2021 年臺灣國際科學展覽會
優勝作品專輯

作品編號　**090027**

參展科別　醫學與健康科學

作品名稱　**Fabrication and Characterization of Biological Electrospinning Nanofiber Scaffold Based on Cellulose Diacetate-Gelatin-Green Tea for Tissue Engineering Applications**

得獎獎項　大會獎　三等獎

國　　家　**Iran**

就讀學校　**Avicenna research center**

指導教師　**Somayeh Baqersad**

作者姓名　**Zohre Mahdavi Sabet**

關鍵詞　**Cellulose di-acetate、Gelatin、Green tea**

作者照片

Abstract

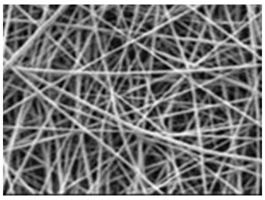
Tissue engineering has developed novel therapies such as many types of wound dressings, bio-pads, scaffolds and bandages, in order to reduce the effects of deep and extensive skin wounds. Here, we have produced an electrospun nanofiber scaffold, based on biodegradable materials such as gelatin (as a natural and hydrophilic polymer) and cellulose diacetate (with optimal biodegradability), in order to increase wound healing using nanotechnology. We also used green tea extract for its anti-oxidant and anti-bacterial effect, to improve the biological properties of the scaffold. In the fabrication process, two polymer solutions: 1. Gelatin (with acetic acid solvent) and 2. Cellulose Diacetate (with acetone solvent) mixed with green tea extract, were prepared. Then they were spun using a two-nozzle electrospinner to produce a hybrid nanofiber scaffold. SEM images showed enough finesse and uniformity of the produced scaffold to simulate the extracellular matrix. Further, measuring the contact angle of water droplet and the web surface, indicated optimal hydrophilicity of the nanofiber scaffold, which controls the level of scaffold degradability and cell adhesion. Also, the results of antibacterial tests for two bacterial strains (E. coli and S. aureus) showed the antibacterial characteristics of the extract-containing scaffold. In addition to previous tests, evaluation of fibroblast morphology on the nanofiber scaffolds, indicated appropriate cell adhesion and expansion, that confirms the biocompatibility of this produced scaffold.

Introduction

Skin is one of the most vulnerable tissues in the human body, and each year, significant skin damages occur to millions of patients, due to an accident or illness. Deep and extensive skin wounds such as burn wounds, deep cuts, surgical wounds, etc. usually do not heal easily and are associated with compilations like infection in the bedsore, excess flesh or skin lesions. Today, the science of tissue engineering has provided new treatment methods, such as various dressings, biological pads, scaffolds, bandages, etc. in order to mitigate those complications.

Basically, tissue engineering is imitating the nature to provide internal and external conditions of the body. It has a special role in repairing or regenerating various kinds of tissues of the human body such as skin, bone, cartilage, cornea, arteries, nerves, etc. Creating a new tissue outside the body, requires a fine and optimal environment for cells to settle, which is capable of mimicking the extracellular matrix of tissues, called the cell scaffold. In the process of tissue engineering, cells are placed on scaffolds, and are grown in culture medium. Interactions between cells and scaffold, could affect cellular activities such as adhesion, migration, proliferation, differentiation and gene expression. Therefore, the more advanced the scaffold can imitate the extracellular matrix, the more likely it would be for cells to detect and approach the desired tissue. The type of the polymer which is used and the method of its synthesis are the two main components of making a perfectly similar scaffold to extracellular matrix. One of the most comparable structures to extracellular matrix in tissue engineering is nanofiber structures. In fact, nanofibers are an imitation from the structure of the extracellular matrix [1].

Nanofiber, is the general term for fibers whose fineness are in the nanometer range. They have a one-dimensional structure, which means they have two dimensions at the nanoscale and one dimension in free.



They have incredible characteristics such as special surface and very high flexibility. One of the main approaches for the production of nanofibers is the electrospinning method. Electrospinning is capable of producing fibers with less than 500 nm diameter. Therefore, the fibers which are produced by this method, have a large surface to volume ratio. Electrospun nanofibers, can be specially used in medical applications such as wound dressing, biological scaffolding and drug delivery [2]. Figure 1 shows a sample of electrospun nanofibers.

Figure1. The electrospun nanofibers [3]

The texture of nanofiber has an important role in simulating a scaffold to the ECM and in tissue engineering. At this time, depending on the polymer characteristics and the purpose of its application, there is a wide range of natural or synthetic biopolymers, available for electrospinning and production of nanofiber scaffolds. Biopolymers are a class of biomaterials, which are easier to produce, compared to other biomaterials. They are formable (in 3D and nanofibers) and cost-effective [4]. Biopolymers are classified in two categories, natural and synthetic, each with different characteristics and applications. According to valid and reliable reports, gelatin and cellulose diacetate polymers have significant properties. In this project, we have used these two materials together under optimal and controlled conditions, with the aim of composing an ideal scaffold with the best simulation of skin and its biocompatibility properties.

Gelatin is a natural active polyamide that has proper biocompatibility, high hydrophilicity and biodegradability. It is made from hydrolyzed collagen protein. Also, it’s translucent, colorless and jelly, and is obtained from collagen found in the skin and bones. Gelatin liquefies at 37° C. Due to the above features; Gelatin is a reasonable alternative for cell scaffolding, as cells could fully attach and differentiate on it [5]. Figure 2 shows an example of a gelatin polymer network.

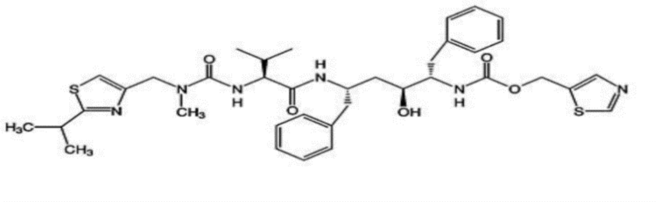


Figure2. Polymer network of gelatin [6]

Cellulose diacetate is a biocompatible polymer and a derivative of cellulose, which is obtained from the reaction of cellulose with acetic acid. Cellulose diacetate has two ester groups and one hydroxyl group which has good biodegradability, optimized hydrophilicity and good mechanical properties [7,8]. Figure 3 shows an example of a cellulose diacetate polymer network.

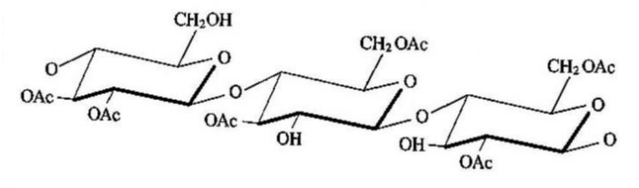


Figure3. Polymer network of cellulose diacetate [8]

Plant extracts, which have been long considered in the field of regenerative medicine, can be a good option in this issue. One of these plants is green tea, which has exclusive properties in traditional medicine. Green tea has antioxidant and antibacterial properties and prevents the formation of cancer cells. It has also been shown beneficial in reducing the skin cell death following sun exposure. In addition, daily consumption of green tea reduces the allergic reactions [3]. In this study, in order to increase the speed of wound healing using nanotechnology, biodegradable polymer nanofibers were produced based on cellulose diacetate, gelatin and green tea extract.

Previous Studies & Research Background

1- Kazimpour, Khademi et al. in 2015, studied the antioxidant properties of oral gelatin coatings enriched with green tea extracts. The results showed the activated oral coating of gelatin had an antibacterial effect and its enrichment with higher levels of green tea extract improved its performance [9].

2- Yousefi, Karimi, Ghare-aghaji, Asgharian et al. in 2015, investigated the morphology of pores in porous electrospun fibers of cellulose diacetate by adjusting the feeding rate. By observing the SEM images, they concluded that by increasing the feed rate, the pore size on the fibers increases. They also found that the depth and uniformity of pores also increased with increasing the feed rate. The increase in pore size has been attributed to the larger diameter of the resulting fibers [8].

3- Zare Khalili et al. in 2015, studied the effect of electrospinning parameters on the diameter and biological properties of polycaprolactone/tragacanth nanofibers. They found that the hydrophilic nature and sufficient elongation of the nanofiber of the produced scaffolds, as well as the antibacterial properties against gram-negative and gram-positive bacteria, make them appropriate for skin tissue repair and wound healing applications [10].

4- In 2016, Baqersad et al. investigated PCL /aloe vera /gelatin electrospinning and the antibacterial properties of the resulting nanofibers. They found that gelatin has excellent hydrophilicity and biological properties, but like many other natural polymers, its mechanical properties are weak; Therefore, in order to eliminate this limitation, they benefited from the addition of polycaprolactone synthetic biopolymer (with the least probability of interaction with other components) to the nanofiber scaffold. Scaffolds with these characteristics were produced using the two-nozzle electrospinning method. They reported the concentration of gelatin as the only factor affecting the morphology of nanofibers. Also, the increase in the natural component in the nanofiber web has increased its hydrophilicity. They proved that electrospun nanofiber scaffolds have a great ability to attach to and grow fibroblast cells, which is due to the presence of various compounds in aloe vera and the hydrophilic properties and sufficient strength of the scaffold [2].

5- Khorshidi, Solouk, Mazinani, Mirzadeh and colleagues conducted a research with the aim of optimizing cell penetration in electrospun nanofiber scaffolds. They concluded that simulating the fibrous arrangement of extracellular matrix in the body, high surface-to-volume ratio, significant porosity and complete disruption continuity are the most important advantages of electrospinning structures [11].

Methods

- **Phase 1- Polymer Solutions Preparation**

In order to prepare a gelatin solution (15% w/v), a certain amount of gelatin powder was added to 90% acetic acid solvent. The next step was to prepare a solution of cellulose diacetate-green tea. For this purpose, cellulose diacetate powder (14% w/v) and green tea extract (5% w/w - relative to the weight of cellulose diacetate) were added to 90% acetone solvent. Then, in order to prepare a homogeneous solution, it was placed on a magnetic stirrer for 3 hours.

- **Phase 2- Electrospinning**

In order to electrospun the hybrid nanofibers, an electrospinning device with two opposite nozzles was used. Solutions of cellulose diacetate, green tea and gelatin were connected separately to feed pumps via 5 ml syringes (with gauge 19). They were then simultaneously and separately electrospun on an aluminum sheet, which was placed on a rotary collector (400rpm).

Electrospinning of the prepared homogeneous solutions was performed for 4 hours in the laboratory at room temperature under the following conditions:

- Gelatin solution: feed rate 0.2 ml/h, spin distance 13 cm, applied voltage 13 kV
- Cellulose Diacetate/Green Tea Solution: feed rate 0.6 ml/h, spin distance 13 cm, applied voltage 14kV

In order to evaluate the efficiency of green tea extract, it was necessary to compare it with a control sample. Therefore, a scaffold without green tea extract was electrospun, as the control sample. This cellulose diacetate-gelatin scaffold, similar to the main scaffold containing green tea extract, was produced by double-nozzle electrospinning and under completely the same conditions.

Results and Discussion

The results of our controlled experiments were compared with an optimal and desirable engineered tissue scaffold. Outcomes are reported both analytically and numerically.

I. Morphology of Nanofibers:

SEM images of nanofibers were prepared to evaluate their similarity to ECM. The fineness of the nanofibers was calculated using Image J software for 20 fibers. Both extract-containing and control scaffolds are appropriate options for ECM simulation. Figure 4 shows the results of this test.

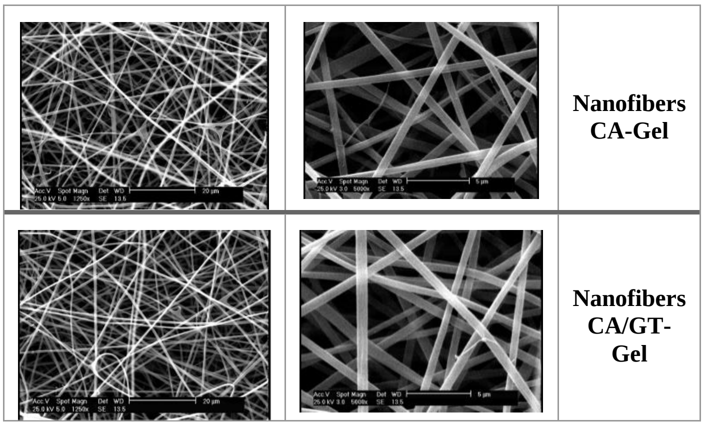


Figure4. SEM images of nanofiber scaffolds with and without extract in two magnifications

Findings from SEM images indicate that the produced nanofiber scaffold can well simulate the extracellular matrix of tissues, especially nanoscale collagen fibers.

II. Level of Hydrophilicity:

After measuring the angle of water droplet with the nanofiber web surface using Image J software, we found that the presence of strong functional groups in gelatin polymer, has led to scaffold hydrophilicity and the presence of this polymer along with cellulose diacetate (with more limited hydrophilicity), has caused the desirable and optimized hydrophilicity of the scaffold. Figure 5 shows the contact angle of a water droplet with a nanofiber web surface.

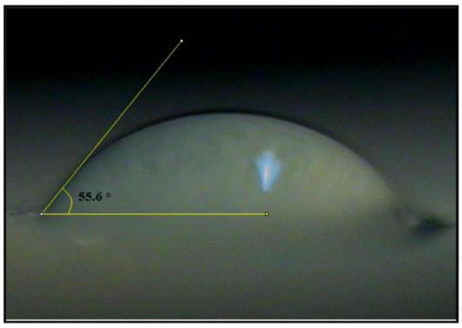


Figure5. Contact angle of water droplet with the surface of nanofiber scaffold containing extract

According to the results of hydrophilicity test, it can be concluded that the presence of gelatin hydrophilic polymer, provides the needed hydrophilicity for the scaffold and the presence of relatively hydrophobic cellulose diacetate, has optimized this hydrophilicity. The results of the cell adhesion test confirm the optimal hydrophilicity of the scaffold. According to the contact angle reported in the hydrophilicity test, it can be concluded that the presence of cellulose diacetate polymer due to its relatively hydrophobic nature, has prevented early destruction of the scaffold (while contacting body fluids). Also, in general, an optimal portion of both types of polymers in the structure of the scaffold controls biodegradability.

III. Antibacterial Tests:

The results of antibacterial test with two gram-negative bacteria of Escherichia coli and gram-positive Staphylococcus aureus, showed that the scaffold containing green tea extract has antibacterial characteristics and the zone of the lack of bacterial growth was observed in both plates.

Table1. Shows the amount of bacterial growth zone for both scaffolds

Sample Image	Bacterial Growth Zone	Bacteria Strain
	0 mm= 1, 2 (without extract) 15 mm= 3, 4 (with extract)	**E. Coli**
	17 mm= 1, 2 (with extract) 0 mm= 3, 4 (without extract)	**S. Aureus**

The results of the antibacterial test on both gram-negative and gram-positive bacterial species, clearly showed the antibacterial power of the sample containing green tea extract, which makes this scaffold beneficial for skin tissue engineering, especially in burn wounds where there is infection in the site of injury.

IV. Cell Attachment and Adhesion to the Scaffolds:

In collaboration with the Pasteur Institute Cell Bank Center, fibroblasts were cultured on both types of scaffolds (containing and non-containing the tea extract). After 2 days, the morphology of the cells was examined by scanning electron microscopy with two different magnifications. It was found that cells were able to grow and expand on both types of scaffolds, which had good biocompatibility. Figure 6 shows the morphology of fibroblast cells on both types of scaffolds.

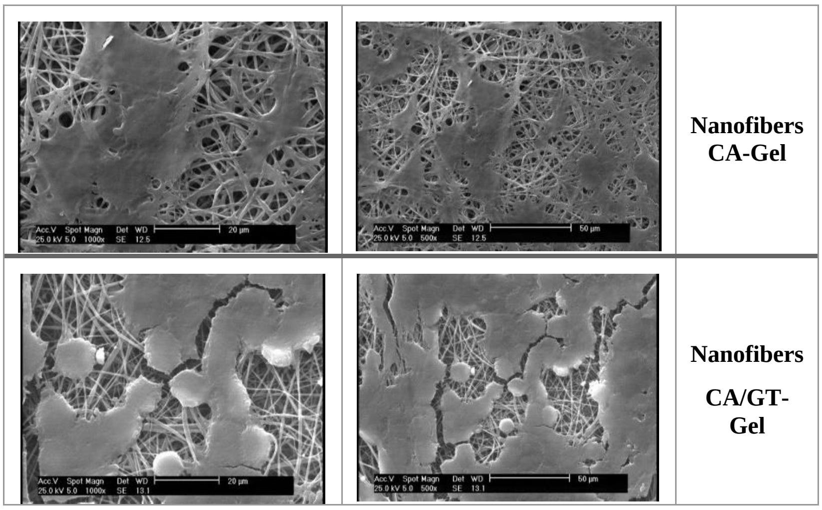


Figure.6 Morphology of fibroblast cells on nanofiber scaffolds in two magnifications

Fibroblast cell cultures and their morphology on the scaffold indicate adhesion, proper growth, full expansion of cells (no cells were found in a spherical state), which confirms its optimal biocompatibility.

Recommendations for Future Studies

1) Investigation of biodegradability of the produced scaffolds.
2) Performing MTT cell cytotoxicity test to ensure scaffold biocompatibility.
3) Performing in-vivo tests to evaluate the response of the living organism to the prepared scaffold.

References

1. Nasuri, Kamil. Azarnia, Abolfazl. Applications of nanofibers in biomedicine. Comprehensive nanotechnology training system: Edu.nano.ir.
2. Baqersad, Somayeh. Electrospun of PCL /Aloe vera and investigation of antimicrobial properties of the produced nanofibers. Amirkabir University of Technology (Tehran Polytechnic) Faculty of Textile Engineering, 2016.
3. Malikdar, Maryam. Nanofibers, Introduction and Opportunities. Tebyan Article.tebyan.net, March 11, 2008.
4. Amirkabir University of Technology, www.aut.ac.ir.
5. Jirofti, Nafiseh. Mohebbi Kalhori, Davood. Kazemzadeh, Gholam Hossein. Taheri, Reza. KhodaDoust, Marzieh. A review of common polymers of artificial vessels used in vascular surgery and dialysis.jms.mums.ac.ir Journal of the School of Medicine, December 2016.
6. www.sigmaaldrich.com.

7. Ebadi, Shahrzad. Specification of the most important variables in the process of cellulose acetate production. Shahid Beheshti University, Faculty of Energy Engineering and New Technologies, 2014.
8. Yousefi, Bahareh. Karimi, Mohammad. Qara-Aghaji, AliAkbar. Asgharian Jedi, Ali Asghar. Alteration of pore morphology in porous electrospun fibers of cellulose acetate by adjusting the feed rate. 10th National Conference on Textile Engineering of Iran, 2015.
9. Khalfi Kazempour, Zahra. Khademi Shurmasti, Dariush. Evaluation of antimicrobial effect of green tea extract using active gelatin coating on the life of rainbow trout fillets. Islamic Azad University, Ayatollah Amoli Branch; Food processing and production, 2015.
10. Khalili Zare, Zahra. Influence of electrospinning parameters on diameter and biological properties produced from polycaprolactone /tragacanth. Amirkabir Polytechnic University of Tehran, Faculty of Textile Engineering, 2015.
11. Khorshidi, Sajedeh. Solouk, Atefeh. Mazinani, Saeedeh. Mirzadeh, Hamid. A review on the methods used to improve cell penetration in electrospun nanofiber scaffolds. Biopathology Research Publications; Miranjafizadeh, Seyed Javad. Winter 2015.

Acknowledgements

As it is said that " If I do not thank the creature, I do not thank the Creator", I have to thank my wise advisors, Dr. Baqersad and Ms. Hayai, who has truly helped me in carrying out this research. I have also to thank prominent professors of Amirkabir Textile College; Dr. Baahrami and Dr. Qolipour Ardakani. Also, I have to acknowledge Dr. Ramezani (Dowlat Laboratory’s lab manager), experts in Cell Bank of Pasteur Institute of Iran, Mrs. Derougar (principal of Farzengan 3rd High School) and Mrs. Faraji (Research Deputy in Farzanegan 3rd High School). I am very grateful to thank all other beloved ones who has helped and accompanied me in this way.

Appendix

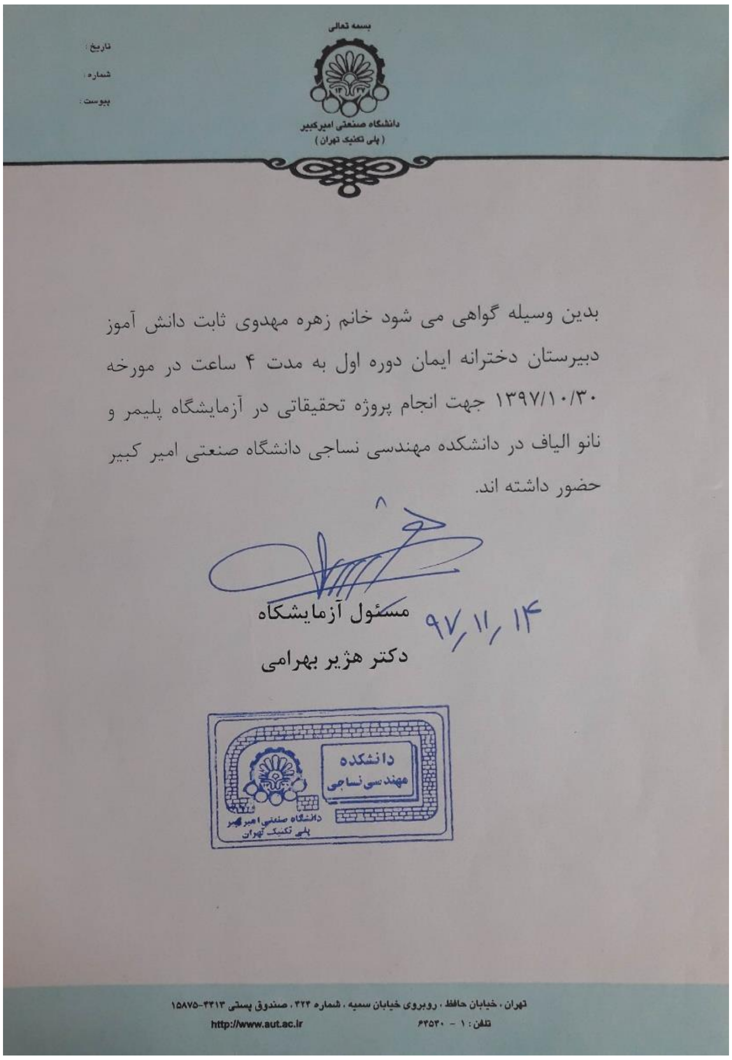
Amirkabir University of Technology
(Tehran Polytechnic)

"AmirKabir University of Technology"

This is to confirm that Ms. Zohre Mahdavi-Sabet, the student of Farzanegan 3rd High School, has participated in a 4-hour session, in the January 20th 2019, to conduct a research project in polymer and nanofibers laboratory in Textile faculty of Amirkabir University of Technology.

Dr. Hazhir Baahrami
Lab Manager

February 3rd 2019



بسمه تعالی

تاریخ :
شماره :
پیوست :

دانشگاه صنعتی امیرکبیر
(پلی تکنیک تهران)

بدین وسیله گواهی می شود خانم زهره مهدوی ثابت دانش آموز دبیرستان دخترانه ایمان دوره اول به مدت ۴ ساعت در مورخه ۱۳۹۷/۱۰/۳۰ جهت انجام پروژه تحقیقاتی در آزمایشگاه پلیمر و نانو الیاف در دانشکده مهندسی نساجی دانشگاه صنعتی امیر کبیر حضور داشته اند.

مسئول آزمایشگاه ۹۷/۱۱/۱۴
دکتر هژیر بهرامی

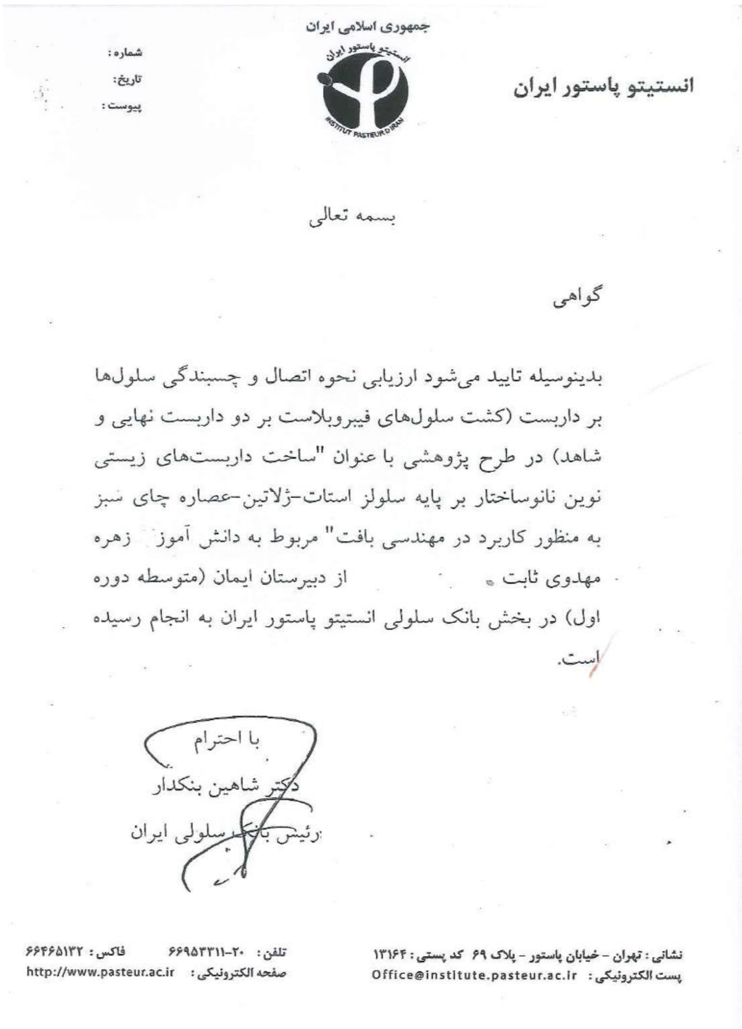
تهران ، خیابان حافظ ، روبروی خیابان سمیه ، شماره ۴۲۴ ، صندوق پستی ۴۴۱۳-۱۵۸۷۵
تلفن : ۱ - ۶۴۵۴۰ http://www.aut.ac.ir

Pasteur Institute of Iran
Research, Production, Education Center

"Pasteur Institute of Iran"

In the Name of God
It is to certify that a research project entitled "Fabrication and Characterization of Biological Electrospinning Nanofiber Scaffold Based on Cellulose Diacetate-Gelatin-Green Tea for Tissue Engineering Applications", concerning the evaluation of cell attachment and adhesion to scaffolds (in particular, the fibroblast cell culture on a specialized and a control scaffold), has been done in Cell Bank Center of Pasteur Institute of Iran by Zohre Mahdavi-Sabet (Student of … Farzanegan 3rd High School).

Regards,
Dr. Shahin … Bonakdar; Head of Iran Cell Bank


جمهوری اسلامی ایران

شماره:
تاریخ:
پیوست:

انستیتو پاستور ایران

بسمه تعالی

گواهی

بدینوسیله تایید می‌شود ارزیابی نحوه اتصال و چسبندگی سلول‌ها بر داربست (کشت سلول‌های فیبروبلاست بر دو داربست نهایی و شاهد) در طرح پژوهشی با عنوان "ساخت داربست‌های زیستی نوین نانوساختار بر پایه سلولز استات-ژلاتین-عصاره چای سبز به منظور کاربرد در مهندسی بافت" مربوط به دانش آموز زهره مهدوی ثابت از دبیرستان ایمان (متوسطه دوره اول) در بخش بانک سلولی انستیتو پاستور ایران به انجام رسیده است.

با احترام
دکتر شاهین بنکدار
رئیس بانک سلولی ایران

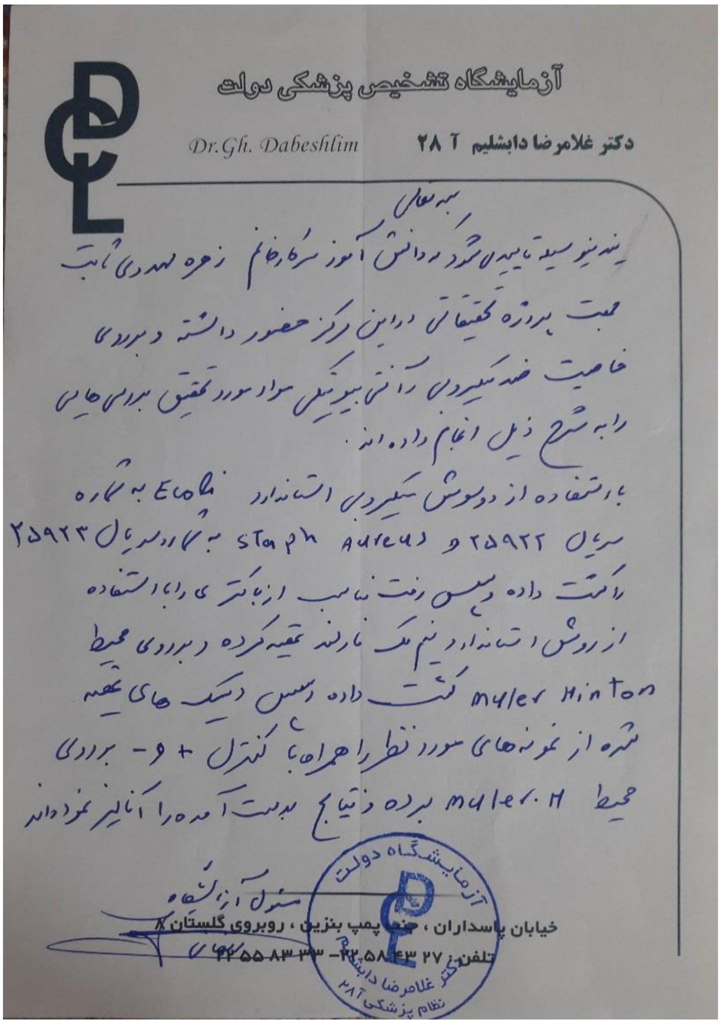
نشانی: تهران - خیابان پاستور - پلاک ۶۹ کد پستی: ۱۳۱۶۴ تلفن: ۲۰-۶۶۹۵۳۳۱۱ فاکس: ۶۶۴۶۵۱۳۲
پست الکترونیکی: Office@institute.pasteur.ac.ir صفحه الکترونیکی: http://www.pasteur.ac.ir

"Dowlat Medical Pathology Laboratory"

To whom it may concern;
It is to certify that Ms. Zohre Mahdavi-Sabet, has been worked in this research center, in order to conduct a project on antibacterial antibiotic effects of a specified extract.
The experiments that she has done are as follows:

1. Culturing two bacterial strains:
   A) E. coli (Serial Number: 25922)
   B) S. aureus (Serial Number: 25923)
2. Preparing the suitable amount of bacteria using McFarland Standard Method
3. Culturing the prepared bacteria on Mueller-Hinton agar medium
4. Transferring the obtained disks (from samples in previous steps) to Mueller-Hinton agar medium, along with positive and negative controls
5. Analyzing the final data

Dr. Gholamreza Dabeshlim
Lab Manager of Dowlat Medical Pathology Laboratory


آزمایشگاه تشخیص پزشکی دولت
Dr.Gh. Dabeshlim — دکتر غلامرضا دابشلیم ۲۸ آ

بسمه تعالی
بدینوسیله تایید می‌گردد دانش آموز سرکار خانم زهره مهدوی ثابت
جهت پروژه تحقیقاتی در این مرکز حضور داشته و بررسی
خاصیت ضد باکتری آنتی بیوتیکی مواد مورد تحقیق برای این
را به شرح ذیل انجام داده اند.
با استفاده از دو سوش میکروبی استاندارد باکتری E coli به شماره
سریال ۲۵۹۲۲ و Staph aureus به شماره سریال ۲۵۹۲۳
را کشت داده و سپس رقت مناسب از باکتری را با استفاده
از روش استاندارد نیم مک فارلند تهیه کرده و بر روی محیط
Muler Hinton کشت داده سپس دیسک های تهیه
شده از نمونه های مورد نظر را همراه با کنترل + و - بر روی
محیط Muler.H برده و نتایج بدست آمده را آنالیز نموده اند
مسئول آزمایشگاه

خیابان پاسداران، جنب پمپ بنزین، روبروی گلستان ۸
تلفن: ۲۲۵۸۴۳۲۷ - ۲۲۵۵۸۳۳۳

【評語】090027

The presentation is quite impressive！The implication of the ECM with PDMS bioengineering, together with green tea antioxidative extract is certainly innovative, which might provide a new paradigm for future medical application.