Viscoelastic behavior of Calcium Phosphate Packed Bacterial Cellulose -Polyvinylpyrrolidone based Hydrogel Scaffolds at Human Fever Temperature

Probal Basu, Nabanita Saha* (Presenting Author, Reference author), Oyunchimeg Zaandra and Petr Saha

Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Trida Tomase Bati 5678, 760 01 Zlín, Czech Republic

***Corresponding/reference author**: nabanita@utb.cz

Abstract

Calcium phosphate (CaP) packed bacterial cellulose (BC) and polyvinylpyrrolidone (PVP) based hydrogel scaffolds (BC-PVP-CaP) are being reported here considering its possible application in bone restoration. The primary focus of this work is to analyze the viscoelastic behavior of 'BC-PVP-CaP' hydrogel scaffolds at human fever temperature condition (39oC) in swelled state. In BC-PVP scaffolds, CaP is present in the form of β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) in different concentrations (i.e. β-TCP/HA_20:80, β-TCP/HA_40:60, β-TCP/HA_50:50). During evaluation of viscoelasticity, BC-PVP scaffold (without CaP) was marked as a control set. The BC-PVP-CaP_50:50 hydrogel scaffold showed the highest degree of swelling at 39°C among the other BC-PVP-CaP scaffolds. However, the other hydrogel scaffolds were also showed notable viscoelasticity at 39oC. Experimental results confirmed that all the CaP filled BC-PVP hydrogel scaffolds (BC-PVP-CaP_20:80, BC-PVP-CaP_40:60, BC-PVP-CaP_50:50) exhibit a promising swelling and viscoelastic behavior at human fever temperature condition (39oC), can be endorsed for its potential application in bone repairing /bone restoration.

INTRODUCTION

Bone is a fundamental unit of human musculoskeletal organ. Notable stressful physiological conditions lead to unwanted bone fracture situation [1]. Aging along with bone related disease also ameliorate the likeliness for bone fracture [2]. Among different well practiced methods, bone autografting has been considered promising due to its facilitation of fracture healing. However, this method also has significant limitations [3]. Hence, a new approach is needed for the potential alternative to this method. In this context, polymeric biomaterial can be efficient alternative approach to this problem. Hydrogel scaffold is polymeric biomaterial which can retain significant amount of water [4]. Due to its similarity with the living tissue in context of its water retaining capacity it can be used in tissue engineering application [5]. Different polymer composites have been used as tissue engineering scaffolds. Synthetic polymers like poly (glycolic acid) [PGA], poly (lactic-co-glycolic) acid [PLGA] and natural polymers like chitosan, gelatin were being utilized [6]. Bacterial cellulose (BC) is biocompatible polymer which has significant mechanical property [5]. Different combinations of biomaterials were prepared with BC; where, the other components were either polymer or other bioactive agents like BC/chitosan, BC/hydroxyapatite. Research indicated that calcium phosphate (CaP) [like hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP)] can be used in bone tissue engineering application [6]. In our earlier work, we have prepared CaP packed BC-polyvinylpyrrolidone (PVP) based hydrogel scaffold, which has the necessary mechanical property and physiochemical property for utilizing in bone tissue engineering [5]. The viscoelastic behavior of the CaP packed BC-PVP based hydrogel scaffold were also analyzed at normal human homeostatic temperature (37°C). However, the analysis of rheological performance of the scaffold material in altered physiological condition is important for the understanding its sustained efficiency. Thus, this study aims to analyze the rheological performance of CaP packed BC-PVP based hydrogel scaffolds in altered physiological condition (i.e. human fever temperature). According to the American College of Critical Care Medicine, and the Infectious Diseases Society of America, a human fever condition can be defined an elevation of the temperature of the human body at 38.3°C or higher [7]. This study focuses the rheological behavior of calcium phosphate filled BC based hydrogel scaffold at human fever temperature condition (39°C).

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EXPERIMENTATION

Preparation of CaP packed BC-PVP based hydrogel scaffold

BC (holding 99% H_2O) was synthesized in presence of basal synthetic Hestrin-Schramm (HS) nutritive medium (pH 7.0) using *Gluconacetobacter xylinus CCM 3611^T* (syn. *Acetobacter xylinum*) at 30°C for 15 days. 100 mL bacteriological culture bottles were inoculated with 5 mL of H.S. medium containing 96×10^8 cells/mL bacteria [bacteria counted at 550 nm wavelength with Grant-Bio McFarland Densitometer DEN-1B, Grant Instruments Ltd, UK]. The freshly prepared BC pellicle is treated with 0.5 N NaOH solution and then heated at 80°C for 1 hours to remove the possible contaminations from the BC pellicle. Thereafter, a homogenous suspension of BC (particle size: 159 nm [SD: ±11.33]) from the obtained BC mat was prepared by grinding (12 minutes) the BC mat in distilled water. Hydrogel scaffolds were first developed by applying the BC (holding 99% water) with polyvinylpyrrolidone (PVP) [5]. 0.5 g BC and 0.5 g PVP is used in each composition. 1 g polyethylene glycol (PEG) was also used in all the solutions to reduce the risk of tissue damage and other significant cytotoxic effects. 2 g Agar used as gelling agent and glycerin (1 mL) used as humectant. Additionally, Calcium phosphate (CaP) is used in the form of β-TCP and HA were applied (Table. 1) in the ratio of 20:80, 40:60 and 50:50 in the hydrogel scaffolds to produce the CaP packed BC based composite hydrogel scaffolds and henceforth will be termed as, "BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50". BC-PVP hydrogel scaffolds was used as base scaffolds (Table 1). All materials were used are reagent grade and majorly purchased from Sigma Aldrich.

Table 1. CaP in BC-PVP based hydrogel scaffolds.

Sample index	CaP (g) [β-TCP/HA]
BC-PVP	0.0/0.0
BC-PVP- CaP_20:80	0.2/0.8
BC-PVP- CaP_40:60	0.4/0.6
BC-PVP- CaP_50:50	0.5/0.5

The BC-PVP based hydrogel based hydrogel scaffolds were prepared following the solvent casting method, applying moist heat and pressure. 100 mL polymer solutions were prepared in 250 mL sealed glass bottles under 15 lbs (107 KPa) pressure and 120°C temperature for 20 minutes [4]. Two sets of polymer solutions were prepared; where one set was without CaP, and another set was with CaP (β-TCP/HA). 25 mL polymer solution from each sealed glass bottles was poured into 75 mm diameter petri-dishes and allowed to cool at room temperature (22-25°C). Finally, smooth, round shaped, off white color BC-PVP based scaffold without CaP (i.e. "BC-PVP", Diameter: 75 mm; Thickness: 6.0-6.3 mm) and with calcium phosphate filled (i.e. "BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50"; Diameter: 75 mm; Thickness: 5.9-6.2 mm) hydrogel scaffolds were attained.

Swelling analysis at human fever temperature (39°C)

Practically, as an implant/scaffold material, the hydrogel scaffolds will be first become swelled after in contact with the biological fluids. Thus, the swelling analysis of CaP packed BC-PVP based hydrogel scaffolds (BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50) was first performed with physiological saline solution (pH 7.40) at human fever temperature (39°C). Circular sections (Diameter: 20 mm) of the hydrogel scaffolds were soaked for specific time intervals (5 min, 30 min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min). The absorptivity of the hydrogel scaffolds is measured by the degree of swelling which is defined by the following equation:

$$\text{Degree of swelling (\%)} = ([W_s - W_d]/W_d) \times 100$$

Where, W_s and W_d are weight of the swollen and dried hydrogel scaffold section.

Viscoelastic behavior study human fever temperature (39°C)

Thereafter, the viscoelastic property of the equilibrium swelled hydrogel samples (20 mm diameter) were studied and by using a modular compact rheometer testing device (Anton Paar, Austria) and "Rheoplus" software package for data analysis. Dynamic frequency sweep analysis was conducted in the linear viscoelasticity region (LVR) at 1% strain amplitude. The rheological measurements was done in the oscillation mode with the angular frequency range from 0.1 to 100 rad.s^{-1} at human fever temperature condition (39°C). The influence of angular frequency on storage (G') and loss (G'') modulus and complex viscosity (η^*) was calculated by the following equation:

$$\eta^* = [(G'/\omega)^2 + (G''/\omega)^2]^{1/2}$$

The tan δ (damping factor) was also studied at specific angular frequencies (i.e. 0.1 rad.s^{-1}, 1 rad.s^{-1}, 10 rad.s^{-1}, 100 rad.s^{-1}.) intervals.

Statistical Analysis

The data are presented as mean ± standard error of the mean (SEM). Statistical differences between the studied samples were assessed using one-way analysis of variance (ANOVA) followed by Bonferonni's post-hoc test by using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

RESULT AND DISCUSSION

The CaP packed BC-PVP based hydrogel scaffolds (BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50) were prepared and their swelling and viscoelastic behavior were studied at human fever temperature (39°C). BC-PVP (without CaP) scaffold is kept as control.

Fig. 1. demonstrates the degree of swelling of the CaP packed BC-PVP based hydrogel scaffolds i.e. "BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50" at human fever temperature (39°C). BC-PVP (without CaP) hydrogel scaffold used as control. It can be seen from the Fig. 1. that, BC-PVP (without CaP) is majorly showing highest degree swelling throughout the study period. On the other hand, BC-PVP-CaP_50:50 hydrogel scaffolds is also showing the highest degree of swelling than BC-PVP-CaP_20:80 and BC-PVP-CaP_40:60. The hydrogel scaffolds reached to equilibrium swelling condition after 300 mins. After equilibrium swelling (i.e. 330 min), BC-PVP (without CaP) hydrogel scaffold is showing highest ($P<0.0001$) degree of swelling. On the hand, the degree of swelling of BC-PVP-CaP_50:50 is also significant ($P<0.0001$). Additionally, the degree of swelling of BC-PVP-CaP_40:60 hydrogel scaffold is also found higher ($P<0.0001$) than BC-PVP-CaP_20:80 in equilibrium swelling condition.

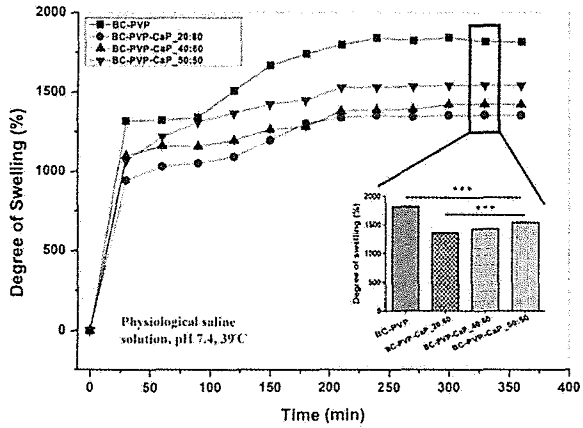

FIGURE 1. Degree of swelling of CaP packed BC-PVP based hydrogel scaffolds: BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50 at human fever temperature (39°C). Asterix (***) indicates level of significance (i.e. P <0.0001).

Fig. 2. represents the viscoelastic behavior of the CaP packed BC-PVP based hydrogel scaffolds i.e. (BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50 at human fever temperature (39°C). It can be seen from Fig. 2(a). that the storage (G') modulus CaP packed BC-PVP based hydrogel scaffolds were higher than the loss (G") modulus which indicates their significant rubbery consistency at 39°C throughout the angular frequency 0.1-100 rad.s^{-1}. BC-PVP (without CaP) scaffold was also used as control. However, the storage modulus values of BC-PVP-CaP_50:50 hydrogel scaffold is found higher at 39°C compared to other scaffolds. In addition, the sustained plateau trend of storage modulus of all of the hydrogel scaffolds is also indicating crosslinked rigid gel like property. Interestingly, the overall trend of loss modulus of the hydrogel scaffolds throughout the angular frequency is increasing. This might be a plausible effect of temperature.

Fig. 2(b). indicates the effect of angular frequency (ω) on complex viscosity (η^*) of the CaP packed BC-PVP based hydrogel scaffolds. It can be observed that the complex viscosity values is decreasing with the increasing angular frequency at 1% strain amplitude, which indicates the significant elastic nature of the hydrogel scaffolds at 39°C.

Additionally, Fig. 2(c). shows the damping factor (tan δ) values of CaP packed BC-PVP based hydrogel scaffolds at specific intervals of angular frequencies: 0.1 rad.s^{-1}, 1 rad.s^{-1}, 10 rad.s^{-1}, 100 rad.s^{-1}. It can be observed that the values of damping factor of all the hydrogel scaffolds are less than 1 (tan $\delta < 1$) at 39°C which indicates the maintenance of gel like elastic behavior throughout the four different angular frequencies in human fever temperature.

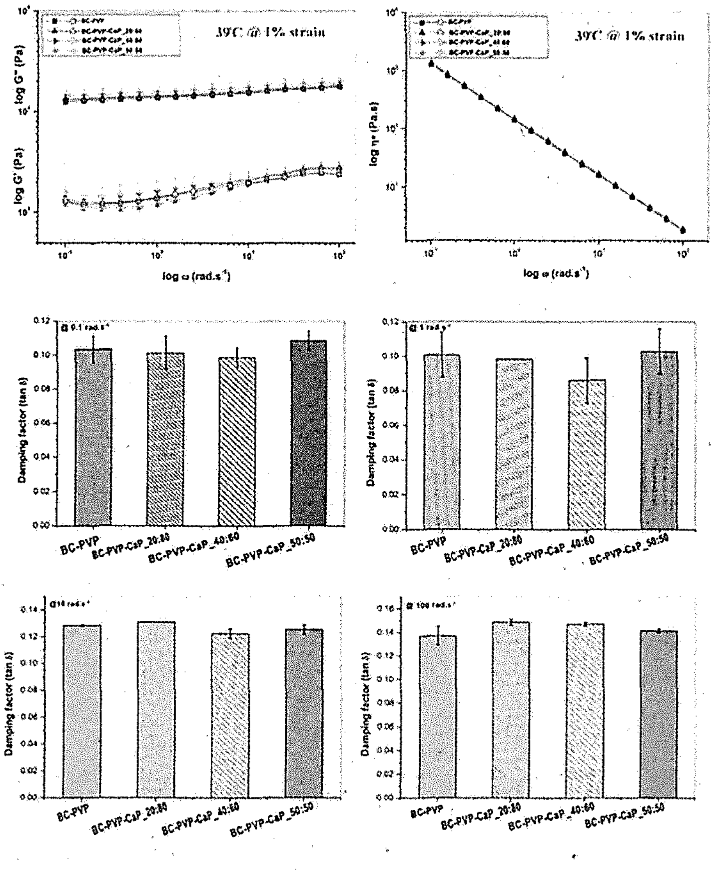

FIGURE 2. Viscoelastic behavior of CaP packed BC-PVP based hydrogel scaffolds i.e. "BC-PVP-CaP_20:80", "BC-PVP-CaP_40:60", "BC-PVP-CaP_50:50" at human fever temperature condition (39°C). (a) Storage (G') and Loss modulus (G"); (b) Complex viscosity (η^*); (c) tan δ values at angular frequencies: 0.1 rad.s^{-1}, 1 rad.s^{-1}, 10 rad.s^{-1}, 100 rad.s^{-1}.

CONCLUSION

This works focuses the swelling and viscoelastic behavior of CaP packed BC-PVP based hydrogel scaffolds i.e. BC-PVP-CaP_20:80, BC-PVP-CaP_40:60, BC-PVP-CaP_50:50 at human fever temperature (39°C). The BC-PVP-CaP_50:50 hydrogel scaffold demonstrated highest degree of swelling after equilibrium swelling condition at 39°C. All the CaP packed BC-PVP based hydrogel scaffolds maintains higher storage modulus throughout the angular frequency at 39°C. Thus, it can be concluded that the swelling and rheological behavior of the three significant CaP packed BC-PVP based hydrogel scaffold i.e. BC-PVP-CaP_20:80, BC-PVP-CaP_40:60, BC-PVP-β-CaP_50:50 are promising at human fever temperature condition (39°C).

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Probal Basu, **Nabanita Saha***, Oyunchimeg Zaandra and Petr Saha
Centre of Polymer Systems, University Institute, Tomas Bata University in Zlín, Trida Tomase Bati 5678, 760 01 Zlín, Czech Republic
***Corresponding author**: nabanita@utb.cz

Introduction

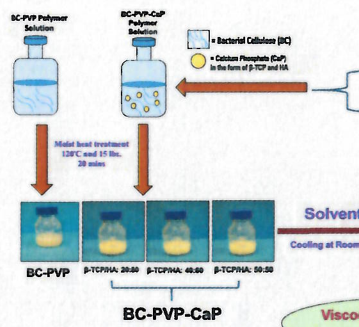
- Bone is a fundamental unit of human skeleton. Notable stressful conditions lead to unwanted bone fracture situation [1].
- Application of polymeric biomaterial (i.e. hydrogel scaffold) prepared in combination with natural polymer (eg., Bacterial cellulose [BC]) and filler material (eg. calcium phosphate like β- tri calcium phosphate [β- TCP] and hydroxyapatite [HA]) can be efficient alternative approach for tissue engineering; especially bone tissue engineering [2].
- The characterization on the basis of swelling and mechanical/ viscoelasticity property of hydrogel scaffold is thus necessary for the proper assessment of its applicability in bone regeneration application.

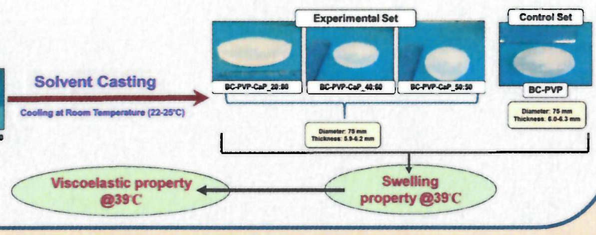
Hydrogel → Tissue engineering — **Study focuses the rheological behavior of calcium phosphate (CaP) filled BC based hydrogel scaffold at human fever temperature condition (39°C).**

Experimentation

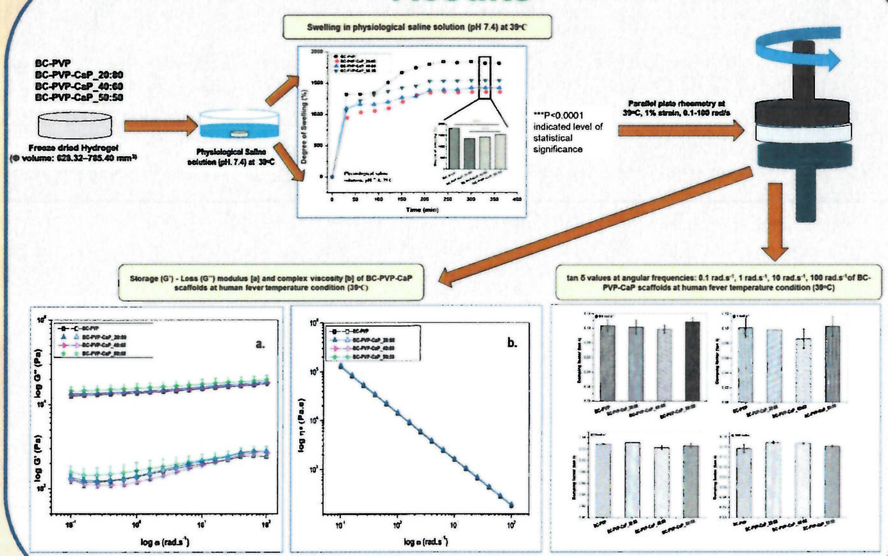
Preparation of CaP filled BC based hydrogel scaffolds [3]

Sample No.	Sample index	PVP	BC	PEG	AGAR	GLYCERINE	CaP (β-TCP/HA)	Water
1	BC-PVP	0.5	0.5	1	2	1	0.0/0.0	95
2	BC-PVP-CaP_20:80	0.5	0.5	1	2	1	0.2/0.8	94
3	BC-PVP-CaP_40:60	0.5	0.5	1	2	1	0.4/0.6	94
4	BC-PVP-CaP_50:50	0.5	0.5	1	2	1	0.5/0.5	94





Results



Conclusion

- This works focuses the swelling and viscoelastic behavior of CaP packed BC-PVP based hydrogel scaffolds i.e. BC-PVP-CaP_20:80, BC-PVP-CaP_40:60, BC-PVP-CaP_50:50 at human fever temperature (39°C).
- The BC-PVP-CaP_50:50 hydrogel scaffold demonstrated highest degree of swelling after equilibrium swelling condition at 39°C.
- All the CaP packed BC-PVP based hydrogel scaffolds maintains higher storage modulus throughout the angular frequency at 39°C.

Thus, the swelling and rheological behavior of the three significant CaP packed BC-PVP based hydrogel scaffold i.e. BC-PVP-CaP_20:80, BC-PVP-CaP_40:60, BC-PVP-β-CaP_50:50 are promising at human fever temperature condition (39°C).

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Nabanita Saha

From: PPS2019 <PPS2019@csir.co.za>
Sent: Wednesday, September 18, 2019 10:40 PM
To: Nabanita Saha
Subject: PPS2019: S03-113 Paper Acceptance

Dear Dr Nabanita Saha,
Your contribution "*Viscoelastic behavior of Calcium Phosphate Packed Bacterial Cellulose -Polyvinylpyrrolidone based Hydrogel Scaffolds at Human Fever Temperature*", with Reference number S03-113, has been accepted as **POSTER** presentation in the symposium S03 - Polymer-based Nanostructured Materials to the **PPS2019** Conference of the Polymer Processing Society.
A very large number of contribution has been submitted and accepted for the PPS20199 which will be held in Pretoria, South Africa during November 18-22 (including postconference tour on November 22nd), 2019. We expect PPS2019 to be a very fruitful event with diverse topics.
In order to allow an optimal organization of the Conference, it would be extremely useful for us if you could finalize your registration as soon as possible. Early bird on or before 30th September 2019.
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