Biointerfaces: What are they and their dynamic evolution

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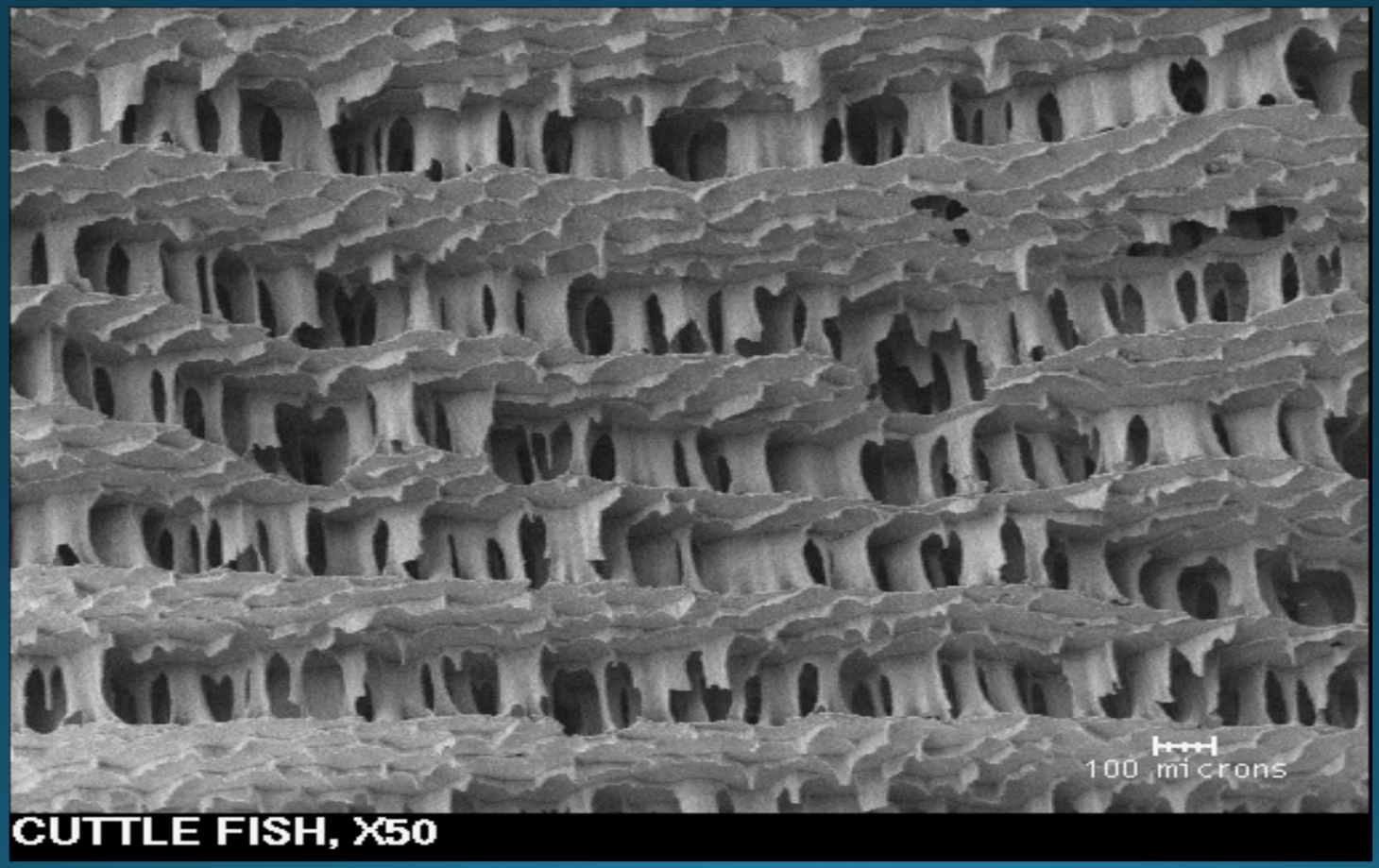
Biological Materials/Systems

- In order to design and produce successful biomaterials we need to appreciate and understand the biological materials and systems we are replacing.
- One of the characteristics of natural materials is their complex architecture/microstructure and their hierachical organisation from molecular structure through to microstructure and macrostructure.

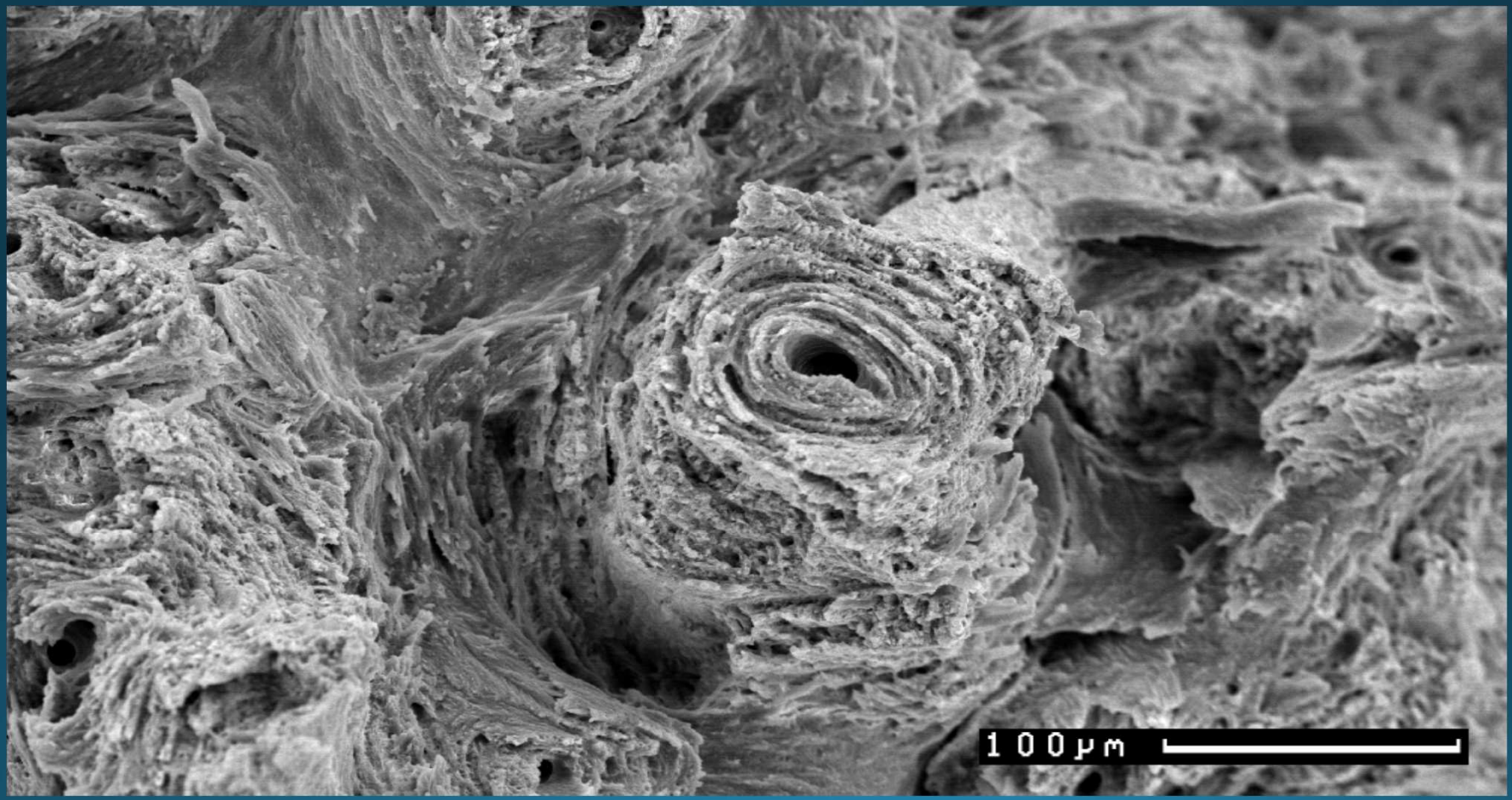
- It is worth looking at the constraints and conditions under which nature produces materials:
- a) **Temperature** - the fabrication temperatures are low and generally over a narrow range between 0 and 45°C.
- b) **Precision Control** - Biological organisms are particularly good at maintaining ionic concentrations of various anions and cations in very small volumes. In man the calcium concentration in blood is kept between 8.5 and 10.3 mg/100ml, yet the calcium concentration can be increased ten fold in a volume of 0.1mm^3 in a fraction of a second.
- c) **Growth Rate** - organisms grow slowly perhaps only a few microns a day.
- These three factors enable biological materials to have the complex and precise architecture referred to previously

- Structural biological materials are optimised in terms of their strength to weight ratio. Millions of years of evolutionary development have been invested in their development.
- Biological materials nearly always have anisotropic properties.
- Engineers and Physicists often regard biological structures as lacking structure and organisation; this is far from reality.

Cuttle Shell- SEM

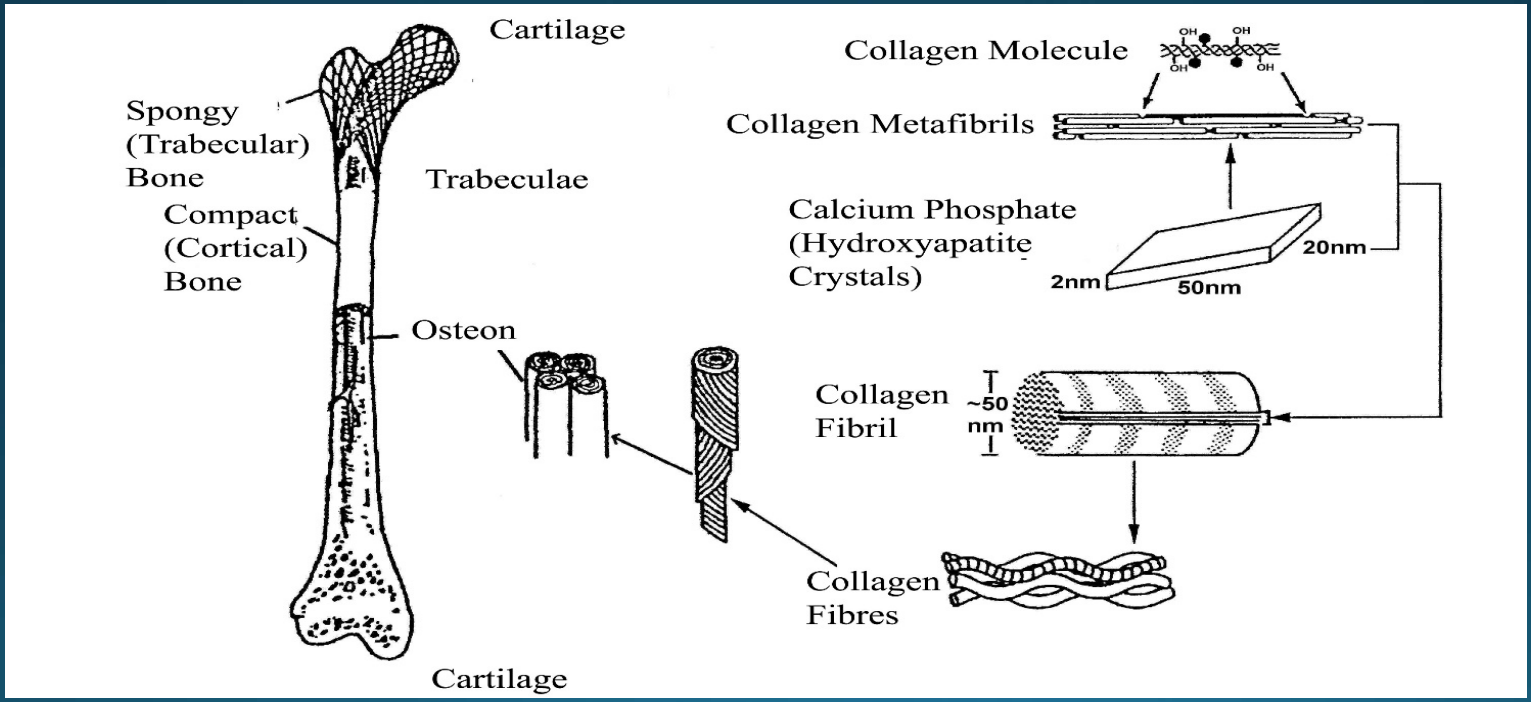


Antler Fracture Surface -SEM

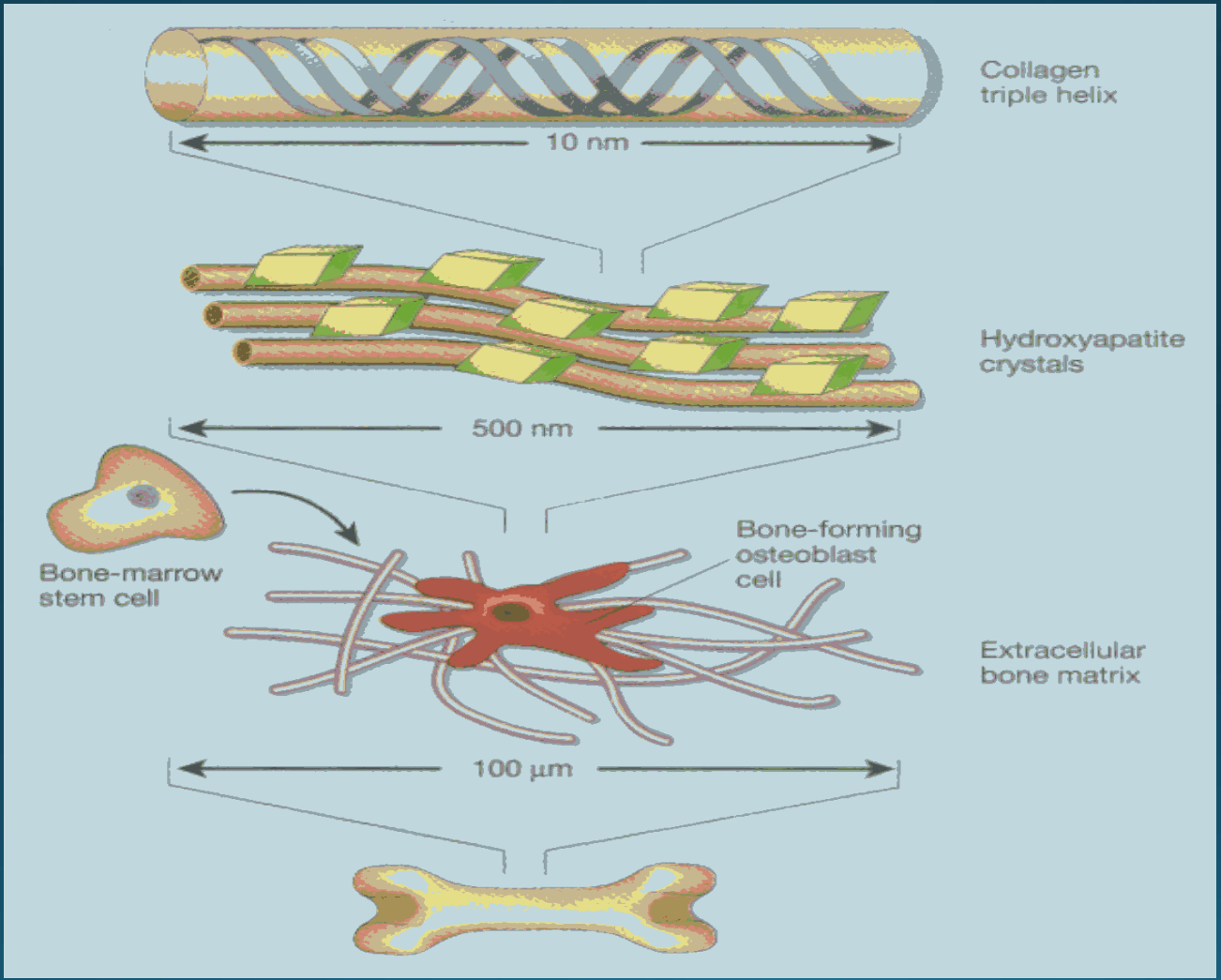


Bone structure

The constituents of bone are organized into three-dimensional structures. The several types of bone structures or morphologies differ, in their relative proportion and organization of collagen and bone mineral.

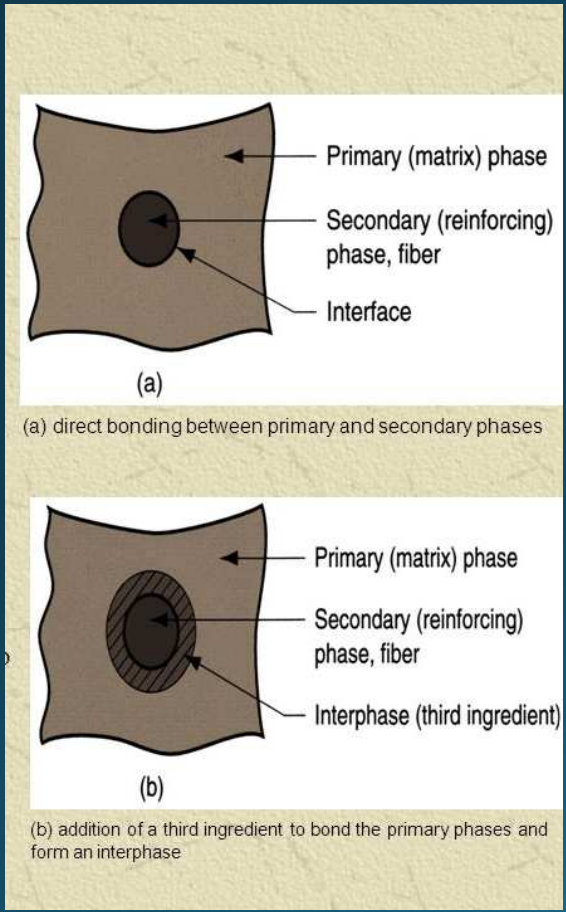


Hierarchical levels of structural organisation in bone



The many scales of organization in natural bone

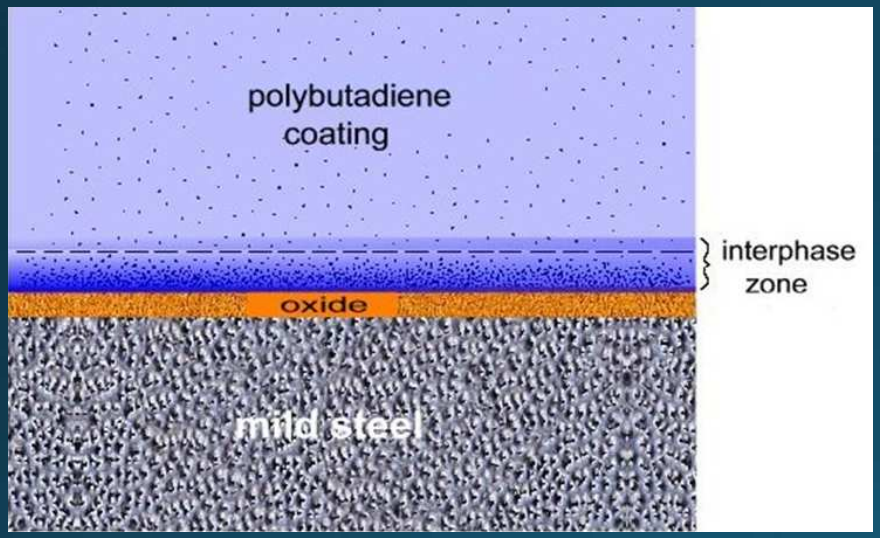
What is an interface and what an interphase?



- **Interface**: Distinct layer between a primary phase and a secondary phase

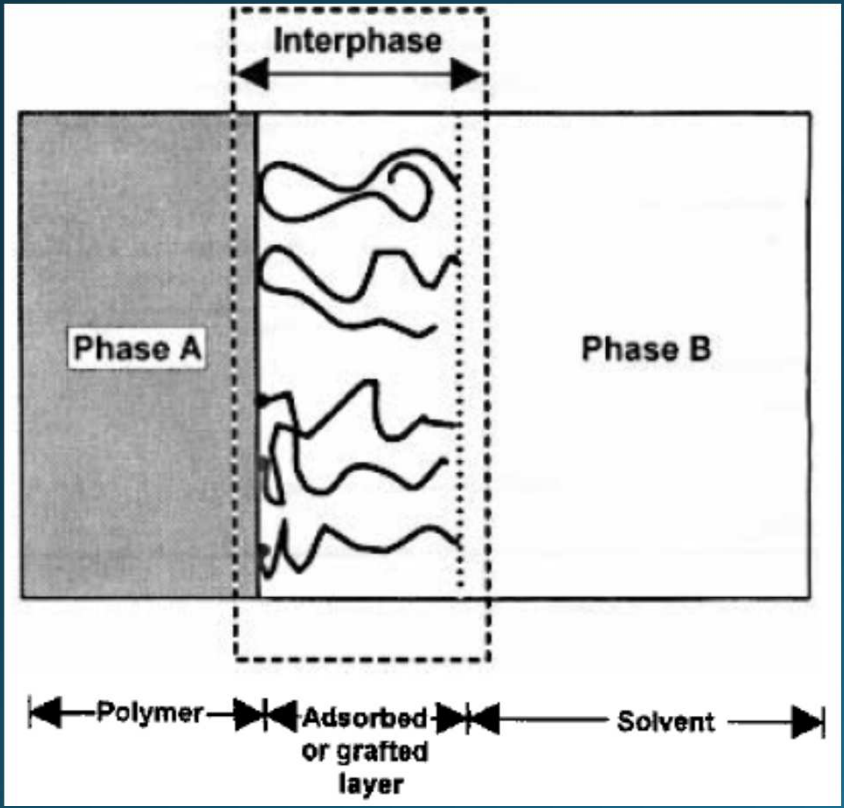
- **Interphase**: Third phase added to achieve bonding of the primary and the secondary phase (adhesive/coating).

In a more complicated case…



- Interphase is fractal in nature and much more complicated than the previous conventional interface.

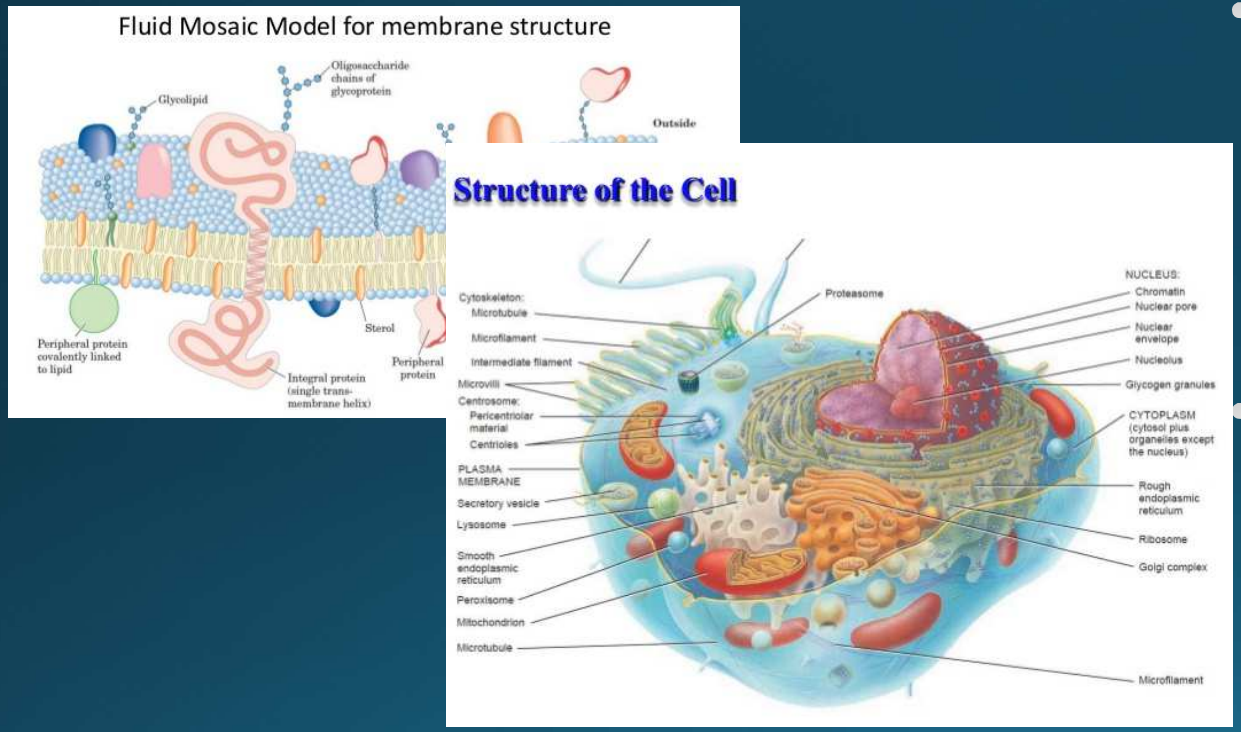
Another interesting interphase



- Layers of adsorbed and grafted molecules can be differentiated on the surface in solution.
- Strong interaction may occur, e.g., chemisorption and covalent bonding.
- Polymer surface modification, offers numerous possibilities of changing surface characteristics and of enhancing the biocompatibility.
- A current research problem is the blood compatibility of implanted materials. Here, surface modification is primarily intended to modify the material-blood interphase to reduce platelet adhesion. One approach to enhancing the blood compatibility of polymer surfaces is plasma deposition of appropriate substrate. In this way a significant change of the chemical composition and dimension of the interphase is achieved.

Geckeler et al, Adv. Mater. 1997, 9, No. 6

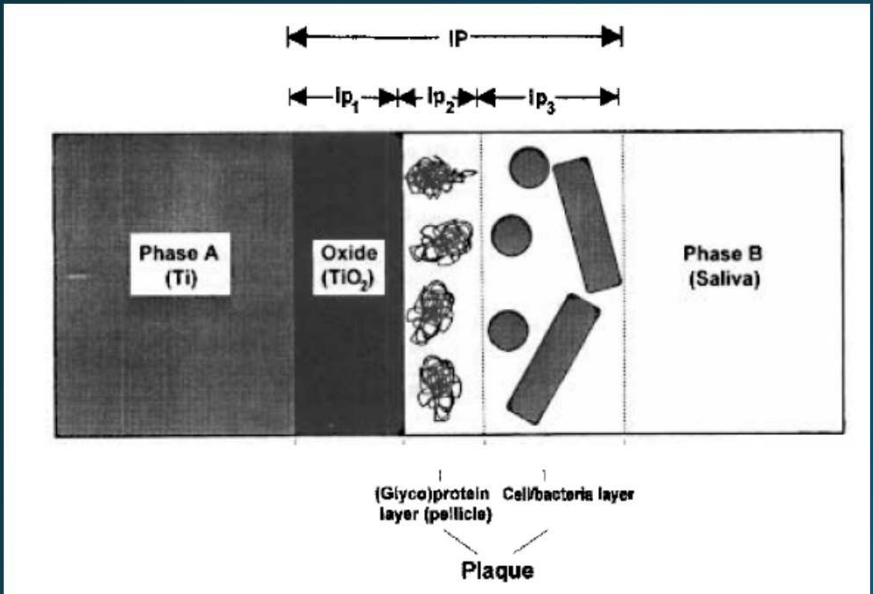
Natural systems



- Biomembranes can be regarded as natural interphases between subcellular compartments or between cells and their environment.
- Further criteria might be active or passive transport processes, as known from biomembranes, which underline their dynamic nature.

https://www.slideshare.net/ashokktt/cell-and-cell-organelles

Mixed artificial-natural systems

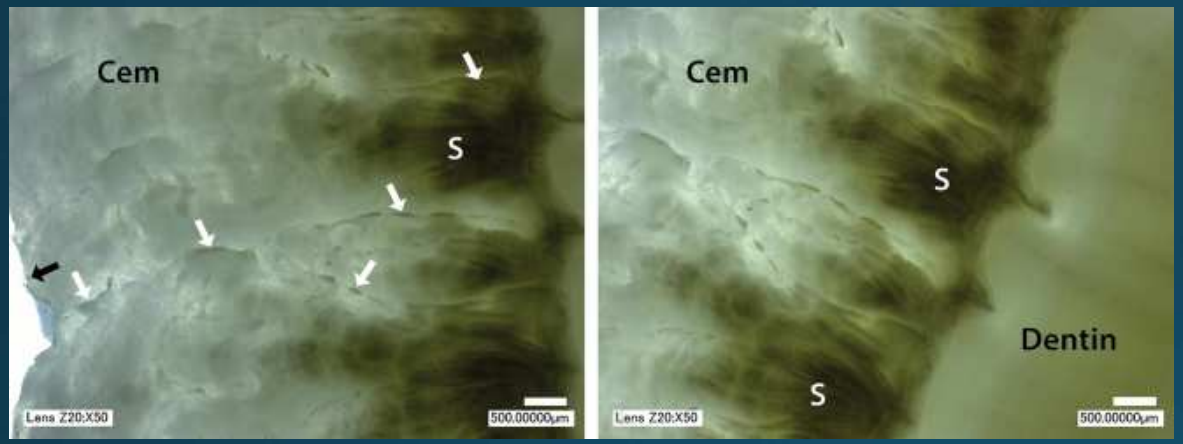


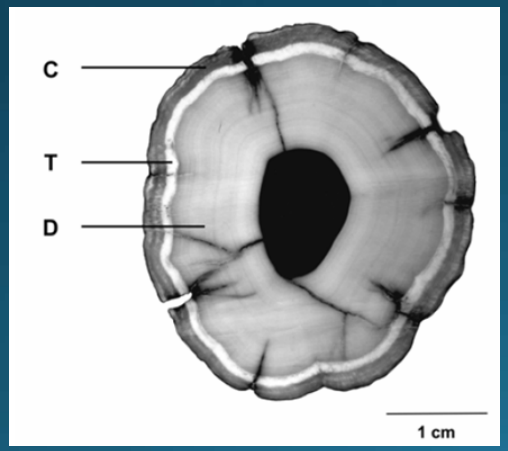
- Time-dependent formation of a macromolecular, protein-rich layer on material surfaces when they are placed in an oral cavity, e.g., dental metallic implants in contact with this primary layer plays the role of a conditioning film for further adhesion phenomena, e.g., the adhesion of oral microorganisms, which causes the formation of dental plaque.
- These phenomena result in a complex constructed interlayer between the material and the bio-liquid. Such an interphase is composed of several sub-interphases evolving between the surface of a dental implant material, e.g., titanium, and saliva. Each distinct phase, i.e., the metal oxide, the macromolecular layer (which in the oral cavity is called pellicle), and the cell layer, can be considered as an interphase, neighboured by sharp interfaces, respectively.

Examples from nature: The Narwhal tusk

New research suggests that the tusk is used as a sensory organ, helping the narwhal pick up changes in its environment. Researchers say males of the species may use the horns to look for food or find mates.

Structure





The outer cementum layer of the tusk is porous, the inner dentin layer has microscopic tubes that channel toward the middle, and the pulp in the centre has nerve endings that connect to the animal's brain

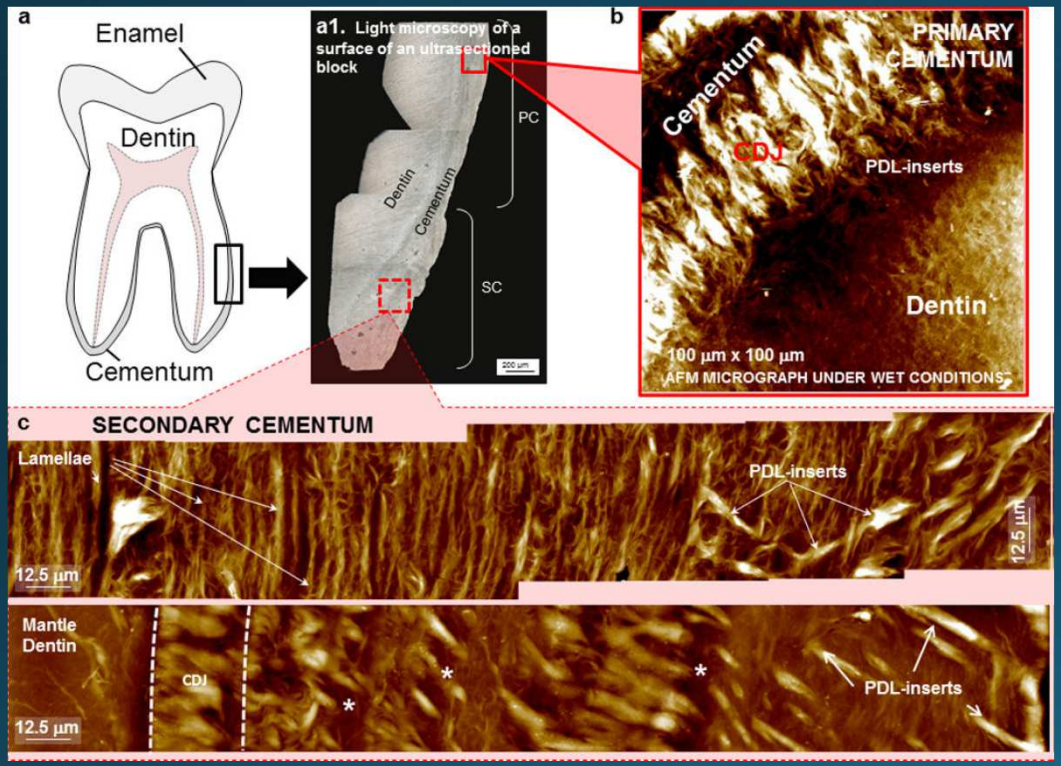
Nweeia et al. , The Anatomical Record Vol297(4) 599- 617, 2014

Cement-Dentin Junction: A graded biointerphase

Grandfield et al., J Engineering in Medicine 2014, Vol. 228(8) 754–767

- The narwhal's hygroscopic collagenous CDJ as a model system to investigate physico-chemical aspects of nature's cushion-like interface between two mineralized tissues: cementum and dentin.
- Resistance to mechanical load of the CDJ due to a change in salinity of the environment was observed.
- Given that the fibrillar and nonfibrillar proteins are the main constituents in the CDJ, it is conceivable that the observed proof-of-concept can be extended to a human CDJ similar in composition and structure but narrower in dimension (a fraction of that of a narwhal CDJ).
- The 1- to 2-mm-wide narwhal CDJ has been proposed as a mechanistic model which could serve as a biomimetic template.

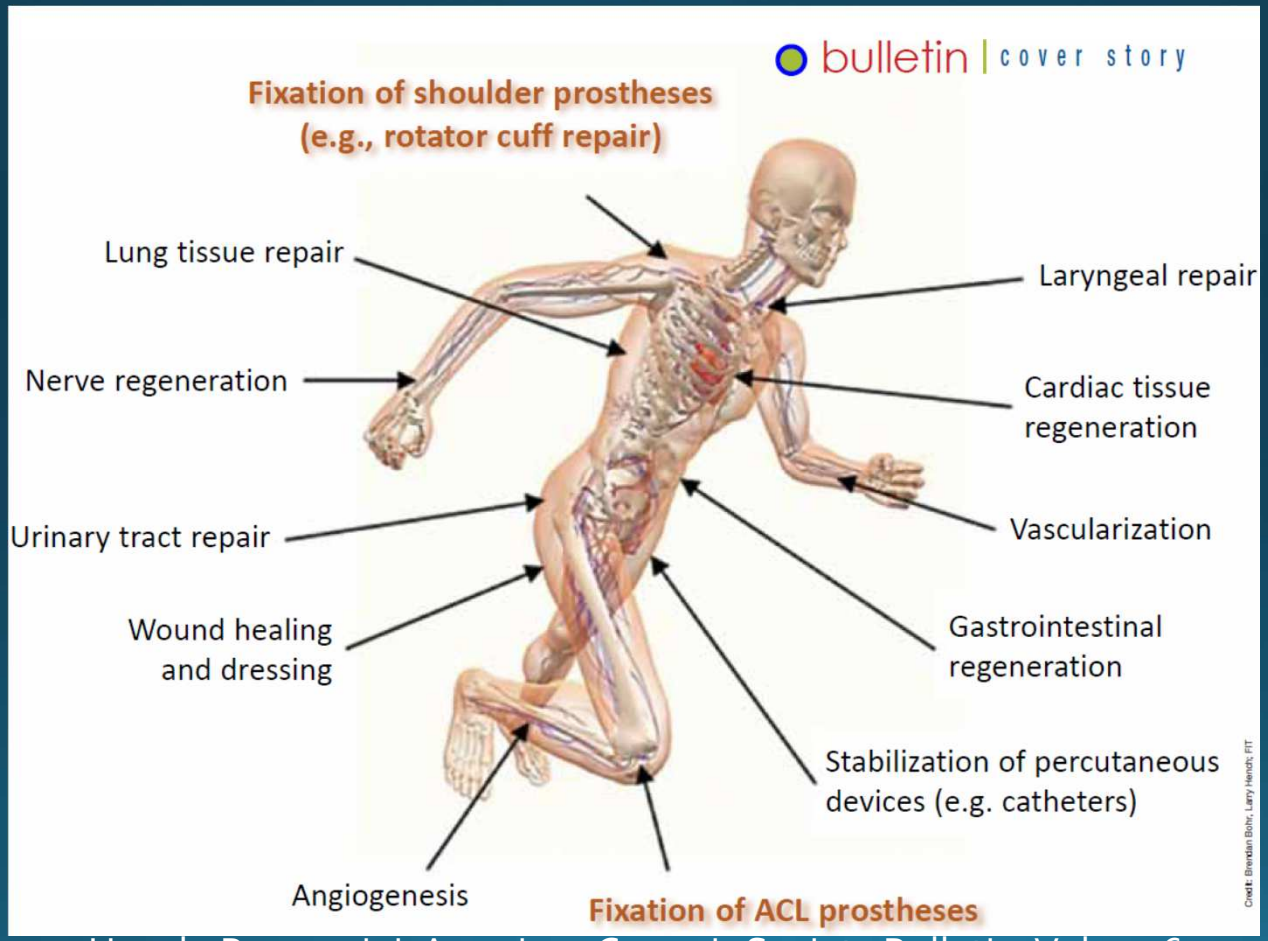
The role of CDJ in human teeth



- Cementum is a mineralized tissue that covers the outermost layer of a tooth root .
- The primary function of cementum is to confine tooth and provide support and load absorption during mastication.
- The cementum is not directly fused to dentin. It is attached to dentin via a 100–200 µm thick interface within which a 10–50 µm wide hygroscopic proteoglycan (PG)-rich layer known as the cementum-dentin junction (CDJ) exists. Within the CDJ, the dominance of collagen fibres that transverse radially to the mantle dentin are tied with proteoglycans and are thought to contribute to an increased ratio of organic to inorganic content.
- The water absorbing fibrous nature of the CDJ may be of importance to transfer loads between adjoining mineralizing tissues implying that, physicochemical changes to the interface could play a key role in the overall biomehanical response to function.
- The collagenous and non-collagenous proteins forming this region exhibit dominant water retention characteristics which have been speculated to help dissipate accumulated function-related stresses. Therefore it is postulated that cementum and its graded interfaces act as biological and mechanical continua allowing for optimum function.

Jang et al. J Mech Behav Biomed Mater. 2014 November ; 39: 184–196

Bioglass- tissue interphases



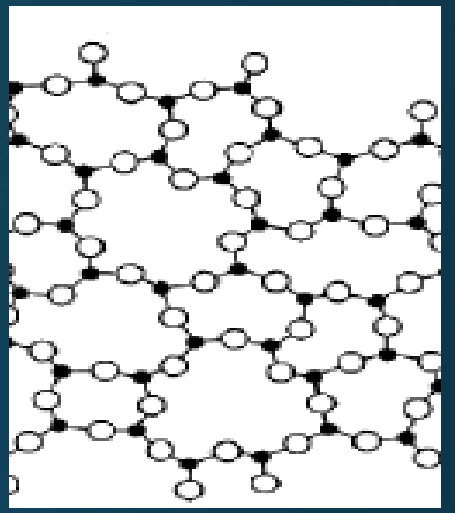
Miguez-Pacheco, Greenspan, Hench, Boccaccini, American Ceramic Society Bulletin, Vol 94, 6

Bioactive Glasses

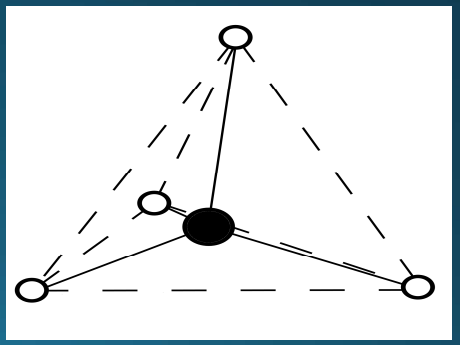
Bioactive glasses are known to:

- consist of a silica (O-Si-O) network structure (+Ca, P, Na, etc. network modifiers)
- form an HCA layer on contact with body fluid
- be class A bioactive materials
- be osteoproductive
- bond to soft tissue and hard tissue
- stimulate genes in osteoblasts (Xynos 2001)
- be brittle in tension

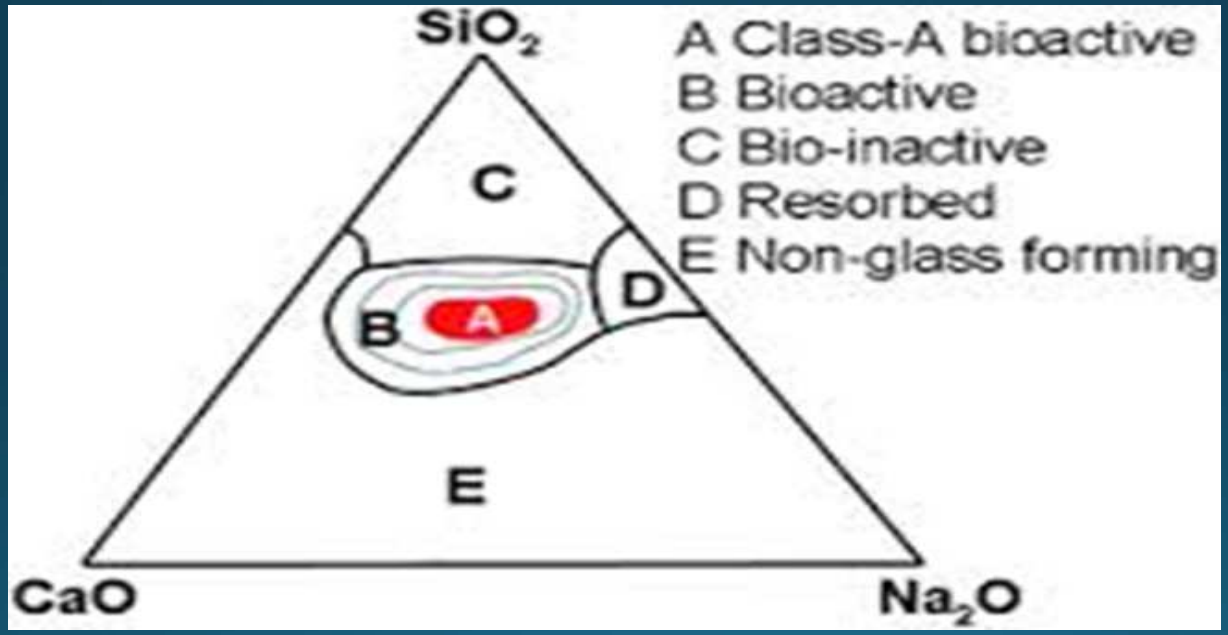
There are 2 types……



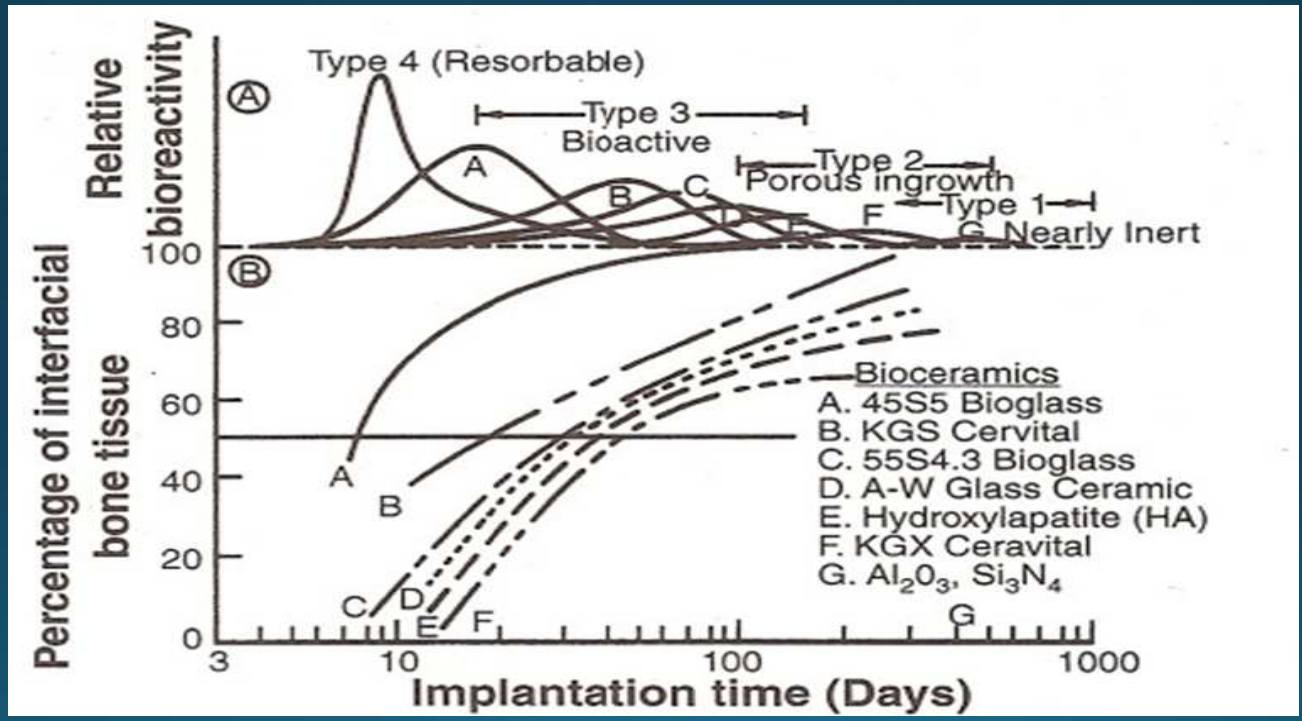
• = Silicon atom
O = Oxygen atom



Phase Diagram



Bioactivity Spectrum



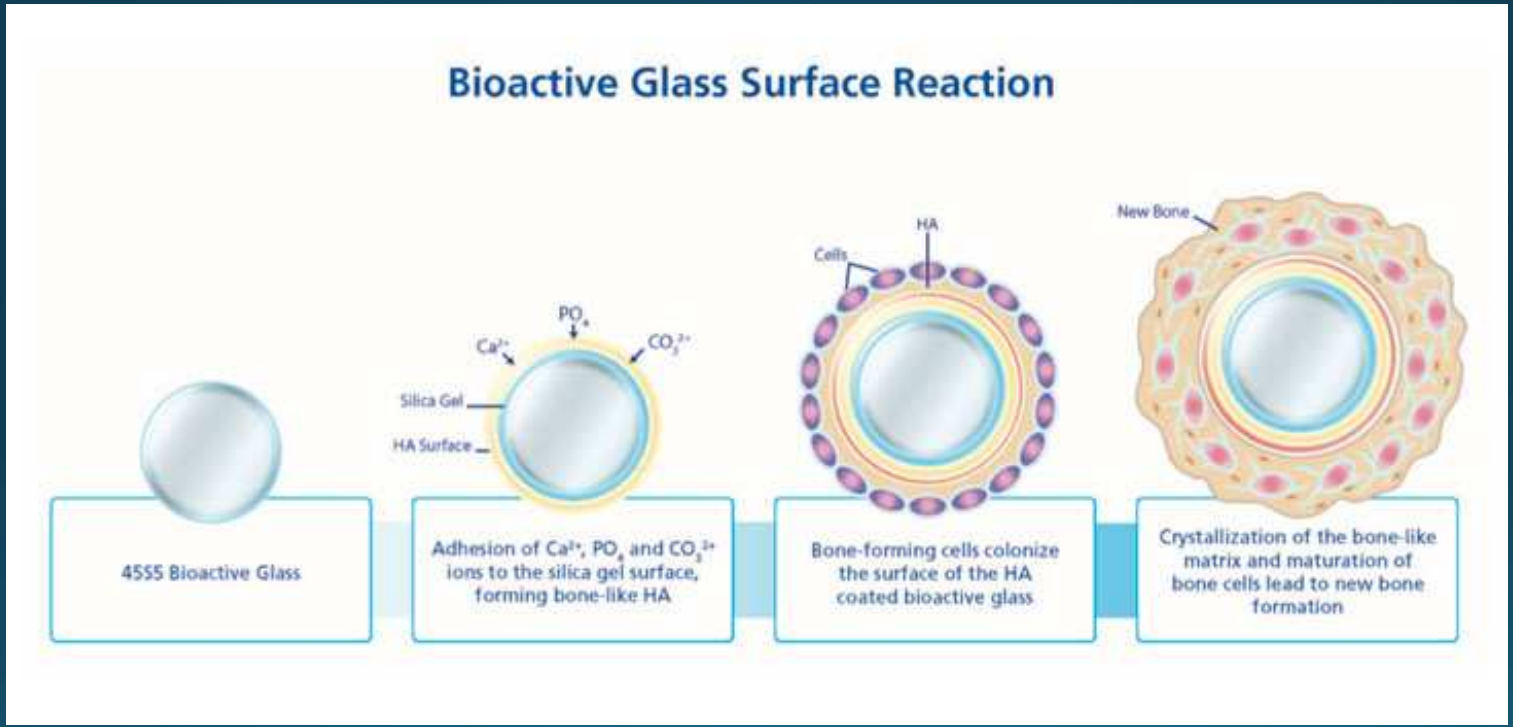
Phases of surface reactions

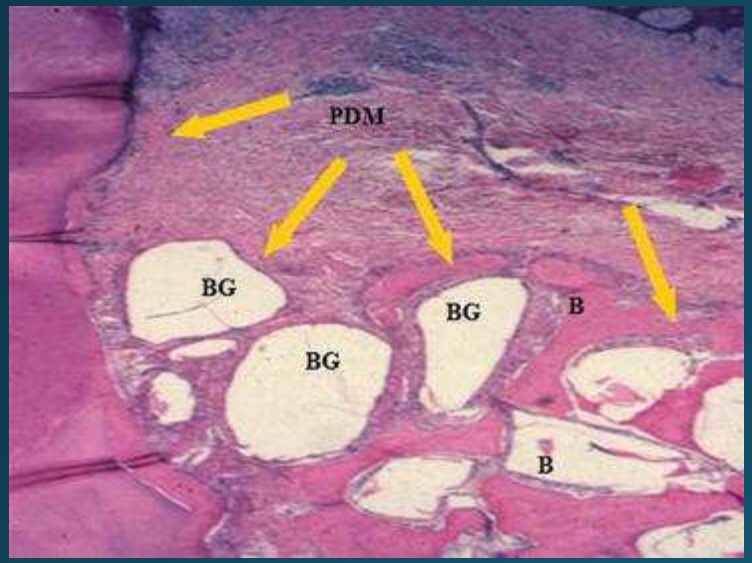
- Crystallization of bone matrix
- Formation of bone matrix
- Differentiation of bone cells
- Attachment of bone cells
- Activity of macrophages
- Adsorption of biological fragments by carbonate hydroxyapatite layer
- Crystallization of carbonate hydroxyapatite
- Adsorption of ions Ca^{2+}, PO_4^{3-}, CO_3^{2-}
- Polycondensation SiOH + HOSi → Si – O – Si
- Formation of Si-OH bonds
- Bioactive glass

Bone mineralisation

Hydrolysis

Surface reaction

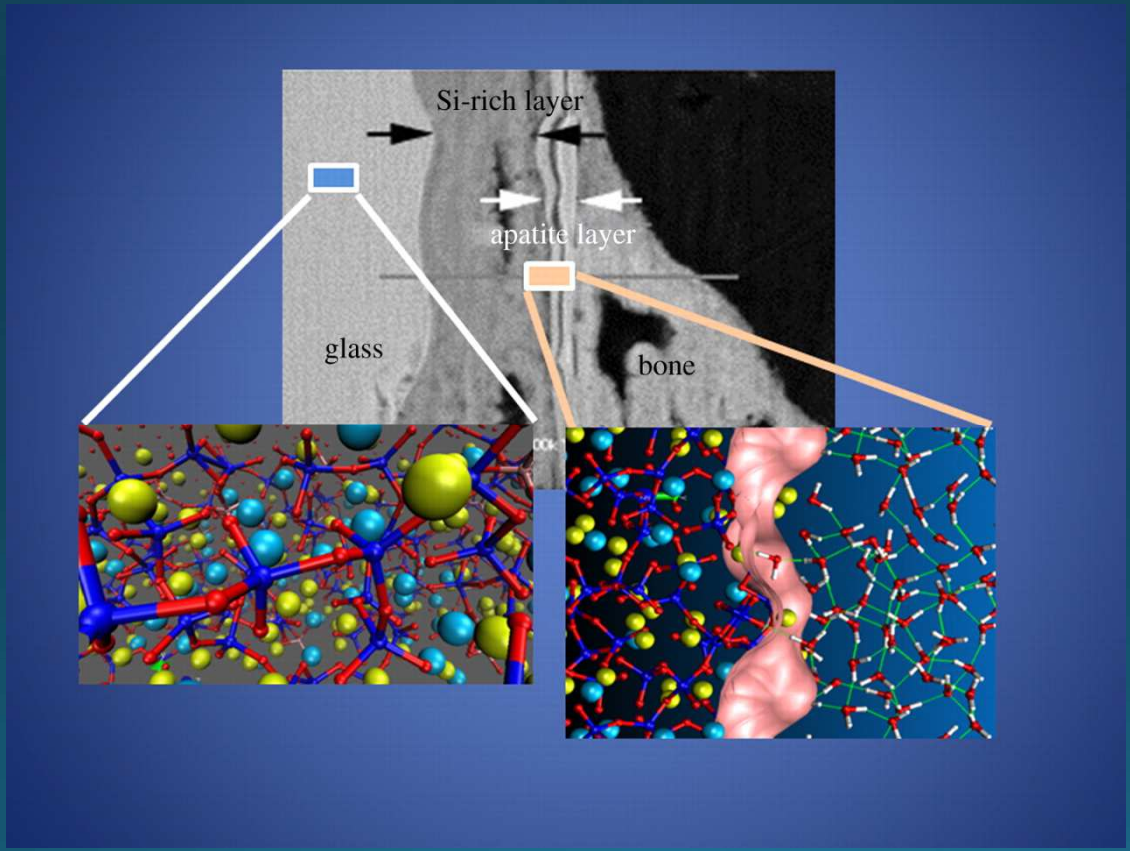




Courtesy of Dr June Wilson

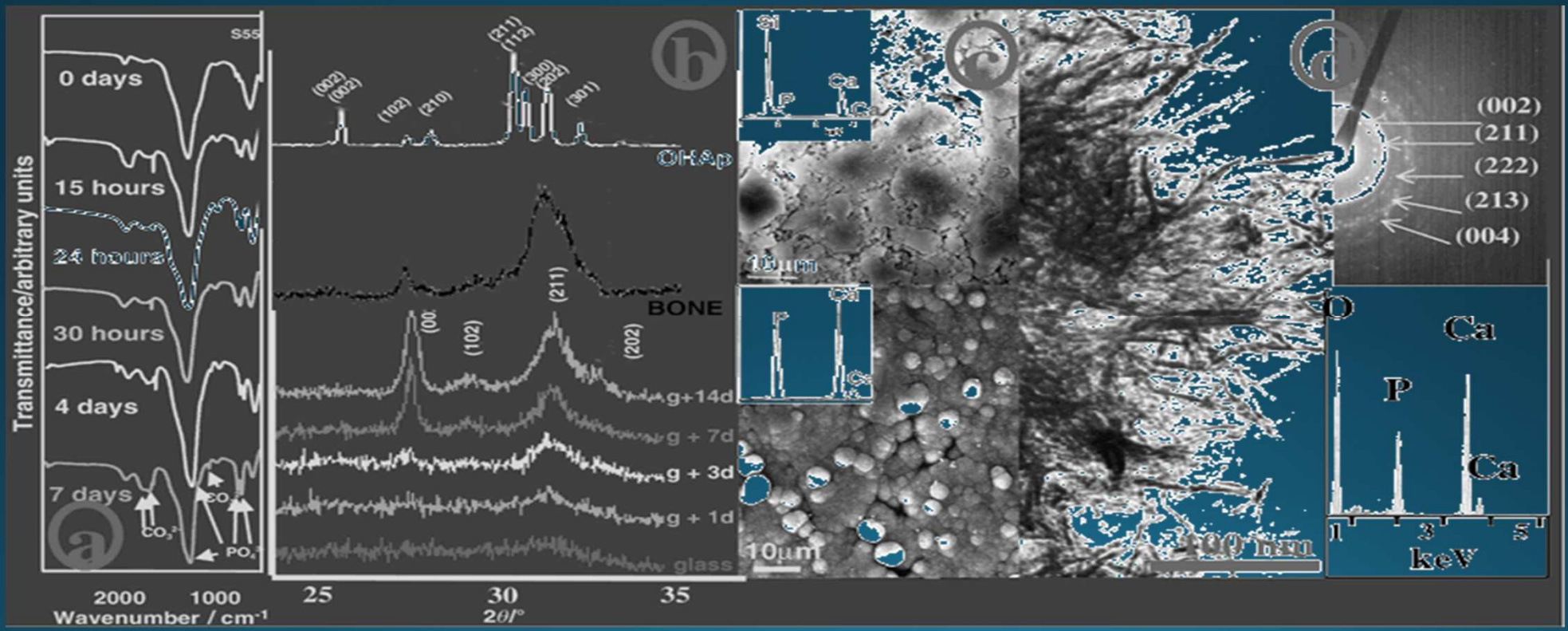
- Bioactive glass particulate used to enhance bone formation around the tooth and thereby restores its function following treatment of periodontal, gum, disease in a patas monkey periodontal defect model. Note the mineralized collagen that surrounds the bioactive glass particles forming a regenerated bone structure. Also note the periodontal ligament labelled PDM that is stable above the regenerated bone.

Interphase

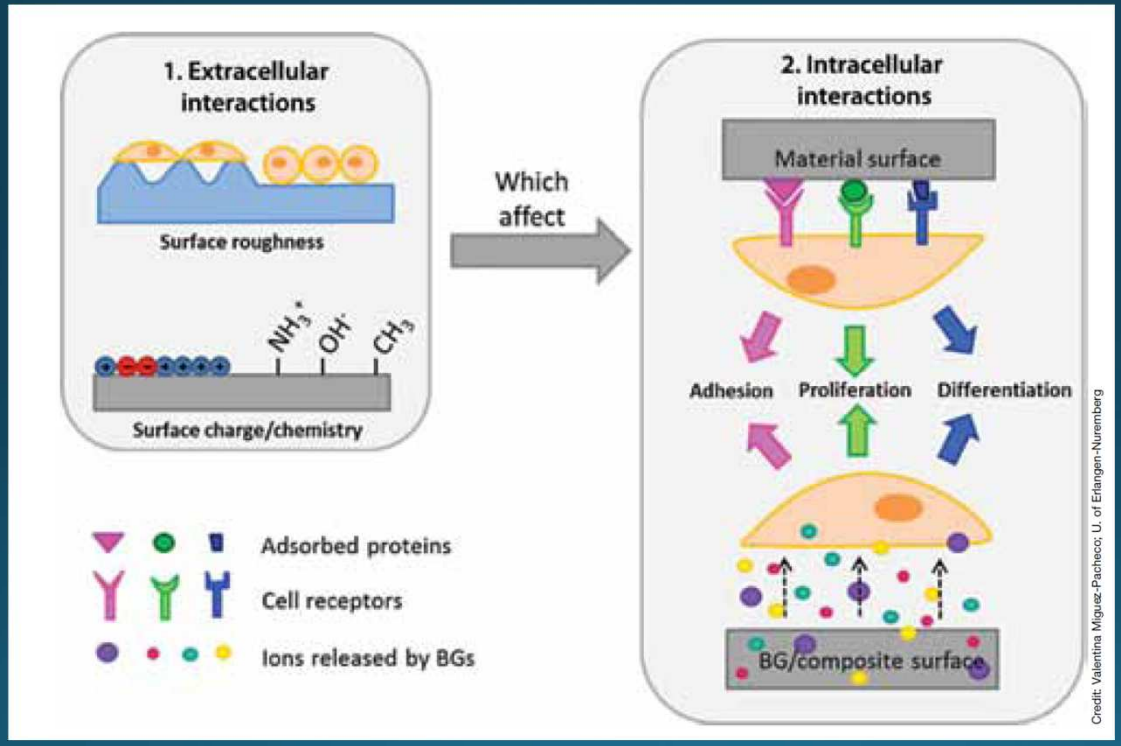


Cormack and Tilocca, Phil. Trans. R. Soc. A (2012) 370, 1271–1280

In vitro evaluation of bioactivity



Two types of interactions



Osteogenesis

- Three basic steps involved in osteogenesis are:

(a) Synthesis of extracellular organic matrix (osteoid)

(b) Matrix mineralization leading to the formation of bone

(c) Remodeling of bone by the process of **resorption** and **reformation**

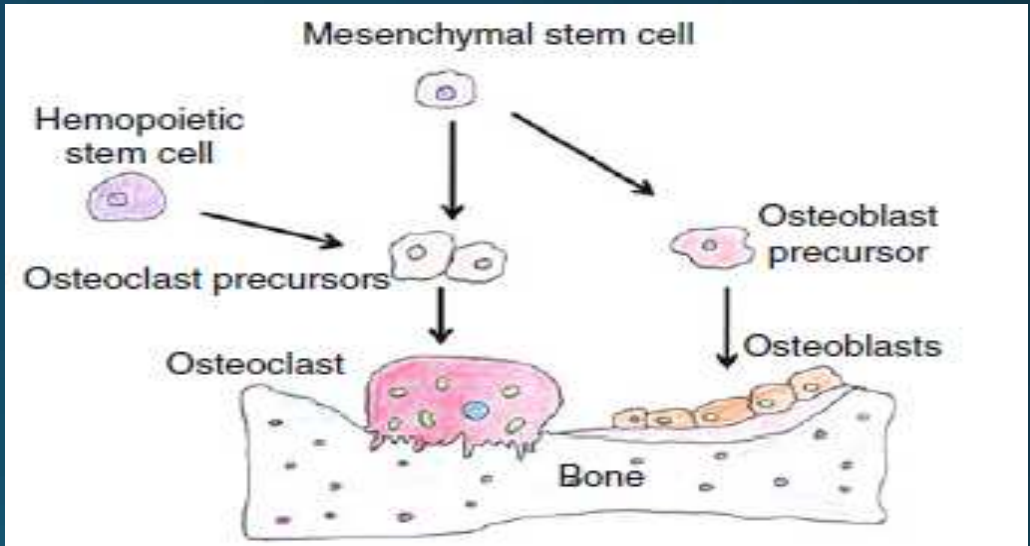


Fig. 2.2 Diagram to show evolution of osteoblasts and osteoclasts in the formation of bone

Intramembranous ossification

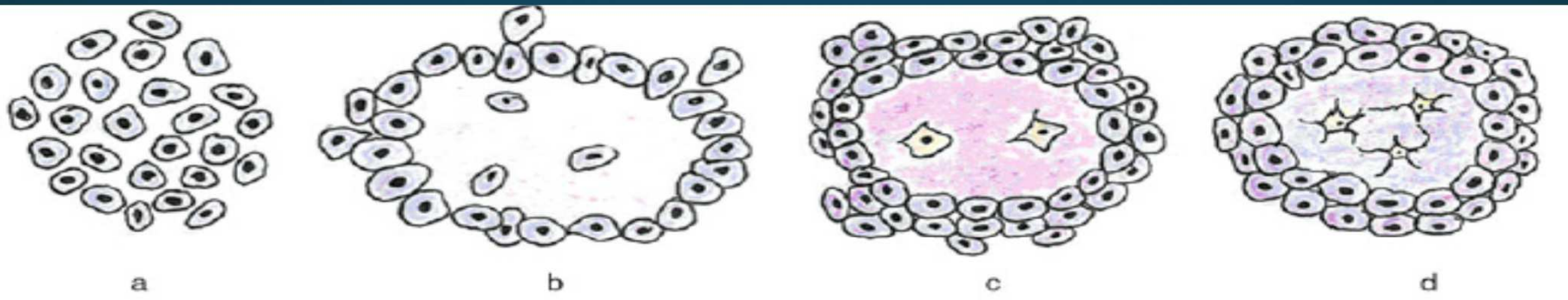


Fig. 2.3 Intramembranous ossification showing: (**a**) Aggregates of osteoprogenotor cells. (**b**) Amorphous ground substance and collagen meshwork formed in the center and in between the cells. (**c**) The mesenchymal stem cell transform to osteoblasts which synthesize osteoid in the center of the aggregate. (**d**) A rudimentary bone tissue formed by the osteoblasts and some of these get incorporated within the osteoid to become osteocytes

Bone resorption and remodelling

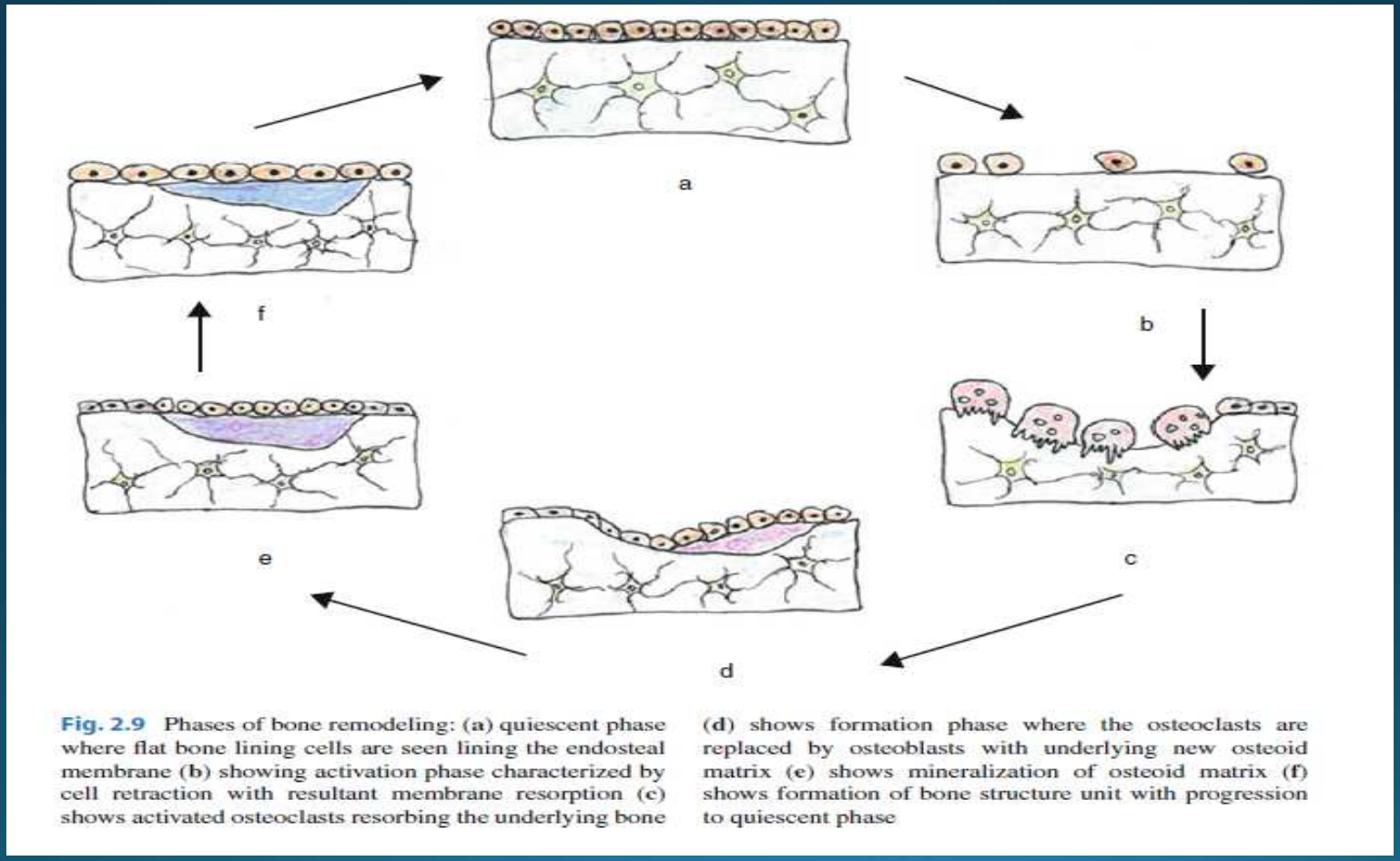


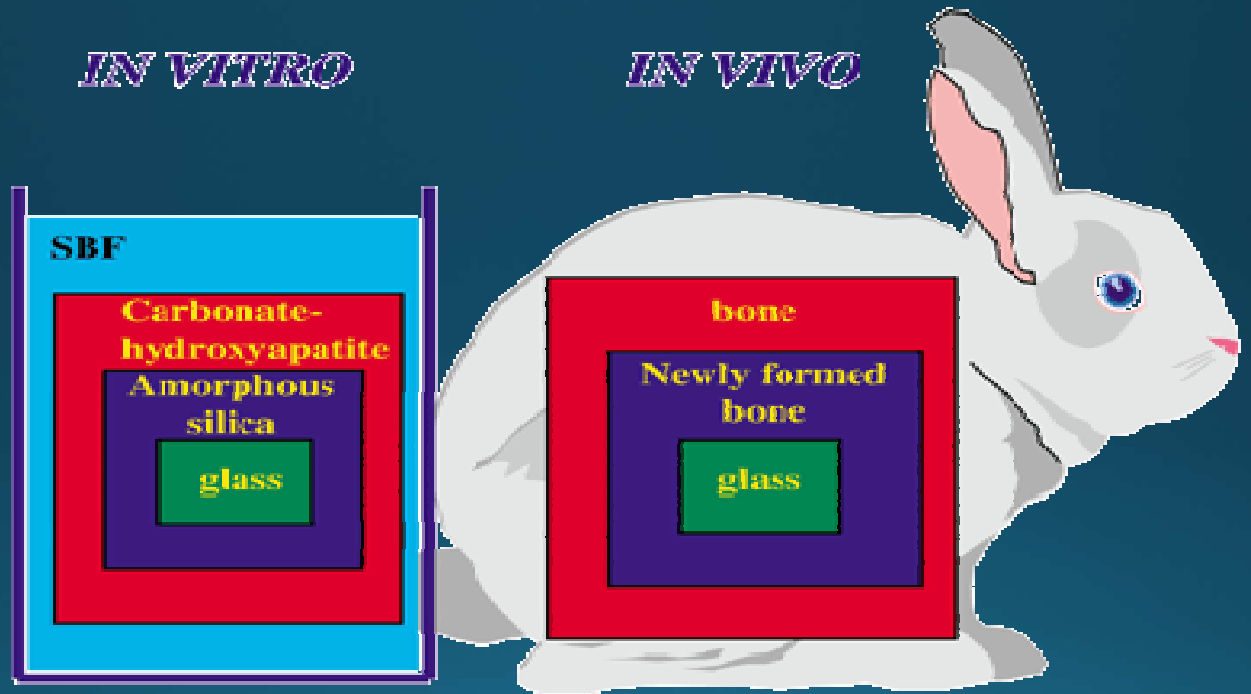
Fig. 2.9 Phases of bone remodeling: (a) quiescent phase where flat bone lining cells are seen lining the endosteal membrane (b) showing activation phase characterized by cell retraction with resultant membrane resorption (c) shows activated osteoclasts resorbing the underlying bone (d) shows formation phase where the osteoclasts are replaced by osteoblasts with underlying new osteoid matrix (e) shows mineralization of osteoid matrix (f) shows formation of bone structure unit with progression to quiescent phase

Requirements for success

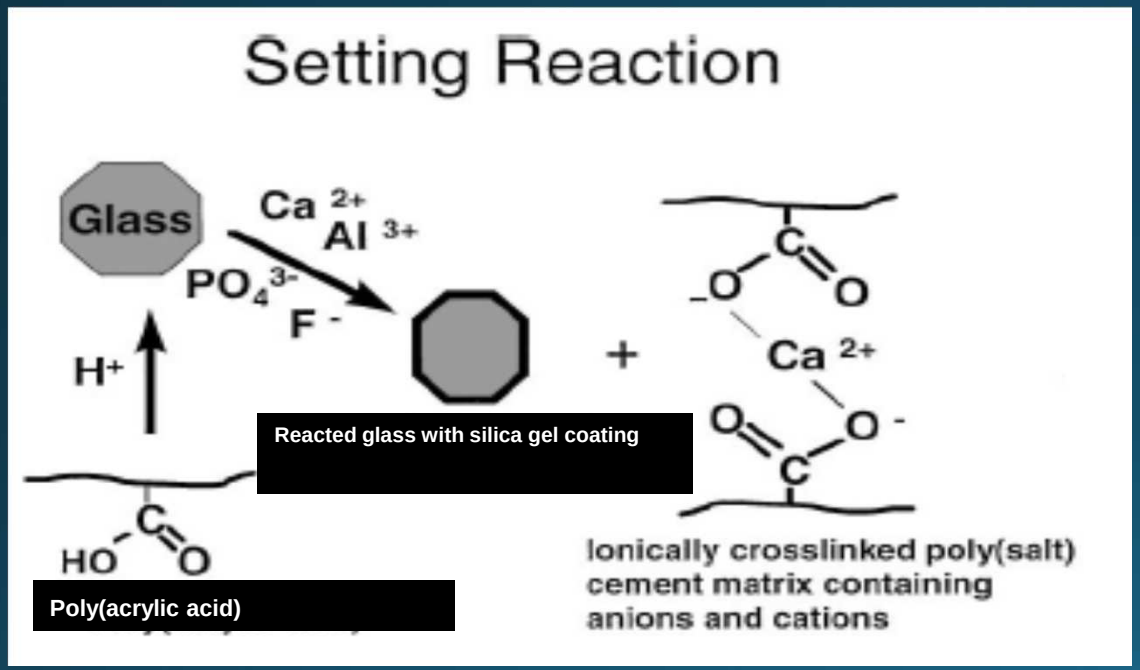
- Rapid formation of an interfacial layer mediated by extracellular matrix molecules; and
- Stable long-term interfacial bonding that prevents micromotion at the interface and inflammatory responses.

Interphase with soft tissue

- Wilson et al. using the same ERMI implants, made it possible to achieve a quantitative histo-morphometric analysis of the hard and soft tissue bonding interfaces of ERMIs. Within three months, bonding stabilized for hard and soft tissues. Soft tissue was bonded by collagen fibres interdigitated within a 150–400-μm-thick bonding gel layer composed of biological HCA and an underlying silica-rich gel layer that began to form on implants within minutes of implantation. It was proposed that the difference in Young's modulus in the interfacial area between soft tissue and bulk BG implant is spread over a substantial interfacial thickness because of an elastically compliant hydrated silica gel (HCA) layer on the BG, which is several hundred micrometers thick.

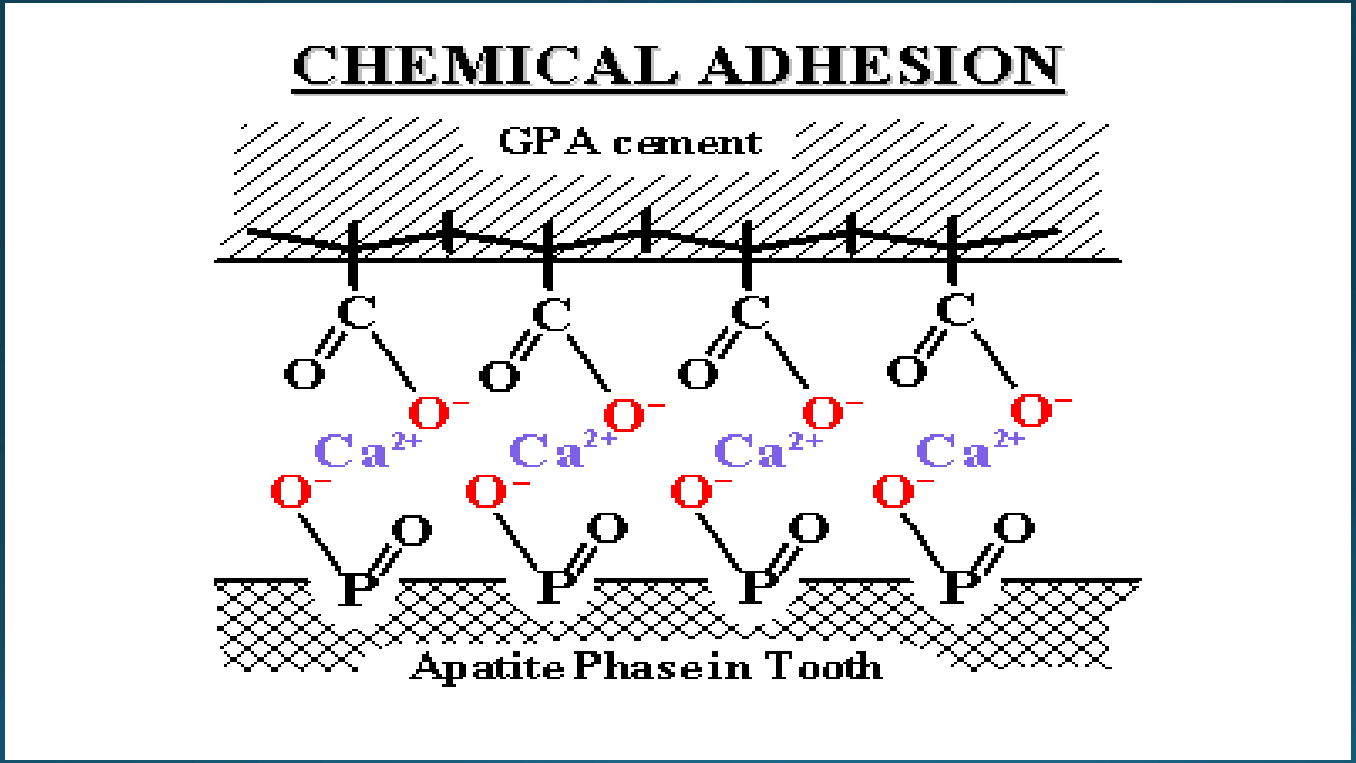

IN VITRO
IN VIVO
SBF
Carbonate-hydroxyapatite
Amorphous silica
glass
bone
Newly formed bone
glass

Glass Ionomer (Polyalkenoate) Cements



- Simplified setting reaction.
- Ions are released from the glass.
- Cations ionically crosslink the polyacrylic acid to give the cement matrix.

Chemical adhesion



Peptide Based Coating with Antibacterial Property

”Smart” Implant

Antibacterial Coating

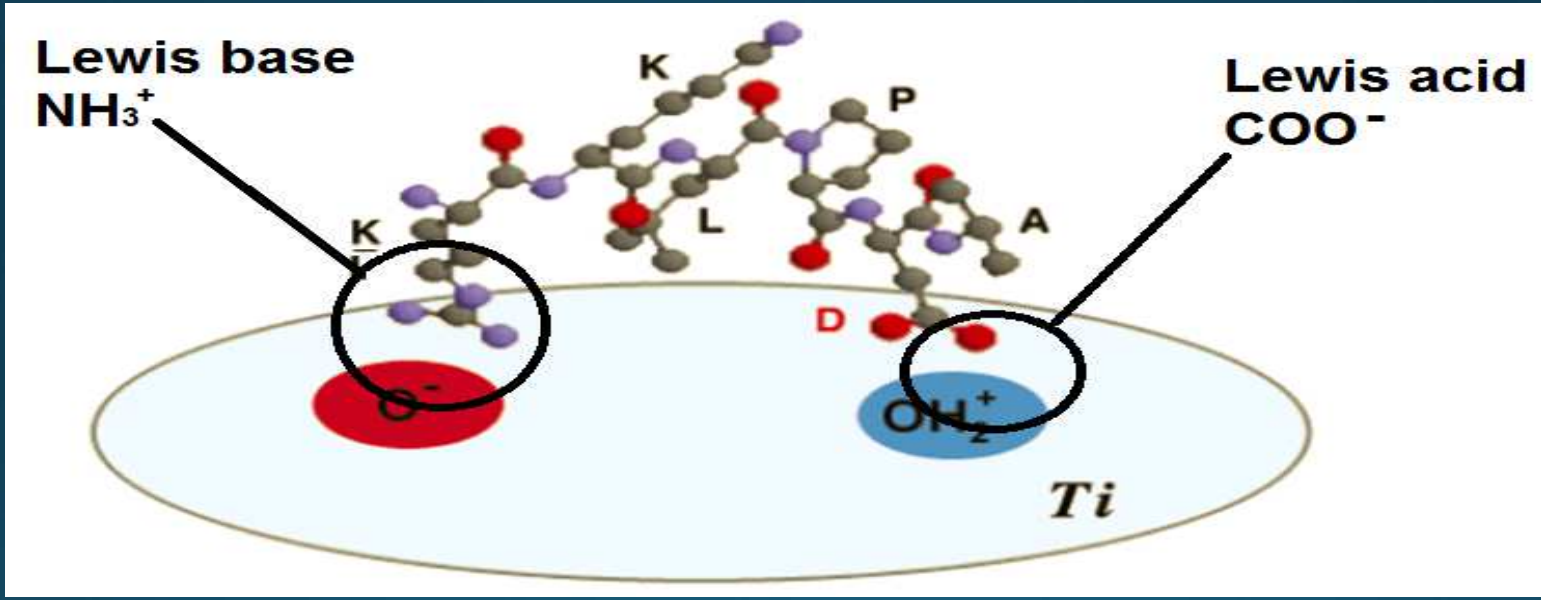
Improved Biocompatibility

Energy Efficient Deposited Coating

Possibility of further functionalisation (by using HA peptide binder functionalisation with antimicrobial peptides)

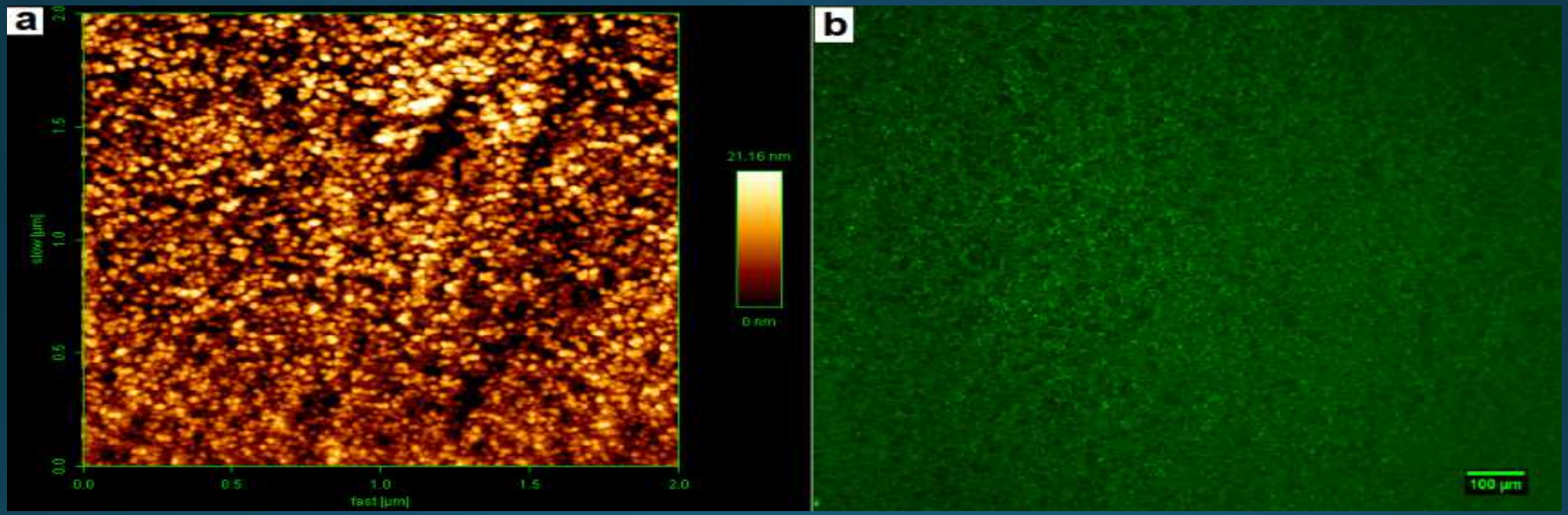
Kelly, M., et al, Peptide aptamers: Novel coatings for orthopaedic implants, Materials Science & Engineering C-Materials for Biological Applications, 54, 84-93 (2015)

Biomedical surfaces

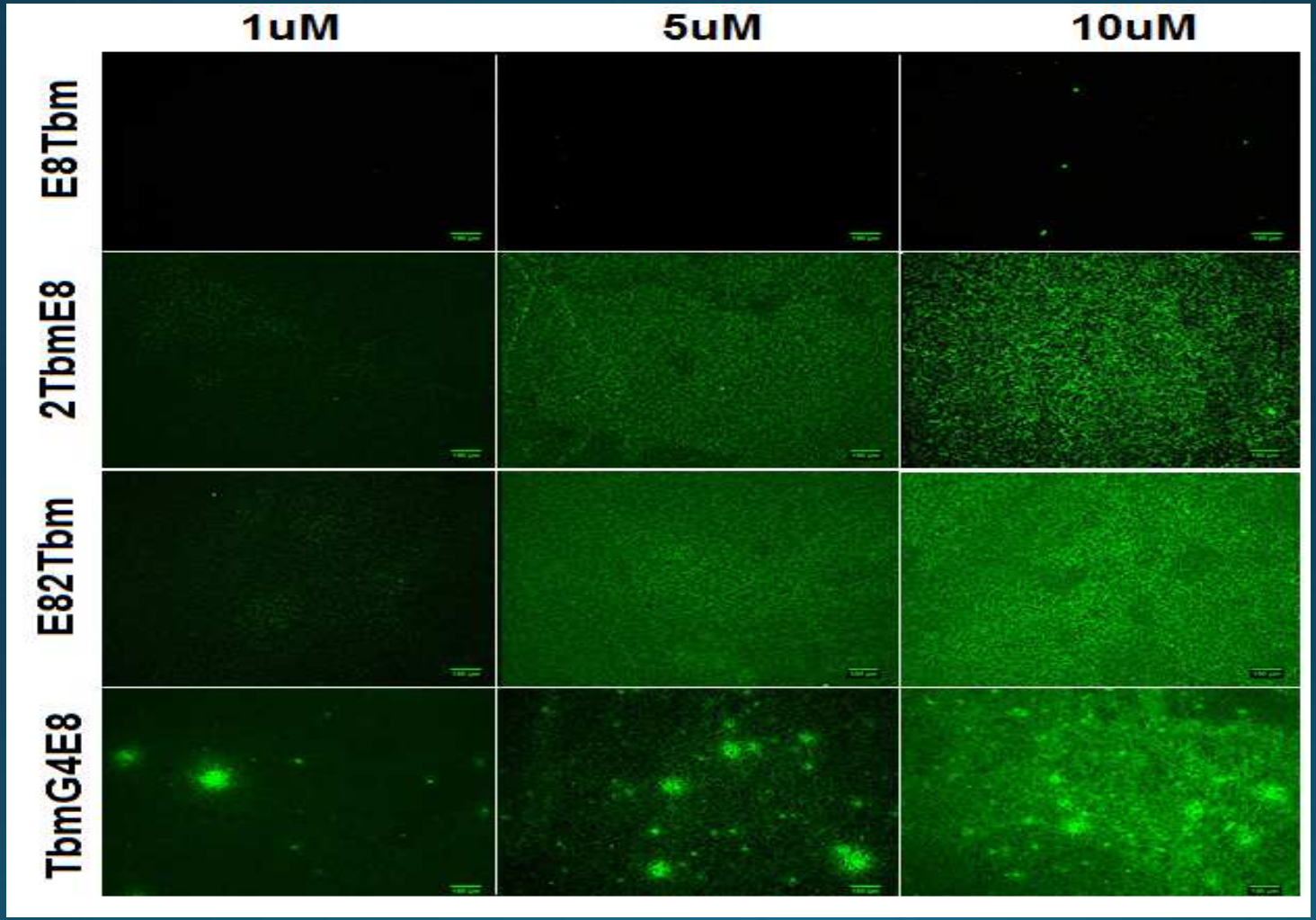


K. I. Sano and K. Shiba, J. Am. Chem. Soc., 2003, 125, 14234

Characteristic titanium oxide nanopilars

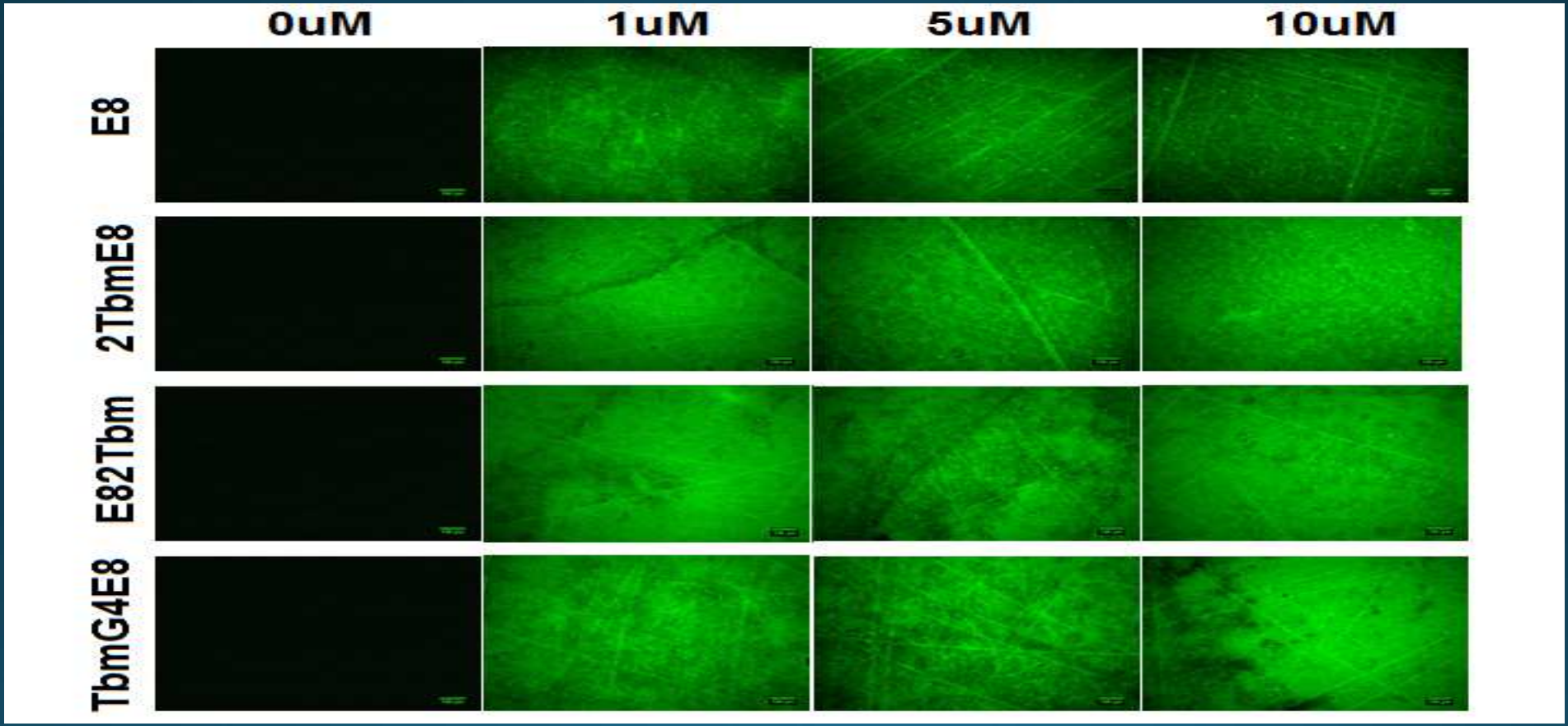


Titanium oxide binder



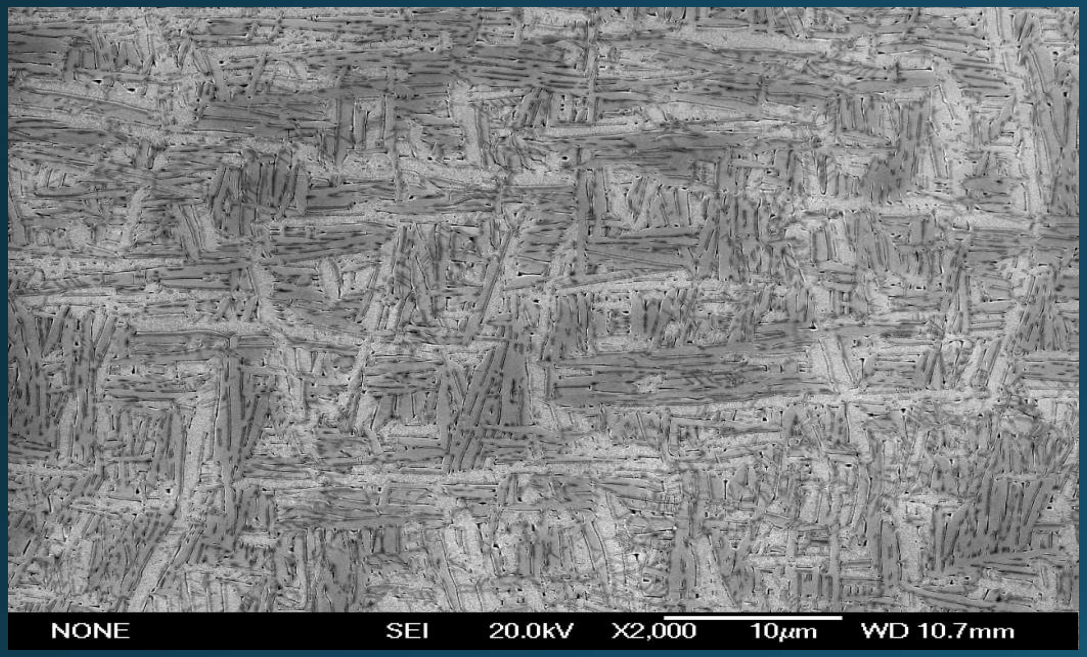
Fluorescence imagines of 10 X of magnification (100µm) of functionalized Ti-6Al-4V with E8Tbm, 2TbmE8, E82Tbm and TbmG4E8 binder at 0, 1, 5, and 10 µM on 24h H_2O_2 etching titanium plates.

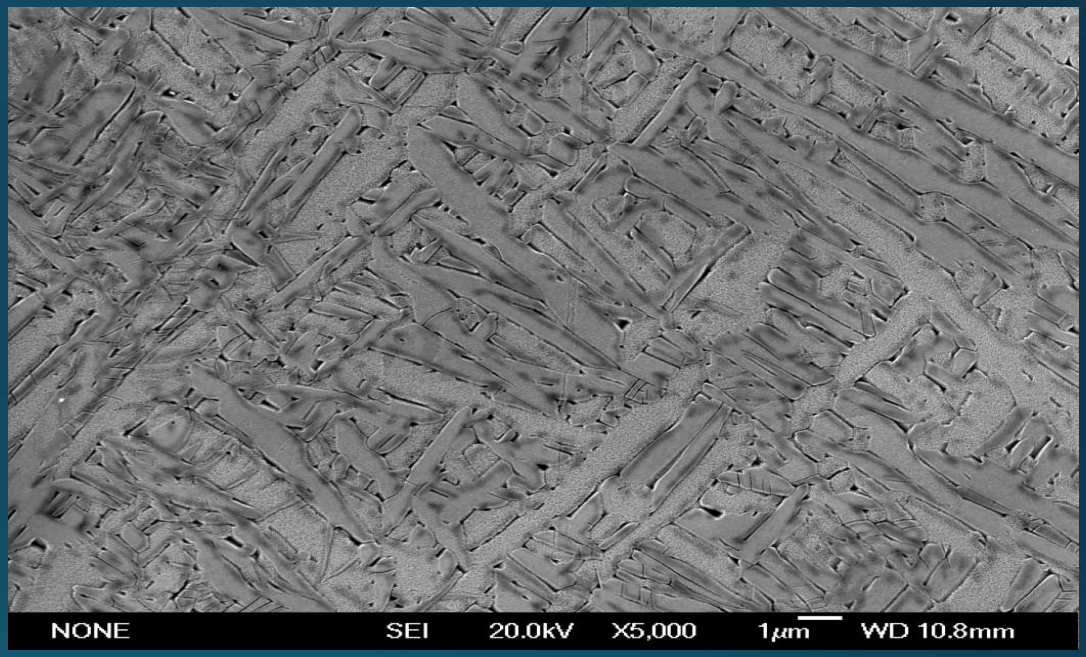
Functionalized HA



Fluorescence picture of 10 X of magnification (100µm) of functionalised Hydroxyapatite withE8, 2TbmE8, E82Tbm and TbmG4E8 binder at 0, 1, 5, and 10 µM.

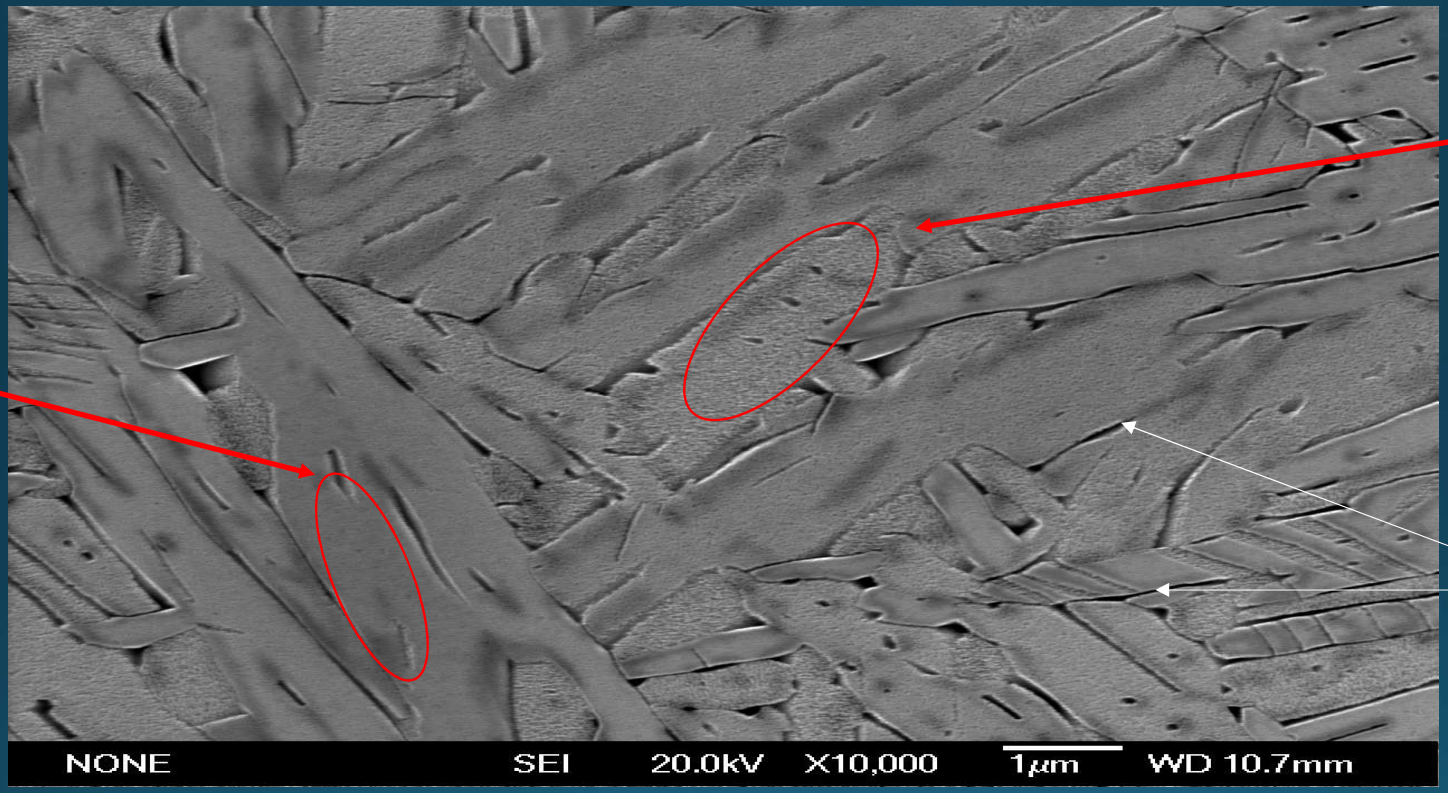
Ti6Al4V 3D printed surface A: Typical grain characteristics





Etching: 8.8 M H_2O_2 + 0.1 M HCl at 80 °C for 30 min and further thermal oxidation at 500 °C

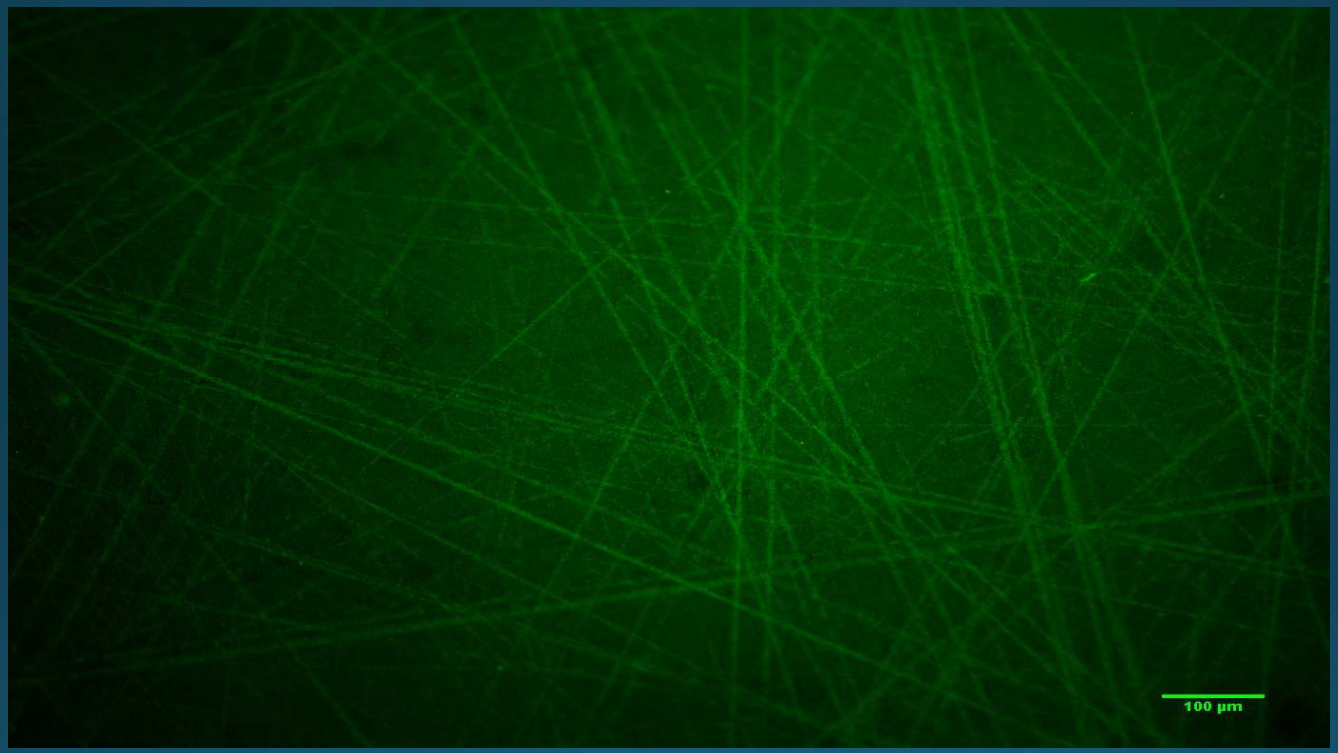
α



ß

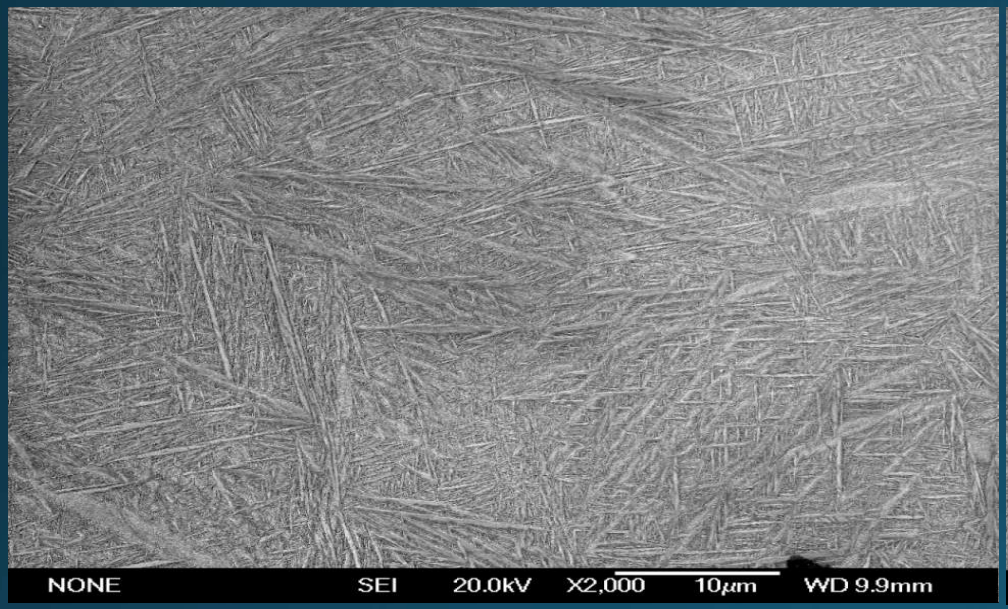
porosity

5FAM-KKLPDAKKLPDAEEEEEEEE.



Fluorescence micrograph (100 µm) of 3D printed Ti6Al4V (surface A) after dip coating in SBF solution at pH4 and concentration of 10 µM of labeled peptide aptamers

In contrast 3D printed Ti6Al4Vsurface B has sharper grains.



Micrograph X2000 and X5000 3D printed Ti6Al4V after etching with 8.8 M H_2O_2 + 0.1 M HCl at 80°C for 30 min and thermal oxidation at 500 °C

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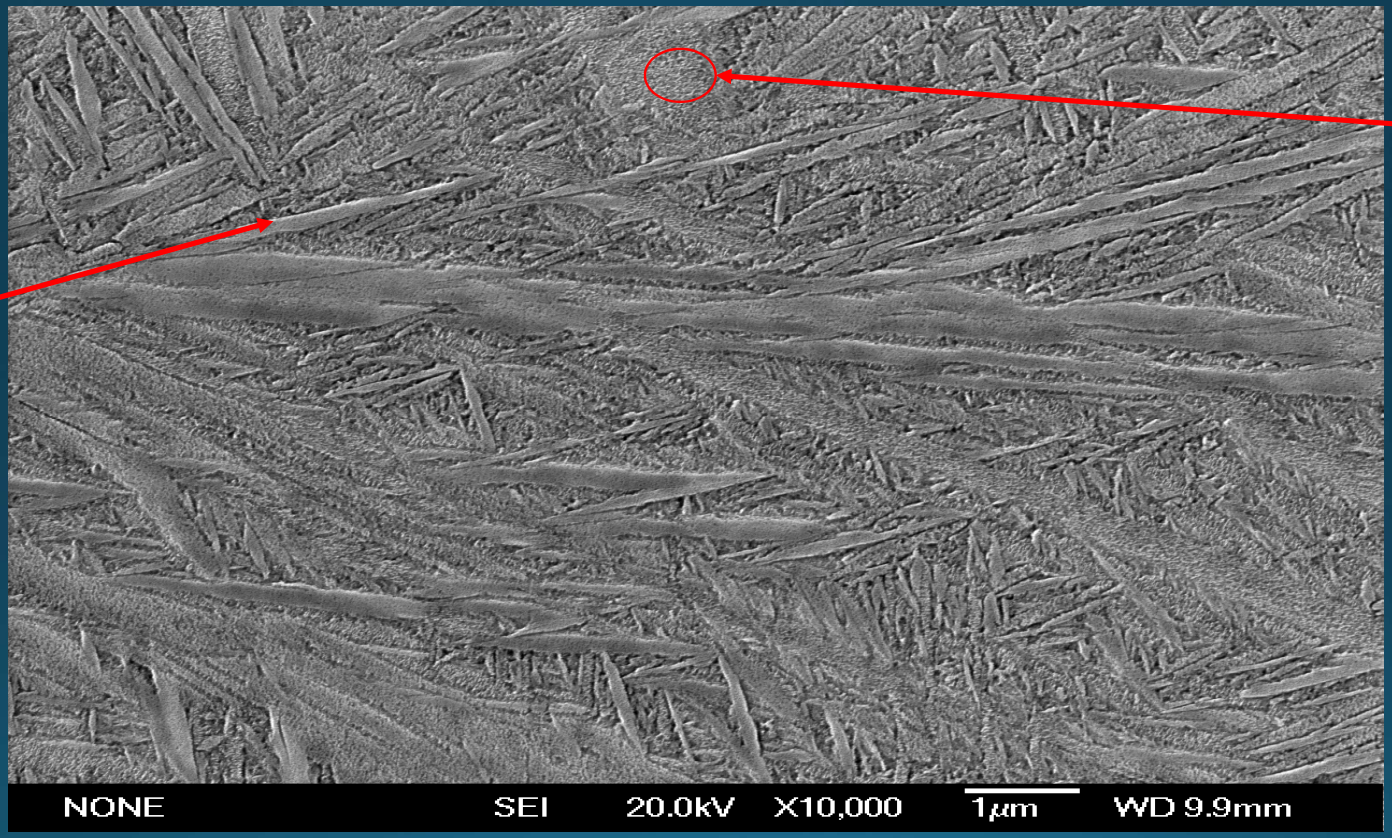
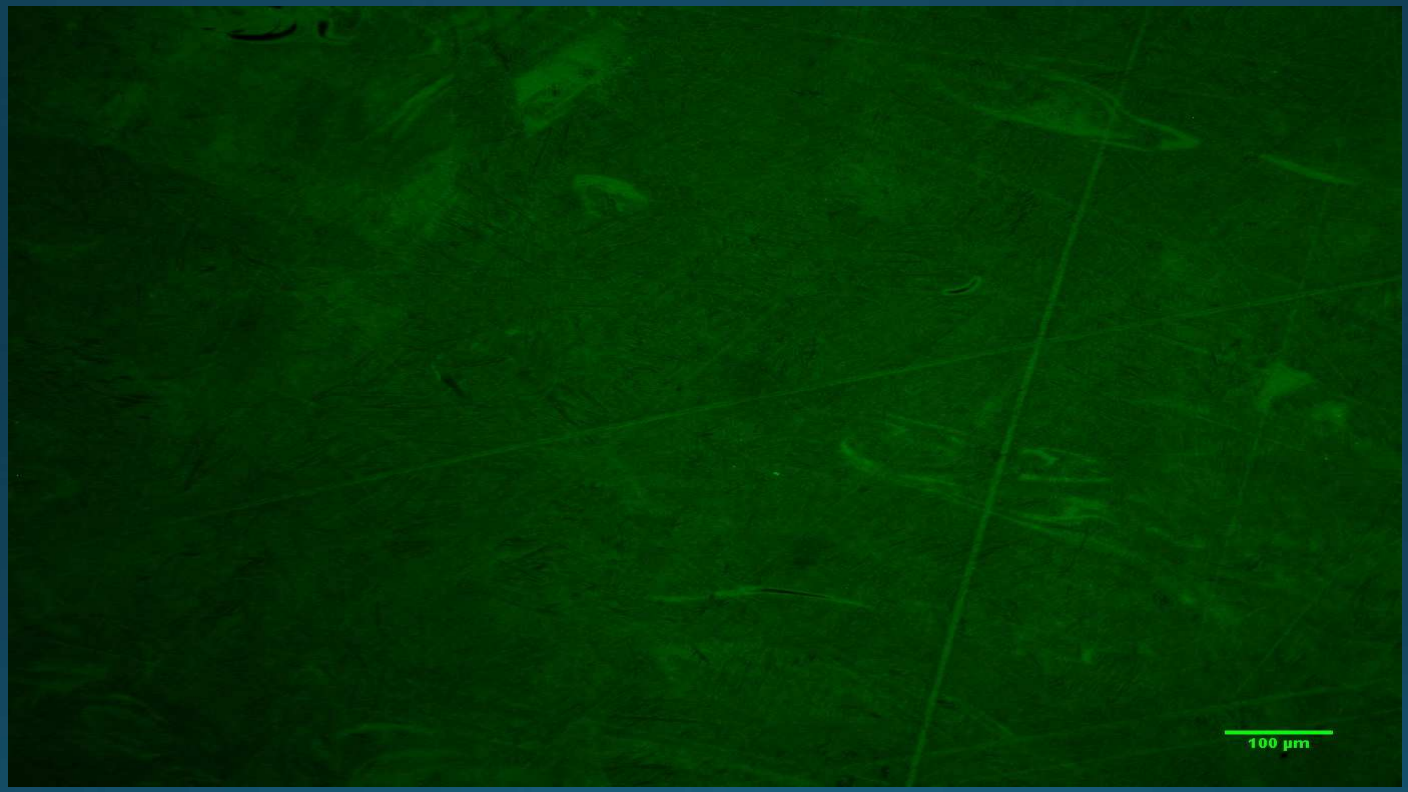


β

5FAM-KKLPDAKKLPDAEEEEEEEE



Fluorescence micrograph (100 µm) of 3D printed Ti6Al4V (surface B) after dip coating in SBF solution at pH4 and concentration of 10 µM of labelled peptide aptamer

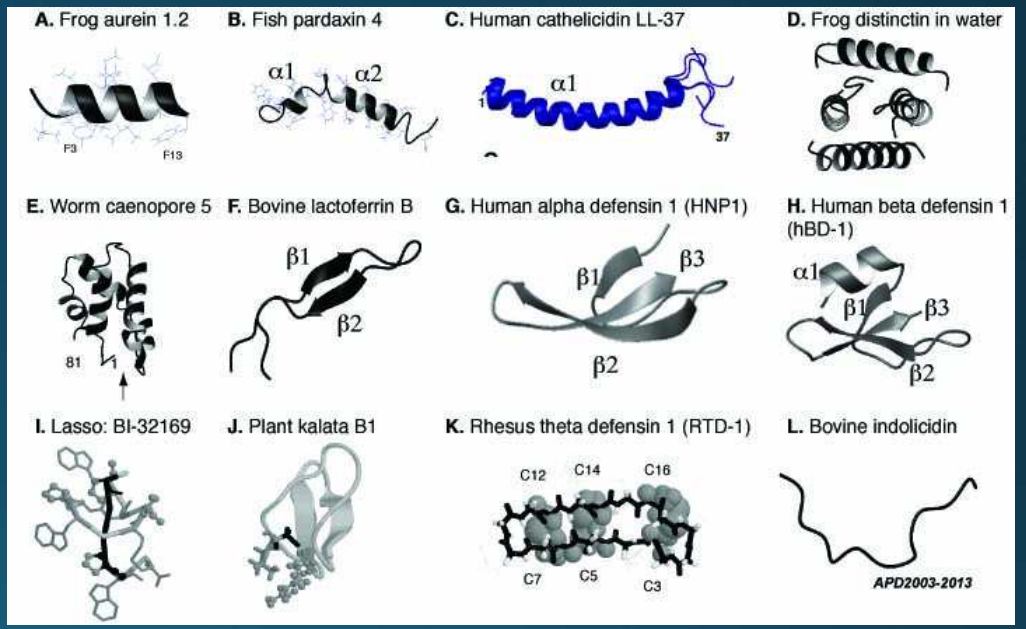
Important Remarks

1) Both surfaces have the composition of α and ß titanium and is possible to obtain porosity in both cases. The topography at nano level will be important for coatings and depositions of bioactive materials such as calcium apatite including hydroxyapatite.
2) After measuring the fluorescence intensities for both samples once the peptide coating was on the surface it was a notable 30% increase in intensity of surface A compared with surface B.

Antimicrobial Peptides

Antimicrobial peptides (AMPs)

- Part of innate immunity of all organisms
- Co-evolved with bacteria
- 12-100 amino acids long sequences
- Mostly cationic and amphipathic
- Classified by secondary structure
- Disrupt bacterial membrane and/or inhibit nucleic acid and protein synthesis



Antimicrobial Peptides

- Clinical applications and commercial development of these compounds is still very limited. **Disadvantages in the production, properties and efficacy of AMPs together with high manufacturing costs** have contributed to slow the transfer from research to clinical practice and development of commercial products.
- There are several AMP compounds that are undergoing clinical trials under the intriguing perspective of joined antimicrobial and immune-modulatory functions
- At present drugs based on AMPs have not been approved yet. Due to their chemical nature (peptides), oral and intravenous administration poses problems due to possible reduction or neutralisation of the active ingredient or induction of allergic reaction.
- AMP derived drugs appear very promising compounds for **topical formulations**, for example for treatment of skin, wounds etc., as well as for the protection of implanted devices ranging from catheters to contact lenses, stents and artificial tissue substitute applications.

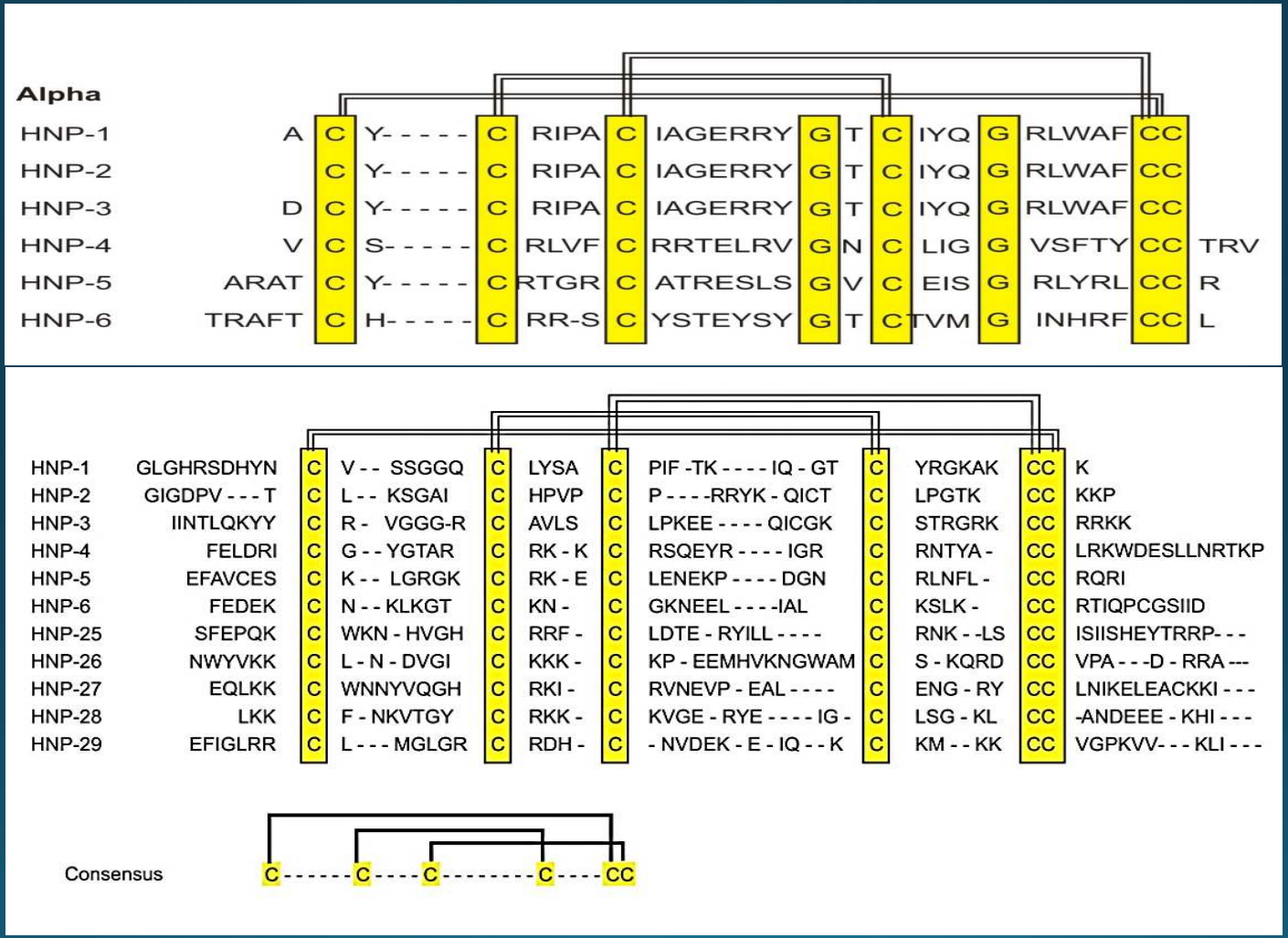
Steinstraesser L et al., Immunobiology, 2011; 216(3):322–333

Wimley et al., J Membrane Biol., 2011;239:27-34

Human defensins

α-defensins

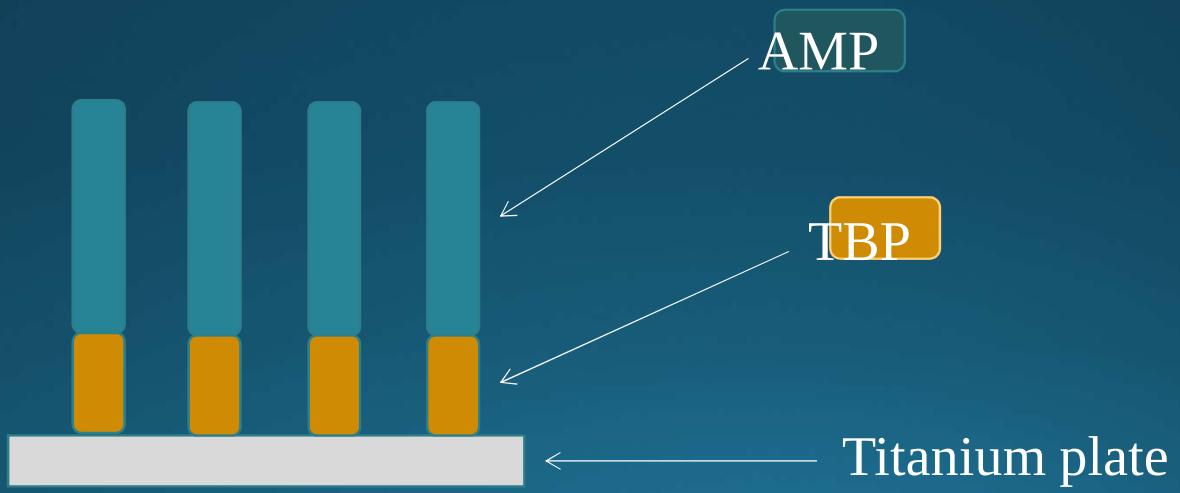
β-defensins



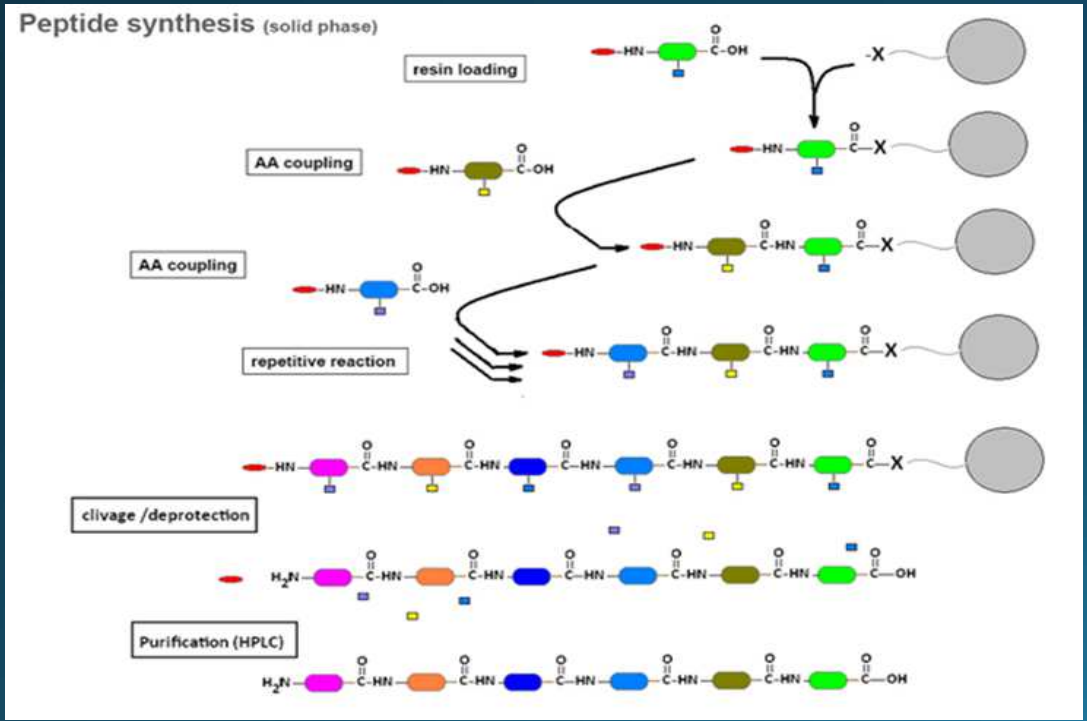
Stamboulis A., De Cogan F., Scott R., Antimicrobial surface, International Patent Application No. PCT/GB2015/050544, Publication No WO2015128643 A1

Antimicrobial peptide coating

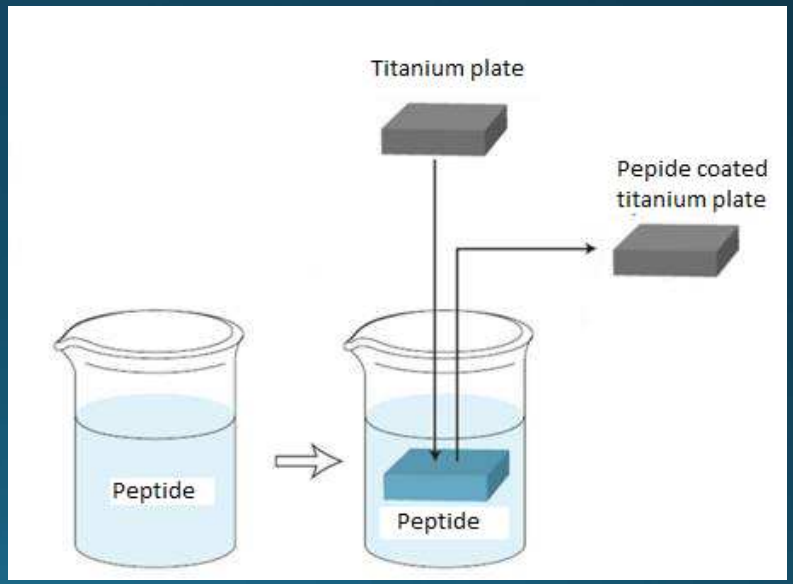
Use of RKLPDA titanium binding peptide (TBP)
Bi-functional hybrid antimicrobial peptide – TBP (AMP-TBP)



Solid Phase Peptide Synthesis



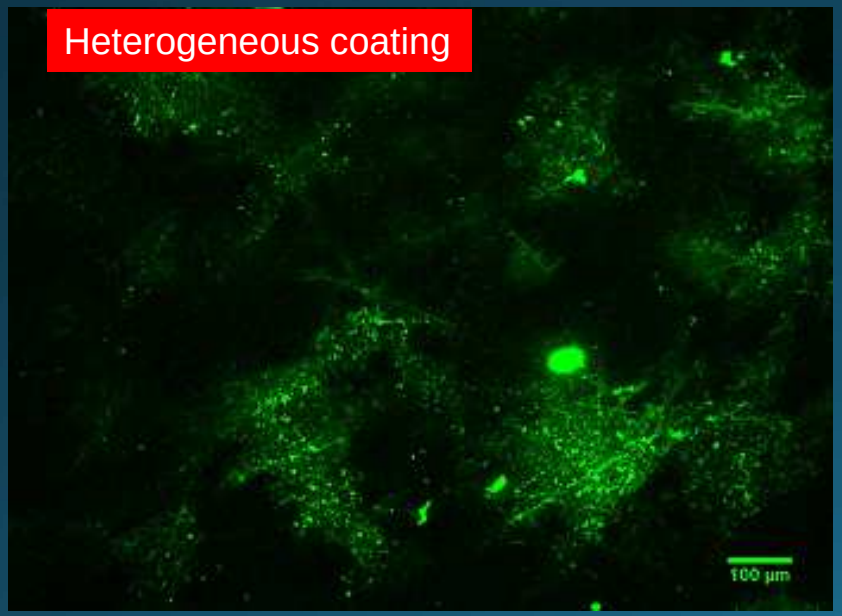
- AMP-TBP peptide attachment to the surface



Synthetic Biology?

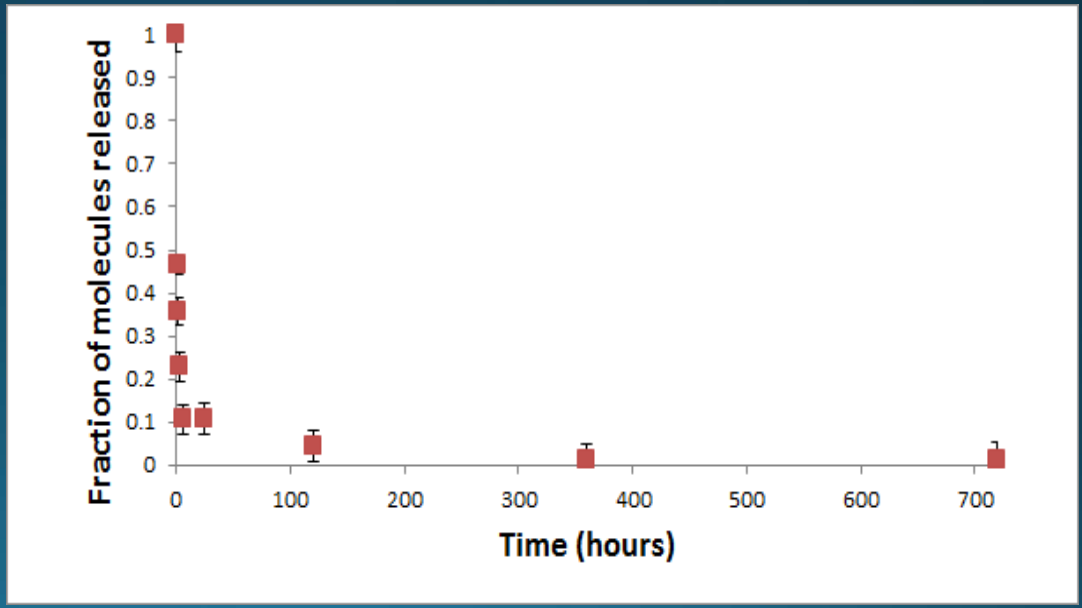
Fluorescence microscopy and Release studies

Fluorescence imaging of fluorescein labelled peptides



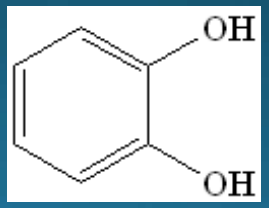
Release studies at pH 7.4, 37°C

67% of peptide was released after 1h



How can we make AMP coating better?

- By replacing peptide aptamers with a polymer mimicking mussel adhesive protein
  - Adheres to virtually all surfaces in aqueous, alkaline and rich in salt conditions
  - Contains a catechol group which strongly binds to organic and inorganic materials



Catechol group

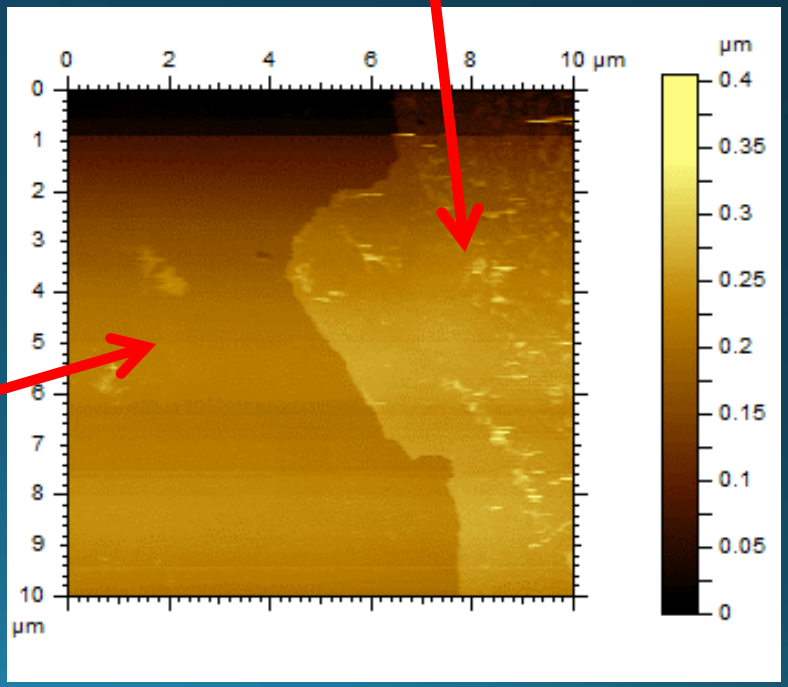
Adhesive polymer antimicrobial coating

AMP conjugated with the adhesive polymer

Film thickness **35.4 ± 4 nm**

Masked surface to allow thickness measurement

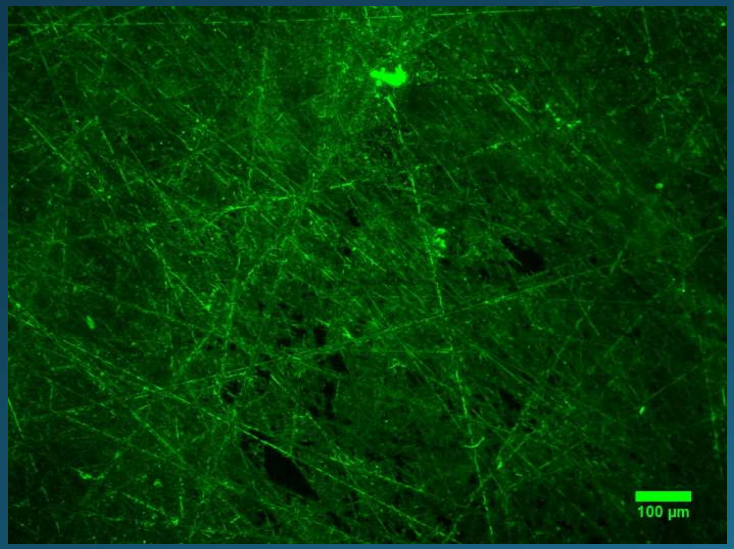
AFM image of AMP conjugated coating



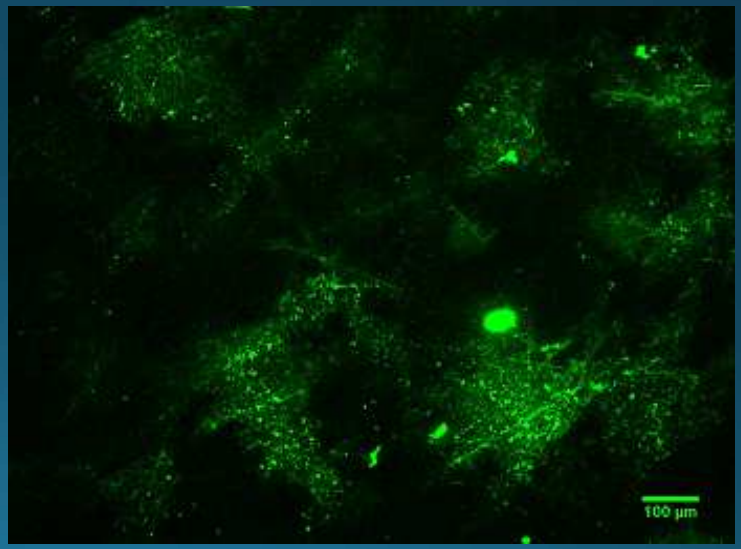
Adhesive polymer antimicrobial coating

- Fluorescence imaging of fluorescein labelled peptides

AMP–adhesive polymer coating



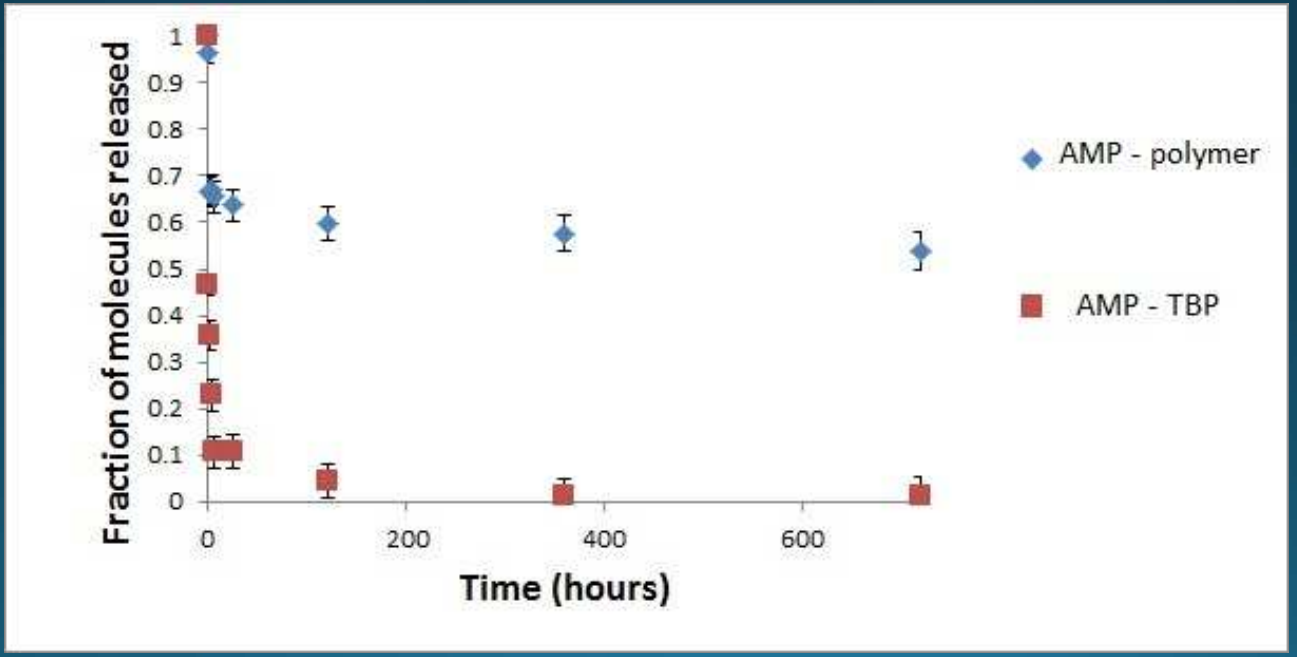
AMP-TBP coating



Adhesive polymer antimicrobial coating

- Release studies at pH 7.4, 37°C

55% of AMPs were present after 30 days



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Engineering and Physical Sciences Research Council

HORIZON 2020

MARIE CURIE ACTIONS