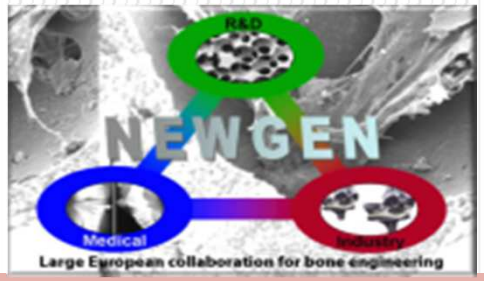


Investigation of biocompatibility and toxicity properties of modified calcium phosphate coatings

Bioactive coatings on medical implants

Monika Furko, Elena Della Bella, Milena Fini, Csaba Balázsi

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico

COST Action MP1301 – NEWGEN, Workshop, 17-18th March, Aveiro, Portugal

Motivation

The main objective of this research is to develop well adherent novel coatings for metallic implant materials which possess simultaneous antimicrobial and biocompatible properties.

The ionic silver, zinc, magnesium and strontium can be incorporated into or co-deposited with different calcium phosphate phases such as hydroxyapatites. One major advantage of silver and other mineral components substituted hydroxyapatite coated implant materials that they promote bone growth and increase the biocompatibility of the implant materials.

Antibacterial and biocompatible coatings on different metallic implant materials such as Ti6Al4V, CoCrMo and stainless steel that are frequently used in orthopaedic surgery, can be prepared by pulse current deposition. The mechanical properties and morphology of deposited layers strongly depend on the parameters of the electrochemical deposition process.

Measurements on calcium phosphate layers

Tasks:

- Optimize the deposition parameters in order to achieve a well adherent thin layer on implant surfaces.

- Optimize the electrolyte composition and concentration in terms of additional elements, such as silver, Zn, Mg, Sr.

- Study and compare the corrosion rate and biodegradable properties of the layers deposited with different parameters by recording potentiodynamic polarization measurements and Electrochemical Impedance Spectroscopy.

- Investigate the biocompatible and cytotoxicity properties of the modified implant materials by cell viability measurements.

Preparation of pure calcium phosphate layers

Electrolyte:

0.49M $Ca(NO_3)_2$, 0.29 M $NH_4H_2PO_4$, 10 ml/l H_2O_2

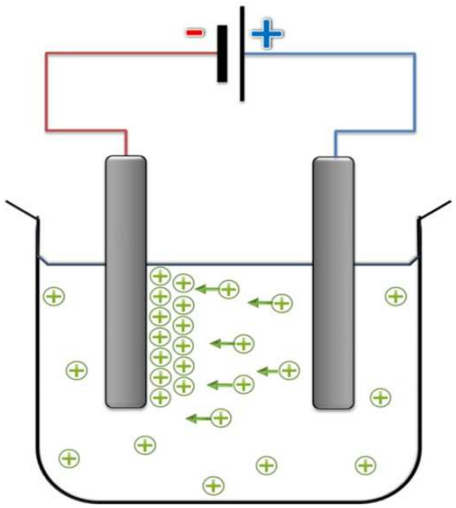
pH:4.5

anode: Pt

cathode: Ti6Al4V alloy

pulse current deposition parameters:

t_{on}: 1ms, t_{off}: 10ms i_p: 1.5A



Treatment after deposition

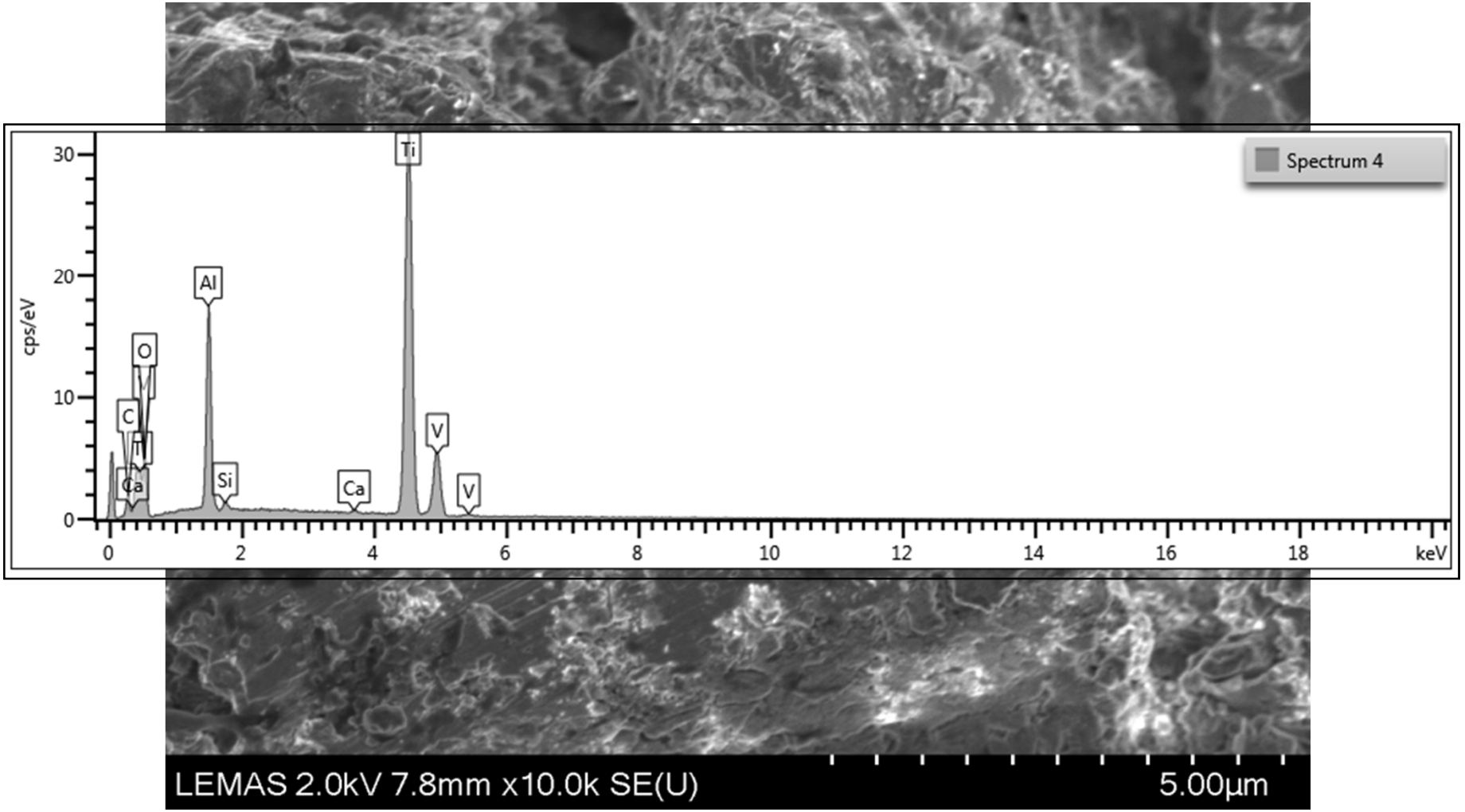
Heat treatment at 900 °C for 30 minutes

P3 set

Treatment in 1M NaOH solution at 70 °C for 2 hours

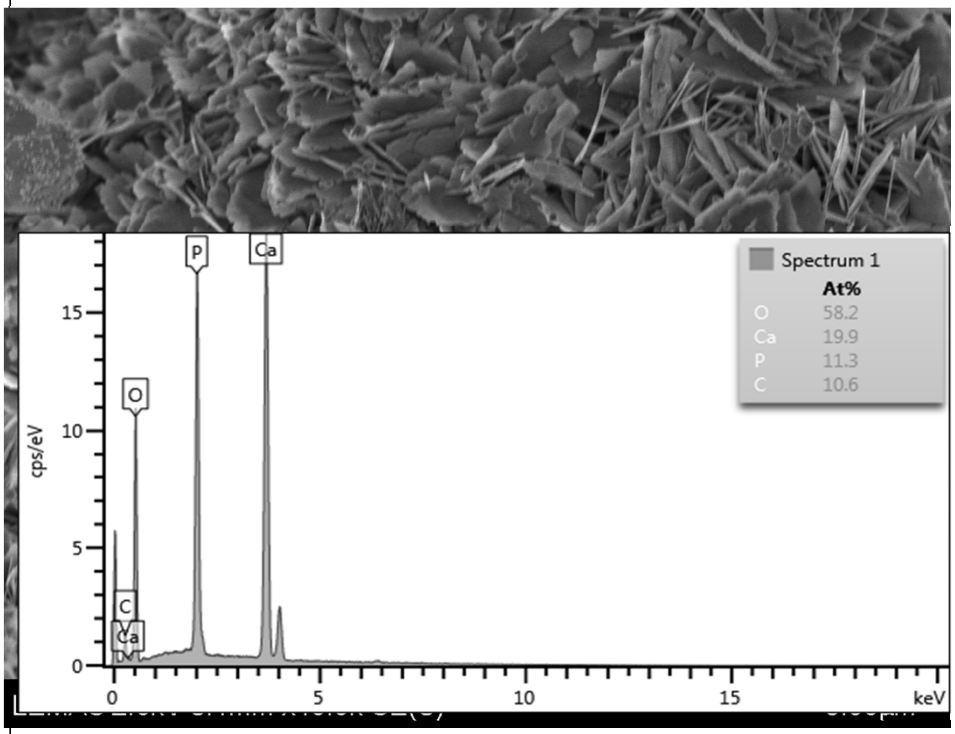
P4 set

SEM images and EDX analysis of sandblasted Ti6Al4V alloy



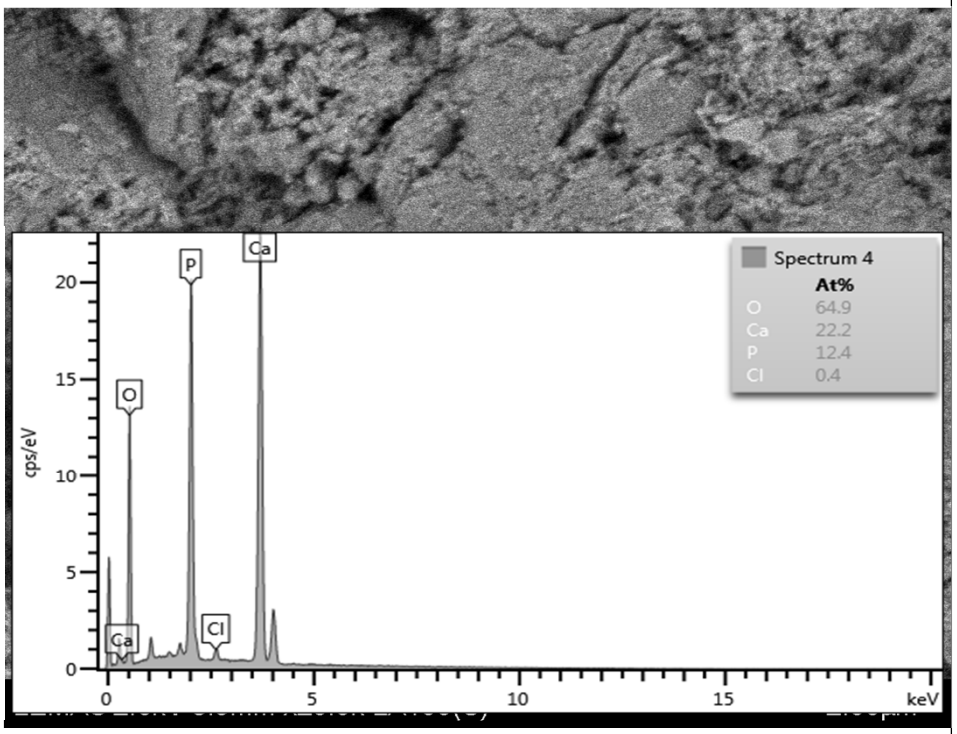
SEM images and EDX analysis on CaP layers

P3 set



Ca/P ratio: 1.76

P4 set



Ca/P ratio: 1.79

Preparation of modified calcium phosphate layers

Methods

- Electrochemical deposition from solution containing appropriate amount of $Ca(NO_3)_2$, $NH_4H_2PO_4$, H_2O_2, $Zn(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, $AgNO_3$ components
- Heat treatment at 900 °C for 30 minutes

P5 and P6 sets

- Electrochemical deposition of pure CaP layers,
- Treatment in 1M NaOH at 70 °C for 2 hours
- Spin coating of solution with following composition: $Zn(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, $AgNO_3$
- Heat treatment at 250 °C for 2 hours.

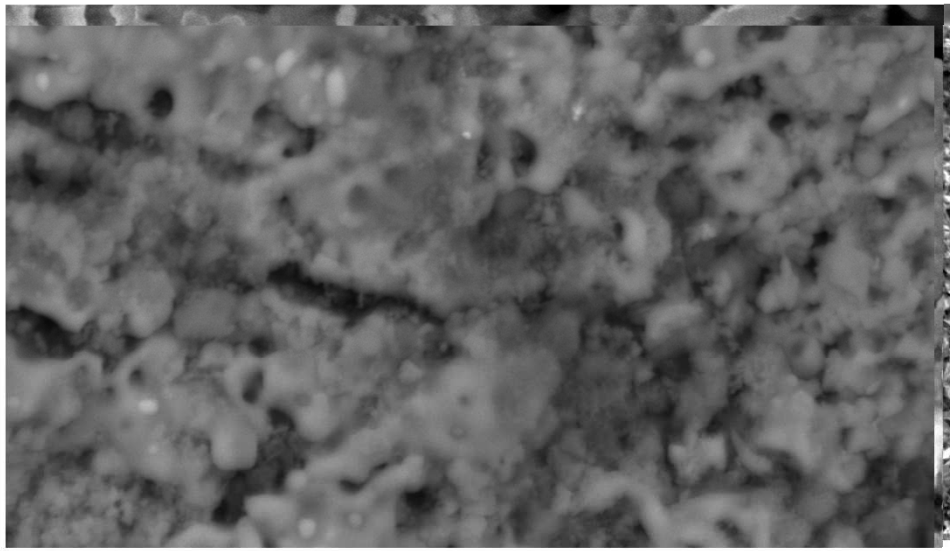
P7 set

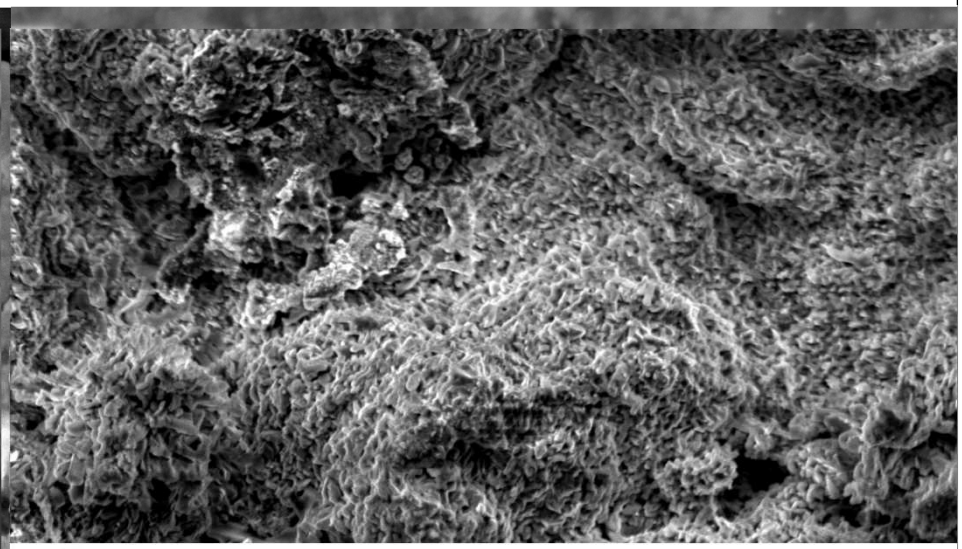
- Electrochemical deposition of pure CaP layers
- Spin coating of solution with following composition: $Zn(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, $AgNO_3$
- Heat treatment at 650 °C for 2 hours.

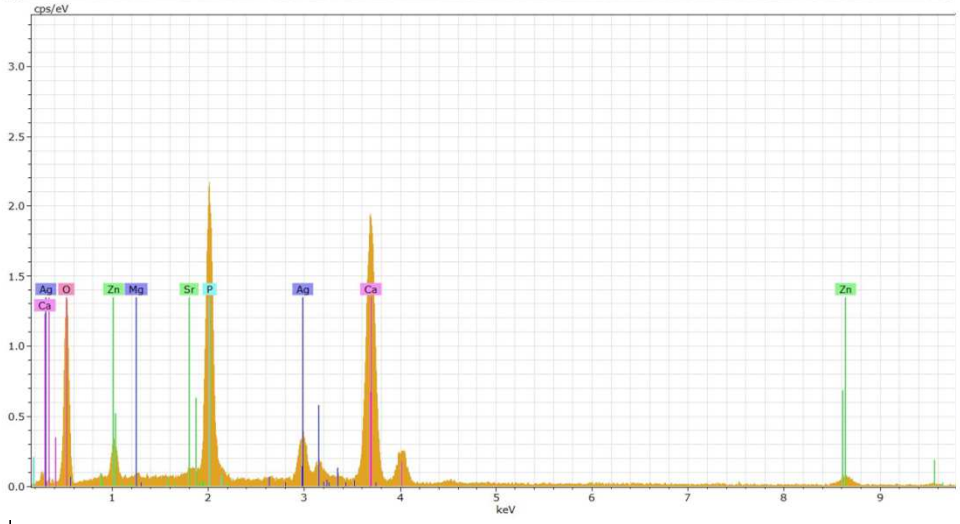
P8 set

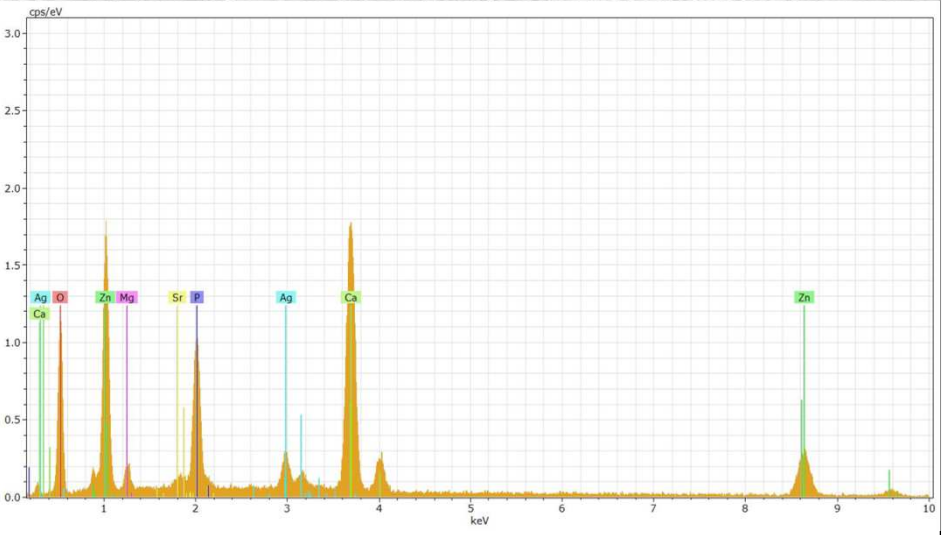
SEM images and EDX analysis on modified CaP layers

P5 set P6 set P7 set P8 set









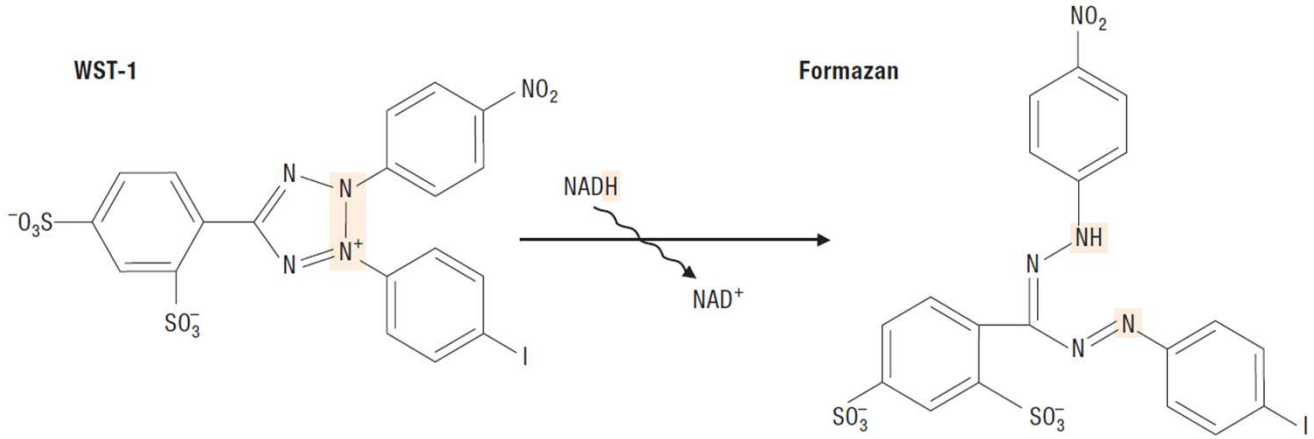
Biocompatible tests

Cells used for the experiments are represented by the MG-63 cell line, which is a line of human osteoblast-like cells. The cultures were maintained at 37°C, 5% CO_2 in a humidified atmosphere in incubator. The cytotoxicity (LDH assay) and WST-1 values were measured after 24 hours and 3 days.

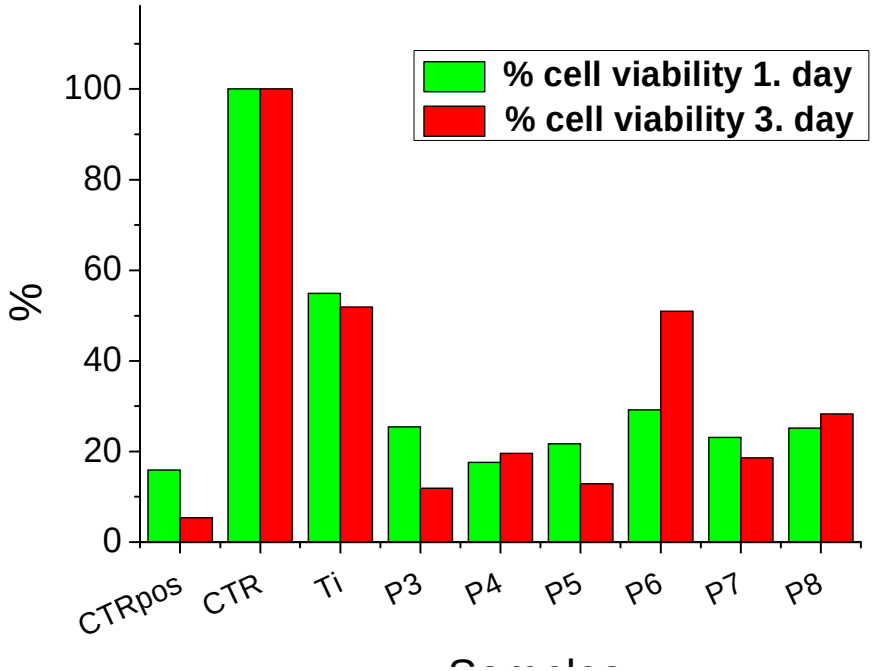
- Cell proliferation tests with reagent WST-1
- LDH test for cytotoxicity
- Live/Dead cell staining experiments
- Gene expression analysis

Cell proliferation tests

Molecular structure of WST-1 and its corresponding reaction product

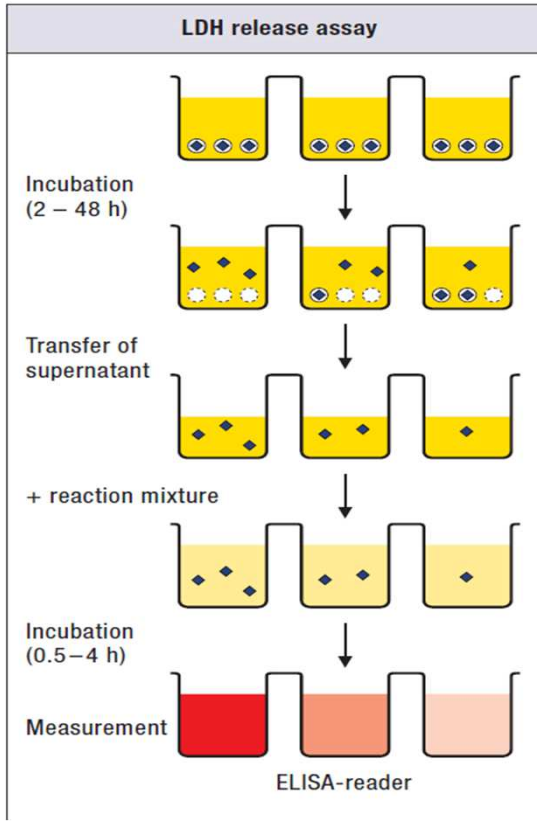


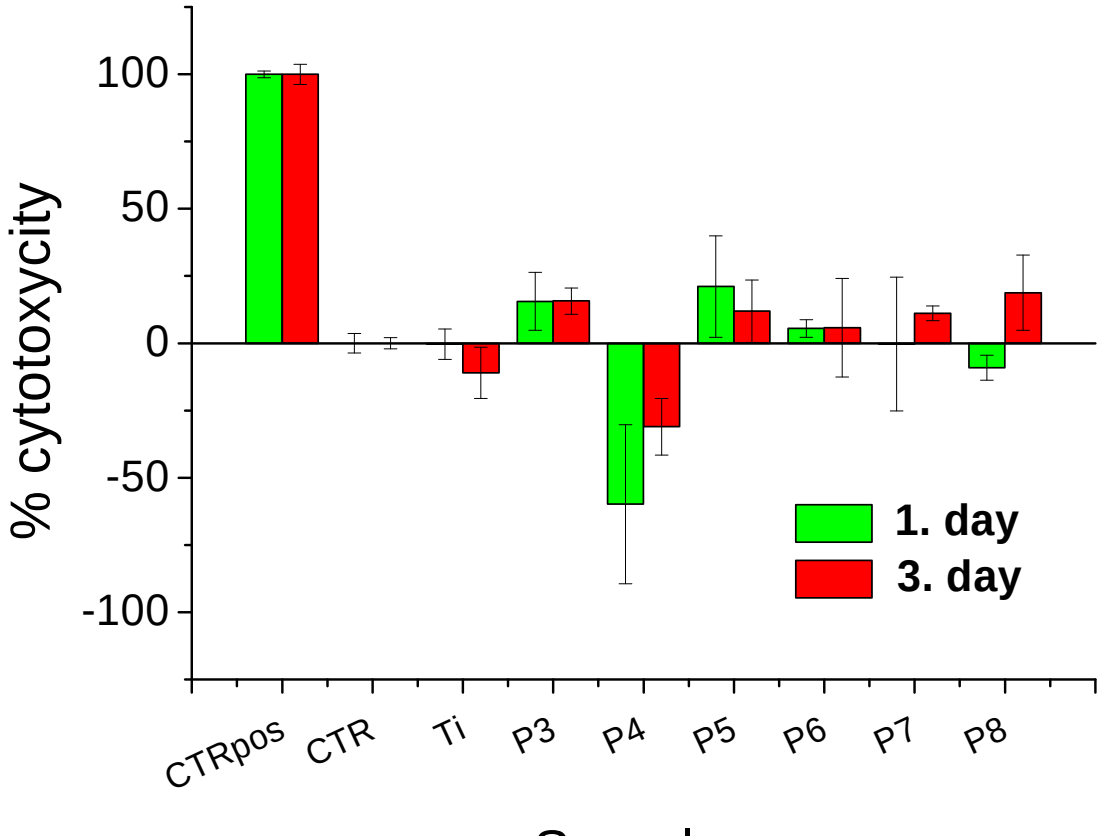
Viable cells convert WST-1 to a water soluble formazan dye. Quantitating the formazan dye in the MTP can be done with an ELISA plate reader. The absorbance directly correlates with the cell number.



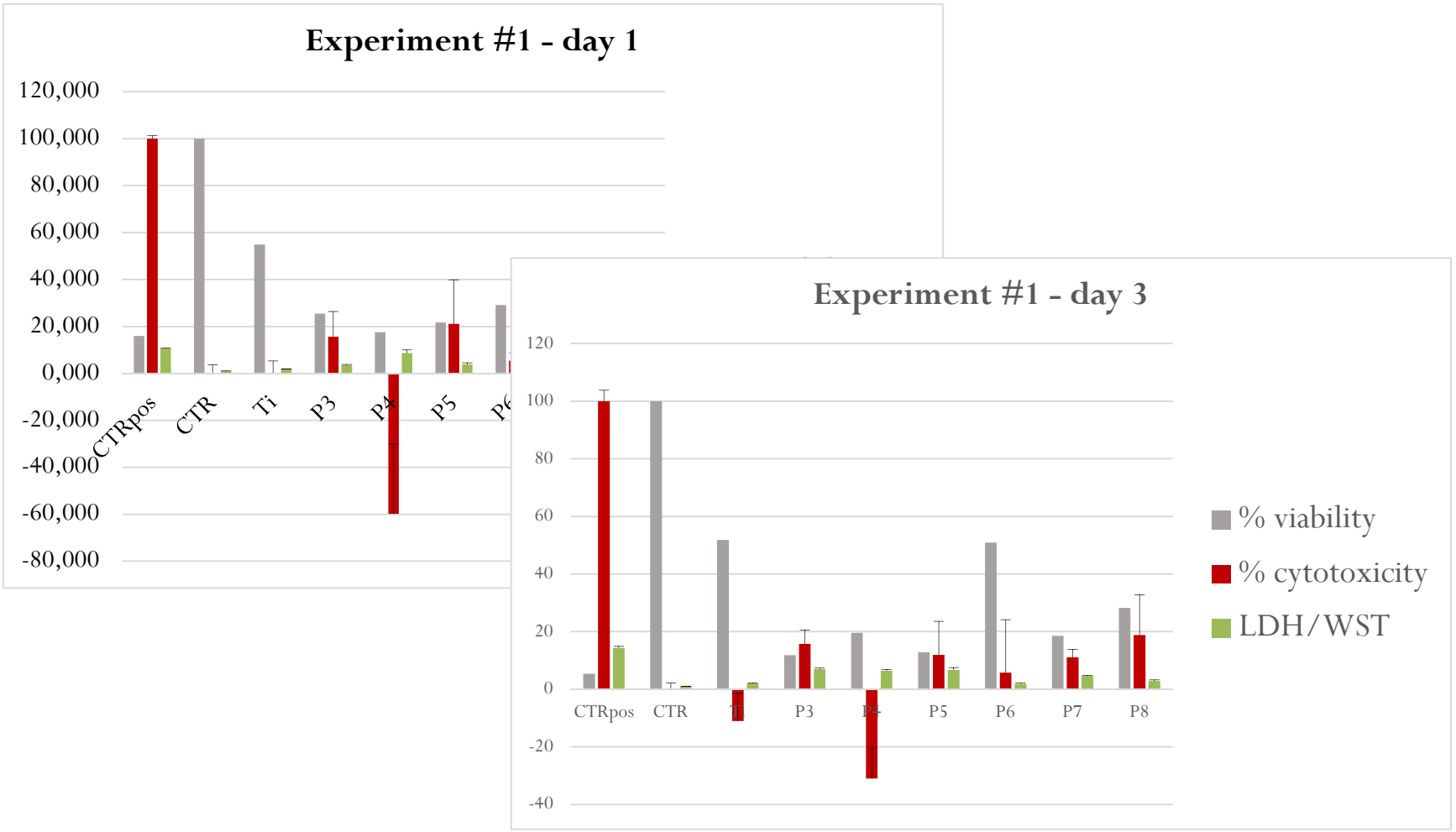
LDH test for cytotoxicity

Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme present in all cells. It is rapidly released into the cell culture supernatant when the plasma membrane is damaged. With the Cytotoxicity Detection Kit, LDH activity can easily be measured in culture supernatants by a single point assay. The use of a spectrophotometric microplate reader (ELISA plate reader) allows the simultaneous measurement of multiple probes and thereby guarantees the easy processing of a large number of samples.





Summary of WST-1 and LDH measurements



Live/Dead cell staining experiments

The Live/Dead assay stain solution is a mixture of two highly fluorescent dyes that differentially label live and dead cells. Live cells are identified on the basis of intracellular esterase activity (generating green fluorescence) and exclusion of the red dye. Dead cells are identified by the lack of esterase activity a
Results after 4 days in cu

MG 63 cells:

Ti6Al4V substrate:

P3 CaP layer:

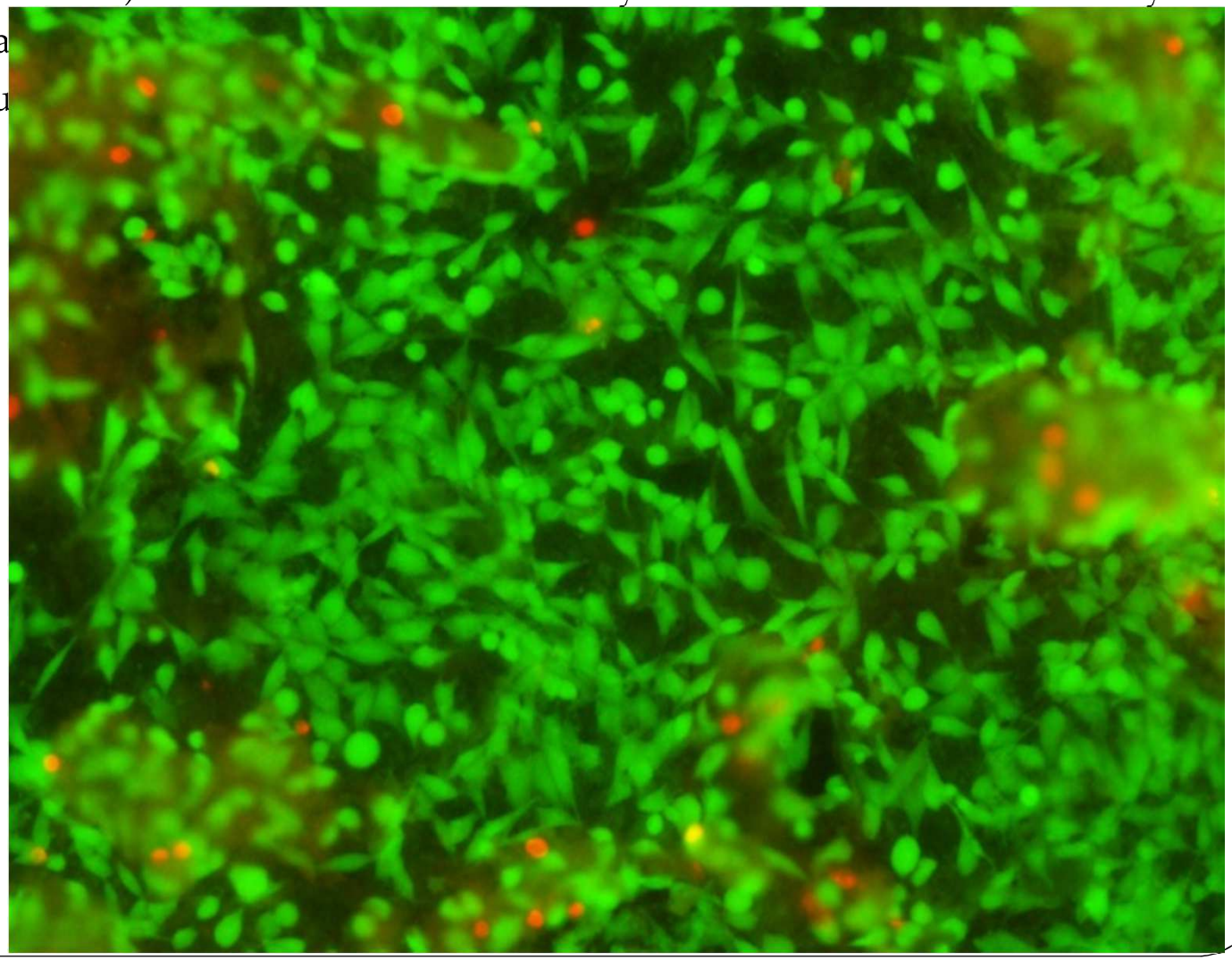
P4 CaP layer:

P5 modified CaP layer:

P6 modified CaP layer:

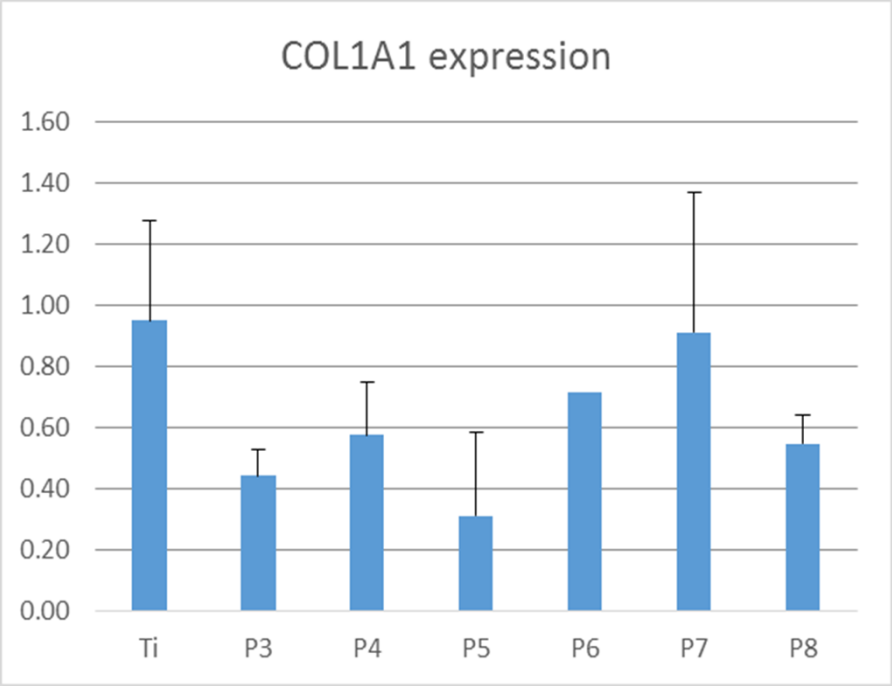
P7 modified CaP layer:

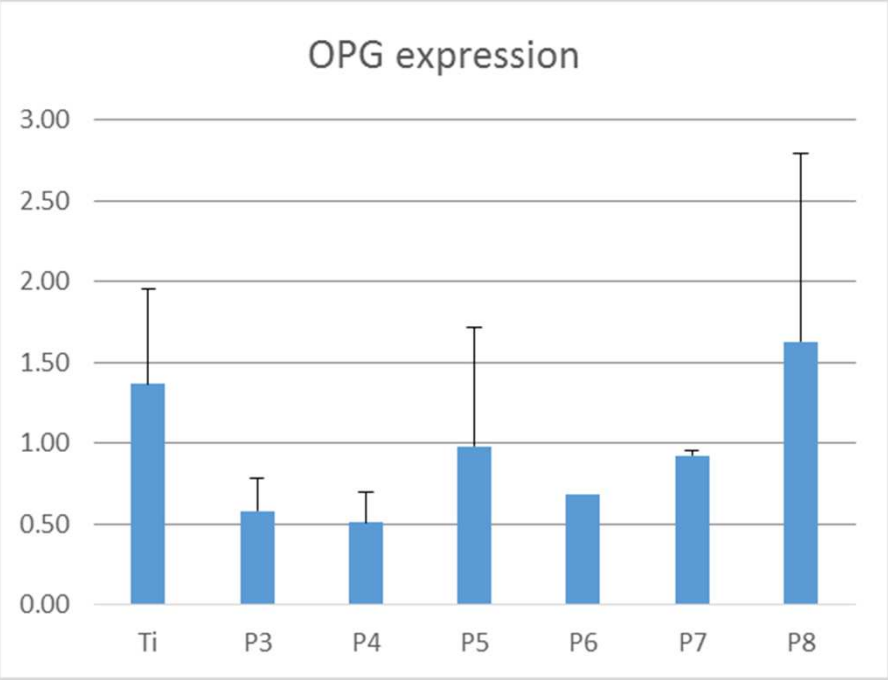
P8 modified CaP layer:



Gene expression analysis

The expression of bone-specific genes was evaluated in cells grown on the different materials, in order to understand whether they may stimulate an osteogenic differentiation





COL1A1: alpha-1 chain of collagen I
OPG: osteoprotegerin

The analyses showed no significant differences in the expression of these genes among the samples. However, the expression levels may vary consistently among the cells grown in different materials, resulting in very high standard deviations.

Expected results

This research might provide innovative solution for prevention of implantation-related infections, promote the bone cell growth and accelerate the wound healing process after surgical operation. It suggests a novel technology with a high potential for the effective and safe replacement of the currently used silver-based technology.

The main aim is to develop stable implant coatings with incorporated antibacterial components. The implant will be able to follow regeneration and to prevent development of infections during this process.

Today's demand for reconstructive surgical procedures is approximately 4 million operations performed annually worldwide. About 2% of them fail because of the infection. Medical implants are very large market capable to absorb high-quality technologies important for improving their quality.

Publications

M. Lakatos-Varsányi, M. Furko, T. Pozman:, *Electrochimica Acta*, 56, (23) 7787-7795. (2011)

M. Furko, M. Lakatos-Varsányi, C. Balazsi, *Materials Science Forum* 812, 41-43 2015.

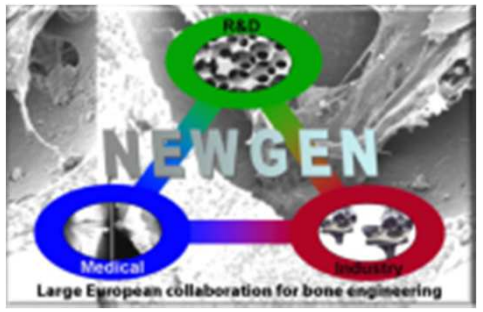
M. Furko, E. Fazakas, E. Takács, *Materials Science Forum* 812, 327-332. (2015)

Nikolett Oláh, Zsolt Fogarassy, Mónika Furkó, Csaba Balázsi, Katalin Balázsi, *Ceramics International* 41 (4) 5863-5871. (2015)

M. Furko, M. Lakatos-Varsányi, C. Balazsi, *J. Sold State Electrochemistry* Volume 20, (2016) 263-271. (DOI 10.1007/s10008-015-3026-1)

M. Furko, Y. Jiang, T. A. Wilkins, C. Balázsi, *Ceramics International* Volume 42, Issue 4, (2016) 4924-4931.

M. Furko, Y. Jiang, T. A. Wilkins, C. Balázsi, *Materials and Science Engineering C* In Press, Accepted Manuscript, 2016,doi:10.1016/j.msec.2016.01.060



Thank you for your attention

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico