

Research Article

Antioxidant Properties of SLA Printed Hydrogels Enriched with Pomegranate Powder

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(First received April 03, 2022 and in final form June 06, 2022)

Reference: Çikrikci Erünsal, S., & Aydoğdu Emir, A. Antioxidant Properties of SLA Printed Hydrogels Enriched with Pomegranate Powder. *The European Journal of Research and Development*, 2(2), 45–52.

Abstract

3D printing (3DP) is a powerful platform for fabrication of complex or personalized geometries. Hydrogels as attractive candidates for wound dressing, soft implant, encapsulation of phenolic compounds, drug delivery, etc. could be manufactured by using different 3D printing techniques. In this study, encapsulation of pomegranate powder into PEGDMA gel by Stereolithography (SLA) printing was investigated and then antioxidant activity and total phenolic content of printed gels with varying shapes were examined to see the effect of different geometry and process conditions. According to the results, pomegranate based blend was successfully incorporated into gel matrix and printed with high printability ratio at optimized 3D printing conditions. UV induced gelation did not prevent existence of phenolic compounds and allowed retention of antioxidant potential in printed samples. Obtained hydrogels represent promising biomaterials with great potential for the use in different applications such as antioxidant gel for tissue engineering, wound healing or as a nutraceutical carrier.

Keywords: SLA print, pomegranate, phenolic compound, hydrogel, antioxidant activity

1. Introduction

Globally, 3D printing (3DP) has started to take a crucial place in different areas from food to tissue engineering applications. Since it is difficult to fabricate complex structures with conventional methods, 3D printing opens a huge way to create personalized shapes depending on demand. Hydrogels having three dimensional network and absorbing a large amount of water have a wide range of applications. For instance, multifunctional hydrogels could be used in wound healing process due to its antioxidant, antimicrobial

and antibacterial activity or wound moistening effect [1]. Similarly, they could function as a soft implant with potential drug composition. With the help of 3DP, they could be designed with a personalized shape.

One of the 3DP is SLA which is based on photopolymerization reaction. Solid form is achieved by solidification of polymer resin upon exposure to UV light [2]. 3DP follows layer by layer process after optimization of process conditions such as layer thickness, UV exposure time, etc. There are so many studies about SLA printed hydrogels such as carboxymethyl cellulose/ ϵ -polylysine hydrogel [1], implant design for ear drug delivery [2], oral polypill dosage forms [3]. However, their polymer compositions and target agents are very different than this study.

Recently, pomegranate has attracted so much attention due to its nutritional value and antioxidant content taking a title as “super food” [4]. It has been grown in various areas of the Middle East, European Mediterranean region, northern Africa and southeast Asia are areas that it is widely grown [5]. Anthocyanins (cyanidin, delphinidin and pelargonidin) and other phenolic compounds (including hydrolyzable tannins such as punicalin, gallic and ellagic acid) are found in its composition. Its antimicrobial potential, its several biological activities such as anti-inflammatory, antioxidant, antihepatotoxic and antigenotoxic activities as well as its antimicrobial potential could make it very good natural alternative to enhance soft implant properties, wound healing or to treat other kind of health related problems [6].

Herein, our previously obtained SLA resin [7] was used for pomegranate powder (PP) encapsulation for biomedical use such as soft implant design with antioxidant property or antioxidant wound dressing. By this way, functional hydrogel design and its function after printing process was evaluated to check the impact of process conditions.

2. Materials and Methods

Poly(ethylene glycol) dimethacrylate (PEGDMA, average MW 550), triethanolamine (TEA) and riboflavin (RF) were purchased from Sigma Aldrich Ltd (St. Louis, Missouri, USA). Pomegranate powder (PP) was obtained from Dohler Group (Istanbul, Turkey). Sodium carbonate, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl, methanol and ethanol were purchased from Sigma-Aldrich.

2.1. Fabrication Procedure

3 units PEGDMA as monomer, 1 unit distilled water with 5% PP, 0.0004 unit RF as photoinitiator and 0.12 unit TEA as co-initiator were combined and stirred until obtaining

a homogenous solution. Polymer resin without PP was also prepared as a control sample. Polymer solution was put into resin vat of SLA printer (Anycubic Photon Mono SE, Hong Kong) and exposed to UV light at 40-Watt 405 nm wavelength LEDs. After preliminary experiments, printing parameters were optimized as follows: layer thickness: 0.1 mm, UV exposure time for first 3 bottom layers: 60 s, exposure time for remaining layers: 90 s. Geometries given in Table 1 was firstly drawn on SOLIDWORKS (2016) and then converted into .STL file. They were uploaded into printer by USB drive and solid gels were obtained approximately within 1 and half hour. Then, the printed models were compared with theoretical models in terms of total cell area. Images of the printed constructs were captured using a high quality phone camera with a ruler beside the construct [8]. The images were firstly set a scale and then measured and compared with theoretical data. Then, printability was expressed as their ratios.

Table 1 3D Hydrogel geometries

Sample	Outer Length (mm)	Thickness (mm)	SA/V ratio
Ring	20	5	0.80
Hexagonal	20	5	0.85
Square	20	5	1.09

2.2. Antioxidant Activity

Antioxidant activity (AA) was measured by 2,2-diphenyl- 1-picrylhydrazyl (DPPH) method described in the study of [9]. Basically, gels were dissolved in ethanol/water solution (80:20). 0.1 ml was taken from that solution and then mixed with 3.9 ml of 25-ppm DPPH solution and finally stored at dark room for 1 h. The absorbance values (A_{gel}) was measured at 517 nm by UV spectrophotometer (UV 2450, Shimadzu, Columbia, USA). For blank preparation, 0.1 ml of methanol ($A_{control}$) was combined and stirred with DPPH solution, and the same procedure was followed for the remaining part. Then, the AA (%) were calculated as:

$$AA (\%) = \frac{(A_{control} - A_{gel})}{A_{control}} \times 100 \quad (1)$$

2.3.Total Phenolic Content

Total phenolic content (TPC) of 3D gels were analyzed by Folin–Ciocalteu method as given in the study of [9]. For this experiment, gels were dissolved in ethanol–water solution (80:20). Diluted sample of 0,5 mL was mixed with 0.2 N Folin-Ciocalteu reagent. After it was stored in dark place for 5 min, 2 mL of 75 g/L sodium carbonate solution was added. Prepared solutions were kept in dark for 1 h. Then, the absorption of solutions was measured at 760 nm by using UV spectrophotometer. Gallic acid was used to prepare calibration curve and then total phenolic content (TPC) of solutions were expressed as gallic acid equivalents (GAE) in milligrams per gram dry weight.

$$TPC (mg\ GAE/g\ gel) = (C \times V \times D)/W_g$$

where C is the concentration (mg GAE/L), V is the volume of solution in L, D is the dilution rate and W_g is the weight of gels (g). The measurements were performed for at least duplicates.

2.4 Statistical Analysis

A one-way analysis of variance (ANOVA) and Tukey's test were conducted to compare the results statistically at the 0.05 significance level using MINITAB (Version 16, State College, PA, USA).

3. Results

A quantitative analysis of the printed shapes is helpful in order to examine the printability of formulated resin with respect to different geometry [10]. The overlap problem could be serious in such kind of techniques. As seen from Fig.1, only some small regions were accumulated and expanded especially in square gels. Ring shape was slightly vulnerable due to thin line thickness.

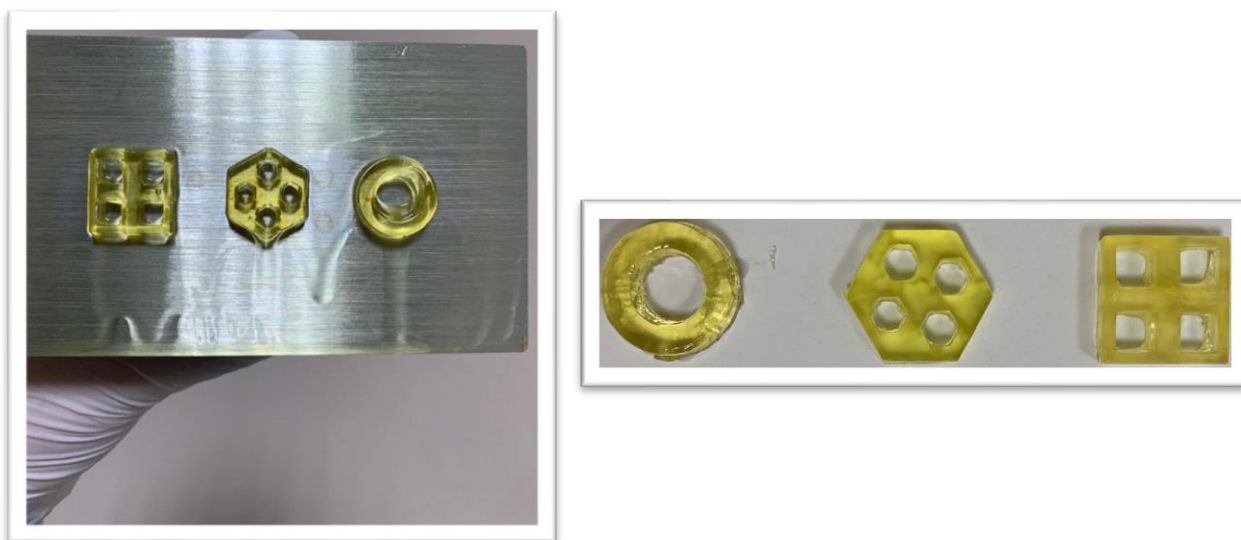


Figure 1 Representative obtained 3D gel images

Obtained 3D tablets with the shape of hexagonal, ring and square (in the reducing order for SA/V ratio, respectively) were displayed in Fig. 1. The highest AA (%) value was found in square geometry, then in hexagonal and finally the lowest percentage in ring shape ($p < 0.05$). TPC values did not significantly differ among the samples except for square structure giving the lowest data ($p < 0.05$). In order to prove the source of this phenolic content, control sample with the absence of PP was also printed as a round shape. AA (%) and TPC values were obtained as $27.52 \pm 6.57\%$ and $77.22 \pm 1.22 \text{ mg GAE/g gel}$ in control gel, respectively. This is the effect of riboflavin found in the main formulation. If control sample was considered, none of them differed as expected ($p < 0.05$).

Table 2 Printability of gels

Sample	Theoretical total cell area(cm ²)	Actual total cell area (cm ²)	Printability
Ring	0.79	0.70 ± 0.03	0.89 ± 0.02
Hexagonal	0.94	1.09 ± 0.03	1.17 ± 0.02
Square	1.44	1.22 ± 0.07	0.85 ± 0.02

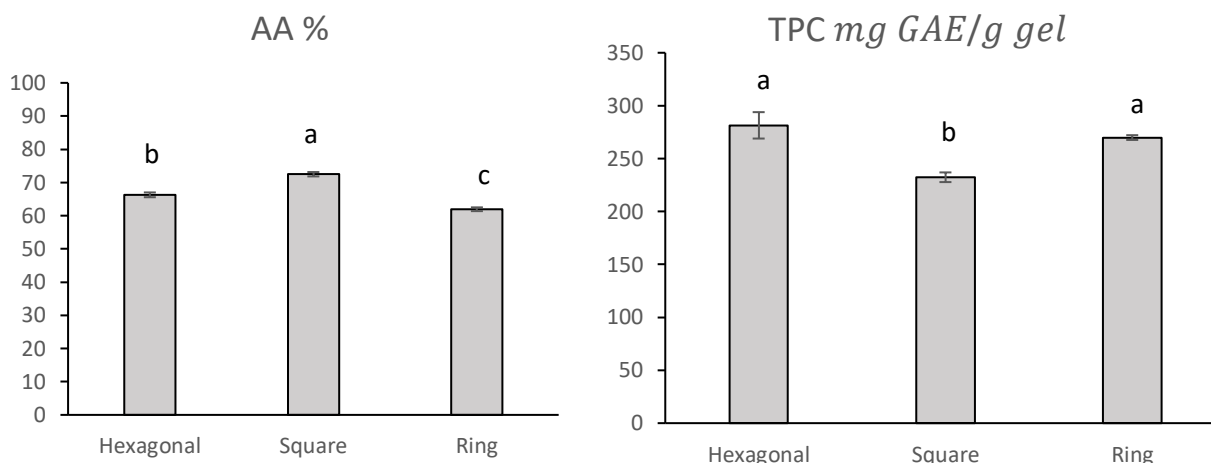


Figure 2 Antioxidant activity (%) and Total Phenolic Content results of PP loaded hydrogels

4. Discussion and Conclusion

When printability values of empty (cell) areas were analyzed, it was seen that they were acceptable ratios when compared to intended area. Printability lower than 1 was due to overlaps after printing whereas printability higher than 1 was mostly owing to some cracks after process.

After successful printing, they were examined regarding to antioxidant properties. Natural antioxidants from plant sources play a crucial role in protecting against the action of free radicals and they are correlated with lower incidence of cardiovascular diseases, diabetes and cancer diseases [11]. The hydroxyl groups in plant extracts facilitate free radical scavenging. Phenolic and flavonoid molecules deactivates free radicals donating hydrogen atoms to free radicals [11].

Since riboflavin could also influence phenolic content of the gels, control sample without PP addition was also evaluated. As given in the result section, control gave half and even lower portion of PP loaded gel results. This proved that PP created a crucial effect on AA and TPC even at low ratios. For PP loaded tablets, antioxidant activity varying between 60 and 75% and phenolic content results of 3D tablets can be clearly ascribed to the presence of phenolic components coming from PP in the formulations. This is in agreement with previous studies [12]. Natural phenolic compounds could be negatively affected from temperature, light, etc. [9]. Despite of UV induced process, samples still gave good antioxidant property indicating efficiency of SLA printing. Although it seems there is a reverse relationship between AA (%) and TPC values, their unit and calculation differences should not be forgotten.

On the other hand, geometry did not cause a huge significant difference in total phenolic content [9]. A poor correlation of antioxidants with other responses such as TPC and total flavonoids content was observed in the study of [6] due to different process applications. Since printed gels had slightly different weights due to the shape, this could affect the amount of PP within gel matrix including the lowest PP in square shape. Additionally, square was the sample with low cross section area. UV irradiation could be limited from the top and other part as a lateral area remained in the dark. This might have affected the observations. Similar results were obtained in other process applications, as well [13]. TPC content analysis on the other hand also confirmed the preservation of phenolics after UV induced 3D process. The presence of quercetin, catechin, gallic acid, cyanidin 3-glucoside, ellagic acid etc. could succeed those advantageous findings for 3D design.

In conclusion, PP loaded PEGDMA gels were successfully printed by SLA technique. The free radical scavenging activity of printed samples using DPPH were obtained at higher percentages giving positive trend with SA/V ratio. This formulation could be proposed for both food industry, tissue engineering and other related fields requiring antioxidant potential and phenolic content composition.

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