The Effect of Vitamin C-Loaded Electrospun Polycaprolactone/Poly(Glycerol Sebacate) Fibers for Peripheral Nerve Tissue Engineering

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Abstract

Vitamin C (VC) is an essential supplement that plays a vital role in cellular processes and functions and has been applied for therapeutic purposes for many years. The beneficial effects of VC on peripheral nerve regeneration have been gained lots of attention. In this study, electrospun polycaprolactone (PCL)/polyglycerol sebacate (PGS) fibers incorporated with different concentrations of VC (5, 10, and 15 wt.%) were developed for peripheral nerve tissue engineering. The morphology of the fibers was investigated using scanning electron microscope (SEM), Fourier-transform infrared spectroscopy (FTIR), tensile analysis (Young’s modulus, ultimate tensile strength (UTS), and elongation at break), release profile of VC from the PCL/PGS fibers, in vitro degradation, water uptake behavior, and contact angle measurements were also studied. MTT assay and SEM were utilized to evaluate the attachment and viability of pheochromocytoma cells (PC12) on the scaffolds. The results showed that all scaffolds had a uniform diameter and mean diameter deceased from 1.24 to 0.88 µm followed by increasing VC. Young’s modulus and UTS enhanced with increasing in VC percentage. MTT assay demonstrated that PCL/PGS containing 5 wt.% VC had a greater viability rate among other scaffolds. Our outcome indicated possible applicability of VC containing scaffolds for nerve tissue engineering.

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Graphical abstract

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Introduction

Peripheral nerve injury (PNI), which occurs mostly due to trauma, could potentially cause lifelong disability and decrease patients’ quality of life [1]. Although axons in the peripheral nervous system (PNS) have relatively more regenerative capacity than those in the central nervous system (CNS), this capability is not sufficient to restore the whole nervous function, and even the current approaches reported to repair peripheral nerve gaps have failed to achieve satisfactory treatment [2, 3]. Therefore, the recruitment of novel therapeutic techniques along with the agents that accelerate the neural repair rate could provide more effective treatment in nerve injuries.

Nowadays, nerve tissue engineering is one of the novel approaches that has attained a lot of attention for peripheral nerve regeneration. In this regard, electrospinning is an appropriate and relatively simple technique for producing fibers in the micro/nanoscale, which can mimic extracellular matrix properties [4]. Some synthetic polymers are appealing candidates in order to fabricate a suitable fiber-based scaffold for repairing the target tissue [5]. The electrospun fibers can provide a pathway for axons’ growth and sprouting [6]. Moreover, fibers could be loaded with therapeutic agents in order to control release. This drug delivery system transports the drugs to the target sites with more accuracy. In addition, sustained release of drugs could potentially decrease their adverse effects [7, 8].

Vitamin C (VC), or ascorbic acid (AA), is an essential water-soluble supplement that plays a vital role in developmental processes, physiological functions, and tissue repairing [9]. Previous studies showed that neural cells absorb a high level of VC that reflects its importance to neuronal function [10]. Some main tasks of VC include gene expression regulation, synthesis of neurotransmitters and peptide hormones, formation of myelin, and neuroprotection against free radical species [9]. This micronutrient is not only necessary to receive day-to-day for the proper function of cellular processes but also has been widely used for therapeutic purposes. Only recently, the effects of VC on regeneration and restoration of damaged nervous tissue have been considered. VC exerts therapeutic effects on neurodegenerative diseases [11, 12]. One of the previous studies indicated that oral administration of VC promoted nerve regeneration and accelerated motor and sensory functional recovery in a mouse model of PNI by increasing the number of regenerated axons and myelin sheets thickness [13]. Furthermore, VC has been shown to accelerate the Wallerian degeneration perhaps by enhancing the macrophages infiltration and phagocytosis, and Schwann dedifferentiation after peripheral nerve-injured [14]. Even though several studies have incorporated VC into fiber structure to repair other tissues like skin [15],...
VC-incorporated electrospun fibers were rarely studied for nerve tissue engineering. Polycaprolactone (PCL) is a popular biocompatible and biodegradable polymer with no toxicity and immunogenicity. Because of easily processing properties and good mechanical properties, PCL has received a great deal of consideration to fabricate efficient scaffolds [16]. The application of PCL in short-term and long-term experimental studies of nerve injuries has proved as a promising material for nerve regeneration [17, 18]. The biomaterial could provide a suitable environment for the migration of supportive cells as well as for the growth of damaged axons stumps due to its appropriate flexibility and rigidity [19]. However, the interaction between cells and the surface of the PCL scaffold is weak as a result of its poor hydrophilicity [20, 21]. Thus, combining PCL polymer with a hydrophilic material is an uncomplicated solution to modify the surface chemistry of scaffolds [22].

PGS is a biodegradable polymeric biomaterial with thermoset elastomeric properties that have been introduced in recent years. Several key features of PGS such as biocompatibility, biodegradability, tunable physical characteristics, and negligible swelling during degradation have made its application attractive in nerve tissue engineering. Furthermore, hydrophilicity and low elastic modulus of PGS have made it more popular [23]. Previous studies indicate that the PGS does not induce apoptosis, has no deleterious effect on the behavior and performance of Schwann cells and induces elongated morphology in PC12 nerve stem cells [24, 25]. However, the low molecular weight of PGS does not allow the prepolymer to electrospin [26]. Nonetheless, this challenge can be removed following the combination of PGS with other polymers like PCL [22]. Moreover, the electrospinning PCL/PGS fibers indicated higher cell viability and proliferation in comparison with PCL alone [22].

In the present study, electrospinning PCL/PGS fibers incorporated with different amounts of VC (5, 10, and 15 wt.%) are developed for nerve tissue engineering. The effect of VC on the morphology and physicochemical properties of the scaffolds is evaluated. The release profile of VC from the PCL/PGS fibers is determined. Moreover, the mechanical properties, surface contact angle, degradation rate, and water uptake of the fibers are studied. Eventually, cell-scaffold interactions are indicated.

**Materials and Methods**

**Materials**

Chemical substitutes include Poly (caprolactone), PCL (Mn = 80,000 g/mol), sebacic acid (purity 99%), glycerol, chloroform, and ethanol were purchased from Sigma-Aldrich (Germany). VC was obtained from Darou Pakhsh Holding Co, Iran. RPMI-1640, dimethyl sulfoxide (DMSO), 3-(4,5- Fetal Bovine serum (FBS), antibiotics, horse serum (HS), and trypsin–ethylenediaminetetraacetic acid (EDTA), dimethylthiazol- 2- yl)-2.5-diphenyltetrazolium bromide (MTT) were obtained from Bioidea (Iran).

**Electrospun Fiber Preparation**

PGS pre-polymer (pPGS) was initially synthesized using the polycondensation method as reported elsewhere [27]. Briefly, sebacic acid and sebacate glycerol (0.8:1 M) were mixed and exposed to inert nitrogen gas for three hours at 170°C. Then, the synthesized polymer and PCL (1:2 w/w) were dissolved in an aqueous solution of ethanol and chloroform (1:9) under magnetic stirring. After that, VC at concentrations of 5, 10, and 15% by weight of PCL/pPGS was added to the polymer solution and stirred for 24 h. Eventually, the prepared mixtures were poured into the plastic syringes with blunt 21-gauge needles and electrospun at the 0.8 ml h−1 feed rate and 20 kV applied voltage while the needle/collection distance was 20 cm. VC-free PCL/PGS sample was also fabricated.

**Morphology and Orientation Evaluation of Fibers**

The produced fibers were examined in terms of morphology and fiber diameter by scanning electron microscopy (SEM; TESCAN-Vega 3, Czech Republic) with an accelerating voltage of 10 kV. The diameter and porosity of electrospun fibers were investigated using ImageJ and MATLAB software program (Wayne Rasband, National Institute of Health, USA), respectively. ImageJ was also utilized to assess the effect of VC on fiber orientation. Followed by superimposing a grid on SEM figures, the mean angle for electrospun fibrous samples was analyzed. The average of 50 fibers was calculated to measure the angle between the vertical grid and a fiber.

**Functional Groups Characterization**

Functional groups in PCL/PGS fibers containing three different concentrations of VC were investigated using Fourier transform infrared spectroscopy (FTIR) (Tensor I, Germany). The 400–4000 cm−1 range wave number was set for this experiment after combining the samples with KBr and pressing into pallets.

**Mechanical Properties**

The mechanical properties of PCL/PGS fibers with/without VC were assessed using a universal testing machine (Santam, Iran) with a 50 N load cell. Tensile test was conducted.
at a constant tensile speed of five mm/min following the preparation of the samples in the form of rectangular shape (3 x 0.5 cm). Young’s modulus, elongation at break, as well as ultimate tensile strength were determined.

In Vitro Release Study

To represent the release profile of VC from the PCL/PGS fibers, the bag diffusion technique was employed. For this purpose, the VC-loaded fibers with the weight of 0.025 g were rested into dialysis membrane bags (cut-off 14 kDa) and each bag was placed in 10 ml of the phosphate buffer solution (PBS) and transported to an oven temperature of 37 °C. One ml of release medium was taken at predetermined time intervals (2, 4, 8, 12, 24, 48 h; 3, 6, 10, and 13 days) and substituted with fresh PBS. Then, the UV-absorbance of each release sample was measured using an ultraviolet–visible spectrometer at the wavelength of 261 nm. The value of the drug release for each sample was calculated using the calibration curve of VC in PBS. The calibration curve for all samples were prepared at a volume ratio of 1 to 10 (1:10) and the final concentration was 1.0 mg/ml. The experiments were carried out three times.

Degradation Rate of the Fibers

To evaluate the stability of PCL/PGS electrospun fibers with and without VC, each sample in the shape of a square (1 x 1 cm^-2) was immersed in PBS (pH = 7.4) after weight determination (W_0) and incubated at 37 °C for 14 days. Then, the samples were removed from the buffer at the end of the specified time (1, 4, 7, 14, 21, 28, 35, 42, 49, and 63 days) and dried in an oven. Dried weights of each sample were recorded (W_d) and weight loss (ΔW) was calculated using Eq. 1:

\[
\Delta W (\%) = \frac{W_0 - W_d}{W_0} \times 100
\]  (1)

Water Uptake

The water absorption of the electrospun fibers was assessed by immersing the scaffolds in saline phosphate with pH = 7.4 at 37 °C. To begin with, the fibers in different groups were cut into pieces of the same size of 1 x 1 cm^-2, and primary weight of each sample (W_t) was recorded. Then, the specimens were immersed in a container containing 5 ml PBS and incubated at 37 °C. After specified time points (1, 3, 5, 7, 24, and 48 h), the samples were taken out of the PBS container and the weight of each wet sample was accurately measured (W_d) after removing their surface excess water via filter paper. Finally, the water absorption percentage was obtained using Eq. 2:

\[
S (\%) = \frac{W_i - W_d}{W_d} \times 100
\]  (2)

Wettability Measurement

The wettability of the PCL/PGS fibers with and without VC was evaluated using water contact angle measurement. For this purpose, a droplet of distilled water with a volume of 4 μl was rested on the surface of the samples and sessile drop technique was carried out using contact angle meter XCA-50 (USA). The mean value of contact angle was calculated following triplicate individual measurements.

Cell Culture and Seeding

In this study, pheochromocytoma cells (PC12) were used to evaluate the cell behavior on the fibrous scaffolds. PC12 cells were isolated from a pheochromocytoma of the rat adrenal medulla and plated in RPMI-1640 (Invitrogen, USA) containing 10% horse serum, 5% fetal bovine serum, and 1% L-glutamine (Invitrogen, USA) and 1% penicillin–streptomycin in cell culture incubator. The PCL/PGS scaffolds with/without VC were prepared in a disc shape and placed on the bottom of 96-well plates after UV sterilization (30 min). These scaffolds were carefully washed with 10% penicillin–streptomycin (1 time) and PBS (3 times) before cell seeding. The cells were detached with trypsin/EDTA (Invitrogen, USA) and seeded on the scaffolds into the 96-well plates with a density of 4000 cells/well and then incubated at 37 °C with 5% CO₂.

Evaluation of Cell Proliferation

Cell viability and cytotoxicity of the fibrous scaffolds in each group were evaluated using MTT assay. After cell culture for each period (1, 4, and 6 days), the culture media was substituted with MTT solution (100 μl of 0.5 mg/ml) and the plates were incubated for 4 h in a dark room. After this time duration, MTT solution was replaced with 300 μl of DMSO that leads to the appearance of purple color due to dissolve the formed formazan crystals. Optical density was measured at 570 nm using an ELISA Reader (Hyperion MPR4). The test was carried out in triplicates.

Evaluation of Cell Attachment

The attachment of PC12 cells on the scaffolds was assessed six days after cells seeding using SEM. For this purpose, following fixation with 3% glutaraldehyde for 2 h at 4 °C,
the cell-seeded scaffolds were dehydrated in ethanol ascending concentrations (30%, 50%, 70%, 80%, 90%, and 100%). Then, SEM imaging was conducted after coating the dried samples with gold.

**Statistical Analysis**

Presented results were shown as mean values ± SD. The data obtained were statistically analyzed using SPSS (version 22). One-way analysis of variance (ANOVA) followed by the LSD post hoc test was performed to compare the means of samples by considering the statistical significance of $P$ values $\leq 0.05$.

**Results and Discussion**

**Morphology of Fibrous Matrices**

As the aligned fibers can provide a suitable pathway for axons growth and sprouting [28], in this study electrospun PCL/PGS aligned fibers containing different amounts of VC were developed in order to facilitate regeneration in damaged peripheral nerves. The morphology and microstructure of the fibers with different concentrations of VC (0–15 wt %) along with their diameter distribution are presented in SEM images (Fig. 1). Moreover, the mean diameter and porosity of electrospun fibers are listed in Table 1. Based on the results, all fibers were smooth and almost bead-free with a uniform diameter (Fig. 1a–h). The diameter distribution of fibers was faced a reduction upon the addition of different concentrations of VC (Fig. 1i–l). The mean diameter of fibers decreased from 1.24 ± 0.6 to 0.88 ± 0.4 µm ($P < 0.5$) with increasing VC from 0 to 15 wt.%. Since VC induces low viscosity to the solution, the decrease of fiber diameter could be assigned to reducing in the solution viscosity by the gradually addition of VC [29]. In addition, the measurement of porosity of fibers using MATLAB software showed that the percentage of porosity in fibers containing vitamin C was decreased from 83 to 81% ($P > 0.5$). However, the porosity in all groups (Table 1) was proper for cell infiltration and distribution and all groups show suitable candidates for nerve bioengineering applications [30].

Created by ImageJ software, similar colors were used to detect the orientation distribution and mean degree of fibers. As can be easily observed in Fig. 2, all samples were aligned as if the scaffolds can be applied as the substrates for axonal sprouting. All broadband of orientation distribution shown in Fig. 2 indicated the same mean angle of fibers and VC had no effect on fiber orientation distribution. No change in the electric field can be a reason why VC concentration did not influence fiber direction [27]. As a matter of fact, VC have no electrical properties.

**FTIR Spectroscopy**

To investigate the possible interactions between polymers and VC in different concentrations, FTIR analysis was
applied. C–H, C–O stretching vibrations, and the asymmetric and symmetric stretch of the carboxyl group were represented at 851, 1124 cm\(^{-1}\). The typical functional groups of PGS appear in 3100–3650 cm\(^{-1}\) for the hydroxyl group (O–H band) and 1600–1800 cm\(^{-1}\) for the ester group (C = O band) which is considered the backbone structure of PGS polymer [27]. Moreover, the molecular interaction of glycerol and sebacic acid appears in 1733 cm\(^{-1}\) [31]. The bands at 2863 and 2931 cm\(^{-1}\) are demonstrating the symmetric and asymmetric stretching of CH\(_2\) group. The peak showed in 1722 cm\(^{-1}\) was considered as carbonyl group (C = O band) [32]. The peak of VC was not observed in the FTIR spectra, which was probably due to the low concentration of VC or the alignment of the VC peak with the PCL/PGS peaks (Fig. 3).

**Mechanical Measurement**

The mechanical properties assessment of fibers including Young’s modulus, elongation percentage at a breakpoint, and tensile strength showed in Table 2. According to the results, Young’s modulus of the PCL/PGS fibers with the different ratios of VC (from 0 to 15 wt.%) was found to be 0.43 ± 0.06, 0.47 ± 0.11, 0.49 ± 0.13, and 0.50 ± 0.12 MPa, respectively. Furthermore, the UTS of PCL/PGS fibers containing 0, 5, 10, and 15 wt.% VC was obtained 0.72 ± 0.1, 0.8 ± 0.15, 1.06 ± 0.14, and 1.14 ± 0.16 MPa one-to-one. Finally, elongation at break was dropped from 34 ± 3.1% to 17 ± 2.4% by increasing VC content. The results indicated that there was no significant difference amongst groups in Young’s modulus (\(P > 0.05\)). Previous studies have estimated suitable Young’s modulus for fiber in order to peripheral nerve regeneration less than 0.5 MPa [23]. So Young’s modulus of the electrospun fibers in this study was in the expected range. On the other hand, UTS was significantly increased and elongation at break was dramatically decreased (\(P < 0.05\)) in PCL/PGS/10% VC and PCL/PGS/15% VC groups while there was no significant difference (\(P > 0.05\)) between PCL/PGS and PCL/PGS/5% VC groups in these indexes. Previous studies showed that using VC in scaffolds could promote mechanical properties. For instance, Janmohammad and colleagues reported that adding ascorbic acid to PCL/hyaluronic acid (HA) scaffold significantly increased Young’s modulus and UTS [33]. In this study, alteration in the mechanical properties could be associated with the

| Sample          | Mean diameter (µm) | Porosity (%) |
|-----------------|--------------------|--------------|
| PCL/PGS         | 1.24 ± 0.6         | 83           |
| PCL/PGS/5% VC   | 1.01 ± 0.4         | 82           |
| PCL/PGS/10% VC  | 0.94 ± 0.3*        | 81           |
| PCL/PGS/15% VC  | 0.88 ± 0.3*        | 81           |

*Significantly different from corresponding parameters of PCL/PGS fibers (\(p < 0.01\))

*Fig. 2 Orientation assessment and distribution of PCL/PGS scaffolds with different amounts of VC. (a) and (b) PCL/PGS fiber with 0%, (c) and (d) 5%, (e) and (f) 10%, (g) and (h) 15% of VC. The figure showed that VC had no significant effect on fiber orientation distribution.*
function of VC as an inactive filler [34]. Borschel and et al. described that the average UTS for rat nerves was between 480 and 810 kPa [35]. According to a study, UTC was increased when VC was gradually added to fiber solution, which corresponds to our results. So among the PCL/PGS fibers containing different amounts of VC those with 5% VC are a more suitable candidate for nerve repair.

### Release Assessment

The release of VC from electrospun PCL/PGS fibers was evaluated at 37 °C and demonstrated in Fig. 4. During the first day, VC released from PCL/PGS fibrous matrices exhibited a burst release manner, and then the gradual release from samples was detectable. Drug release in a constant manner and during a prolonged period of time can mediate more beneficial therapeutic effects [7, 8]. Following peripheral nerve injury, free radical species rapidly increase at the site of injury which can lead to further cell damage [36]. The long-term presence of VC as a powerful free radical scavenger can mediate cell protective effects and attenuate tissue damages. Furthermore, VC is required for some

![Fig. 3 FTIR spectra of PCL/PGS scaffolds containing different amounts of VC from 400 to 4000 cm⁻¹. Fourier transform infrared (FTIR) spectra of a PCL/PGS fiber with 0%, b 5%, c 10%, and d 15% of VC is shown. IR spectra of pPGS and PCL polymer are illustrated as e and f, respectively.](image)

### Table 2 Young’s modulus, UTS, and elongation of the electrospun fibers

| Samples          | Young’s modulus (MPa) | UTS* (MPa)  | Elongation (%) |
|------------------|-----------------------|-------------|----------------|
| PCL/PGS          | 0.43 ± 0.06           | 0.72 ± 0.1  | 34 ± 3.1       |
| PCL/PGS/5% VC    | 0.47 ± 0.11           | 0.8 ± 0.15  | 28 ± 3.2       |
| PCL/PGS/10% VC   | 0.49 ± 0.13           | 1.06 ± 0.14*| 20 ± 2.5*      |
| PCL/PGS/15% VC   | 0.50 ± 0.12           | 1.14 ± 0.16*| 17 ± 2.4*      |

*Significantly different from corresponding parameters of PCL/PGS fibers (p < 0.05)

![Fig. 4 Release profile of VC from PCL/PGS electrospun fibers. Almost 58% of VC was slowly released from the fibers within 6 days and over 40% was release in the first 24 h.](image)
cellular processes such as myelin formation which is crucial for nerve regeneration [9].

**Degradation Behavior**

The in vitro degradation pattern of electrospun PCL/PGS fibers containing different amounts of VC was evaluated by incubation the scaffolds in PBS at 37 °C for nine weeks. On the basis of the results, the mean weight loss of the PCL/PGS fibers containing 0, 5, 10, and 15 wt.% VC was 37 ± 1.8, 41 ± 2, 43 ± 2, and 46 ± 2.5%, after four weeks, respectively. It also increased to 43 ± 2.5, 47 ± 2.5, 49 ± 2 and 51 ± 2.5 at the end of the ninth week, respectively (Fig. 5). All fibers showed a slow degradation rate and proper biodegradability. Previous studies demonstrated that axons require a stable substrate for growth and sprouting and so scaffolds with low-speed degradability are an excellent candidate for nerve regeneration [24, 37]. On the other hand, the results showed that by increasing the amount of VC a higher weight loss was observed. This increase in weight loss could be associated with the hydrophilicity properties of VC [29].

**Water Uptake**

Since hydrolysis is the primary mechanism for degradation, scaffolds must therefore absorb sufficient water so as to have a proper degradation rate inside the body. Moreover, water absorption improves cell nutrition and growth and prevents the scaffold from collapsing [27]. Therefore, crucial information about structural integrity and deformation of scaffolds can be achieved through evaluating the water uptake capacity [38]. In this study, the water uptake behavior of the PCL/PGS fibers containing 0, 5, 10, and 15 wt.% VC was evaluated in PBS solution at 37 °C for 48 h (Fig. 6). The results indicate that the fibers had the highest water uptake in the first hour of incubation. After one hour, all samples reached a plateau. At this time point, the water uptake percentage for the PCL/PGS fibers was 240%. By increasing VC loading up to 15%, the water uptake percentages raised from 250 to 264%. Then, all scaffolds showed the plateau up to 48 h. This increase in water-uptake capacity after adding VC could be related to the hydrophilicity of VC [33].

**Surface Hydrophilicity Evaluation**

Contact angle measurement is a suitable method to evaluate the surface hydrophilicity and predict physical contact behavior between cells and scaffolds. Hydrophilic surfaces usually show high levels of protein uptake and adhesion which are the two main factors in cell interaction with scaffolds. By increasing protein absorption, cell behavior on the scaffold can be improved [39]. Accordingly, by modifying the surface of the scaffold, the interaction of the cell with the scaffold can be indirectly regulated. Hydrophilic scaffolds accelerate cell growth, adhesion, proliferation, migration, and differentiation [40]. In this regard, axons require hydrophilic substrates in order to grow and sprout [41]. Polar groups formed on the surface of fibers absorb large amounts of water molecules, which can be the main reason for improving the hydrophilicity of the surface of these fiber scaffolds. As shown in Table 3, the presence of VC in the sample increases the hydrophilicity of the sample surface. VC forms several hydrophilic groups (OH and COOH) on the surface of PCL/PGS fibers, which cause the fiber scaffold surface to become wettable. The results of the present study showed that the contact angle degree for electrospun PCL/
PGS fibers was significantly decreased from 31.4° ± 3.2° to 13.6° ± 3.8° following incorporation of VC (P < 0.05). This result is in accordance with other studies about employing VC in the fabricated scaffolds. For example, Jannmohammad and colleagues reported that VC decreased water contact-angle and increased the hydrophilicity of PCL/HA due to the presence of hydrophilic groups of VC [33].

**Cell Viability and Attachment**

Evaluating fiber cytotoxicity and cell viability and proliferation on them are important aspects to investigate the effectiveness of the scaffold in nerve regeneration. This matter is evaluated by the MTT test as a proper analysis for viable cells [42]. PC12 cell line as a useful model for neurobiological and neurochemical studies was selected throughout this study [43]. As seen in Fig. 7, cells viability on the surface of fibers containing 5 wt.% VC was greater compared to the PCL/PGS fibers with increasing incubation time and became significant on the third and seventh days of cell culture. The observed difference may be correlated with improvement in surface hydrophilicity of fibers after adding VC as discussed above as well as direct and positive effects of VC on cell viability and growth [10]. The presence of hydroxyl groups in the structure of VC might be responsible for the antioxidant properties. These results indicate that VC in this dose as a safe and non-toxic substance is well present in the structure of fibers. On the other hand, the results showed that with increasing the VC percentage from 10 to 15%, cell viability significantly decreased on the third and seventh days of culture in comparison with other groups. It could be associated with an increase in the pH of the cell environment with an increase in the percentage of vitamin C [44]. The morphology and adhesion of PC12 cells on the electrospun fibers were assessed after 7 days of cell culture using SEM (Fig. 8, (a) PCL/PGS fiber with 0%, (b) 5%, (c) 10%, and (d) 15% of VC). A suitable scaffold must provide proper surface chemistry for cell attachment and growth [45]. As illustrated in Fig. 8, scaffolds containing 5 wt.% VC exhibited better cell growth and attachment than the other groups.

**Conclusions**

In the present study, PCL/PGS electrospun fibers containing different concentrations of VC were developed and different in vitro tests were conducted to characterize them and determine their biocompatibility. Aligned and non-beaded electrospun fibers with uniform diameter were fabricated in order to use in neural tissue engineering. The fibers exhibited diameter, mechanical properties, degradation, water uptake behaviors, and surface hydrophilicity in the acceptable range for nerve applications. However, only the fibers containing 5% VC provided a more suitable environment for PC12 cell viability and growth than VC-free PCL/PGS fibers. A higher concentration of VC is probably due

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**Table 3** Contact angle degree of the electrospun fibers containing 0, 5, 10 and 15 wt.% VC

| Samples               | Contact angle (Degree) |
|-----------------------|------------------------|
| PCL/PGS               | 31.4 ± 3.2             |
| PCL/PGS/5 wt. % VC    | 18.1 ± 3.1             |
| PCL/PGS/10 wt. % VC   | 13.9 ± 2.9             |
| PCL/PGS/15 wt. % VC   | 13.6 ± 3.8             |

By increasing gradually VC content from 5 to 15 wt.%, the water contact angle of scaffolds decreased.
to enhance in the pH of the cell environment reducing the rate of cell viability and proliferation. Therefore, PCL/PGS containing 5 wt.% VC seems to be an appropriate candidate for nerve tissue engineering.

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**Declarations**

**Conflict of Interest** The authors have not disclosed any competing interests.

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