RESEARCH ARTICLE

# The study of biocompatibility of electrospun polycaprolactone/gelatin scaffold containing double layer hydroxide nanoparticles on L929 mouse fibroblast cells

***Mahsa Mirzavand[1], Tahereh Foroutan[1*], Seyedeh Sara Shafiei[2]***

*[1] Department of Animal Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*
*[2] Department of Stem Cells and Regenerative Medicine, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

**ARTICLE INFO**

***Article History:***
*Received 13 Jun 2024*
*Accepted 07 Sep 2024*
*Published 01 Dec 2024*

***Keywords:***
*polycaprolactone/gelatin*
*layered double hydroxide nanoparticles*
*fibroblast cells*
*L929*

**ABSTRACT**

**Objective(s):** Nanofibrous scaffolds provide an environment similar to the body's extracellular matrix for cells, which affects cell morphology, adhesion, proliferation, and function. The present study aimed to investigate the biocompatibility properties of electrospun polycaprolactone-gelatin (PCL/Gel) scaffold containing layered double hydroxide (LDH) nanoparticles on mouse L929 fibroblast cells.

**Methods:** In this study, LDH nanoparticles were synthesized by co-precipitation method. A scaffold made of polycaprolactone and gelatin with 1% wt of optimized nanoclay was fabricated using electrospinning method. The synthesized nanoparticles were evaluated by XRD method and electron microscopy study. 2×104 L929 fibroblast cells were seeded on the scaffolds for 3 to 5 days. Then, different grades of ethanol were used to dehydrate the scaffolds. After drying the scaffolds, scanning electron microscopy was used to characterize and examine the fixed cells, and MTT test was used to evaluate the toxicity of the scaffolds.

**Results:** The results of the present study showed that with the addition of LDH reduced the fiber diameter and increased the biocompatibility of the scaffold after culturing mouse L929 fibroblast cells. Also, scanning electron microscope images indicated better interaction and proliferation of L929 cells on PCL/Gel/LDH compared to the PCL/Gel group.

**Conclusions:** Adding LDH to PCL/Gel nanofibers is effective on the adhesion and viability of cells.

**How to cite this article**
*Mirzavand M., Foroutan T., Shafiei S.S. The study of biocompatibility of electrospun polycaprolactone/gelatin scaffold containing double layer hydroxide nanoparticles on L929 mouse fibroblast cells. Nanomed Res J, 2024; 9(4): 424-431. DOI: 10.22034/nmrj.2024.04.009*

## INTRODUCTION

Nanofiber scaffolds with many pores provide an environment similar to the body's extracellular matrix for cells, which affects the morphology, adhesion, migration, proliferation, differentiation, and function of cells (1). The type of biomaterials used in the production of nanofiber scaffolds is important and the electrospinning method is used as an effective method in the production of nanofibers (2). Raw materials made of scaffolds are natural and synthetic polymers that are composited with other compounds such as nanoparticles and provide optimal scaffolding (3).

An efficient scaffold is biocompatible and biodegradable. This scaffold does not cause immune reactions in the body, and is not toxic to cells. It degrades over time and is excreted from the body without causing damage or harmful substances. It has appropriate porosity and supports the release of nutrients to cells, angiogenesis, and migration (4). Polycaprolactone (PCL) is a synthetic polymer that can be combined with a variety of polymers. It is hydrophobic and has a low melting point (5).

* Corresponding Author Email: *tataforoutan@gmail.com*

It is biocompatible and non-toxic to cells. PCL is easily processed due to its low melting point and exceptional compatibility in mixing with other polymers, so it is important in the field of biomedicine and the production of cellular scaffolds (6).

The hydrophilicity and proteinaceousness of the PCL surface are known to be a promoter of cell growth. For this reason, researchers are focusing on immobilizing biomolecules such as gelatin on electrospun scaffolds and using this method to improve the scaffold's compatibility (7). Gelatin, a natural polymer, has properties such as high-speed biodegradability, good biocompatibility, water solubility, flexibility, and very low immunogenicity. It contains 18 amino acids, of which glycine is the predominant amino acid in gelatin and modulates cellular adhesion. Gelatin-based scaffolds are used for a variety of medical applications such as bone regeneration, skin tissue engineering, neural tissue engineering, and drug delivery (8-10). Gelatin is combined with PCL to improve cell adhesion and biodegradation rate. Many studies have shown the application of PCL/Gel-based electrospun scaffolds in the fields of wound dressing and soft tissue engineering such as nerve, cartilage, and skin.

layered double hydroxide nanoparticles (LDH) are classified in the category of bioceramics and are used together with polymers in the manufacture of nanocomposite scaffolds. The functional and important features of these materials are biocompatibility, low cytotoxicity, high chemical stability, solubility or pH-controlled release property in acidic environments, unique anion exchange properties, as well as easy and low-cost synthesis method (11-13). The aim of this study is to fabricate polycaprolactone-gelatin electrospun scaffolds, which have benefited from both synthetic and natural polymer properties. Also, in order to enhance the physical and biological properties of this scaffold, LDH nanoparticles will be added to it at specific concentrations. the biocompatibility properties, the cell viability, and morphology of L929 fibroblast cells on scaffolds were evaluated.

## MATERIALS AND METHODS

*Materials used in LDH synthesis*

For the synthesis of LDH and the manufacture of scaffolds, magnesium chloride (105833) and aluminum chloride (101084) and gelatin (1040700500) made by Merck company were used. Polycaprolactone (100141014) from the company Sigma was prepared, all solvents and consumables were used in the laboratory grade.

*Synthesis of LDH*

First, magnesium chloride and aluminum chloride salts were dissolved in a ratio of 2:1 in 50 ml of deionized water, and then the pH of the solution was adjusted to 10 by adding 0.5 M sodium hydroxide solution dropwise. Then, the samples were left on a magnetic stirrer for 24 hours for the aging process at room temperature. After the required time, the LDH precipitate was collected by centrifugation for 6 minutes at 4500 rpm, and the washing process continued until the pH reached 7. Finally, the sample was transferred to a freeze dry for drying to prepare powder (14).

*Fabrication of electrospun scaffolds*

Electrospun scaffolds were fabricated with a specific weight percentage of LDH relative to the polymer phase (the optimum obtained was considered to be 1%wt). The Soluble to solvent ratio was fixed in all samples and was considered to be 10%wt. Also, the ratio of PCL to gelatin was considered to be 50:50. First, all solid materials were weighed on a scale, then the materials were placed in the solvent on a magnetic stirrer for a specific time to dissolve the polymer phase. Then LDH was added to the polymer solution. In order to uniformly and stably disperse the LDH particles in the solution, an ultrasonic bath was used for one hour. Next, 5 ml of the prepared solutions were poured into a syringe with a needle with an internal diameter of 21G, and then the prepared syringe was placed in a special nozzle holder at a distance of 17 cm from the collector plate. At this stage, the electrospinning process was started by applying a voltage of 21 kV and an injection rate of 1 ml/h. The electrospun fibers were collected on foil and stored in the refrigerator for longer shelf life (15).

*LDH nanoclay characterization*

In order to evaluate the synthesized nanoparticles, the X-ray diffraction (XRD) method was used. X-ray diffraction is a method for studying the structure of crystalline materials. X-ray diffraction or XRD is used for phase analysis and to examine the size of grains and particles of nanomaterials. This is possible through the processing and analysis of X-rays returned from the sample surface. In this experiment, the phases in the synthesized powder were identified by the

X-ray diffraction technique. The X-ray diffraction pattern was prepared using a D5000 diffractometer manufactured by SIEMENS with CuKα radiation at a wavelength of 1.54051 and The accelerator voltage of 35 kV was prepared in the range of 2 out of 5 with a length of 0.02 steps.

The obtained data were plotted using Excel software. The morphology of nanoparticles and fibers was evaluated by scanning electron microscopy.

*Scaffolds characterization*

In this study, the ALS2100 scanning electron microscope made by Seron of South

Korea was used to evaluate the structure of nanofibers and also to evaluate the shape and morphology of cells cultured on scaffolds. In this microscope, an electron column must first be created, for which electron guns are used. The larger the number of electrons and the smaller the diameter of this column, the more desirable it will be, while the similar speed of these electrons is considered as their other positive feature. After the production of this column of electrons, according to the conditions desired by the user, they are accelerated by creating an electric field and with the help of several electromagnetic lenses, its radius is reduced to the desired level. In this way, holes embedded in the passage of electrons are also used. After the electrons have reached the desired speed and the radius of the column has been adjusted, these electrons collide with a specific point of the object under complete control, and the result of their interaction with the sample is recorded by special sensors. Of course, it is clear that a specific sensor is required to record each interaction. After registering these works, the electron column is directed to the point adjacent to the current point and the effects of the interaction of this point are also recorded, and this is done for a 2D network on the surface of the body and for each point (and of course at a very high speed).

*L929 fibroblast cells*

In the present study, L929 cell line, which is of mouse fibroblast origin, was used to investigate the biocompatibility and adhesion of cells to scaffolds. The desired cells were prepared from the National Center for Genetic and Biological Resources of Iran and for their cultivation on scaffolds from the DMEM (Dulbecco's modified Eagle Medium medium), 10% FBS and (Fetal bovine serum) and 1% Antibiotics were used.

*Biocompatibility and investigation cell adhesion to the scaffold*

At this stage, cells are seeded on the scaffold. For this purpose, the scaffolds are cut into 2×1 cm dimensions and sterilization steps are performed on them. Then, 20,000 cells are counted and seeded on the scaffolds for 3 to 5 days. After the specified time, the scaffolds were washed with PBS and immersed in 2.5% glutaraldehyde for 3 days to stabilize the cells. After 3 days, the glutaraldehyde is removed and the scaffolds are washed with PBS. Then, to dehydrate the scaffolds, they are placed in 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% alcohols for 20 minutes, respectively. After the scaffolds were dried at room temperature, a scanning electron microscope was used to characterize and examine the stabilized cells.

*Assessment of scaffolds toxicity by MTT method*

The MTT test is based on a colorimetric method designed and based on the reduction and breakdown of material methyl thiazole tetrazolium by the mitochondrial enzyme succinate dehydrogenase present in living cells. The result of this process is the production of formazan blue crystal in the cytoplasm. 100 microliters of medium containing MTT were added to $10^5$ cells at 37°C. After removing the supernatant, 100 microliters of isopropanol were added. After 10 minutes, the absorbance was measured using an Elisa Reader Anthos 2020 ver1.8, Anthos Lab Tec (Instruments®, Austria) at a wavelength of 620 nm.

**RESULTS**

In order to verify the synthesis of LDH nanoparticles, XRD test was used. The main feature of the XRD pattern of clay minerals includes: a spectrum with a small number of peaks, sharp and symmetrical peaks at low angles, and relatively wide and asymmetric peaks at high angles. The XRD pattern for the synthesized LDH is shown in Figure 1-a. Based on the fact that X-ray diffraction is unique to each crystalline material, it can be identified based on the standard XRD pattern of the material. LDH X-ray diffraction pattern, at low angles, has peaks of (009), (003), (006), indicating that nanoparticles have been successfully synthesized. SEM was also used to investigate the morphology of nanoparticles. According to Figure 1-b, the morphology of LDH can be observed as

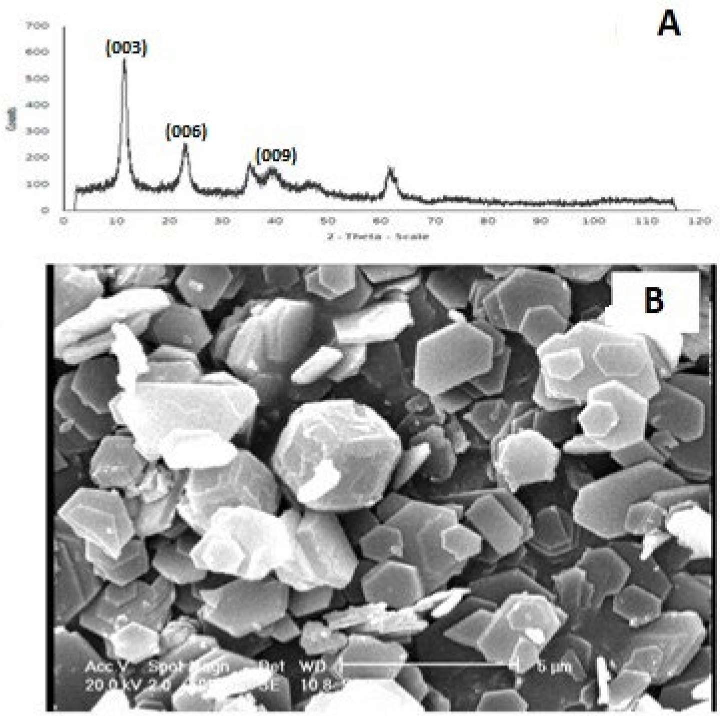


Fig. 1. A) XRD test on LDH nanoparticles. B) SEM image of synthesized nanoparticles.

hexagonal plates and disks. After making the scaffolds, a scanning electron microscope was used to evaluate the shape and diameter of the fibers. Figure 2 shows the SEM images of the fibers. The fiber diameter was measured using Image J software. As can be seen in the figure, all fibers have a smooth structure without beads. The average fiber diameter for the PCL/GEL scaffold is about 285 nm. This scaffold has uniform fibers. By adding LDH, the fiber diameter size decreased so that the average fiber diameter in the 1%wt LDH scaffold was about 230 nm. As the amount of LDH increases, fibers with non-uniform diameters and containing beads are formed, and agglomerated LDH particles are seen on part of the fibers. The biocompatibility of electrospun polycaprolactone/gelatin scaffold containing LDH nanoparticles was evaluated after culture of L929 mouse fibroblast cells using the MTT assay and the results showed that the survival rate of cells seeded on PCL/GEL/LDH was significantly increased compared to PCL/GEL ($P<0.01$) (Figure 3). Scanning electron microscopy images also indicated better interaction and proliferation of L929 cells on PCL/GEL/LDH (Figure 4a) compared to PCL/GEL (Figure 4b). In the first case, the cells penetrated into different layers, while in the lack of LDH, the cells were observed in more superficial layers.

## DISCUSSION AND CONCLUSION

Almost all cells in the body, except blood cells, tend to attach to a semi-solid substrate called the extracellular matrix (ECM). The extracellular matrix provides tissue-like mechanical properties, such as flexibility, tensile strength, and facilitates and manages the exchange of substances and electrolytes essential for tissue nutrition. Also, by having the property of biodegradability and regeneration by secretion of tissue cells, it promotes repair, migration, and the availability of growth factors for cells. In tissue engineering, the presence of a scaffold similar to the extracellular matrix, which can support cell attachment and proliferation and the release of growth factors, is considered essential. The results mentioned in this study indicate that the PCL/Gel scaffolds are suitable candidates for serving as tissue engineering scaffolds. In general, a suitable scaffold should have the following characteristics:

- **Biocompatibility:** Scaffolds must be biocompatible and degradable. Cells seeded on scaffolds should be able to function normally,

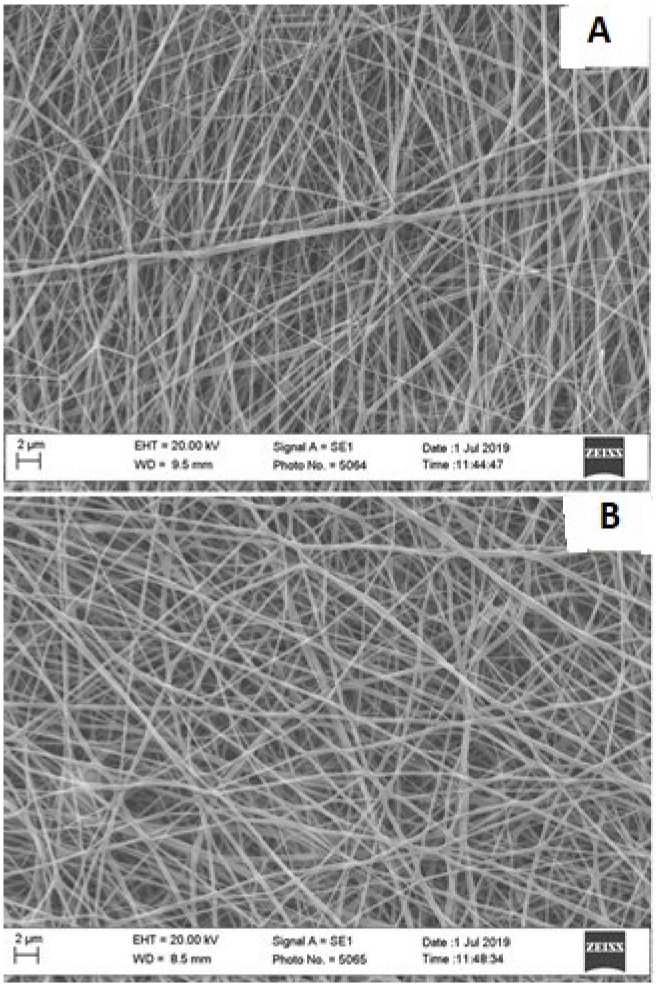


Fig. 2. Examining the morphology and diameter of fibers using SEM.

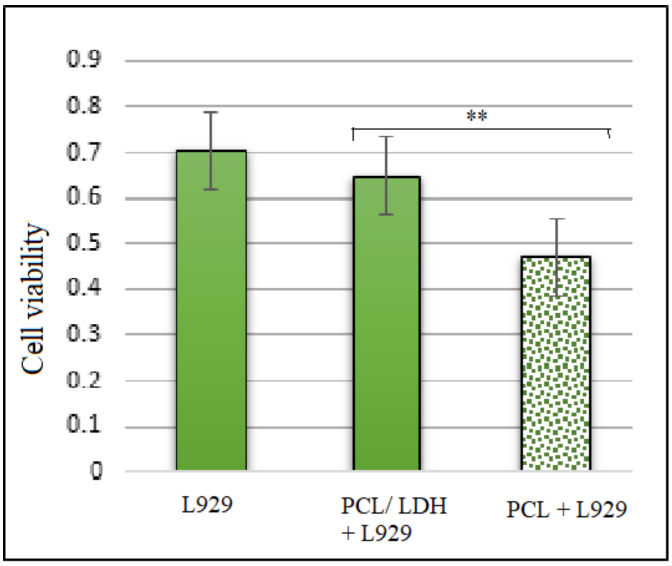


Fig. 3. Biocompatibility of electrospun polycaprolactone scaffold containing LDH nanoparticles after culture of L929 mouse fibroblast cells using MTT assay. Increased viability rate of cells seeded on PCL/LDH compared to PCL ($P<0.01$).

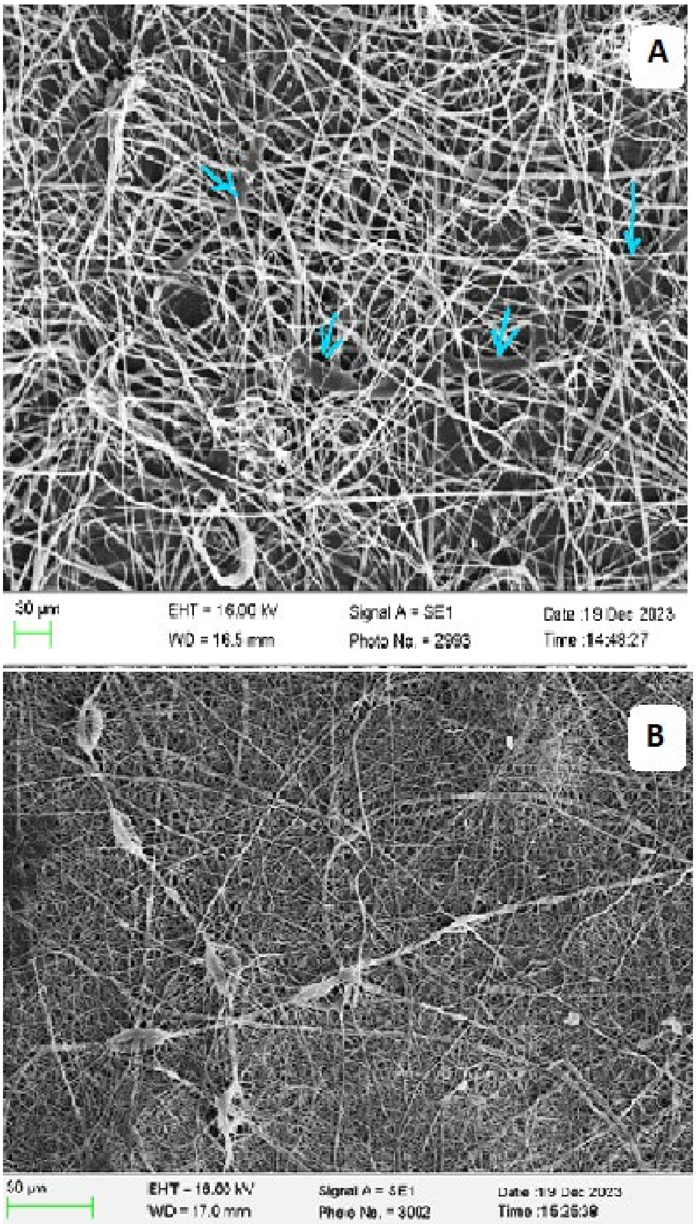


Fig. 4. Investigating the biocompatibility of L929 fibroblast cells (blue arrows) seeded on (a)PCL/Gel/LDH and (b) PCL/Gel electrospun scaffolds using SEM.

adhere to the surface, and begin to proliferate and migrate before the new matrix is produced. The scaffold should not cause any immune response in the body, and it should not be toxic to the cells.

• **Biodegradability:** Since scaffolds are not intended to be permanent, they must degrade over time and be able to be removed from the body without causing harm.

• **Mechanical properties and appropriate porosity:** The architecture of the scaffold should resemble the characteristics of the tissue in which it is to be implanted. A suitable scaffold has a highly porous structure. These pores support the diffusion of nutrients to the cells as well as angiogenesis and migration.

In this study, biocompatible polycaprolactone polymer with natural gelatin polymer with equal weight ratio was used to fabricate scaffolds. In general, PCL polymer, along with its numerous advantages in fabricating scaffolds, has disadvantages such as hydrophobicity, low degradability and poor interaction with the cell. For this reason, this polymer is commonly used in combination with other polymers and compounds. In a study in 2005, PCL and gelatin fiber scaffolds were fabricated separately, as well as in combination. The final results showed that the composite fibers are more desirable than gelatin or PCL alone. In addition to combining polymers with each other, adding nanoparticles and ceramics can have multiple effects on the final scaffold. Nanoparticles by combining and interacting with a variety of polymers reduce or increase the mechanical properties of scaffolds. scaffolds can also affect cells and cause them to send various types of messages to the cell, leading to growth and differentiation. Considering the results obtained from previous studies, as well as the lack of reports on the use of PCL/GEL scaffolds combined with LDH nanoclay on L929 fibroblast cells, in this study, Mg-Al LDH nanoparticles were selected as a suitable candidate for addition to PCL/GEL polymers to fabricate scaffolds. LDH nanoparticles were prepared by co-precipitation method and the necessary analyses were performed on them. XRD analysis was performed to verify the synthesis of LDH. Considering the consistent and Specific pattern of LDH in X-ray diffraction, the obtained spectrum indicates good synthesis of LDH. The presence of sharp peaks is due to the high crystallinity of LDH crystals. In this study, SEM images were used to examine the morphology of the synthesized fibers. SEM images showed uniform fibers without beads. The results of measuring the average fiber diameter using IMAGE J software showed that the fiber diameter decreased by adding LDH to the polymers. The main reason for this is due to the parameters affecting electrospinning. First, by adding LDH, the Solution conductivity coefficient increases. This action allows the solution in the needle tip to more easily overcome its surface tension and be affected by the voltage force, resulting in a decrease in fiber diameter (16). MTT assay and cell morphology studies using electron microscopy confirm good cell adhesion and growth on the scaffold. The results from scanning electron microscopy clearly show an increase in cell number after 3 days, indicating that LDH does not have a negative effect on cell proliferation. The MTT assay also showed that none of the scaffolds were toxic and that the addition of LDH increased cell viability and adhesion. As a result, it can be said that adding LDH is effective in adhesion and viability of cells. Finally, it can be concluded that adding LDH nanoparticles to the polymer matrix can provide a suitable substrate for cell growth and proliferation and be a good candidate for tissue engineering applications.

## CONFLICT OF INTEREST

The authors of this article have no conflict of interest.

## REFERENCES


1. Elsner JJ, et al. Novel biodegradable composite wound dressings with controlled release of antibiotics: Results in a guinea pig burn model. Burns. 2011;37(5):896-904. https://doi.org/10.1016/j.burns.2011.02.010
2. Doi Y, Steinbuchel A, Chen G. Biopolymers, Polyesters III - Applications and Commercial Products. Wiley-VCH; 2002.
3. Pazyar N, et al. Skin wound healing and phytomedicine: a review. Skin Pharmacol Physiol. 2014;27(6):303-10. https://doi.org/10.1159/000357477
4. Ghasemi-Mobarakeh L, et al. Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. Biomaterials. 2008;29(34):4532-9. https://doi.org/10.1016/j.biomaterials.2008.08.007
5. Khandwekar AP, et al. In vivo modulation of foreign body response on polyurethane by surface entrapment technique. J Biomed Mater Res A. 2010;94(2):227-52. https://doi.org/10.1002/jbm.a.32852
6. Hajiali F, Tajbakhsh S, Shojaei A. Fabrication and properties of polycaprolactone composites containing calcium phosphate-based ceramics and bioactive glasses in bone tissue engineering: a review. Polym Rev. 2018;58(1):164-207. https://doi.org/10.1080/15583724.2017.1332640
7. Liu X, Won Y, Ma PX. Surface modification of interconnected porous scaffolds. J Biomed Mater Res A. 2005;74(1):84-91. https://doi.org/10.1002/jbm.a.30367
8. Alihosseini F. Plant-based compounds for antimicrobial textiles. In: Antimicrobial Textiles. Elsevier; 2016:155-95. https://doi.org/10.1016/B978-0-08-100576-7.00010-9
9. Ghasemi-Mobarakeh L, et al. Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. Biomaterials. 2008;29(34):4532-9. https://doi.org/10.1016/j.biomaterials.2008.08.007
10. Mattioli-Belmonte M, et al. Tuning polycaprolactone-carbon nanotube composites for bone tissue engineering scaffolds. Mater Sci Eng C. 2012;32(2):152-9. https://doi.org/10.1016/j.msec.2011.10.010
11. Jin W, et al. Biocompatible hydrotalcite nanohybrids for medical functions. Minerals. 2020;10:172. https://doi.org/10.3390/min10020172
12. Lv F, et al. Layered double hydroxide assemblies with controllable drug loading capacity and release behavior as well as stabilized layer-by-layer polymer multilayers. ACS Appl Mater Interfaces. 2015;7(34):19104-11. https://doi.org/10.1021/acsami.5b04569
13. Senapati S, et al. Layered double hydroxides as effective carrier for anticancer drugs and tailoring of release rate through interlayer anions. J Control Release. 2016;224:186-

98. https://doi.org/10.1016/j.jconrel.2016.01.016
14. Baradaran T, et al. Poly(ε-caprolactone)/layered double hydroxide microspheres-aggregated nanocomposite scaffold for osteogenic differentiation of mesenchymal stem cell. Mater Today Commun. 2020;24:100913. https://doi.org/10.1016/j.mtcomm.2020.100913
15. Ahmadi S, et al. Electrospun nanofibrous scaffolds of polycaprolactone/gelatin reinforced with layered double hydroxide nanoclay for nerve tissue engineering applications. ACS Omega. 2022;7(22):18519-31. https://doi.org/10.1021/acsomega.2c02863
16. Mohamadi F, et al. Electrospun nerve guide scaffold of poly(ε-caprolactone)/collagen/nanobioglass: an in vitro study in peripheral nerve tissue engineering. J Biomed Mater Res A. 2017;105(7):1960-72. https://doi.org/10.1002/jbm.a.36068