האיגוד הישראלי לכירורגית לב וחזה
THE ISRAEL SOCIETY OF CARDIOTHORACIC SURGERY
האיגוד הקרדיולוגי בישראל
ISRAEL HEART SOCIETY
The 60th International Conference of the Israel Heart Society
in association with the Israel Society of Cardiothoracic Surgery
22-23 April 2013, ICC International Convention Center, Jerusalem

Cell –Matrix Interactions In The Pathobiology of Calcific Aortic Valve Disease.

Lena Naser[1] ; Suzan Abedat [1] ; Ronen Beeri [1] ; Abeer Risheq[1]; Zvi Bar-Shavit[2]; Chaim Lotan[1], Mony Shuvy [1]

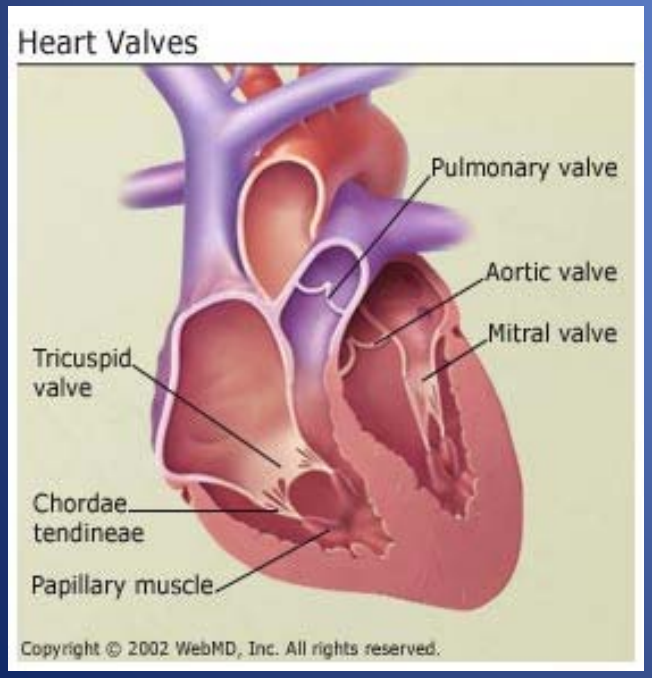
[1]Heart Institute, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

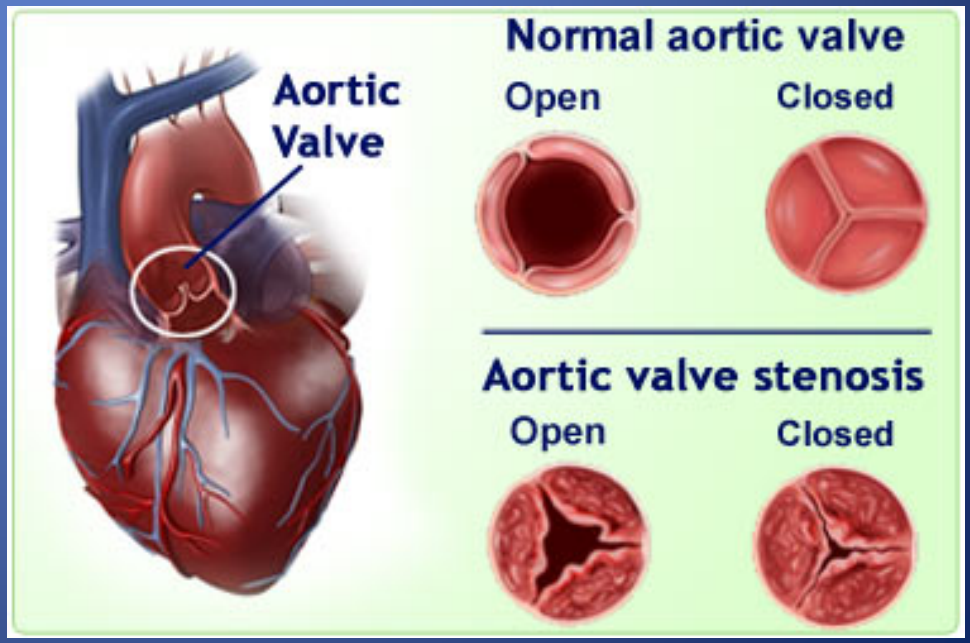
Calcific Aortic Stenosis

• Calcific Aortic Stenosis(AS) occurs when a valve opening is smaller than normal, due to stiff or fused leaflets.

•Its commonly caused by progressive calcification of aortic valve.

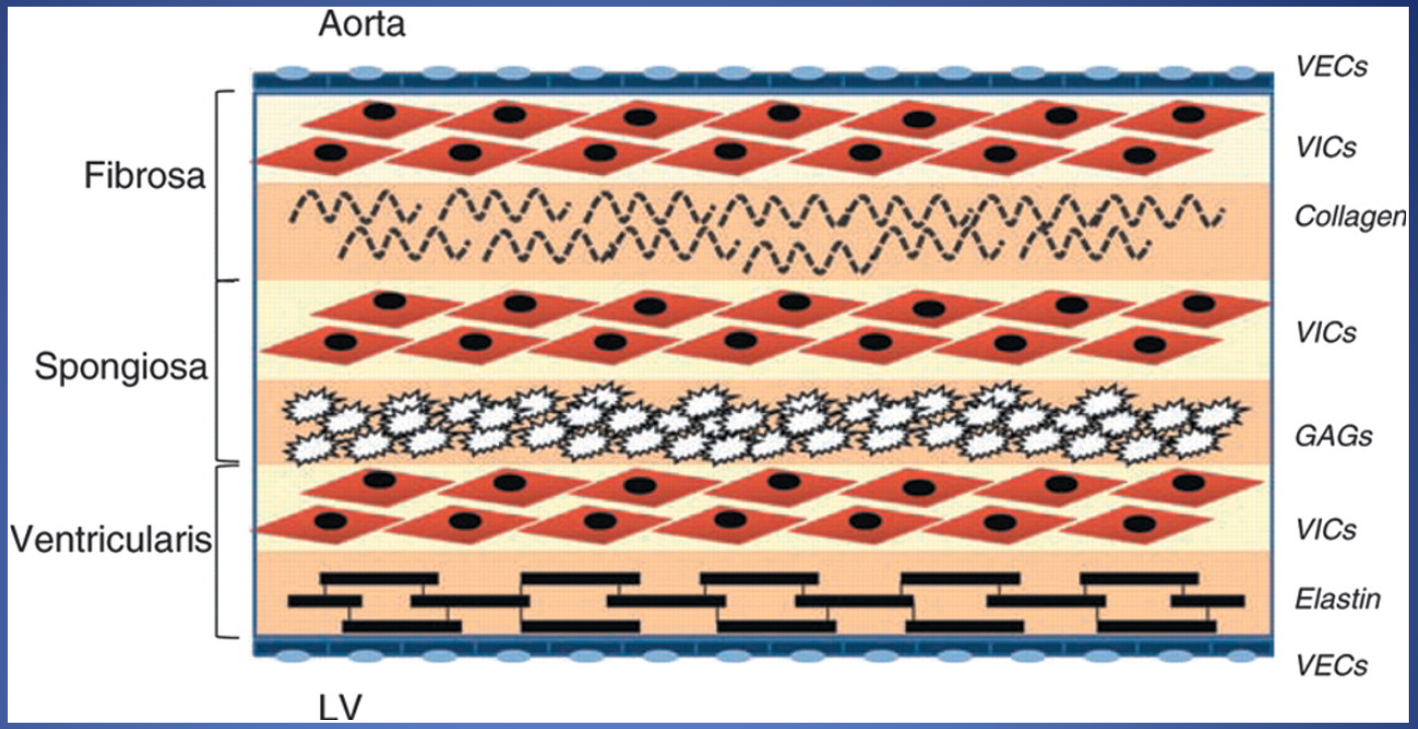
•AS is the most common cause of aortic valve replacement .





Normal Aortic Valve structure and ECM

Aortic valve



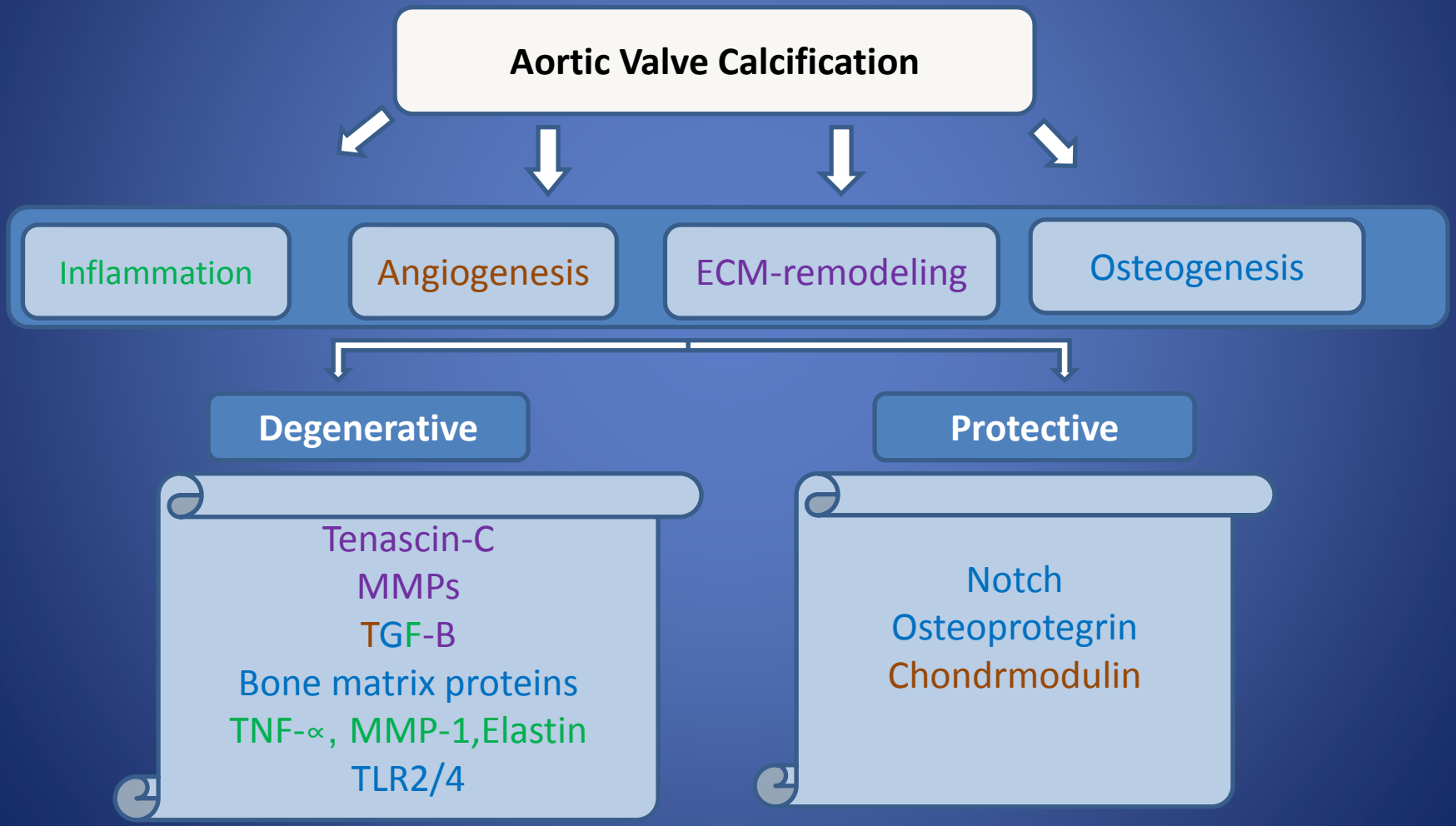
Functions of ECM :

1)Structural support

2) mechanical support

3) Provide biological signalling during tissue remodeling.

Possible molecular mechanisms underlying the onset of degenerative aortic valve disease



Ertan Y, Johannes ,Molecular and cellular mechanisms of aortic stenosis. International Journal of Cardiology (2009) 135(1):4-13

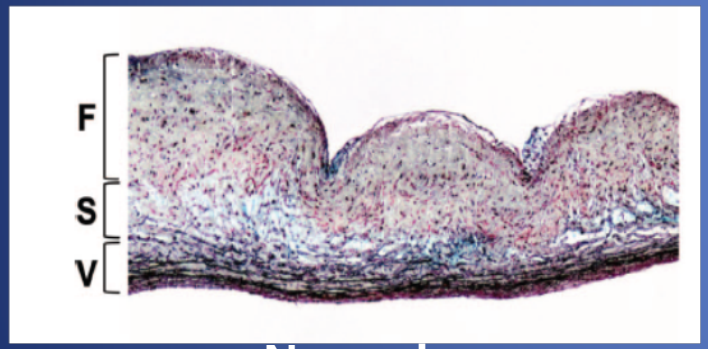
Daihiko H, Naritaka K, Masatoyo Y, Keiichi F. Molecular mechanisms underlying the onset of degenerative aortic valve disease. J Mol Med (2009) 87:17–24

ECM Changes in CAVD

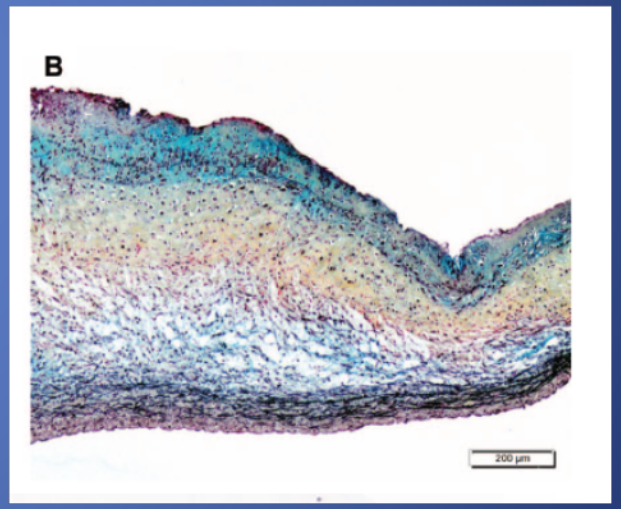
•The trilayer structure of the valve ECM is disrupted in CAVD.

• Disorganized ECM protein synthesis and degradation, changes in the localization and expression of ECM components contribute to development of CAVD.

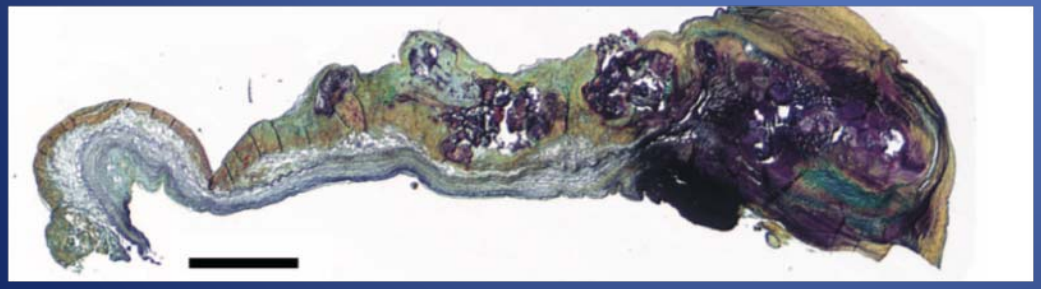
•lesions and calcification occur preferentially in the fibrosa layer.



Normal



abnormal



abnormal

[Circulation Research]

Valve ECM

1) Collagen

2) Proteoglycan and glycosaminoglycans

3) Elastin

4) Chondromodulin-I(gLycoprotien)

5) Periostin

6) Tenascin-C

7) Fibronectin

8) Laminin

The main goal of our study

To study the role of the extracellular matrix protein in aortic valve interstitial cells calcification

Phase I

Explant

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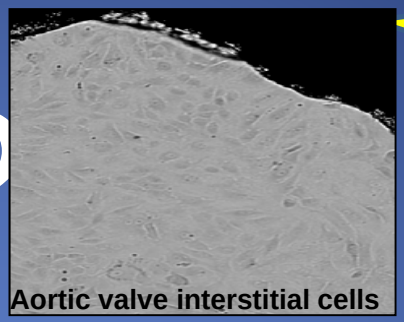


Aortic valve interstitial cells

Phosphate

Which one of the ECM proteins involved in the calcification process

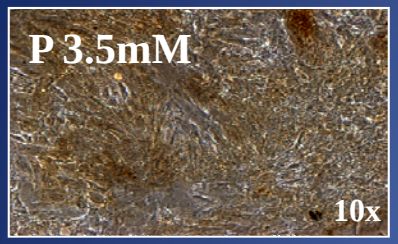
Culture the AVICs on different ECM coating + P 3.5mM

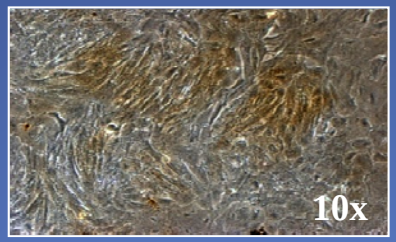
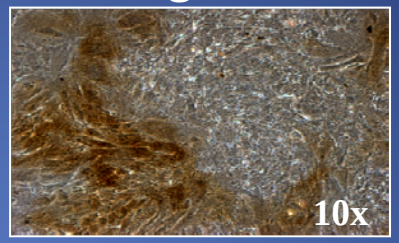
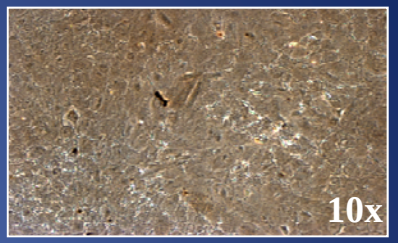
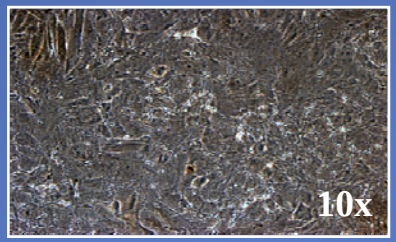
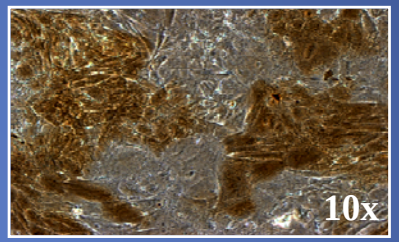
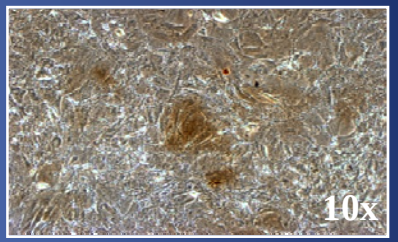
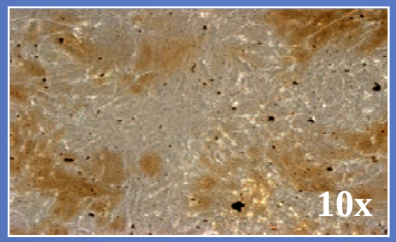
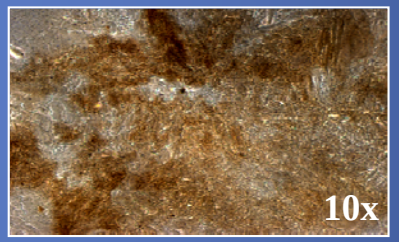
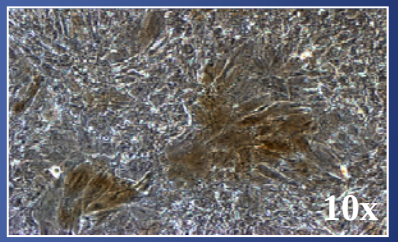
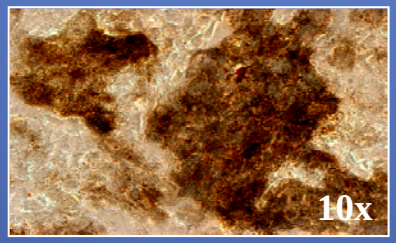
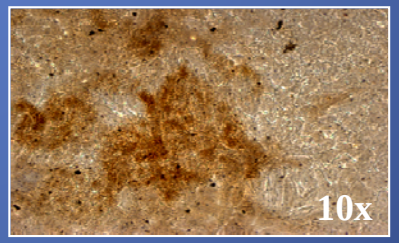
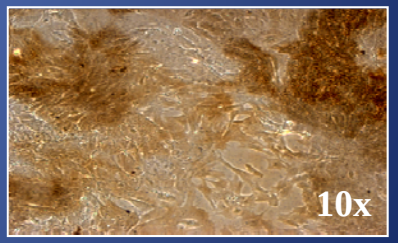
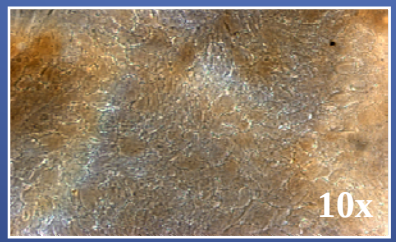
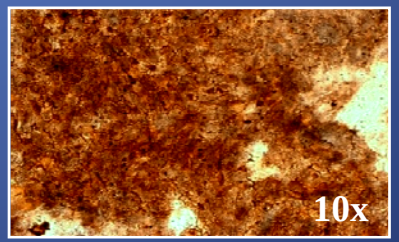
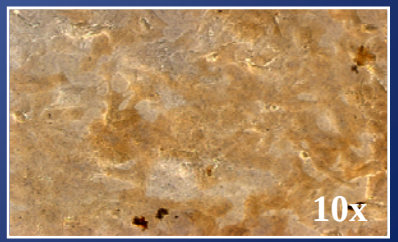
Without coating	Fibronectin	Periosten	Elastin	Tenascin	Collagen type III

14 days

Calcification
von Kossa Staining

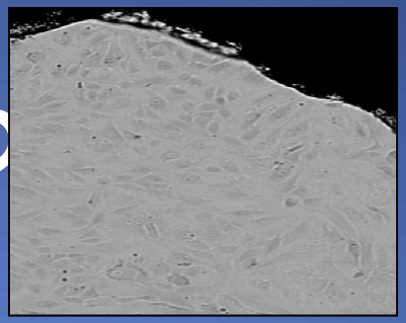
von Kossa staining



	0.1ug/ml	1ug/ml	10ug/ml
Periosten			
Collagen type III			
Elastin			
Tenascin			
Fibronectin			

Phase II

Explant



Aortic valve interstitial cells

Can Tenascin and Fibronectin enhanced calcification without p tion an Calcif sphate

Without phosphate

- Control
- Fibronectin 1ug/ml
- Tenascin 0.1 ug/ml

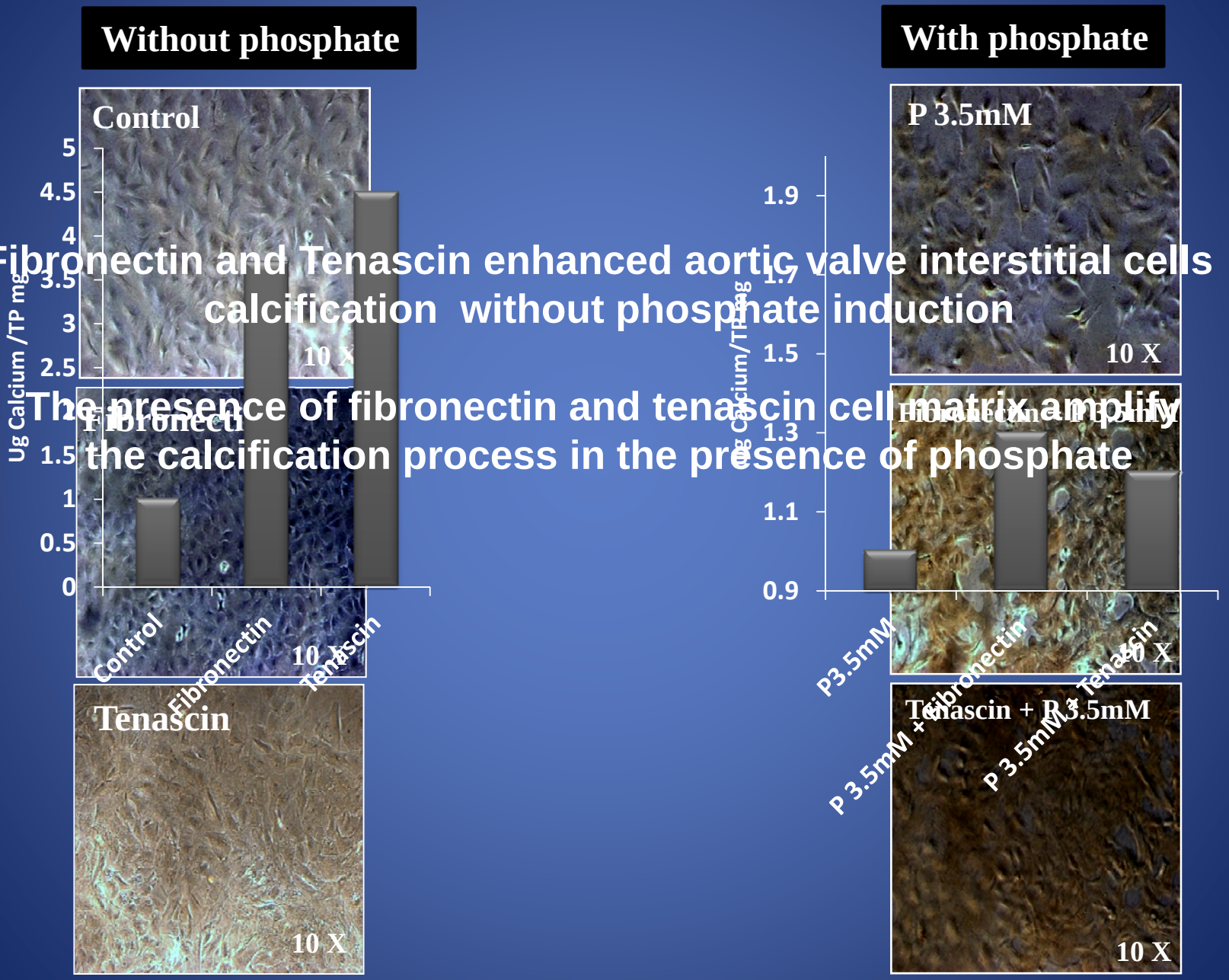
With phosphate

- Phosphate 3.5 mM
- Phosphate 3.5 mM +Tenascin
- Phosphate 3.5 mM +Fibronectin

7 day

von Kossa staining and calcium quantification

von Kossa staining and calcium quantification



Fibronectin and Tenascin enhanced aortic valve interstitial cells calcification without phosphate induction

The presence of fibronectin and tenascin cell matrix amplify the calcification process in the presence of phosphate

Osteogenesis

- **Tenascin production increased in the fibrosa parts of the valves, especially adjacent to the calcified areas**
- **Cells of the osteoblast express TNC from the onset of Osteogenesis , then TN-C modulate osteoblast behavior by stimulating cell differentiation** ***[Journal of the American College of Cardiology 2002]***
- **Fibronectin is known to be critical in the formation of calcified structures and is associated with osteoblast differentiation.** ***[Journal of Cell Science, 2006]***

?

Does osteoblast transformation process involved in ECM induced calcification in aortic valve interstitial cells

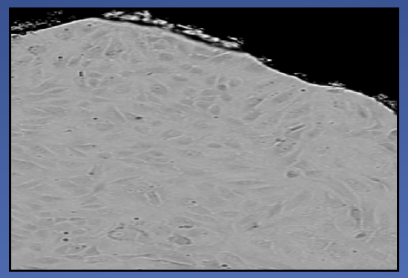
Osteoblast markers

- **Runx2** regulates bone development, bone maturation, and bone maintenance through the regulation of osteoblast differentiation and function

- **Osteopontin** is the most abundant glycoprotein produced by osteoblasts which compose the organic part of the bone and are essential for calcification.

- **Osteocalcin** late osteoblast marker , abundant in osteocyte

Phase III



Aortic valve interstitial cells

Without phosphate

- Control
- Fibronectin 1ug/ml
- Tenascin 0.1 ug/ml

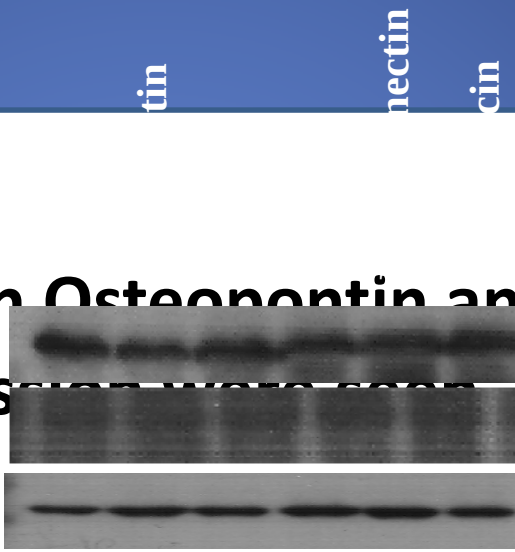
With phosphate

- Phosphate 3.5 mM
- Phosphate 3.5 mM +Tenascin
- Phosphate 3.5 mM +Fibronectin

7 day

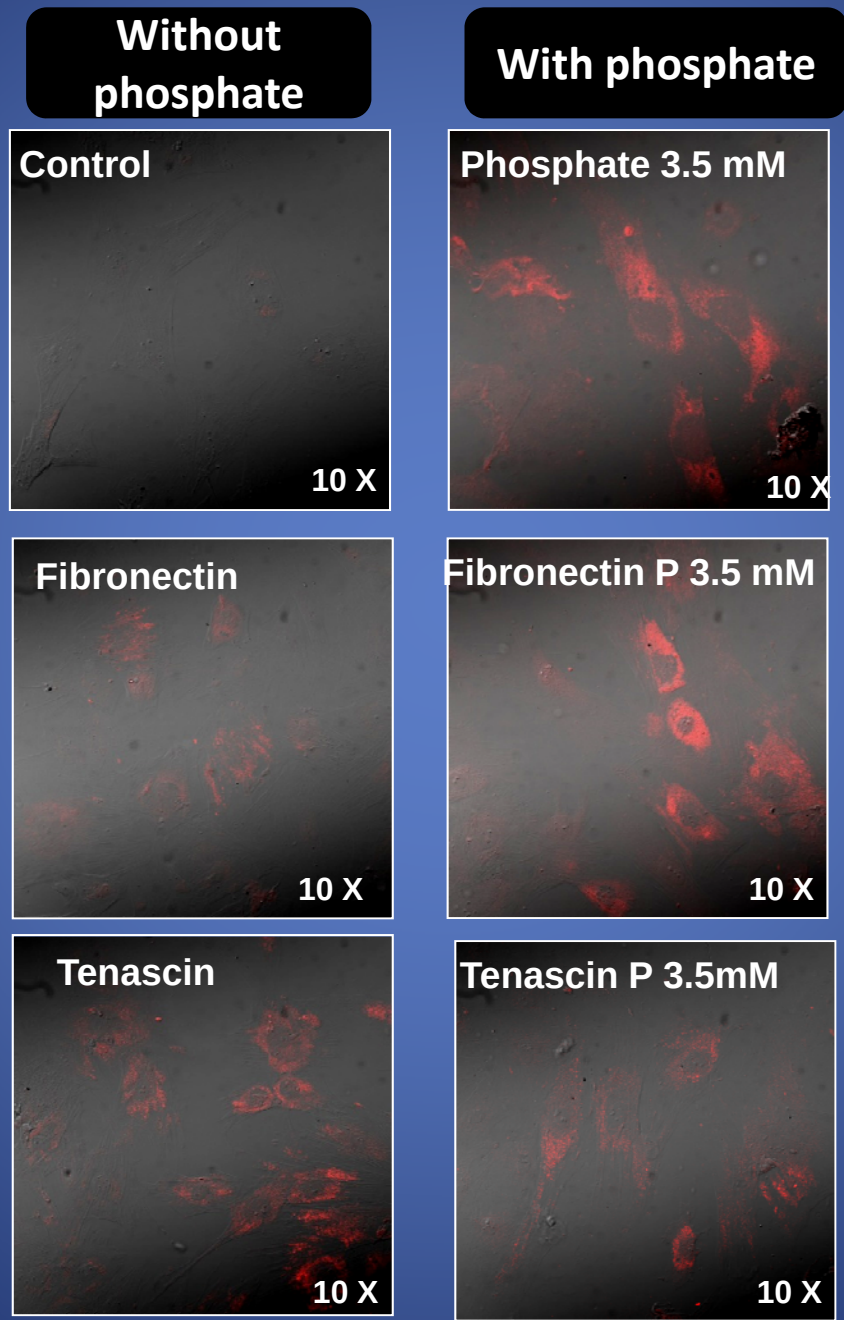
- Western Blot
  - Runx-2
  - Osteopontin
- Immunostaining
  - Osteocalcin

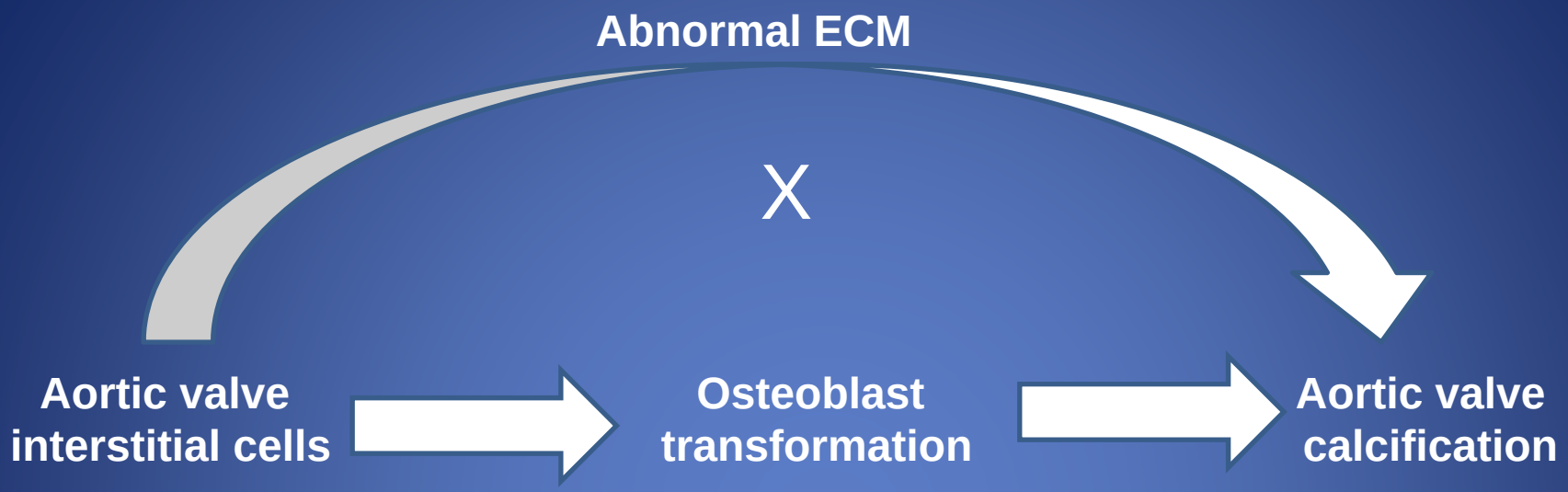
Western Blot of Runx-2 and Osteopontin



No difference in Osteopontin and Runx-2 expression were seen

Osteocalcin Immunostaining





ECM remodeling induced aortic valve interstitial cell calcification independent of osteoblast transformation process

Summary

- Fibronectin and Tenascin enhanced aortic valve interstitial cell calcification

- Fibronectin and Tenascin increased the calcification of the cells in the presence of Phosphate

- Fibronectin and Tenascin enhanced calcification independent on osteoblast transformation process

Future planes

To determine the pathways which involved in ECM changing and its effect on the VIC regulation and calcification .

Thank you for your listening

Lena Nasser