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DESIGN OF CELLULOSE ACETATE-COLLAGEN NANOFIBER AND ITS IN VITRO ASSESSMENT AS WOUND DRESSING CANDIDATE

M. F. DZIKRI^{a,*}, T. P. ARMEDYA^a, S. Q. KHAIRUNISA^b, S. C. W. SAKTI^a, Y. RAHARJO^a, W. PURNAMASARI^c, N NASRONUDIN^b, . M. Z. FAHMI^{a,*}

^aDepartment of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya 61115, Indonesia

^b Institute of Tropical Disease, Airlangga University, Surabaya 61115, Indonesia ^cDepartment of Biomedical Engineering, Faculty of Science and Technology, Universitas Airlangga, Surabaya 61115, Indonesia

In this study, we developed nanofiber composite membrane of cellulose acetate and collagen via electrospinning. Several variations on electrospinning process such as time, flow rate and collector optimizations have been done. The result showed that the optimum conditions were reached with flow rate at 0.05 μ L/h using a drum or cylinder-shaped collector and optimum time for membrane formation for 3 h. The results of the CA-collagen membrane performed good results with the modulus young 1,237 x10⁻⁵ GPa. The entire membrane has elongation according to human skin so it can be used as a candidate for wound dressing. The MTT assay reveals that all membrane was non-toxic with viability all over 80%.

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1. Introduction

Skin is an external organ of the human body that has a very important role in protecting the inside of the body against the environment that potentially harmful. Skin serves as the first line of defence against the entry of chemicals and microorganisms as well as providing a barrier to prevent fluid loss and regulate body temperature. The appearance of skin lesions will trigger the healing process. Wounds are defined as damage or disturbance of epithelia continuity of the skin or mucosa due to physical or thermal contact. Based on the time in the healing process naturally, it can be categorized into acute and chronic wounds [1]. If the healing process is continuous and normal within the range of 8-12 weeks, then it includes the acute wound category. However, delay on healing processes due to local or systemic factors that occur within months or years include chronic wound categories [2]. Although injuries are categorized as acute or chronic, the basic function of the dressings used in the treatments remains the same, to provide a barrier or protector to prevent bacterial contamination and to absorb exudates [3].

Among the polymers used as wound dressings, cellulose acetate (CA) and polycaprolactone (PCL) perform advantages. PCL is used in the mixture for wound dressing applications because it provides mechanical strength to the dressing, and has been used as a drug carrier in drug delivery system. PCL has good biocompatibility and biodegradation properties so it is potential to be applied in biomedical field [4]. However, PCL also has a very hydrophobic property [5]. Application of electrospinning techniques has succeeded in preparation of nanofiber from CA and has potential for application in the biomedical field. The CA is a biopolymer commonly used for medication purpose due to its high hydrophilicity, good fluid transport and

^{*}Corresponding author: m.zakki.fahmi@fst.unair.ac.id

water absorption capability. The CA, an acetate esters of cellulose, has been used extensively in the preparation nanofiber by electrospinning that the CA has also some favorable properties, such as good biocompatibility, biodegradation, regenerative properties, high affinity toward other substances, and tensile strength [6]. In addition, CA also has excellent biocompatibility properties with the human body environment [7]. In contrast to PCL, CA have more hydrophilicity properties [8]. The properties of hydrophobicity and hydrophilicity need to be known to examine the wettability of biomaterials as biomedical applications such as wound healing. This is because they will be in contact with blood, water, and other body fluids during their use. The nanofiber membrane with good hydrophilicity and high porosity facilitate wound healing, especially in the early healing phase [9-12].

One of the most clinically effective ingredients used for wound healing and skin regeneration is collagen which is the main protein of the extracellular matrix [10]. The main form of collagen-based wound dressings include: films, gels, sponges and fibers [11]. Collagen provides structural integrity and tensile strength to tissues. Tissue damage after injury requires collagen to repair and restore its structure and function. Collagen has several advantages such as biocompatibility, biodegradability, and low antigenicity [13]. There is no research has been done with the addition of collagen in CA nanofiber composites. The addition of collagen should be considered due to its important role on healing process.

Electrospinning is an interesting technique for synthesizing nanofiber from various biodegradable polymers because of the simplicity of technique and the ability to effectively control the process. The main components of electrospinning consist of high voltage power supply, syringe and syringe pump, and metal collector [14]. Electrospinning is an effective technique because of its flexibility to obtain nanofiber from a wide selection of polymers, the ability to control nanofiber diameters, morphology and fibrous structures, easy modification by adding various solutes or nanomaterials to solutions for electrospinning, possibly obtaining nanofiber with bio-component configurations, and porous [15]. This study tries to develop cellulose acetate-collagen nanofiber membrane (CCM) with electrospinning method in the hope that membrane will enhance its biocompatibility characteristic. Treatment with chemical and physical crosslinking agents was also investigated. The resulted membrane was then characterized and tested in order to obtain valuable information of its biomedical application.

2. Experimental

Materials

Material used in this experiment are cellulose acetate 15 wt% (CA, Sigma Aldrich, Mw : 30 kDa), acetone (Merck), citric acid (Merck). Collagen was obtained from BATAN Jakarta. Analytical grade formic acid were purchased from Merck. Huh7 cell was obtained from Institute of Tropical Disease Airlangga University. Sodium hydroxide, Dulbecco's Modified Eagle Medium (DMEM) cell culture medium, Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), MTT (3-(4,5- dimethyltiazole-2- yl)-2,5- diphenyltetrazrazine bromide), and dimethyl sulfoxide (DMSO) were obtained from Sigma Aldrich.

Electrospinning preparation of cellulose Acetate-collagen membrane

Cellulose acetate solution in acetone (15 wt%) and collagen in formic acid (0.05 wt%) then were blended using a magnetic stirrer to form a homogeneous solution. Then, 0.05 g of citric acid was added into the solution. The physical crosslink is carried out by heating at a temperature of 80 ° C on the already formed nanofiber membrane. Electrospinning equipped with a flat/cylinder collector was operated in a high voltage power supply (12 kV). The cellulose acetate-collagen solution was fed into the syringe with a certain flow rate (0.1, 0.3, 0.5, and 0.7 μ L/h).

Meanwhile the optimization of running time was done with variations of time 1, 3, 5, and 7 h.

Characterization of cellulose acetate-collagen membrane (CCM)

Cellulose acetate-collagen membrane with 1x1 cm² in size was observed its surface structure by using scanning electron microscope (SEM, Zeiss EVO MA-10). Gold layer was conducted prior to observation. Mechanical properties of nanofibers were analyzed by using universal testing machine, Shimadzu Autograph AG-X (Shimadzu, Japan). Mechanical properties test was performed in order to determine mechanical strength of CCM against the force given from the outside. This tensile test data was used for determining stress, strain, and nanofiber membrane moduli.

Cytotoxicity properties analysis

Toxicity test of CCM was conducted by MTT [3 - (4,5 - dimethylthiazol - 2yl) - 5 (3 - carboxymethoxyphenyl) - 2 - (4 - sulfophenyl) - 2H tetrazolium] assay (Sigma - Aldrich). Prior to in vitro assay with Huh7 cells, CCM was sterilized under UV light. Huh7 cell was seeding at wells with a density of 5.4 x 104, incubate for 24 h at 37°C, 5% in CO₂ incubator. The CCM sample was put at each well with 0.5x0.5 cm² in size and add 200µL medium and incubated for 48 h. A 300µl medium containing MTT (DMEM 270 µL + MTT 30 µL) and incubated for other 4 h was added. The precipitate formed by the MTT result was dissolved by the addition of 200 ml of DMSO. Absorbance measured at 560 nm and 750 nm wavelengths using GloMax-Multi Microplate Multimode Reader (Promega). Measurement results are compared with controls. MTT Assay controls are Huh7 cells which had been seeded in culture medium at wells without the addition of CCM sample. The absorbance data obtained was used to determine the percentage of living cells (cell viability). If the percentage less than 60% it indicates CCM is toxic and can kill living cells.

The MTT reagent used is a tetrazolium salt, which can be broken down into formazan crystals by the succinate tetrazolium reductase system present in the respiration pathway of the active mitochondria in living cells. The intensity of the purple colour that is formed is proportional to the number of living cells.

3. Results

Parameter Optimization of Electrospinning

The experimental result showed that 0.05 μ l/h is the most optimum flow rate for formation of CCM membrane as shown in Fig. 1. Taylor Cone, which formed at this flow rate has perfectly conical shape and produces continuous fibers. The dope solution attracted and attached on the surface of collector.



Fig. 1. The shape of taylor cone with flow rate (a) 0.01 μl / h, (b) 0.05 μl / h, (c) 0.1 μl / h, (d) 0.15 μl / h, (E) 0.20 μl / h.

This study also show that longer electrospinning process produce thicker nanofibers (Fig. 2). Meanwhile, variation of running time in the electrospinning process did not give any significant difference in the diameter of the formed nanofiber.



Fig. 2. Variation of running time (a) 1 h, (b) 3 h, (c) 5 h, and (d) 7 h.

The optimization of collector shows that the use of rotating cylinder collectors resulted membrane with uniform thickness on the entire surface. Optimization using flat collector forms uneven and thicker membranes in the center of plate Fig. 3.



Fig. 3. Collector variation results (a) rotating cylinder and (b) flat.

Characterization of Nanofiber

SEM results of the nanofiber was performed on Fig. 4 resulted that CCM with electrospinning technique has been formed with the fiber diameter of 200-250 nm. Fig. 4a is CA nanofiber membrane with cylinder collector (CMC) is constructed with a randomly oriented fiber which are not tied each other and inhomogeneous diameter, meanwhile 4b and 4c representing CA-collagen nanofiber membrane with a cylinder (CCC) and flat collector (CCF). In the other side, Fig. 4d and 4e, show CA-collagen nanofiber membrane after heating at 80°C with cylinder collector (CCD). As the Fig. 5 exhibit cross section formation from the addition of citric acid. This cross section indicates the bond between cellulose acetate and citric acid through chemical bonding. SEM EDX analysis as show in Fig. 6 exhibit atomic elements and ratio between cross section compared nun-cross section indicates high O levels.



Fig. 4. The SEM test results of nanofiber membrane (a) CA, (b) CA-collagen cylinder collector, (c) CA-collagen flat collector, (d) CA-collagen with crosslink citric acid, and (e) CA-collagen with heating.



Fig. 5. Cross section formed by the addition of citric acid crosslink agent.



Fig. 6. SEM EDX results (a) section of cross section and (b) non cross-section.



Fig. 7. Stress value on various nanofiber membranes.



Fig. 8. Elongation measurement results (%) on various nanofiber membranes.

Further mechanical properties study reveals that CMC has the lowest stress value compare with other membranes due to unlinked fiber structure. Meanwhile the CCD has a stress value of 6.129×10^{-4} MPa, which has a lower value than the membrane with a heating treatment or without treatment (Fig. 7). Fig. 8 shows that the percent value of elongate from the nanofiber membrane: CMC, CCC, CCF, CCH, CCD are 17.08%, 11.56%, 12.13%, 10.48%, 15.83% respectively.

Cytotoxicity assay

CA-collagen membrane with heating gives lower value than CA membrane that is 84,97%. The low degree of viability (%) gives meaning that the membrane with the addition of citric acid are more toxic compared with the other membranes Fig. 9.



Fig. 9. Cell viability (%) of various membranes.

4. Discussion

Initially, the polymer solution forms a half-spherical surface as a result of surface tension. High voltage is applied between the spinnerets and the metal collector will convert the half-spherical surface of the polymer solution into a cone, called taylor cone [16]. At a flow rate of 0.01 μ l / h, the resulting taylor cone dried quickly before the fibers reached the collector. The applied voltage of 12 kV with slower flow rate cause the polymer dope solution dried quickly. More stable taylor cone was formed at higher flow rate (0.05 μ l / h). The dope solution attracted and attached on the surface of collector. The higher flow rate causes the balance between the released polymer solutions to the replacement of the polymer solution from within the syringe during jet formation become uncontrolled. The taylor cone to form elongated and the force of gravity to make its shape downward. The flow rate affects the formation of the nanofiber diameter; the minimum flow rate is preferable to maintain a balance between the released polymer solution and the replacement of the solution with the new one during jet formation [17]. Unstable formed taylor cone can be found at flow rate of 0.1; 0.15; 0.2 μ l /h as shown in Fig. 1. The experimental result showed that 0.05 μ l/h is the best flow rate for formation of CCM membrane.

Formed membrane with operating time 1 h, cannot be peeled from the aluminum foil due to its thin layer. The variation of running time in the electrospinning process did not give any

significant difference in the diameter of the formed nanofiber [18]. The result of running time variation can be seen in Fig. 2.

It has been found that the use of rotating cylinder collectors resulted membrane with uniform thickness on the entire surface as shown in Fig. 3(a). Uniform thickness only can be found at the center of aluminum foil when flat collector is used (Fig. 3(b)). Fiber with smaller diameter was observed at membrane obtained from cylinder collector. Fiber spread to all direction during rotation of collector, which resulted film with homogenous thickness and smaller diameter. For static collectors, the electrostatic forces give the effect of stretching the fibers transversely to form a fiber density perpendicular between one fiber and the other [19].

As presented in Fig. 4, it can be seen that the CCM with electrospinning technique has been formed with the fiber diameter of 200-250 nm. The CA nanofiber membrane with cylinder collector (CMC) is constructed with a randomly oriented fiber which are not tied each other and inhomogeneous diameter (Fig. 4a). Unlike the case with Fig. (b) and (c) sequentially representing CA-collagen nanofiber membrane with a cylinder (CCC) and flat collector (CCF). The CMC have a relatively short distance between fibers and the fibers diameter distribution is inhomogeneous. On the other hand, between Fig. 4 (b) and (c) gives unequal morphological structures due to the use of different collector. Membrane obtained from cylinder collector has more homogenous on its diameter and comparison in that from flat collector. As seen in Fig. 4c, flat collector produce membrane, which have defect and inhomogeneous fiber diameter. Fig. 4d represents the morphological structure of the CA-collagen nanofiber membrane after heating at 80°C with cylinder collector (CCH) for 2 h. Heating step disrupt the balance of H_2O content in the membrane and lead to dehydration. Exposure to high temperatures results membrane with compressed structure due to denaturation of collagen [20-21].

Morphological structure of CA-collagen with addition of citric acid with cylinder collector (CCD) can be seen in Fig. 4e. Nanofiber is connected each other with citric acid as crosslink agents. The cross section image formed from the addition of citric acid can be seen in Fig. 5. Citric acid connects one cellulose acetate with others via chemical bonding. Both of citric acid and cellulose acetate contain C and O atoms. In the cross section the content of O atom is greater than in the non-cross section. It is possible that in addition to the cellulose acetate itself the presence of the O atom is from citric acid, which also has much O on its structure. The number of O atoms in the cross section compared with the non-cross section (Fig. 6a) is 2.19. While the ratio of percentage C and O in the non-cross section (Fig. 6b) is 5.61. It shows that the small value of the ratio between atom C and O in the cross section indicates high O levels due to chemical bonds between citric acid with cellulose acetate.

It's seen in Fig. 7, the CMC has the lowest stress value due to unlinked fiber structure. The highest stress value is obtained from CCF, but the membrane has an inhomogeneous thickness. CA-collagen membrane collected from cylinder collector has a high stress value and the membrane has a homogenous thickness. Heating process has damaged the collagen structure, which decreases the mechanical properties of the membrane. CCD has a stress value of 6.129×10^{-4} MPa, which has a lower value than the membrane with a heating treatment or without treatment.

Fig. 8 shows that the percent value of elongate from the nanofiber membrane: CMC, CCC, CCF, CCH, CCD are 17.08%, 11.56%, 12.13%, 10.48%, 15.83% respectively. The experiment data are in agreement with Maganaris and Paul (1999) that the mechanical properties of human skin have a percent elongation between 1-25%, so then obtained membrane can be applied as wound dressing. It's found that the modulus young value of CA-Collagen (flat collector) > CA-collagen (cylinder collector) > CA-collagen (heating treatment) > CA-collagen-citric acid > CA. The ratio between stress and strain will give the value of young modulus which is in the range of hooke law is still valid [22].

From the results of the MTT cytotoxicity test, the CA nanofiber membrane provides the lowest cell toxicity value indicated by the high percentage cell viability obtained. Viability (%) is a value that indicates the presence of living cells. In this study, the viability value was determined from the absorbance of the treatment group compared with the control group. CA-collagen membrane with heating gives lower value than CA membrane that is 84,97%, while membrane nanofiber CA-collagen with addition of citric acid show the degree of viability (%) lowest equal to

71,28%. The low degree of viability (%) gives meaning that the membrane with the addition of citric acid are more toxic compared with the other membranes. The viability (%) result can be seen in Fig. 9.

5. Conclusions

Electrospinning is an effective technique in preparation of nanofiber membrane. The CCM is optimally made at a flow rate of 0.05 μ l / h with a drum or cylinder collector for 3 h running. The characterization results show morphologically the diameter of nanofiber fiber measuring 200-250 nm. The tensile test gives results that the CA-collagen membrane with the cylinder collector gives a high yield. While the 80 ° C heating treatment did not give better results because the collagen has been denatured. Citric acid crosslinked the cellulose acetate via chemical bonding that can be observed through SEM instruments. The percent value of elongate from the nanofiber is in agreement with the mechanical properties of human skin, so then obtained membrane can be applied as wound dressing. The amount of modulus young values obtained from the largest was the CCF, CCC, CCD, CCH, and CMC. The MTT toxicity test shows that the entire membrane is not toxic with percentage viability of CMC, CCH, CCD, are 89.96%, 84.97%, and 71.28%, respectively.

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Dig. J. Nanomater. Bios. - International Editorial Board

Page 3 of 4

\$ -

Article Index

Dig. J. Nanomater. Bios. (/index.php/journals/digest-journal-of-nanomaterials-and-biostructures)

Local Editorial Board (/index.php/journals/digest-journal-of-nanomaterials-and-biostructures?start=1)

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Back to Top



(/)

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Dig. J. Nanomater. Bios. - Local Editorial Board

Page 2 of 4

\$ -

Article Index

Dig. J. Nanomater. Bios. (/index.php/journals/digest-journal-of-nanomaterials-and-biostructures)

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Digest Journal of Nanomaterials and Biostructures 8

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The proforma invoice is available one month starting with it's issue date. Your paper will be visible on the main site only after the publication tax will be paid, otherwise after one month the proforma invoice will be cancelled and your paper will be automatically rejected.

May I remind you that as specified on the proforma invoice, the BANK CHARGES FOR WIRE TRANSFER ARE SUPPORTED BY THE CUSTOMER !

Our bank does not have any charges for incoming transfers, the charges are deducted by the senders bank and by an intermediary bank (if it is the case of such bank), you must ask at your local bank the fees for the transfer(your bank and intermediary bank). On the payment instructions form you have 3 options BEN, SHA, OUR on "Details of Charges" field, there you must selected OUR (OUR instruction means you will pay all the transfer charges. We will receive all your payment).

Debit/Credit Card transfer is also available through online electronic payment. Please request this option if it is more suitable for you compared to wire transfer. You can pay in any currency, the conversion from your currency to USD or EURO is made automatically by the system. The transfer charges for the online electronic payment are included in the final publication fee requested.

Best Regards, Virtual Company of Physics Technical Manager and Marketing Dr. I. D. Simandan Copyright Transfer Statement DJNB.pdf 105K

P 60 M. F. DZIKRI.pdf 167K

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: MIHAI POPESCU <pop_al.mihai@yahoo.com>

Dear Prof Popescu,

Thank you for the email. I prefer patennya via credit card, please inform me guideline for this. Thank you [Quoted text hidden] --Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Airlangga University Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: MIHAI POPESCU <pop_al.mihai@yahoo.com> Mon, Mar 11, 2019 at 10:54 AM

Mon, Mar 11, 2019 at 10:37 AM

Sorry form my unclear word on previous email. I just prefer to transfer via credit card and need your guideline for this way.

Moreover, is this any final proofing for my manuscript? Because I plan to change my co-author name. Thank you [Quoted text hidden]

Virtual Company of Physics <marketing@chalcogen.ro> To: Muhammad.fathan.dzikri-17@fst.unair.ac.id Cc: m.zakki.fahmi@fst.unair.ac.id Wed, Mar 13, 2019 at 1:55 PM

Dear author,

You will receive a email from PayPal service with the transfer request in the name of Virtual Company of Physics for the transfer of the publication tax regarding your accepted article for publication.

The transfer charges for the online electronic transfer will be included in the final fee requested (* 5.2% + 3 EURO is the PayPal transfer fee per transaction).

Please reply if you wish to transfer the publication fee by credit/debit card to send you the link for payment.

Virtual Company of Physics Technical Manager and Marketing Dr. I.D. Simandan

[Quoted text hidden]

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: Virtual Company of Physics <marketing@chalcogen.ro> Wed, Mar 13, 2019 at 2:10 PM

Dear

Yes I prefer credit card [Quoted text hidden] Dear author,

We have send you the electronic link for payment. The e-mail is from a PayPal address not from my e-mail address. Please also check your spam folder.

Virtual Company of Physics Technical Manager and Marketing Dr. I.D. Simandan

[Quoted text hidden]

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: Virtual Company of Physics <marketing@chalcogen.ro> Wed, Mar 13, 2019 at 8:54 PM

Thank you, I got and paid it [Quoted text hidden]



Copy proof of accepted paper for publication

7 messages

Virtual Company of Physics <marketing@chalcogen.ro> To: Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> Thu, Mar 14, 2019 at 4:48 PM

Dear author,

We have received your transfer concerning the publication fee, you can find attached the copy proof of the paper accepted for publication. Please send it back after the required modifications(if necessary),together with the copyright form. You also have attached the invoice regarding the transfer you have just made.

Best Regards, Virtual Company of Physics Technical Manager and Marketing Dr. I. D. Simandan

On 13-Mar-19 15:54, Mochamad Zakki Fahmi wrote:

Thank you, I got and paid it

On Wed, 13 Mar 2019 at 17.28 Virtual Company of Physics <marketing@chalcogen.ro> wrote:

Dear author,

We have send you the electronic link for payment. The e-mail is from a PayPal address not from my e-mail address. Please also check your spam folder.

Virtual Company of Physics Technical Manager and Marketing Dr. I.D. Simandan

On 13-Mar-19 09:10, Mochamad Zakki Fahmi wrote:

Dear

Yes I prefer credit card

On Wed, 13 Mar 2019 at 13.56 Virtual Company of Physics <marketing@chalcogen.ro> wrote: Dear author.

Dear autrior,

You will receive a email from PayPal service with the transfer request in the name of Virtual Company of Physics for the transfer of the publication tax regarding your accepted article for publication. The transfer charges for the online electronic transfer will be included in the final fee

requested (* 5.2% + 3 EURO is the PayPal transfer fee per transaction). Please reply if you wish to transfer the publication fee by credit/debit card to send you the link for payment.

Virtual Company of Physics

Technical Manager and Marketing Dr. I.D. Simandan

On 12-Mar-19 22:39, MIHAI POPESCU wrote:

----- Forwarded Message -----From: Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id>

To: MIHAI POPESCU <pop_al.mihai@yahoo.com> **Sent:** Monday, March 11, 2019, 5:54:44 AM GMT+2 **Subject:** Re: Fw: Proforma Invoice and Copyright statement for the accepted article for publication

Sorry form my unclear word on previous email. I just prefer to transfer via credit card and need your guideline for this way.

Moreover, is this any final proofing for my manuscript? Because I plan to change my co-author name. Thank you

On Mon, 11 Mar 2019 at 10.37 Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> wrote: Dear Prof Popescu,

Thank you for the email. I prefer patennya via credit card, please inform me guideline for this. Thank you

On Mon, 11 Mar 2019 at 02.04 MIHAI POPESCU <pop_al.mihai@yahoo.com> wrote:

----- Forwarded Message -----From: Virtual Company of Physics <marketing@chalcogen.ro> To: MIHAI POPESCU <pop_al.mihai@yahoo.com> Sent: Monday, March 4, 2019, 9:40:49 AM GMT+2 Subject: Fwd: Proforma Invoice and Copyright statement for the accepted article for publication

------ Forwarded Message ------Subject:Proforma Invoice and Copyright statement for the accepted article for publication Date:Thu, 28 Feb 2019 12:43:06 +0200 From:Virtual Company of Physics <marketing@chalcogen.ro> To:Muhammad.fathan.dzikri-17@fst.unair.ac.id

Dear author,

Your paper has been accepted for publication. You can find attached the copyright form which must be sent back filled in and the proforma invoice for the payment of the publication tax. Only wire transfer is accepted at one of the accounts specified in the Proforma Invoice to Beneficiary: "Virtual Company of Physics".

Please specify in sender* and description(details)** fields of the bank transfer form:

* customer name, affiliation and personal address;
** number and date of the received Proforma Invoice.
If you do not specify this details, we can not link the bank transfer to you hence the article will not be published and the transfer tax will be unidentified, blocked and returned to the sender's bank.

The proforma invoice is available one month starting with it's issue date. Your paper will be visible on the main site only after the publication tax will be paid, otherwise after one month the proforma invoice will be cancelled and your paper will be automatically rejected.

May I remind you that as specified on the proforma invoice, the BANK CHARGES FOR WIRE TRANSFER ARE SUPPORTED BY THE CUSTOMER !

Our bank does not have any charges for incoming transfers, the charges are deducted by the senders bank and by an intermediary bank (if it is the case of such bank), you must ask at your local bank the fees for the transfer(your bank and intermediary bank). On the payment instructions form you have 3 options BEN, SHA, OUR on "Details of Charges" field, there you must selected OUR (OUR instruction means you will pay all the transfer charges. We will receive all your payment).

Debit/Credit Card transfer is also available through online electronic payment. Please request this option if it is more suitable for you compared to wire transfer. You can pay in any currency, the conversion from your currency to USD or EURO is made automatically by the system. The transfer charges for the online electronic payment are included in the final publication fee requested.

Best Regards, Virtual Company of Physics Technical Manager and Marketing Dr. I. D. Simandan

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Airlangga University Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Airlangga University Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Airlangga University Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Airlangga University Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id

2 attachments



DJNB_DzikriMF.docx

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: Virtual Company of Physics <marketing@chalcogen.ro> Fri, Mar 15, 2019 at 3:04 PM

Please find the attached file for proofed manuscript and copyright transfer Statement. Thank You

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Universitas Airlangga Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id



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2 attachments

DJNB_DzikriMF.docx 1970K

IMG_20190315_0001.pdf 762K

Virtual Company of Physics <marketing@chalcogen.ro> To: Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id>

Dear author,

We have received you final form of the paper. The paper will be posted online in a couple of days.

Best regards, Virtual Company of Physics Technical Manager and Marketing Dr. I. D. Simandan Fri, Mar 15, 2019 at 4:57 PM

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: Virtual Company of Physics <marketing@chalcogen.ro>

Thank you Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Universitas Airlangga Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id



[Quoted text hidden]

Virtual Company of Physics <marketing@chalcogen.ro> To: Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id>

Dear author,.

What is the full name of author NASRONUDIN?

The name of the authors must be as specified next: The forename (first name) and second name (middle name) must be just a letter and only the last name (surname/family name) must be written in full, as in this example: FirstName MiddleName LastName = F. M. Lastname

Please send us the names of the authors as specified.

Regards.

[Quoted text hidden]

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: Virtual Company of Physics <marketing@chalcogen.ro>

The full Author I listed below:

1. MUHAMAD FATHAN DZIKRI = M.F. DZIKRI

- 2. TRI PRASETYO ARMEDYA = T.P. ARMEDYA
- 3. SITI QOMARIYAH KHAIRUNISA = S.Q. KHAIRUNNISA
- 4. SATYA CANDRA WIBAWA SAKTI = S.C.W. SAKTI
- 5. YANUARDI RAHARJO = Y. RAHARJO
- 6. WULAN PURNAMASARI = W. PURNAMASARI
- 7. NASRONUDIN NASRONUDIN = N. NASRONUDIN

Thank you

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Universitas Airlangga Phone : +62-838-32901697 Mon, Mar 18, 2019 at 9:30 AM

Mon, Mar 18, 2019 at 2:15 PM

Mon, Mar 18, 2019 at 4:47 PM



[Quoted text hidden]

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: Virtual Company of Physics <marketing@chalcogen.ro> Sat, Mar 23, 2019 at 8:35 AM

Dear Dr. Simandan,

There are a word mistake on title of my paper, namely, "Cellulosa" should be change with "Cellulose". Could you change it? Thank you [Quoted text hidden]



Re: Your publication in Digest Journal Of Nanomaterials And Biostructures

1 message

j.b.schofield@labstep.com <j.b.schofield@labstep.com> To: m.zakki.fahmi@fst.unair.ac.id Mon, Jun 10, 2019 at 7:16 PM

Hi there,

Congratulations on publishing your work "DESIGN OF CELLULOSE ACETATE-COLLAGEN NANOFIBER AND ITS IN VITRO ASSESSMENT AS WOUND DRESSING CANDIDATE" in Digest Journal Of Nanomaterials And Biostructures !

My name is Jake and I'm the cofounder and CEO of Labstep, a research data and inventory management platform for scientists. We are a team of researchers building tools to make your life in the lab easier. Based on the team's personal frustrations in the lab, Labstep offers features that streamline data documentation and knowledge transfer. Our mission is to increase the reproducibility and continuity of scientific studies.

I want to show you how Labstep can demonstrably save you time. It's free and always will be for academia. We only charge biotech companies.

Do you have 10 minutes for a short demo over videocall? Alternatively, we can send you a link for a recorded video demo.

Kind regards, Jake CEO & Co-founder Labstep.com



Manuscript Submission

4 messages

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: pop_al.mihai@yahoo.com Sat, Jan 26, 2019 at 9:05 AM

[January 25th, 2019]

Mihai Popescu Editor-In-Chief *Digest Journal of Nanomaterials and Biostructures*

Dear Prof. Popescu:

I would like to submit an Article, titled "DESIGN OF CELLULOSA ACETATE-COLLAGEN NANOFIBER AND ITS IN VITRO ASSESSMENT AS WOUND DRESSING CANDIDATE", for consideration for publication as full paper in *Digest Journal of Nanomaterials and Biostructures* (word count: 4.032; figures 9; references 21). The paper was co-authored by Muhammad Fathan Dzikri, Tri Prasetyo Armedya, Yanuardi Raharjo, Satya Candra Wibawa Sakti, and Puwanti

In this study, we prepared nanofiber composite membrane of cellulose acetate and collagen via electrospinning. Optimization of electrospinning process was reached by varying flow rate and design of collector. Further characterization of obtained the membrane was furnished with SEM-EDX and mechanical characterizations. By this observation we found that composite nanofiber with flat collector performed highest tensile strength value compared with modified (citric acid and heating treatment membrane). Cytotoxicity evaluation of the membrane reveals that all of membranes are non-toxic and suitable for clinical application.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership. This manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. All the authors have approved the manuscript and agree with submission to your esteemed journal. There are no conflicts of interest to be declared.

Thank you for your consideration. We hope our manuscript is suitable for publication in your journal.

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Universitas Airlangga Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id



2 attachments

cover_letter by M.Z. Fahmi.pdf 106K MIHAI POPESCU <pop_al.mihai@yahoo.com> Reply-To: MIHAI POPESCU <pop_al.mihai@yahoo.com> To: Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> Sun, Feb 3, 2019 at 4:23 PM

Dear author,

This is a confirmation that we have received your submitted article for publication in DJNB. After evaluation you will be informed if it is accepted for publication and you will receive a confirmation email together with a copyright statement and a proforma invoice for the payment of the publication tax. From receiving the paper it usually takes about three months for acknowledgement of acceptance for publication or not.

Thank you for submitting your paper to our journal, Editorial Team

Digest Journal of Nanomaterials and Biostructures

Chalcogenide Letters

Journal of Ovonic Research

Journal of Optoelectronic and Biomedical Materials

Journal of Non - Oxide Glasses

[Quoted text hidden]

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: MIHAI POPESCU <pop_al.mihai@yahoo.com>

Dear Prof. Popescu,

Is there any update for my submission? thank you. Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Universitas Airlangga Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id



[Quoted text hidden]

Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> To: ahmadi jaya permana <ahmadi-j-permana@fst.unair.ac.id> Thu, Feb 21, 2019 at 12:36 PM

Thu, Oct 17, 2019 at 10:33 AM

[Quoted text hidden]

Best Regards,

Mochamad Zakki Fahmi, Ph.D (張家其) Assistant Professor, Departement of Chemistry Airlangga University Phone : +62-838-32901697 Email : m.zakki.fahmi@fst.unair.ac.id



Auto Response: Manuscript Submission

1 message

MIHAI POPESCU <pop_al.mihai@yahoo.com> To: Mochamad Zakki Fahmi <m.zakki.fahmi@fst.unair.ac.id> Sat, Jan 26, 2019 at 9:05 AM

Dear author,

We have received your email. We will get back to you in a couple of days with further details and instructions.

Regards, Editorial Team