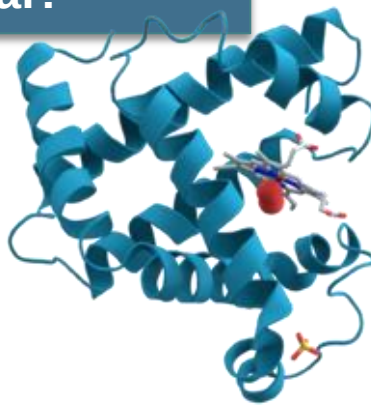
Adsorción de proteínas de la sangre en la superficie del implante

(Andersson, 2005).





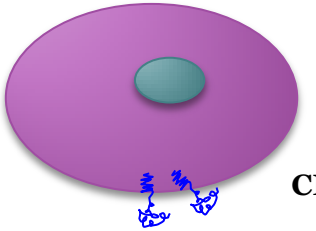
¿Por qué son importantes las proteínas adsorbidas en el biomaterial?



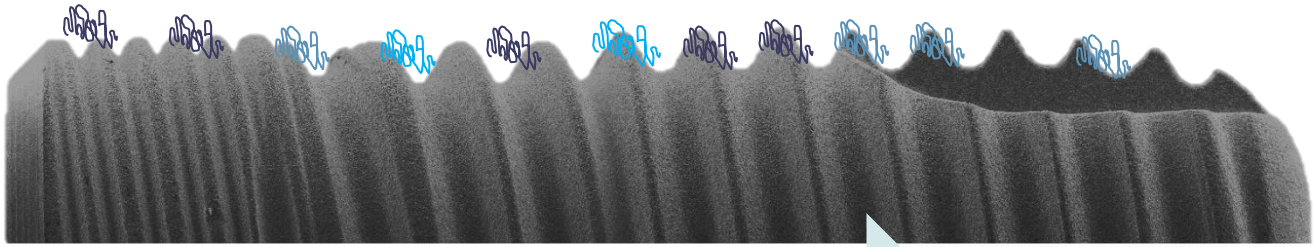
La primera capa de proteínas adsorbidas

determinará la respuesta biológica al biomaterial

(Engberg, 2015)



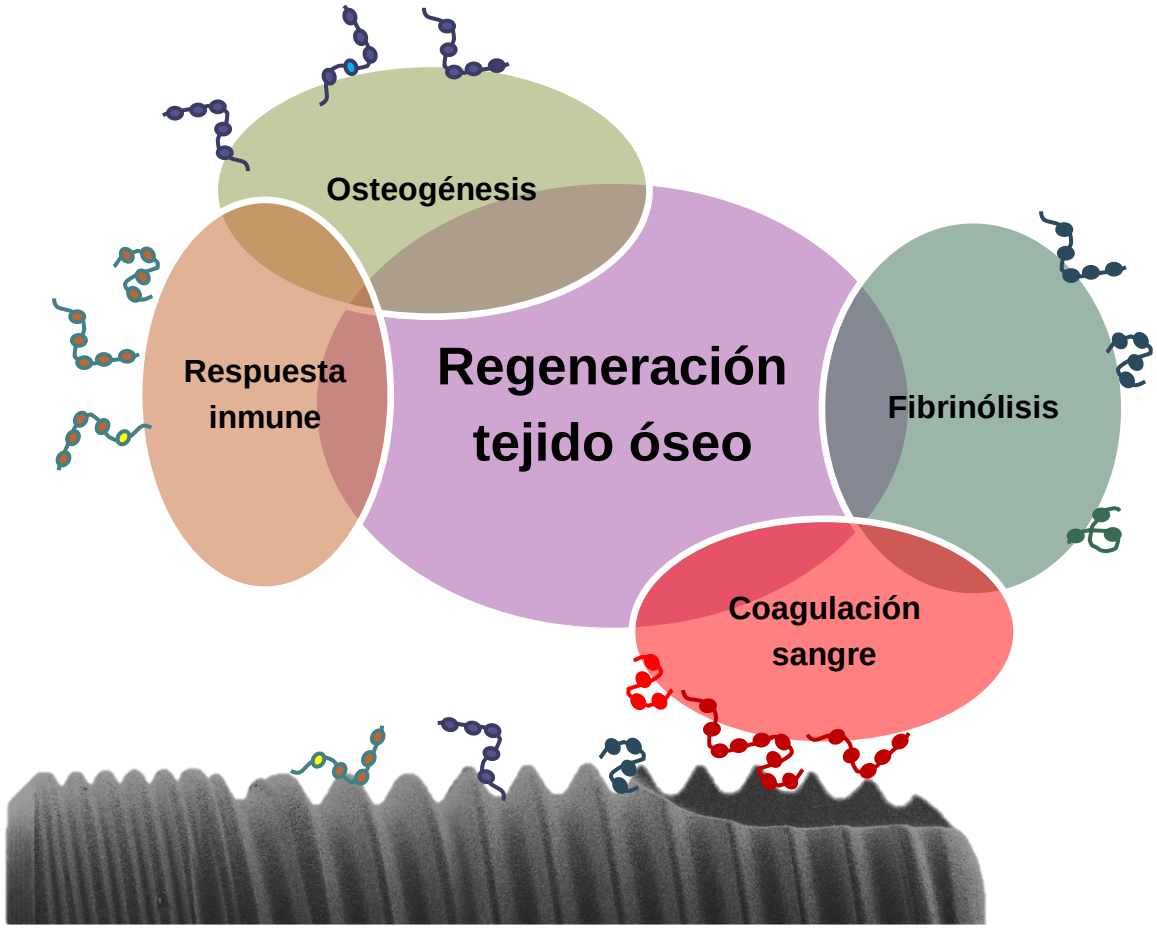
CÉLULA



INTERACCIÓN: Célula-Proteína-Material

Regeneración

Estudio proteómico comparativo de GMI



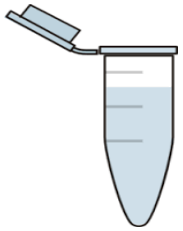
Proteómica: metodología

1

Incubación con suero humano(3h 37ºC)

Lavados consecutivos para eliminar las proteínas no adsorbidas

H_2O Mili-Q
100mM NaCl Tris-HCl pH 7.1
N=4 (4 implantes)



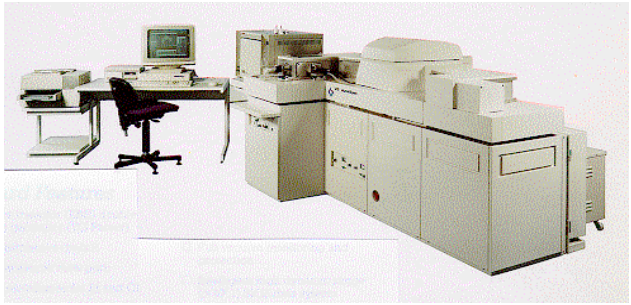
ELUCIÓN FINAL

2

Elución de las proteínas adsorbidas en el biomaterial
4% SDS, 100 mM DTT en 0.5M TEAB

3

Cuantificación e identificación:
Espectroscopía de masas (MS/MS)



4

Análisis de datos: **PROGENESIS**

DAVID (**D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery)
Base de datos de familias de proteínas, útiles para la identificación y clasificación de funciones de proteínas

DAVID BIOINFORMATICS DATABASE
DAVID Bioinformatics Resources 6.7
National Institute of Allergy and Infectious Diseases (NIAID), NIH

ESTUDIO PROTEÓMICO COMPARATIVO :

GMI

ST

gmi
ilerimplant group

Comparativa GMI vs ST

Proteínas con mayor afinidad con implantes GMI

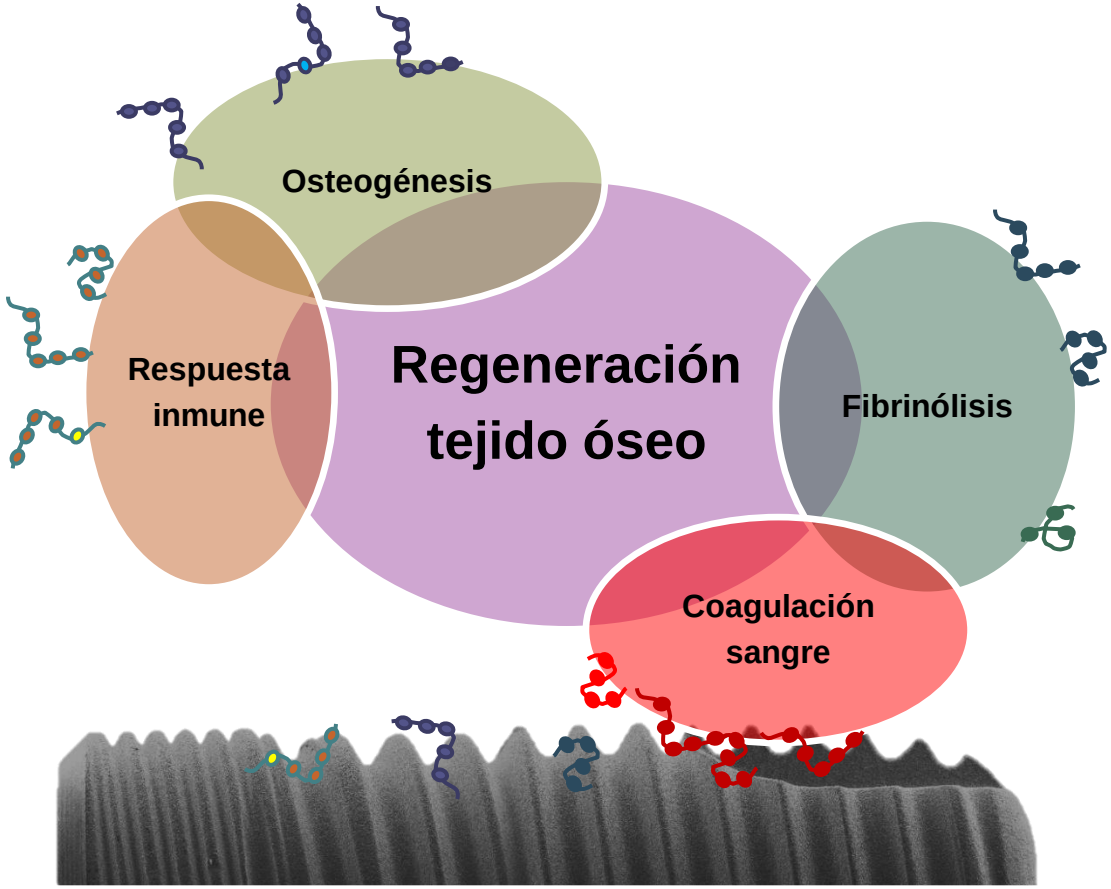
Accesión	Péptidos contados	Descripción	GMI/ST Valor p	GMI/ST Ratio
PROC_HUMAN	8	Vitamin K-dependent protein C	1,17E-06	27,21
FA10_HUMAN	8	Coagulation factor X	1,86E-04	17,21
FA7_HUMAN	3	Coagulation factor VII	1,04E-04	16,27
TAGL2_HUMAN	2	Transgelin-2	2,40E-05	11,54
SAA1_HUMAN	2	Serum amyloid A-1 protein	1,46E-02	5,48
FA9_HUMAN	7	Coagulation factor IX	2,44E-04	5,47
APOC1_HUMAN	3	Apolipoprotein C-I	3,58E-04	5,05
FCN3_HUMAN	2	Ficolin-3	5,42E-03	4,65
CAMP_HUMAN	2	Cathelicidin antimicrobial peptide	3,93E-02	4,37
APOE_HUMAN	26	Apolipoprotein E	1,17E-03	3,61
APOC4_HUMAN	5	Apolipoprotein C-IV	2,27E-05	3,60
APOA4_HUMAN	28	Apolipoprotein A-IV	1,83E-05	3,54
CXCL7_HUMAN	6	Platelet basic protein	5,79E-03	3,05
PROS_HUMAN	13	Vitamin K-dependent protein S	2,07E-03	2,87
PLEK_HUMAN	4	Pleckstrin	1,34E-02	2,69
SEPP1_HUMAN	5	Selenoprotein P	3,31E-03	2,65
C4BPB_HUMAN	2	C4b-binding protein beta chain	4,79E-02	2,54
APOA1_HUMAN	22	Apolipoprotein A-I	2,38E-04	2,49
LBP_HUMAN	4	Lipopolysaccharide-binding protein	3,72E-02	2,38
ANT3_HUMAN	26	Antithrombin-III	6,32E-04	2,33
THRB_HUMAN	25	Prothrombin	9,76E-04	2,31
DESP_HUMAN	4	Desmoplakin	1,97E-02	2,29
FA12_HUMAN	12	Coagulation factor XII	1,15E-03	2,29
C4BPA_HUMAN	19	C4b-binding protein alpha chain	9,04E-03	2,25
FHR5_HUMAN	6	Complement factor H-related protein 5	7,01E-03	2,16
FA5_HUMAN	7	Coagulation factor V	2,80E-02	2,12
RAP1B_HUMAN	2	Ras-related protein Rap-1b	2,17E-02	2,00
CO4A_HUMAN	60	Complement C4-A	2,94E-02	1,95
CO4B_HUMAN	60	Complement C4-B	2,94E-02	1,95
GELS_HUMAN	19	Gelsolin	4,75E-04	1,94
PROP_HUMAN	3	Properdin	4,48E-02	1,87
IBP4_HUMAN	2	Insulin-like growth factor-binding protein 4	1,47E-02	1,84
APOC2_HUMAN	5	Apolipoprotein C-II	1,99E-02	1,83
MOES_HUMAN	2	Moesin	3,81E-02	1,80
PON1_HUMAN	10	Serum paraoxonase/arylesterase 1	3,35E-03	1,75
PPIB_HUMAN	2	Peptidyl-prolyl cis-trans isomerase B	2,51E-02	1,73
LIMS1_HUMAN	2	LIM and senescent cell antigen-like-containing domain protein 1	4,01E-02	1,73
CETP_HUMAN	2	Cholesteryl ester transfer protein	3,03E-02	1,53
QSOX1_HUMAN	4	Sulfhydryl oxidase 1	1,92E-02	1,50

Comparison GMI vs ST

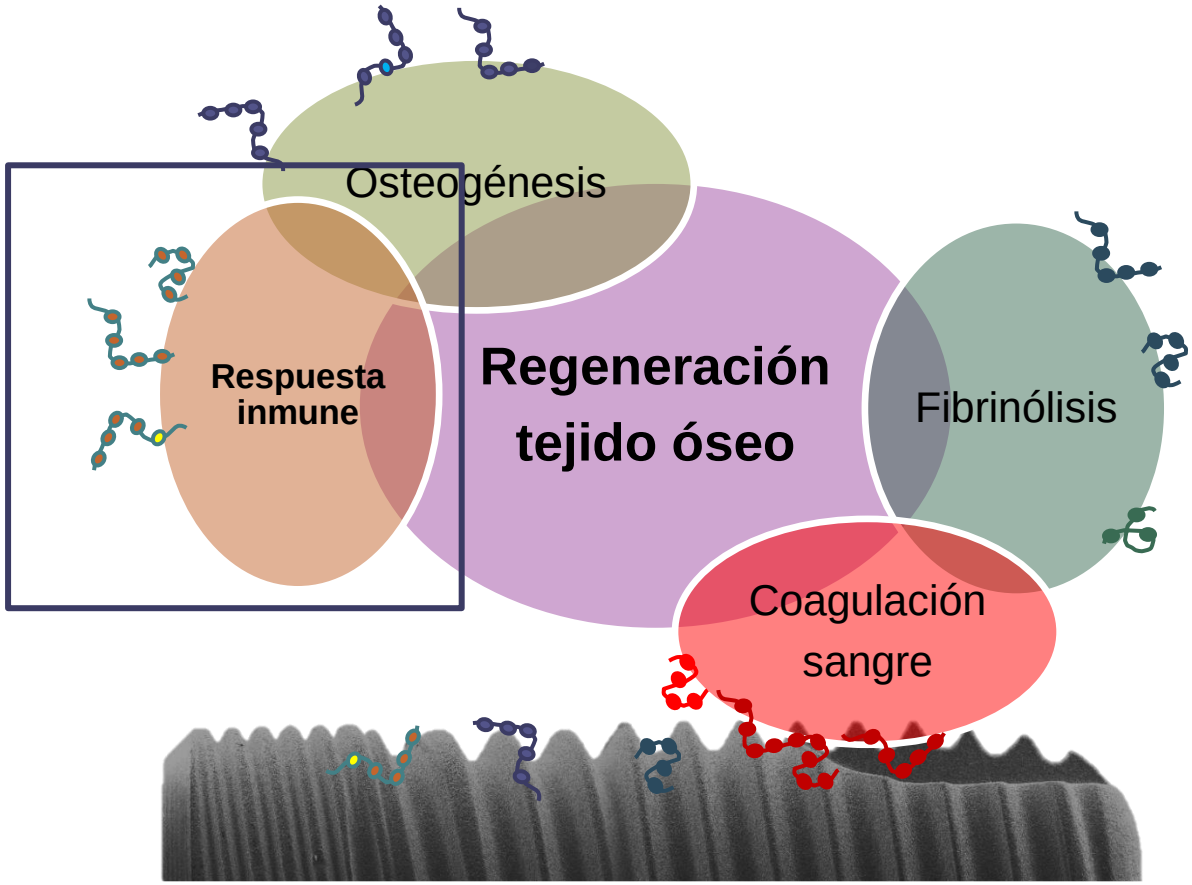
Proteínas con menor afinidad con implantes GMI

			GMI/ST	
			p value	Ratio
CO8B_HUMAN	10	Complement component C8 beta chain	3,64E-02	0,64
IGJ_HUMAN	3	Immunoglobulin J chain	4,59E-02	0,62
HV320_HUMAN	4	Ig heavy chain V-III region GAL	2,57E-02	0,62
TTHY_HUMAN	5	Transthyretin	1,70E-02	0,61
HBA_HUMAN	2	Hemoglobin subunit alpha	8,32E-03	0,53
ITIH4_HUMAN	18	Inter-alpha-trypsin inhibitor heavy chain H4	6,49E-03	0,51
CO8A_HUMAN	6	Complement component C8 alpha chain	2,48E-02	0,51
CO5_HUMAN	22	Complement C5	4,78E-02	0,49
IGHG2_HUMAN	14	Ig gamma-2 chain C region	1,38E-02	0,47
IGHG4_HUMAN	8	Ig gamma-4 chain C region	1,42E-02	0,44
FETUA_HUMAN	7	Alpha-2-HS-glycoprotein	1,08E-03	0,40
PGRP2_HUMAN	8	N-acetylmuramoyl-L-alanine amidase	1,81E-02	0,37
KNG1_HUMAN	17	Kininogen-1	6,98E-05	0,33
CFAB_HUMAN	19	Complement factor B	2,06E-02	0,33
FA11_HUMAN	20	Coagulation factor XI	6,27E-03	0,33
AMBP_HUMAN	8	Protein AMBP	5,48E-03	0,32
RET4_HUMAN	5	Retinol-binding protein	1,62E-03	0,31
VTDB_HUMAN	16	Vitamin D-binding protein	1,34E-02	0,30
IGHA1_HUMAN	11	Ig alpha-1 chain C region	1,34E-03	0,28
FCN1_HUMAN	2	Ficolin-1	7,85E-03	0,26
IGHA2_HUMAN	8	Ig alpha-2 chain C region	5,53E-03	0,24
HPT_HUMAN	21	Haptoglobin	4,07E-03	0,22
CERU_HUMAN	24	Ceruloplasmin	3,71E-04	0,22
CBG_HUMAN	2	Corticosteroid-binding globulin	5,97E-03	0,22
ANGT_HUMAN	6	Angiotensinogen	2,07E-03	0,21
AACT_HUMAN	11	Alpha-1-antichymotrypsin	4,84E-04	0,20
ITIH1_HUMAN	10	Inter-alpha-trypsin inhibitor heavy chain H1	2,50E-03	0,19
ALBU_HUMAN	57	Serum albumin	1,13E-02	0,18
A1AT_HUMAN	20	Alpha-1-antitrypsin	1,05E-03	0,17
TRFE_HUMAN	42	Serotransferrin	5,42E-03	0,15
A1AG2_HUMAN	7	Alpha-1-acid glycoprotein 2	2,30E-02	0,12
A1AG1_HUMAN	8	Alpha-1-acid glycoprotein 1	3,51E-03	0,12
ZA2G_HUMAN	11	Zinc-alpha-2-glycoprotein	7,13E-04	0,12
AFAM_HUMAN	10	Afamin	1,22E-03	0,12
A2MG_HUMAN	50	Alpha-2-macroglobulin	3,84E-03	0,11
HEMO_HUMAN	16	Hemopexin	4,91E-03	0,10
ITIH3_HUMAN	2	Inter-alpha-trypsin inhibitor heavy chain H3	1,47E-03	0,10
A1BG_HUMAN	9	Alpha-1B-glycoprotein	2,28E-03	0,09
A2GL_HUMAN	4	Leucine-rich alpha-2-glycoprotein	6,02E-03	0,08

Proceso complejo



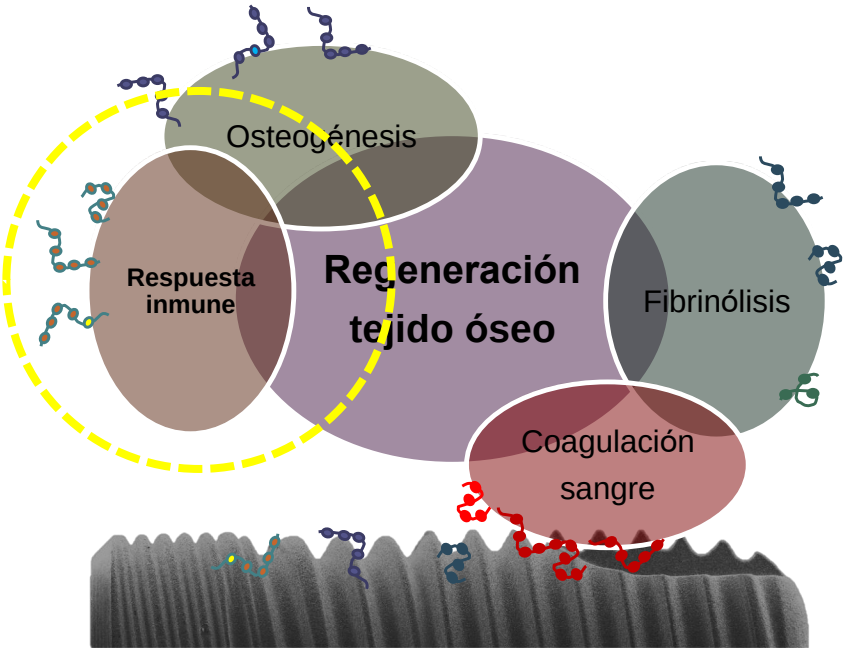
1º: RESPUESTA INMUNE

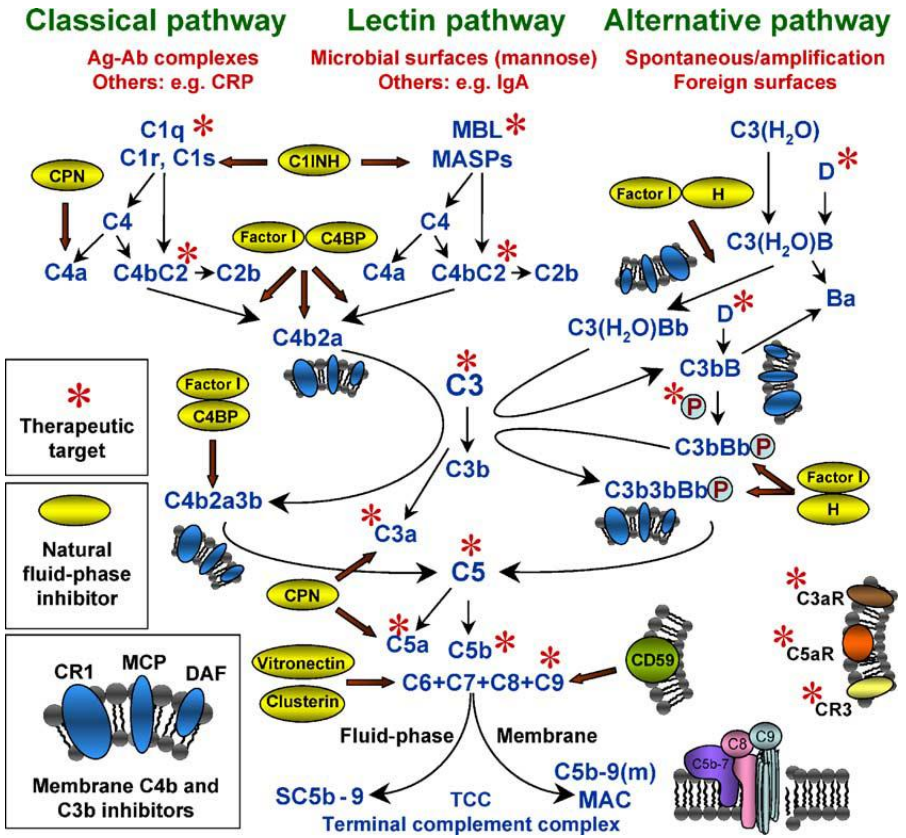


Cascada del sistema del complemento

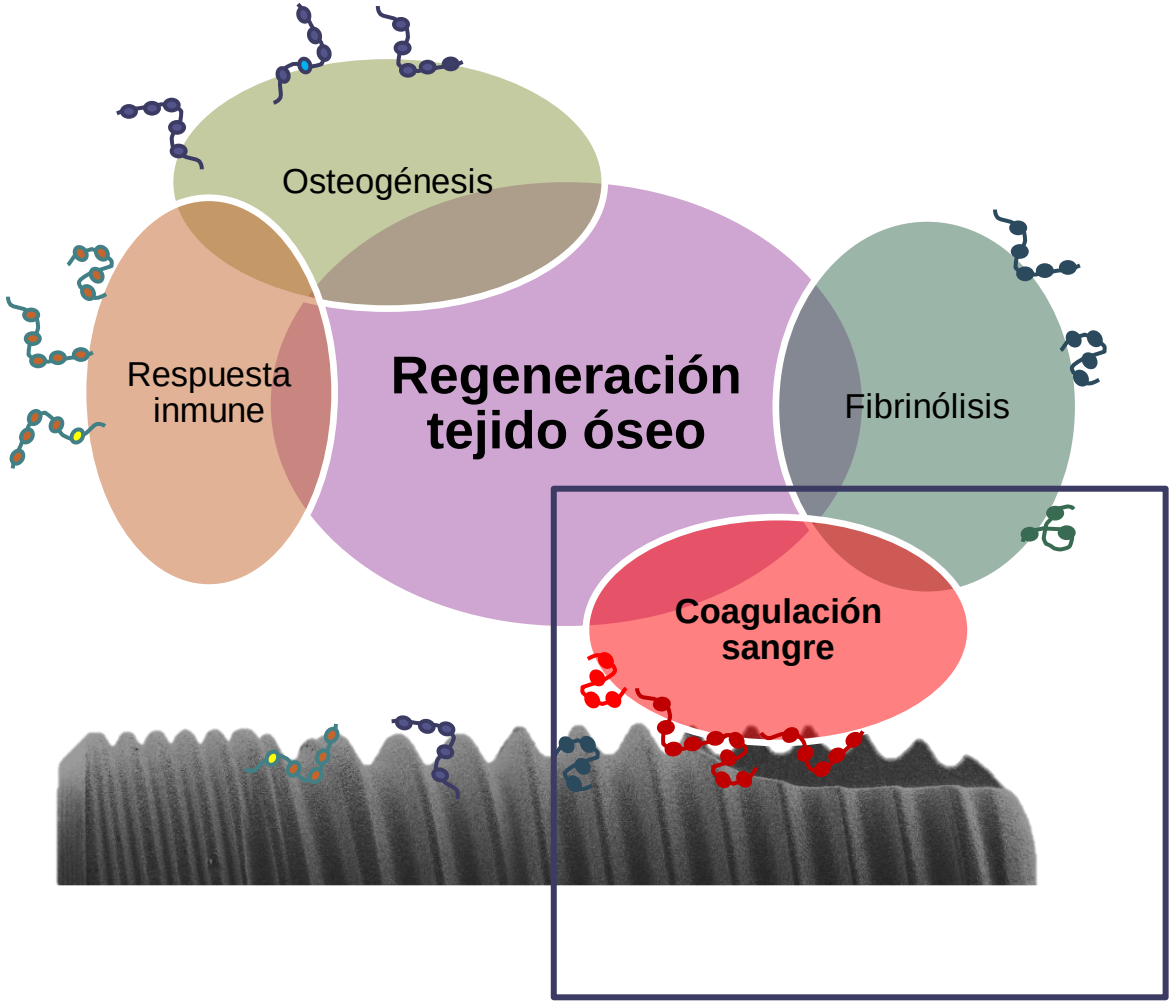
No se detecta una adsorción desproporcionada de proteínas del sistema del complemento, ni en los implantes de GMI, ni en los de Straumann.

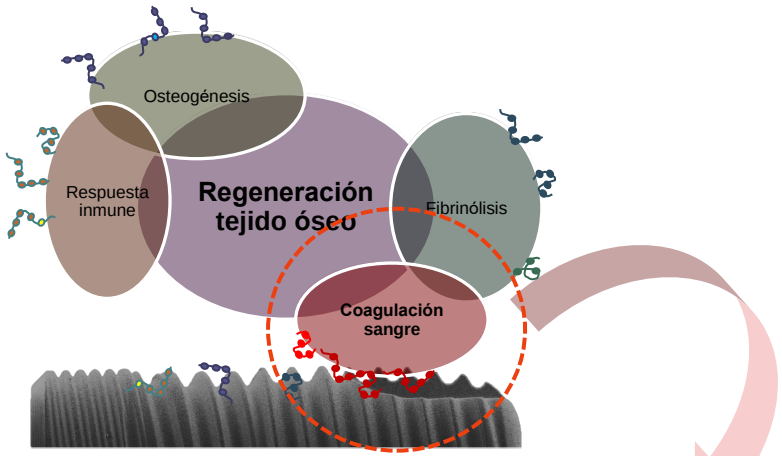
No hay proteínas relacioandas con la inflamación crónica (GMI & Straumann)





2º: COAGULACIÓN DE LA SANGRE



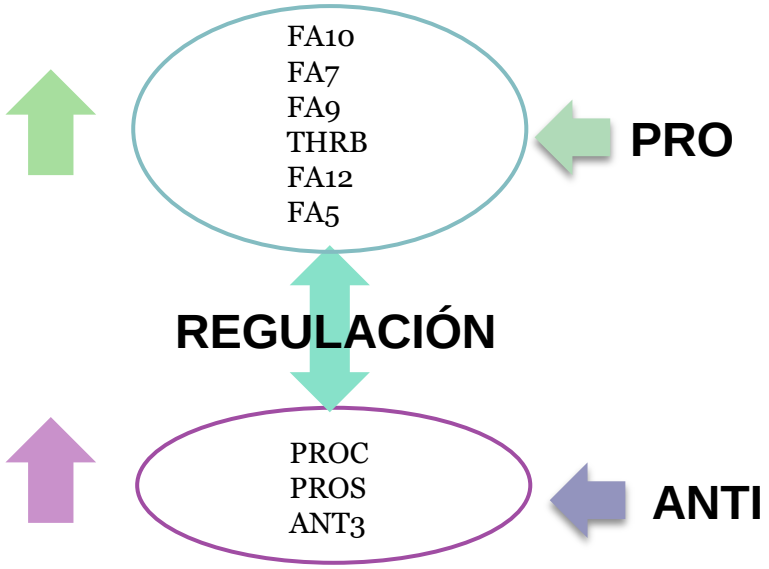


Comparación GMI vs ST

GMI: Alta actividad de coagulación:

Proteínas pro- y anti- coagulantes se adhieren más a la superficie de los implantes de GMI.

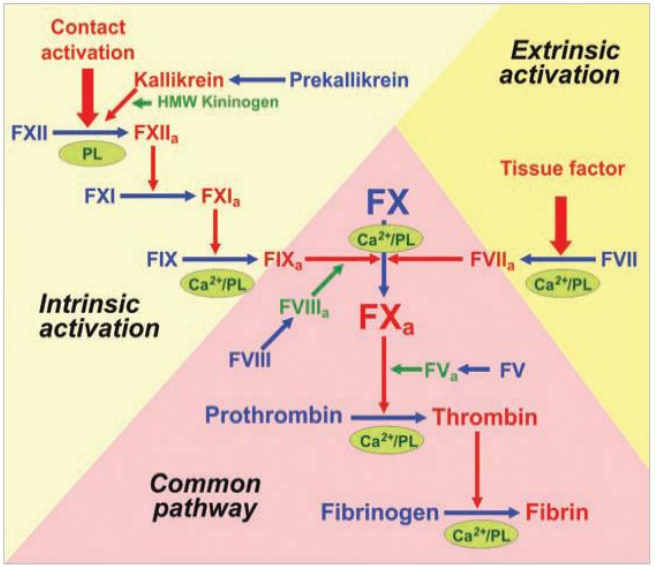
Accesión	Descripción	Valor p	Ratio
PROC_HUMAN	Vitamin K-dependent protein C	1,17E-06	27,21
FA10_HUMAN	Coagulation factor X	1,86E-04	17,21
FA7_HUMAN	Coagulation factor VII	1,04E-04	16,27
FA9_HUMAN	Coagulation factor IX	2,44E-04	5,47
PROS_HUMAN	Vitamin K-dependent protein S	2,07E-03	2,87
ANT3_HUMAN	Antithrombin-III	6,32E-04	2,33
THRB_HUMAN	Prothrombin	9,76E-04	2,31
FA12_HUMAN	Coagulation factor XII	1,15E-03	2,29
FA5_HUMAN	Coagulation factor V	2,80E-02	2,12
KNG1_HUMAN	Kininogen-1	6,98E-05	0,33
FA11_HUMAN	Coagulation factor XI	6,27E-03	0,33
A2MG_HUMAN	Alpha-2-macroglobulin	3,84E-03	0,11
FA11_HUMAN	Coagulation factor XI	6,27E-03	0,33
A2MG_HUMAN	Alpha-2-macroglobulin	3,84E-03	0,11

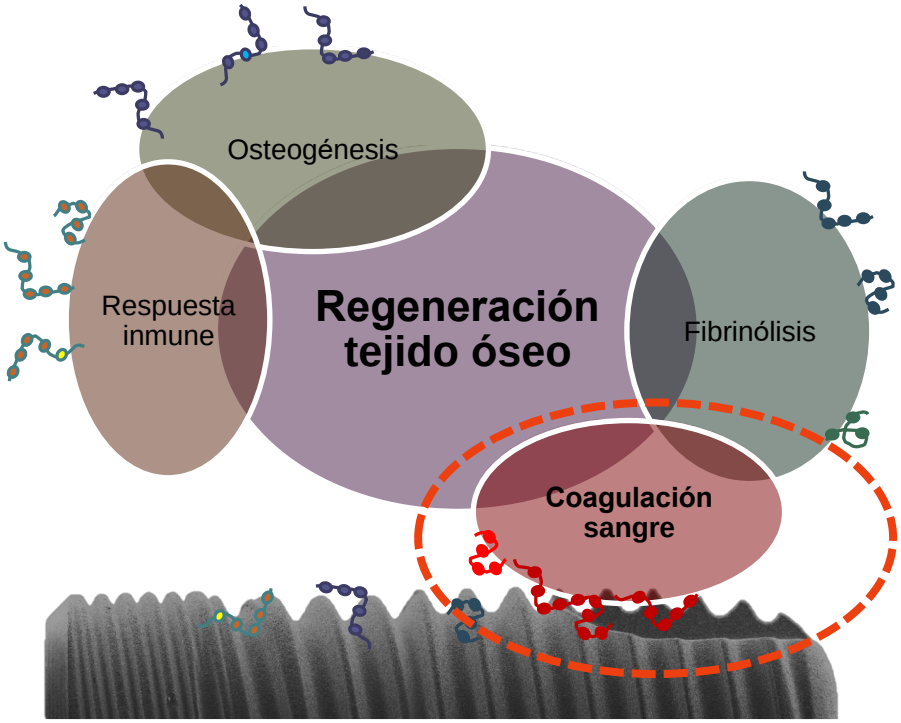


Los implantes GMI mejoran el proceso de coagulación

Prothrombin

Esta proteína juega un papel clave en la coagulación sanguínea

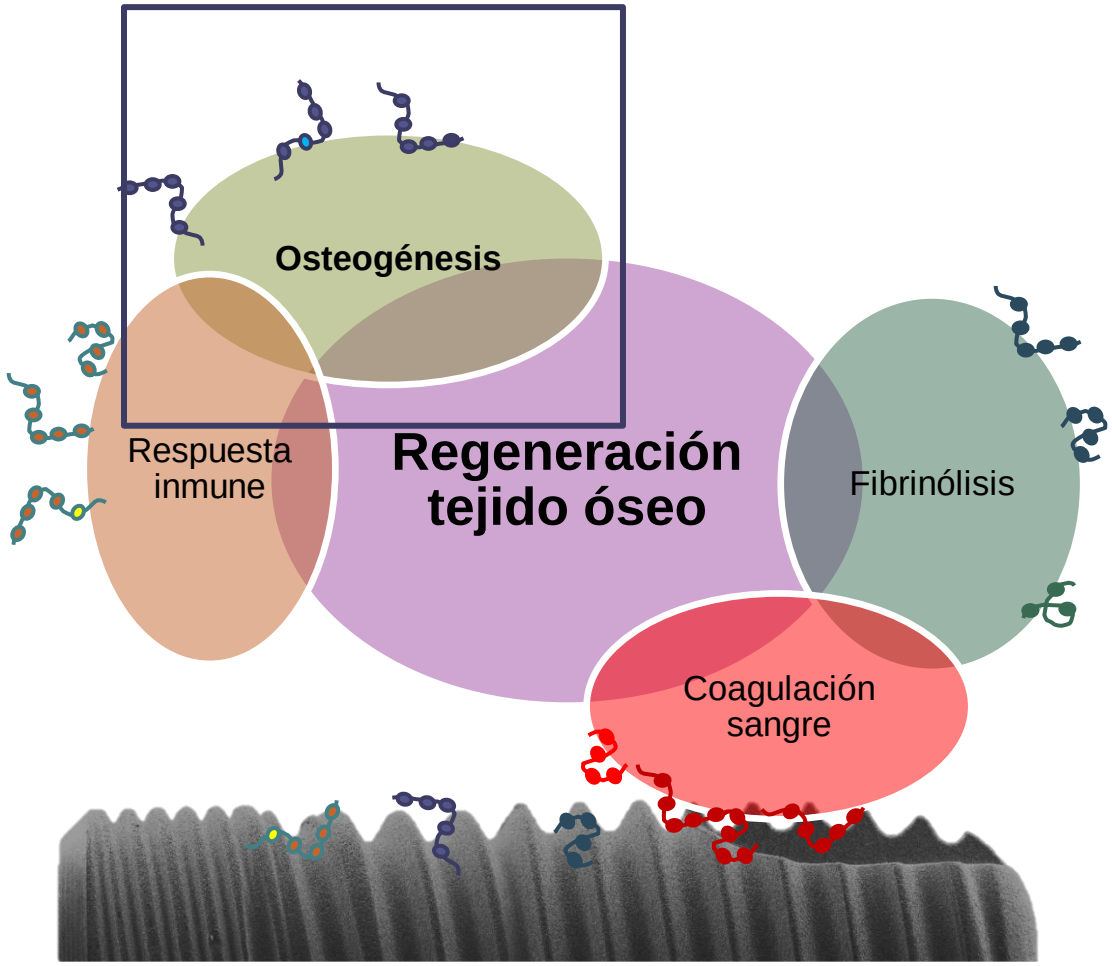




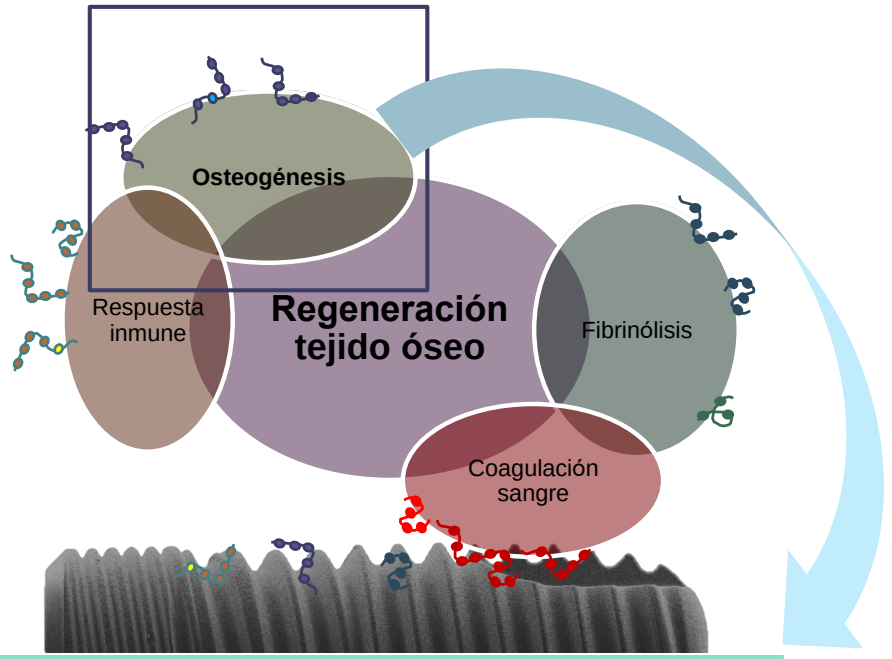
Sin embargo, la coagulación sanguínea es un sistema altamente regulado.

ANT3, PROC, PROS **podrían asegurar el desarrollo de un proceso correcto, evitando la inflamación aguda y la trombosis.**

3º: OSTEOGÉNESIS



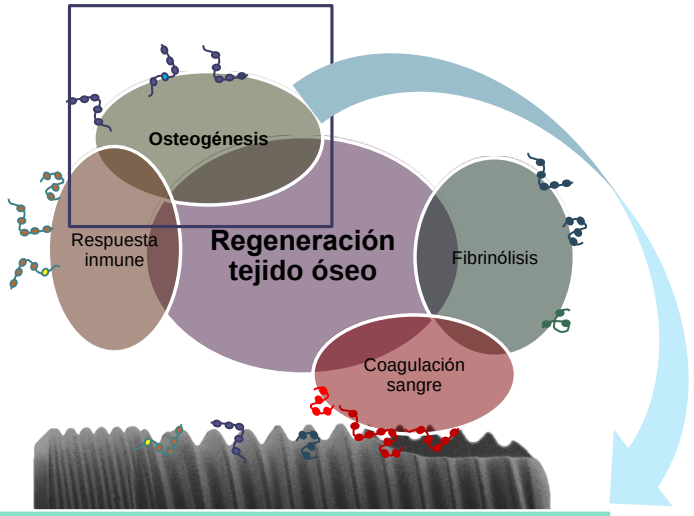
LOS IMPLANTES GMI MEJORAN LA OSTEOGÉNESIS



Mayor afinidad con implantes GMI

Accesión	Description
APOE_HUMAN	Apolipoprotein E
SEPP1_HUMAN	Selenoprotein P

GMI



Comparación GMI vs ST

Mayor afinidad con implantes GMI

GMI

Accesión	Description
APOE_HUMAN	Apolipoprotein E
SEPP1_HUMAN	Selenoprotein P

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Review

Selenoproteins and selenium status in bone physiology and pathology

Zhichao Zhang [a], Jinsong Zhang [b,*], Jianru Xiao [a,**]

[a] Department of Orthopaedic Oncology, Changzheng Hospital, The Second Military Medical University, Shanghai 200003, PR China
[b] School of Tea Food Science, Anhui Agricultural University, Hefei 230036, Anhui, PR China

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ABSTRACT

Background: Emerging evidence supports the view that selenoproteins are essential for maintaining bone health.
Scope of review: The current state of knowledge concerning selenoproteins and Se status in bone physiology and pathology is summarized.
Major conclusions: Antioxidant selenoproteins including glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), as a whole, play a pivotal role in maintaining bone homeostasis and protecting against bone loss. GPx1, a major antioxidant enzyme in osteoclasts, is up-regulated by estrogen, an endogenous inhibitor of osteoclastogenesis. TrxR1 is an immediate early gene in response to 1α,25-dihydroxyvitamin D3, an osteoblastic differentiation agent. The combination of 1α,25-dihydroxyvitamin D3 and Se generates a synergistic elevation of TrxR activity in Se-deficient osteoblasts. Of particular concern, pleiotropic TrxR1 is implicated in promoting NFκB activation. Coincidentally, TrxR inhibitors such as curcumin and gold compounds exhibit potent osteoclastogenesis inhibitory activity. Studies in patients with the mutations of selenocysteine insertion sequence-binding protein 2, a key trans-acting factor for the co-translational insertion of selenocysteine into selenoproteins have clearly established a causal link of selenoproteins in bone development. Se transport to bone relies on selenoprotein P. Plasma selenoprotein P concentrations have been found to be positively correlated with bone mineral density in elderly women.
General significance: A full understanding of the role and function of selenoproteins and Se status on bone physiology and pathology may lead to effectively prevent against or modify bone diseases by using Se.

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

The role of Apolipoprotein E in bone metabolism

Andreas Niemeier [a,b,*], Thorsten Schinke [c], Joerg Heeren [b], Michael Amling [c]

[a] Department of Orthopaedics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[b] Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[c] Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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ABSTRACT

Apolipoprotein E (apoE) is a major structural apolipoprotein of several lipoprotein classes. Over the last 13 years, numerous studies have focused on the question whether human apoE affects bone phenotypes and, more recently, whether apoE regulates bone metabolism in mice. Here, we first provide a brief background introduction into the structure, established physiological and pathophysiological functions of apoE, and will then discuss the new aspects of the emerging role of apoE in bone.

Potencial osteogénico de la proteína ApoE

Aumenta la diferenciación osteoblástica

Disminuye la diferenciación osteoclástica

54 BioScience Trends. 2016; 10(1):54-66.

Original Article

DOI: 10.5582/bst.2016.01006

Multifarious effects of 17-β-estradiol on apolipoprotein E receptors gene expression during osteoblast differentiation *in vitro*

Yuyan Gui[1,2,*], Zhongliang Duan[1,2,*], Xuemin Qiu[1,2], Wei Tang[3], Hans-Jürgen Gober[4], Dajin Li[1], Ling Wang[1,2,**]

[1] *Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, IBS, Fudan University Shanghai Medical College, Shanghai, China;*
[2] *Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Shanghai, China;*
[3] *Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;*
[4] *Department of Pharmacy, Wagner Jauregg Hospital and Children's Hospital, Linz, Austria.*

Summary **Apolipoprotein E (ApoE) regulated bone metabolism in mice might mediate uptake of lipid particles into target cells such as osteoblasts *via* receptor-mediated endocytosis by apoE receptors, which includes the low-density lipoprotein receptor (LDLR) family and heparan sulfate proteoglycans (HSPGs). There is no report regarding the expression of ApoE receptors mRNA induced by estrogen during osteoblast differentiation *in vitro*. Primary osteoblasts were collected from the calvaria of newborn mice and were subjected to osteoblast mineralization culture with serial concentrations of 17-β-estradiol (E2) *in vitro*. RNA was isolated at days 0, 5 and 25 of differentiation. Real-time PCR was conducted to analyze apoE receptors mRNA levels. We found that most LDLR family members genes were induced during osteoblast differentiation *in vitro*. The effect of E2 on apoE receptors gene expression during osteoblast differentiation was multifarious. The most noted members of the LDLR family involved in the maintenance of bone metabolism were LRP5, LRP6, LRP4, and Apoer2. LRP6 was up-regulated, while LRP5, LRP4, and Apoer2 were down-regulated by E2. Given that LRP6 is required for early stages of differentiation, we speculate E2 promotes osteoblast differentiation mainly in the early stage.**

Keywords: 17-β-estradiol,Apolipoprotein E receptors, Low-density Lipoprotein Receptors Family, Heparan sulfate proteoglycans, Osteoblast differentiation, Reproductive endocrine metabolic network

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Research Article

Apolipoprotein E inhibits osteoclast differentiation via regulation of c-Fos, NFATc1 and NF-κB

Woo-Shin Kim[a], Hyung Joon Kim[a], Zang Hee Lee[a], Youngkyun Lee[b], Hong-Hee Kim[a,]*

[a] *Department of Cell and Developmental Biology, BK21 Program and Dental Research Institute, Seoul National University, 28 Yeongon-Dong, Chongno-Gu, Seoul 110-749, Republic of Korea*
[b] *Department of Biochemistry, School of Dentistry, Kyungpook National University, Daegu 700-422, Republic of Korea*

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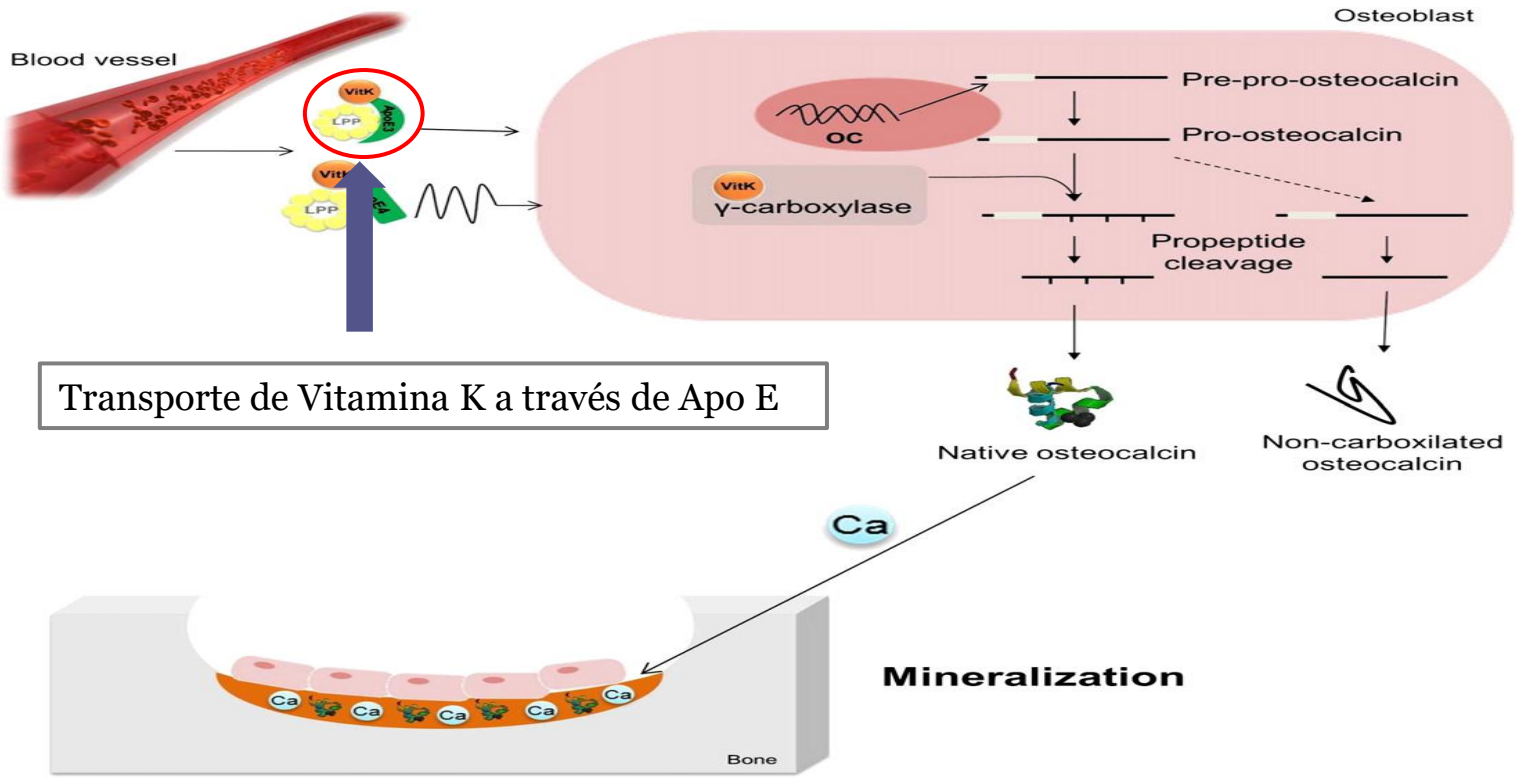
NF-κB

ABSTRACT

Apolipoprotein E (ApoE) plays a major role in the transport and metabolism of lipid. Other functions of ApoE include modulation of innate and adaptive immune responses. The expression of ApoE in osteoblasts and its relevance with bone formation have also been reported. However, the effect of ApoE on osteoclasts has not yet been examined. Here, we investigated the role of ApoE in osteoclast differentiation using bone marrow-derived macrophages (BMMs) and RAW264.7 cells. We found a down-regulation of ApoE gene expression during osteoclastic differentiation of those cells. Overexpression of ApoE in BMMs and RAW264.7 cells significantly blocked the induction of c-Fos and nuclear factor of activated T cell c1 (NFATc1), transcription factors critical for expression of osteoclast marker genes, by receptor activator of nuclear factor κB ligand (RANKL), the osteoclast differentiation factor. ApoE inhibited osteoclast differentiation, as measured by decreased number of tartrate-resistant acid phosphatase (TRAP)-positive multinuclear cells (MNCs). In addition, ApoE reduced the expression of dendritic cell-specific transmembrane protein (DC-STAMP) and ATPase, H^+ transporting, lysosomal 38 kDa, V0 subunit d2 (ATP6v0d2), genes involved in cell–cell fusion during osteoclastogenesis. Knock-down of ApoE using a specific siRNA promoted the RANKL-mediated induction of osteoclast differentiation. While ApoE did not affect the activation of ERK, JNK, and p38 MAPK signaling pathways by RANKL, the phosphorylation of p65 trans-activation domain on serine 536 and transcription activity of NF-κB were reduced by ApoE overexpression. These findings suggest that ApoE plays an inhibitory role in osteoclast differentiation via the suppression of RANKL-dependent activation of NF-κB and induction of c-Fos and NFATc1.

Papel de la vitamina K y la ApoE: importancia en el metabolismo óseo



Transporte de Vitamina K a través de Apo E

Rodrigues, A. *et al.* Low osteocalcin/collagen type I bone gene expression ratio is associated with hip fragility fractures. Bone 51, 981–9 (2012)

CONCLUSIONES

ACTIVIDAD	GMI	STRAUMMAN
RESPUESTA INMUNE	—	—
COAGULACIÓN SANGRE	↑	↓
OSTEOGÉNESIS	↑	↓