SZLOVÁKIAI MAGYAR AKADÉMIAI TANÁCS

SZMAT

Fiatal kutatók a szlovákiai magyar tudományosságért díj

Bioüveg – kalcium foszfát kompozit cementek elkészítése és tanulmányozása

Somorja, 2016.12.09.

RNDr. Tibor Sopčák, PhD.

Background

1.9.2012 - 24.8.2016/ Institute of Materials Research of the Slovak Academy of Sciences (IMR SAS)

PhD. Student,
Field of study: Materials Science and Engineering.

2003 - 2006 /Pavol Jozef Šafárik University in Košice (UPJŠ)

Faculty of Science,
Field of study:
organic chemistry
“Master's Degree”

2003 - 2006 /Pavol Jozef Šafárik University in Košice (UPJŠ)

Faculty of Science,
Field of study: chemistry
“Bachelor´s degree“

1999 - 2003 /Secondary school for medical assistants in Košice

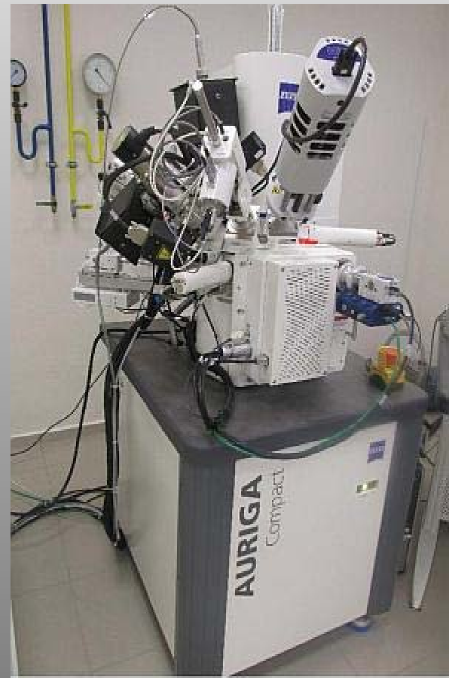
Field of study:
pharmaceutical assistant

Outline - IMR SAS

- Advanced powder technologies/materials
- Nano-structured materials
- Structural and fuctional ceramics
- Advanced steels

Experimental equipments

AURIGATM CrossBeam®: GEMINI® electron beam SEM



Biomaterials

synergistic interaction of materials science, biological science, chemical science, medical science and mechanical science

………… number of definitions

- synthetic or natural materials to be used to replace parts of a living system or to function in intimate contact with living tissues (NIH)

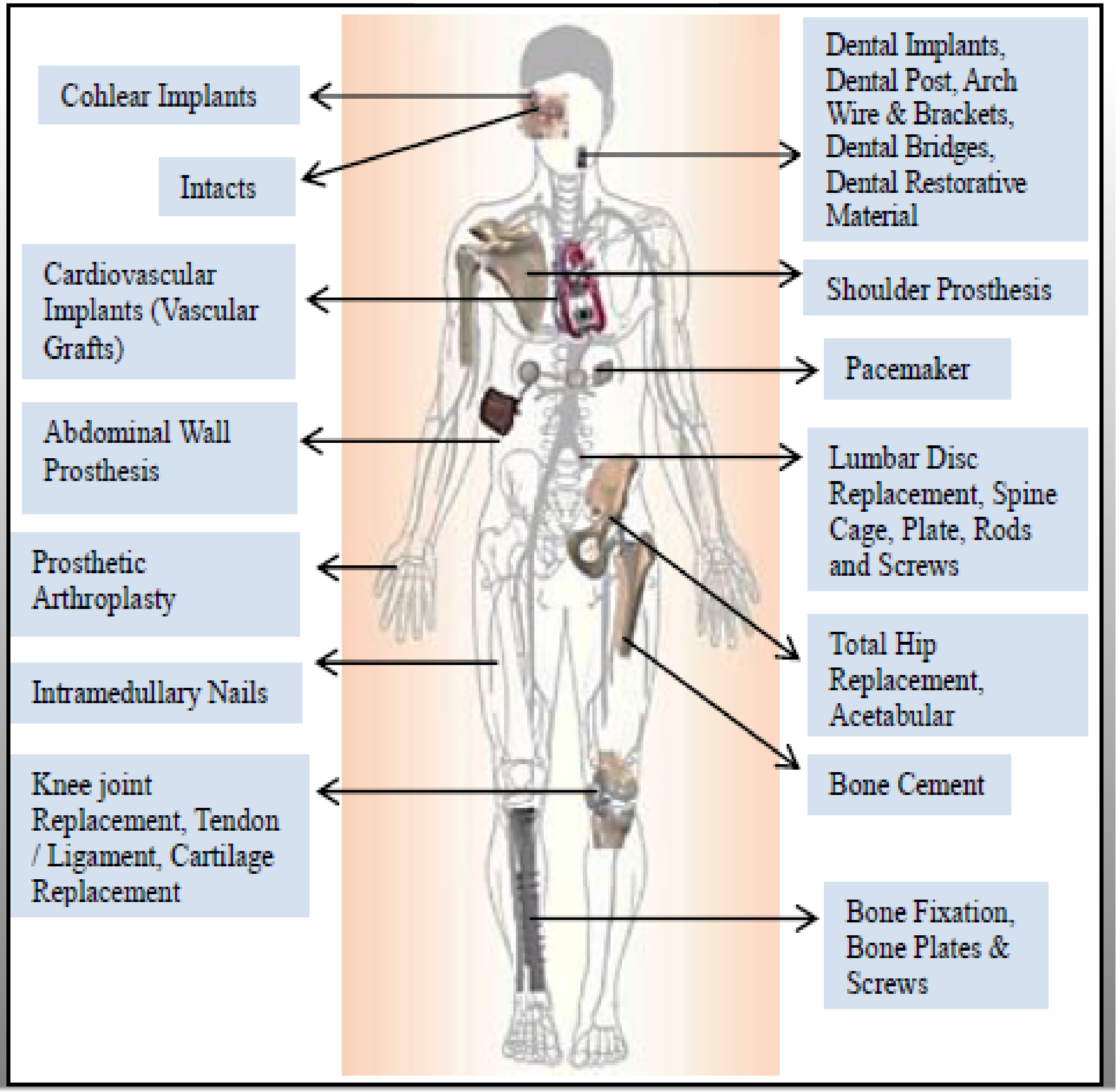
Composites

Biomaterials

Metallic

Polymeric

Ceramic



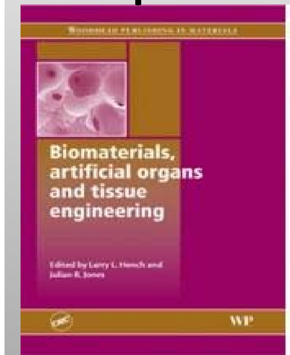
Bioceramics

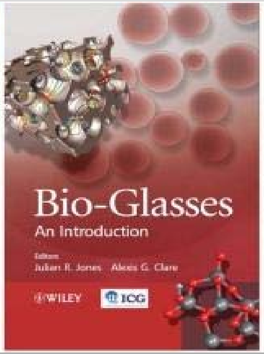
Bioinert
Al_2O_3
ZrO_2

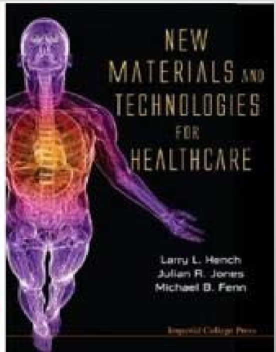
Bioactive
hydroxyapatite
Bioglass

Resorbable
α,β - TCP

1969 prof. Hench







- amorphous glasses consisting solely of elements found in the body

- materials that are specially developed for the repair and reconstruction of diseased or damaged parts of the musculo-skeletal system

Biocements (CPC)

1980-Brown, Chow, LeGeros

- mixture of one or more calcium phosphate powders which upon mixing with liquid phase sets in vivo at body temperature

PhD Thesis „ Effect of bioactive glass addition on properties of calcium phosphate composite systems“

Applications

http://rotamed.com/en/index.php?option=com_content&view=article&id=87&Itemid=106

//www.vjortho.com/2009/12/endothermic-calcium-phosphate-cement-for-defect-augmentation/

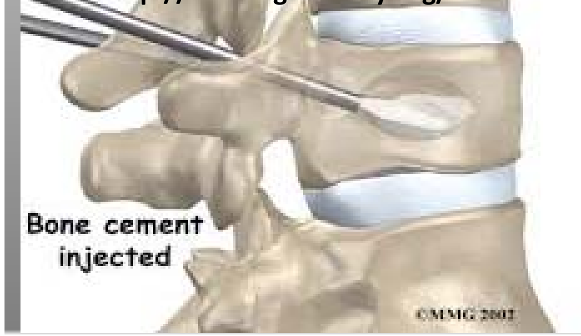
cc.scu.edu.cn

reinforcement of osteoporotic bones

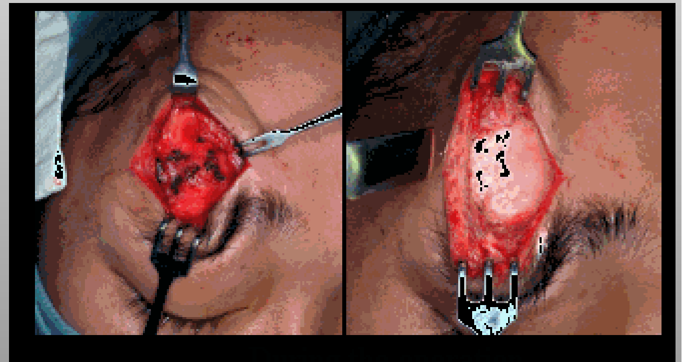
http://www.ijdr.in/

filling bone defects, connecting bone fragments

filling root canal, periodontal defects, dental pulp



http://buildingindustry.org/web



https://asps.confex.com/asps/2003am/techprogram/images/3389-0.gif

craniofacial and maxillofacial applications

Objective of my work ⇨ preparation and characterization of composite bone cements

Composite cements

Bioactive glass (BG)

$CaO-SiO_2$

$CaO-SiO_2-P_2O_5$

Calcium phosphate

Brushite

$CaHPO4 \, . \, 2H_2O$

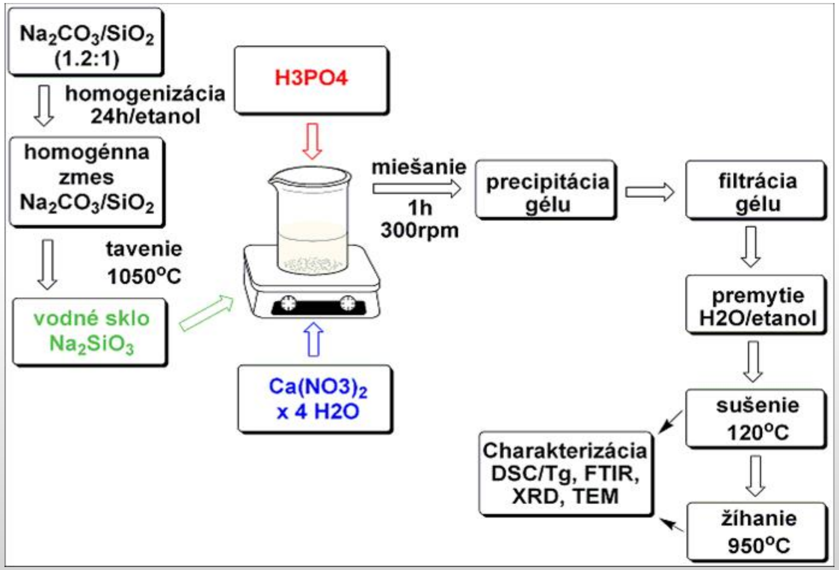
1) **Synthesis of bioactive glasses by means of precipitation method**

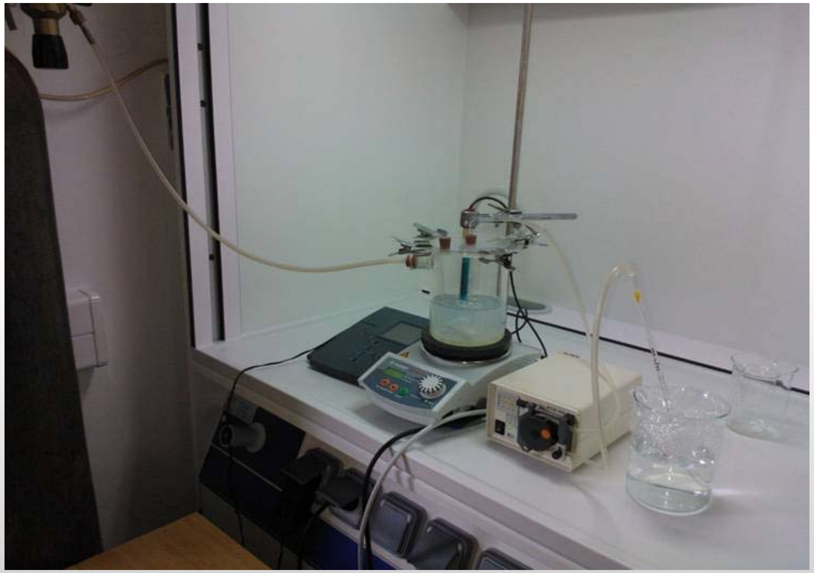
2) **Characterization of chemical composition, phase evolution and particle morphology as well as clarification of precipitation mechanism of prepared glass systems**

3) **Preparation and characterization of composite bone cements based on brushite with the addition of binary $CaO-SiO_2$ and ternary $CaO-SiO_2-P_2O_5$ bioactive glass systems of different crystallinity.**

1) Synthesis of BG

Sol-gel precipitation method





hydrated calcium silicate and calcium silicate phosphate (CSH/CSHP)

Mechanism of precipitation and phase composition of CaO-SiO2-P2O5 systems synthesized by sol-gel method

Journal of Non-Crystalline Solids (2015)

$CaO-SiO_2$

1. System „final pH 12.3“
   Ca/Si = 1

- diff. chem. composit.
- phase composition
- morphology

$CaO-SiO_2-P_2O_5$

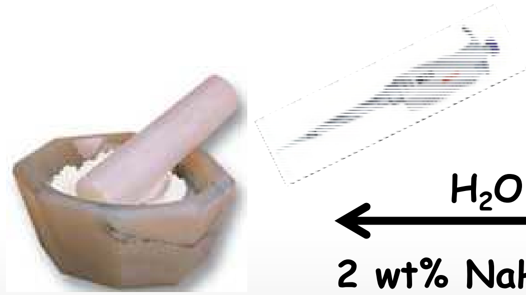
1. System „final pH 11.4“
2. System „final pH 10 con.“
3. System „final pH 7.4“
4. System „final pH 5.5“

2) Preparation of cement composites

Brushite (B) + (CSPH)120°C / (CSP)950°C ; (CSH)120°C / (β-woll)950°C

(10, 30, 50 hm%)

mech.homog. → planetary ball millling



H_2O ← 2 wt% NaH_2PO_4

pressing

Mechanism of setting - Hydrolysis of B and woll

$CaSiO_3$ (CSH) + $3H_2O \rightarrow Ca(OH)_2 + H_4SiO_4$

$6CaHPO_4 + 3Ca(OH)_2 \rightarrow Ca_9(PO_4)_5(HPO_4)OH + 5H_2O$

$9CaHPO_4 + H_2O \rightarrow Ca_9(PO_4)_5(HPO_4)OH + 3H_3PO_4$

(6 mm D ×12 mm H)

100% (RH),37°C
45 min

hardening
v PBS, 37°C
1 a 7 days

15 min H_2O
drying, 80°C/2h

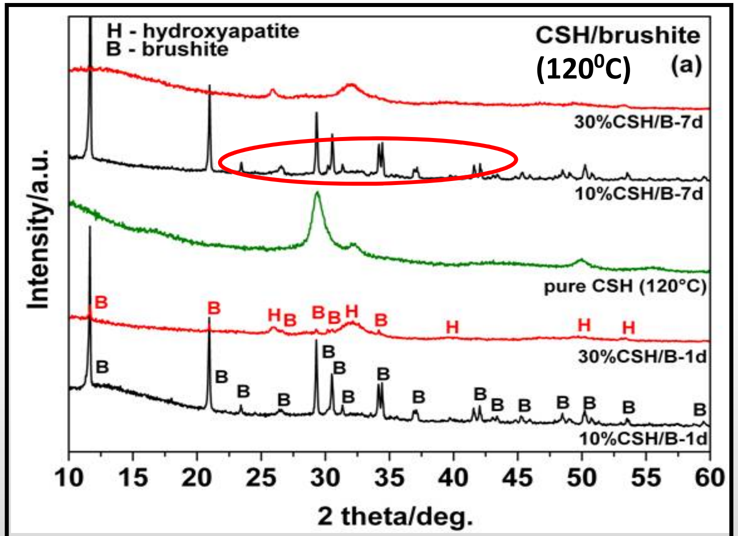
Compressive strength (CS)

Microstructure, morphology, particle size (SEM, TEM)

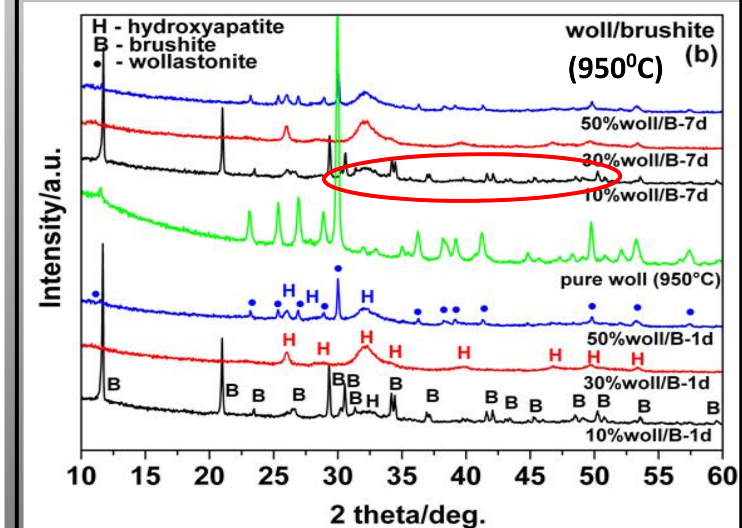
Phase composition identifying functional groups (XRD, FTIR)

Chem.analysis, porosity

3) XRD analysis of biocements after hardening



CaO-SiO_2 / brushite



Bioactive glass content

10 wt%

majority brushite

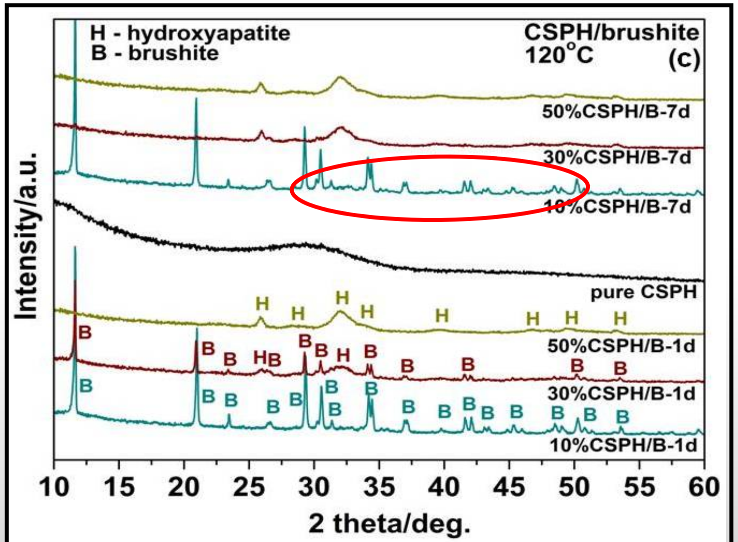
30 wt%

biphasic. systems:
major. nanoHap,
minor.brushite

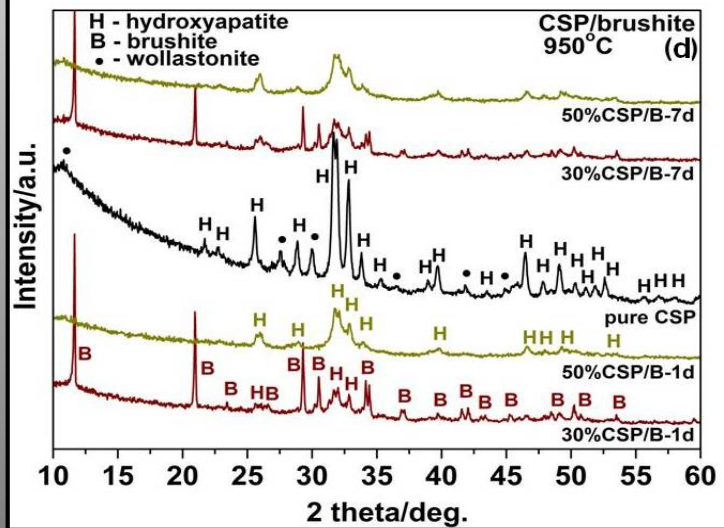
50 wt%

CSPH,CSP/brushite
nanoHap

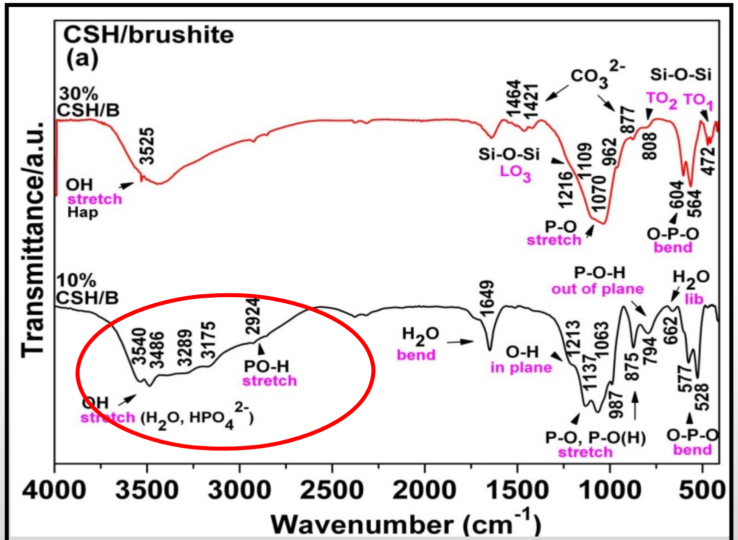
woll/brushite
nano Hap/ b-woll



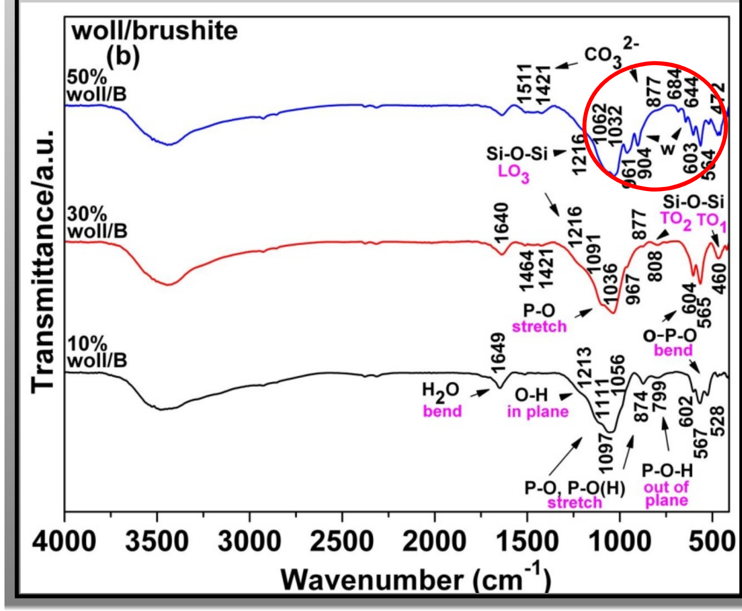
CaO-SiO_2-P_2O_5 / brushite



4) FTIR analysis of biocements after hardening



$CaO-SiO_2$ / brushite



Bioactive glass content

10 wt%

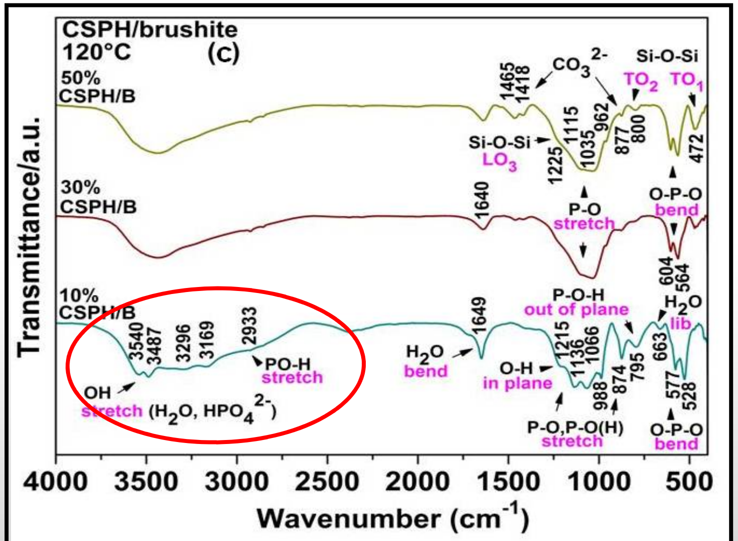
brushite vibrations
(2900 - 3550 cm^{-1})
(1215 - 795 cm^{-1})

30 wt%

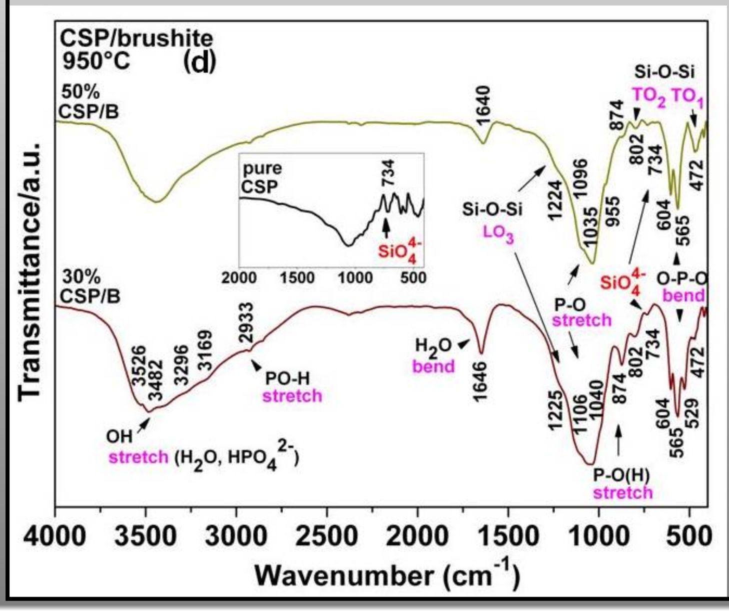
Hap vibrations, vibrations assoc. with the increase of Si in the sample

50 wt%

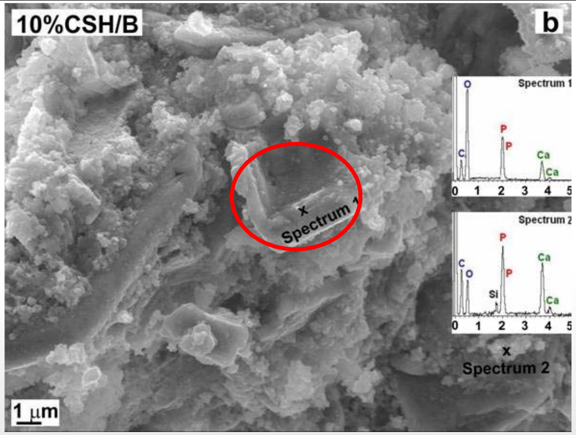
Hap vibrations,
woll/brushite
β-woll vibrations
(961, 904, 684, 644 cm^{-1})



$CaO-SiO_2-P_2O_5$ / brushite

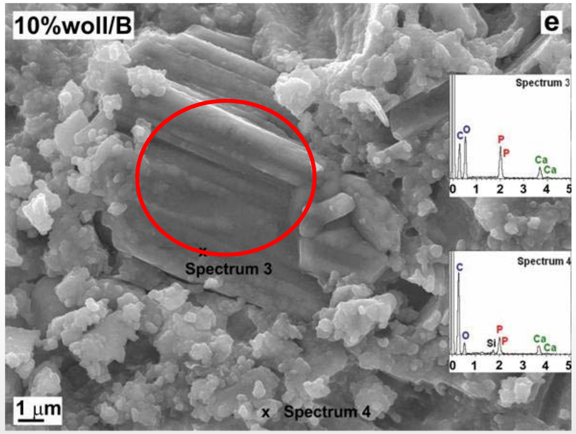


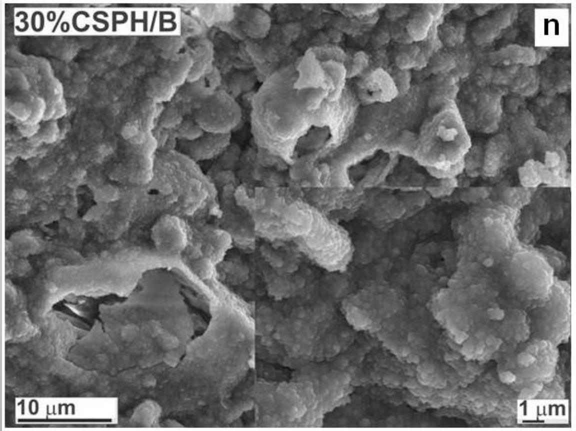
5) Microstructure of biocements after hardening



10 wt%

coarser irregular particles of brushite (~10 µm) surrounded by finer spherical nanoparticles



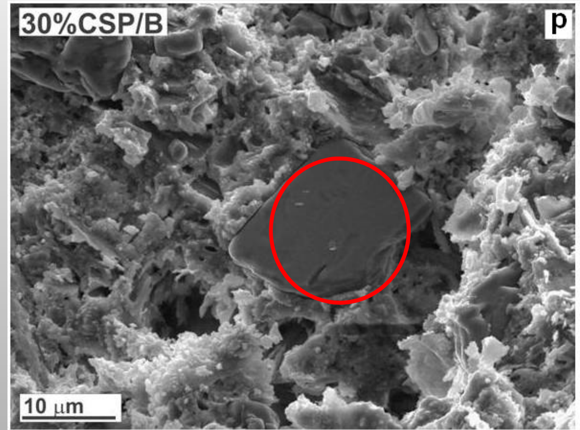


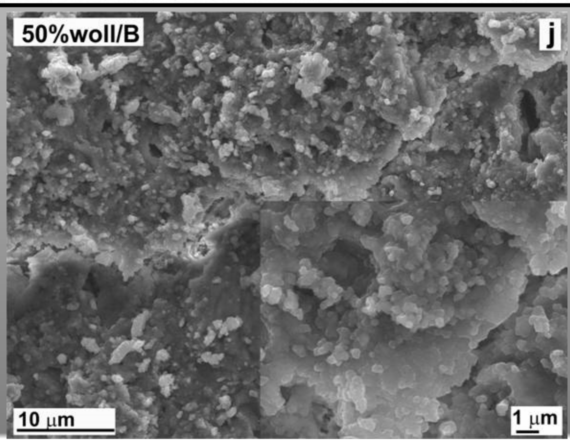
30 wt%

~ 100 nm Hap nanoparticles

brushite phase still preserved

irregular pores of different sizes

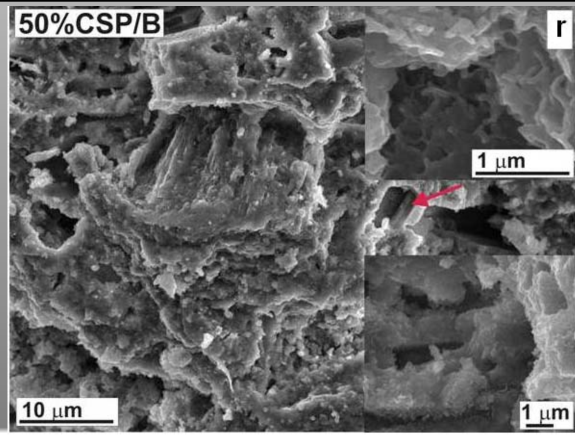




50 wt%

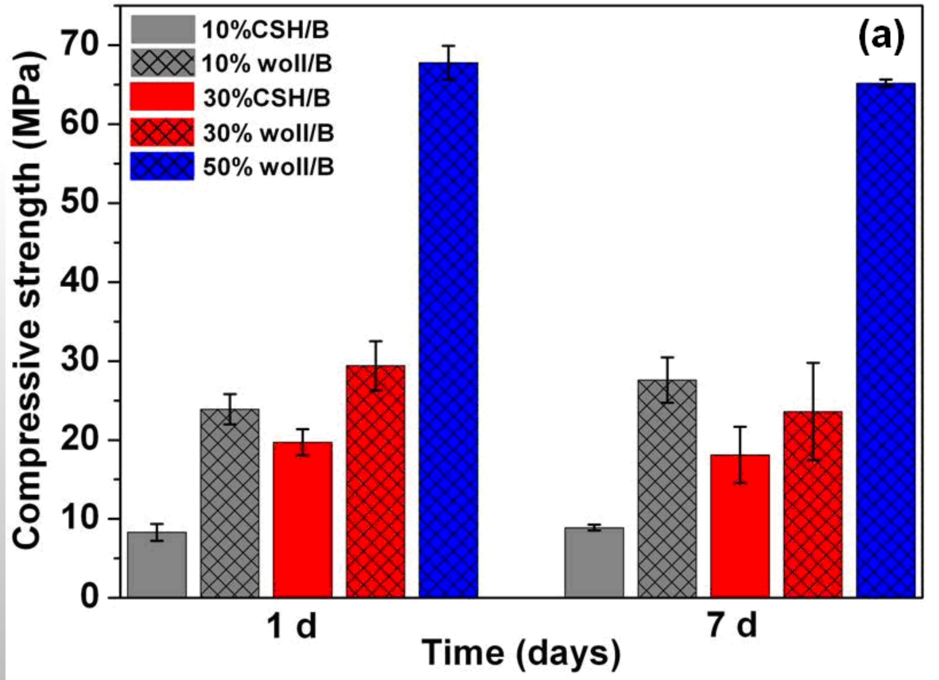
empty places after the origin woll particles

microstructure with higher density, only Hap nanoparticles were visible

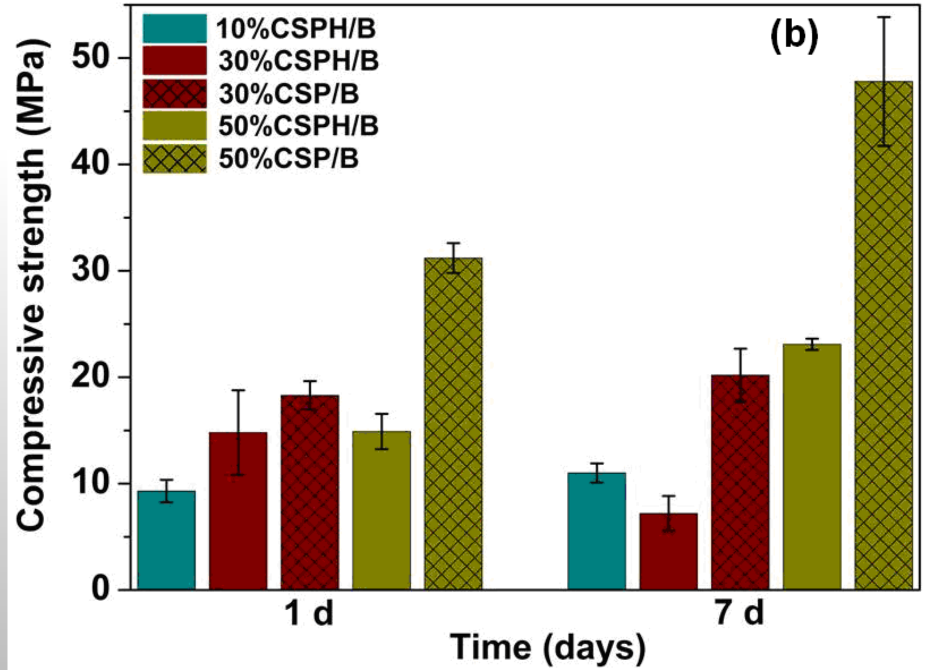


6) Compressive strength of bioc. after hardening

CaO-SiO_2 / brushite



CaO-SiO_2-P_2O_5 / brushite



Composites with crystalline woll and CSP phase had ↑ CS

10 wt% ⇨ high content of untransformed brushite = ↓ CS ~ 10 MPa

50 wt% ⇨ (~ 67 Mpa), strengthening the fine and reasonably compact cement matrix by hard untransf. woll or CSP particles

Guo et al.
$CaSiO_3$/CPC → 5-20%,↓38-30 MPa

Huan and Chang
$Ca_3SiO_5/\beta-TCP$/MCPM
30%, 42.5 MPa

Wang et al.
β - Ca_2SiO_4/ACP/DCPD
8%, 47.5 MPa

7) Setting time of biocements after hardening

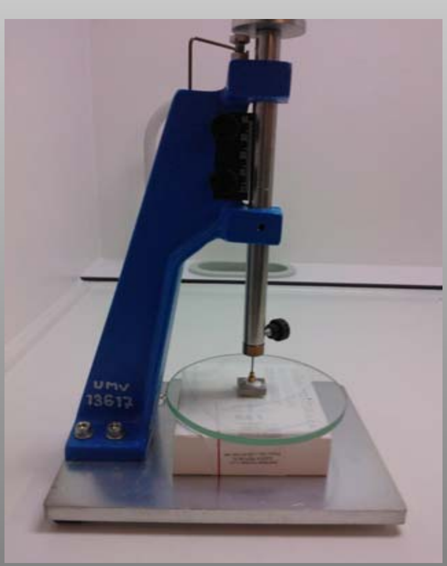
Optimum P/L ratio, final setting times total porosity

$CaO-SiO_2$ / brushite

Sample	P/L (mg/ml)	Final sett. time (min)	Porosity (%)
10CSH/B	2.50	12 ± 1.4	45 ± 1.7
30CSH/B	2.00	43 ± 2.8	58 ± 1.1
10woll/B	2.80	8 ± 1.8	45 ± 0.7
30woll/B	2.70	5 ± 2.1	44 ± 0.5
50woll/B	2.67	7 ± 0.7	44 ± 0.3

$CaO-SiO_2-P_2O_5$ / brushite

Sample	P/L (mg/ml)	Final sett. time (min)	Porosity (%)
10CSPH/B	2.22	25 ± 1.1	46 ± 0.3
30CSPH/B	1.70	20 ± 1.0	60 ± 0.6
50CSPH/B	1.21	17 ± 0.7	64 ± 0.4
30CSP/B	3.14	28 ± 1.4	64 ± 0.4
50CSP/B	3.18	30 ± 2.1	43 ± 0.8



Setting time

Vicat aparatus
(ISO standard 1566)

The cements were considered set until the Vicat needle (400 g) no longer leaves a visible print on the surface of the pastes

Guo et al.
$CaSiO_3$/CPC → 5-20%, 21-30 min

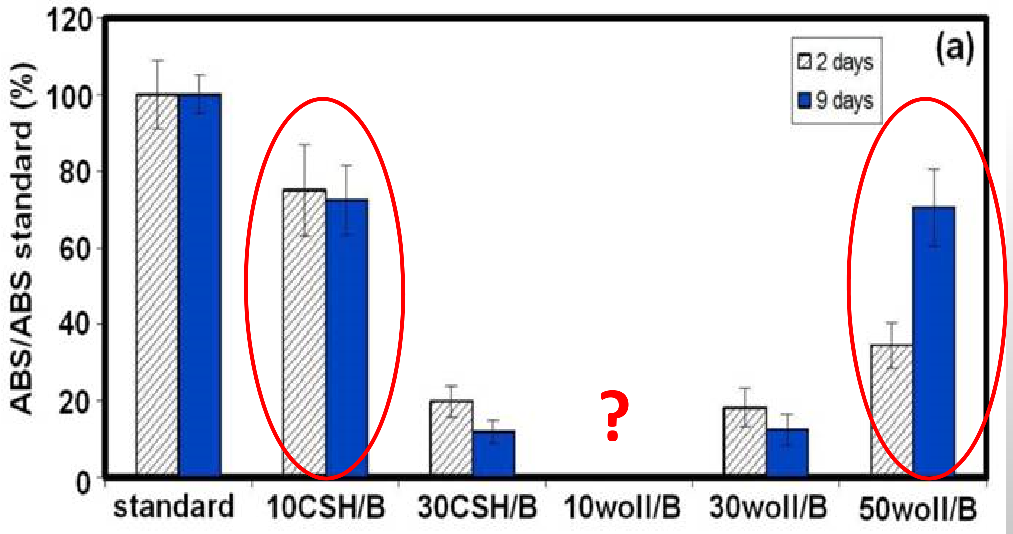
Huan and Chang
$Ca_3SiO_5/\beta-TCP$/MCPM
30%, 35 min

Wang et al.
$\beta - Ca_2SiO_4$/ACP/DCPD
8%, 42 min

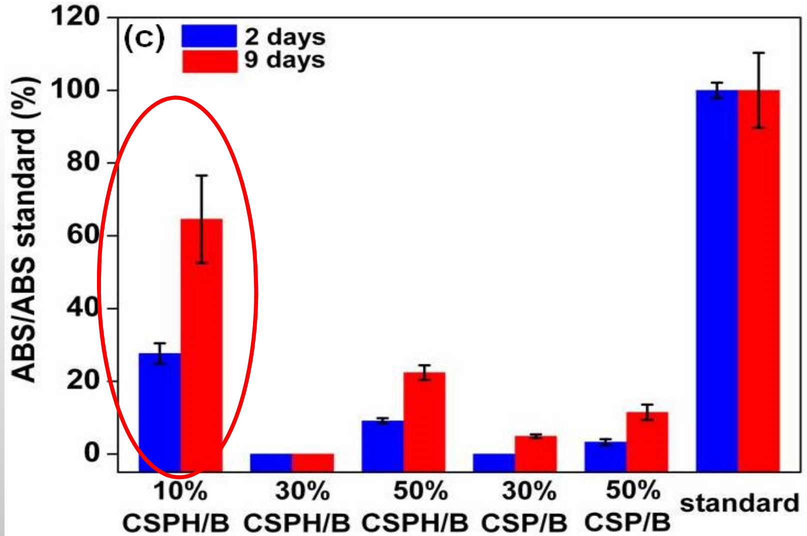
8) In vitro cytotoxicity of biocomposites

Relative proliferation osteoblast activities and viability on composite substrates

$CaO-SiO_2$ / brushite



$CaO-SiO_2-P_2O_5$ / brushite



<u>Composite substrates</u>

Pellets (6 mm D×0.5 mm H), soaked in PBS solution at 37 °C for 3 d were sterilized in an autoclave at 121 °C.

MC3T3E1 preosteoblastic murine cells

<u>MTS assay:</u> is a colorimetric assay for assessing cell metabolic activity

…enzymes are capable of reducing the yellow tetrazolium dye to its insoluble formazan, which has a purple color. Determination of formazan in wells at a wavelength of 490 nm

10 wt% ➡ CSH,CSPH ~ 70% prolif.

10woll/B 0 % viability and prolif.

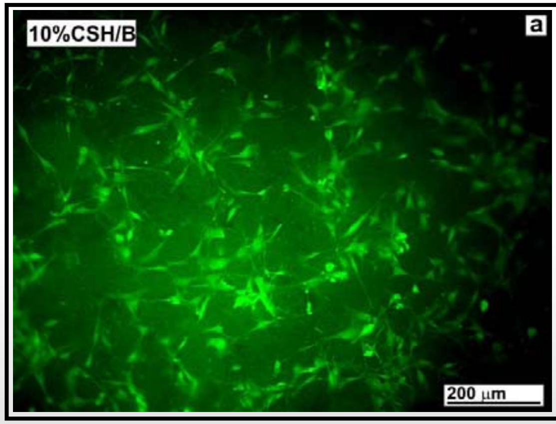
<u>cytotoxic</u>

30 wt%
50 wt% ➡ ~ 20% prolif.

50woll/B → increase of prolif. from ~ 40% to 70% after 9 d cultivation

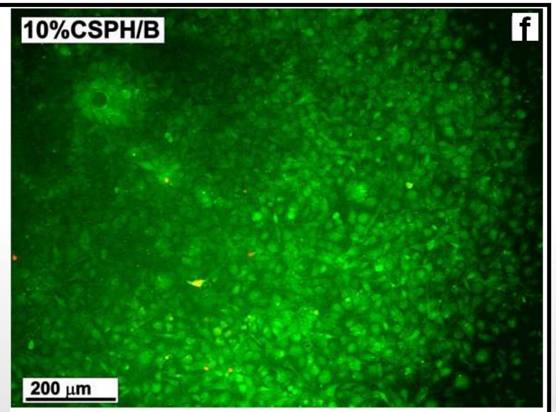
9) Morphology and osteoblast density

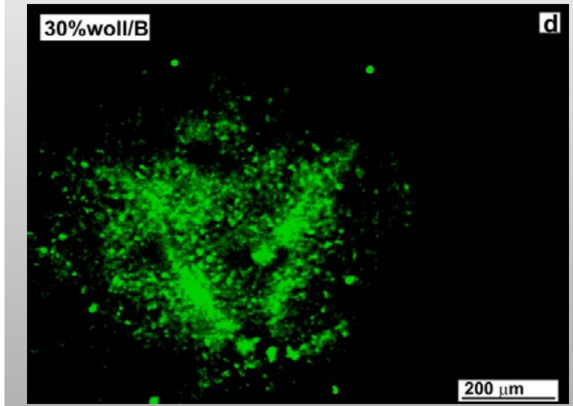
Morphology and osteoblast density on composite substrates after 9 d culture



10 wt%

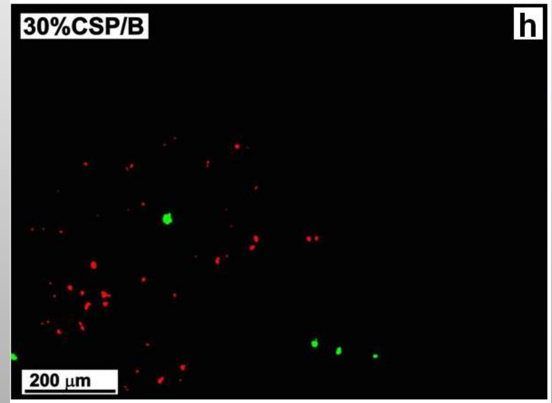
high density of well-spread live cells with clearly distinguished filopodia

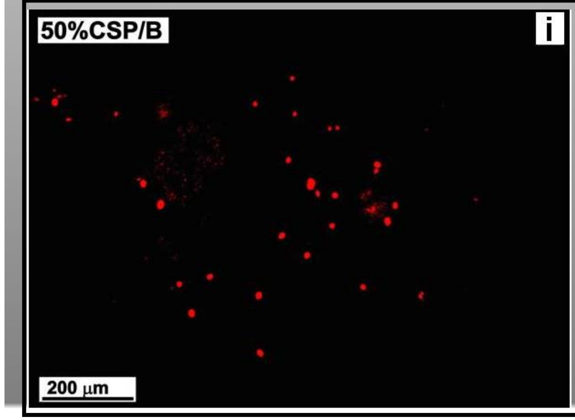




30 wt%

lower number and density of live cells without filopodia, low adherence with the sample surafces

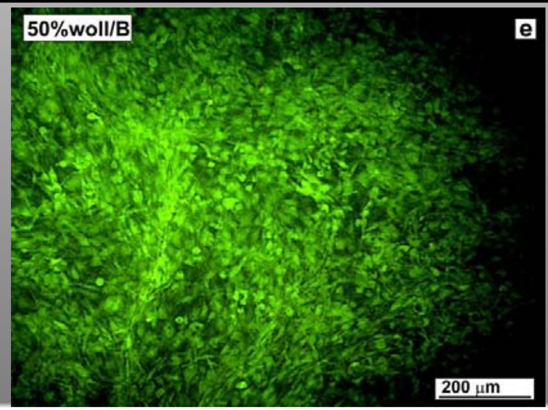




50 wt%

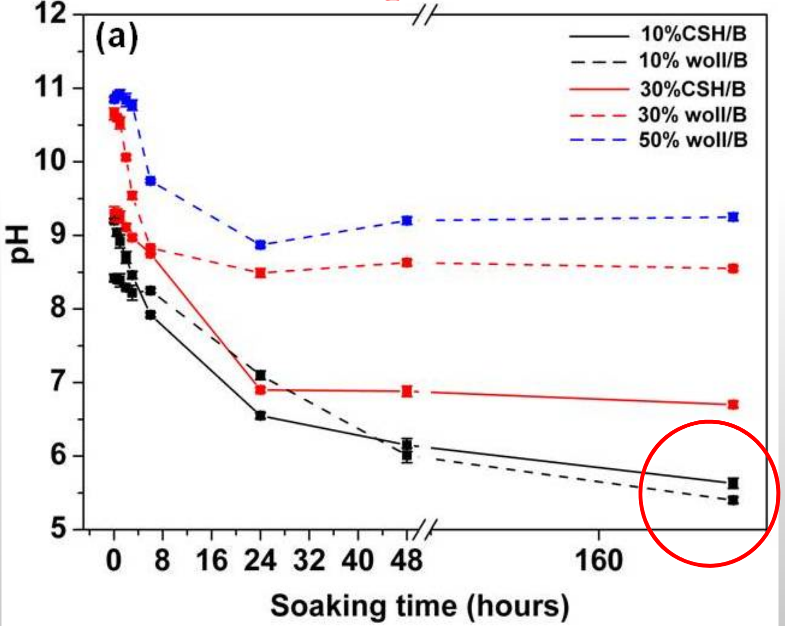
dead globular cells

high density of the mutually interconnected adjacent live osteoblasts

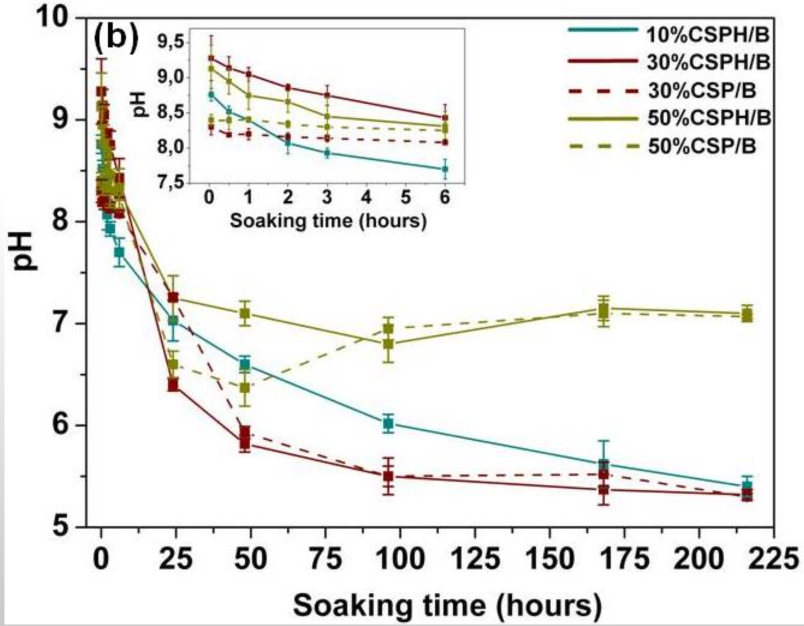


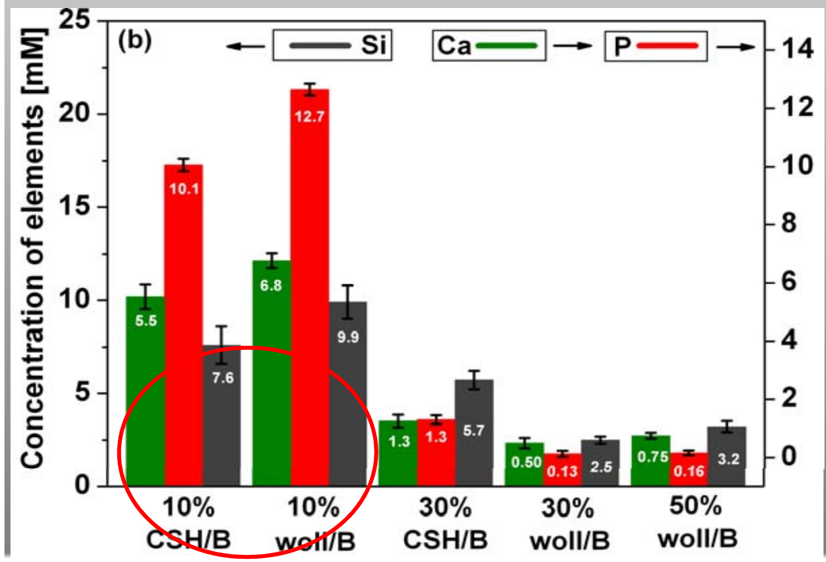
10) Variations of pH and concentrat. of elements

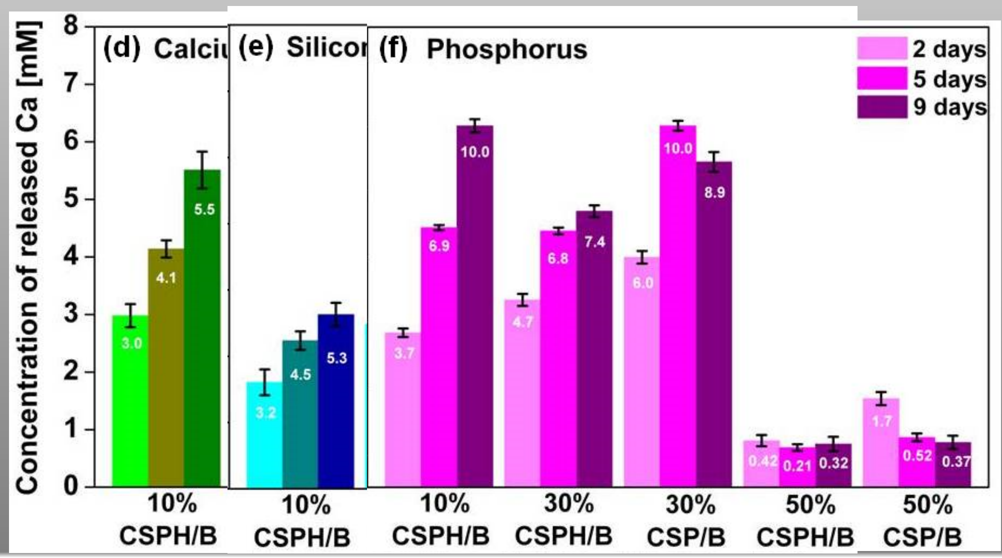
CaO-SiO$_2$ / brushite



CaO-SiO$_2$-P$_2$O$_5$ / brushite

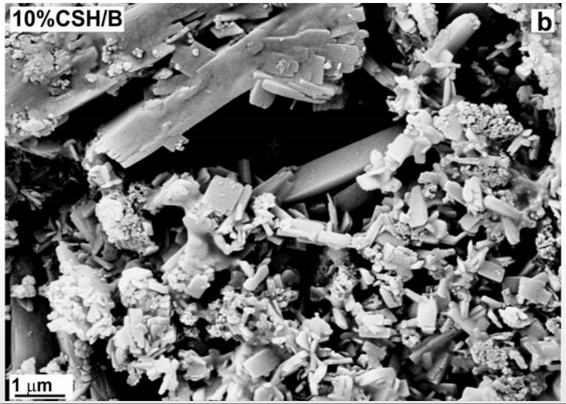






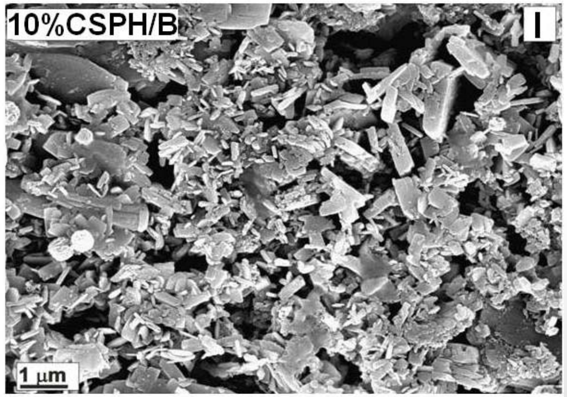
11) Surface microtexture of biocomposites

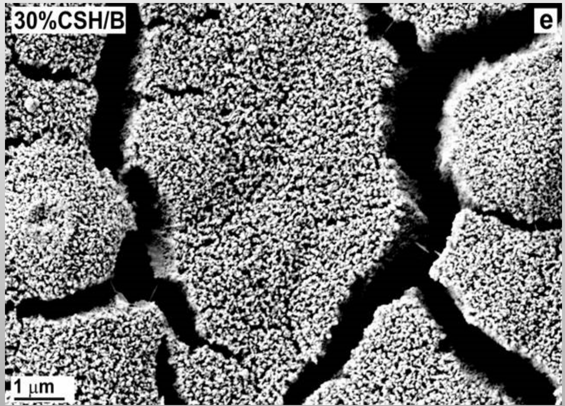
Microstructure of composite substrates after 9 d cultivation



10 wt%

plate like brushite particles with adges oriented parallel to the surface of the sample
better adherence and proliferation of osteoblasts!!!

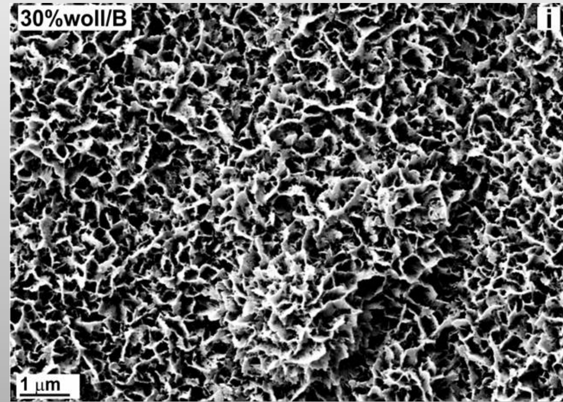


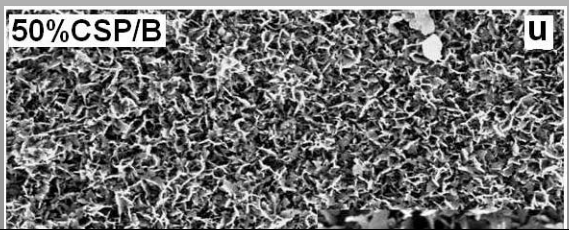


30 hm%

fine spherical particles

extremely thin needle-like particles oriented perpendicularly to the origin surface of sample

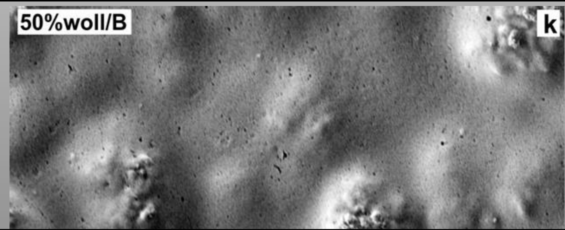




Phase transformations, microstructure formation and in vitro osteoblast response in calcium silicate/brushite cement composites
Biomedical materials (2016)

50 wt%

rough, very dense and compact micro-texture composed of globular calcium phosphate islands



Effect of phase composition of calcium silicate phosphate component on properties of brushite based composite cements
Materials characterization (2016)

Conclusions

- **Composite cements were prepared by mechanical mixing of CaO-SiO_2 and CaO-SiO_2-P_2O_5 systems of different crystallinity with brushite as calcium phosphate source of the cmenet matrix. The results showed a significant effect bioactive glass addition on the properties of calcium phosphate composite systems**

- **The phase composition in the final cements and transformation of brushite to calcium deficient hydroxyapatite were controlled by the Ca/P ratio in the initial cement powder mixture and by dissolution of calcium silicate and calcium phospho-silicate phase.**

- **Setting time of cement composites were in the range of 5-43 minutes depending on the bioactive glass content and the rate of transformation of the origin brushite phase.**

- **The compressive strength of the set cements increased with the filler addition and the highest value (exhibited composite cements with 50 wt% crystalline phase content.**

- **A strong relationship between the composite in vitro cytotoxicity and surface microtopography was demonstrated. The rougher microstructure and plate like morphology of coarser particles in cements allowed cells to better adhere and proliferate contrary to other samples with particles in the form of nanoneedles or thin plates perpendicularly oriented to sample surface.**

Future perspective

Hungarian Academy of Sciences

Centre for Energy Research

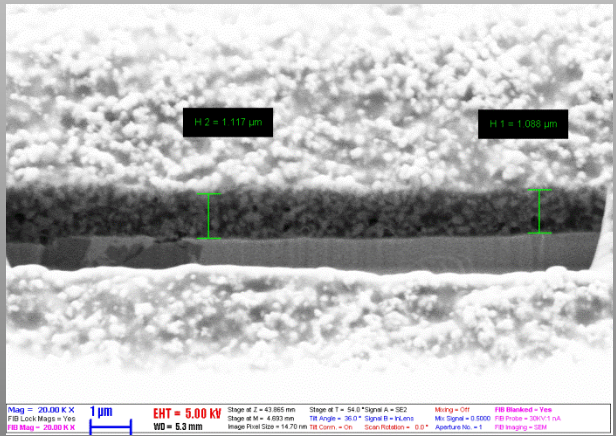
Konkoly-Thege Miklós út 29-33

Budapest

Thin Film Physics Department

The goal of research:

Electrospray deposition (ESD) of bioactive calcium phosphate thin films on Ti substrates




Thanks for your attention