PRODUCTION OF BICOMPONENT GELATIN/OLIVE OIL NANOFIBERS FOR BIOMEDICAL APPLICATIONS USING COAXIAL SPINNERET

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Abstract

In this study, production of electrospun nanofibers containing olive oil was carried out using gelatin polymer. Olive oil is a natural green vegetable oil, which is abundant in vitamin, carotene and many trace elements. Olive oil has many functions in nutrition and health care and it is rich in essential fatty acids, including Vitamin A, D, E, K and other antioxidant substances, which can be rapidly absorbed by the body and can maintain skin elasticity and moisture. For this reason, in this study we focused on fabrication of nanofibrous mat containing olive oil that can be used in medical field. Nanofibers spun by bicomponent electrospinnig method using coaxial spinneret.

Gelatin polymer was dissolved in distilled water/acetic acid at concentration 10%. Then olive oil was added to solution at a rate of 9:1 wt. Electrospinning from solution was carried out at varying process parameters such as feeding rate and applied voltage and also different mixture of polymer and olive oil. Bicomponent electrospun fibers were characterized by FT-IR spectroscopy and SEM instrument. FT-IR spectroscopy was used to prove the presence of olive oil in fiber structure.

Introduction

Nanofibers can be produced using a solution via electrospinning setup that could be assembled at a reasonable cost. Electrospinning setup is comprised of a power source that is able to work at high voltages, electronic syringe pump, syringe and a collector plate. The principle of the system based on transferring the polymer solution into the electrostatic drawing area through a needle at a feeding rate and latter to be collected in the form of nanofibers onto the collector plate after exposure to a high drawing force in this area [1, 2].

Electrospinning is a versatile technique to produce nanofibers for medical, filtration, transport and energy applications. It can be utilized using co-axial and/or different spinneret designs to produce bicomponent nanofibers [3].

McCann et al. conducted a research on availability of co-axial electrospinning to produce core-sheath, hollow, or porous nanofibers from mineral oil as a core component and PVP $(TiOiPr)_4$ as a sheath component using co-axial spinneret. They have concluded that electrospinning will become an essential and useful top-down technique for the fabrication of one-dimensional nanostructures with hollow interiors [4].

Wang et al. investigated the morphological and structural properties of multifunctional scaffolds for bone tissue engineering that were constructed via dual-source dual-power electrospinning (DSDPES). Emulsion electropinning used to produce rhBMP-2 (recombinant human bone morphogenetic protein)/PDLLA fibers and a composite suspension containing Ca–P/PLGA nanocomposite fibers were prepared and then bicomponent scaffolds were constructed by DSDPES. Further investigations showed that bicomponent scaffolds developed using this method could provide balanced osteoinductivity and osteoconductivity [5].

Apart from specific spinneret designs, bicomponent nanofibers can be produced by preparing a bicomponent solution. Mincheva et al. prepared a bicomponent solution including N carboxyethylchitosan and poly(vinyl alcohol) polymers and developed a tissue engineering scaffold for biomedical applications via electrospinning. They found that their bicomponent nanofibers can be used as biomedical scaffolds due to their bicompatibility, water-resistance of cross-linked mats and aligned nanofibrous structure [6].

As it could be seen from present literature, bicomponent nanofibers developed for medical applications intensified on tissue engineering and scaffolds. In this study, instead, we have designed a bicomponent nanofibrous material that could be used in therapeutics and wound care within medical field.

1 Materials and Methods

1.1 Materials

Type A gelatin was purchased from Sigma company. Olive oil used as purchased. Acetic acid as a solvent from Sigma company used as received. Collector plate was covered by an aluminum foil.

1.2 Electrospinning of Gelatin/Olive Oil

Gelatin of 10 g (10 wt%) was dissolved in acetic acid (80 wt%) and 20 ml pure water mixture and the solution was stirred for 6 hours using a magnetic stirrer (WiseStir) and then stayed overnight in laboratory. To the comparison aim, bicomponent nanofibers were produced both preparing a bicomponent solution and using coaxial spinneret in different feeding ratios of Gelatin polymer and olive oil.

1.2.1 Electrospinning of Bicomponent Solution of Gelatin/Olive Oil

Three different concentrations of Gelatin (G)/Olive Oil (OO) bicomponent solution were prepared and electrospun using standard electrospinning apparatus in different conditions given in Table 1:

Tab. 1: *Electrospinning of 1:9 OO/G solution at 12 kV and 8 cm distance and varying feeding rates*

Feeding Rate	**Status of Electrospinning**
1.5 ml/hr	Regular nanofiber production
2 ml/hr	Regular nanofiber production
2.5 ml/hr	Dripping over collector plate plus nanofiber production due to higher feeding rate

Source: Own

1:9 OO/G was the first concentration prepared by adding 2 ml olive oil into the 18 ml Gelatin solution (10wt%) and total solution volume defined as 20 ml. Finally, 0.2 g spun 20 surfactant (0.1 ml spun 20 for 10 ml) added to the solution and the whole solution was stirred and materials within solution were well dispersed. The other concentrations of 2:8 OO/G and 3:7 OO/G were prepared in the same manner. The only electrospinning process could be achieved by concentration of 1:9 OO/G while it could not be achieved for other solutions due to phase separation. The best voltage applied defined as 12 kV and distance between grounded collector and needle tip fixed at 8 cm.

1.2.2 Co-axial Electrospinning of Gelatin/Olive Oil

In this part, Gelatin of 10 wt%, whose preparation had been previously explained, and pure olive oil were co-axially electrospun (Gelatin polymer was fed from outer side of spinneret and olive oil was fed from inner/core side of the spinneret) in varying feeding and voltage rates using a co-axial spinneret apparatus. The distance between grounded collector and needle tip fixed at 8 cm. Firstly, bicomponent nanofibers co-axially electrospun in five different solution feeding rates given table 2.

Tab. 2: *Co-axial Electrospinning of Gelatin (G) /Olive Oil (OO) at 18 kV and 8 cm distance*

G Feeding Rate (ml/hr)	**OO Feeding Rate (ml/hr)**	**Status of Electrospinning**
0.7	0.2	Nanofiber production at 30 seconds intervals with a dripping a few drops of olive oil
1	0.2	Nanofiber production couldn’t be achieved
1.3	0.2	Almost regular nanofiber production
1.6	0.2	Almost regular nanofiber production plus dripping a few drops of olive oil
1.9	0.2	Poor nanofiber production

Source: Own

Secondly, after defining the best feeding rate of Gelatin polymer as 1.3 ml/hr bicomponent nanofibers co-axially electrospun in four different voltages is given in table 3.

__Tab. 3:__ Table 3. Co-axial Electrospinning at a feeding rate of 1,3 ml/hr Gelatin (G) / 0,2 ml Olive Oil (OO) and 8 cm distance

Voltage Applied (KV)	**Status of Electrospinning**
14	Poor nanofiber production plus dripping a few drops of olive oil
16	Almost regular nanofiber production plus dripping a few drops of olive oil
18	Almost regular nanofiber production
19	Regular nanofiber production

Source: Own

1.3 SEM Analysis

Bicomponent nanofibers produced using bicomponent solution and co-axial spinneret were characterized using a Scanning Electron Microscopy (SEM) to define morphological properties.

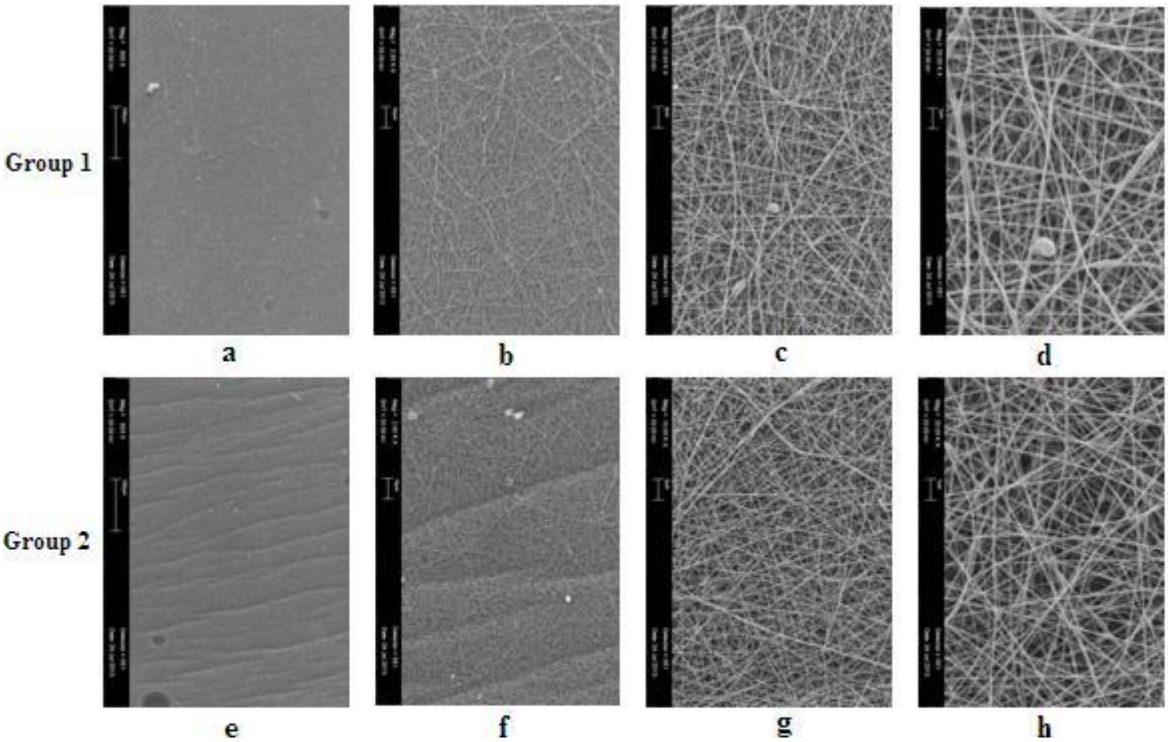
1.4 FT-IR Analysis

Olive oil and gelatin content of bicomponent nanofibers and chemical bonds within structure were analyzed using FT-IR.

2 Results and Discussion

2.1 SEM Investigation

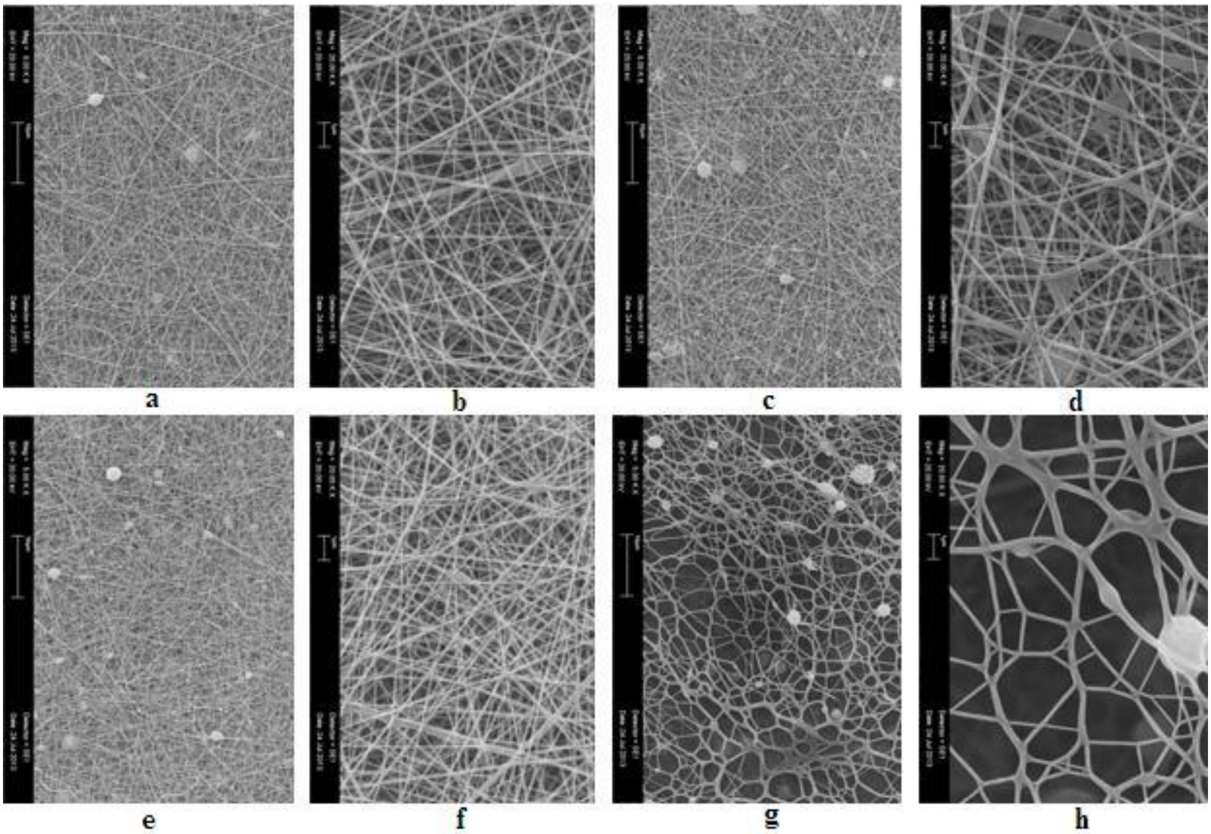
Fig. 1 and Fig. 2 have revealed the morphological information for bicomponent nanofibers from bicomponent solution and coaxial spinneret, respectively.



Source: Own

Fig. 1: *SEM images of bicomponent nanofibers electrospun from bicomponent solution of Gelatin/Olive Oil in 1:9 ratio divided in 2 groups by different process parameters; Group1: Electrospun at 11.2 kV, 1.5 ml/hr solution feeding rate, 8 cm collector distance, SEM magnification rates: (a) 500x, (b) 2kx, (c) 10kx, (d). Group 2: Electrospun at 11.2 kV, 2 ml/hr solution feeding rate, 8 cm collector distance, SEM magnification rates: (a) 500x, (b) 2kx, (c) 10kx, (d) 20 kx*

Fig. 1 shows bicomponent solution yielded nanofibers in a defined orientation and changing feeding rate has leaded a little change on morphology of the nanofibers such as small lines seen in image within Group 2, e and f.



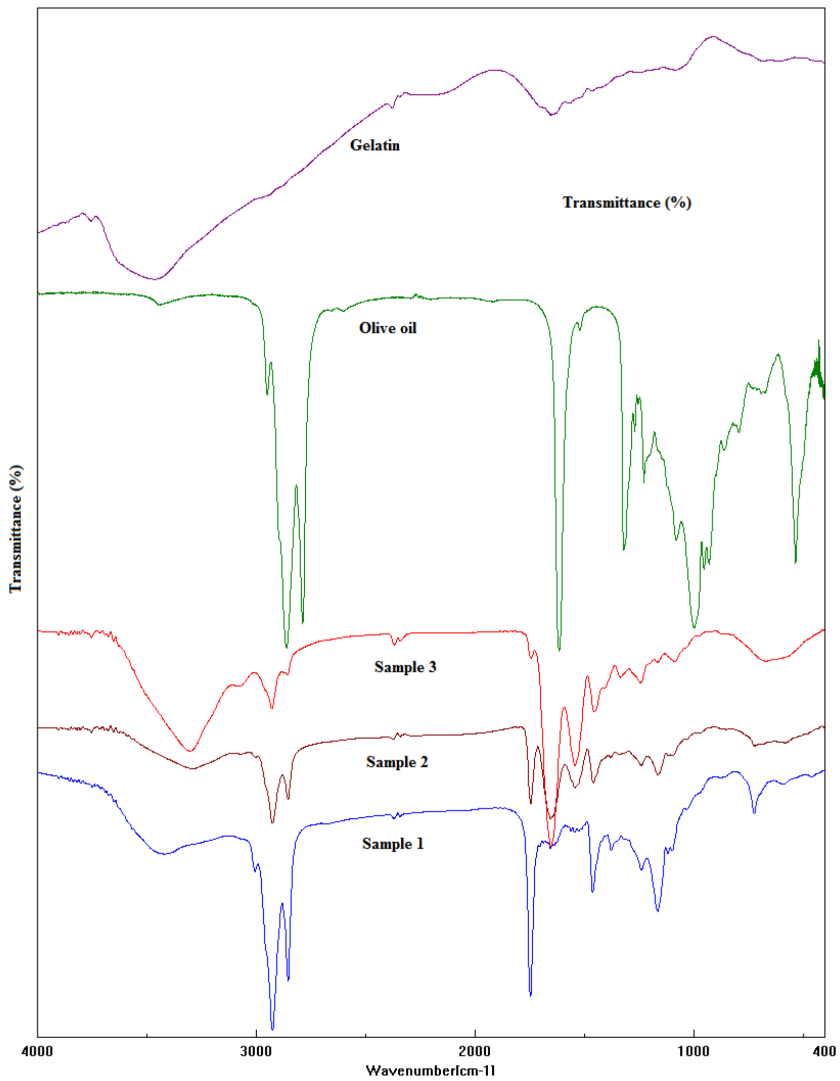
Source: Own

Fig. 2: *SEM images of bicomponent nanofibers electrospun using co-axial spinneret in different process parameters and feeding rates of Gelatin and Olive Oil; nanofibers electrospun at 18 kV with a gelatin feeding rate of 1.6 ml/hr and olive oil feeding rate of 0.2 ml/hr a:5 kx and b:20 kx; nanofibers electrospun at 18 kV with a gelatin feeding rate of 1.3 ml/hr and olive oil feeding rate of 0.2 ml/hr c:5 kx and d:20 kx; nanofibers electrospun at 19 kV with a gelatin feeding rate of 1.3 ml/hr and olive oil feeding rate of 0.2 ml/hr e:5 kx and f:20 kx; nanofibers electrospun at 18 kV with a gelatin feeding rate of 1.9 ml/hr and olive oil feeding rate of 0.2 ml/hr g:5 kx and h:20 kx,8 cm distance from collector for all samples.*

Fig. 2 demonstrated that voltage change has not changed the morphology much, but when feeding rate of gelatin increased to 1,9 ml/hr, rapid phase separation occurred and soap bubble-like or spider web-like structures have been generated. In addition, diameters of the nanofibers have remarkably decreased. Related literature assumes that formation of such structures may result from rapid phase separation and minimal energy principle [7].

2.2 FT-IR Analysis

FT-IR spectrum seen at Fig. 3 shows olive oil content trapped into the bicomponent nanofibrous structures produced using bicomponent solution and coaxial spinneret. The steep peak seen at 2924 and 2852 cm^{-1} belong to the symmetrical and asymmetrical -C-H stretching within olive oil structure. These peaks are respectively seen at 2925-2854 cm^{-1} in the spectra of sample 1 and sample 2, which are bicomponent nanofibers produced using coaxial spinneret respectively at 18 kV and 19 kV with a Gelatin feeding rate of 1.3 ml/hr and olive oil feeding rate of 0.2 ml/hr. these peaks also seen at 2928-2857 cm^{-1} in the spectra of sample 3, which is bicomponent nanofibers produced using bicomponent solution at 11.2 kV and 1.5 ml/h feeding rate, within FT-IR spectrum belongs to all three samples that produced 8 cm collector distance. It proves that all three bicomponent nanofiber samples contain olive oil.



Source: Own

Fig. 3: *FT-IR Analysis of Gelatin/Olive Oil Bicomponent Nanofibers*

Conclusion

To answer the question whether olive oil added to bicomponent nanofibers can be produced using either a bicomponent solution or a coaxial spinneret for medical field and therapeutic applications, the production trials were carried out at changing applied voltages, solution feeding rates while distance between needle tip and grounded collector plate was fixed at 8 cm. Changing applied voltage did not have a crucial effect over morphology of the bicomponent nanofibers produced using a bicomponent solution, while little changes have occured within the structure such as small lines occurrence due to an increase in feeding rate of solution from 1.5 ml/hr to 2 ml/hr. Again, voltage change did not have a crucial effect on morphology of the bicomponent nanofibers produced using a coaxial spinneret while increasing the feeding rate of Gelatin polymer caused rapid phase separation, which concluded with development of soap bubble-like structures or spider web-like structures and fibers with lower diameter. FT-IR analyzes proved that olive oil content in both bicomponent nanofibers which means these nanofibers could be used in medical field such as wound closure materials due to their therapeutic activity. Finally, use of coaxial spinneret has provided different morphological possibilities such as the development of biomimetic nanofibers and nanofibers with lower diameters and containing active agents.

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VÝROBA NANOVLÁKEN ELEKTROSPUNINGEM ZA POUŽITÍ KOAXIÁLNÍ SNOVACÍ TRYSKY, VYUŽITÍ OLIVOVÉHO OLEJE A ŽELATINOVÉHO POLYMERU

Tato studie pojednává o výrobě nanovláken elektrospuningem (elektrostatickým zvlákňováním) obsahujícím olivový olej s pomocí želatinového polymeru. Olivový olej je přírodní zelený rostlinný olej, který je bohatý na vitaminy, karoten a mnoho stopových prvků. Olivový olej má mnoho funkcí ve výživě a zdravotní péči a je bohatý na esenciální mastné kyseliny, včetně vitamínu A, D, E, K a dalších antioxidačních látek, které mohou být rychle vstřebávány v těle a pomáhají udržovat pokožku pružnou a vlhkou. Z tohoto důvodu jsme se v této studii zaměřili na výrobu nanovlákenné vrstvy obsahující olivový olej, která může být využita v oblasti medicíny. Nanovlákna jsou tažena metodou dvousložkového elektrostatického zvlákňování za použití koaxiální snovací trysky.

PRODUKTION VON NANOFASERN DURCH ELEKTROSTATISCHE VERSPINNUNG UNTER VERWENDUNG VON KOAXIALEN SCHÄRDÜSEN, NUTZUNG VON OLIVENÖL UND GELATINEPOLYMEREN

Diese Studie befasst sich mit der Produktion von Nanofasern durch elektrostatische Verspinnung unter Einsatz von Olivenöl mit Hilfe von Gelatinepolymeren. Beim Olivenöl handelt es sich um ein natürliches grünes Pflanzenöl, das reich an Vitaminen, Karotin und vielen Spurenelementen ist. Olivenöl hat viele Funktionen innerhalb der Ernährung und der Gesundheitspflege und ist auch reich an essenziellen Fettsäuren. Darunter befinden sich die Vitamine A, D, E, K und andere Antioxidantien, die vom Körper schnell absorbiert werden können und dazu beitragen, die Haut elastisch und feucht zu halten. Aus diesem Grunde konzentrieren wir uns in dieser Studie auf die Erzeugung einer Nanofaserschicht, die Olivenöl enthält und auch auf medizinischem Gebiet Verwendung finden kann. Nanofasern werden mit der Methode der zweikomponentigen elektriostatischen Verspinnung unter dem Einsatz von koaxialen Schärdrüsen gezogen.

PRODUKCJA NANOWŁÓKIEN METODĄ ELEKTROPRZĘDZENIA PRZY WYKORZYSTANIU IGŁY KOAKSJALNEJ, Z ZASTOSOWANIEM OLIWY Z OLIWEK ORAZ POLIMERU ŻELATYNOWEGO

Niniejsze opracowanie poświęcone jest produkcji nanowłókien metodą elektroprzędzenia przy wykorzystaniu oliwy z oliwek i polimeru żelatynowego. Oliwa z oliwek jest naturalnym zielonym olejem roślinnym, bogatym w witaminy, karoten oraz wiele pierwiastków śladowych. Oliwa z oliwek ma wiele funkcji w żywieniu i opiece zdrowotnej oraz zawiera wiele esencjalnych kwasów tłuszczowych, w tym witaminy A, D, E, K oraz inne przeciwutleniacze, które mogą się szybko wchłaniać, pomagając w utrzymaniu elastycznej i nawilżonej skóry. Z tego powodu w prowadzonych badaniach skupiliśmy się na produkcji warstwy nanowłóknowej zawierającej oliwę z oliwek, która może być wykorzystana w dziedzinie medycyny. Nanowłókna wytwarzane są metodą dwuskładnikowego elektroprzędzenia przy wykorzystaniu dyszy (igły) koaksjalnej.