Experimental study of 3D-printable biocomposite of [HA/PMMA/Sericin] materials

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Abstract

In the previous study, a biocomposite material of [HA/Bioplastic/Sericin] was developed as a printable material. The highest strength was only 3.89 MPa which was achieved by the composite with 60/40 ratio of HA/Bioplastic and additional sericin of 0.3%w/w of HA. The present of sericin within the biocomposite could improve cell attachment. However, since cassava starch based bioplastic as a matrix was degraded in PBF, the dimensional accuracy could not be maintained. In the present study, the matrix of bioplastic was replaced by PMMA with various P/L ratios of 2/1.8, 2/1.185 and 2/1.9. A series test was carried out to investigate the printable characteristic in 3D printer with an optimum printing parameter such as curing time window, flow rate through a nozzle, tensile strength of the printed sample, microstructure and x-ray diffraction. Response Surface Method (RSM) was employed to optimize the printing process parameter of the 3D-Bioprinter, predict the tensile strength of the sample and it was validated by experiment. The flow rate of pasta was 78.5mm^3/s, the highest predicted tensile strength was 6.01 MPa and experiment was 5.12 MPa. This lower strength might be caused by the existence of porosity as conformed by SEM, while hydroxyapatite still exist as indicated by the XRD. Copyright © 2017 VBRI Press.

Keywords: Optimum setting, printing parameter, bio composite, printable, 3D-bioprinter.

Introduction

In the previous study, a printable biocomposite material that composed by hydroxyapatite (HA), bioplastic-cassava starch based and silk sericin (Bombyx mori) have been developed. It was found that biocomposite with ratio of [HA/Bioplastic] 60/40 with sericin of 0.3%w/w of HA showed the highest tensile (3.89 MPa) and flexural strength (2.51 MPa) [**1, 2**]. In the biology aspect test, the present of sericin 0.32%w of hydroxyapatite within the biocomposite could improve cell attachment. However, although, this biocomposite material was printable using 3D printer, it was degraded easily in PBF. In addition, its strength was also lower than that needed for bone implant [**3, 4**]. Hydroxyapatite [**6, 7**] powder is a material which is a potential material to be used in bone tissue engineering, because HA has the property of osteointegration, the ability to fuse with human bone. The function of HA as the subtance that gives the similar characteristic to bone. PMMA [**8**, **9**] is widely used as bone cement to secure orthopedic implants, but it still has many disadvantages, such as a poor biocompatibility with bone. Several studies concerning [HA/PMMA] as bone cement have also been done by previous investigators [**10-12**].

The addition of 2.5%w/w HA in PMMA bone cement can result the maximum value of ultimate compressive strength, elastic modulus strength, elastic modulus of compression, and compression yield strength. One of the materials that will be developed is biocomposites pasta [HA/PMMA/Sericin]. Sericin [**13**] is ecofriendly and natural biopolymer. It has been used in many industries as well as in the production of functional biomaterials. But nowadays, since demand of variability of product is high, rapid prototyping technology such as a 3D printer machine and ABEF are becoming suitable technology to do that [**14-16**]. There is various commercial 3D printer machines-FDM based technology available in the market place, but it could only be used to print a product with limited materials such as PLA or ABS filament. In the present study, the current 3D printer machine was modified to be a 3D-Bioprinter allowing to print biocomposite pasta, and thus optimization process parameters need to be carried out. The optimization was performed by Response Surface Method (RSM). RSM [**17**, **18**] was selected as it can determine level and the optimum variable values that might be exist. Using this method, it can determine an accurate estimate optimization, if the mathematical models meet statistical assumptions.

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Experimental

Materials and preparation

Materials used in this study were hot curing Polymethyl methacrylate (PMMA powder and MMA liquid; ADM England), hydroxyapatite powder (HA; Sigma Aldrich), and silk sericin powder extracted from the local silk cocoon (Bombyx mori, MW=25-150 kDa). The biocomposite pasta was prepared as follows: firstly, mixing of powder of PMMA (10g), HA (1g) and sericin (0.0032g); secondly the mixed powder was blended with liquid of MMA (9ml) at room temperature with P/L ratios of PMMA/MMA of 2/1.8, 2/1.85 and 2/1.90. All process of mixing was done manually till the color of mixed was white-like. The pasta was then taken to fill the attached syringe at a 3D-Bioprinter as seen in **Fig. 1**. The syringe has inner diameter of 28mm, height of 100mm and nozzle diameter of 2mm.

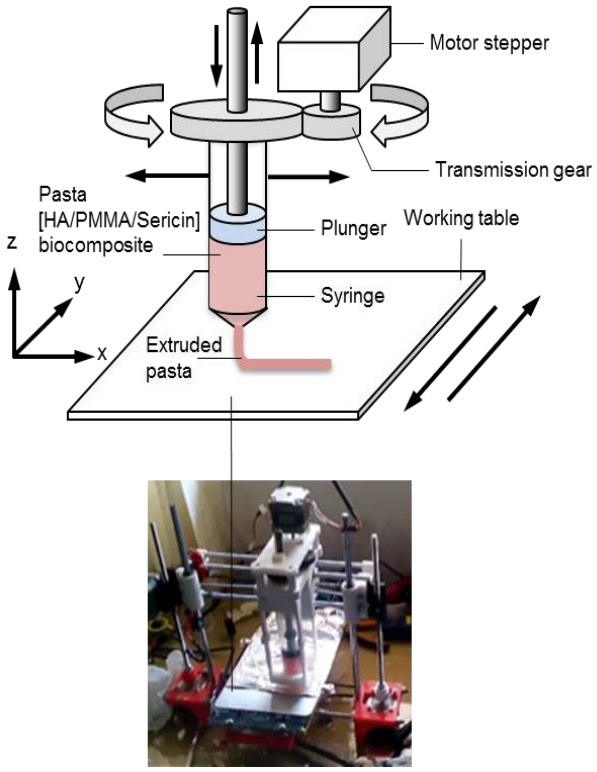

Fig 1. Printing process of sample at the 3D-Bioprinter.

The 3D-Bioprinter with optimum printing process parameters set up was run to print test samples. The printing process on the 3D-Bioprinter was carried out with three processing factors including infill speed (Vi, mm/s), perimeter speed (Vp, mm/s) and layer height (h) and scenario of printing for the first, second layer and so on shown in **Fig. 2.** Tests were performed into 2 steps. The first step was focused on performance of the 3D-Bioprinter including investigating time window for printing base on material states, pasta flow rate (mm^3/s) and optimum setting of printing parameter using Response Surface Method (RSM). The second step was measurement of sample tensile strength as shown in **Fig 3** (3.4mm thick) produced by 3D-Bioprinter based on printing parameter (Vp, Vi and h) in **Table 3,** observation of microstructure and identify existence of hydroxyapatite in the composite. All samples were dried up in oven at 95 ^oC for 2 hours [**19**] before testing at Universal Testing Machine and scanning electron microscope. Three printing parameters as factors and levels (low and high) for optimum set up processing parameter were shown in **Table 1**.

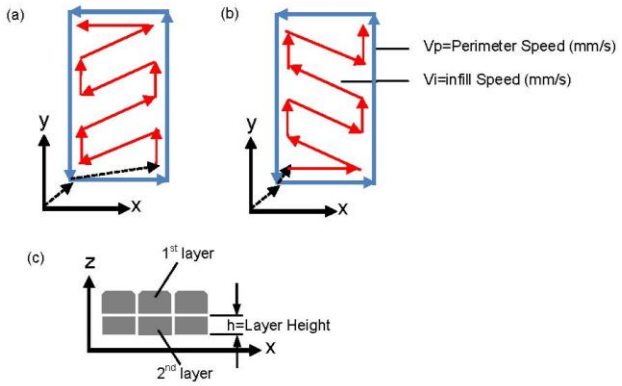

Fig 2. Printing process parameters: infill speed (Vi), perimetric speed (Vp) and layer height (h): (a) First layer, (b) Second layer and (c) Cross-section of sample that consists of 2 layers (1st and 2nd layer).

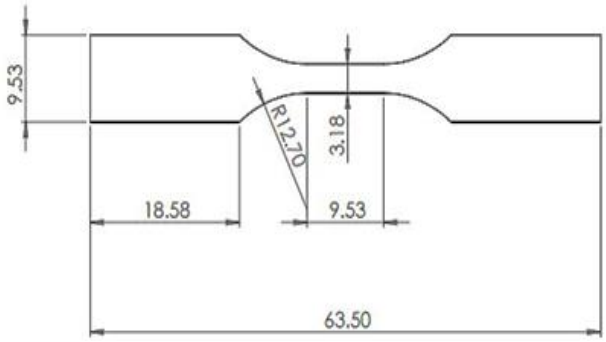

Fig 3. Sample of tensile strength (unit in mm).

Table 1. Printing process parameter of 3D-Bioprinter: factors and levels.

Factors	Levels		
	-1	**0**	**+1**
Infill speed, Vi (mm/s)	80	100	120
Perimeter speed, Vp (mm/s)	60	80	100
Layer height, h (mm)	0.3	0.4	0.5

Optimization of printing process parameters

Process of optimization was performed by Response Surface Method with two design of experiments. The first was 2^k (k= 3) with additional of 4 center points and the second was 2^k with additional of 4 center points, 6 center points and 6 axial points. Therefore, there would be 12 test run for the first order and 20 test run for the second order.

The highest tensile strength of the printed sample was predicted according to the optimum printing parameter.

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This tensile strength was predicted to follow regression equation-1 where β is constant:

$$Y_{TS} = \beta_0 + \beta_1 V_p + \beta_2 V_i + \beta_3 h + \beta_4 V_p V_i + \beta_5 V_p h + \beta_6 V_i h + \beta_7 V_p V_i h + Error \quad (1)$$

Table 2. First Order: 2k factorial with 4 center points (Test run, Coded Variable, Actual Variable and Parameter Code).

Run Test	Coded Variable			Actual Variable			Parameter Code
	x1	x2	x3	Vp (mm/s)	Vi (mm/s)	h (mm)	
1	-1	-1	-1	60	80	0.3	PSISLH608003
2	+1	-1	-1	100	80	0.3	PSISLH1008003
3	-1	+1	-1	60	120	0.3	PSISLH6012003
4	+1	+1	-1	100	120	0.3	PSISLH10012003
5	-1	-1	+1	60	80	0.5	PSISLH1008005
6	+1	-1	+1	100	80	0.5	PSISLH1008005
7	-1	+1	+1	60	120	0.5	PSISLH6012005
8	+1	+1	+1	100	120	0.5	PSISLH10012005
9	0	0	0	80	100	0.4	PSISLH8010004
10	0	0	0	80	100	0.4	PSISLH8010004
11	0	0	0	80	100	0.4	PSISLH8010004
12	0	0	0	80	100	0.4	PSISLH8010004

Table 3. Second Order: Central point (Test run, Coded Variable, Actual Variable and Parameter Code)

Run Test	Coded Variable			Actual Variable			Parameter Code
	x1	x2	x3	Vp (mm/s)	Vi (mm/s)	h (mm)	
1	-1	-1	-1	60	80	0.3	PSISLH608003
2	+1	-1	-1	100	80	0.3	PSISLH1008003
3	-1	+1	-1	60	120	0.3	PSISLH6012003
4	+1	+1	-1	100	120	0.3	PSISLH10012003
5	-1	-1	+1	60	80	0.5	PSISLH1008005
6	+1	-1	+1	100	80	0.5	PSISLH1008005
7	-1	+1	+1	60	120	0.5	PSISLH6012005
8	+1	+1	+1	100	120	0.5	PSISLH10012005
9	0	0	0	80	100	0.4	PSISLH8010004
10	0	0	0	80	100	0.4	PSISLH8010004
11	0	0	0	80	100	0.4	PSISLH8010004
12	0	0	0	80	100	0.4	PSISLH8010004
13	1.682	0	0	46,36	100	0.4	PSISLH434610004
14	1.682	0	0	113,6	100	0.4	PSISLH1136410004
15	0	-1.682	0	80	66,36	0.4	PSISLH80663604
16	0	1.682	0	80	133,64	0.4	PSISLH801336404
17	0	0	-1.682	80	100	0.23	PSISLH80100023
18	0	0	1.682	80	100	0.57	PSISLH80100057
19	0	0	0	80	100	0.4	PSISLH8010004
20	0	0	0	80	100	0,4	PSISLH8010004

Results and discussion

A printable biocomposite material has been developed using composition of hydroxyapatite (HA), PMMA and Sericin. Varying composition of P/L ratios (PMMA) including 2/1.8, 2/1.85 and 2/1.90 have been applied as a matrix. While 3 various HA powder of 10, 15 and 25%w/w of PMMA have been contented in the biocomposite in order to gain a better biocompatibility and bioactivity. In this study, sericin of 0.32% w/w of HA has also been added for improvement of cell attachment as indicated by the previous study. Two series test have been carried out to investigate the printability of the biocomposite that has been developed on the basis of the performance of the 3D-Bioprinter (curing time window based on material state, the flow rate of biocomposite pasta through nozzle and optimum printing parameter), and other tests to find property of the printed samples (UTM/tensile strength, SEM/microstructure and XRD/the existence of HA). Curing time window of the biocomposite for various HA contents which is in this study 10, 15 and 25 %w/w and P/L ratios of PMMA can be seen in the **Figs 4, 5** and **6.** There were time stages starting from mixing of the composite to form pasta (pasta stage), dough state and solid stage. Here, curing time window was defined starting from pasta stage when the pasta of the biocomposite was in the syringe at the 3D-Bioprinter till dough stage. Printing process was carried out during pasta stage until dough stage or until pasta in the syringe was run out. In this study, the volume of pasta in the syringe was determined following the end of the dough state.

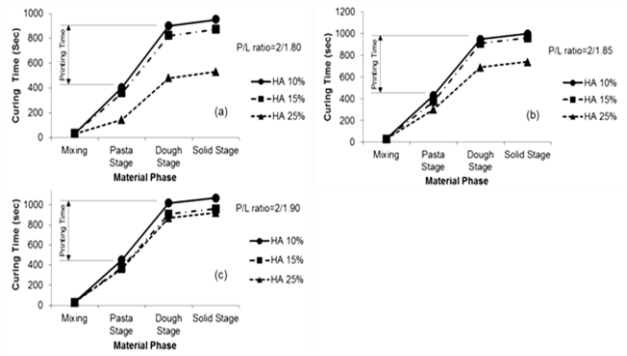

Fig. 4. Time window for biocomposite with various HA contents 10, 15 and 25 %w/w and P/L ratios of PMMA: (a) P/L=2/1.80 (b) P/L=2/1.85 (c) P/L=2/1.90.

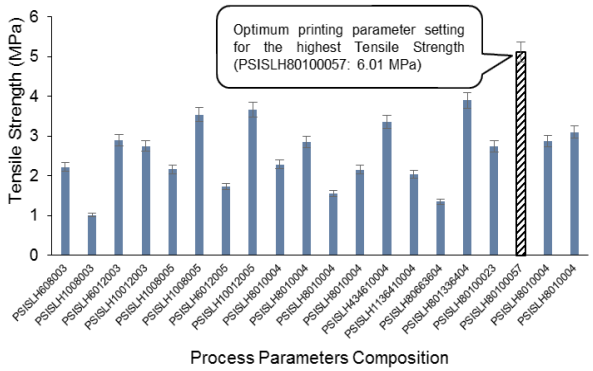

Fig. 5. Tensile strength of biomaterial for various process parameters (perimeter speed, infill speed and layer height.

As seen in those Figures, by increasing HA content in the biocomposite with various P/L ratios, printing time was similar but time to achieve dough state decreases. In another word, time for preparation of pasta was shorter compared to that other pasta with HA content lower. Since hydroxyapatite in the biocomposite of [HA/PMMA/Sericin] was ceramic particle (size of 150-200 μm), this ceramic would determine the ease of flow of the biocomposite through the nozzle. Increasing HA particles also made the pasta difficult to flow through the nozzle. Concerning the flowability of the biocomposite through the nozzle, to decide which the best biocomposite with composition P/L ratios of PMMA and HA contents, it needs another consideration such as its tensile strength shown in **Fig. 5**. The best flow rate of pasta through the nozzle was 78.5mm^3/s that achieved by the biocomposite with P/L ratio of PMMA 2/1.8 and HA content of 10% w/w. Beyond the flow rate, printable properties was also indicated by optimum printing parameters. There were three printing parameters that need to be optimized using Response Surface Method (RSM), including infill speed (Vi, mm/s), perimeter speed (Vp, mm/s) and layer height (h, mm). The samples test were printed following the optimum printing parameter set up of the 3D-Bioprinter to obtain the highest tensile strength. Applying Response Surface Method, the predicted of the highest tensile strength (Y_{TS}) of the biocomposite was 6.01 MPa that obtained for setting of processing parameters factors of Vi=130.92mm/s, Vp=113.64mm/s and h=0.57mm as indicated by equation-2. Meanwhile, based on the experiment of tensile test, with the same parameters factors and levels, the highest tensile strength was only 5.12 MPa (**Fig. 5,** material code of PSISLH80100057). It was about 14.8% lower than that of model prediction using Response Surface Method. The lower strength of the experiment result occurs may be due to the existence of porosity within the samples made by 3D-Bioprinter as indicated by the microstructure image obtained by scanning electron microscope depicted in **Fig. 6.**

$$Y_{TS}=8.5-0.149\ V_p+0.089\ V_i-32.6\ h-0.000124\ V_p^2-0.000193\ V_i^2+38.4\ h^2+0.000508\ V_pV_i+0.292\ V_ph-0.170\ V_i.h \quad (2)$$

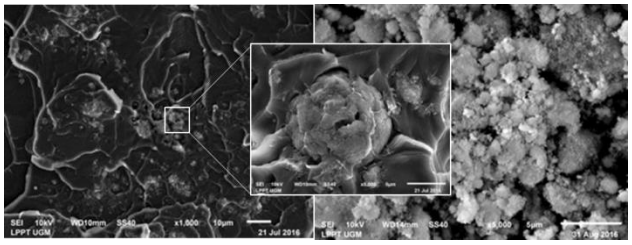

Fig. 6. Microstructure of the biomaterial with 10%w/w of HA.

Conclusions

A printable biocomposite pasta made of [HA/PMMA/ Sericin] has been successful developed. Test samples have been prepared by using 3D-Bioprinter with three printing parameters (3 factors: perimeter speed, infill speed and layer height and each factor have 2 levels). Three various HA contents (10, 15, 25%w/w) and P/L ratios of PMMA (2/1.80, 2/1.85 and 2/1.90) have been tested to investigate the best composition that fit for printing process. In this 10% w/w HA was selected and it would be applied for making composition pasta. Following the Response Surface Method (RSM), the predicted of the highest tensile strength of the printed sample was 6.01 MPa which was achieved by optimum setting of processing parameter (infill speed=130.92mm/s, perimeter speed=113.64mm/s and layer height=0.57mm) for predicted equation-2. This strength was 14.8% higher than that obtained by experiment. This might be due to the existence of porosity that confirmed by SEM results. Allowing the measurement data of the experiment and predicting tensile strength of the dried sample with RSM, the flow rate of pasta with 10%w/w HA and P/L ratio of 2/1.85 PMMA through the nozzle was 75.8 mm^3/s. Successfully of the present work would open for the possibility of fabricating human organ (hard tissue) using 3D-Bioprinter.

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References

1. Tontowi, A.E.; Setiawan, A.; *AIP Conference Proceedings*. **2016**, *1746*, 020035. **DOI:** 10.1063/1.4953960
2. Nugraha, F.K.A; Tontowi, A.E.; *AIP Conference Proceedings*, **2016**, *1746*, 020037. **DOI:** 10.1063/1.4953962
3. Tontowi, A.E.; Perkasa, D.P.; Mahulauw, A.; Erizal; *Conference NanoCON*, **2014**, Pune.
4. Tontowi, A. E.; dan Setiawan, A.; *The 2016 Conference on Fundamental and Applied Science for Advanced Technology*, **2016**, 1.
5. Tontowi, A.E.; Shafiqy, D. I.; Triyono, J.; *International Journal of Applied Engineering Research*, **2015**, *10*.
6. Tontowi, A.E.; Kuswanto, J.; Sihaloho, R.I; Sosiaty, H.; *Proceeding of ICST2015*; **2015**.
7. Mondal, S.; Mondal, B.; Dey, A.; Mukhopadhyay, S.S.; *Journal of Minerals and Materials Characterization & Engineering*, **2012**, *11*, 55.
8. Hosseinzadeh, H. R. S.; Emami, M.; Lahiji, F.; Shahi, A.S.; Masoudi, A.; Emami, S. The Acrylic Bone Cement in Arthroplasty, *INTECH* Chapter 5, **2013**, pp.101.
9. Walsh, W. R.; Svehla, M. J.; Russell, J.; Saito, M.; Nakashimac, T.; Gilliesa, R. M.; Brucea, W.; Hori, R.; *Biomaterials*, **2004**, *25*, 4929.
10. Shinzato, S.; Kobayashi, M.; Mousa, W. F.; Kamimura, M.; Neo, M.; Kitamura, Y.; Kokubo, T.; Nakamura, T.; *Journal of Biomedical Materials Research*, **2000**, *51*, 258.
11. Itokawaa, H.; Hiraideb, T.; Moriyaa, M.; Fujimotoa, M.; Nagashimaa, G.; Suzukia, R.; Fujimoto, T.; *Biomaterials*, **2007**, *28*, 4922.
12. Zebarjad, S. M.; Sajaadi, S. A.; Sdrabadi, T. E.; Yaghmaei, A.; Naderi, B.; *Journal of Engineering*, **2011**, *3*, 795.
13. Rangi, A.; Jajpura, L.; *Journal Textile Science and Engineering*, **2015**, *5*, 1.
14. Cooper, K.G.; Rapid Prototyping Technology Selection and Application; Marcel Dekker Inc. New York; **2001**; 1.
15. Mason, M. S.; Huang, T.; Landers, R. G.; Leu, M. C.; Hilmas, G.E.; Hayes, M.W.; Aqueous-Based Extrusion Fabrication of

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Ceramics on Demand, Proceedings of Solid Freeform Fabrication Symposium, Austin TX, 2007, pp. 124.
16. Chandramohan, D.; Marimuthu, K.; *International Journal of Advanced Engineering Technology*, **2011**, *2*, 435.
17. Montgomery, D.C.; Runger, G.C.; Applied Statistics and Probability for Engineers; 3rd Ed., John Wiley & Sons Inc., New York, **2003**.
18. Montgomery, D. C.; Design and Analysis of Experiments; 4th Ed., John Wiley & Sons, New York; **1997**.
19. Nejatian, T.; Sefat, F.; Johson, T.; *Journal of Materials*, **2015**, *8*, 2093-2109.

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