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ORIGINAL ARTICLE

Preparation of antibacterial electrospun Polylactic-co–glycolic acid nanofibers containing Hypericum Perforatum with bedsore healing property and evaluation of its drug release performance

Faegheh Pourhojat[1]; *Shahab Shariati*[2,*]; *Mahmoodreza Sohrabi*[1]; *Hamid Mahdavi*[3]; *Leila Asadpour*[4]

[1]*Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Chemistry, Rasht Branch, Islamic Azad University, Rasht, Iran*
[3]*Iran Polymer and Petrochemical Institute Polymer Engineering*
[4]*Department of Veterinary, Rasht Branch, Islamic Azad University, Rasht, Iran*

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Abstract

Skin drug delivery systems with controlled release are suitable means for the local transfer of pharmaceutical compounds to the damaged and healthy layers of skin. Nanofibrous membrane prepares uniform moisture in the wound environment with less accumulation of fluid secretion due to its variable pore size. Electrospinning takes advantage of using herbal extracts in the form of electrospun nanofibrous mats as an alternative for skin grafts. In this study, after preparing optimized fibrous mats of Poly lactic-co – glycolic acid (PLGA), characterization and identification of their chemical structure was carried out by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). The drug release of the optimized electrospun nanofibrous mats and the kinetics of drug release were investigated in vitro. Then, wound dressing performance, antibacterial property, cell adhesion and biocompatibility of nanofibrous scaffolds prepared by Methyl Thiazolyl Tetrazolium (MTT) assays as well as important features of suitable wound dressing including porosity, Water Vapor Transmission Rate, and swelling percentage of nanofibrous mats were evaluated in vitro. Nanofibrous mats containing *Hypericum perforatum* extract without any bead with burst drug release kinetics followed Higuchi kinetic model with proper regression coefficient ($PLGA\text{-}E_{10}$ = 0.8, $PLGA\text{-}E_{30}$ = 0.76). The results of the antibacterial activity of nanofibrous mats against Gram positive bacteria (*S. aureus*) as well as the results of the cell culture test and in vitro biodegradability tests on these mats showed good potential of composite scaffolds as antimicrobial coverage for wounds with Gram positive bacteria infectious agent.

Keywords: *Drug Release; Kinetic; Electrospinning; Hypericum Perforatum; Poly Lactic-co–glycolic Acid.*

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INTRODUCTION

Wound is the gap of cell prolongation and natural anatomic operation of skin. Wound dressing containing wound healing agents is used as therapeutic methods to speed up the repairing process of injured skin. Direct delivery of drugs is beneficial because lower amount of antibiotics need to be used compared to systemic administration, which usually involves usage of excessive doses to achieve substantial local effects [1]. Nanofibrous wound dressing due to unique properties like high surface to volume ratio, protection of wound against contamination and air penetration into the wound has shown good potential in wound dressing [2, 3]. There are many reports on application of effective pharmaceutical plants during different wound healing phases such as coagulation, inflammation, fibroblast, epithelization, condensation and wound closure in different literature [4, 5]. Poly lactic-co–glycolic

* Corresponding Author Email: *shariaty@iaurasht.ac.ir*

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acid (PLGA) as a synthetic polymer has a slower rate of biodegradation and a more reliable mechanical property compared to the natural polymers [6]. FDA approved-aliphatic polyesters such as poly lactic acid (PLA), polyglycolic acid (PGA), polycaprolactone *(*PCL) and their copolymers have being widely applied in skin tissue engineering and as a wound dressing [7]. PLGA is used for controlled drug delivery due to its high biocompatibility. Nanofibers of PLGA containing Cefazolin were electrospun successfully [8]. PLGA nanofibers containing Mefoxin showed higher burst release than PLGA/PLA-poly ethylene glycol [9]. PLGA, PLGA/PLA fibers decreased abdominal adhesion up to 40% and 22%, respectively [9]. PLGA wound dressing loaded with Fiusidic Acid prevented the colonial bacterial formation obviously [10]. PLGA fibers with Tetracycline Hydrochloride showed very severe primary burst release and following it after 3 hours showed negligible release [11].

Other studies have shown the great potential of several biodegradable copolymers as wound healing agents including curcumin-loaded PLA nanofibers [12], PGA/collagen composite nanofibrous scaffolds [13], nanofibrous scaffolds containing gum tragacanth/PCL [14] and PLGA/silk fibroin hybrid scaffolds [15]. Sun *et al.* showed that the use of ginsenoside Rg3-loaded electrospun PLGA fibrous membranes as wound covers induce healing and suppression of the formation of hypertrophic scar [6]. Moreover, silk fibroin/PLGA electrospun nano-dressings displayed improved wound closure compared to alone PLGA dressings and to control samples in an *in vivo* wound model of diabetic rats due to a demonstrated improved re-epithelialization process [15].

Regarding slight side effects caused by using therapeutic plants and also shortage in effective drug for curing wounds, studying the effect of therapeutic plants is essential for facilitating wound repair procedure. Using plant in the form of wound dressing and putting it directly on the wound position in comparison to other methods of using plant (ointment, cream and tincture) is useful and causes decrease in side effects of chemical compounds existing in plant. The defect of using plant extracts in the form of ointment and hydrogel is that they are unable to maintain the stable form and keep the moisture of healthy tissue around the wound. If ointment and hydrogels are not covered, they will dry and cannot be used on slough wound [16]. The purpose of this study was to make PLGA polymer nanofibrous membrane containing alcoholic extract of *Hypericum Perforatum* using electrospinning method and to investigate the practical efficiency of the prepared meats.

EXPERIMENTAL

Materials

PLGA (M_W=36,000 Da) (RG504H, lot # 1020751 with the 50:50 mole ratio of glycolic acid to L-lactic acid) was supplied by Boehringer Ingelheim. Dichloromethane (DCM), *N, N*-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO) and Triton X-100 were supplied from Merck (Darmstadt, Germany). *Hypericum Perforatum* alcoholic extract was obtained from Zardband Pharmaceuticals (Tehran, Iran). The salts of NaCl, KCl, Na_2HPO_4, $NaHCO_3$, Na_2CO_3 and K_2HPO_4 as well as Muller Hinton Agar (MHA) were purchased from Merck. Bacterial strains containing *Staphylococcus aureus* (*S. aureus*) (PTCC 1113) were prepared from the Persian Type Culture Collection. 3-[4, 5-Dimethylthiazol-2-yl] diphenyl tetrazolium bromide (MTT) was purchased from Amresco (Solon, Ohio, USA). All materials were used directly without any further purification. Phosphate buffer saline solution (PBS) with pH 7.4 was prepared by disolving 0.137 g sodium chloride (NaCl), 0.01 g disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), 0.0025 g potassium chloride (KCl) and 0.002 g potassium dihydrogen phosphate (KH_2PO_4) in 800 mL distilled water. pH of solution was adjusted to 7.4 using 0.1 M HCl. Solutions were then diluted with distilled water to 1000 mL [17]. The pH of PBS was measured with pH meter (GP353EDT direction model, England).

Preparation of PLGA-Extract solution

The PLGA solution was prepared by dissolving PLGA powder at a concentration of 10% (w/v) in DCM: DMF [80:20 (v/v)]. PLGA: Extract (Ex) blend solutions with 10 and 30% (v/v) of alcoholic *Hypericum Perforatum* extract were obtained by mixing the prepared PLGA solution with the alcoholic extract at different volumetric ratios (90:10 and 70:30) while stirring vigorously for 10 min until complete dissolution. To obtain homogeneous solutions, Triton X-100 (1% (v/v)) was used as a non-ionic surfactant. To improve the conductivity of polymeric solution, potassium hydrogen phosphate salt (K_2HPO_4) was added to solution at 0.78 % (w/v) concentration.

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Electrospinning process for preparation of the PLGA-Ex fibers

Electrospinning of PLGA fibers was performed at room temperature with a 5 mL glass syringe with a blunt-tip stainless steel spinneret as positive electrode and a woody plate covered with aluminum sheet as a collector. The electrospinning process condition such as voltage in the range of 10-25 kV (High voltage power supply; Gamma High Voltage Research, Ormond, Fanavaran Nano Meghyas, Co, Tehran, Iran) and the distance between the needle and collector in the range of 100-200 mm, also flow rate of polymeric solution in the range of 0.1-4 mL h^{-1} was changed to achieve optimized nanofibrous mats. In the following sections, fiber mats will be described as PLGA-E_n that n is the volumetric percentage of *Hypericum Perforatum* extract. Fiber diameter and optimum conditions of electrospun PLGA and PLGA-E_n nanofibrous membranes are reported in Table 1.

Characterization of PLGA-E_n fibrous mats

The morphology of PLGA-E_n fibrous mats was observed by scanning electron microscopy (SEM; JEOL JSM-T300, USA). The mean diameter and diameter distribution of the nanofibers were measured by Image - J software. For this purpose, a small section of the synthesized fiber mats was placed on the SEM sample holder and sputter - coated with gold prior to the analysis. The Fourier transform infrared Nicolet™iS™50 (FT-IR) spectrometer was applied in the range of 400–4000 cm^{-1} to identify the presence of functional groups and to confirm the incorporation of herbal drug in the membranes. Total *Hypericin* content in plant alcoholic extract was determined by spectrophotometry method and expressed in *Hypericin percent* (% w/w) [18].

Quantification of total Hypericin content in plant alcoholic extract

The content of *Hypericin* in the alcoholic extract as one of the antibacterial agents available in herbal extract was determined by spectrophotometry method [18]. According to the calibration equation ($y = 8\times10^{-5} X + 0.001$) with $r^2 = 0.99$, a linear relationship was achieved between the absorbance at 587 nm and concentration of *Hypericin*.

The concentration of *Hypericin* in extract was evaluated by using the Eq. (1):

$$\text{Hyp \%} = \frac{A}{780}\frac{100}{m} \quad (1)$$

Where A= the measured absorbance at 587 nm, m= grams of residual after drying of 25 mL of extract, 780=specific absorbance of *Hypericin* at 587 nm. The total *Hypericin* content of *Hypericum Perforatum* was obtained as 0.2342 ± 0.063 (% w/w).

Measuring water vapor transmission rate of PLGA-E_n fibrous mats

The water vapor transmission rate (WVTR) across the membranes was measured by utilizing a bottle with an exposure area of 1.76 cm^2 which was filled with 10 mL PBS and its opening was covered by a prepared circular nanofibrous mat. The bottles were placed in an oven at 37˚C. The weight of the assembly was measured every hour during 12 h and a graph of evaporated water versus time was plotted. WVTR was calculated using the Eq. (2):

$$\text{WVTR} = \frac{Slope \times 24}{area}\left[\frac{g}{day\ .m^2}\right] \quad (2)$$

The data were reported as mean ± SD for three replicate measurements [19].

Measuring swelling percentage (%) of PLGA-E_n fibrous mats

Water uptake ability or swelling percentage of nanofibrous mats is one of the properties of wound dressing that is needed to study the assessment of wound dressing. To perform this test, glass vials containing 10 mL of PBS (pH = 7.4 ± 0.2) were used. For this purpose, prepared PLGA nanofibrous mats were weighted in the form of 2 cm^2 discs, put into the vials and kept at 37 ˚C for 24 h in the oven. Then, samples were taken out of solution and by using filter paper, the remained solution on the membrane surface was removed and mats weighed once more and swelling percentage of nanofibrous mats was calculated according to Eq. (3).

$$\text{Swelling percentage (\%)} = \frac{W_s - W_o}{W_o} \times 100 \quad (3)$$

Where W_s is the weight of the swollen mat and W_o is the initial dry mat weight. The data were reported as mean ± SD of three parallel runs [20].

Determination of porosity percentage (%) of PLGA-E_n fibrous mats

To determine the porosity of nanofibrous membranes, dimensional approach was used in ambient condition according to Eq. (4). For this purpose, membranes were first weighed in the

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form of discs with 150 mm diameter and thickness of layers was measured by micrometer.

$$\text{Porosity (\%)} = 1 - \frac{\rho}{\rho_0} \times 100 \quad (4)$$

where ρ is the density of the electrospun membrane and ρ_o is the density of the bulk PLGA polymer that is 1.34 g cm^{-3} [21].

In vitro drug release measurement of fibrous mats

In release studies, nanofibrous mats were cut in 5×5 cm^2 dimension and put in containers containing 25 mL PBS solution (pH = 7.4) and were sampled at 100 rpm at various time intervals. 2 mL of release environment containing antibacterial *Hypericin* active substance was withdrawn and its absorption at ʎ= 587 nm was read using an UV−Vis spectrophotometer (model UV−2401; Shimadzu, Japan) and the release environment was replaced with 2 mL fresh PBS solution. After that, release percentage was calculated and reported using calibration curve and surface correction equation. Meanwhile, the *Hypericin* amount released from nanofiber was calculated according to the Eq. (5):

$$C_n^{'} = C_n \left(\frac{V_T}{V_T - V_S}\right)\left(\frac{C_{n-1}^{'}}{C_{n-1}}\right) \quad (5)$$

where is the corrected concentration of the n^{th} sample (mg L^{-1}); C_n is the measured concentration of *Hypericin* in the n^{th} sample in mg L^{-1}; C_{n-1} is the measured concentration of the $(n-1)^{th}$ sample in mg L^{-1}; V_T is the volume of receiving fluid (mL) and V_S is the volume of withdrawn sample (2 mL) [22]. By considering the Eq. (5), the release percentage was calculated and reported.

Analysis of the drug-release kinetics

For evaluation of drug-release kinetics and determination of drug-release mechanism in all the samples, the Peppas– Korsmeyer equation (Eq. 6) was used [23]:

$$\frac{M_t}{M_\infty} = Kt^n \quad (6)$$

Where M_t is the cumulative amount of the drug released at time t, M is the initial drug loading, K is a constant characteristic of the drug – polymer system, and n is the diffusion exponent, which suggests the nature of the release mechanism. In addition to Fickian theory, four more models were used to further analyze the drug-release profile, including the zero-order, first order, Higuchi, and Hixon–Crowell models. These model equations are listed as follows:

$$\text{Zero order: } Q_t = Q_0 + K_0 t \quad (7)$$

$$\text{First order: } \ln Q_t = \ln Q_0 + K_1 t \quad (8)$$

$$\text{Hixson–Crowell: } Q_0^{1/3} - Q_t^{1/3} = K_s t \quad (9)$$

$$\text{Higuchi: } Q_t = K_H \sqrt{t} \quad (10)$$

Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most of the time, $Q_0^{1/4}$), K_0 is the zero-order release constant, K_1 is the first-order release constant, K_H is the Higuchi dissolution constant and K_S is a constant incorporating the surface–volume relation.

In vitro study of antibacterial activity of fibrous mats

The antibacterial activity of the electrospun PLGA mats with and without extract was evaluated against *S. aureus* (Gram-positive bacteria) by the disc diffusion method. The amount of inoculated bacteria on each plate is equivalent to half Mc Farland. Nanofibrous membranes (with a diameter of 1 cm) were sterilized under UV light for 2 h, and put on Muller -Hinton agar plates incubated with bacteria. Then, plates were inverted in the 37 °C incubator for 24 and 48 h. PLGA nanofibrous discs without extract and Vancomycin antibiogram disc were used as negative and positive controls, respectively. The inhibition zones were recorded for 3 times.

Cell line and sub culturing

Due to the use of prepared nanofibrous membranes as wound dressing, the adhesion and the proliferation ability of human skin fibroblast cells (HSF) were examined. For this purpose, at first, nanofibrous mats (in dimension of 1×2 cm^2) were sterilized using Gamma ray and samples were put in 24−well plates. To remove residual organic solvents, each of the samples was washed three times with PBS solution.

Cells at a concentration of 5000 cell mL^{-1} were cultured on nanofibrous mats into the wells and Dulbeco′ s Modified Eagle′ s medium (DMEM) containing 10% fetal bovine serum (FBS), streptomycin 1% and penicillin 1% was poured on it and the mats were placed in an incubator containing 5% CO_2 for 4 days. Then, to stabilize the cells, solutions on top of the mats were removed and the contents of the wells were washed with PBS and then glutaraldehyde solution was placed on mats for 45 min. After this period, water on the mats was taken by ethanol 35 %, 70 % and 96 % and the samples were dried for SEM studies.

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Biocompatibility study (MTT assay) of fibrous mats

This study was carried out to evaluate biocompatibility of nanofibrous mats, neat PLGA, and PLGA-E_{10} according to the international standard ISO 10993-1 method [24]. For this purpose, nanofibrous mats (1×2 cm^2) were put in 24−well culture plate after sterilization with Gamma ray. To remove residual solvent, nanofibrous mats were washed three times with PBS solution. Then, 2.5 mL of DMEM culture medium without FBS was poured onto each sample and were incubated at 37 ˚C for 1 and 5 days. Also, two wells containing culture medium without sample as a control well were incubated for 1 and 5 days. Then, skin fibroblast cells were counted after trypsinizing the flask, centrifuged and were poured in each 96−well culture plate and incubated with a concentration of 1000 cells in each well with 100 µL of culture medium containing FBS for one day. After that, cell culture medium was removed and instead, 90 µL of nanofibrous sample extract in culture medium that was incubated for 1 and 5 days was poured onto cells. 10 µL of FBS was added to each well and incubated for 24 h. After this period, medium on top of the cells was removed and instead, 100 µL of MTT solution in the concentration of 0.5 mg mL^{-1} was poured in PBS solution in each well and incubated for 4 h. Then, the wells were empty of MTT solution and instead, 100 µL of DMSO was poured into each well. The culture medium was put in a shaker incubator for 15 min so that formed formazan crystals are dissolved completely in DMSO. Finally, the optical density of the wells was measured using ELISA Reader (at λ= 570 nm), six wells were filled with extract and the data obtained were averaged. The biocompatibility percent of the samples was calculated by dividing the amount of absorption of wells containing extract to control wells.

RESULTS AND DISCUSSION

Structural characterization of nanofibrous mats

Morphological study:

According to the influential variables, uniform nanofibrous mats were produced by electrospinning process in the following conditions. In Table 1, diameter and diameter distribution range of prepared fibers for producing large amounts of nanofibers were summarized (Fig. 1).

The nanofibrous structure of the pristine PLGA sample was characterized as individual, uniform, and randomly oriented fibers with an average diameter of 364 nm. The addition of *Hypericum Perforatum* extract changed the diameter of nanofibers dramatically. The fiber dimension distributions were evaluated by Image J software that showed fiber dimension distribution is shifted towards higher dimensions after addition of *Hypericum Perforatum*. This increase in the fiber diameters and junctions may be due to the incorporation of extract which reduces the solvent evaporation rate. When the solvent evaporation rate reduces, excessive solvent may cause the fibers to merge where they contact to form junction resulting in inter and intra layer bonding [25].

Fourier transforms infrared spectroscopy (FT- IR):

As is evident in comparing the IR spectrum of the pure PLGA fibers and PLGA-E_n fibers, the absorption bands around 1700-1800 cm^{-1} corresponded to C=O vibrations. Also, C−O−C stretching vibrations occurred at 1000-1260 cm^{-1} which is indicative of ester groups. Absorption bands at 2850−3000 cm^{-1} are related to the stretching vibration of alkyl group −CH, $-CH_2$, $-CH_3$ and peak of the end hydroxyl group is seen at 3400−3500 cm^{-1}. Also, absorption bands at 850-1450 cm^{-1} are related to bending vibration of C−H. There was variation in 2928 cm^{-1} related to the plant extract in mixed nanofiber mats in (PLGA-E_{30} and PLGA-E_{10}), but by increasing the volume of extract, its intensity has increased, that is an important indication of the existence of extract in the matrix. Absorption band below 880 cm^{-1} is for aromatic rings of plant extract. The C=C stretching vibrations occurred in 1500, 1600 cm^{-1} and peak at 1200 cm^{-1} is related to C−O phenolic groups. Broad peak at 3400 cm^{-1} is due to the existing stretching vibration of the hydroxyl group. FT-IR spectrum of PLGA membranes loaded with plant extract is shown in Fig. 2 (a, b, c, d).

Porosity Measurements:

The result of the porosity test showed that scaffold containing 30 % extract has more porosity than scaffolds with 10% extract and pure PLGA nanofibers. When polymer concentration is lower, diameter and weight of gathered samples on collector plate are lower. The average thickness of mats decreases in comparison to solution with a higher concentration and as a result, the porosity of PLGA-E_{30} scaffold was higher than other

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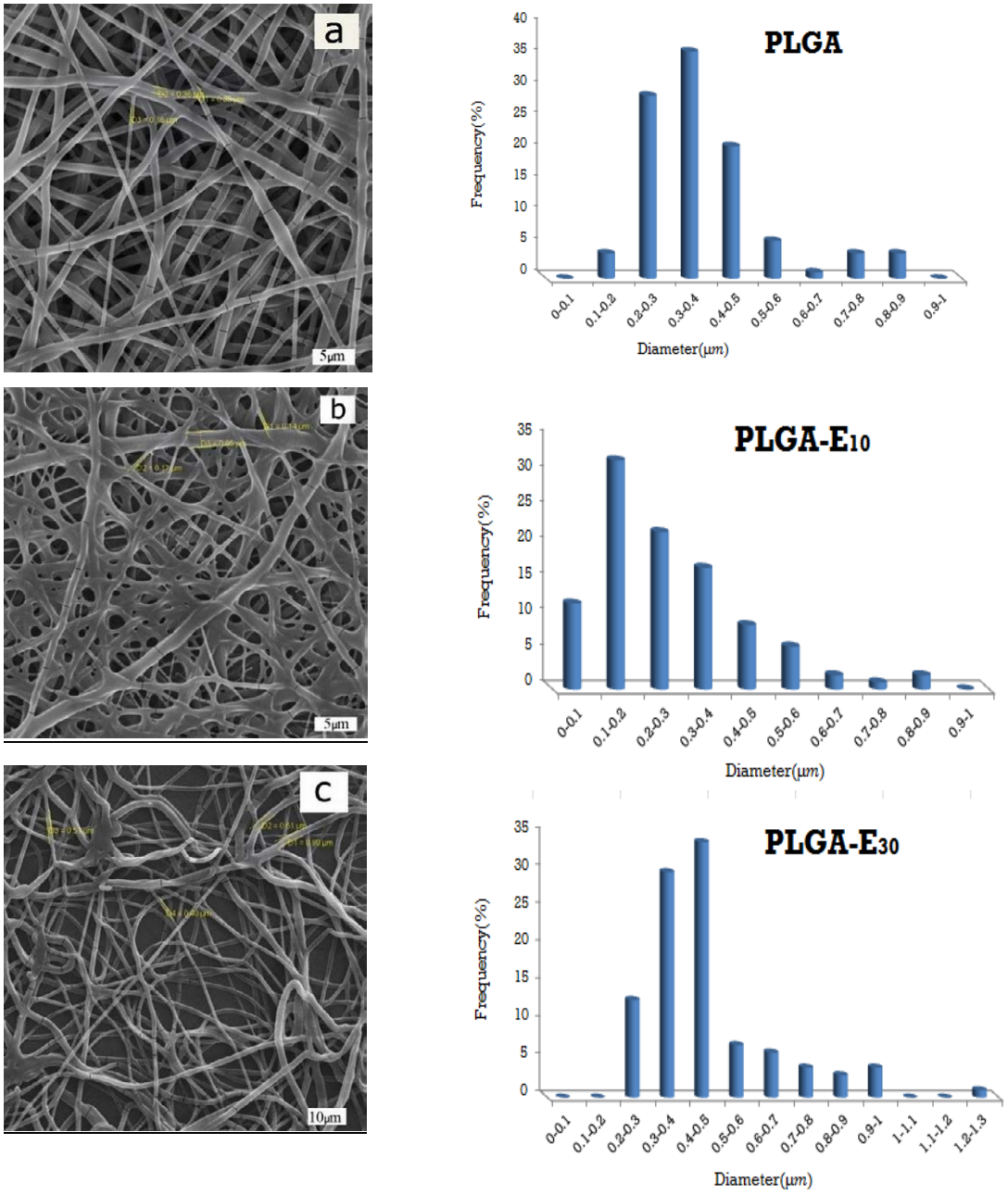

Fig. 1: SEM micrographs and diameter distribution of (a) PLGA, (b) PLGA-E_{10}, (c) PLGA-E_{30}.

Table 1: Fiber diameter and optimum conditions of electrospun PLGA and PLGA-E_n nanofiber membranes.

Samples	Average diameter (nm)	Diameter distribution (nm)	experimental condition for obtaining optimal membranes		
			KV (V)	Distance (mm) (D)	Flow rate (mL h^{-1})
Pure PLGA	364 ± 0.12	100-500	15	120	0.1
PLGA-E_{10}	270 ± 0.17	100-400	20	100	1
PLGA-E_{30}	464 ± 0.2	200-500	10	100	2

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samples. By considering the obtained results, it is observed that the porosity of both scaffolds with the concentration of 10% (v/v) and PLGA without extract is ideal for attachment and cell growth (Table 2).

Performance evaluation of prepared nanofibrous mats as wound dressing

Water Vapor Transmission Rate (WVTR):

In this section, WVTR in a wide range of 1200−3057 (g m^{-2} day^{-1}) was obtained for WVTR dressing property with the incorporation of extract. WVTR was dramatically increased and this was due to the incorporation of drug in the membrane that makes PLGA-E_{30} with WVTR=3057 and 91% porosity of nanofibrous mat as a suitable wound healer dressing. Fig. 3 shows the results of WVTR measurements for the membranes.

Swelling Degree (%):

Swelling process depends on polymer hydrophilic tendency. PLGA is not hydrophilic like hydrogels or other natural polymers and its swelling takes place slowly. But, due to polymer degradation during hydrolysis, the number of end groups increases and therefore, inflammation process increases and causes more water absorption. From the results presented in Fig. 4, *Hypericum Perforatum* extract loaded membrane performed a better absorption of PBS than the pure PLGA fibers indicating the better exudate absorption capacity of the drug-loaded membrane. Because PLGA is a hydrophilic polymer, increasing the herbal extract of *Hypericum Perforatum* in combination with K_2HPO_4 salt results in increasing the hydrophilicity of polymeric mat. In this research, by adding alcoholic extract of *Hypericum Perforatum*, better absorption for polymeric mat was observed.

Drug release studies:

As seen in Fig. 5, in the first 24 h of burst release, 68% and 69% of extract was released in PLGA−E_{10} and PLGA−E_{30}, respectively. This effect can be explained by two main factors. First, the physical interaction of the drug-polymer, i.e. accumulation of the drug (*Hypericin*) on the surface of hydrophobic PLGA nanofibers during the electrospinning process occurs. In the next 24 h, only 7 % of *Hypericin* release has been increased and up to 240 h, only 12 % has been added to the previous release percent that shows a release observed could be due to drug-polymer interaction and this shows that *Hypericin* can be physically trapped on the surface of PLGA polymeric nanofibers. The results of the herbal extract release of PLGA-E_n nanofibrous mats were shown in Fig. 5. The explosive release and very short initial release phase could be due to the drugs trapped near the surface of the fibers. Burst release encourages rapid inhibitory effect of antimicrobial agents in the open wound area and in the later stages of the controlled release prevents the reproduction and growth of pathogenic factors. But the adverse effect of accumulation of pharmacological agents in wound area due to side effects should not be ignored. However, PLGA-E_{30} system despite the greater diameter of synthesized fibers (464 ± 0.2 mm) causes the controlled release in comparison to PLGA-E_{10} (270 ± 0.17 mm).

Analysis of the drug-release kinetics:

According to the results, the only mechanism of drug release from nanofibrous mats in PBS solution was Fickian model and matrix degradation didn't have the main role in the release of *Hypericin*. The early high level release is probably due to the release of *Hypericin* on the surface of fibers that was matched with Higuchi model (Table 3). So, because of long-term degradation of the polymer, release mechanism isn't match with Hixon-Crowell model. The greatest value of correlation coefficient (r^2) was obtained from Higuchi model. In addition, the correlation coefficient for different release rate of drug from various mats explains relative adaptation to the zero order model that this model is related to the delivery of poorly soluble drugs into the release medium. The calculated variables of release kinetic models are shown in Table 3.

Biological study of prepared nanofibrous mats as wound dressing

Antimicrobial activity:

The inhibitory effect of PLGA-E_n against pathogenic bacteria by disc diffusion was carried out. Fig. 6 shows the inhibitory zone formation around the nanofibrous discs containing the extract in agar plates of *S. aureus.* The results are summarized in Table 4. The inhibitory effect of *Hypericum Perforatum* extract was shown against Gram-positive bacteria. The sample with wider inhibition zone clearly identifies the inhibitory effect of the extract against *S. aureus* comparing the antibacterial activity of the standard

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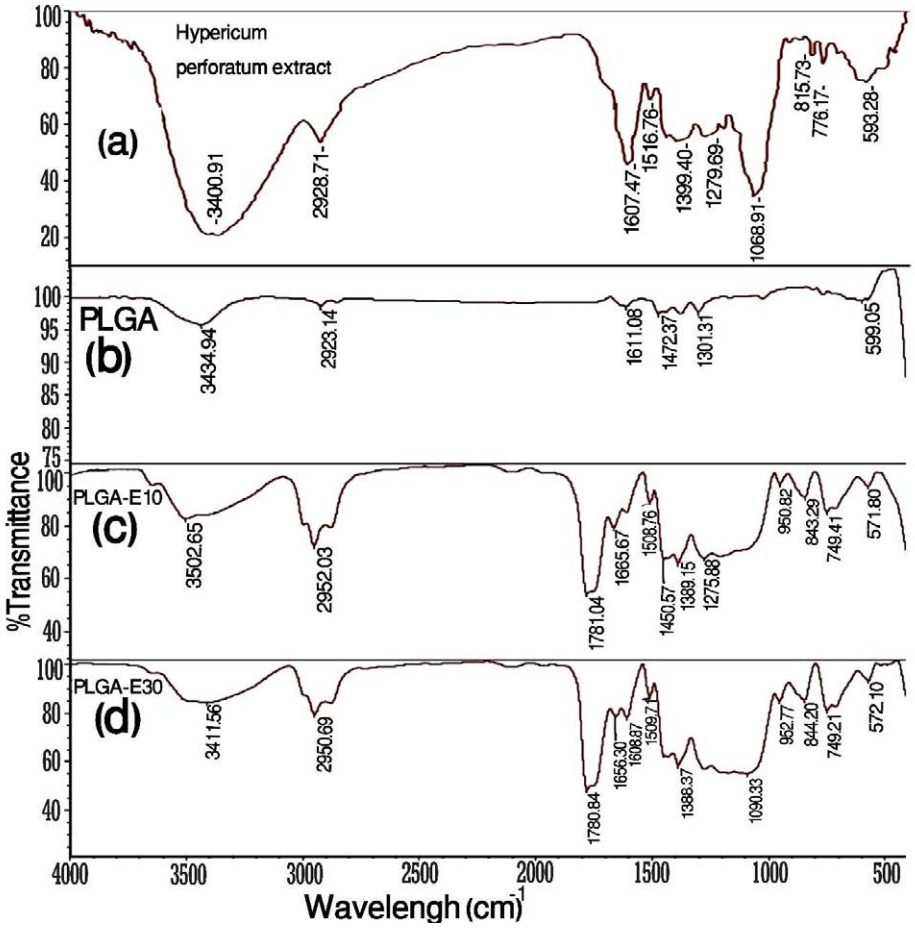

Fig. 2: FT-IR spectra of Hypericum Perforatum extract (a), FT-IR spectra of PLGA (b), PLGA-E10 (c), PLGA-E30 (d) nanofibrous membranes.

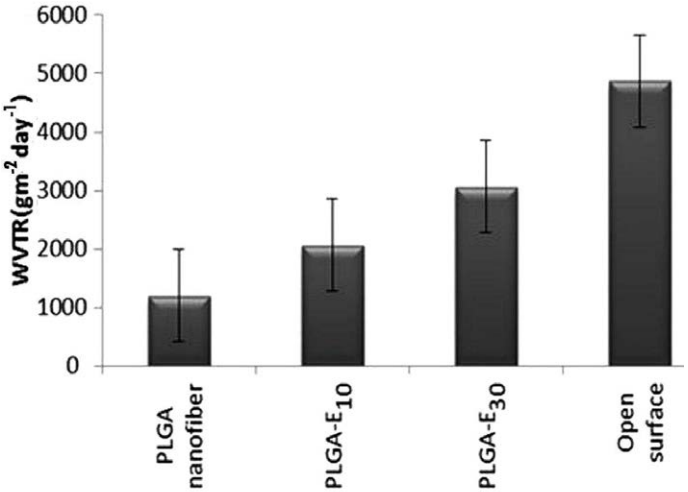

Fig. 3: Water vapor transmission rate (WVTR) of the membranes.

Table 2: The physical characterization of PLGA-En nanofibrous scaffold.

Mats	Bulk polymer density ($g.cm^{-3}$)	Porosity (%)	Thickness average (µm)	diameter (cm)	Mat mass (g)	PLGA concentration (wt %)
PLGA	1.34	79	0.034	15	1.69	10
PLGA-E_{10}	1.34	84	0.035	15	1.32	10
PLGA-E_{30}	1.34	91	0.032	15	0.68	10

Table 3: Regression coefficients of mathematical models fitted to the release of Hypericin from the PLGA-En nanofibrous mats.

samples	Zero order	First order	Hixson–Crowell	Higuchi
PLGA-E_{10}	0.57 ± 0.03	0.31 ± 0.06	0.4 ± 0.01	0.8 ± 0.09
PLGA-E_{30}	0.5 ± 0.06	0.23 ± 0.03	0.28 ± 0.04	0.76 ± 0.05

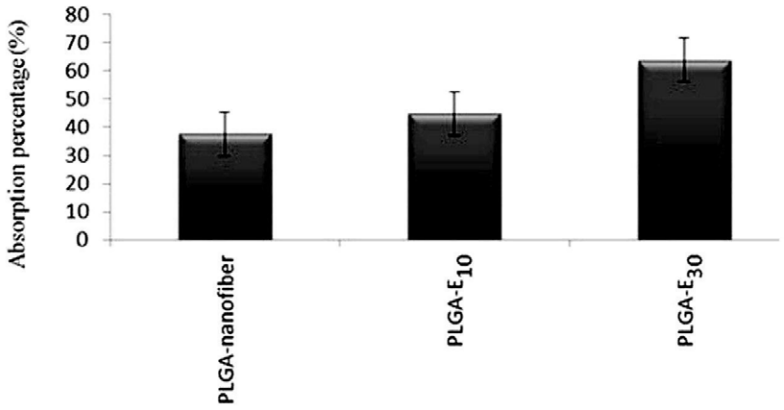

Fig. 4: Swelling percentage (%).

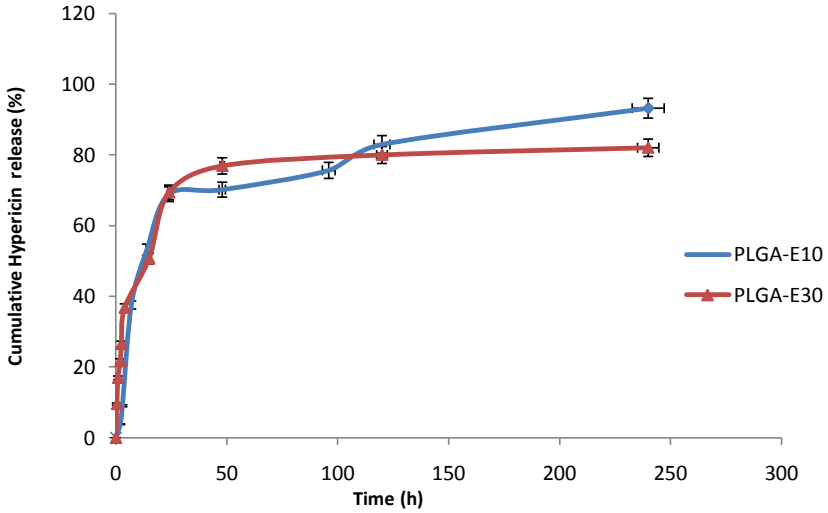

Fig. 5: Drug release from PLGA-En nanofibrous mat.

Table 4: Inhibition zone in mm ± SD for PLGA-En nanofibrous mats (results shown are the mean of three measurements).

Samples	*S. aureuse* (mm ± SD)	*E. Coli* (mm ± SD)
PLGA	-	-
PLGA-E_{10}	12.00 ± 0.23	-
PLGA-E_{30}	20.00 ± 0.12	-

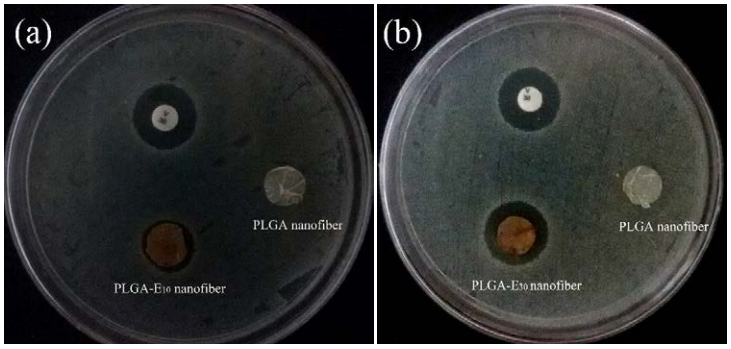

Fig. 6: Inhibition zone of PLGA-E10 (a), PLGA-E30 (b) nanofibrous mats against S.aureuse.

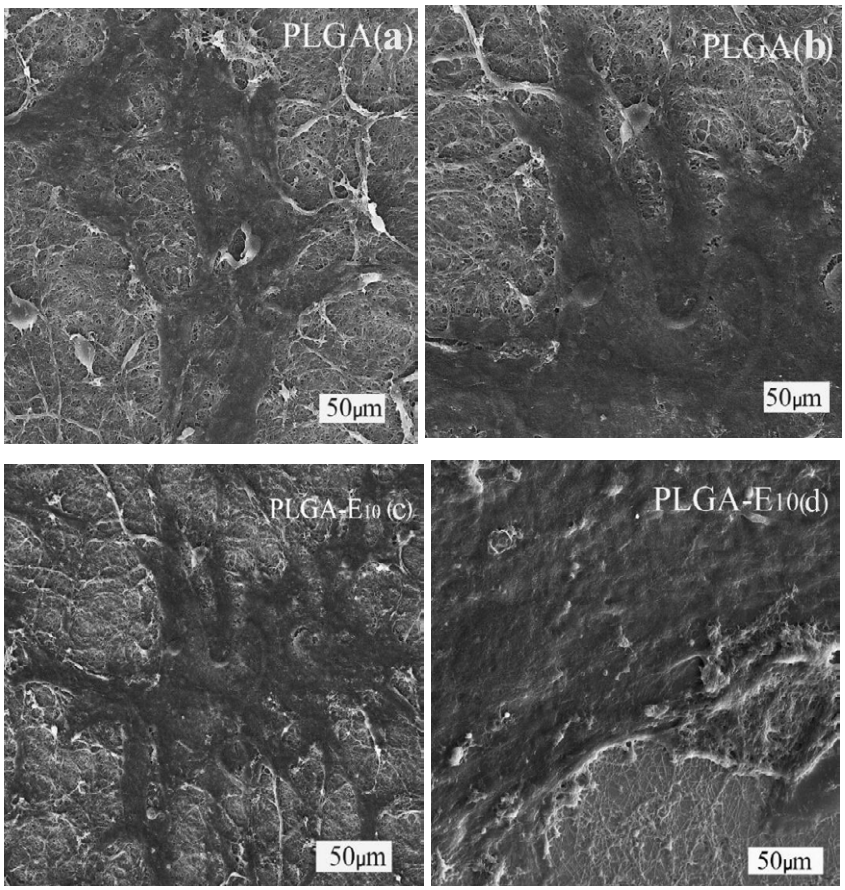

Fig. 7: SEM image of interactions between HSF cells and (a) PLGA (The first day of cell culturing), (b) PLGA (The third day of cell culturing, (c) PLGA-E10 (The first day of cell culturing), (d) PLGA-E10 (The third day of cell culturing) nanofibrous membranes mats.

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antibiogram disc. Inhibition zone was obtained about 12 ± 0.23 mm for PLGA-E_{10} and 20 ± 0.15 mm for PLGA-E_{30}.

Culture of human dermal fibroblasts (HDFs):

As seen in Fig. 7, all the scaffolds are good substrates for fibroblast cell growth in both times and have prepared a suitable bed for fibroblast connection and as a result, produce a better growth and reproduction for them. Fig. 7 (a to d) show that by comparing cell adhesion on two above scaffolds, in the third day, the amount of cell adhesion on PLGA scaffold is more than PLGA-E_{10} mat. In this study, due to the very high biocompatibility of polymer, cell adhesion and culture was better than PLGA**.**

Determination of cell viability by MTT assay:

As shown in Fig. 8, HSF cells are attached to both of graft types. So, scaffolds have compatibility with fibroblast cell, the reason is very high biocompatibility of PLGA polymer. Toxicity of PLGA-E_{10} membrane loaded by plant extract was lower than the control group. Although, materials loaded with plant but don′ t release harmful compounds for cell. In evaluating the compatibility of nanofibrous mats, the reason for low biocompatibility percent could be due to the solvents used for making nanofibrous mats that solvent effect is not completely removed in mats and some of it is left in mat field. But, in biocompatibility test after 5 days, the cells showed a survival rate of 80%. As microbial test approved, this bed is promising for drug delivery systems for those surfaces to Gram-positive pathogenic microorganisms.

CONCLUSIONS

Using polymeric fibers in wound dressings in combination with suitable additives for faster treatment of wounds like antibiotics, cause acceleration in wound healing. In this study, the production of PLGA nanofibrous scaffolds containing alcoholic extract of *Hypericum Perforatum* was carried out by electrospinning method and their compatibility was evaluated with HSF cells.

By observing nanofibers morphology by SEM and characterization of mats by FT-IR, two scaffolds were selected as optimized scaffolds for carrying out cell culture studies. The results of HSF cell culture showed more absorption amount than negative control group (without mat) by considering SEM images of samples after 1 and 3 days of cell culture.

Also, nanofibrous scaffold didn't have cytotoxicity effect. Because of high biocompatibility of PLGA polymer, it is a good substrate for fibroblast cell growth for cell attachment, growth and proliferation. The cells penetrate into the scaffolds and these scaffolds prepare more extensive layers for fibroblast attachment due to the unique property of nanofibers (high surface to volume ratio).

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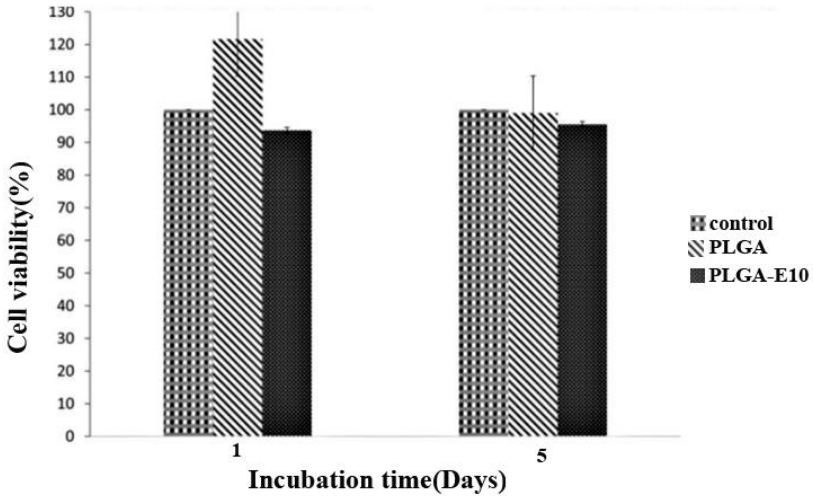

Fig. 8: In vitro biocompatible nature of PLGA and PLGA-E10 in fibroblasts ($p < 0.05$).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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