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Quercetin and curcumin in nanofibers of polycaprolactone and poly(hydroxybutyrate-*co*-hydroxyvalerate): Assessment of *in vitro* antioxidant activity

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ABSTRACT: Polymeric nanofibers are materials that can be used as scaffolds in tissue engineering. Quercetin and curcumin are antioxidants because of scavenge free radicals and chelate metal ions properties, protecting tissues of lipid peroxidation. The objective of this study was to develop a scaffold with potential antioxidant activity that was produced from nanofibers consisting of polycaprolactone (PCL) and a blend of PCL/poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHB-HV) with the addition of quercetin or curcumin as the bioactive compound. Curcumin and quercetin were integrated into the solution at a concentration of 3%. The electrospun nanofibers were characterized using calorimetry and thermogravimetric analysis, and the addition of bioactive compounds did not alter the thermal properties of the biomaterial. The antioxidant activity of scaffolds with the active compounds was evaluated by hydrate 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) methods. The scaffolds with PCL and PCL/PHB-HV blend with quercetin exhibited higher antioxidant activity than curcumin with both methods. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43712.

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INTRODUCTION

Electrospun nanofibers are three-dimensional structures with large contact surface compared to the diameter and high porosity, also is an attractive topic in tissue engineering field. The characteristics and properties enable restructuring of the native extracellular matrix, which is necessary for creating a specific tissue or organ.[1]

To assist and accelerate the healing process of tissues using scaffolds, some researchers have studied the incorporation of bioactive compounds into these three-dimensional structures. The addition of these compounds can provide scaffolds with antibacterial,[2] antifungal,[3] anticancer,[4] anti-inflammatory,[5] and antioxidant.[6]

Quercetin is a flavonoid, which is found in various foods, such as onions, apples, black tea and citrus fruits, and curcumin is a diarylheptanoid, which has been isolated from turmeric (*Curcuma longa* L.) and is found in spicy foods, such as curry, both compounds have the potential to act as bioactive compounds. These components have antioxidant, anti-inflammatory and antibacterial properties that stimulate the recovery of injured tissue. Furthermore, because quercetin and curcumin can be integrated into the interstices of nanofibers, the polymeric material can protect against bioactive degradation due to its photosensitivity.[7]

Polymeric nanofibers containing bioactive compounds, such as quercetin and curcumin, have become the focus of much research. According to Wu *et al.*,[8] agents, such as quercetin, may be of interest in the development of new drugs that can act as both an anti-inflammatory agent and an antioxidant. Martin[9] studied the *in vivo* topical application of quercetin and reported improvement in wound healing and protection of

tissue from oxidative damage. Li *et al.*,[10] developed ethyl cellulose nanofibers with quercetin using an electrospinning method and assessed the *in vitro* release of the drug. Despite the importance of these results, these studies have not evaluated the content of bioactive compounds added to these scaffolds but only focused on the pharmacological action of the biomaterial. In this study, we determined the content and antioxidant potential of quercetin and curcumin in the scaffolds to justify the application of these new biomaterials in tissue engineering.

The objective of this study was to develop a scaffold with potential antioxidant activity that was produced from nanofibers consisting of polycaprolactone (PCL) and a blend of PCL/poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHB-HV) with the addition of quercetin or curcumin as the bioactive compound.

EXPERIMENTAL

Preparation of Polymer Solutions for Electrospinning

The polymers consisted of PHB-HV [containing 12% mol of poly(3-hydroxyvalerate)] and PCL (molecular mass 80,000 g mol^{-1}) (Sigma-Aldrich®, USA). Two polymer solutions were prepared for electrospinning. The first solution consisted of 12% PCL and 1.4% NaCl (w v^{-1}), and the other solution contained 5% PCL, 10% PHB-HV and 1.4% sodium chloride (NaCl) (w v^{-1}). Chloroform was used as the solvent for both solutions. The commercial bioactive compounds (curcumin and quercetin) (Sigma-Aldrich®, USA) were integrated into the solutions at a concentration of 3% (w v^{-1}). NaCl and chloroform (as a solvent) were used in the polymer solutions. All of the solutions were homogenized using a magnetic stirrer (Fisatom, Brazil) for 16 h.

Electrospinning

For the electrospinning technique, the solutions were injected via a capillary with a diameter of 0.8 mm, and the distance between the capillary and the collector was 120 mm. In addition, an electric potential of 25 kV was used with a feed rate of 2000 μL h^{-1} to the PCL solution and 200 μL h^{-1} for the solution with the PCL/PHB-HV blend. All of the tests were carried out at 22 °C with the relative humidity maintained at 65 ± 1%.[11]

Physical-Chemical Analysis in the Developed Nanofibers

Viscosity of the Polymer Solutions. The viscosity of the polymer solutions with and without the addition of 3% quercetin or curcumin were determined using a rheometer (Brookfield DV-III Ultra Programmable Rheometer, USA), and 0.5 mL of each sample was employed.

Shape and Diameter of the Nanofibers. Images and 30 measurements of the diameters of the nanofibers were obtained using a scanning electron microscope (SEM) (JEOL JSM-6610 LV, Japan). The 20 samples were fractured in liquid nitrogen to suppress the mobility of the polymer chains. Before the analyses, the samples were fixed in a metallic holder with carbon tape and coated with gold using a sputtering diode (Denton Vacuum CAR001-0038, USA).

Endothermic and Exothermic Transitions of Nanofibers. Differential scanning calorimetry (DSC) (Shimadzu DSC-60, Japan) was used to determine the melting temperature of the scaffolds (12% PCL, 1.4% NaCl and 3% quercetin or curcumin solution; blend 5% PCL, 10% PHB-HV, 1.4% NaCl and 3% quercetin or curcumin solution) and quercetin and curcumin. Approximately 4 mg of the sample was placed in a closed aluminum capsule under a nitrogen atmosphere at a flow rate of 50 mL min^{-1}. The analyses were carried out at room temperature up to 180 °C with a heating rate of 10 °C min^{-1}. The melting temperature was determined from the peak melting temperature in the DSC curve according to the ASTM (American Society for Testing and Materials) D7426-08[12] method.

Thermal Degradation of Nanofibers and Residual Solvent Content. The thermal stability of the scaffolds (12% PCL, 1.4% NaCl and 3% quercetin or curcumin solution; blend 5% PCL, 10% PHB-HV, 1.4% NaCl and 3% quercetin or curcumin solution) and the bioactive compounds (quercetin and curcumin) as well as the residual solvent contents were determined by thermogravimetric analysis (TGA) (Shimadzu DTG-60, Japan) according to the ASTM D3850-12[13] method. The analyses of 2 − 6 mg samples were carried out at room temperature to 500 °C in an inert atmosphere consisting of nitrogen with a flow rate of 30 mL min^{-1} and a heating rate of 10 °C min^{-1}.

Evaluation of Content of the Bioactive Compounds Incorporated into the Nanofibers. The bioactive content incorporated into the samples (12% PCL, 1.4% NaCl and 3% quercetin or curcumin solution, blend 5% PCL, 10% PHB-HV, 1.4% NaCl and 3% quercetin or curcumin solution) was estimated using UV/visible (UV/Vis) spectroscopy after extraction of the bioactive compounds from the nanofibers. To obtain the samples, 1 mL of chloroform and 1 mL of dimethylsulfoxide (DMSO) were added to 10 mg of each nanofibers, and these dispersions were placed in an ultrasonic bath (Unique-USC 1400A, Brazil) for 10 min. Next, 8 mL of methanol were added to the nanofibers with quercetin, and 8 mL of acetonitrile were added to the nanofibers with curcumin.

The samples were placed in an ultrasonic bath for 10 min and centrifuged (Quimis Q222T216, Brazil) at 2500 rpm. The resulting solutions were analyzed with a UV/Vis spectrophotometer (PerkinElmer Lambda25, Brazil) at 425 and 375 nm for curcumin and quercetin, respectively.

The standard curves were constructed from stock solution of quercetin and curcumin (1 mg mL^{-1}) diluted in methanol and acetonitrile, respectively. The standard solutions were prepared (50 mg mL^{-1}) to construct calibration curves for spectrophotometry. The standard solutions were diluted to concentrations of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 μg mL^{-1}. The quercetin and curcumin solutions were prepared and homogenized in an ultrasonic bath, and the absorbance was read using a UV/Vis spectrophotometer (PerkinElmer Lambda25, Brazil).

The validation of the method for determining the bioactive content was carried out based on the guidelines of the International Conference on Harmonization (ICH) American Pharmacopeia, and the specificity, linearity and limits of detection and quantification parameters were evaluated. The specificity was assessed by analysis of the solution containing quercetin and curcumin

as well as the white solution (without the bioactive) to investigate any possible interference in the quantification of the drugs.

To assess the linearity, the standard solutions were analyzed in triplicate on three different days, and the results were compared to a previously constructed standard curve using linear regression.

The detection limit (DL) and quantification limit (QL) were calculated directly from the straight slope (S) and the standard deviation of the intercept (SD), which were obtained after construction of three calibration curves. The DL and QL were calculated using Equations (1) and (2), respectively.

$$DL = 3,3SD/S \quad (1)$$

$$QL = 10SD/S \quad (2)$$

Confocal Microscopy. To assess the incorporation of the bioactive compounds into the nanofibers, a confocal microscope was used to determine the fluorescence emission profile of the scaffolds produced from solution of 12% PCL and a blend of 5% PCL blend/10% PHB-HV, which contained NaCl (1.4%) with and without the bioactive compound (3%). Initially, slides were prepared with the samples and water, and coverslips were placed over the samples. Then, the samples were visualized on a fluorescence confocal microscope (Leica TCS SP8, Germany). The analyses were carried out at a wavelength of 425 nm.

Test of Antioxidant Activity with Hydrate 2,2-diphenyl-2-picrylhydrazyl (DPPH). The assay was carried out according to the methodology reported by Miliauskas *et al.*[14] The samples of the extracts of each compound (quercetin, and curcumin) that were obtained from the nanofibers and reacted with the DPPH radical were read using a spectrophotometer (Quimis Q898DRM, Brazil) at 515 nm.

To extract the bioactive compounds from the nanofibers (12% PCL, 1.4% NaCl and 3% quercetin or curcumin solution; blend 5% PCL, 10% PHB-HV, 1.4% NaCl and 3% quercetin or curcumin solution), 1 mL of chloroform and 1 mL of DMSO were added to 10 mg of each nanofiber sample, and these dispersions were placed in an ultrasonic bath (Unique-USC 1400A, Brazil) for 10 min. Next, 8 mL of methanol were added to the nanofibers with quercetin, and 8 mL of acetonitrile were added to the nanofibers with curcumin. The samples were again placed in an ultrasonic bath for 10 min and centrifuged (Quimis Q222T216, Brazil) at 2500 rpm.

The DPPH solution was prepared on the day of analysis at a concentration of 0.024 g L^{-1} of methanol. To carry out the assay, 3.9 mL of the DPPH solution were added to 0.1 mL of the bioactive compound sample extracted from the nanofibers. In addition, two control solutions were prepared using 0.1 mL of a solution of the mixture of solvents of nanofibers without bioactive compounds and 3.9 mL of a DPPH solution. The first solution contained chloroform, DMSO and methanol, and the second solution contained chloroform, acetonitrile and DMSO. The samples were maintained in the dark at room temperature. Then, the samples were read at 10 min intervals using a spectrophotometer until 40 min of reaction were completed to monitor the reduction of absorbances. All of the determinations were obtained in triplicate, and Tukey's test was carried out to determine the statistically significant differences ($p < 0.05$) among the means.

Test of Antioxidant Activity with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) Diammonium Salt (ABTS). The assay was carried out according to the methodology reported by Miliauskas *et al.*[14] To prepare the samples, the bioactive compounds were extracted for the DPPH assay.

After extraction, 30 μL of the samples containing the bioactive compounds were added to 3 mL of a $ABTS^+$ solution. The $ABTS^+$ radical was generated by oxidation of ABTS with potassium persulfate. Two control solutions were prepared from 30 μL of the solution of the mixture of solvents of nanofibers with and without the extracts and 3 mL of the $ABTS^+$ solution. One solution contained chloroform, DMSO and methanol, and the other solution contained chloroform, acetonitrile and DMSO. The samples were maintained in the dark at room temperature and analyzed on a spectrophotometer at 734 nm at 6 min intervals for 18 min. All of the measurements were carried out in triplicate, and Tukey's test was used to determine the statistically significant differences ($p < 0.05$) among the means.

RESULTS AND DISCUSSION

Shape and Diameter of the Nanofibers

The Figure 1 shows the SEM images of PCL and PCL/PHB-HV blend electrospun nanofibers containing quercetin and curcumin. It was demonstrated that nanofibers were uniform without the presence of any beads. The electrospun nanofibers diameter with PCL showed 332 ± 86 and 434 ± 65 nm to quercetin (a) and curcumin (b), respectively. The electrospun nanofibers diameter with PCL/PHB-HV showed 556 ± 101 and 515 ± 109 nm to quercetin (c) and curcumin (d), respectively.

The NaCl was added in the solutions due to salt affects the diameter of the nanofibers because its components confer electrical conductivity to the solution, providing greater ion mobility, and thus, the nanofibers become more elongated between the capillary tip and the collector. According Beachley and Wen[15] reported that the addition of NaCl to a PCL solution resulted in nanofibers with small diameters that were uniform when compared with those produced without the addition of salt.

Viscosity of the Polymer Solutions

The electrospinning experiments demonstrated that it was possible to develop nanofibers without droplets from solution with viscosity values that ranged between 2.2 and 3.4 Pa s (Table I). The concentrations of the solutions also influenced the formation of the nanofibers because the low viscosity in the low concentration solutions led to nanofibers with droplets. By increasing the concentration, the viscosity of the solution increased due to improvement in the molecular mesh, which enabled a continuous jet during electrospinning.[16]

Endothermic and Exothermic Transitions of the Nanofibers

In the curcumin sample, the transition calorimetry curves had a transition in the form of a peak, representing a first order endothermic event with a melting point of 175 °C and an enthalpy variation of 124 mJ mg^{-1}. In the quercetin samples, only a shift in the baselines of the curve was observed, representing a

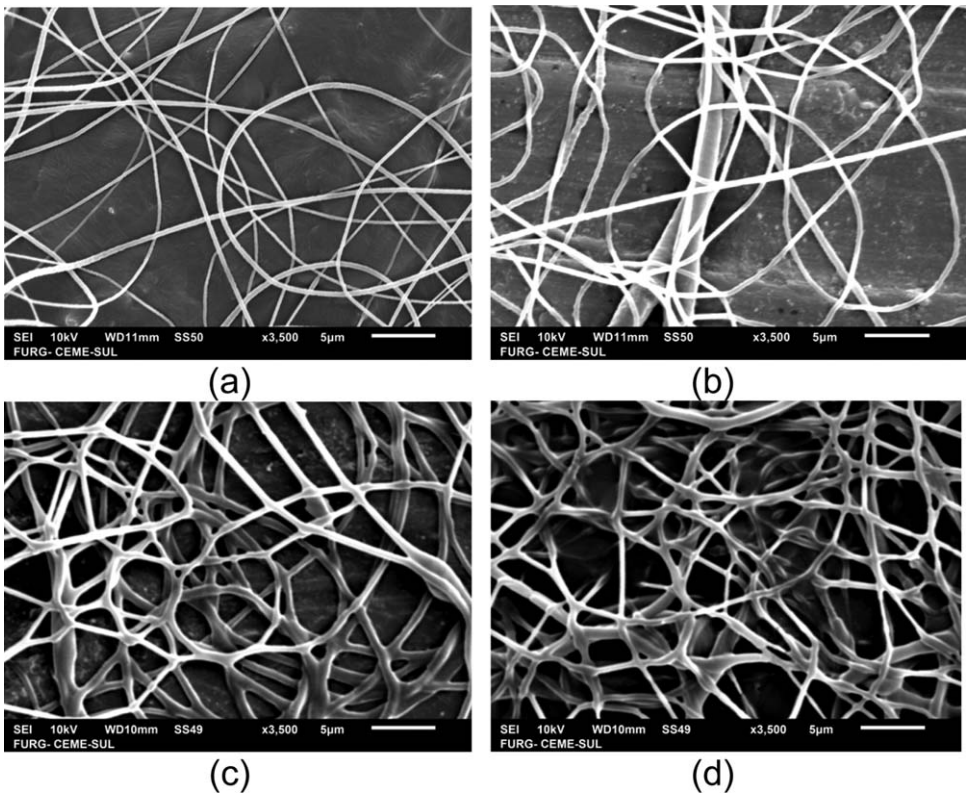

Figure 1. SEM images of PCL electrospun nanofibers containing quercetin (a) and curcumin (b), PCL/PHB-HV containing quercetin (c) and curcumin (d) (×3500 times).

second order endothermic event at 100 °C. This result indicated that the melting point of this compound was greater than 180 °C.

The scaffolds of PCL and PCL with the bioactive compounds exhibited first order endothermic events with melting points of ~58 °C. The enthalpies of the samples were 55 mJ mg^{-1}, 47 mJ mg^{-1}, and 47 mJ mg^{-1} for pure PCL scaffolds, PCL with curcumin and PCL with quercetin, respectively, and these values are relatively similar.

Furthermore, in comparison to the PCL/PHB-HV blend, the PCL scaffold presented a difference in the transition reactions. Two types of events occurred including a first order event that was characterized by endothermic peaks and a second order one that was characterized by a shift in the baselines of the curves from 112 °C to 160 °C. The first order event was also observed in the DSC of the PCL nanofibers but the formation of the blend reduced the melting point to approximately 54 °C in both the pure nanofibers and in those with the added compounds. The second order event was most likely due to the addition of PHB-HV to the nanofibers, which had a melting point of approximately 150 °C. The enthalpies of the samples were 11 mJ mg^{-1}, 7 mJ mg^{-1}, and 11 mJ mg^{-1} for the scaffolds consisting of a PCL/PHB-HV blend, PCL, and PHB-HV with curcumin and quercetin, respectively.

Table I. Results from the Analyses of Polymer Solutions and Scaffolds of Nanofibers Containing Curcumin and Quercetin

Sample	Parameters							
	C	Q	P1	P1C	P1Q	P2	P2C	P2Q
Viscosity (Pa s)	-	-	2.2	3.4	2.3	3.3	3.4	3.0
Melting point (°C)	175	>180	58	58	58	54	54	55
Enthalpy (mJ mg^{-1})	124	34	55	47	47	11	7	11
Initial degradation temperature (°C)	287	300	333	330	344	228	225	229
Final degradation temperature (°C)	400	384	434	439	449	294	290	286
Maximum degradation temperature (°C)	353	352	403	399	404	268	262	267

(-): unanalyzed sample; C: curcumin; Q: quercetin; P1: 12% PCL + 1.4% NaCl; P1C: 12% PCL + 1.4% NaCl + 3% curcumin; P1Q: 12% PCL + 1.4% NaCl + 3% quercetin; P2: 5% PCL + 10% PHB-HV + 1.4% NaCl; P2C: 5% PCL + 10% PHB-HV + 1.4% NaCl + 3% curcumin; P2Q: 5% PCL + 10% PHB-HV + 1.4% NaCl + 3% quercetin.

PCL is a biodegradable and bioreabsorbable synthetic polymer that has been used in tissue engineering. In addition, PCL has a melting point between 58 and 63 °C. The melting temperatures of the manufactured scaffolds (54, 55, and 58 °C) were close to the value of the pure polymer.[17]

Thermal Degradation of the Nanofibers and Residual Solvent Content

Quercetin and curcumin exhibited maximum degradation temperatures of 352 to 353 °C, respectively. The thermogravimetric curves generated when the active compounds were integrated into the PCL nanofibers had a mass loss behavior that was nearly identical to the curve observed for the nanofibers produced with only PCL. This result demonstrates that the addition of the compounds did not change the thermal stability of the nanofibers because the degradation temperature (403 °C) of the PCL polymer remained similar to that of the polymer with the compounds (399 and 404 °C, with curcumin and quercetin, respectively).

The addition of PCL to the bioactive compounds resulted in a higher initial degradation temperature compared to that of the pure compounds, demonstrating that the polymer confers greater protection to the bioactive compounds. According to Shin *et al.*,[18] PCL exhibits an initial degradation temperature of 365 °C. The TGA curves obtained by Machado *et al.*[19] indicated that PHB-HV has an initial thermal degradation temperature of 250 °C. Because this polymer is more easily degraded, the temperature for blends of PCL and PHB-HV decreased compared to that of the scaffold containing PCL.

The thermal behavior of the scaffolds for use in tissue engineering has been assessed by Patrício *et al.*,[20] who reported mean degradation temperatures between 300 and 400 °C. These results are similar to those determined in this study. TGA analysis confirmed that the nanofibers contained no trace of the solvent used in the polymer solution. Chloroform has an evaporation point of 61 °C. Therefore, no change in this temperature range was observed in any of the curves.

Assessment of the Content of Bioactive Compounds Incorporated into the Nanofibers

The content of quercetin and curcumin in 10 mg of scaffolds was measured using spectroscopy UV/Vis. The quercetin and curcumin contents for PCL were 174.2 ± 0.1 and 159.3 ± 0.2 μg, respectively. For the PCL/PHB-HV blend, the quercetin and curcumin contents were 155.7 ± 0.1 and 137.9 ± 1.2 μg, respectively. A larger amount of quercetin was incorporated into the nanofibers compared to the amount of incorporated curcumin for both the PCL scaffolds and the PCL/PHB-HV blend.

Brahatheeswaran *et al.*[21] studied the *in vitro* release of curcumin in zein nanofibers for application as scaffolds in the biomedical field. The release of this compound increased over exposure time in fibroblast cells even though they did not measure the curcumin release. Therefore, a quantitative analysis of the curcumin in the nanofibers could not be performed.

Validation of the Method of Determination of the Bioactive Content

Analysis of the samples without the bioactive compounds indicated that the method is specific, and no interference in the extraction of bioactive compounds containing quercetin and curcumin was observed. The linearity of the technique was determined after construction of three calibration curves for three days, and the limits of detection and quantification were calculated from the curve data (ICH). With a determination coefficient close to 1.0 and straight upwards of calibration curves, the data indicated that the technique has a positive linear correlation between the concentration of drugs and absorbance [eqs. (3) and (4)].

The limits of detection and quantification for quercetin using the UV/Vis spectrophotometer were 0.7 mg mL^{-1} and 2.0 mg mL^{-1}, respectively. For curcumin, the detection and quantification limits were 0.03 mg mL^{-1} and 0.4 mg mL^{-1}, respectively.

$$\text{Absorbance} = 0.0594 \times \text{quercetin concentration } (\mu g\ mL^{-1}) - 0.0026 \quad R^2 = 0.9999 \tag{3}$$

$$\text{Absorbance} = 0.1402 \times \text{curcumin concentration } (\mu g\ mL^{-1}) - 0.042 \quad R^2 = 0.9984 \tag{4}$$

Confocal Microscopy

Pure curcumin and the curcumin-containing nanofibers were observed using a confocal microscope to analyze its incorporation into the scaffolds. The nanofibers containing only the polymers were analyzed using two wavelengths (375 and 425 nm) to confirm that no fluorescence emission was observed in the absence of curcumin. The images obtained from the microscopy are shown in Figure 2. The results indicated the presence of curcumin in nanofiber scaffolds, confirming the results observed in the bioactive content analysis.

The microscopic images of the samples containing quercetin are not shown because this compound is not fluorescent. According to Frederice *et al.*,[22] quercetin exhibits low fluorescence due to a lack of significant emission at room temperature. Furthermore, quercetin only absorbs light in the $400 - 500$ nm range when aluminum cations are added to the solution.

Test of Antioxidant Activity with DPPH

Quercetin presented the highest antioxidant activity in the PCL nanofibers and the PCL/PHB-HV blend (Table II). The PCL samples containing quercetin exhibited higher ($p < 0.05$) antioxidant activity than that of the blend with quercetin. This result may be associated with the quercetin content in the nanofibers because a higher concentration of this compound was observed in the PCL scaffolds than in the PCL/PHB-HV blends.

The equal lowercase letters indicate that there is no significant difference ($p < 0.05$) among the times. The equal capital letters indicate that there is no significant difference ($p < 0.05$) among the samples.

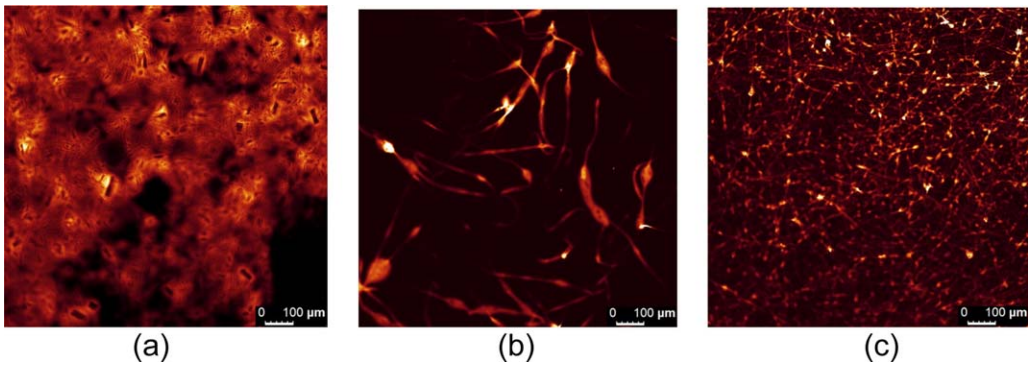

Figure 2. Confocal microscopy of curcumin (a), PCL nanofibers with curcumin (b), and PCL/PHB-HV nanofibers with curcumin (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table II. Antioxidant Activity Expressed in % DPPH Inhibition

	% DPPH inhibition			
Time (min)	P1Q	P1C	P2Q	P2C
10	35.3 ± 3.7^{dC}	39.5 ± 3.3^{eB}	23.6 ± 2.3^{iA}	44.7 ± 1.1^{jB}
20	51.1 ± 2.1^{cD}	41.6 ± 5.8^{eE}	41.2 ± 2.2^{hE}	49.8 ± 2.3^{lD}
30	68.2 ± 2.0^{bF}	42.3 ± 6.7^{eG}	56.4 ± 1.5^{lgH}	53.1 ± 3.1^{lH}
40	87.3 ± 2.0^{aI}	43.6 ± 6.5^{eL}	70.0 ± 1.5^{fJ}	53.3 ± 2.7^{lK}

P1Q: 12% PCL + 1.4% NaCl + 3% quercetin; P1C: 12% PCL + 1.4% NaCl + 3% curcumin; P2Q: 5% PCL + 10% PHB-HV + 1.4% NaCl + 3% quercetin; P2C: 5% PCL + 10% PHB-HV + 1.4% NaCl + 3% curcumin.

An increase in the antioxidant activity was observed as a function of time. Therefore, to stabilize the action of the bioactive compound with DPPH, the analysis must be performed for more than 40 min.

According to Cullen *et al.*,[23] wound dressing materials that exhibit antioxidant activity can be classified according to the percentage of DPPH inhibition. Between 25% and 50% is considered the median range, and a DPPH inhibition of more than 75% provides the best antioxidant activity for dressings. The PCL scaffold with quercetin exhibited a DPPH inhibition of 87.3%, which is a high value for dressings.

Wu *et al.*[8] developed polymeric nanoparticles containing quercetin, and *in vitro* studies on the sequestering ability of DPPH radicals demonstrated that the antioxidant activity of the nanoparticles was greater than that of free quercetin. Parize *et al.*[24] demonstrated that chitosan microparticles containing curcumin had a higher antioxidant activity (33%), which was measured by DPPH radicals, than chitosan films and microspheres containing curcumin. Based on these studies, the use of nanobiotechnology provides greater interaction and protection of bioactive compounds. In addition, electrospinning does not hinder the antioxidant activity of the bioactive compounds present in the polymer solutions. Therefore, scaffolds are effective when applied directly to the area to be treated.

Test of Antioxidant Activity with ABTS

No statistically significant difference was observed for the same compound over time ($p < 0.05$) (Table III), indicating that no changes were observed in the $ABTS^+$ radical sequestration. When different nanofibers were compared, a statistically significant difference ($p < 0.05$) was observed between samples of PCL containing quercetin and curcumin as well as when these compounds were added to the PCL/PHB-HV blend.

The equal lowercase letters indicate that there is no significant difference ($p < 0.05$) among the times. The equal capital letters indicate that there is no significant difference ($p < 0.05$) among the samples.

Table III. Antioxidant Activity Expressed as % $ABTS^+$ Reduction

	% $ABTS^+$ reduction			
Time (min)	P1Q	P1C	P2Q	P2C
6	60.2 ± 3.8^{aA}	29.6 ± 2.6^{bB}	61.5 ± 6.8^{cA}	33.9 ± 2.2^{dB}
12	62.1 ± 3.7^{aC}	31.3 ± 2.4^{bE}	63.1 ± 7.3^{cD}	34.7 ± 2.3^{dE}
18	63.6 ± 4.2^{aF}	31.5 ± 3.0^{bG}	64.5 ± 7.1^{cF}	35.5 ± 2.2^{dG}

P1Q: 12% PCL + 1.4% NaCl + 3% quercetin; P1C: 12% PCL + 1.4% NaCl + 3% curcumin; P2Q: 5% PCL + 10% PHB-HV + 1.4% NaCl + 3% quercetin; P2C: 5% PCL + 10% PHB-HV + 1.4% NaCl + 3% curcumin.

The compound with the highest antioxidant activity was quercetin (i.e., 63.6% reduction for the PCL scaffold and 64.5% for the PCL/PHB-HV blend). The same result was observed with the DPPH antioxidant activity method and the dosage of bioactive compounds in the scaffold. This result may be due to the higher level of quercetin incorporation into the nanofibers. Both polymers enable the bioactive compound to perform its antioxidant action on the $ABTS^{+}$ radical without any changes due to the composition.

Antioxidant compounds reduce the adverse effects found in damaged tissue by the removing products of inflammation. Moreover, biomaterials with antioxidant activity help to control the oxidative stress of damaged tissue, which accelerates the healing process. Therefore, scaffolds that are employed in tissue engineering should have a high antioxidant activity.

CONCLUSIONS

The PCL containing quercetin and curcumin nanofibers, with 332 ± 86 and 434 ± 65 nm average diameters, respectively, were successfully prepared. The scaffolds that showed higher antioxidant activity were PCL and PCL/PHB-HV with quercetin (87.3 ± 2.0 and 64.5 ± 7.1 nm, respectively) than curcumin in both methods tested. The addition of quercetin provided the best results for PCL and PCL/PHB-HV blend scaffolds and presented a bioactive content of 174.2 ± 0.1 and 155.7 ± 0.1 μg in 10 mg of scaffold, respectively. The demonstrated that the addition of active compounds in scaffold provides antioxidant activity to the material and simultaneously these compounds protects against environmental degradation agents such as light, humidity, radiation, pH, and oxygen. The best results with antioxidant activity of quercetin scaffolds were expected because of the greater incorporation of this bioactive in nanofibers was identified in the assays. From the experimental results it was suggested that the developed material is important in tissue engineering applications. However, it is possible for this application, further studies are needed to determine important parameters such as the compound release in the time scale and cytotoxicity of the material.

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