Phenolic Compound, Antioxidant and Antibacterial properties of Electrospun PVP Nanofiber loaded with Bassela rubra linn extract and Alginate from Sargassum sp.

Eka Lutfi Septiani¹*, Okky Putri Prastuti¹, Siti Machmudah², Sugeng Winardi², Wahyudiono³, Hideki Kanda³ and Motonobu Goto⁴

¹Department of Chemical Engineering, Universitas Internasional Semen Indonesia, Gresik 61122 Indonesia
²Department of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Surabaya 60111 Indonesia
³Department of Material process Engineering, Nagoya University, Nagoya 464-8603 Japan

*Corresponding Author’s E-mail: eka.septiani@uisi.ac.id

Abstract. An alternative wound dressing based on nanofiber mats have been developed recently. The antioxidant and antibacterial activity play an important role in wound healing process. This study aims to combine the properties of Bassela rubra linn extract (BRLE) and Alginate from brown macroalgae into Polivynil Pyrrolidone (PVP) nanofiber using electrospinning method. Firstly, the optimization step was conducted to obtain the best operation voltage and distance. Afterwards, the nanofiber composites of PVP/BRLE/Alginate were injected into electrospinning tool in various Alginate concentrations of 1%, 2%, and 3% (w/w) respectively. The morphology of PVP nanofiber observed by Scanning Electron Microscopy (SEM) conveys that the closest distance and the highest voltage were the best. Meanwhile, the in vitro analysis through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and antibacterial activity to S. aureus and E. choli bacteria show a quite strong radical scavenging ability around 60% and adequate antibacterial property as good as reference a well-known material consecutively.

Keywords: Nanofiber, Electrospinning, Total Phenolic Compound, Antioxidant, Antibacterial.

1. Introduction

A serious wound generally appears in the skin because of surgery, accident, and disease such as diabetes. This wound may alter to a chronic wound once the healing mechanism experiences a failure in which the wound cannot heal completely in 12 weeks [1]. Regarding to this issue, wound dressing become an importance part in a proper healing process by maintaining moist environment at the wound area. As a result, the closed wound would contribute re-epithelialization, collagen synthesis, and angiogenesis faster than the open ones [2]. A cotton based wound dressing is widely used since a long time ago due to its advantages in cost and absorbing exudate from the wound. However, it is not only give a small supporting in bacterial prevention and oxygen exchange, but also it should be removed oftentimes which lead to a more painful feeling, sometimes even cause re-injury [3]. Therefore, a cheaper and more adequate wound dressing development is required.
Nanofiber through electrospinning method is one of promising wound dressing materials with many benefits, particularly, in its simply, versatile, cheap cost production and its properties [4]. In general, polymer nanofiber is used as drugs carrier while it is applied as a wound dressing. Several research reveal superiority of polymer nanofiber combined with antiinflammation compound from plants extract. A good enhancement in healing process was represented by *Lawsonia inermis*/gelatin/starch by means of in vivo test [5]. Garcinia mangostana [6], Green tea extract [7], the other plant extracts in nanofiber mats may speed up healing process [8]. Meanwhile, *Basella rubra linn* (as known as Malabar spinach, Gendola, red Binahong) was confirmed has an anti-inflammatory compound [9] that is believed to accelerate an injury recovery. Polyninyl Pirrolydone/ *Basella rubra linn* extract (PVP/BRLE) was evaluated in this previous research through in vitro analysis [10]. Nevertheless, its antioxidant property is relatively low, below 50%. Hence, recent research needs to propose Alginate as an added material to raise the antioxidant activity which is related to healing process acceleration potency. Lavender-Alginate nanofiber indicates alleviation of inflammatory response to wound burn on animal body [11]. The electrospun carboxymethylcellulose/sodium alginate/CaCl2/polyethylene oxide polymeric solution also can provide significant percentage of oxidant inhibition compared to the mats without alginate [12]. A comparison study between extract spirulina-Polycaprolactone (Spi-PCL) and extract spirulina-Alginate-Polycaprolactone (Spi-Alg-PCL) was conducted by Choi et. al. (2017). The result reveals that the alginate presence promotes eminent wound recovery in a rat model, especially in 5 days observation [13]. Accordingly, this research is purposing a nanofiber composite of PVP/BRLE/Alginate in order to understand its potency as a wound dressing via free radical scavenging activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and antibacterial tests towards *Staphylococcus aureus* and *Escherichia coli*. This study carried several operation parameters to gain prominent nanofiber size.

2. Experimental Section

2.1. Material

Dried *Bassela rubra linn* (Binahong) leaves with Premium Herb brand was acquired from Madiun City, East Java, Indonesia. The leaves, then, were leached by Soxhlet extractor to get its phenolic and alkaloid compounds. Further, the *Bassela rubra linn* extract (BRLE) solution was concentrated using vacuum evaporation process. Fresh *Sargassum sp.* (brown macroalgae) was purchased from local market in Lampung City, Sumatera island, Indonesia. Alginate compound from the macroalgae was obtained from extraction process by hydrothermal auto-clave reactor with the ratio of the macroalgae mass to water 1:20 w/v [14]. 1,300,000 g/mol MW PolyvynilPyrrolidone (PVP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were produced by Aldrich Chemistry. Methanol, ethanol, and sodim carbonate were manufactured by Wako Pure Chemical Corporation. Folin-Ciocalteu’s agent was supplied from Sima-alorich. Deionized water was used in the experiment. Commercial Povidone-iodine (Betadine) was bought from a local market in gresik City, East Java, Indonesia.

2.2. Experimetal setup and method

At the first, the 8% (w/w) of PVP in the ethanol solvent was synthesized by electrospinning method as can be found somewhere [10] with the tip-to-collector distances of 10 cm and 12 cm. Various voltage of 8, 10, 12 kV by a power supply (Matsusada) were applied to obtain the best process parameter. Furthermore, the BRLE and alginate solution were mixed into 8% (w/w) PVP solution to get a precursor solution that was changed into nanofiber composite of PVP/BRLE/Alginate using optimized parameters. The composition of BRLE was fixed in the smallest percentage of previous study [10], 2% (w/w). Meanwhile, the percentage of Alginate solution was adjusted in the variation of 1, 2, and 3% (w/w) of the total solution. The different PVP/BRLE/Alginate solutions were injected from syringe into electrospinning chamber by mean of tiny capillary pipe to produce the nanofiber mats. To set the rate at 1 mL/hour, a syringe pump (HARVARD APARATUS) was employed. As the solution move out from the tip of capillary spinneret, the solution was spinning to generate nanofiber sheet.
Scanning Electron Microscopy (SEM, Hitachi S-4300, Japan) analysis with carbon coating was conducted to determine the best distance and voltage of the electrospinning process of PVP through the nanofiber morphology. Moreover, in vitro analysis was conducted by Total Phenolic Compound, DPPH, and antibacterial assay. The total content of phenolic compounds (TPC) of the PVP/BRLE/Alginate nanofiber composite was analyzed by Folin-Ciocalteu method in 1 mL of the sample solutions [15], generated from 50 ppm of PVP/BRLE/Alginate nanofiber mat, were mixed with Folin-Ciocalteu solution and left for 3 minutes. Afterwards, 1 ml of 7.5% (w/w) sodium carbonate and 7 ml pure water were added into the the solution. The total phenolic compound of the sample solutions was measured by UV-VIS Spectrophotometer (V-550, Jasco, Japan) using wavelength of 750 nm after 2 hours incubation. Besides that, the radical scavenging ability was specified by using DPPH assay. The absorbance of the mixture from 50 ppm DPPH solution and 50 ppm sample solution in the ratio of 1:1 (v/v) was measured as A sample while the absorbance of 25 ppm DPPH solution was measured as A control, thus, the antioxidant activity can be calculated by equation (1) [16]

\[
\text{%AA} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]  

(1)

The antibacterial activity of the PVP/BRLE/Alginate nanofiber composite was conducted using streak plate method. An agar solution was poured into a plate which is signed with four quadrants. Then, 6-mm in diameter of nanofiber was placed onto a streak of the strain drawn on each quadrant of the agar plate using metal loop, respectively. The inhibit zone, appear once the bacteria become susceptible, were observed in this study [17]. Povidone-iodine was used as reference in this assay.

3. Results

3.1. Optimization process

The optimum parameter process is determined by 8% PVP nanofiber from SEM image as displayed as Figure 2. The morphology from 9000 magnificent of the pictures shows a nanofiber form with different range of diameter distribution in which determined by ImageJ software analysis. As can be seen, the longer distance shows the larger nanofiber mean diameter at all the voltages used. However, the general behavior is the increasing distance should cause the smaller the average diameter of nanofiber due to the orientation and the elasticity that preserved by the polymer jets [18]. This deviation may be affected by the weakening of the electric field at a certain distance [19] so that the force becomes unbalance and the jet becomes unstable [20]. On the other hand, the applied voltages in the electrospinning process also provide a prominent effect to the nanofiber mean diameter. According to the Figure 2, it conveys that the highest voltage produces the smallest mean diameter and standard deviation of 372 ± 132 nm and 407 ± 161 nm for the tip-to-collector distance of 10 cm and 12 cm successively. In term of 10 cm distance, however, the average diameter of nanofiber elevates from 428 ± 121 nm at 8 kV to 463 ± 169 nm at 10 kV before decrease at 12 kV. The lowest voltage maintains the polymer jet elongation due to oscillation until reaching the collector whereas the middle voltage (12 kV) encourages a rapid spinning movement in the process [21]. Hence, the polymer solution under 10 kV has longer drying time and stretching range than those of the solution under 12 kV [22]. Furthermore, the increase of voltage results a multiple jet that causes similar nanofiber diameter even though the spinning process runs promptly [23]. Regarding another distance, the higher the employed voltage, the smaller nanofiber average diameter. It can be led by sufficient stretching of the solution during electrospinning process [24]. In the wound dressing application, the smaller and the more homogeneous mean diameter of nanofiber are important because these characteristics can enhance the proliferation process during wound healing mechanism [25] and hasten the adhesive process [26]. Thus, the best parameter condition for the experiment is applying the tip-to-collector distance at 10 cm and the voltage of 12 kV.
3.2 In vitro analysis

As a result of the optimizing the operation condition, the electrospinning process of PVP/BRLE/Alginate nanofiber was conducted at the tip-to-collector distance at 10 cm as well as the voltage of 12 kV since the little amount addition of the organic extract may affect inconsiderably [7]. According to the Table 1, the evaluated phenolic compound of the nanofiber composite of PVP/BRLE/Alginate shows a fluctuating result while the antioxidant activity result describes an escalating tendency as the increase of Alginate concentration. The data reveals that both total phenolic content and antioxidant activity cannot be parallelized. The radical scavenging ability levels up by the addition of Alginate concentration. Nevertheless, the highest total phenolic compound is not indicating the highest antioxidant activity as well. It may be caused by prooxidant activity from the brown macro algae [27] which allows the maximum percentage of antioxidant activity at 60.7 ± 0.06 with the increase of phenolic compound.

| Sample         | Total Phenolic Content (mg GAE/mL precursor) | Antioxidant activity (%) |
|----------------|---------------------------------------------|--------------------------|
| 8/2/1 PVP/BRLE/Alginate | 32 ± 0.74                                    | 58.6 ± 0.31               |
| 8/2/2 PVP/BRLE/Alginate | 34 ± 1.19                                    | 60.7 ± 1.34               |
| 8/2/3 PVP/BRLE/Alginate | 32 ± 0.37                                    | 60.7 ± 0.06               |

The antibacterial test against S. aureus as shown as the Figure 3 conveys that the raising of the Alginate percentage from 1% to 2% in the nanofiber composite can broaden the inhibit zone, yet the more addition cannot extend the inhibit zone. This data may figure out the relevance with the antioxidant activity of the nanofiber mats.

Table 2 represents the antibacterial zone from the reference material and the PVP/BRLE/Alginate nanofiber composite from S. aureus and E. coli. All materials show better antibacterial activity to resist E. coli (gram-negative) growth than S. aureus ones (gram-positive). It occur because the gram-negative bacteria have a thin layer of cell wall no more than 10 nm although they have three layers of wall whilst the gram-positive bacteria have a thick layer, peptidoglycan, between 20 and 80 nm [28].
Based on the antibacterial susceptibility, the nanofiber composite of PVP/BRLE/Alginate exhibits an adequate property as good as a commercial medicine even better, especially in S. aureus prevention.

Figure 2. Morphology of PVP nanofiber (a),(c),(e) 8 kV, 10 kV, 12 kV at the distance of 10 cm. (b),(d),(f) 8 kV, 10 kV, 12 kV at the distance of 12 cm.

Figure 3. Resistance zone of PVP/BRLE/Alginate towards S. aureus in various Alginate composition (a) 1%, (b) 2%, (c) 3% w/w.
Table 2. Inhibit zone of reference material and PVP/BRLE/Alginate nanofiber.

| Sample                  | Inhibit zone (mm) |
|-------------------------|-------------------|
|                         | E. coli | S. aureus |
| Commercial Povidone-iodine | 6       | 2         |
| 8/2/1 PVP/BRLE/Alginate  | 5       | 3         |
| 8/2/2 PVP/BRLE/Alginate  | 5       | 4         |
| 8/2/3 PVP/BRLE/Alginate  | 6       | 4         |

4. Conclusion

Synthesis of the PVP/BRLE/Alginate nanofiber composite was conducted at the superlative operation condition, achieved from different distances and voltages, tip-to-collector distance at 10 cm and the voltage of 12 kV. The variation of Alginate proportion in the composites was evaluated through in vitro analysis, Total Phenolic Compound, antioxidant ability, antibacterial susceptibility. The results show that the phenolic compound and antioxidant properties are not analogous. However, the antioxidant activity can be related to the antibacterial activity which reveal that the higher antioxidant activity in percentage, the wider resistance zone. The highest antioxidant activity and the largest area of bacterial resistance are 60.7 ± 0.06% and 6 mm in turn.

Reference

[1] Sheehan P, Jones P, Caselli A, Giurini J M, Veves A 2003 Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial Diabetes care 26(6) 1879-1882
[2] Dhivya S, Padma V V, Santhini E 2015 Wound dressings–a review BioMedicine 5(4) 24–28
[3] Ovington L G 2001 There ’ s More to Dressings Than Gauze Wet-to-Dry and Wet-to-Moist — What ’ s Gauze Dressings : Patient Issues 19 (8) 477–484
[4] Agarwal S, Wendorff J H, Greiner A 2008 Use of electrospinning technique for biomedical applications Polymer (Guildf) 49 (26) 5603–5621
[5] Hadisi Z, Nourmohammadi J, Nassiri S M 2018 The antibacterial and anti-inflammatory investigation of Lawsonia Inermis gelatin-starch nano-fibrous dressing in burn wound International journal of biological macromolecules 107 2008-2019
[6] Charernsriwilaiwat N, Rojanarata T, Ngawhirunpat T, Sukma M, Opanasopit P 2013 Electrospun chitosan-based nanofiber mats loaded with Garcinia mangostana extracts International journal of pharmaceutics 452 (1-2) 333-343
[7] Pusporini P, Edikresnha D, Sryianti I, Sucitati T, Munir M M, Khairurrijal K 2018 Electrospun polyvinylpyrrolidone (PVP)/green tea extract composite nanofiber mats and their antioxidant activities Materials Research Express 5(5) 054001
[8] Zhang W, Ronca S, Mele E. 2017 Electrospun nanofibres containing antimicrobial plant extracts Nanomaterials 7(2) 42
[9] Singh M, Kumari R, Nandini D, Kotecha M 2016 Preliminary phytochemical screening of Basella rubra Linn Journal of Pharmacognosy and Phytochemistry 5(4) 224
[10] Septiani E L, Gabriel A A, Prastuti O P, Indalsari D N, Arijanti E D, Machmudah S, Goto M 2020 Correlation of Extract Composition on Antioxidant Activity of ELECTROSPUN PolyvinylPyrrolidone/Bassela rubra linn Leaf Extract Composite In Key Engineering Materials 851 122-127 Trans Tech Publications Ltd.
[11] Summa M, Russo D, Armiriotii A, Brunetti V 2016 Materials Chemistry B
[12] Persin Z, Ravber M, Stana Kleinscpek K, Knez Ž, Škerget M, Kurečič M 2017 Bio-nanofibrous mats as potential delivering systems of natural substances Textile research journal 87(4)
[13] Choi J I, Kim M S, Chung G Y, Shin H S 2017 Spirulina extract-impregnated alginate-PCL nanofiber wound dressing for skin regeneration Biotechnology and Bioprocess Engineering 22 (6) 679-685

[14] Rajauria G, Jaiswal A K, Abu-Ghannam N, Gupta S 2010 Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds International journal of food science & technology 45(12) 2485-2493

[15] Chhouk K, Uemori C, Kanda H, Goto M 2017 Extraction of phenolic compounds and antioxidant activity from garlic husk using carbon dioxide expanded ethanol Chemical Engineering and Processing: Process Intensification 117 113-119

[16] Suwantong O, Pankongadisak P, Deachathai S, Supaphol P 2013 The potential of electrospun poly (L-lactic acid) fiber mats containing a crude Garcinia dulcis extract for use as wound dressings Chiang Mai J Sci 40 (3) 517-33

[17] Sanders E R 2012 Aseptic Laboratory Techniques : Plating Methods 2. Streak Plate Procedure : Isolation of Bacterial Colonies Using the Quadrant Method May 1–18

[18] Doshi J and Reneker D H 1995 Electrospinning process and applications of electrospun fibers Journal of electrostatics 35 (2-3) 151-160

[19] Baumgarten P K 1971 Electrostatic spinning of acrylic microfibers Journal of colloid and interface science 36 (1) 71-79

[20] Ding W, Wei S, Zhu J, Chen X, Rutman D, Guo Z 2010 Manipulated electrospun PVA nanofibers with inexpensive salts Macromolecular Materials and Engineering 295 (10) 958-965

[21] Mazoochi T, Hamadanian M, Ahmadi M, Jabbari V 2012 Investigation on the morphological characteristics of nanofibrous membrane as electrospun in the different processing parameters International Journal of Industrial Chemistry 3(1) 1-8

[22] Reneker D H and Yarin A L 2008 Electrospinning jets and polymer nanofibers Polymer 49(10) 2387-2425

[23] Liu Z, Ju K, Wang Z, Li W, Ke H, He J 2019 Electrospun jets number and nanofiber morphology effected by voltage value: numerical simulation and experimental verification Nanoscale research letters 14 (1) 1-9

[24] Sharifi L, Assa F, Ajamein H, Mirhosseini S H 2017 Effect of Voltage and Distance on Synthesis of Boehmite Nanofibers with PVP by the Electrospinning Method 98 63–74

[25] Hodgkinson T, Yuan X F, Bayat A 2014 Electrospun silk fibroin fiber diameter influences in vitro dermal fibroblast behavior and promotes healing of ex vivo wound models Journal of tissue engineering 2016

[26] Chen S, Liu B, Carlson M A, Gombart A F, Reilly D A, Xie J 2017 Recent advances in electrospun nanofibers for wound healing Nanomedicine 12 (11) 1335-1352

[27] Kılıçgün H and Altuner D 2010 Correlation between antioxidant effect mechanisms and polyphenol content of Rosa canina Pharmacognosy magazine 6 (23) 238

[28] Mai-Prochnow A, Clauson M, Hong J, Murphy A B 2016 Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma Scientific reports 6 (1) 1-11