14th Multinational Congress on Microscopy

15.-20.9.2019, Beograd, Srbija

″Formation of calcium phosphates TiO_2 nanotubes in the presence of albumine: insight in formation of multifunctional nanocoposites″

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Formation of calcium phosphates on TiO_2 nanotubes in the presence of albumine: insight in formation of multifunctional nanocomposites

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Among largest health issues in modern society increased frequency of hard tissue chronical diseases takes special place [1]. These diseases could be a consequence of physical trauma, birth defects, other diseases (cancer), etc. In response to this problem, World health organization has appointed years 2000-2010 a Bone and Joint Decade [2]. Often the only treatment of such diseases is implantation with the aim to regenerate damaged or diseased tissue. However, only in the USA, approximately 10% of implants fail prematurely [3]. In addition, due to the continuous population ageing, many patients are outliving their implants [4]. These problems clearly urge the need to develop new, innovative implant materials. The solution of such problems is sought in development of multifunctional materials, which in addition to replacing missing tissue or enabling its regeneration, as well as having improved mechanical properties, will act as local drug delivery system [4-6]. New hard tissue regeneration biomaterials emerging in recent years are composite materials based on calcium phosphates (CaPs) and different inorganic nanomaterials as they offer possible ways of preparing multifunctional materials because inorganic nanomaterials embedded within CaP can increase their mechanical strength [7] and can be used as drug delivery systems, like TiO_2 nanotubes (TiNTs). Nowadays it is possible to produce arrays of TiNTs with precisely controlled diameter, length, wall thickness and phase [8]. *In vitro* cells response to nanotubular surface is better compared to not treated surfaces and can be modulated by changing the diameter of TiNTs [9]. TiNTs also offer a possibility to covalently immobilize bioactive molecules, and in that way act as drug delivery system [10]. Modifying TiNTs arrays with CaP can be achieved by different techniques, biomimetic, electrochemical and magnetron sputtering being mostly used [11]. However, to the best of our knowledge TiNTs are not used as fiber reinforcements of CaP ceramics and cements.

In this research we have investigated the impact of TiO_2 nanotubes (TiNT) on properties of formed CaP solid phase at conditions closed to physiological in the presence of bovine serum albumine (BSA). BSA is the most abudant protein in human plasma and is one of first macromolecules that will interact with synthesised nanocomposites in human body. It is one of three soluble proteins which are immediately adsorbed on the surface of the implant, influencing its *in vivo* performance [12]. However, its role in mineralization of titanium implants is still controversial [13].

All experiment systems were preformed in a thermostated double-walled glass vessel with a 50 mL capacity at 25 °C. Precipitation systems were not additionaly stirred. Control precipitation system (CS) was prepared by fast mixing of equal volumes (20 mL) of equimolar $CaCl_2$ and Na_2HPO_4 reactant solutions (c = 4 mmol dm^{-3}), so-called anionic and cationic solution. The pH of anionic solution was adjusted to 7.4 with HCl. Precipitation systems containing TiNT and/or BSA were prepared by adding TiNT suspension or BSA solution to the anionic reactant solution. Before mixing the reactant solutions the pH was readjusted when necessary. The progress of reaction was followed by measuring the pH of the precipitation systems. Based on pH curves induction time (t_{ind}), the time passed from initiation of precipitation process untill the begining of amorphous CaP (ACP) transforms into more stable crystalline phase was calculated. Prepared precipitates were filtered after 60 minutes of reaction time. The influence of TiNT and BSA on structure and morphology of precipitated CaP phase was investigated by Fourier – Transform Infrared spectroscopy (FTIR), Raman spectroscopy, Poxwder Diffaction (XRD), Scanning Electron microscopy (SEM), Transmission Electron Microscopy (TEM).

Representative pH vs time curves are shown in Fig. 1. Curves obtained in all investigated systems showed typical sigmoidal shape reflecting three stages of the precipitation process: i) initial slight pH decrease associated with the formation of ACP, during which the changes in pH and calcium concentrations are small or absent, ii) an abrupt decrease in pH associated with the secondary precipitation of crystalline phase upon ACP, iii) final slight pH change associated with solution-mediated growth and phase transformation [14,15]. Induction times were obtained from intersection of the tangents drawn on the second and third section of pH *vs.* time curve (Table 1.). In the presence of TiNTs a significant reduction of induction time was observed, indicating promotion of ACP transformation, which was not concentration dependent in the investigate concentration region. Contrary to TiNTs, in the presence of BSA transformation is inhibited and the influence is concentration dependent. In the precipitatuion systems containing both TiNTs and BSA the transformation was faster at higher TiNTs concentrations.

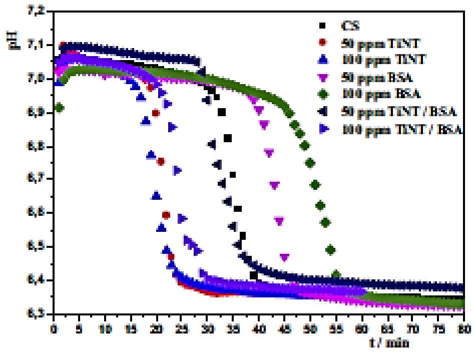

Figure 1.Representative pH vs. time curves of investigated precipitation systems. pH_{init} = 7.4, θ / °C = (25 ±0.1).

Table 1. Average induction times (t_{ind}) obtained from pH vs. time (t) curves (Fig. 1) from 5 measurements with standard deviations (SD). pH_{init} = 7.4, θ / °C = (25 ±0.1).

	(t_{ind} ± SD) / min		
γ / mg L^{-1}	TiNT	BSA	TiNT / BSA
0		29.4 ± 3.2	
50	17.4 ± 0.9	39.7 ± 1.0	28.2 ± 1.1
100	17.9 ± 1.9	45.1 ± 0.9	20.3 ± 1.5

PXRD patterns and FTIR spectra showed that presence of either TiNTs or BSA, or both of them does not influence the composition of the formed precipitate. In all systems calcium deficient hydroxyapatite (CaDHA, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, $0 < x < 2$) was formed. CaDHA is considered to be a promising material for manufacturing biomaterials for hard tissue regeneration as biological apatite, the main inorganic part of animal hard tissues, is in fact ion-substituted CaDHA [16]. Althought TiNTs do not influence the morphology, SEM investigation have shown that CaDHA grows on TiNT surface in linear layout (Fig. 2).

The obtained results point to a biomimetic preparation route of multifunctional CaP based biomaterials.

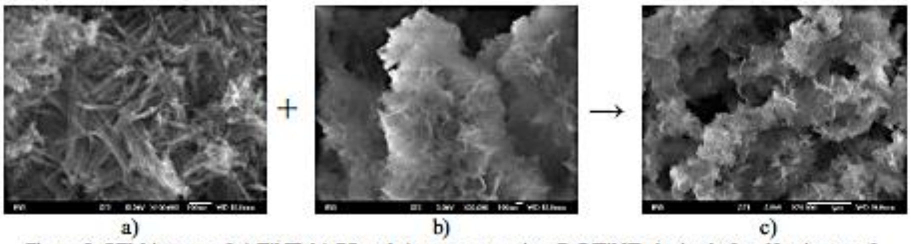

Figure 2. SEM images of a) TiNT, b) CS and c) nanocomposites CaP/TiNT obtained after 60 minutes of reaction time.

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Acknowledgment

This work has been financially supported by the Croatian Science Foundation under project IP-2018-01-1493.

FORMATION OF CALCIUM PHOSPHATES ON TiO_2 NANOTUBES IN THE PRESENCE OF ALBUMINE:
Insight in formation of multifunctional nanocomposites

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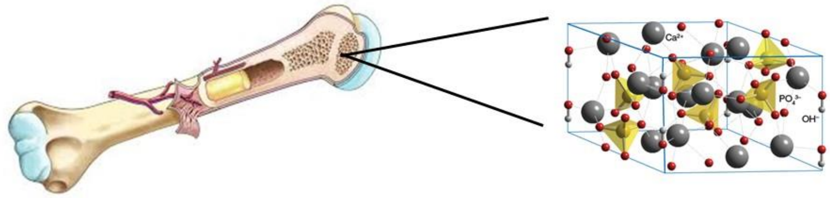
2000-2010 Bone and Joint Decade

- Hard tissue chronical diseases are among the largest health issues in modern society
- Treatment of such diseases – implantation
- Requirement for new implantation materials – multifunctional materials
- Calcium phosphate and inorganic nanomaterials nanocomposites

Moroni, A. Nandakumar, F. B. De Groot, C. A. van Blitterswijk and P. Habibovic, *J. Tissue Eng. Regen. Med.* **9** (2015) 745–759.

Calcium Phosphates and TiO_2 nanotubes

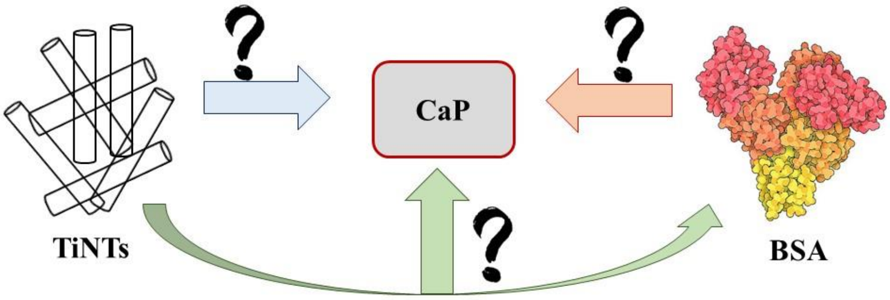
- Calcium Phosphates (CaPs) are main inorganic component of hard tissues
- In the biological systems they occur mainly in the form of "biological apatite" - poorly crystallized nonstoichiometric hydroxyapatite or CaDHA doped with sodium, magnesium or carbonate ions
- CaPs have poor mechanical properties – doping with inorganic nanoparticles
- TiO_2 nanotubes (TiNT) acts as drug delivery systems



C. Canal and M. P. Ginebra, *J. Mech. Behav. Biomed. Mater.* **4** (2011) 1658–1671
E. Beltrán-Partida, et. al., *J. Nanobiotechnology*, **15** (2017) 10-15.

Aim of the study

- Investigation of the effect of TiNT on properties of formed CaP solid phase at conditions closed to physiological in the presence of Bovine Serum Albumine (BSA)
- HSA is the most abundant protein in human plasma



Materials and Methods

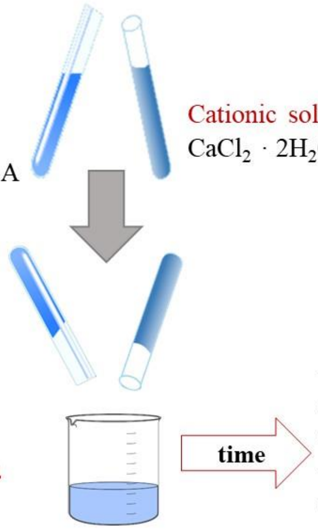
Anionic solution

a) Na_2HPO_4
b) Na_2HPO_4 + TiNT
c) Na_2HPO_4 + BSA
d) Na_2HPO_4 + TiNT/BSA

Cationic solution

$CaCl_2 \cdot 2H_2O$

Solution	Concentration
$CaCl_2 \cdot 2H_2O$	4 mmol dm^{-3}
Na_2HPO_4	4 mmol dm^{-3}
TiNT	50 or 100 mg dm^{-3}
BSA	50 or 100 mg dm^{-3}

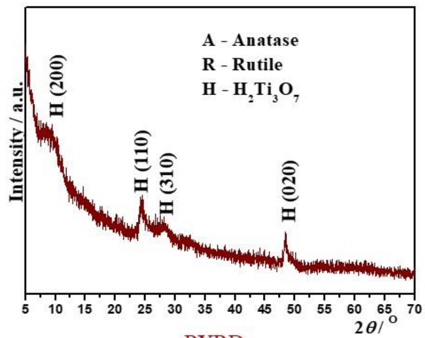


25 °C
$pH_{int} \approx 7.4$
60 min

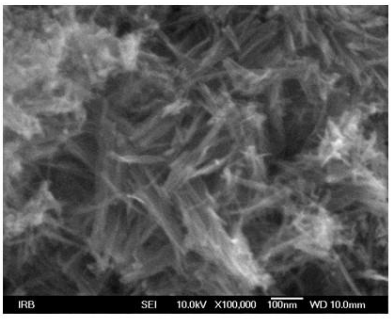
- Potentiometry
- Fourier Transform Infrared Spectroscopy (**FTIR**)
- Powder X-ray diffraction (**PXRD**)
- Scanning Electron Microscopy (**SEM**)
- Transmission Electron Microscopy (**TEM**)

TiNT Synthesis and Characterization

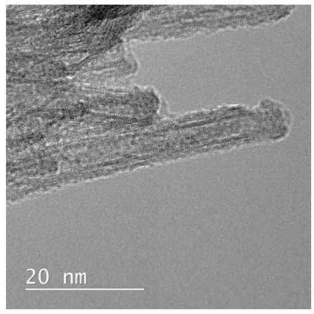
- TiNTs were prepared using a hydrothermal method similar to that described by Kasuga and co-workers



PXRD



SEM



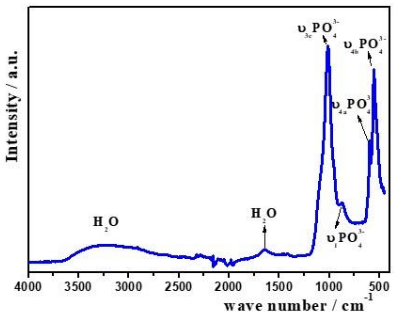
TEM

- TiNTs are mixture of rutile, anatase and $H_2Ti_3O_7$
- Average length 100 nm and diameter 5-10 nm

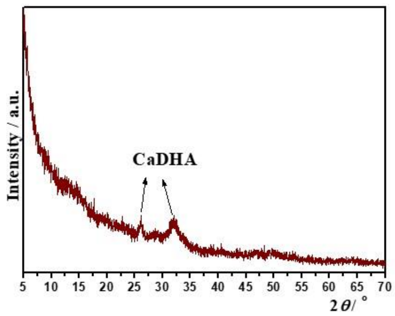
T. Kasuga, M. Hiramatsu, A. Hoson, T. Sekino, K. Niihara, *Adv. Mater.* **15** (1999) 1307.

Control system (CS)

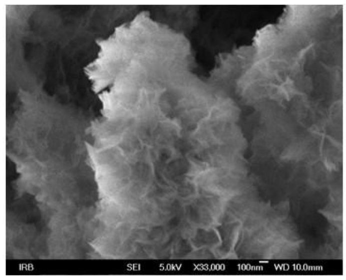
- System without TiNT or BSA: $Na_2HPO_4 + CaCl_2$



FTIR



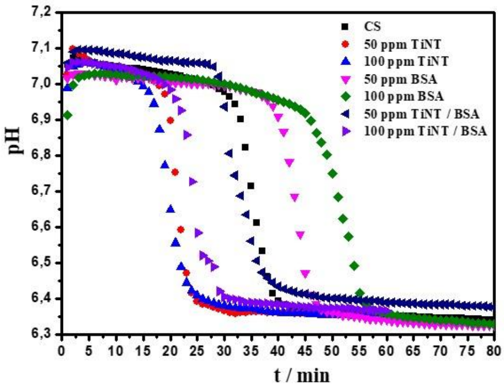
PXRD



SEM

- Calcium deficient hydroxyapatite (CaDHA, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, $0 < x < 2$)
- Leaf-like morphology

CaP/TiNT nanocomposites Characterization – Induction time



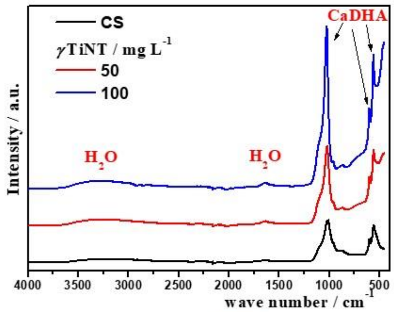
Representative pH vs. time curves

- The induction time is time passed from initiation of precipitation process till the amorphous phase transformation
- It is determined from intersection of the tangents drawn on the first two parts of pH vs. time curve

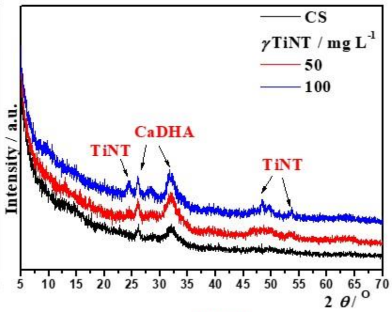
Induction time of investigated systems

γ / mg L^{-1}	0	50	100	
		17.4 ± 1.0	17.9 ± 2.0	TiNT
t_{ind} / min	29.4 ± 3.2	39.7 ± 1.0	45.1 ± 0.9	BSA
		28.2 ± 1.1	20.3 ± 1.5	TiNT/BSA

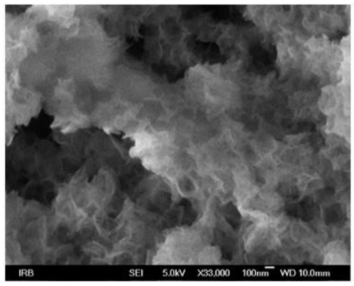
CaP/TiNT nanocomposites characterization



FTIR



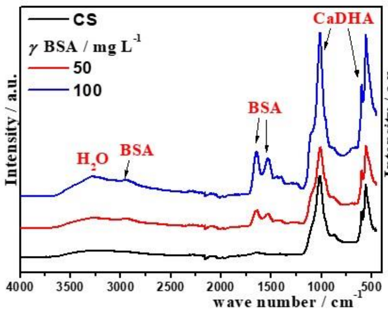
PXRD



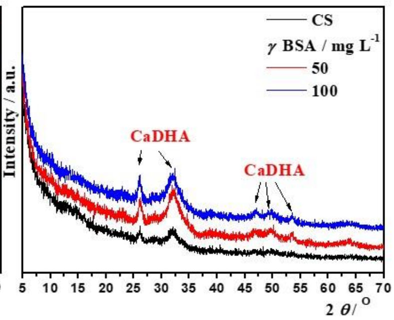
SEM

- TiNT does not influence the composition of precipitated CaP form
- SEM investigation has shown that CaDHA grows on TiNT surface in a linear layout

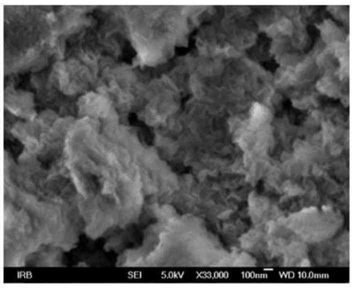
CaP/BSA nanocomposites Characterization



FTIR



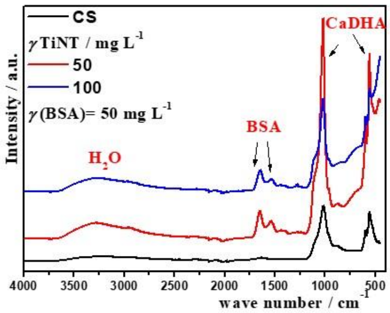
PXRD



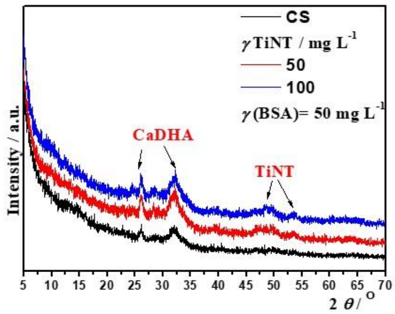
SEM

- BSA does not influence the composition of precipitated CaP form nor the morphology of CaDHA

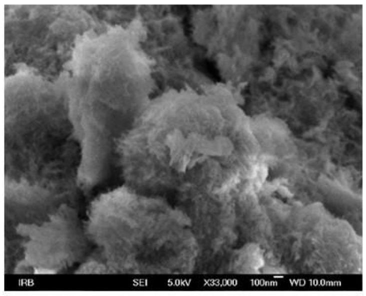
CaP/TiNT nanocomposites in the presence of BSA Characterization



FTIR



PXRD



SEM

- BSA does not influence the composition of precipitated nanocomposites
- In the presence of BSA, the linear layout of CaP on TiNT surface is not observed

To conclude…

- TiNTs act as promotors while BSA acts as inhibitor of CaDHA formation
- Neither of the additives influence the composition of the formed precipitate
- Changes of morphology were observed
- Combined effect of both additives is intermediate between the effects observed in the presence of TiNT or BSA alone
- The obtained results point to a biomimetic preparation route of multifunctional CaP based biomaterials

Acknowledgement

Ruđer Bošković Institute

Andreja Gajović, Ivana Panžić
Nadica Maltar Strmečki
Krunoslav Užarević

Institute of Physics

Iva Šrut Rakić

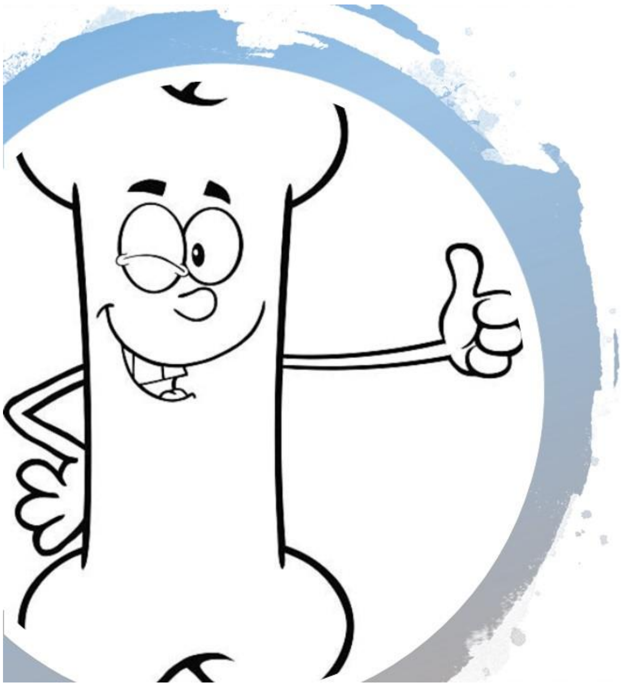
Faculty of chemical engineering and technology

Fabio Faraguna

Jožef Štefan Institute

Goran Dražić

This work has been financially supported by the Croatian science foundation project Mechanisms of calcium phosphates formation on inorganic nanomaterials. A biomimetic synthetic route for multifunctional nanocomposites for hard tissue regeneration



Thank you for your attention!