GMI STUDY.
COMPARATIVE PROTEOMIC STUDY BETWEEN GMI and STRAUMANN DENTAL IMPLANTS

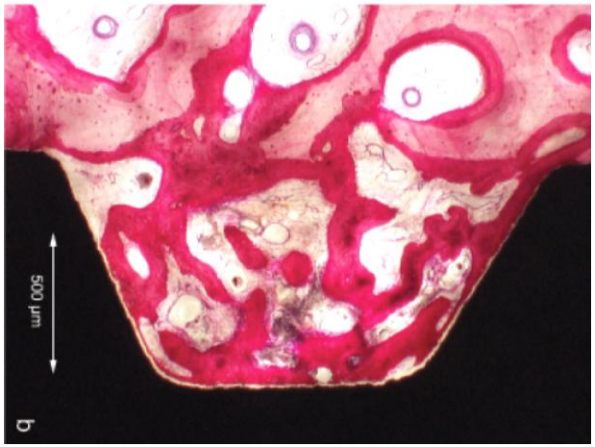
PIMA Grup de Polímers i Materials Avançats | Universitat Jaume I | Universidad del País Vasco - Euskal Herriko Unibertsitatea | CIC bioGUNE Bilbao

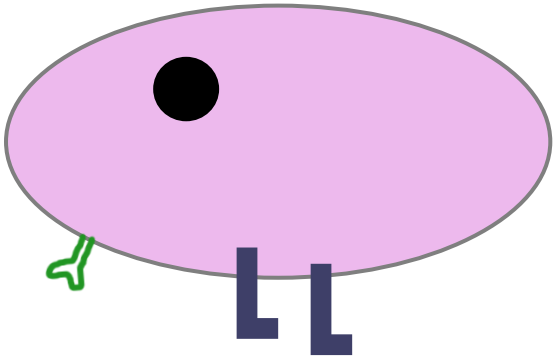
Hypothesis. Proteomic study of first protein layer

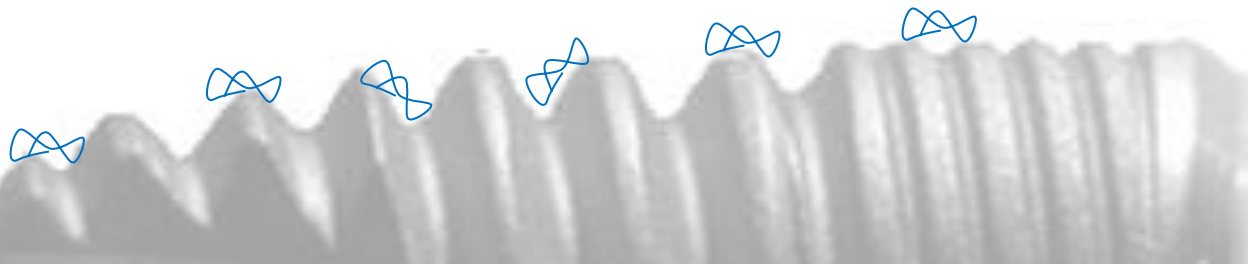
Post-implantation, the biomaterial becomes in contact with the blood,

Protein adsorption on its surface

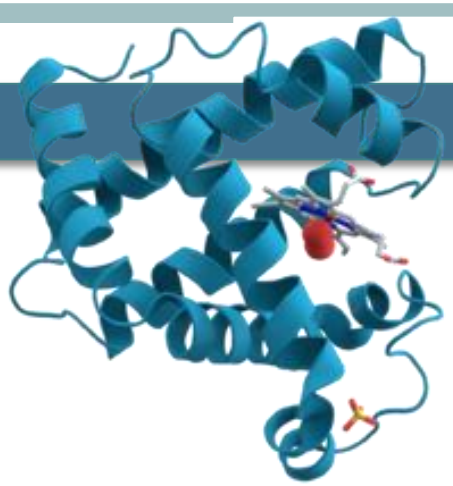
(Andersson, 2005).







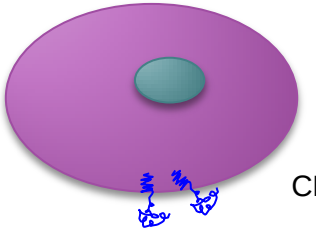
Protein importance on biomaterial

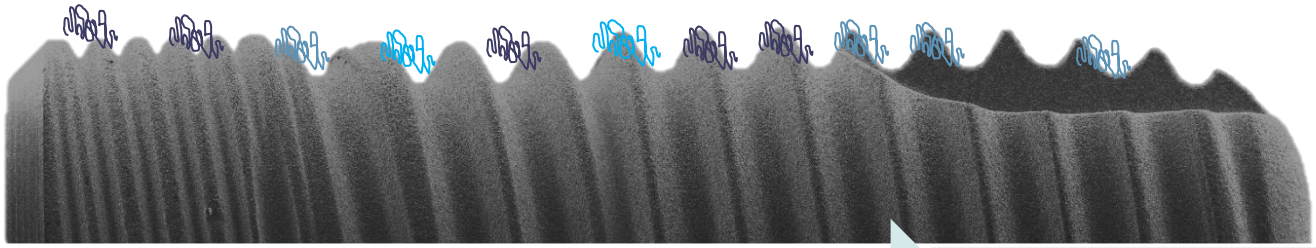


The first layer of adsorbed proteins will define

The biological response to the material

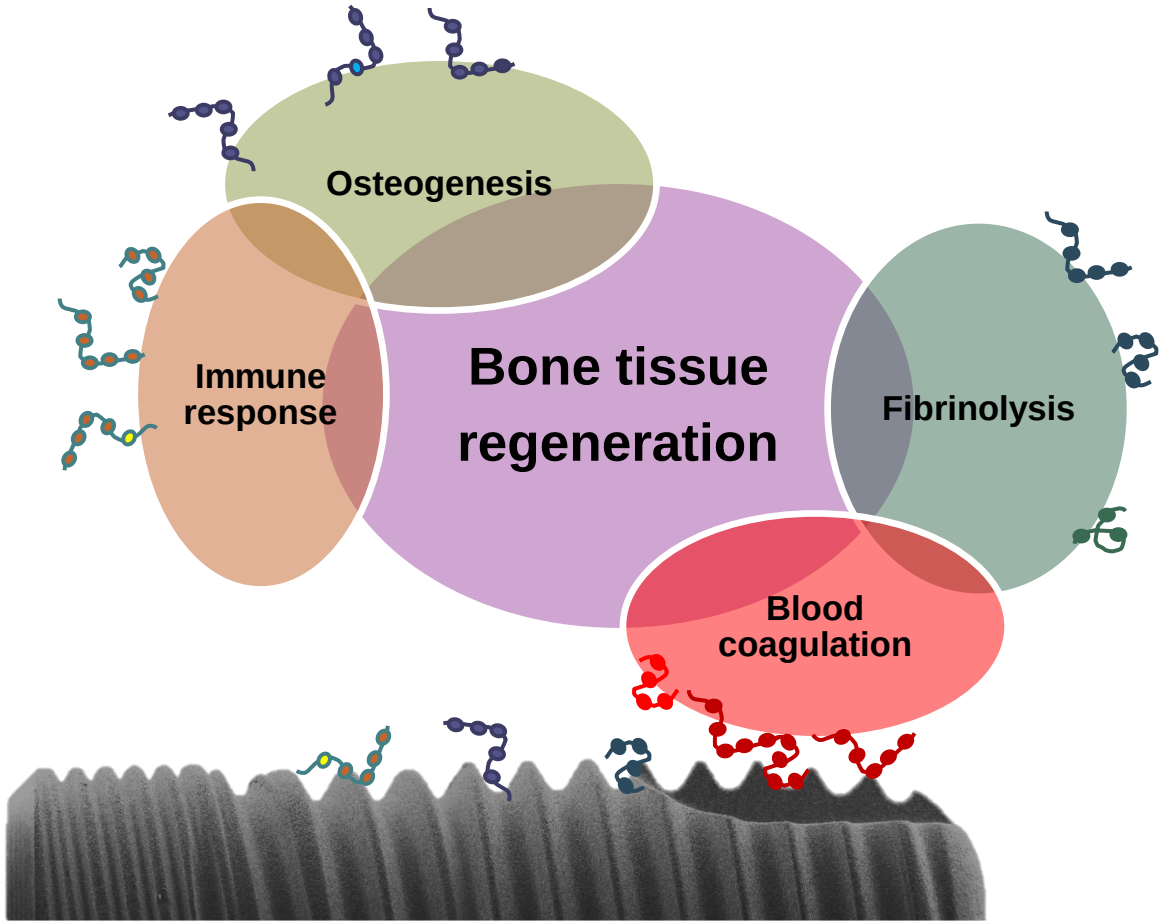
(Engberg, 2015)





Cell-Protein-Material interaction → **Regeneration**

GMI proteomic comparative study



Proteomics: methodology

1

Incubation on human serum (3h 37ºC)

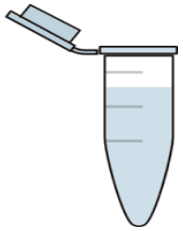
Consecutive washes to eliminate the non-adsorbed proteins

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

Biomaterial surface-attached protein elution

2

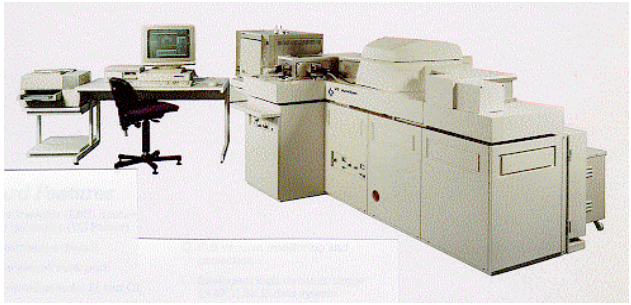
4% SDS, 100 mM DTT in 0.5M TEAB



Final elution

3

Quantification and identification:
Mass Spectrometry (MS/MS)



Data analysis: **PROGENESIS**

DAVID (**D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery)
Biological data base of protein families, useful to protein function identification and classification

4

COMPARATIVE PROTEOMIC STUDY:

ST

GMI

gmi
ilerimplant group

Comparison GMI vs ST

Proteins with higher affinity with GMI implants

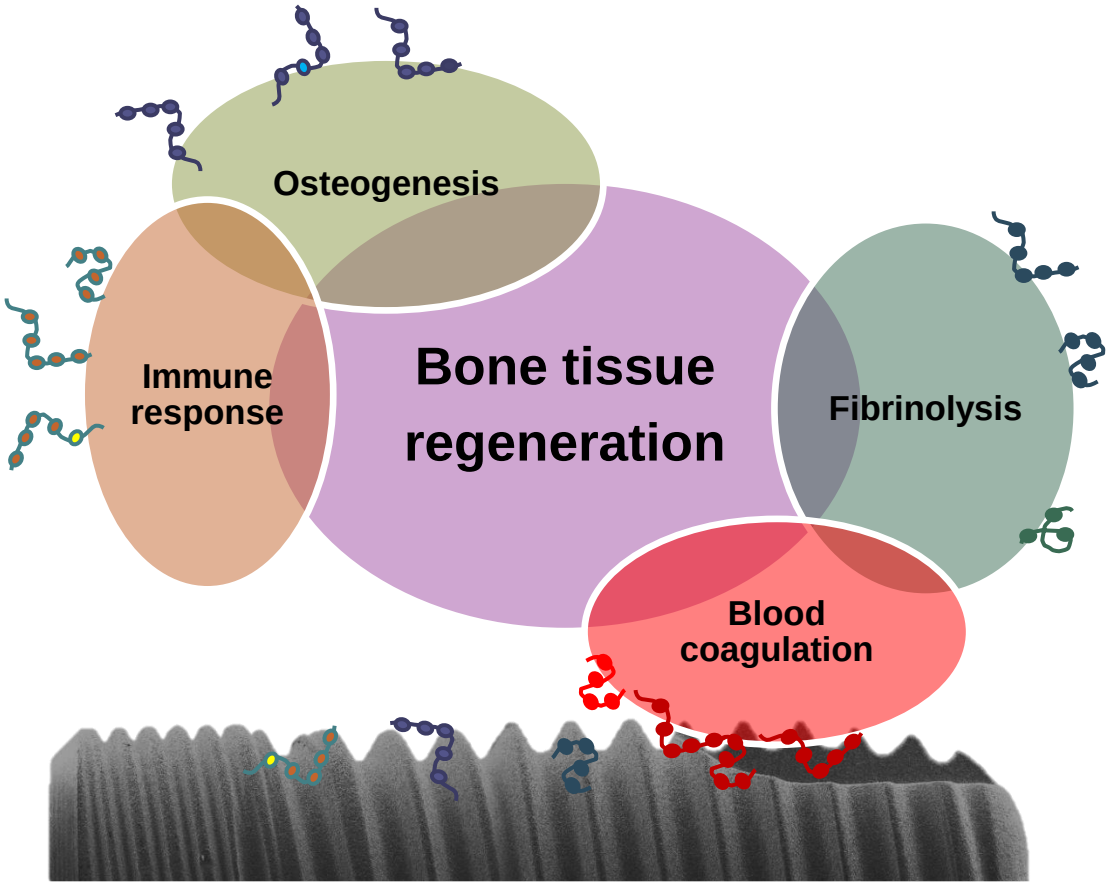
Accession	Peptide count	Description	GMI/ST p value	GMI/ST Ratio
PROC_HUMAN	8	Vitamin K-dependent protein C	1,17E-06	27,21
FA10_HUMAN	8	Coagulation factor X	1,86E-04	17,21
FA7_HUMAN	3	Coagulation factor VII	1,04E-04	16,27
TAGL2_HUMAN	2	Transgelin-2	2,40E-05	11,54
SAA1_HUMAN	2	Serum amyloid A-1 protein	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

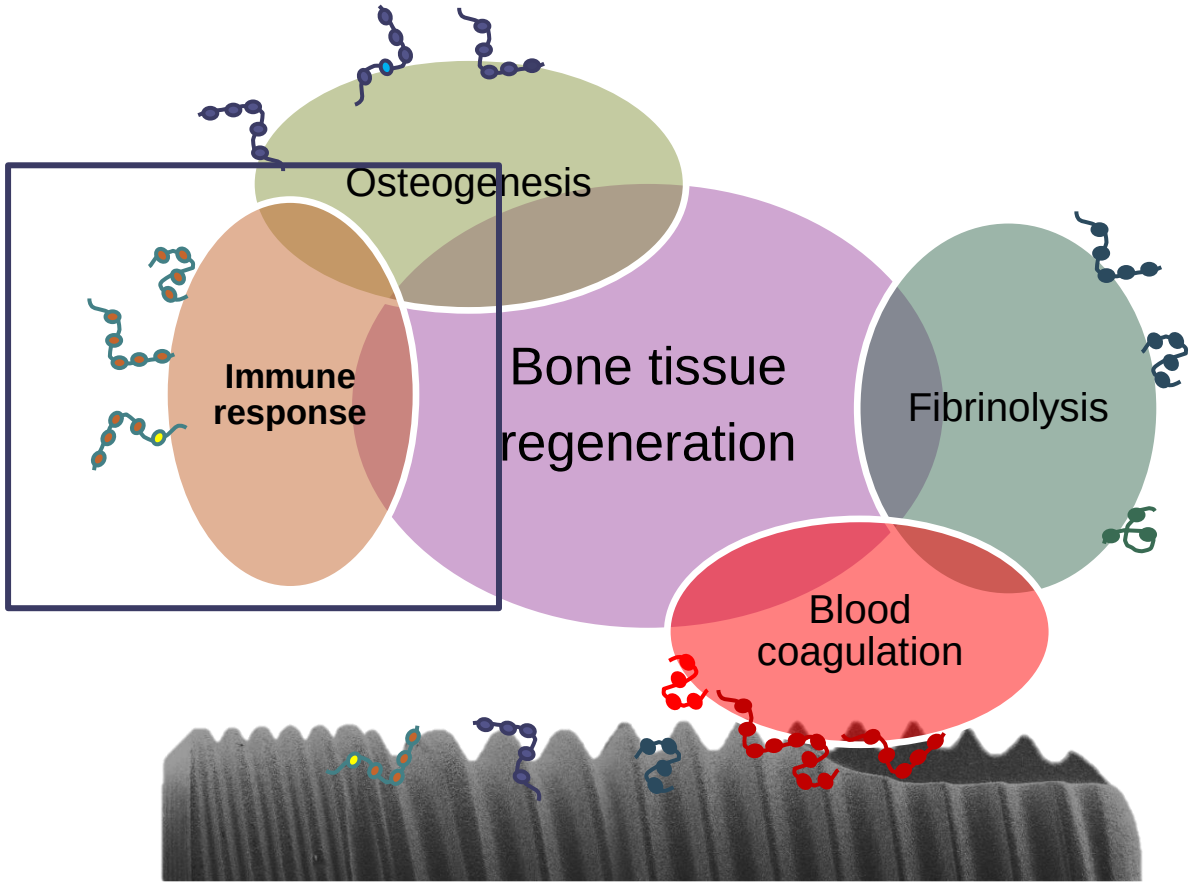
Proteins with lower affinity with GMI implants

			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

Complex process



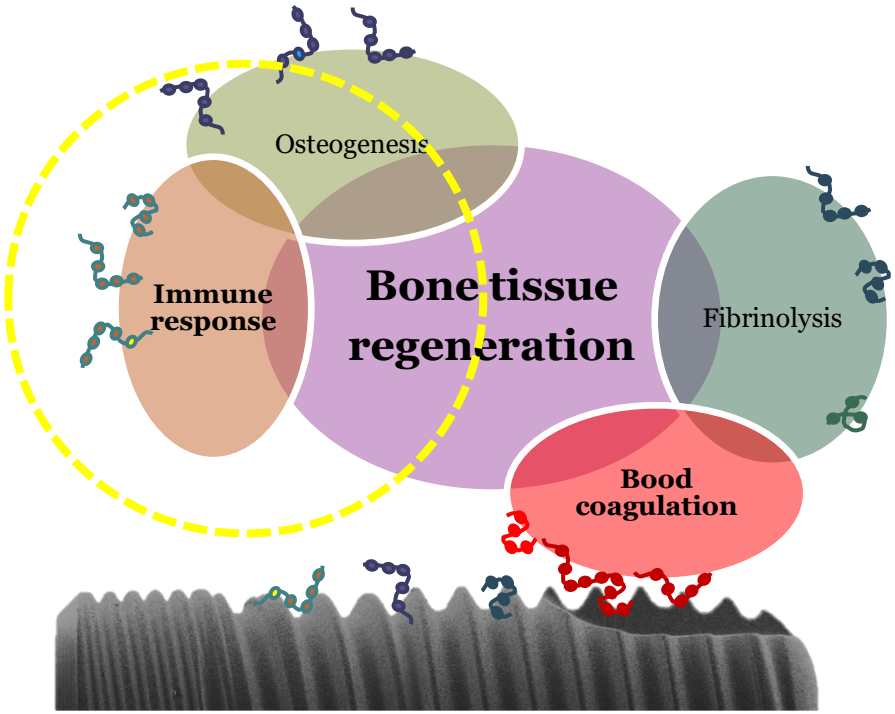
1º: IMMUNE RESPONSE

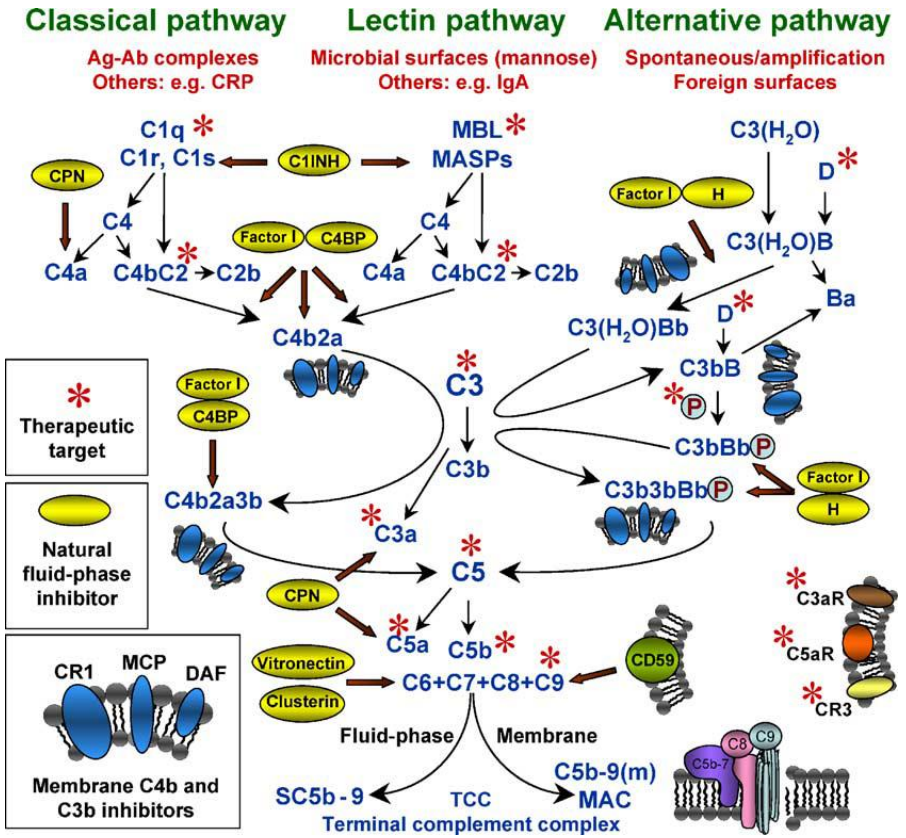


Complement system cascade

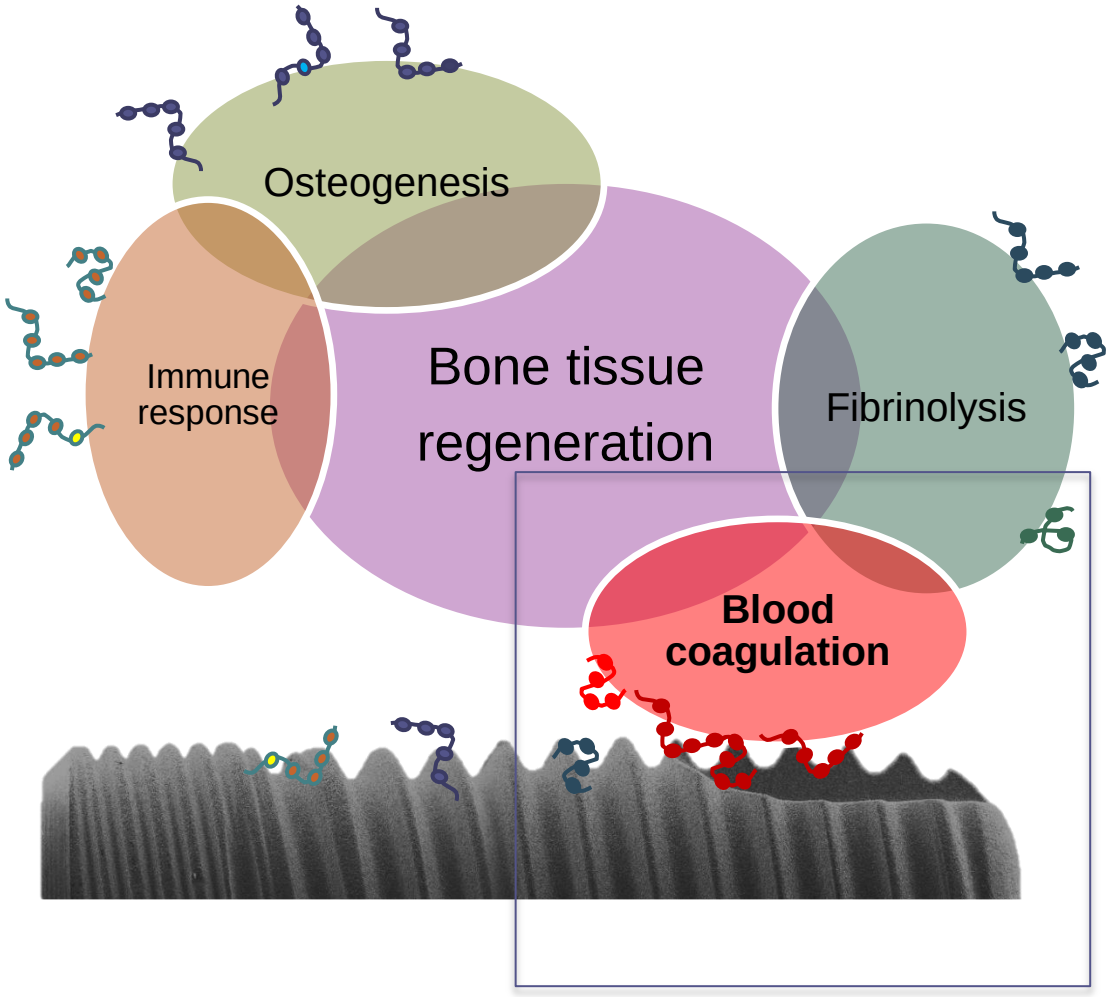
Desproportionated adsoption of complement system proteins was not detected in GMI or Straumann.

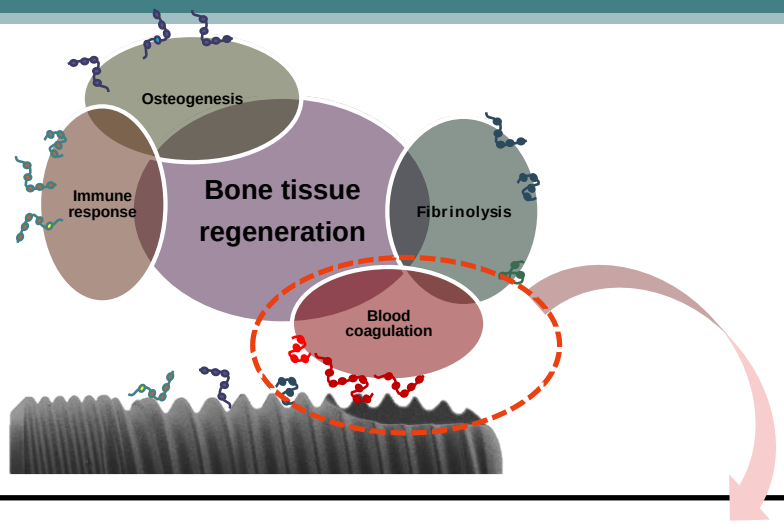
No proteins related to chronic inflammation(GMI & Straumann)





2º: BLOOD COAGULATION



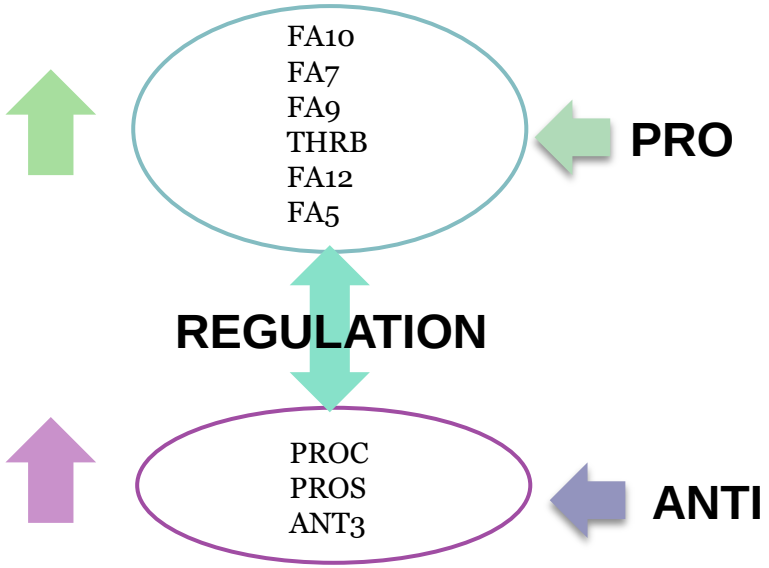


Comparison GMI vs ST

GMI: Higher coagulation activity:

Pro- and anti- coagulant proteins were found more attached onto GMI surface

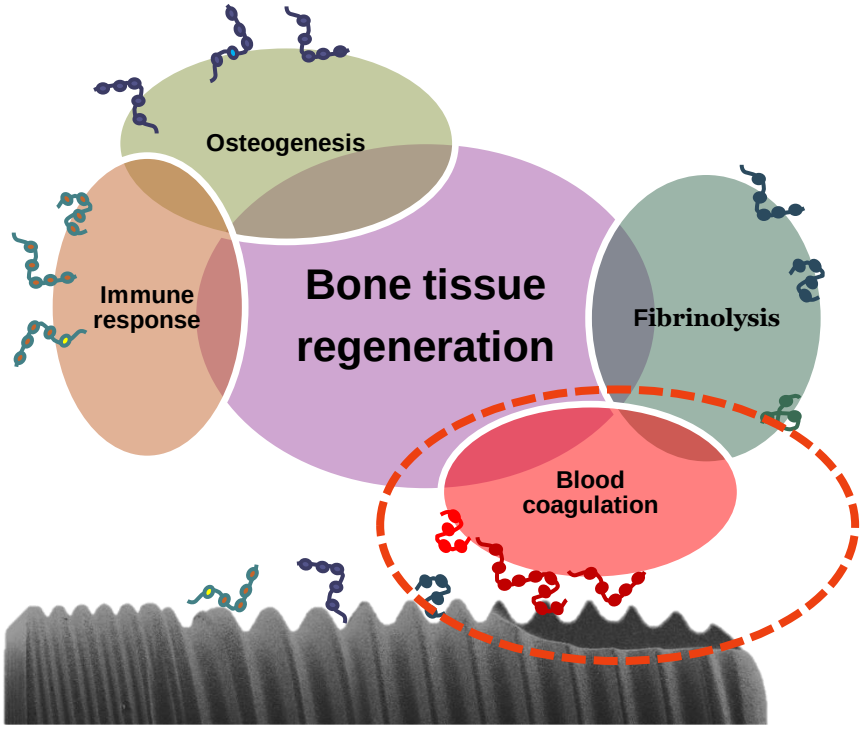
Accession	Description	p value	Ratio
PROC_HUMAN	Vitamin K-dependent protein C	1,17E-06	27,21
FA10_HUMAN	Coagulation factor X	1,86E-04	17,21
FA7_HUMAN	Coagulation factor VII	1,04E-04	16,27
FA9_HUMAN	Coagulation factor IX	2,44E-04	5,47
PROS_HUMAN	Vitamin K-dependent protein S	2,07E-03	2,87
ANT3_HUMAN	Antithrombin-III	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

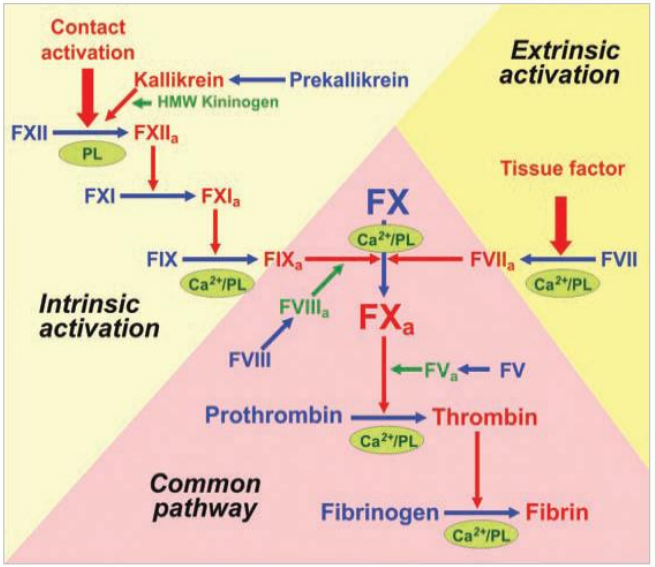


GMI implants IMPROVE coagulation process

Prothrombin

This protein display a key role in blood clotting

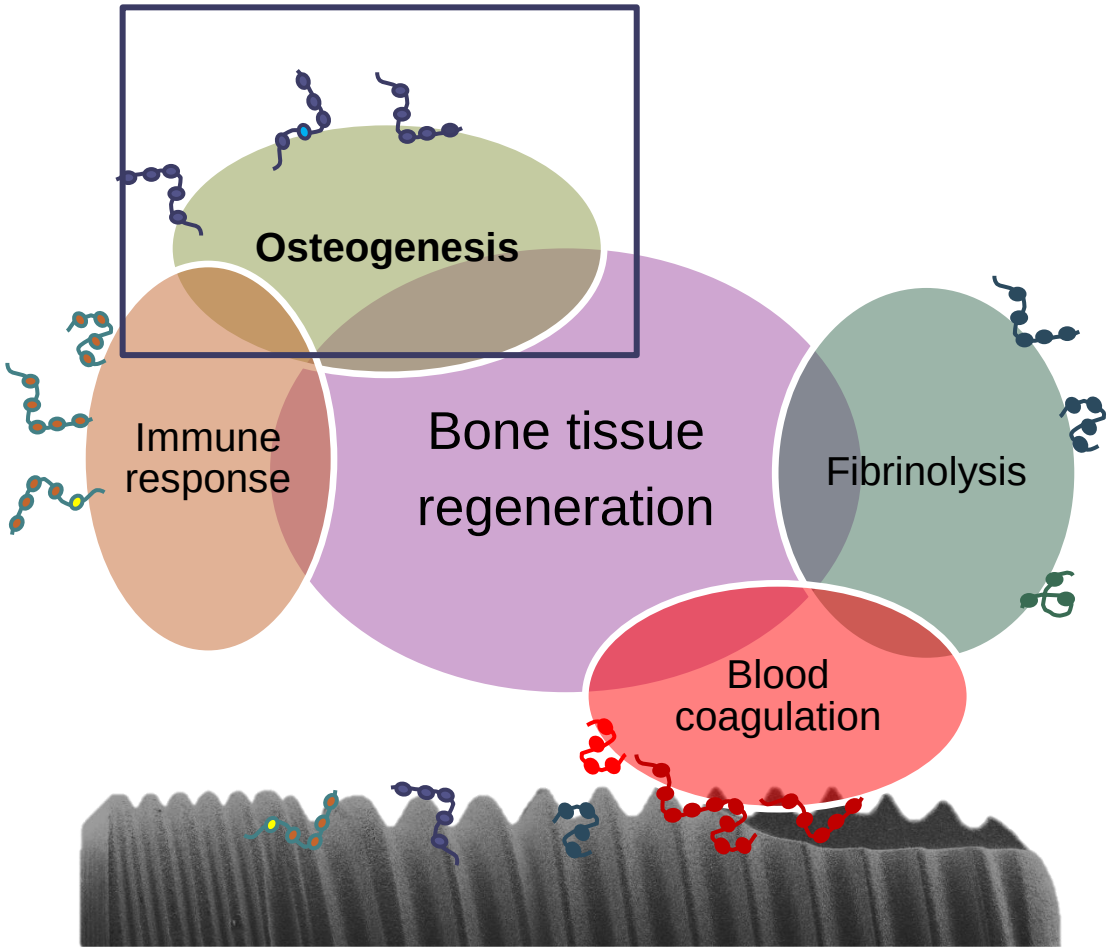




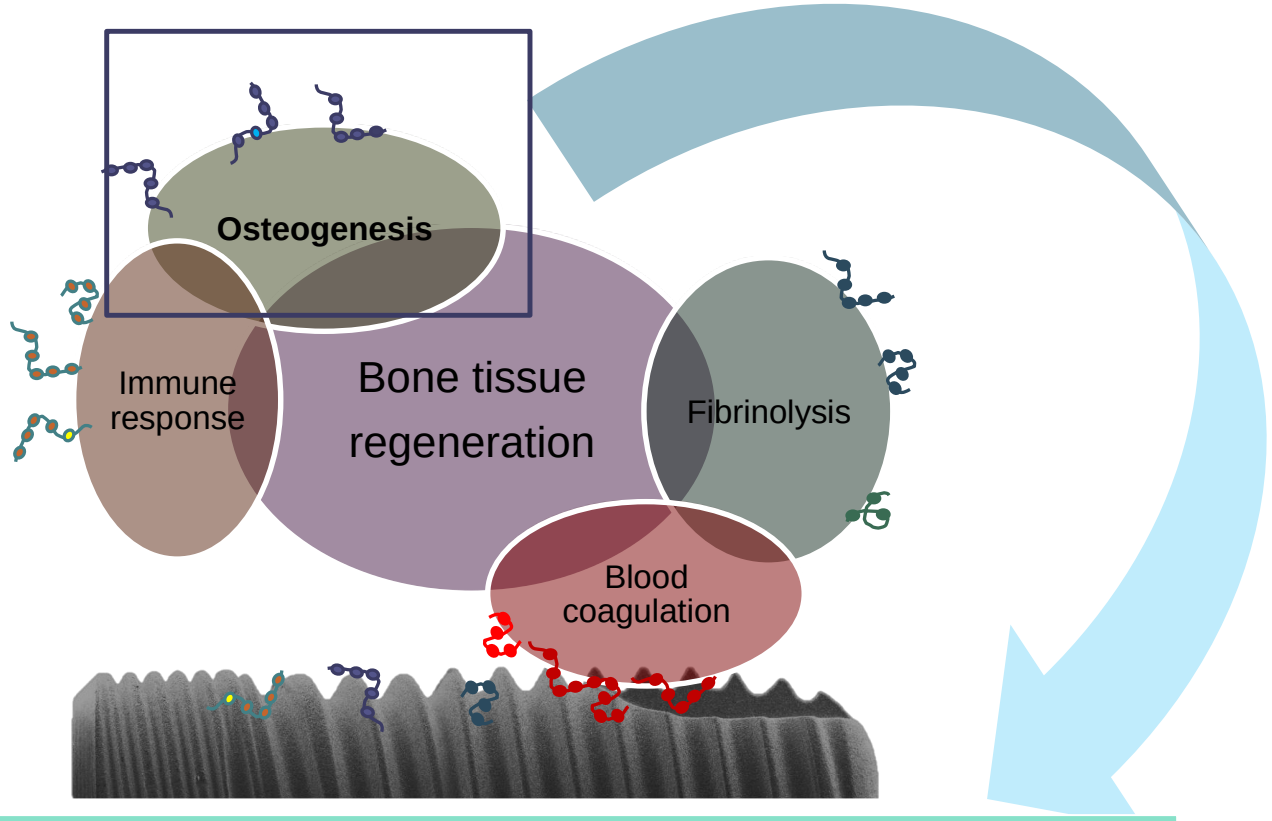
However, blood coagulation is a high regulated system.

ANT3, PROC, PROS **could ensure the correct process development in order to avoid an acute inflammation and thrombosis**

3º: OSTEOGENESIS



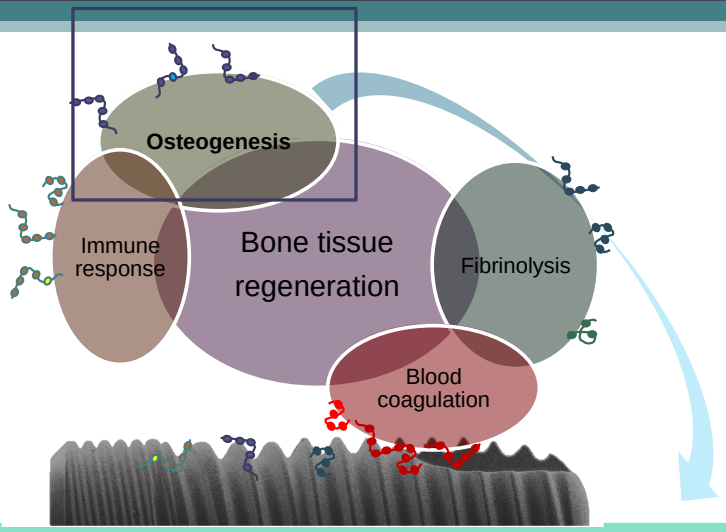
GMI IMPLANTS IMPROVE OSTEOGENESIS



Osteogenic proteins, higher affinity to GMI implants

Accession	Description
APOE_HUMAN	Apolipoprotein E
SEPP1_HUMAN	Selenoprotein P

GMI



Comparison GMI vs ST

Higher affinity to GMI

GMI

Accession	Description
APOE_HUMAN	Apolipoprotein E
SEPP1_HUMAN	Selenoprotein P

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Review

Selenoproteins and selenium status in bone physiology and pathology

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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.

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Bone

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Original Full Length Article

The role of Apolipoprotein E in bone metabolism

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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.

Osteogenic potential of ApoE protein

Increase osteoblastic differentiation

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*

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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

Decrease osteoclastic differentiation

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Research Article

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

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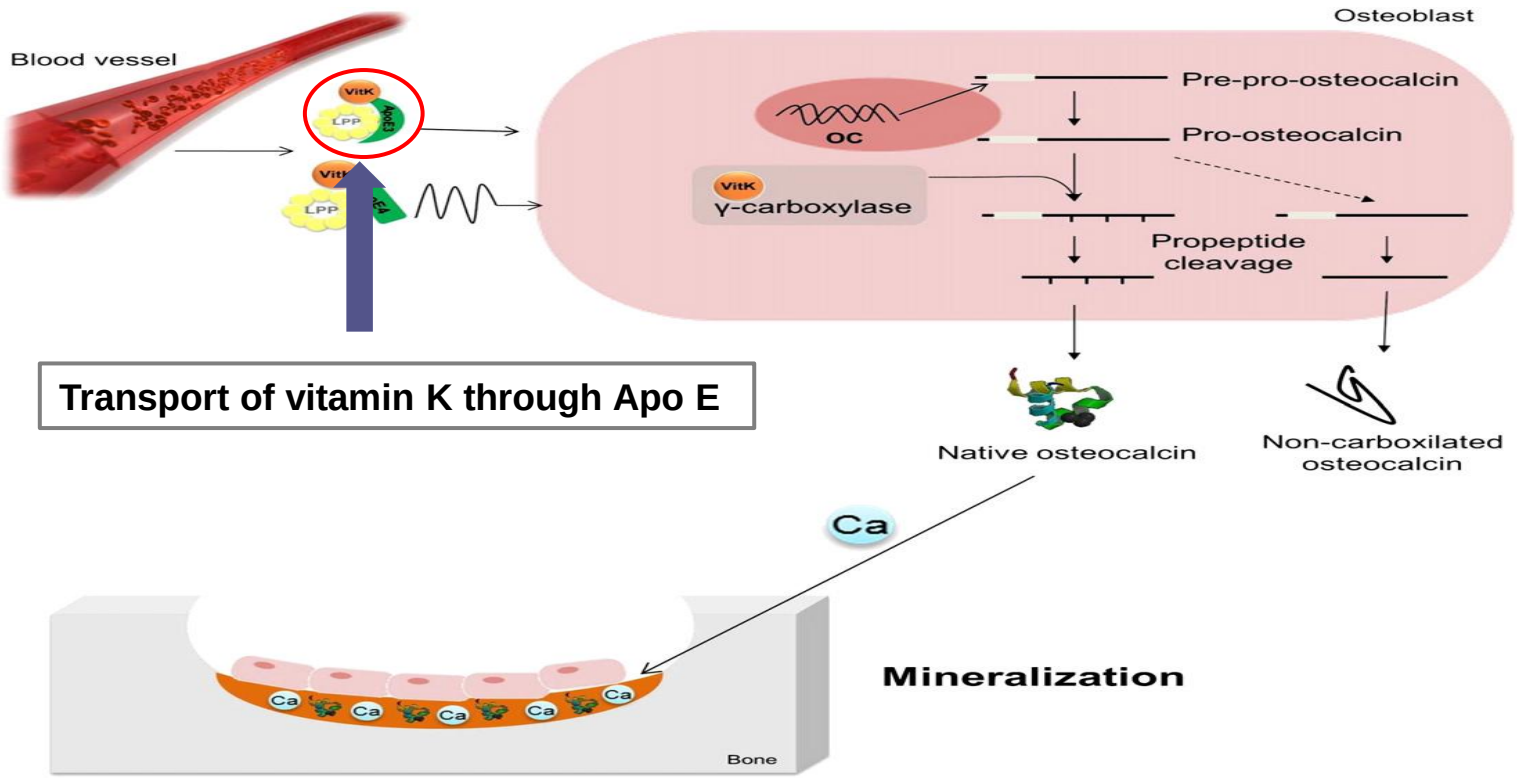
NFATc1
NF-κB

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.

Role of vitamin K and ApoE: importance in bone metabolism



Transport of vitamin K through 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)

CONCLUSIONS

ACTIVITY	GMI	STRAUMMAN
IMMUNE RESPONSE	—	—
BLOOD COAGULATION	↑	↓
OSTEOGENESIS	↑	↓