Cumhuriyet Sci. J., 45(1) (2024) 88-93
DOI: https://doi.org/10.17776/csj.1383556

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Electrospun Poly(ϵ-caprolactone) Nanofibers Containing Pomegranate Peel Extract and Bioactive Glass as Potential Wound Dressings

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Research Article

History
Received: 30/10/2023
Accepted: 12/03/2024

ABSTRACT

This study focuses on the effect of pomegranate peel extract (PPE) as a natural medicinal substance and 45S5 bioglass (BG) particles as a bioactive material on the microstructure, antioxidant properties, and fibroblast cell cytotoxicity of biocompatible poly(ε-caprolactone) (PCL) nanofiber scaffolds. The hybrid nanofibers were fabricated via the electrospinning technique. The microstructure of nanofiber scaffolds was characterized by using scanning electron microscopy (SEM). The results indicated that the incorporation of PPE and BG particles did not change the morphology of the fibrous structure of the PCL nanofiber scaffolds. The DPPH analysis was performed to determine the antioxidant properties of nanofiber scaffolds and demonstrated that the incorporation of PPE improves the antioxidant properties of scaffolds. Cell cytotoxicity studies using fibroblast L929 cells also showed that high cell viability values were observed for hybrid PPE and BG loaded PCL nanofiber scaffolds. The findings proved that the integration of PPE and BG particles into PCL nanofibers yielded favorable characteristics suitable for wound dressing purposes, involving improved antioxidant capacity.

Keywords: Electrospinning, Wound dressings, Polycaprolactone, Pomegranate peel extract, Bioactive glass.

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Introduction

Wound dressings have been suggested to be one of the most advanced treatment methods for various skin injuries caused by external damage or accident [1,2]. The increase in the prevalence of non-healing wounds has also led to the development of new wound dressing materials for tissue engineering applications [2]. Wound healing is a complex process involving blood coagulation, epithelialization, wound contraction, collagen synthesis, and blood vessel formation. The healing process involves an accurate pattern of synchronized steps involving migratory and resident cell populations, extracellular matrix components, and soluble mediators [3]. Therefore, biologically inspired wound dressing materials have been developed to mimic the extracellular matrix's (ECM) structural function as well as serve as a platform for cell migration and proliferation during wound healing [2]. Diverse forms of wound dressings, including hydrogels, films, membranes, sponges, and nanofibers, have been developed in order to accelerate the healing process [3]. Electrospun nanofibrous dressings have drawn the most interest among these types of wound dressings due to their structural resemblance to the ECM, high specific surface area, exceptional mechanical qualities, and high porosity [4].

Bioactive glasses (BGs) have shown promising results in soft tissue applications. The original 45S5 Bioglass® promoted angiogenesis [5]. The composition of 45S5 Bioglass® consists of 45 wt% SiO_2, 24.5 wt% CaO, 24.5 wt% Na_2O, and 6.0 wt% P_2O_5 [6,7]. Silica functions as a network modifier, while calcium, potassium, and sodium ions are network formers. Si facilitates precipitation or surface reconstruction by non-bridging oxygen sites. It promotes bone bonding and mechanical stability by attaching other metal ions and functional groups. These features make BGs better for soft tissue engineering than hard tissue engineering or implants. The primary advantage that BGs provide to the field of soft tissue engineering is their high degree of bioactivity under physiological conditions as well as their high surface reactivity for the formation of hydroxyl carbonate apatite (HCA) layers on soft tissue [7]. Due to their osteogenicity, angiogenesis, proliferators, and biocompatibility, BGs were extensively used in soft tissue engineering for wound healing [3,4,7–10]. However, cell injury and inflammatory responses may result from the intrinsic brittleness and sharp morphology of BG particles and fibers. Moreover, if glass powder is placed directly on a wound without a secondary dressing, it could be quickly removed from the surrounding area. Thus, combining polymers (i.e., chitosan, poly(lactic acid), poly(ϵ-caprolactone), and polyvinyl alcohol) with inorganic materials like glass particles is an efficient way to make use of both the therapeutic properties of glasses and the adaptability of polymers [2,11].

Poly(ϵ-caprolactone) (PCL) is often employed as the matrix in many biomedical applications due to its semi-crystalline microstructure, high mechanical flexibility, intrinsic non-toxicity, low biodegradability, high biocompatibility, and high ability to imitate the ECM.

Furthermore, the low hydrophilic nature of PCL, which affects adhesion and cell proliferation, makes this biopolymer a unique choice [12]. But due to its low stiffness, hydrophobic nature, and lack of bioactivity, it has limits in biological applications [8]. The usage of BG particles as modifiers of PCL matrix has been extensively documented in the literature. This approach enables the fabrication of bioactive composites with enhanced mechanical behavior and osteostimulative characteristics [13].

Pomegranate has a wide variety of different types of bioactive compounds, is native to the nations of the Mediterranean region, and may be consumed in the form of the fruit itself as juice, flower extract, seed oil, roots, leaves, or peel [14]. It contains polyphenols, flavonoids, anthocyanin, ascorbic acid, ellagic acid, carotenoids, tannins, and other compounds [15]. As modern medicine explores phytochemicals with minimal side effects, pomegranate emerges as a valuable natural resource with antibacterial properties and high concentrations of flavonoids and tannins [16–18]. There has also been a long-standing interest in the use of pomegranate peel extract for therapeutic purposes due to its high phenolic content [18]. Several studies combined pomegranate peel extract with different materials to improve the properties of scaffold materials for various tissue engineering applications. Garcia et al. combined collagen, nanohydroxyapatite and pomegranate peel extract to design gels for bone tissue regeneration [19]. Azarfam et al. fabricated gelatin/agarose/zeolite hydrogel composites for tissue engineering applications [18]. Costa et al. prepared PVA/starch/polyacrylic acid films with pomegranate peel extract by using the solvent casting method [20]. Karabulut et al. fabricated 3D-printed scaffolds by combining pomegranate peel extract, pomegranate seed extract, starch, and polyvinyl alcohol [21].

The novelty of the present study is the fabrication of nanofibrous scaffolds for wound dressing applications made by a natural compound (pomegranate peel extract), which has advantages over common PCL-based scaffolds and improves the wound dressing properties such as microstructure, antioxidant, and cytotoxicity behaviors. This study aims to investigate the effect of pomegranate peel extract and bioactive glass as combined additives on the wound dressing application of PCL-based scaffolds. Hence, the bioactive glass particles were synthesized using the melt quenching method, and hybrid scaffolds were produced using the electrospinning method. The synergetic effect of pomegranate peel extract and bioactive glass was studied.

Materials and Methods

Materials

Poly(ε-caprolactone) (PCL, average Mn 80,000, Sigma Aldrich), N, N-dimethylformamide (DMF, for gas chromatography ECD and FID, Merck), and dichloromethane (DCM, extra pure, Merck) were used to prepare electrospinning polymer solutions. Silicon dioxide (SiO_2, Sand, white quartz, purum p.a., powder, Sigma-Aldrich), di-sodium hydrogen phosphate (Na_2HPO_4, anhydrous for analysis, Merck), calcium carbonate ($CaCO_3$, precipitated for analysis, Merck), and sodium carbonate (Na_2CO_3, anhydrous, puriss, Riedel-de Haën) were used to prepare 45S5 bioactive glass. Pomegranates were purchased from the local market to prepare pomegranate peel extract. Ethanol (C_2H_5OH, absolute for analysis, Merck) and deionized water were used to prepare pomegranate peel extract (PPE).

Bioactive Glass Preparation

Traditional melt quenching was used for the production of bioactive glass particles with the following compositions: 45 w% SiO_2, 24.5 w% CaO, 24.5 w% Na_2O, and 6.0 w% P_2O_5. Precursor compounds were heated to 1250 °C in a platinum crucible. The melt was rapidly quenched into deionized water after two hours. The produced frits were then remelted for a second time for two hours at 1250 °C, and the melt was then quenched to produce a homogenous and amorphous structure. By grinding and sieving below 45 µm, glass particles were produced. In our prior study, the characterization of these BGs was carried out [22].

Pomegranate Peel Extract Preparation

Pomegranate peel extract (PPE) was prepared by the method described by Bodbodak et al. [23]. Briefly, fresh pomegranates were purchased from local markets in Istanbul, Türkiye. Pomegranates were peeled and dried in an oven at 40 °C for 48 hours. Then, the dried peels were powdered using a grinder. The PPE was then obtained by soaking 5g of powder in 250 mL of 80% (v/v) ethanol and stirring for 48 hours at room temperature. The obtained solution was centrifuged and filtered through Whatman No:1 filter paper. The supernatant was concentrated with a rotary evaporator at 50 °C. For further usage, the concentrated supernatant was freeze dried to obtain PPE.

Scaffold Preparation

The polymer solution (10% w/v) was provided by dissolving PCL in the DMF/DCM mixture (1:4 v/v) under continuous stirring for two hours. BG particles were added to this solution at a 15% w/w concentration with respect to the polymer. The quantities of BG and PCL utilized in this study were determined in accordance with the quantities specified in our previous study [10]. Then, PPE with different concentrations (5, 10, and 15 w% with respect to polymer) was introduced into the polymer solution and stirred overnight. PCL/BG/PPE solutions were placed in syringes. An electrospinning device (Nanospinner 24 Touch, Inovenso Co.) was used to prepare scaffolds. The applied voltage of 25 kV was applied to the tip of the needle. The distance between tip to collector was adjusted to 17 cm, and a polymer flow rate of 2 ml/h was used. An aluminium foil was wrapped around the collector, positioned perpendicular to the tip. The electrospinning process was performed under

ambient conditions. The samples with BG particles and PPE with contents of 5%, 10%, and 15% were named PCL/BG/5PPE, PCL/BG/10PPE, and PCL/BG/15PPE, respectively. The scaffold fabrication process was illustrated in Figure 1.

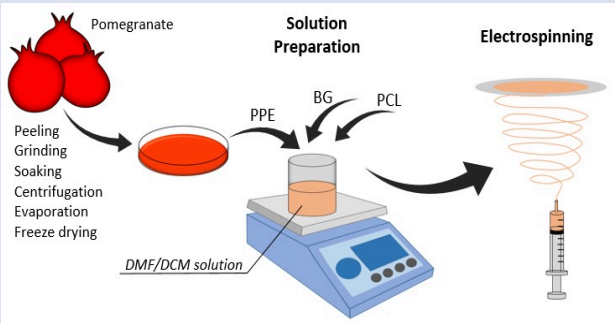

Figure 1. The scaffold fabrication process

Characterization Studies

A scanning electron microscope (SEM, JSM-5410, Jeol) was used to examine the morphology of electrospun scaffolds. A Matlab-based image analysis program called SIMPoly was utilized to estimate the diameters of nanofibers and their standard deviations [24]. Functional molecular groups of PPE were analyzed in the 450–4000 cm^{-1} range using a Fourier transform infrared (FTIR) spectrometer (Perkin-Elmer, Spectrum Two).

Antioxidant Activity

The DPPH assay was employed to evaluate the free radical scavenging activity of each nanofiber mat. A DDPH assay solution was prepared by mixing 1 mg of nanofiber mat and 3 mL of DPPH methanol solution (0.3 mM). The mixture was shaken for a minute before being incubated for 30 minutes. Following this, the UV absorbance of the DPPH test solution was determined at 517 nm. The average values were taken from three specimen tests. The antioxidant activity was calculated by:

$$Antioxidant\ activity\ = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100 \qquad (1)$$

where A_{DPPH} is the absorbance of the DPPH methanol solution at 517 nm and A_S is the absorbance of the DPPH assay solution at 517 nm [25].

Cell Culture Study

Mouse L929 fibroblast cells were used for the MTT cytotoxicity assay. Nanofibers were placed on a 96-well plate and sterilized with UV light for 20 minutes before the test. After that, 10^5 cells per well were seeded on the scaffolds and incubated for 72 h at 37 °C with 85% humidity. MTT (3-(4,5)-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) was used to determine the in vitro cytotoxicity of scaffolds after 72 h. MTT solution was added in each well, and crystallized formazon substance dissolved in dimethyl sulfoxide were formed after incubation for 4 h at 37 °C and with 5% CO_2. Optical densities of the obtained solutions were measured at 570 nm.

Results and Discussion

Characterization of PPE

FTIR spectroscopy was employed to identify and analyse the characteristic peaks associated with PPE (Figure 2). As shown in the figure, the FTIR spectrum of PPE exhibited an apparent peak at 3293.70 cm^{-1}, indicating the presence of alcohol and carboxylic acid functional groups. This peak corresponds to the stretching vibrations of OH groups. The CH stretching vibration peak was detected at 2932.22 cm^{-1}. A distinct peak at 1717.22 cm^{-1} was observed, representing the existence of a carbonyl group (C=O). This confirms the presence of aldehydes, ketones, and carboxylic acid groups in the extract. The peak observed at 1611.05 cm^{-1} signifies the occurrence of H-O-H bending vibrations. The peak at 1441.00 cm^{-1} corresponds to the O-CH_3 deformation, while the peak at 1338.61 cm^{-1} represents CH bending. Additionally, the band identified at 1222.72 cm^{-1} indicates the presence of esters and ether functional groups, specifically CH_2CO stretching. The C-O-C vibration was displayed by the peak at 1029.09 cm^{-1} [26]. Based on the similarity between the identified peaks and the existing literature, it can be inferred that the preparation of PPE was accomplished effectively.

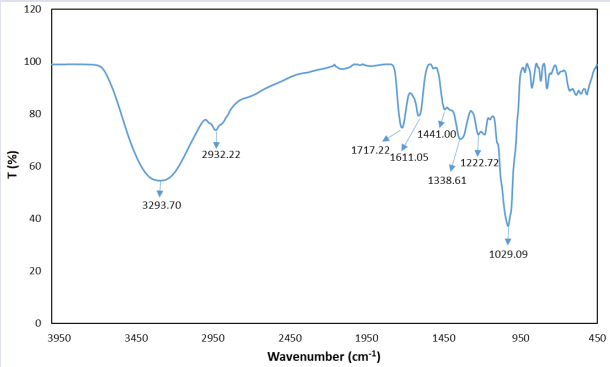

Figure 2. FTIR Spectrum of PPE

Surface Morphology of Scaffolds

In this study, a series of PCL/BG/PPE nanofibrous scaffolds with biocompatibility and antioxidant activity were prepared for wound healing applications. SEM analysis was utilized to investigate the surface morphology and size distribution of nanofibers. As illustrated in Figure 3, fibers with homogenous, bead-free, and randomly oriented architectures were formed by incorporating BG with varying concentrations of PPE into PCL nanofibers. The nanofiber diameters of composite nanofibers were 799 ± 128 nm (PCL/BG/5PPE), 709 ± 109 nm (PCL/BG/10PPE), and 741 ± 109 nm (PCL/BG/15PPE), respectively. The results showed that the incorporation of PPE and BG had no adverse impact on fiber formation. The incorporation of PPE was found to result in an increase in fiber diameter compared to the fiber diameter size

(389±72 nm) of the PCL/BG nanofiber structure in our previous study [10]. This increase in fiber diameter is caused by the reduction of the electrical conductivity of the polymer solutions with the incorporation of extracts. Similar results were observed in the studies using several extracts as additives for electrospun nanofibers [27,28].

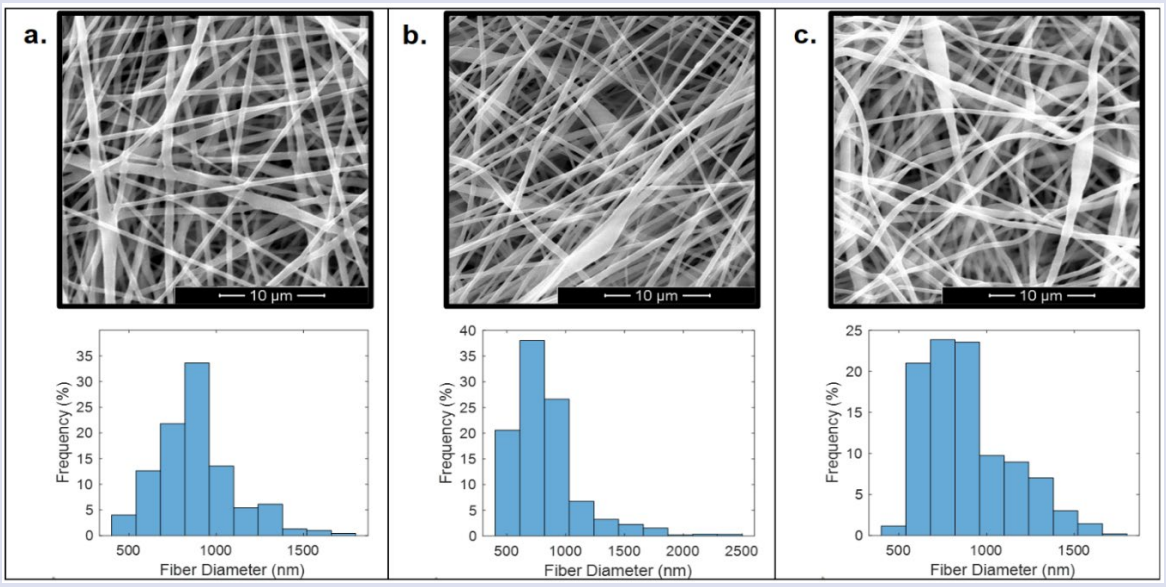

Figure 3. SEM images and fiber distributions of PCL/BG/5PPE (a), PCL/BG/10PPE (b), and PCL/BG/15PPE (c) scaffolds

Antioxidant Activity of Scaffolds

During wound injury, the excessive generation of free radical molecules known as reactive oxygen species (ROS) damages the extracellular matrix and cell membranes, hindering wound healing [29]. Plants' wound healing properties are linked to their antioxidant activities, mainly involving phenolic compounds like phenolic acids and flavonoids, which scavenge free radicals [30,31]. These antioxidants protect cells from ROS's harmful effects and regulate the wound healing process. Physical skin injuries trigger inflammation and immune cell responses to restore tissue. However, ROS's presence can impede healing by damaging cells and promoting infections. Plant-derived antioxidants, such as tannins, phenolic acids, flavones, flavonols, catechins, and others, act as natural free radical scavengers, helping deactivate and remove ROS, safeguarding cells, and supporting effective wound healing [31]. The hydrolysable tannins (ellagic acid, gallic acid, punicalin, pedunculagin, and punicalagin) are largely responsible for the biological activities of pomegranate peel extract [16,32,33]. The integration of these compounds into fibrous PCL based materials could serve to impart antioxidant properties to them. As a result, the antioxidant activity of PCL/BG/5PPE, PCL/BG/10PPE, and PCL/BG/15PPE scaffolds was assessed through the utilization of the DPPH radical scavenging assay. PCL/BG/5PPE, PCL/BG/10PPE, and PCL/BG/15PPE scaffolds exhibited antioxidant activities of 46.21%, 50.24%, and 62.61%, respectively (Figure 4). The results revealed an increase in correlation between the quantity of PPE incorporated into scaffolds and the observed improvement in antioxidant activity.

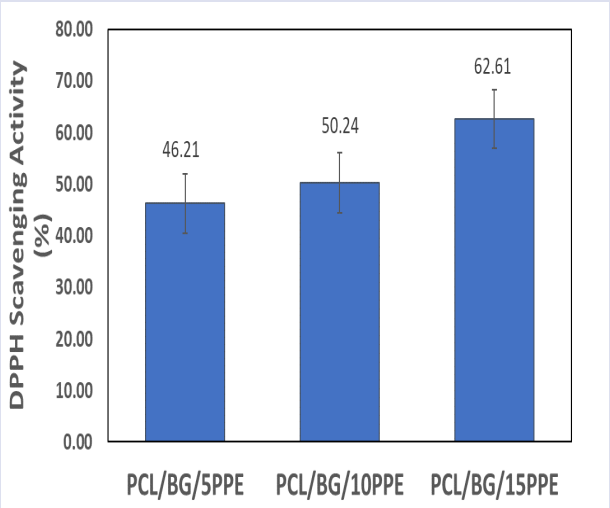

Figure 4. Antioxidant activity of PCL/BG/5PPE, PCL/BG/10PPE, and PCL/BG/15PPE scaffolds.

Cytotoxicity of Scaffolds

Fibroblasts are the primary cells of the dermis that play a vital role in tissue repair [34]. Hence, the L929 cell line is employed in a 72-hour direct MTT assay (Figure 5). The cell viability (expressed in%) observed with the varying extract concentrations was 103.35 ± 3.72 for PCL/BG/5PPE, 95.52 ± 3.16 for PCL/BG/10PPE, and 85.23 ± 5.66 for PCL/BG/15PPE. In accordance with EN ISO-10993-5/12, cell viability greater than 80% is classified as non-toxic [35,36]. The viability of the L929 cells incubated at all the extract concentrations was above 80%. Therefore, the biocompatibility of the scaffold made it acceptable for usage in wound dressing applications.

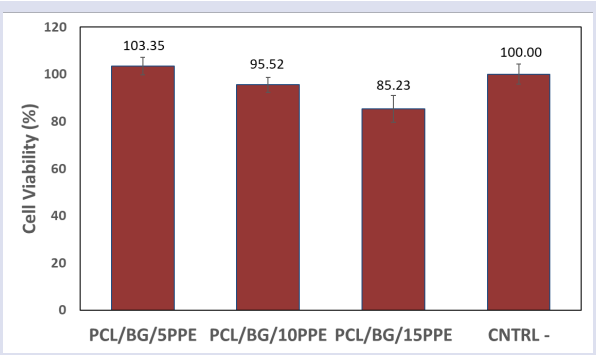

Figure 5. Viability percentage of L929 fibroblast cells treated with PCL/BG/5PPE, PCL/BG/10PPE, and PCL/BG/15PPE scaffolds for 72h incubation.

Conclusion

In summary, poly(ϵ-caprolactone) (PCL) fibers functionalized with pomegranate peel extract (PPE) and 45S5 bioglass particles (BG) were successfully obtained by electrospinning using PCL as polymer in N,N-dimethylformamide/dichloromethane (1:4 v/v) as organic solvents to prepare polymeric solutions. The electrospun fibers produced from PCL/BG/PPE were homogeneous, bead-free and randomly oriented structures and had no toxic effect on L929 cell cultures. This study demonstrated that electrospinning is a promising advance for the production of scaffolds composed of nanofibers containing recovered bioactive compounds of pomegranate peel extracted with deionized water/ethanol (1:4 v/v) solution. Antioxidant tests have revealed that PPE imparts antioxidant properties to the scaffolds. Moreover, cell toxicity studies showed that biocompatible structures can be obtained by using BG prepared by traditional melt quenching method and PPE in PCL nanofiber structures. This study is novel in its investigation of the utilization of nanofibers produced through the electrospinning technique, which involves the incorporation of biologically functional components derived from pomegranate peel and 45S5 bioactive glass into the PCL polymer for the purpose of developing wound dressings.

Conflicts of interest

There are no conflicts of interest in this work.

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