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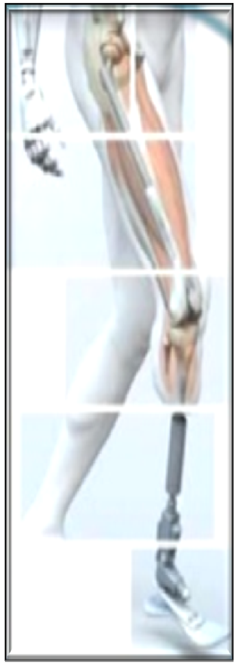
Calcium carbonate filled hydrogel as scaffold for bone tissue engineering



Presented under the program

COSTMP1301: New generation biomimetic and customized implants for bone engineering
Biomaterials (design, processing, characterization, modelling) and applications to regenerative medicine
Agenda : Workshop COST Action MP1301, Bulgarian Academy of Science, 13-14 October , Sofia, Bulgaria

by

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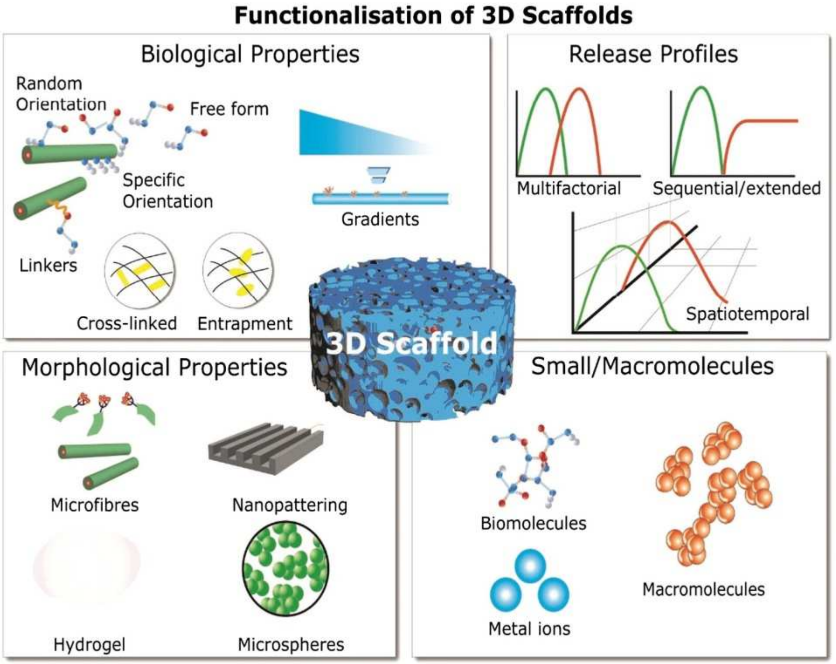
Outline

- Introduction
- Goal
- Experiments
- Conclusion



- **Tissue engineering scaffolds** play a vital role in regenerative medicine.
- **It** not only provides a temporary 3 dimensional (3D) support during tissue repair, but also regulates the cell behaviour, such as:

-Cell adhesion
-Cell proliferation and
-Cell differentiation

- **Scaffold** should have the key characteristics:

- highly interconnected porous structure (which allows cell penetration and nutrient & waste transportation)
- Biocompatibility and biodegradability (which are the basic requirement for the biomaterials used for scaffolds)
- Suitable mechanical properties to meet the specific applications
- Appropriate surface modification and topography to support cell adhesion and growth

Source: Advanced Drug Delivery Review 84 (2015) 1-29

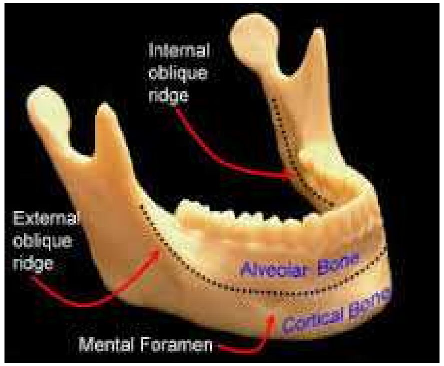
Why Calcium Carbonate filled Hydrogel as Scaffold ?

- Guided bone regeneration (GBR) method is a well established therapy to repair mandible and alveolar bone defects infected by periodontal diseases.

- The principle of GBR method is to prevent an invasion of non-functional scar tissues.

- GBR membranes also have an important function which encourages bone growth.

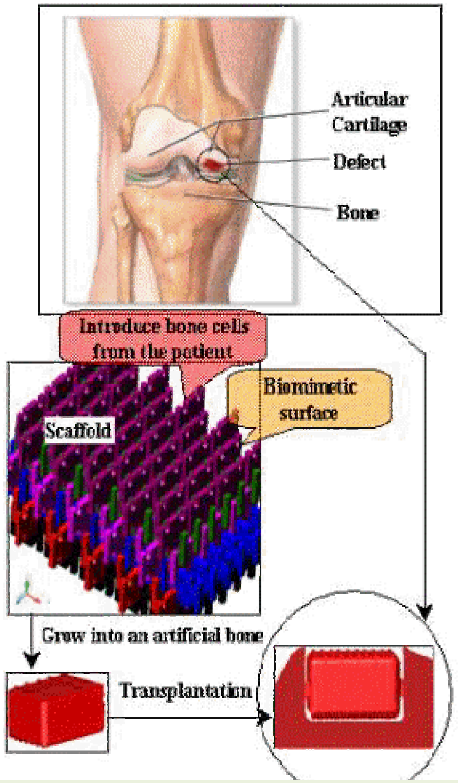
- GBR membranes are occasionally utilized with dental implant or bone grafting materials.



The basic principle of Guided Bone Regeneration (GBR) involves the placement of mechanical barriers to protect blood clots and to isolate the bone defect from the surrounding connective tissue, thus providing bone-forming cells with access to a secluded space intended for bone regeneration.

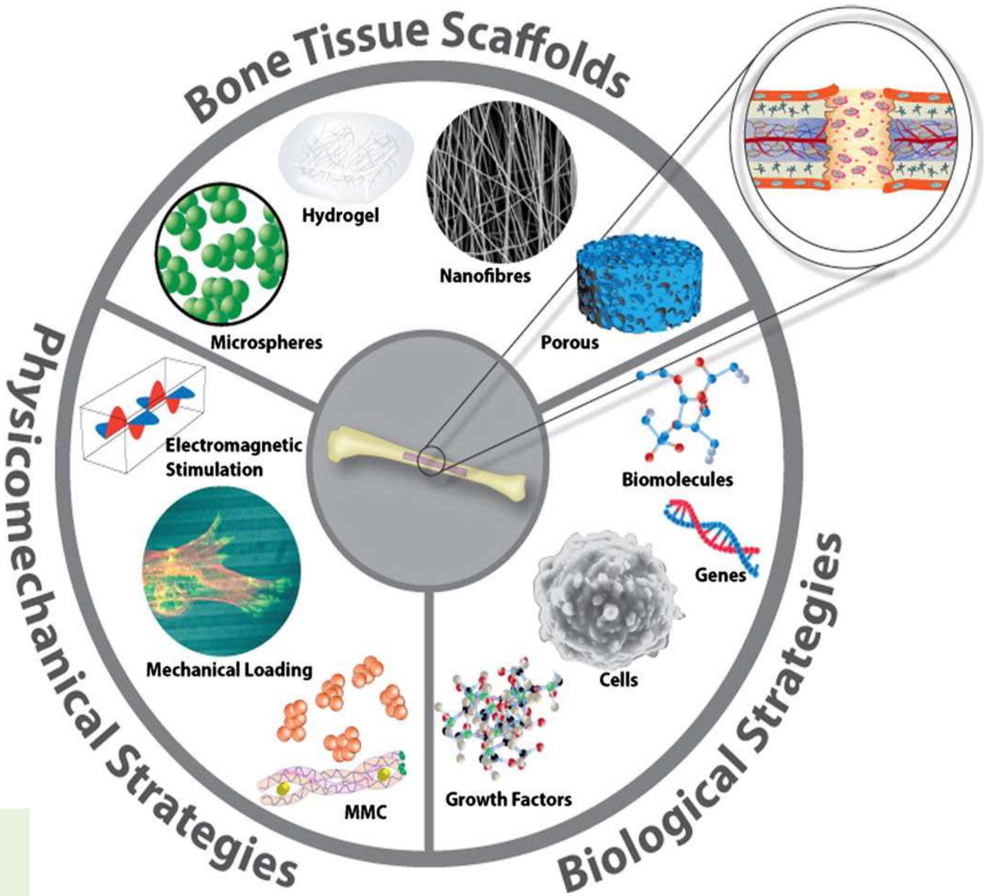
Ref: El Haddad E, Lauritano D, Candotto V, Carinci F. *Guided bone regeneration is a reliable technique in implant dentistry: An overview and a case report*. OA Dentistry 2014 Mar 10;2(1):5.

Why Calcium Carbonate filled Hydrogel as Scaffold ?

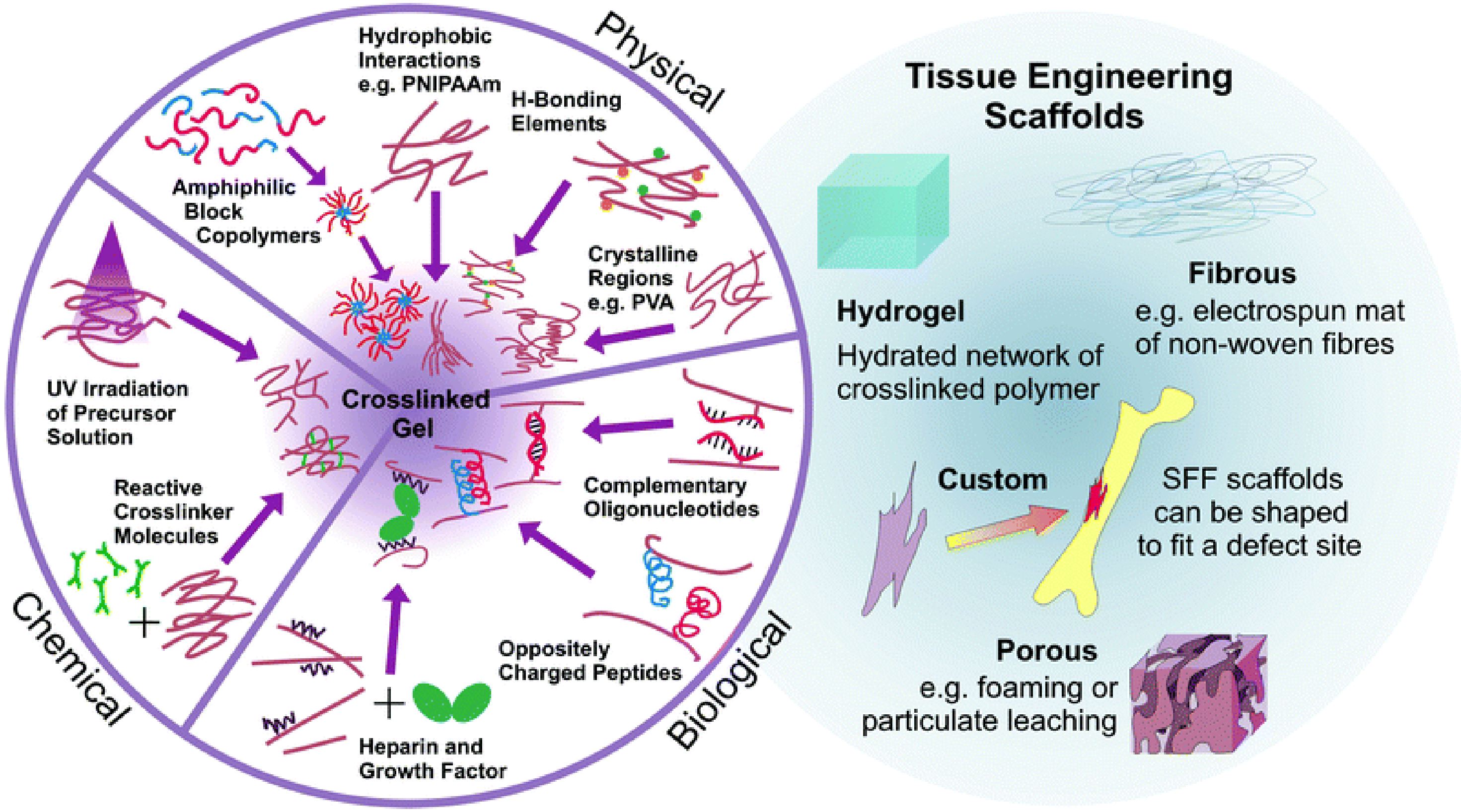


Bone tissue engineering using a scaffold
as a template for tissue regeneration

Source: http://www.eng.nus.edu.sg/EResnews/0210/rd/rd_10.html



Why Calcium Carbonate filled Hydrogel as Scaffold ?



Typical current practice expectation

A bioabsorbable polymer “filled” with:

- Ceramic e.g. HA, TCP, BCP
- Calcium Carbonate ($CaCO_3$) or Calcium Sulfate ($CaSO_4$)
- Bioactive glass particulate or fiber
- Bioabsorbable polymeric fibers

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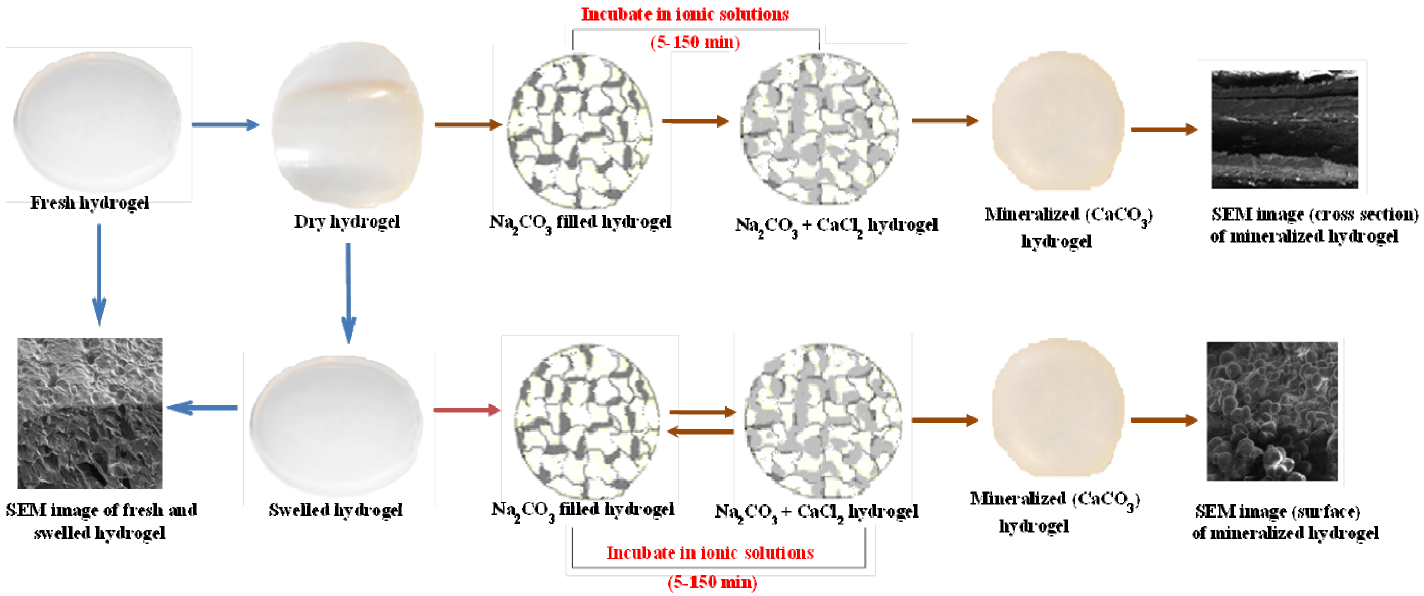
- The development of **responsive biomaterials** capable of demonstrating modulated function in response to dynamic physiological and mechanical changes in vivo remains an important challenge in **bone tissue engineering**.

- Many **natural and synthetic hydrogels** are already reported as having potential biological applications and considered as promising material for tissue engineering, as **scaffolds** for bone-tissue and dental transplants.

- Further, inspired by **natural biogenic minerals**, many researchers have attempted to **mimic natural biomineralization** through organic-inorganic hybridization in order to develop **a new material** for **medical applications**.

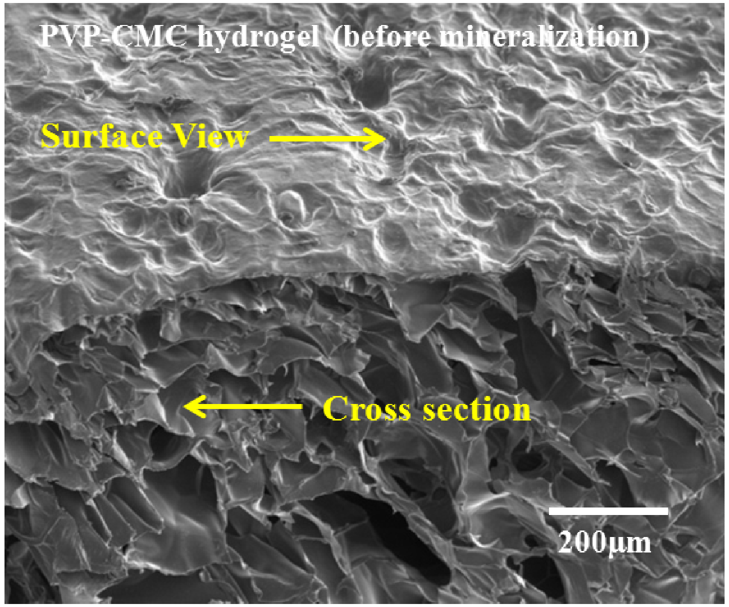
- Actually, **biomimetic chemical compositions** and **structures** in the **bone-regenerative scaffolds** have shown enhanced ability for cell biomineralization and osteogenesis.

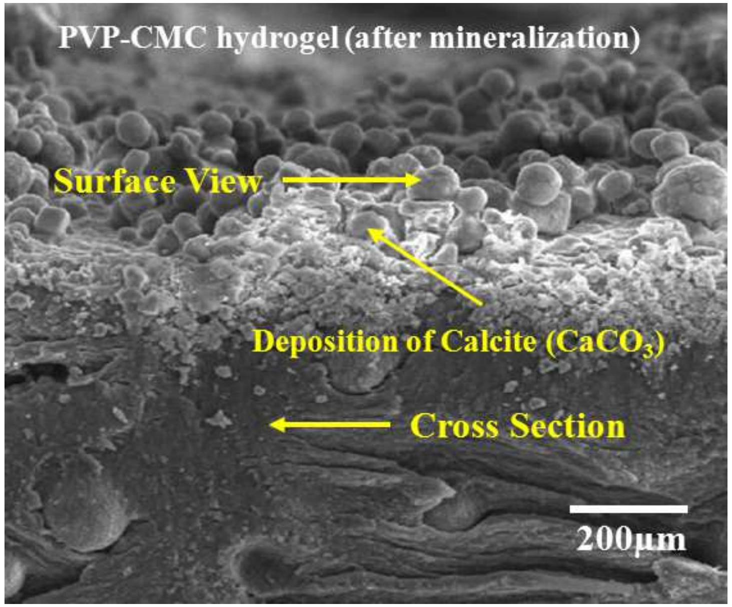
Thus, attempted has been taken for the preparation of biomimetic composition based biomaterial scaffold (i.e. **Biomineralized PVP-CMC Hydrogel**) for biomedical applications



Schematic diagram for Biomineralization of PVP-CMC hydrogel

Ref. Shah R. et.al: Preparation of $CaCO_3$-based Biomineralized Polyvinylpyrrolidone–Carboxymethylcellulose Hydrogels and their Viscoelastic Behavior (2014) J. Appl. Poly. Sci. DOI: 10.1002/APP.40237.





Morphology of PVP-CMC hydrogel (before and after mineralization)

Ref. Shah R. et.al: Preparation of $CaCO_3$-based Biomineralized Polyvinylpyrrolidone–Carboxymethylcellulose Hydrogels and their Viscoelastic Behavior (2014), J. Appl. Poly. Sc. DOI: 10.1002/APP.40237.

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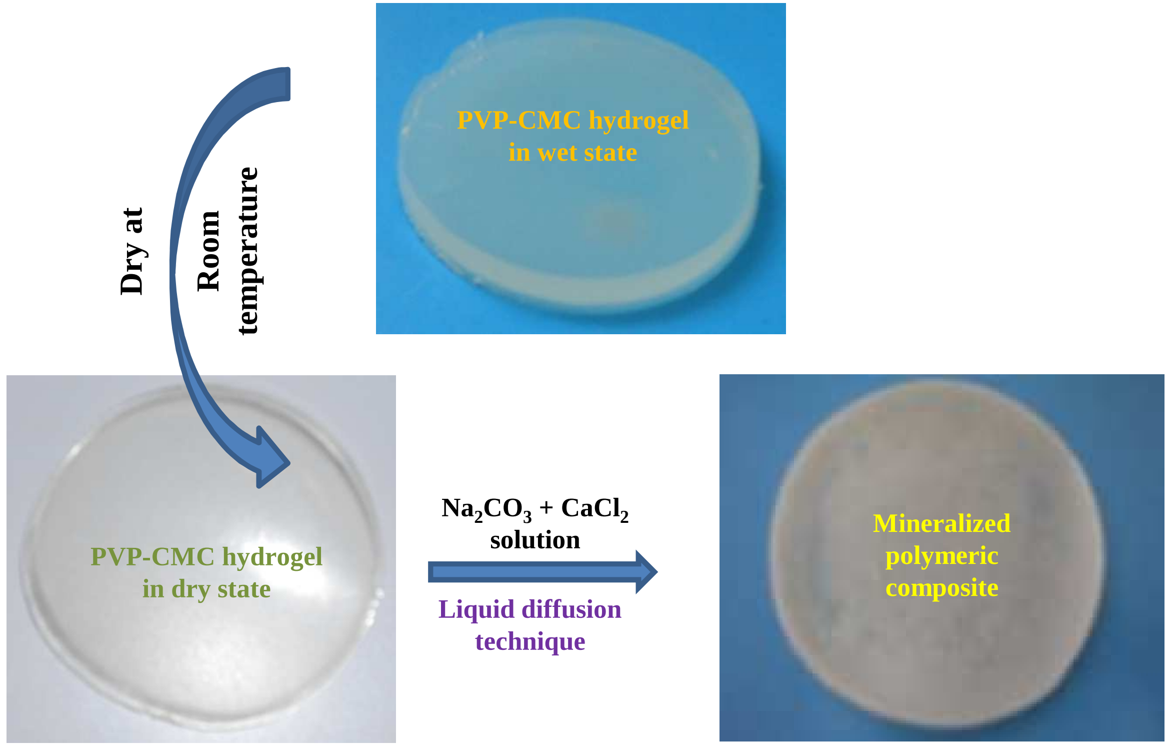
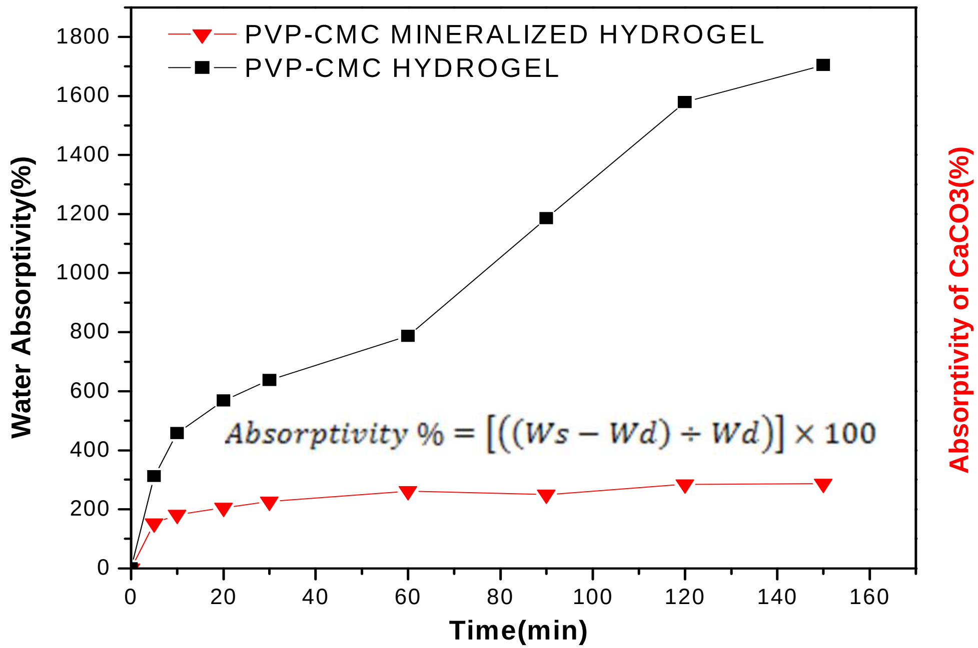


Organization of Calcite ($CaCO_3$) crystal
inside the pores of hydrogel matrix

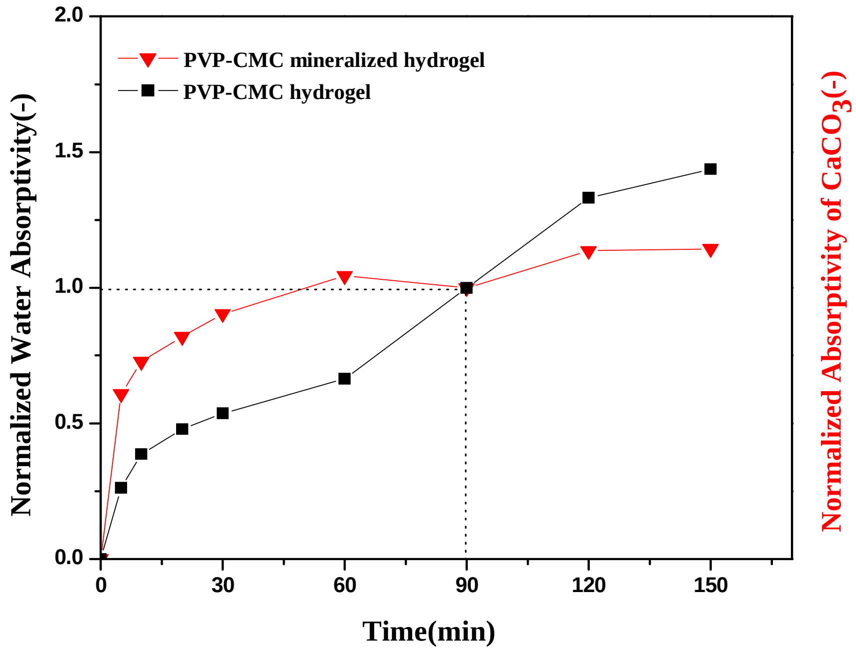
Preparation of mineralized polymer Composites

Ref. Shah, R.; Saha, N.; Kitano, T.; Saha, P. : Mineralized polymer composites as biogenic bone substitute material, *AIP Conf. Proc*. 2015, 1664, DOI: 10.1063/1.4918447.



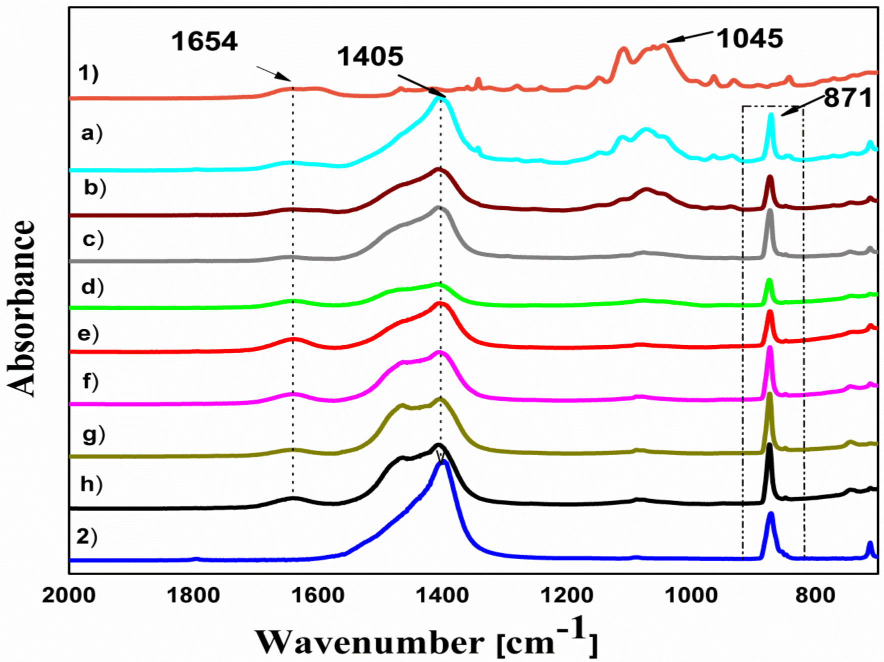
Absorptivity of H_2O and $CaCO_3$ within PVP-CMC Hydrogels

Ref. Shah R. et.al: Preparation of $CaCO_3$-based Biomineralized Polyvinylpyrrolidone–Carboxymethylcellulose Hydrogels and their Viscoelastic Behavior (2014) DOI: 10.1002/APP.40237.



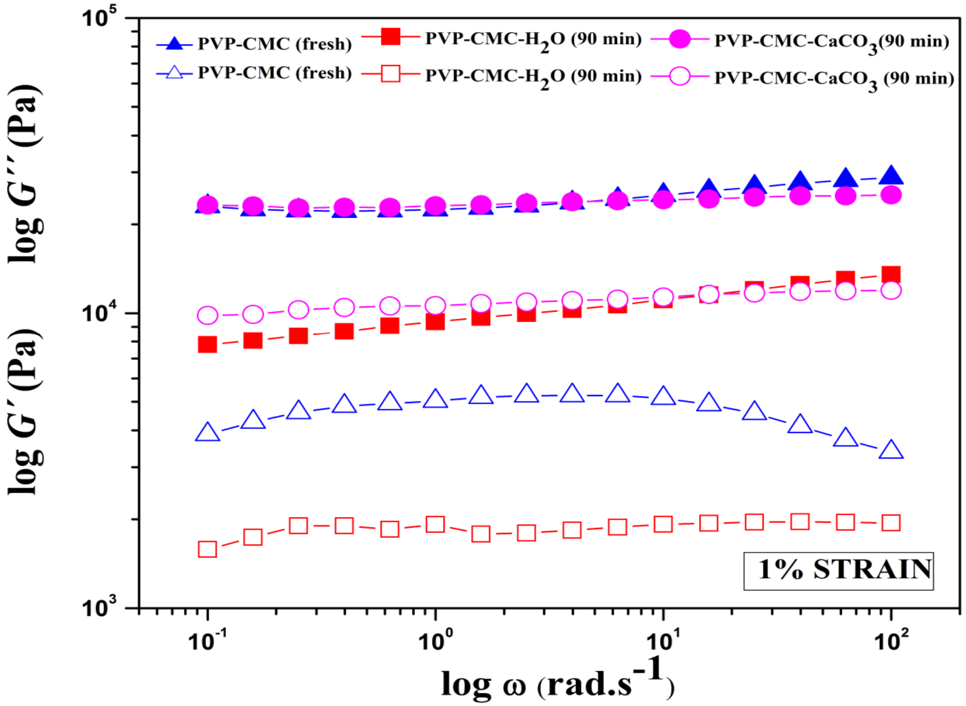
Time dependent absorptivity behaviour of calcite and water filled PVP-CMC hydrogels. Both absorptivity of water and of $CaCO_3$ are normalized by the each value at the time of 90 min.

Ref. Shah, R.; Saha, N.; Kitano, T.; Saha, P.: Influence of Strain on Dynamic Viscoelastic Properties of Swelled (H_2O) and Biomineralized ($CaCO_3$) PVP-CMC hydrogels, *Appl. Rheol.* 2015, *25*, *33979* DOI: 10.3933/ApplRheol-25-33979



FTIR spectra of (1) PVP-CMC hydrogel, (2) pure $CaCO_3$ and biomineralized ($CaCO_3$) PVP-CMC hydrogel in the order time of mineralization i.e. 5, 10, 20, 30, 60, 90, 120 and 150 mins.

Ref. Shah R. et.al: Preparation of $CaCO_3$-based Biomineralized Polyvinylpyrrolidone–Carboxymethylcellulose Hydrogels and their Viscoelastic Behavior (2014), J .Appl. Poly. Sc., DOI: 10.1002/APP.40237.



Rheological properties of fresh (before drying)**, swelled in water** (90 min) **and mineralized with $CaCO_3$** (90 min) **PVP-CMC hydrogels**

Ref. Shah R. et.al: Preparation of $CaCO_3$-based Biomineralized Polyvinylpyrrolidone–Carboxymethylcellulose Hydrogels and their Viscoelastic Behavior (2014), J. Appl. Rheo. DOI: 10.1002/APP.40237.

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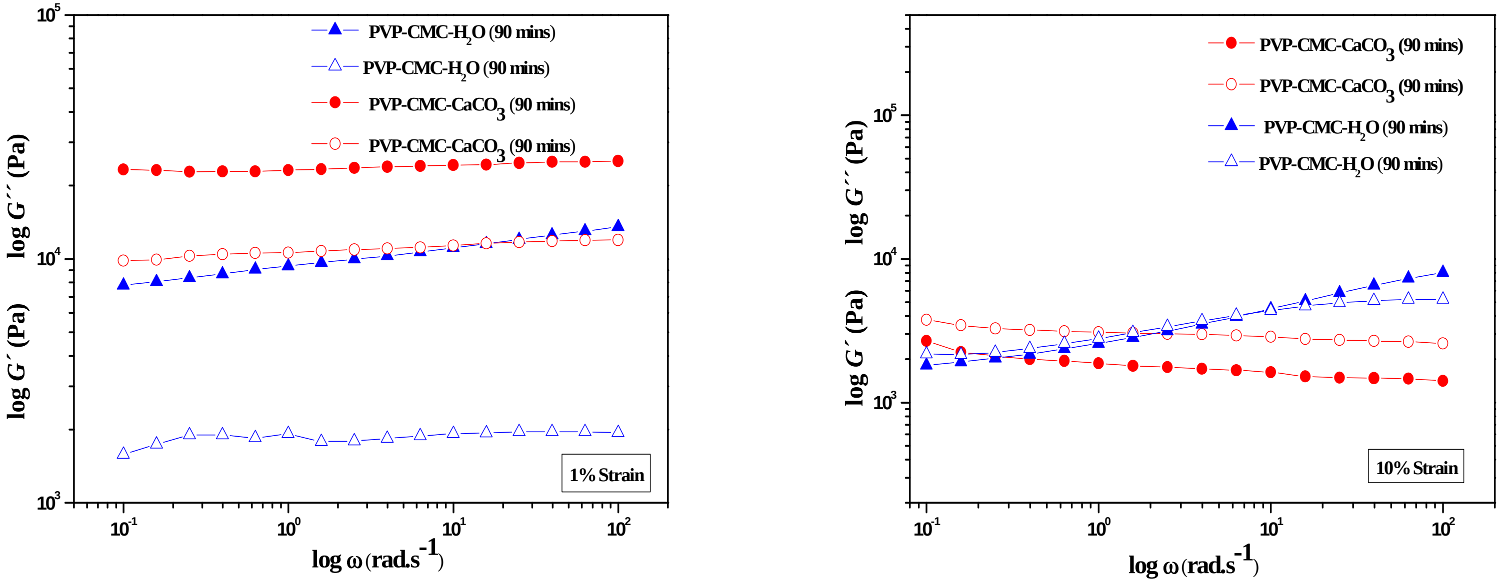

Dynamic viscoelasticity (storage (filled symbol) and loss moduli (open symbol) of PVP-CMC hydrogel and its mineralized hydrogel at 90 min. water absorption or mineralization as a function of angular frequency under 1% strain and 10% strain

Ref. Shah, R.; Saha, N.; Kitano, T.; Saha, P.: Influence of Strain on Dynamic Viscoelastic Properties of Swelled (H_2O) and Biomineralized ($CaCO_3$) PVP-CMC hydrogels, *J. Appl. Rheol.* 2015, *25*, *33979* DOI: 10.3933/ApplRheol-25-33979

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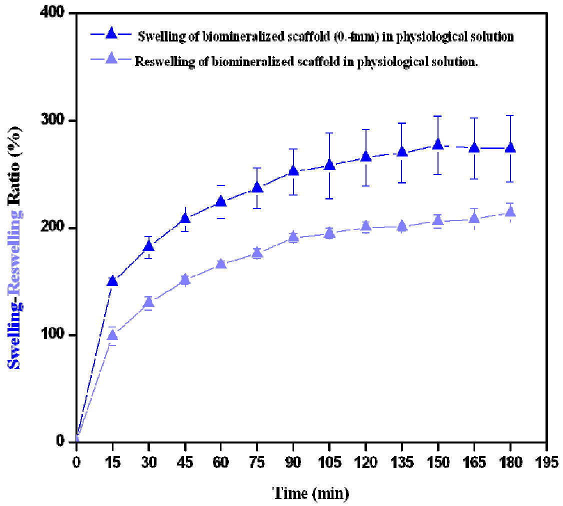
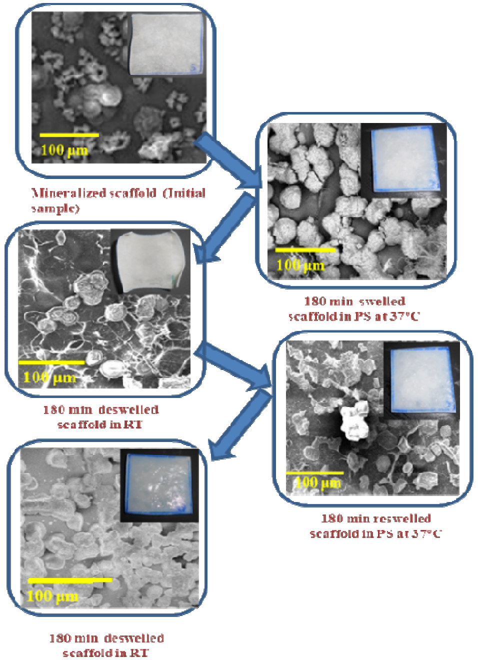
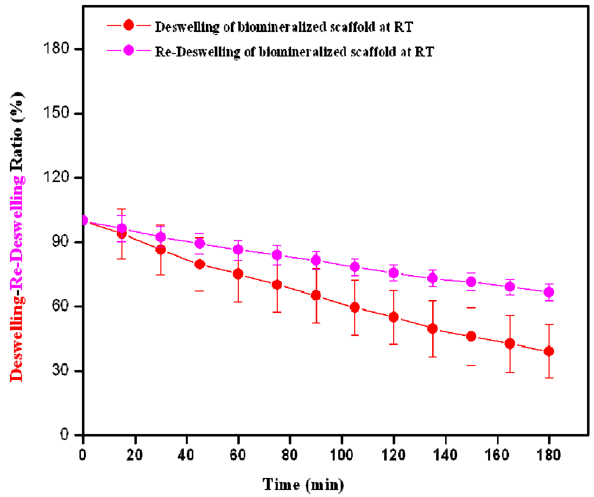


Swelling behavior of biomineralized scaffold in physiological solution
(0.9%NaCl, 37ºC and pH=7.5)

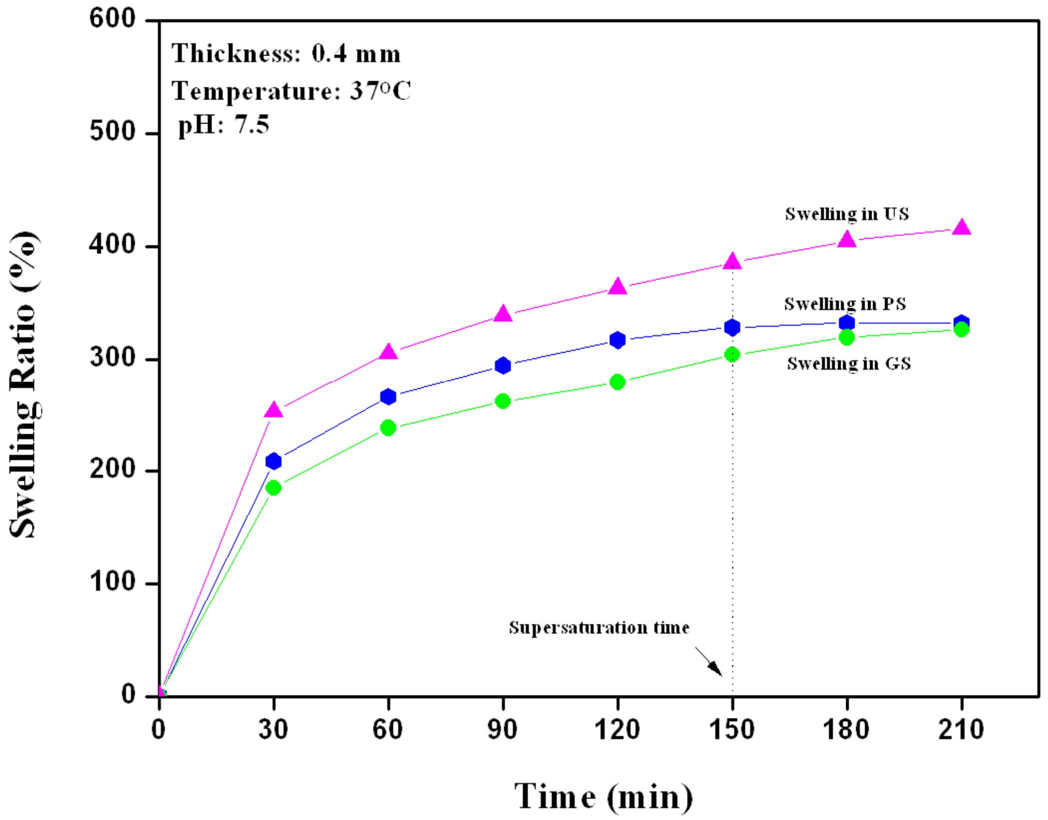


Surface view of biomineralized scaffolds after swelling-deswelling -reswelling -deswelling process in PS
(PS: physiological solution, RT: room temperature)



De-swelling behavior of biomineralized scaffold at room temperature.

Ref. Shah, R.; Saha, N.; Zukermann R.N.; Saha, P. :Stimuli responsive and biomineralized scaffold: an implant for bone-tissue engineering, SPE ANTEC 2015, *Conf. Proc*, Orlando, Florida, USA.



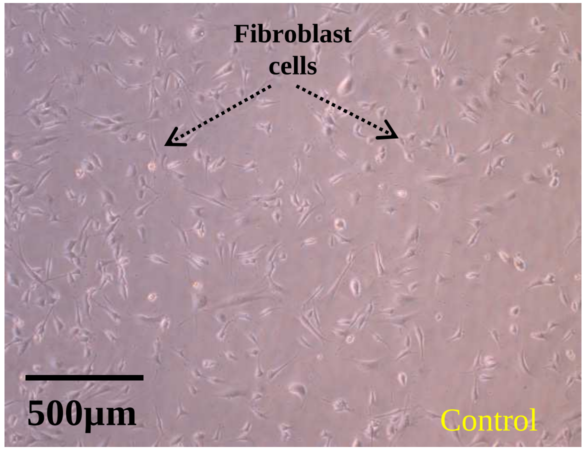
Swelling behaviour and super saturation time of biomineralized ($CaCO_3$) PVP-CMC hydrogel in simulated biological solutions

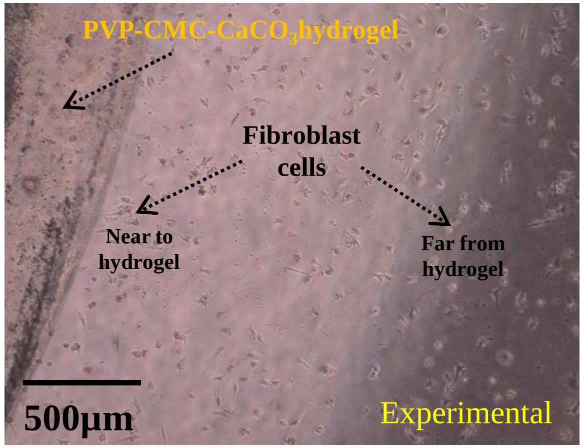
Manuscript under revision

Brief information of biomineralized ($CaCO_3$) PVP-CMC hydrogel

Sample Index	Concentration of Ionic Solution (g/100ml)		Biomineralized ($CaCO_3$) PVP-CMC hydrogel (Diameter = 80mm)			
			Wet Sample		Dry Sample	
	Na_2CO_3	$CaCl_2$	Weight (gm)	Thickness (mm)	Weight (gm)	Thickness (mm)
*I		-	43.762	-	2.507	0.41
II	10.50	14.70	9.450	1.15	2.681	0.46
III	5.25	7.35	9.802	1.21	2.289	0.44
IV	4.20	5.88	9.989	1.28	2.239	0.43
V	2.10	2.94	12.033	1.37	1.990	0.41
VI	1.05	1.47	13.105	1.42	1.810	0.40
*VII	0.55	0.55	17.320	1.59	1.757	0.40
*VIII	0.27	0.27	20.327	1.87	1.664	0.37
*IX	0.13	0.13	22.16	2.14	1.558	0.34
*X	0.07	0.07	24.16	2.31	1.518	0.34

Manuscript submitted





Fibroblast cell growth in presence of biomineralized ($CaCO_3$) PVP-CMC hydrogel

Manuscript submitted

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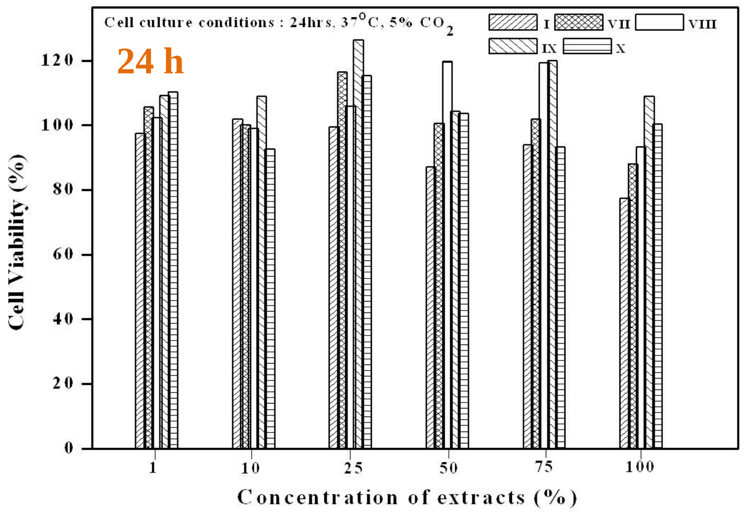

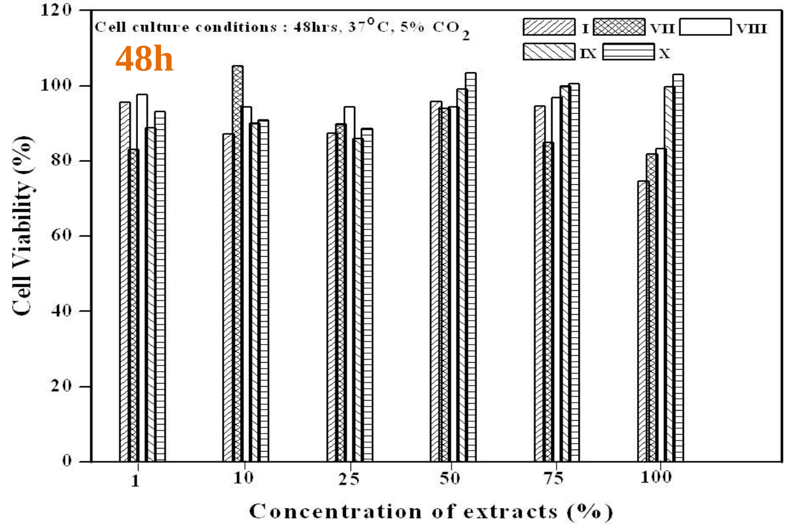

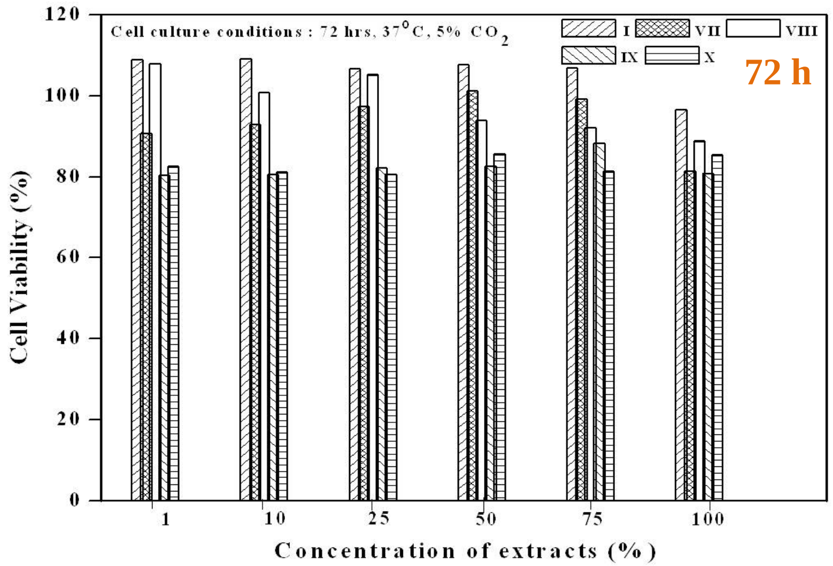

Manuscript submitted

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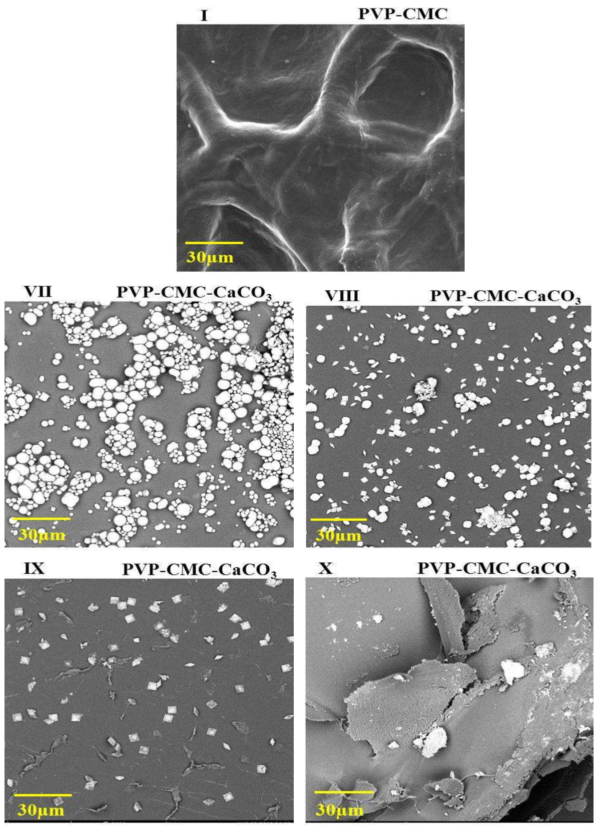
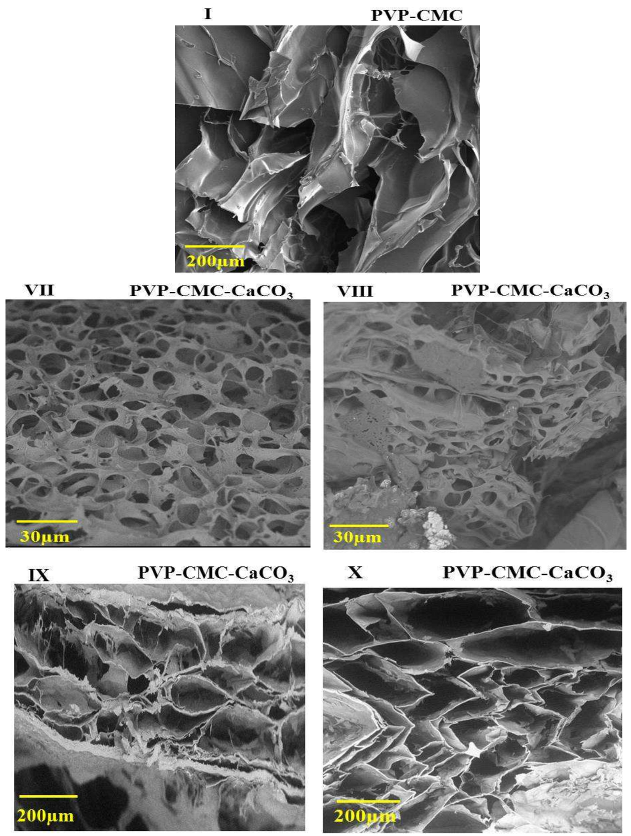


Surface images



Cross sectional images

SEM micrographs of biomineralized ($CaCO_3$) PVP-CMC hydrogel

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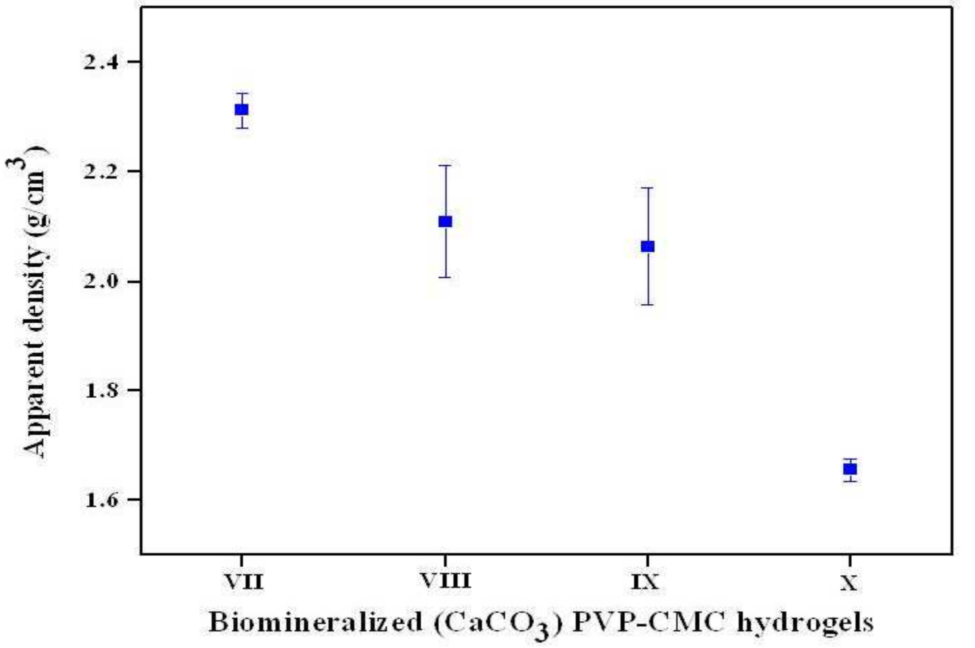
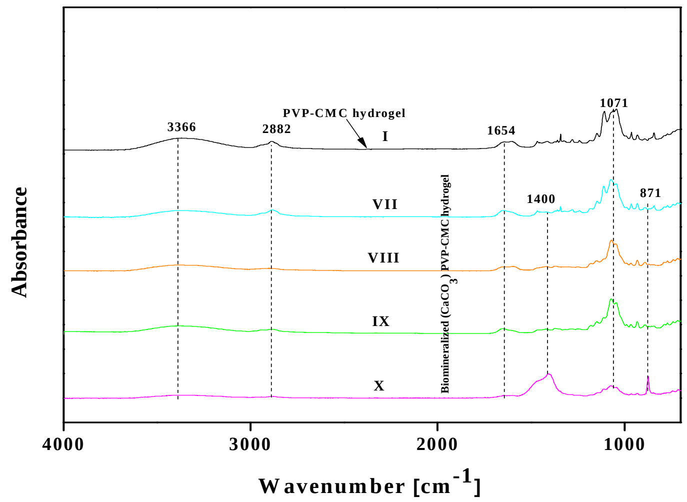
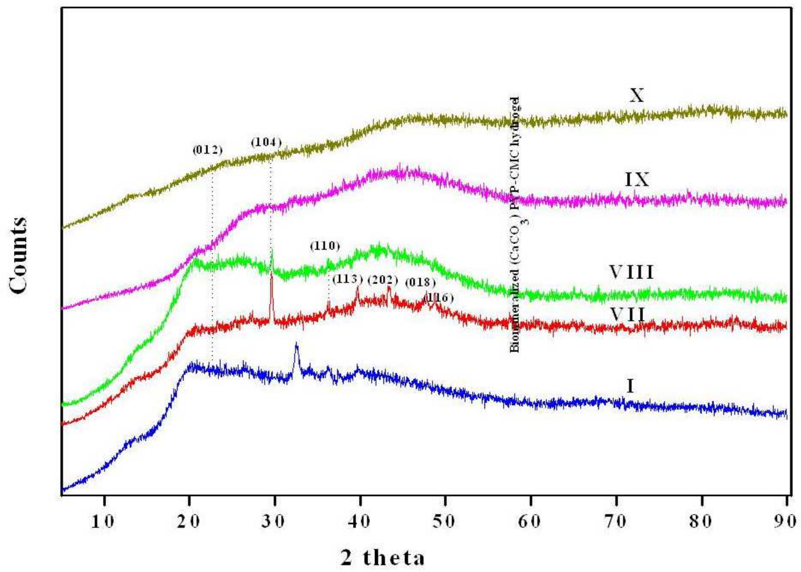


Apparent density of biomineralized ($CaCO_3$) PVP-CMC hydrogel

Manuscript submitted



FTIR Analysis of biomineralized ($CaCO_3$) PVP-CMC hydrogel



XRD Analysis of biomineralized ($CaCO_3$) PVP-CMC hydrogel

Manuscript submitted

List of published article on Mineralized ($CaCO_3$)filled PVP-CMC Hydrogel

Saha N, Shah R, Vyroubal R, Kitano T, Saha P: Morphology, Absorptivity and Viscoelastic Properties of Mineralized PVP-CMC Hydrogel, Novel Trends in Rheology V Book Series: ***AIP Conf. Proc.*** 1526 **2013,** 292 – 300. ISSN: 0094-243X, ISBN: 978-073541151-7.

Saha N, Vyroubal R, Shah R, Kitano T, Saha P: Effect of Strain on Viscoelastic Behavior of Fresh, Swelled and Mineralized PVP-CMC Hydrogel, Novel Trends in Rheology V Book Series: ***AIP Conf. Proc.*** 1526,**2013**, 301-309, ISSN: 0094-243X, ISBN: 978-073541151-7.

Shah R, **Saha N**, Kitano T, Saha P: Preparation of $CaCO_3$-based Biomineralized Polyvinylpyrrolidone–Carboxymethylcellulose Hydrogels and their Viscoelastic Behavior ***J. App. Poly. Sci.,*** **2014,** DOI: 10.1002/APP.40237.

Shah, R.; **Saha, N**.; Kitano, T.; Saha, P.: Influence of Strain on Dynamic Viscoelastic Properties of Swelled (H_2O) and Biomineralized ($CaCO_3$) PVP-CMC hydrogels***, Appl. Rheol***. **2015**, *25*, *33979* DOI: 10.3933/ApplRheol-25-33979.

Shah, R.; **Saha, N**.; Kitano, T.; Saha, P. : Mineralized polymer composites as biogenic bone substitute material, ***AIP Conf. Proc***. **2015,** 1664, DOI: 10.1063/1.4918447.

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Conclusions

- The morphological image confirms the deposition of calcium ions in the PVP-CMC matrix. The crystals obtained are granular in shape which increases with mineralization time. After filling up the pores of hydrogel matrix, the $CaCO_3$ are deposited on the surface.

- Swelling study of MPC (varying thickness) was performed in presence of physiological solution at 37^{o}C to explore the potential biomedical applications like drug delivery and /or for the treatment of a dynamic bone disorder etc.

- Further, the rheological properties of the MPC explained the elastic and viscous nature of the developed material at low and high strain. The linear nature clearly indicates the solidity of the polymeric material. Also, when the strain increases from 1% to 10% the elasticity of the material reduces and slowly viscous nature is observed.

Conclusion remarks to biomineralization and biomineralized composites

- Biomineralization is a compendium of current topics focusing on processes of formation, organization, as well as mineralization of novel structural materials.

- Biomineralized materials / composites have promising approach to several biomedical fields.

- By understanding Nature's tools to make strong and tough materials, similar properties can be endowed into man-made materials in the near future.

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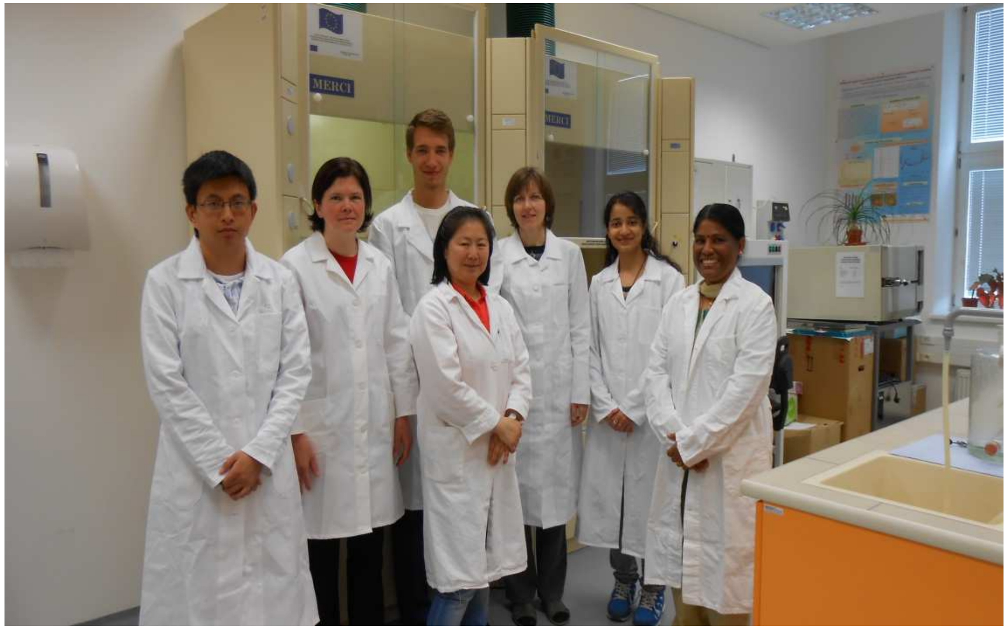
ACKNOWLEDGEMENTS

This work was conducted within the framework of COST Action MP1301 “New Generation Biomimetic and Customized Implants for Bone Engineering” – www.cost.eu. And the work was supported by the Ministry of Education, Youth and Sports of the Czech Republic - NPU Program I (LO1504).



Research Team Members

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