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Electrospinning of Gelatin/Poly (Vinyl Pyrrolidone) Blends from Water/Acetic Acid Solutions

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Electrospinning is a versatile and efficient technique for obtaining polymeric microfibers and nanofibers with great potential for applications in tissue engineering, biosensors, filtration, wound dressings, controlled drug release and enzyme immobilization. Electrospun fibers are obtained by applying an electric field in a polymer solution resulting in non-woven fibrous mats with high surface area relative to volume and high porosity. In this work, the electrospinning of gelatin/poly(vinyl pyrrolidone) (PVP) blends was investigated. The polymers were electrospun from solutions containing different concentrations of water and acetic acid. Solutions were characterized by measuring the pH, electrical conductivity, surface tension and viscosity. The influence of acetic acid concentration in solution properties and its influence in the spinnability were investigated. The resulting non-woven membranes were characterized by using scanning electron microscopy (SEM), thermogravimetric analysis (TG) and cytotoxicity.

Keywords: *electrospinning, gelatin, PVP, tissue engineering, biomaterials*

1. Introduction

The artificial replacement of damaged and diseased tissues is a promising avenue of research in the field of regenerative medicine. Tissue engineering is a multidisciplinary field which aims tissue regeneration by using biomaterials¹. Recently, the study of micro and nanofibers have attracted great interest due to their properties such as large surface to volume ratio and ability to form fibers^{2,3}.

Electrospinning has been recognized as a versatile technique for obtaining micrometer and nanometer fibers, since it is possible to obtain fibers with a wide range of polymers, both natural and synthetic, as well as combinations of both polymers³⁻⁸. Electrospinning plays an important role for manufacturing fibrous biomaterial scaffolds for tissue engineering applications with fiber diameters ranging from few microns to less than 100 nm. These materials, such as proteins (gelatin, collagen and silk fibroin) and polysaccharides (chitosan, hyaluronic acid and cellulose) can be designed to mimic the extracellular matrix due to the fibrous structure with diameters in micrometer and nanometer scales^{1,3,7,9}. Applications can be found in a wide variety of biomedical applications such as scaffolds for tissue engineering, wound dressings and drug delivery systems^{1-3,5,7,8,10-13}.

Basically, four components are required for polymer solution electrospinning: a high voltage supply; a capillary tube, which can be a small diameter needle; an infusion pump, and a grounded collector plate^{2,3}. One electrode is attached to a needle connected to a syringe filled with the

polymer solution, whereas the other is fixed to the collector connected to ground. The electric field is applied to the end of the capillary tube containing the fluid that induces an electric charge on the liquid surface, resulting in a force opposite to the surface tension. As the electric field intensity is increased, the fluid surface at the tip of the capillary tube elongates to form a conical shape known as Taylor cone¹¹⁻¹⁴. The jet is stable only at the region close to the tip of the needle, after which jet instabilities are observed. In the jet trajectory from the needle to the collector, the solvent evaporates and dry fibers are deposited on the collector plate as an interconnected network^{1,11,15}. The collected fibers are typically in the form of a blanket^{5,7,11}. Figure 1 shows a scheme of an electrospinning system.

The electrospinning process depends on many parameters, classified broadly into solution, process and environmental parameters. These parameters can influence the processing of polymer solutions into fibers by electrospinning^{1,5,10,16}. The main solution parameters are viscosity, surface tension and electrical conductivity; whereas the main process parameters are applied voltage, flow rate and distance from the needle tip to the collector plate. Environmental parameters include temperature and humidity of the environment^{1,11,13,17}.

Polymeric materials are widely applied in the biomedical area. Although it is much easier to obtain electrospun fibers from synthetic polymers, natural polymers are desired due to its biocompatibility and biodegradability¹⁸. One can use blends of synthetic and natural polymers, aiming the

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development of new materials with properties intermediate or even superior to those observed in pure constituents⁹.

Gelatin is a derivative of collagen obtained by denaturing its triple helical structure^{13,19}. There are two types of gelatin extracted from collagenous tissue: Type A, which is pre-processed by acid treatment; and Type B, which is pre-processed by an alkaline treatment. The main difference between the two is that type B has higher carboxylic acid content than type A. The molecular weight distribution of type A and B gelatin is slightly different. Type B gelatin usually has a greater proportion of high molecular weight fractions, while type A has a more homogeneous distribution, containing larger amount of low molecular weight polypeptides²⁰.

Due to the similarities between collagen and gelatin, the latter became an attractive polymer for applications in tissue engineering²¹. Electrospun gelatin mats have been used for different applications such as artificial skin, bone graft substitutes, cartilage, blood vessels, and tendon implants. In medicine, gelatin can be used as sponges and bleeding stakes which are then absorbed by the human body^{5,13,18,22}.

The ideal solvent for gelatin for medical applications is water in the absence of toxic residues. However, at room temperature gelatin is partially soluble in water and usually presents high viscosity, which makes it not suitable for electrospinning. This occurs because at room temperature there is an increase in hydrogen bonding, which leads to a transition in the individual helices that were separated in the collagen denaturation. The helices rearrange themselves over time to form a three-dimensional gel network²³. Therefore, in order to obtain electrospun gelatin mats at room temperature, it is necessary to use other solvents in order to achieve solution properties that are suitable for electrospinning.

Some studies on gelatin electrospinning reported that nanofibers were obtained from solutions using solvents such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), 2,2,2-trifluoroethanol (TFE) that can be used to dissolve natural polymers^{6,24,25}. However, these solvents are highly toxic and residues of these solvents in which the fibers were electrospun may be released by degradation in the body and be detrimental to the host. Thus acid aqueous systems are preferable for biomedical applications such as water / formic acid and water / acetic acid²⁵.

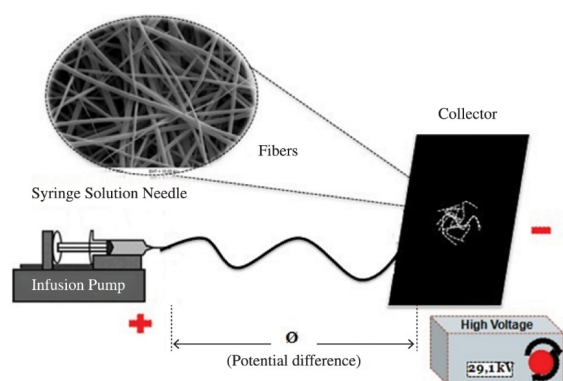


Figure 1. Schematic representation of the typical assembly of an electrospinning apparatus.

Poly(vinyl pyrrolidone) (PVP) is a synthetic polymer, water soluble, biocompatible, hemocompatible and has been applied as a biomaterial for many years²⁶. PVP is remarkable due to its ability to interact with a wide variety of hydrophilic and hydrophobic materials and has properties similar to those of a protein due to its pyrrolidone structure. This material exhibits low immunogenicity, antigenicity and very low toxicity²⁷⁻²⁹. This material is used in a wide variety of applications and it is interesting from a biological point of view, since it has structural features similar to those of proteins, and has great potential for applications in the medical field²⁸⁻³⁰.

Gelatin and PVP are miscible and have interesting biological properties because the mixture of collagen/PVP gives the possibility of producing new materials for the treatment of wound healing^{31,32}. These materials are miscible due to the strong interactions between the synthetic and biological component, mainly due to hydrogen bonds. PVP can be used in the preparation of membranes and fibers in the electrospinning process in order to adjust the morphology, solution viscosity, size and size distribution of pores, increase the permeability of the membrane, and prevent the formation of defects^{24,29}.

This study investigated the electrospinning of gelatin / PVP blends from of acetic acid/water solutions. The effect of acetic acid concentration in the solution properties and in the fiber morphology was investigated. The use of acetic acid in the solutions ensured obtaining solutions with properties suitable for electrospinning. It was found that the average diameters of the resulting fibers vary depending on the solvent composition. The solutions properties such as viscosity, surface tension, electrical conductivity and pH were measured. The morphology of the membranes was evaluated by Scanning Electron Microscopy (SEM) and analyzed by thermogravimetric analysis (TG). Cytotoxicity of gelatin/PVP mats was evaluated with Vero cells.

2. Material and Methods

The materials used in this study were gelatin type B from bovine skin (Sigma Aldrich), PVP (MW = 360,000, Sigma Aldrich), acetic acid (CH₃COOH, 99.7% Ecibra) and deionized water. Solutions were prepared in different mass ratios of water/acetic acid, without any treatment or purification. Polymer solutions were prepared by dissolving 9.2% by weight of gelatin and 7.4% by weight of PVP at various concentrations of water/ acetic acid. Compositions of each solution are shown in Table 1. All solutions were stirred for 2 hours (Nova Ética agitator, Model 114) at 26°C and electrospun at the same day of preparation.

2.1. Solution characterization

All polymer solutions were characterized by measuring pH, viscosity, surface tension and electrical conductivity. The pH, electrical conductivity and surface tension were measured using a digital pH meter (Digimed, Model MD-22), a digital conductivity meter (Digimed, Model DM-32) and a ring tensiometer (Krüss, K-12 and Sigma, 701), respectively. For each solution, average values of these properties were obtained from at least three measurements.

Solution viscosity as a function of the shear rate was measured using a Haake RS1 rheometer, with cone and plate geometry PP35Ti (size 35 mm) in steady shear, with shear rates from 0.01 to 100 s⁻¹. All measurements were performed at 25°C.

2.2. Electrospinning

Electrospinning was performed at room temperature, using a 10 ml disposable syringe with a metal needle of 0.55 mm internal diameter. The positive pole of the high voltage supply (0-30kV, Testtech) was connected to the tip of the metallic needle of the syringe, while the ground electrode was used to ground the copper collector plate with dimensions of 5.0 × 10.0 cm. The flow rate was controlled by an infusion pump (KD-Scientific, KDS Model-100), connected to the syringe. The distance from the needle tip to the collector was 10 cm. Solutions were processed with voltage of 27.1 to 27.2 kV and flow rate of 1 mL/h. Electrospun mats were collected on a copper plate covered with aluminum foil. In each test, approximately 5 ml of the polymer solution were used.

2.3. Characterization of electrospun membranes

Scanning electron microscopy-SEM (Zeiss, Evoma-15) was used to analyze gelatin/PVP electrospun fibers. Samples were coated with gold using a Sputter Coater equipment (Bal-Tec, SCD-O50). Average fiber diameters were determined from SEM images using the image analysis software ImageJ from at least 50 measurements randomly chosen.

Thermogravimetric analysis TG was performed using samples of approximately 10 mg using a STA 409C (Netzsch, Germany). The carrier gas was nitrogen, at a flow rate of 50 mL / min. The samples were heated from 18 to 500°C in a single heating rate of 10°C/min.

2.4. Cytotoxicity

Tests for indirect cytotoxicity of the biomaterial gelatin/PVP fluid extract were performed according to the ISO-10993-5^[33] standard. Vero cell line (Adolfo Lutz/São Paulo) were cultured in medium 199, with 10% fetal bovine serum and 10% antibiotic solution (penicillin / streptomycin), and were maintained at 37°C and 5% CO₂.

Samples were sterilized using ethylene oxide according to ISO 11135^[34] standard and incubated in culture medium for 24 hours to obtain the fluid extract. The cell seeding was performed with density of 3 × 10³ cells/well, in culture plates of 24 wells. Cells were cultured for 24 hours in this

condition until reaching approximately 80% monolayer confluence. Then the culture medium was replaced by the extraction fluid. As a negative control (not cytotoxic) the standard culture medium was used, and as positive control (cytotoxic) a phenol solution 0.4% in culture medium was used. The tests were performed in triplicate.

After 24 hours of incubation the cell morphology was evaluated by phase contrast microscopy. The analysis were complemented by cell fixation in glutaraldehyde (2.5%), followed by staining with Toluidine Blue 0.1%. Observations were made by inverted light microscopy (A1 Axiovert, Zeiss).

For the observation of the cells in direct contact with the biomaterial the culture conditions and processing was the same described above.

3. Results and Discussion

Figure 2 shows a plot of pH versus acetic acid concentration, showing that the pH decreases monotonically with increasing acetic acid concentration from 3.3 to 1.9. This behavior was expected, since the concentration of hydrogen ions (H⁺) is higher in the acidic environment³⁵. Monitoring the solution pH is important since gelatin can degrade in acidic environment at pH less than 1.5^[6]. Thus, the solutions used in this work presented admissible pH values.

Figure 3 shows a plot of surface tension versus acetic acid concentration. It can be seen that the surface tension varied between 33 mN/m and 40 mN/m, and presented a tendency to decrease with increasing the acetic acid concentration.

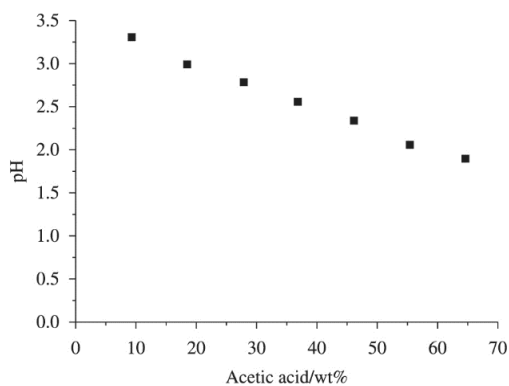


Figure 2. pH as a function of acetic acid concentration.

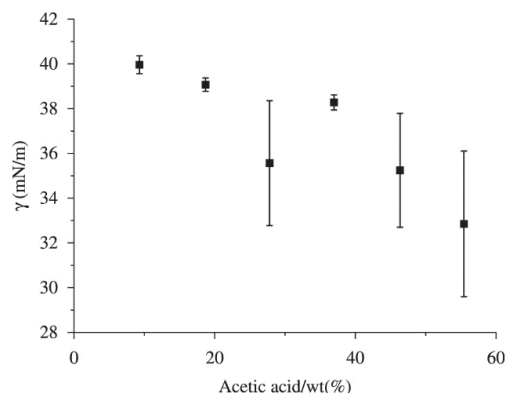


Figure 3. Surface tension as a function of acetic acid concentration in gelatin/PVP solutions.

Table 1. Solution concentration of water/acetic acid (wt%)

Sample	Acetic acid concentration (wt%)
1	9.3
2	18.6
3	27.8
4	37
5	46.3
6	55.5
7	64.8

The decrease in surface tension with the increase of acetic acid concentration was expected since acetic acid has surface tension of about 28.8 mN / m, while water has surface tension of about 72 mN / m at room temperature^{6,36}. However, there is evidence that the polymers also contributed to surface tension decrease. According to the literature, the water / acetic acid solution surface tension at a concentration range of 10 to 40 wt% have values of approximately 55.0 to 41.0, respectively³⁷. The influence of the polymers can be attributed to the fact that they might act as surfactants, being adsorbed at the interface, due to the fact that both gelatin and PVP has hydrophobic and hydrophilic molecular segments^{21,29}.

Figure 4 shows a graph of electrical conductivity as a function of acetic acid concentration. It can be seen in the figure that the conductivity varied from 658.1 $\mu\text{S}/\text{cm}$ and 2001.9 $\mu\text{S}/\text{cm}$ and decreased with increasing acetic acid concentration. The decrease in electrical conductivity occurred differently to what was expected, due to higher concentration of H^+ ions in the solution³⁶. However, it has been reported by Vieira et al.³⁸ that the ionic conductivity decreases with higher levels of acetic acid, which can be due to the formation of multiple ions. On the other hand, there is also evidence that the polymers have an influence on the electrical conductivity of the solution, since this property is mainly determined by the type of polymer and solvent used^{11,39}.

Figure 5 shows a plot of viscosity η versus shear rate. It can be observed that the viscosity presented a slightly shear thinning behavior at low shear rates ($< 1\text{ s}^{-1}$) and at high shear rates ($> 10\text{ s}^{-1}$). Newtonian behavior can be observed in the range of shear rates from 1 to 10 s^{-1} .

A shear rate estimate at the exit of the capillary during the electrospinning process can be obtained from the internal diameter and flow rate given in the previous section. For a Poiseuille flow of a Newtonian fluid, the shear rate can be calculated by where Q is the flow rate and R is the internal diameter of the capillary. Therefore, the solution viscosity can be considered to be Newtonian, since at the end of the capillary, the characteristic shear rate is within the Newtonian range for all solutions.

Figure 6 shows the Newtonian viscosity η_N as a function of acetic acid concentration. The Newtonian viscosity values were taken as an average value of viscosities at shear rates from 1 to 3 s^{-1} .

It can be observed in Figure 6 that η_N ranges from 0.42 to 1.45 Pa.s, and it tends to increase with increasing the concentration of acetic acid. However, η_N of the solution with lower acid concentration (solution 1) is higher than η_N of solutions 2, 3 and 4. According to Davanço et al.⁴⁰, the increased solution viscosity at acidic pH can be related to increased molecular aggregation.

Figure 7 shows a TG thermogram for pure gelatin and PVP. For pure gelatin, it can be observed a weight loss of 20% in the sample curve in the range of temperature of 18 to 100°C before initiating the degradation process, which corresponds to water evaporation present in the sample. Degradation starts at a temperature of approximately 250°C and proceeds up to 420°C with weight loss of approximately 70%. In this process the samples undergo endothermic reactions of hydrolysis and oxidation⁴¹. In the final step

above 420 to 500°C exothermic reactions occur at the end of pyrolysis derived collagen with a mass loss of 89% leaving around 11% ash formed by carbon residues⁴¹.

For pure PVP, TG curve shows an initial weight loss of about 20% from 18 to 80°C , loss of water, the weight loss up to this temperature range is assigned to oligomers, low molecular weight, loss of moisture and residual solvent in

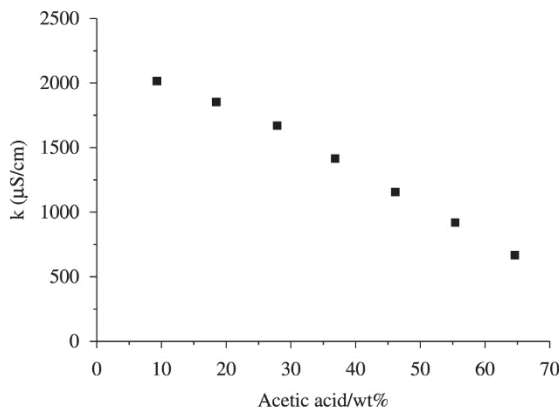


Figure 4. Solution electrical conductivity as a function of acetic acid concentration.

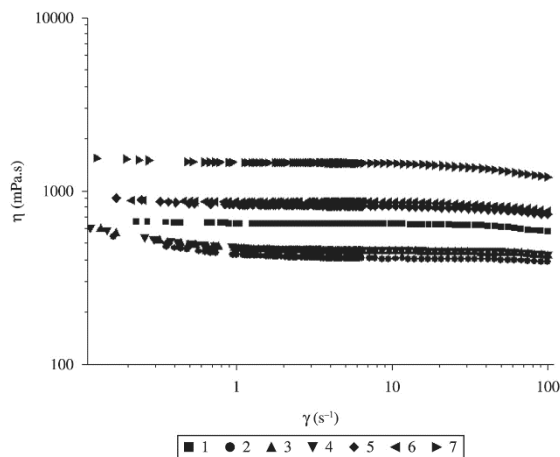


Figure 5. Viscosity as a function of shear rate of the solutions with different concentrations of acetic acid.

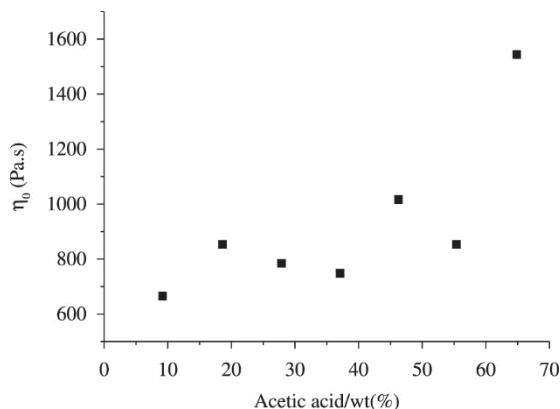


Figure 6. Zero-shear viscosity versus acetic acid concentration of gelatin / PVP

this temperature range PVP degradation can be observed above 330°C, where the weight loss can be attributed to decomposition of the structural polymer⁴².

Figure 8 shows the TG thermogram of electrospun gelatin/PVP membranes obtained from solutions 4 and 7 (see Table 1), which are the membranes used in the cytotoxicity tests. It can be observed that the decomposition begins at approximately 350°C and continues up to 480°C. This shows that the thermal stability of the gelatin was improved due to the presence of PVP. This observation is consistent with the results observed in Figure 7, where it was shown that pure gelatin begins to decompose at about 250°C and the

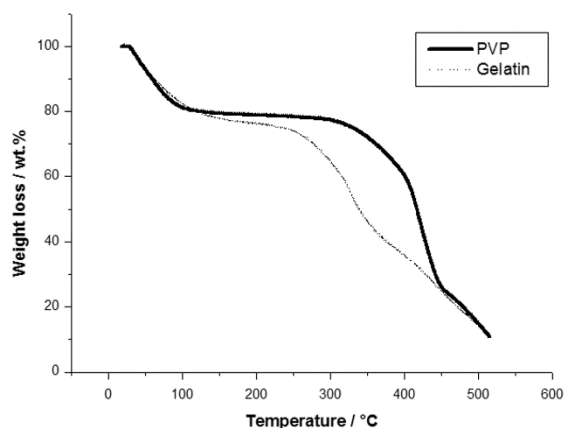


Figure 7. Thermogravimetric curves (TGA) of pure gelatin and pure PVP.

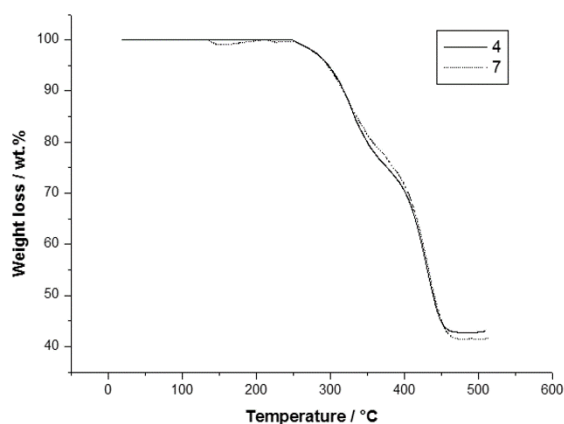


Figure 8. TGA thermal degradation of the polymer blend gelatin / PVP.

polymer blends begin to decompose at higher temperatures above 350 °C. The improved thermal stability of the gelatin is attributed to the interaction between gelatin/PVP, thus leading to higher heat resistance of the resulting polymer blend in comparison with pure gelatin, such as reducing the mass loss of pure gelatin and PVP 11% to about 40% of the polymer blend.

Figure 9 shows SEM images of electrospun gelatin/PVP membranes. It can be observed that uniform fibers were obtained for solutions 4 (Figure 9d), 5 (Figure 9e) and 7 (Figure 9g). For electrospinning from solutions 1, 2 and 3 (Figures 9a-c, respectively) the formation of fibers in some regions was observed, but interconnected fibers in the area analyzed were not observed and it can also be observed the presence of defects. Interestingly, for the case of electrospinning from solution 6 (Figure 9f) it is observed an evidence of fiber formation, but complete solvent evaporation did not occur, leading to the dissolution of the fibers on the collector. It is also important to highlight that this spinning behavior was reproduced.

Figure 10 shows fiber diameter histograms from the electrospun membranes obtained from solutions 4, 5 and 7 (Figures 10a-c, respectively). It can be observed a decrease in average diameter with the decrease of acetic acid concentration. Also, it can be observed that in Figure 10a (solution 4) the histogram peak is in the range of 100-200 nm, where in figure 10b the peak is the range of 300-400 nm (solution 5), and in Figure 10c (solution 7) the peak is in the range of 480-510 nm with significant broader distribution.

It is known that high solution surface tension inhibits the electrospinning process, since jet instabilities lead to jet breakup in droplets⁴³. From the results presented in Table 2, it can be seen that fibers were obtained for higher values of surface tension (solution 4), thus, it can be concluded that the surface tension, within the range of values obtained, was not the determining factor for the formation of fibers.

From Figures 5 and 6 and Table 2, it can be seen that the viscosity depends on the acetic acid concentration. Studies indicate that higher viscosity values often give rise to uniform fibers with larger average diameter due to polymer chain entanglements, which makes jet stretching more difficult^{1,17}. However, as observed in Table 2, the solution with lowest viscosity (solution 7) resulted in fibers with larger diameters when compared with fibers obtained from solution 5, which has higher viscosity. Therefore, this

Table 2. Values of solution properties and the average diameter of the fibers gelatin / PVP obtained by electrospinning.

Number	pH	γ (mN/m)	k (μ S/cm)	η_0 (mPa.s)	Average diameter (nm)*
1	3.30	40.24	2001.9	662.3	-
2	2.98	38.88	1839.4	846.6	-
3	2.78	33.56	1661.2	782.2	-
4	2.54	38.04	1409.2	746.6	154 (38)
5	2.33	33.43	1138.5	1014	355 (121)
6	2.04	30.53	916.1	851.9	-
7	1.89	33.25	658.1	1541	519 (192)

*The value in parentheses corresponds to the standard deviation.

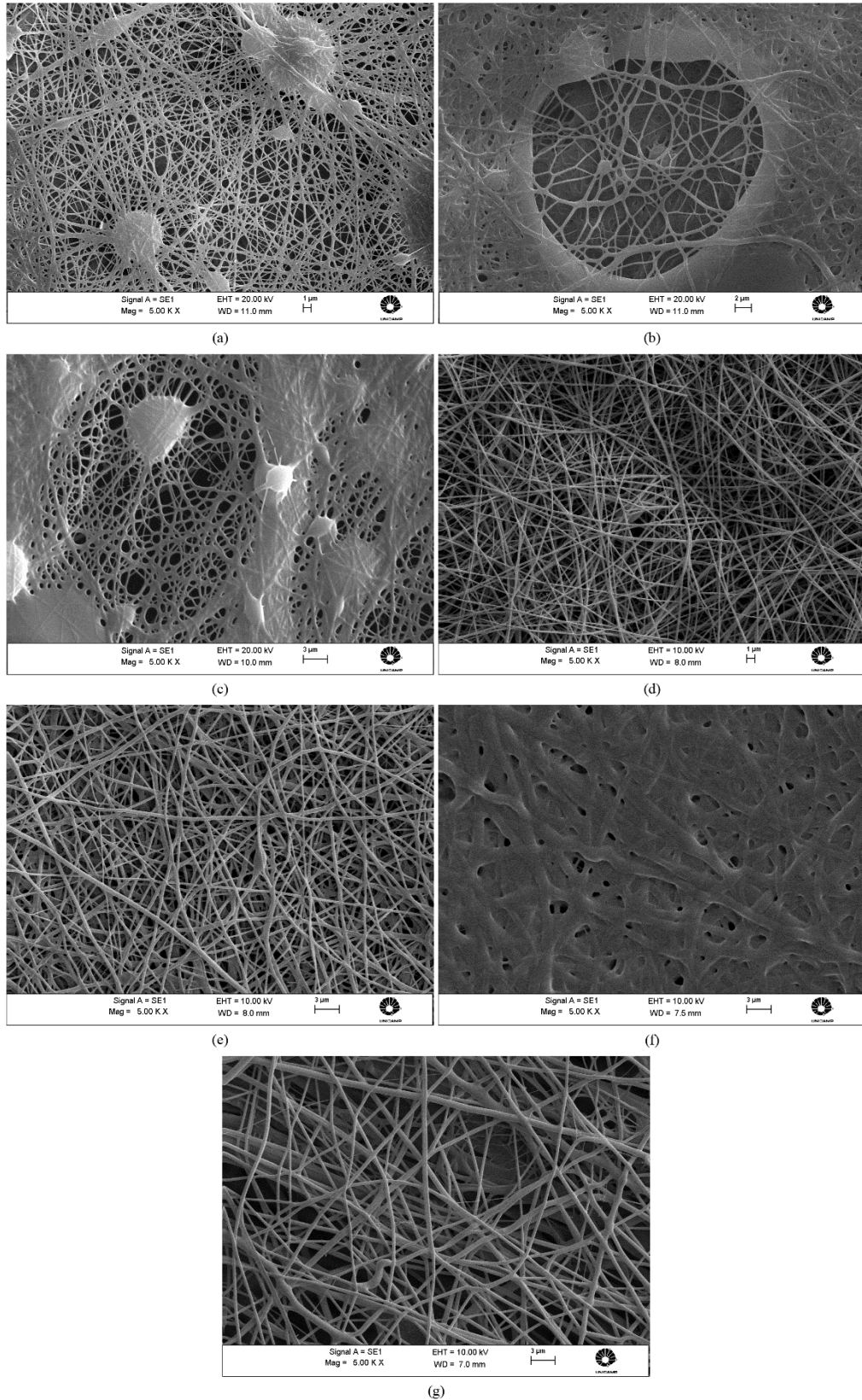
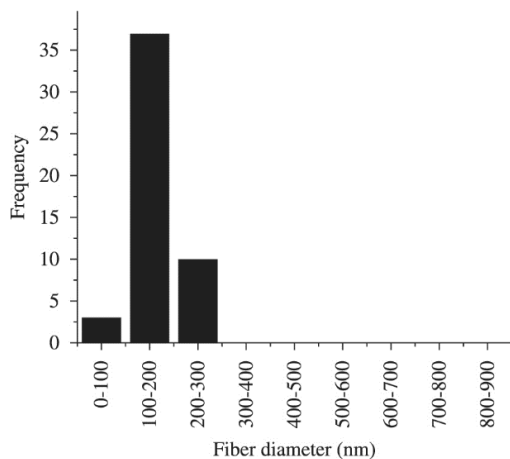


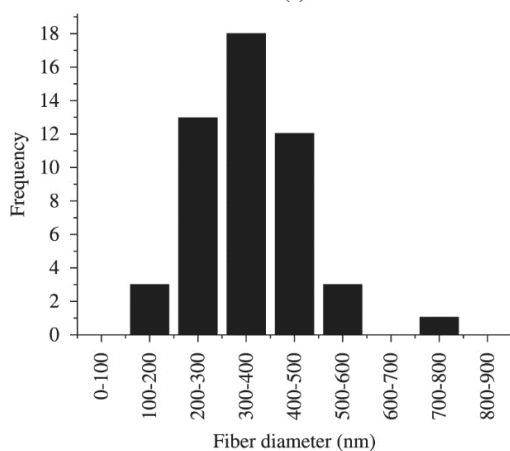
Figure 9. SEM images of the electrospun fibers from different solutions with magnification of 5000x: (a) 1 (9.3wt%), (b) 2 (18.6wt%), (c) 3 (27.8wt%), (d) 4 (37.0wt%), (e) 5 (46.3wt%), (f) 6 (55.5wt%) and (g) 7 (64.8wt%).

result indicates that the viscosity was not the main solution property which determines the fiber diameter.

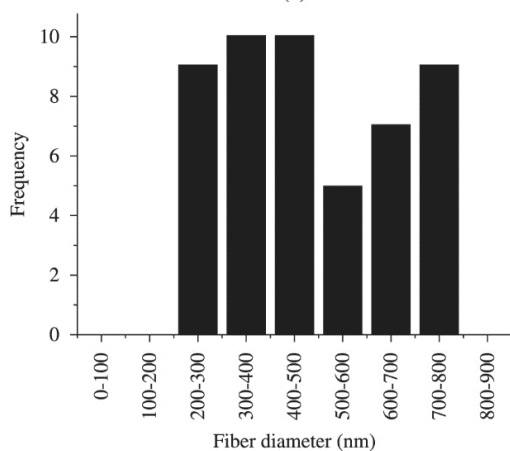
It can be seen in Table 2, that the average fiber diameter decreased with electrical conductivity increase, although for values of electrical conductivity above 1661.2 $\mu\text{S}/\text{cm}$, the fibers were not uniform and had many defects. Thus, there is



(a)



(b)



(c)

Figure 10. Histogram of fiber diameters of gelatin / PVP electrospun. With different concentrations of acetic acid (%) weight: (a) Solution 4 (37.0), (b) Solution 6 (46.3), (c) Solution 7 (64.8).

a maximum limit for electrical conductivity in order to obtain uniform fibers, where above this limit jet breakup occurs.

From the results presented it can be concluded that the solution electrical conductivity, which depends on acetic acid concentration has a significant effect on the fiber formation and morphology.

Figure 11 shows a photograph of an electrospun gelatin/PVP membrane. Typical membranes presented dimensions of approximately 3.5×3.5 cm, and thickness of approximately 3 mm.

In vitro cytotoxicity tests are made as an initial verification of the biomaterials biocompatibility evaluation, aiming future applications of a specific biomaterial in tissue engineering. The cytotoxicity is an *in vitro* technique that can be morphologically detected by cell observation, or can be measured quantitatively by cell lysis (cell death), the inhibition of cell growth and other possible effects on cells caused by the devices, materials and / or their extracts. In this investigation the morphology of cells exposed to elution biomaterial extracts was observed.

In Figure 12a it is noted a confluent monolayer scattered of cells, used as non cytotoxic control. We observed the basophilic cytoplasm and the individual nucleus, containing 1 to 3 nucleoli, typical of this cell line. In Figure 12b it can be observed that the solution of phenol showed cytotoxic effects, and in this condition the few cells were adhered to the culture plate, showing various extensions and very dense nucleus; it is not possible to identify the nucleoli, indicative of degeneration process. Debris were observed in the culture medium.

In Figure 13a and Figure 13c cultured cells provided indirect cytotoxicity which showed growth pattern expected to Vero cells, and consistent with non cytotoxic control. The cells had become quite spread, forming a confluent monolayer with fibroblastic appearance, well-defined nucleus and clear nucleolus identification. There was no cell debris or expressive cell degeneration. In Figure 13b and Figure 13d the same result can be observed in the test cell culture in direct contact with the biomaterial gelatin / PVP. The cells in the culture dish, and even in areas of direct contact with the biomaterial, kept the standard typical fibroblast without significant signs of degeneration and cell death^{44,45}.

The growth of Vero cells in confluent monolayer with typical fibroblast morphology, and similar to non cytotoxic control, shows that the biomaterial gelatin / PVP can be

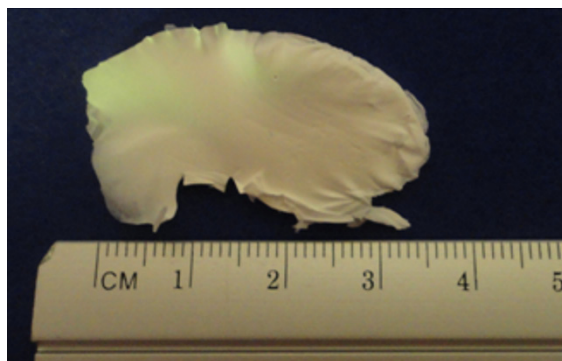


Figure 11. Electrospun gelatin/PVP membrane.

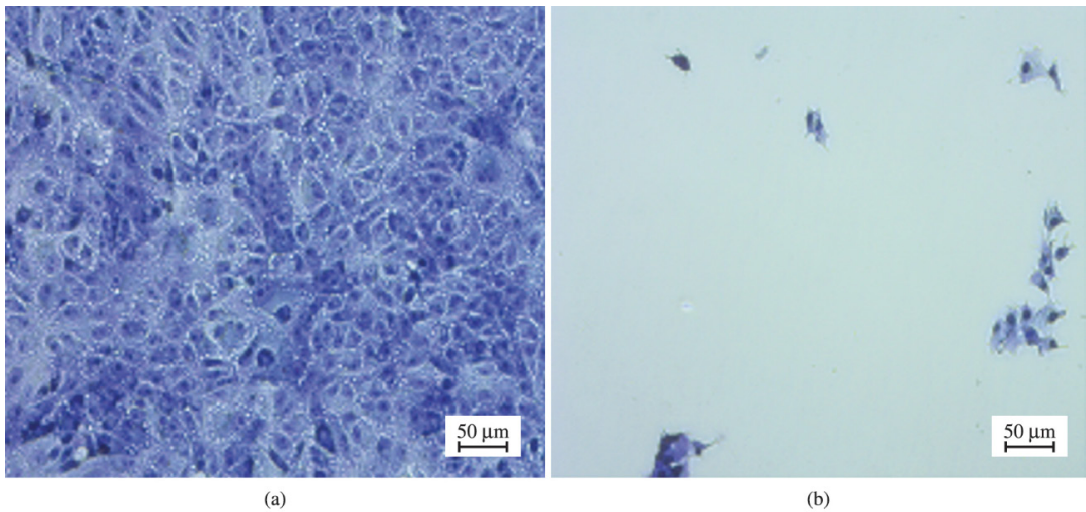


Figure 12. Test of cytotoxicity controls. (a). noncytotoxic control, confluent monolayer of cells. (b) Control cytotoxic few cells present, with very dense nucleus, cellular prolongations and presence of debris. Cells stained with toluidine blue, increased 200x.

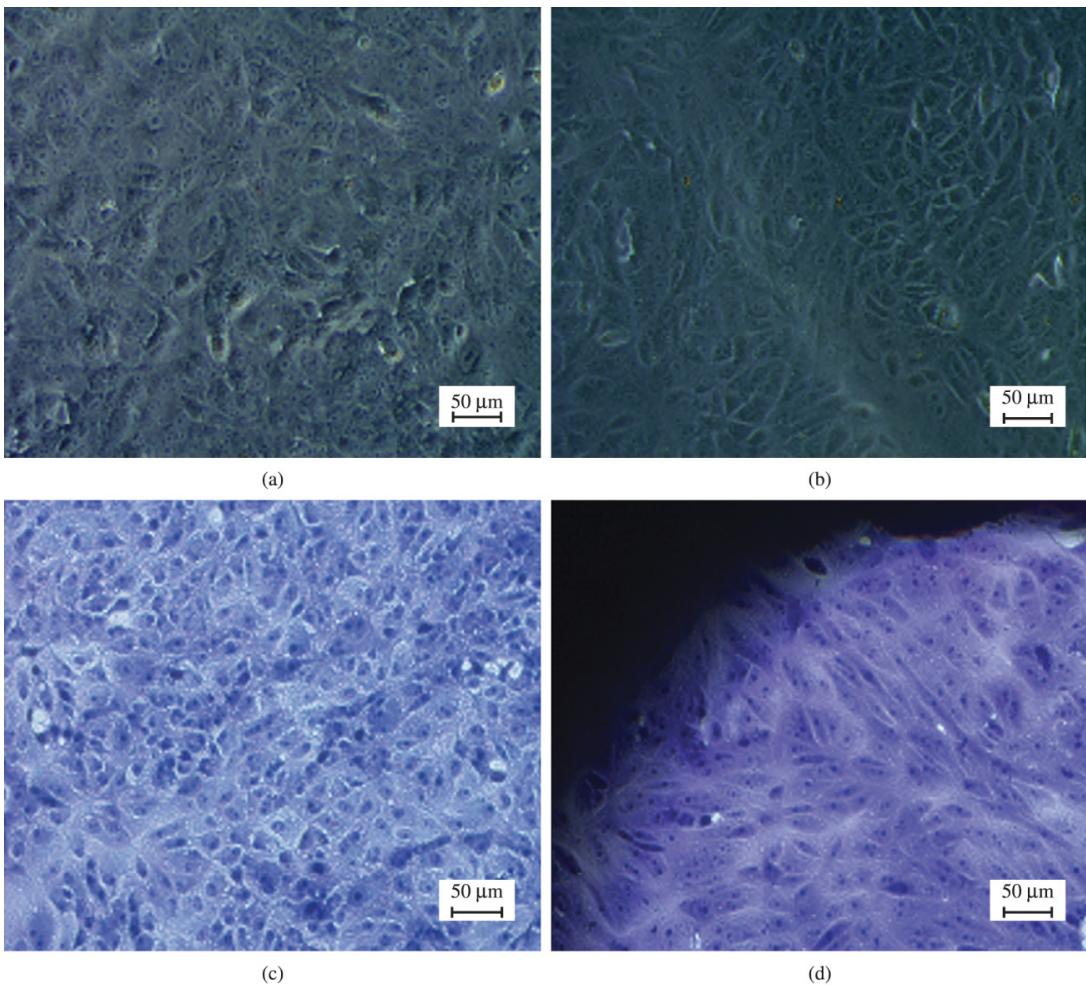


Figure 13. Test of cytotoxicity biomaterial. (a) Cytotoxicity indirect, phase contrast, (b) Direct contact of the cells with the biomaterial, phase contrast. (c) indirect cytotoxicity, cells stained with toluidine blue (d) Direct contact of the cells with biomaterials, cells stained with toluidine blue. It can be observed the cell monolayer widely scattered, with typical morphology of fibroblasts, even in the limit contact with the biomaterial gelatin / PVP. Increase: 200.

considered non cytotoxic^{44,45}. The growth pattern cytotoxic, with few cells, with extensions, dense core, debris in medium and signs of cellular degeneration was not observed in samples of indirect cytotoxicity of the gelatin biomaterial / PVP. The results obtained with the cell culture in direct contact with the biomaterial proved remarks indirect cytotoxicity. It was possible to observe the same confluent monolayer on the surface of direct contact with the biomaterial with typical fibroblast cells. These results enable future trials to guide the study of cellular interaction with the biomaterial.

In Figure 13a and Figure 13c cultured cells provided indirect cytotoxicity showing growth pattern expected to Vero cells, and consistent with control noncytotoxic. The cells had become quite spread, forming a confluent monolayer with fibroblastic appearance, well-defined nucleus and nucleolus identification of between 1 to 3 per core. There was no cell debris or cell degeneration expressive. In Figures 13b and 13d the same result can be observed in the test cell culture in direct contact with the biomaterial gelatin / PVP. The cells in the culture dish, and even in areas of direct contact with the biomaterial, kept the typical fibroblast standard without significant signs of degeneration and cell death^{44,45}.

The growth of Vero cells in confluent monolayer with typical fibroblast morphology, and similar to control non cytotoxic, shows no cytotoxicity of the gelatin biomaterial / PVP^{44,45}. The growth pattern cytotoxic, with few cells, with extensions, dense core, debris in solution and signs of cellular degeneration was not observed in samples of indirect cytotoxicity of the gelatin biomaterial / PVP. The results obtained with the cultivation of cells in direct contact with the biomaterial proved remarks indirect cytotoxicity. It was possible to observe the same confluent monolayer on the surface of direct contact with the biomaterial with

typical fibroblast cells. These results enable future trials to guide the study of cellular interaction with the biomaterial.

4. Conclusions

In this work, the influence of acetic acid concentration in the electrospinning of galatin/PVP blends from aqueous solutions containing acetic acid was investigated. From the results obtained, it was found that lower concentrations of acetic acid promoted the formation of fibers with smaller diameters, within the concentration range studied. Therefore, the presence of acetic acid has a significant effect on fiber morphology and average diameter.

Solution surface tension and viscosity did not significantly affect the formation of gelatin/PVP fibers. It was found that electrical conductivity was the decisive parameter that highly influenced the resulting fiber diameter.

As expected, it was observed that solutions with higher electrical conductivity produced fibers with smaller diameters. Nanofibers obtained were cylindrical and were randomly distributed in a fibrous mat. In the thermogravimetric analysis of gelatin/PVP, it was observed that the improved thermal stability of the gelatin is attributed to the interaction between gelatin/PVP.

The results obtained from cytotoxicity assays showed that the biomaterial gelatin/PVP showed the expected pattern of growth to Vero cells and no significant signs of degeneration and cell death, which is consistent with the non cytotoxic standard.

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