Polímeros
ISSN: 0104-1428
ISSN: 1678-5169
Associação Brasileira de Polímeros

Pimentel, Cristiane Agra; Souza, José William de Lima; Santos, Flávia Suzany Ferreira dos; Sá, Mayelli Dantas de; Ferreira, Valéria Pereira; Barreto, Gislaine Bezerra de Carvalho; Rodrigues, José Filipe Bacalhau; Sousa, Wladymyr Jefferson Bacalhau de; Britto, Cláudio Orestes; Sousa, Francisco Kegenaldo Alves de; Fook, Marcus Vinicius Lia

Sulfonated poly(ether ether ketone)/hydroxyapatite membrane as biomaterials: process evaluation

Polímeros, vol. 29, no. 1, 2019
Associação Brasileira de Polímeros

DOI: 10.1590/0104-1428.01018

Polímeros
ISSN 1678-5169 (Online)

https://doi.org/10.1590/0104-1428.01018

ORIGINAL ARTICLE

Sulfonated poly(ether ether ketone)/hydroxyapatite membrane as biomaterials: process evaluation

Cristiane Agra Pimentel[1]*, José William de Lima Souza[1], Flávia Suzany Ferreira dos Santos[1], Mayelli Dantas de Sá[1], Valéria Pereira Ferreira[1], Gislaine Bezerra de Carvalho Barreto[1], José Filipe Bacalhau Rodrigues[1], Wladymyr Jefferson Bacalhau de Sousa[1], Cláudio Orestes Britto Filho[1], Francisco Kegenaldo Alves de Sousa[1] and Marcus Vinicius Lia Fook[1]

[1]Laboratório de Avaliação e Desenvolvimento de Biomateriais do Nordeste – CERTBIO, Unidade Acadêmica de Engenharia de Materiais – UAEMa, Centro de Ciências e Tecnologia – CCT, Universidade Federal de Campina Grande – UFCG, Campina Grande, PB, Brasil

**cristiane.pimentel@certbio.ufcg.edu.br*

Abstract

Poly(ether ether ketone) (PEEK) has excellent properties, such as high biocompatibility and an elastic modulus similar to bone, which makes it a suitable biomaterial. When modified with sulfuric acid (H_2SO_4) and hydroxyapatite (HA), its workability and bioactivity is enhanced, and this makes it of great importance in medicine. This study investigates a better combination of process parameters to manufacture sulfonated PEEK/HA (SPEEK/HA) membranes for biomaterials. Chemical, thermal, and biological analyses were carried out on all samples. The sulfonated structure was observed to enhance wettability, adhesion, and cell viability. Furthermore, an increase in the degree of sulfonation facilitated their workability as required for biomaterials; making them suitable for osseointegration. Besides, the SPEEK/HA membranes presented cell adhesion, confirming the viability to use as biomaterial. This study presents a cheap alternative method to easily process biomaterials of improved workability.

Keywords: *biomaterial, chemical modification, hydroxyapatite, membrane, SPEEK.*

How to cite: Pimentel, C. A., Souza, J. W. L., Santos, F. S. F., Sá, M. D., Ferreira, V. P., Barreto, G. B. C., Rodrigues, J. F. B., Sousa, W. J. B., Britto Filho, C. O., Sousa, F. K. A., & Fook, M. V. L. (2019). Sulfonated poly(ether ether ketone)/hydroxyapatite membrane as biomaterials: process evaluation. *Polímeros: Ciência e Tecnologia*, *29*(1), e2019009. https://doi.org/10.1590/0104-1428.01018

1. Introduction

Poly(ether ether ketone) (PEEK) has attracted interest in medicine. This polymer has been used in orthopedics, neurosurgery, and traumatology because of its favorable mechanical, chemical, and tribological properties[1]. The benefits of this material include excellent mechanical resistance, high biocompatibility, improved biological inertia, low friction coefficient, elastic modulus similar to bone, reusability, and resterilization[2,3]. However, its high processing temperature makes it difficult to be worked into shape.

Recently, research has been done to chemically modify PEEK with sulfuric acid (H_2SO_4) to improve its workability and processing. Studies were initially geared towards its use in fuel cells, which convert chemical energy to electrical energy[4]. However, more recently, it has been studied to be used as biomaterials. Zhao et al.[5] studied sulfonated PEEK (SPEEK) applications in orthopedic implants. Kalambettu and Dharmalingam[6] studied the fabrication of SPEEK membranes incorporated with hydroxyapatite (HA), and Montero et al.[7] researched biofilms fabricated with SPEEK.

Although SPEEK is commonly applied in fuel cells and biomaterials, further studies for other possible applications are necessary[5], as it possesses some limitations. Some of these are its inability for direct bone apposition, which could result in poor osseointegration[8]; and a possible presence of acid residue, which makes it risky for medical applications[6].

SPEEK is a relevant biomaterial in terms of medicine devices and scientific prospects because of its simple processing and requirement of ordinary equipment. It is also easy to be worked into shape and incorporated with drugs[7].

This paper focuses on a detailed evaluation of the influence of process parameters on the performance of a SPEEK/HA membrane. The chemical, thermal, and wetting properties of SPEEK/HA were evaluated. In addition, cell viability and adhesion were studied to be applied in biomaterials.

2. Materials and Methods

2.1 SPEEK/HA membranes preparation

Two grams of PEEK (Victrex USA Inc – Vicote 702) were dissolved in 50 mL of H_2SO_4 98% (VETEC Química Fina Ltda). The mixture was heated up to 50 °C and mechanically stirred for 3 h. After 1.5 h of stirring, 0.6 g of HA (Labsynth) was added (during stirring). After 4 days, the solution was poured on a plate and frozen at -80 °C.

It was placed into a freeze drier for 24 h and allowed to stand for another 24 h before it was decanted to isolate the membrane. The product was then filtered, washed with distilled water until complete removal of H_2SO_4, and dried at 50 °C in an oven for 3 h.

2.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was carried out in a Perkin Elmer spectrophotometer (Spectrum 400), and the spectra were recorded in absorbance mode, to detect any chemical bonding at the SPEEK/HA membranes and to calculate the degree of sulfonation (DS). The DS of PEEK was calculated using the following Equation 1:

$$\%DS = \left[1 - \frac{SPEEK\ peak\ height}{PEEK\ peak\ height}\right] \times 100 \quad (1)$$

where the normalized SPEEK peak height (1484 cm^{-1}) is compared with the PEEK peak height (1494 cm^{-1}) to obtain the %DS. The PEEK sulfonation can be confirmed by the division of the aromatic C=C absorption band at 1494 cm^{-1} and the appearance of a new band at 1484 cm^{-1}. Therefore, it is necessary to measure the heights of the characteristic bands of both materials[4].

2.3 Thermogravimetric Analysis (TGA)

The thermal properties of SPEEK/HA membranes were examined using TGA. Thermal analysis was carried out in a nitrogen atmosphere between 25 °C (room temperature) and 600 °C at a heating rate and flow rate of 10 °C/min and 20 mL/min, respectively, using a TG 50H (Shimadzu) analyzer.

2.4 Wettability analysis

The wettability analysis was done by the static drop method. A contact angle goniometer (CERTBIO) was used to measure the contact angle of the membrane. Deionized water was used as the liquid. A total of three points of the contact angle was measured in each sample within 30 s after the drop.

2.5 Cytotoxicity study

The cytotoxicity analysis was performed according to ISO 10993-5: 2009 (Biological evaluation of medical devices -- Part 5: Tests for *in vitro* cytotoxicity)[9]. An L929 fibroblast cell line (ATCC NCTC clone 929) was grown in RPMI culture medium (RPMI 1640 Medium, Gibco - Invitrogen Corporation, Grad Island, USA) and was supplemented with 10% bovine fetal serum (Gibco, Life Technologies) and 1% antibiotic-antimycotic (Gibco, Life Technologies). These cells were preserved in CO_2 incubators at 37 °C in 5% atmosphere. The cell suspension (100 µL per well) was added to a 96-well plate at 1×10^5 cells/mL in the RPMI 1640 culture medium. The plate was transferred to a CO_2 oven (5%) at 37 °C and incubated for 24 h. The culture medium was aspirated from all wells, and then 170 µL RPMI 1640 culture medium and 30 µL sample extract were added to each well. The plate was incubated again in a CO_2 oven (5%) at 37 °C for 24 h. The culture medium was aspirated from all wells and 100 µL of MTT solution (1 mg/mL) was added. The plates were incubated again for 3 h in a CO_2 oven (5%) at 37 °C. The supernatant was discarded and 100 µL of isopropyl alcohol was added per well. Optical density was read on a microplate reader (VictorX3 - PerkinElmer) at 570 nm with 650 nm reference filters. Cell viability was calculated as a percentage of the modified z-Score test for outliers detection. High density polyethylene (HDPE) and natural latex were used as a negative and positive control, respectively.

2.6 Cell adhesion

To evaluate the cell adhesion of the membrane, the samples were preserved in 70% ethanol for 24 h. They were then washed thrice in sterile PBS and dried at 40 °C for 24 h. They were placed into a 48-well tissue culture plate, and 500 µL of OFCOL II cell suspension was added per well at 1 × 105 cells/mL in RPMI 1640 culture medium (RPMI 1640 Medium, Gibco - Invitrogen Corporation, Grad Island, USA) supplemented with 10% bovine fetal serum (Gibco, Life Technologies) and 1% antibiotic-antimycotic (Gibco, Life Technologies). The plate was transferred to the CO_2 incubator (5%) at 37 °C and incubated for 7 days. After incubation, the culture medium was aspirated from all the wells and the samples were washed with PBS. The PBS was aspirated, and formaldehyde solution was added to each well at 10% for 10 min for cell attachment. The formaldehyde was removed and the samples were washed with PBS. The PBS was removed and the samples were dried at 40 °C for 24 h. Cell adhesion was evaluated through the surfaces of the samples by scanning electron microscopy (SEM) (WORLD PHENOM - PRO-X 800-07334 model); the samples for which were coated with gold for better visualization.

2.7 Design of Experiment (DOE)

After defining and validating the methodology of other studies, this research chose DOE to study the influence of process parameters on SPEEK/HA membrane properties. The studied parameters (inputs) were PEEK sulfonation time, HA addition time, and freeze-drying time. Duplicates of a 2^3 factorial design without center points were conducted, and Minitab 18 was used to analyze the results.

Table 1 shows the experimental planning matrix with minimum and maximum values for each variable.

Table 2 shows the samples and input parameters, including the duplicates (E9 to E16). All experiments were performed randomly.

The output parameters to evaluate the experimental design were based on characterizations that could greatly influence the membrane properties of biomaterials. The characterizations are FTIR analysis, defined by %DS; TGA, defined by the

Table 1. Experimental planning matrix.

Input Parameters	Unit	-1	+1
Freeze-drying time	H	24	48
Sulfonation time	H	2.0	3.0
HA addition time	H	0.5	1.5

Table 2. Sample identification per experiment.

Experiment	Sample	Freeze-drying time	Sulfonation time	HA addition time
1	E1	24	2	0.5
2	E2	48	2	0.5
3	E3	24	3	0.5
4	E4	48	3	0.5
5	E5	24	2	1.5
6	E6	48	2	1.5
7	E7	24	3	1.5
8	E8	48	3	1.5
9	E9	24	2	0.5
10	E10	48	2	0.5
11	E11	24	3	0.5
12	E12	48	3	0.5
13	E13	24	2	1.5
14	E14	48	2	1.5
15	E15	24	3	1.5
16	E16	48	3	1.5

weight loss during a change from 300 to 400 °C, which is due to the removal of sulfonic acid groups[7]; and wettability analysis, defined by the water contact angle. A higher %DS increases the wettability, which would facilitate workability of the membrane and osseointegration[10-12]. Table 3 presents the output parameters per characterization at experimental planning.

Table 3. Output parameter per characterization.

Characterization	Unit	Output parameter
1 - FTIR	%	Degree of sulfonation (DS)
2 - Wettability	Degree	Water contact angle
3 - TGA	%	Weight loss - ranged from 300 to 400 °C (removal of sulfonic acid groups)

3. Results and Discussions

3.1 FTIR analysis

FTIR spectra of the membranes are shown in Figure 1 and 2. PEEK was used as the target material and reference for identifying functional groups.

The sulfonation of PEEK is confirmed by the division of the aromatic C=C absorption band at 1494 cm^{-1} and the appearance of a new band at 1484 cm^{-1}. This peak was used to calculate %DS during DOE (Figure 1a). A sharp peak at 1024 cm^{-1} confirms the presence of a H_2SO_4 group, which is due to the S=O group in all samples and duplicates (Figure 1a). A peak at 3425 cm^{-1} confirms the presence of the OH group, which is bonded to an SO_3H group. The peak intensities in all samples are different because of varied process parameters. The highest peak intensity corresponded to a higher degree of sulfonation, as observed in E8, E12, and E15 (Figure 1 and 2). Previous studies have reported similar bands[7,13,14].

A shift of the peak from 3447 cm^{-1} to 3425 cm^{-1} was observed for samples where HA was deposited on the SPEEK surface (Figure 1). The coordinate bond of the HA polar group and OH group of the SPEEK was weak as a result of the stretching of OH bonds[15], and this led to a low peak frequency.

3.2 TGA

TGA curves are shown in Figure 3 and 4. PEEK was used as the target material. SPEEK/HA membranes of all samples were thermally stable up to 350 °C and exhibited two distinct weight loss stages, depending on the process parameters used, as shown in Figure 3 and 4, whereas PEEK samples had one weight loss at 550 °C (Figure 3 and 4).

The mass loss at 100 °C is due to the removal of water molecules absorbed by the material. The first stage of thermal degradation of samples during a shift from 300 to 400 °C is due to the removal of SO_3H. The second stage of thermal degradation ranging from 500 to 600 °C is due to polymer degradation. All these results are corroborated by literature[4,7].

In the final degradation stage around 550 °C, the samples approach a final mass at 600 °C, except samples E5 and E13, as seen in Figures 3 and 4; however this does not happen in the PEEK sample. Zaidi et al.[16] explained that when H_2SO_4 (95-98%) is used for sulfonation, material degradation and crosslinking reactions are avoided.

TGA was used to estimate the weight loss of the samples on DOE by assuming that the first stage of thermal degradation is entirely caused by elimination of sulfonic acid groups.

3.3 DOE results

Table 4 presents the DOE results, outlining all samples and parameters. The mass loss of samples E5 and E13 could not be obtained probably because of reaction problems, such as problems at sulfonation reaction.

The main objective of DOE was to obtain the best combination of output parameters for optimum performance of the samples as biomaterials. The best combination was a high degree of sulfonation, high wettability, and higher mass loss at 350 °C. An increase in %DS facilitated the wettability of the materials, improving their workability as required for biomaterials; making them suitable for osseointegration[17].

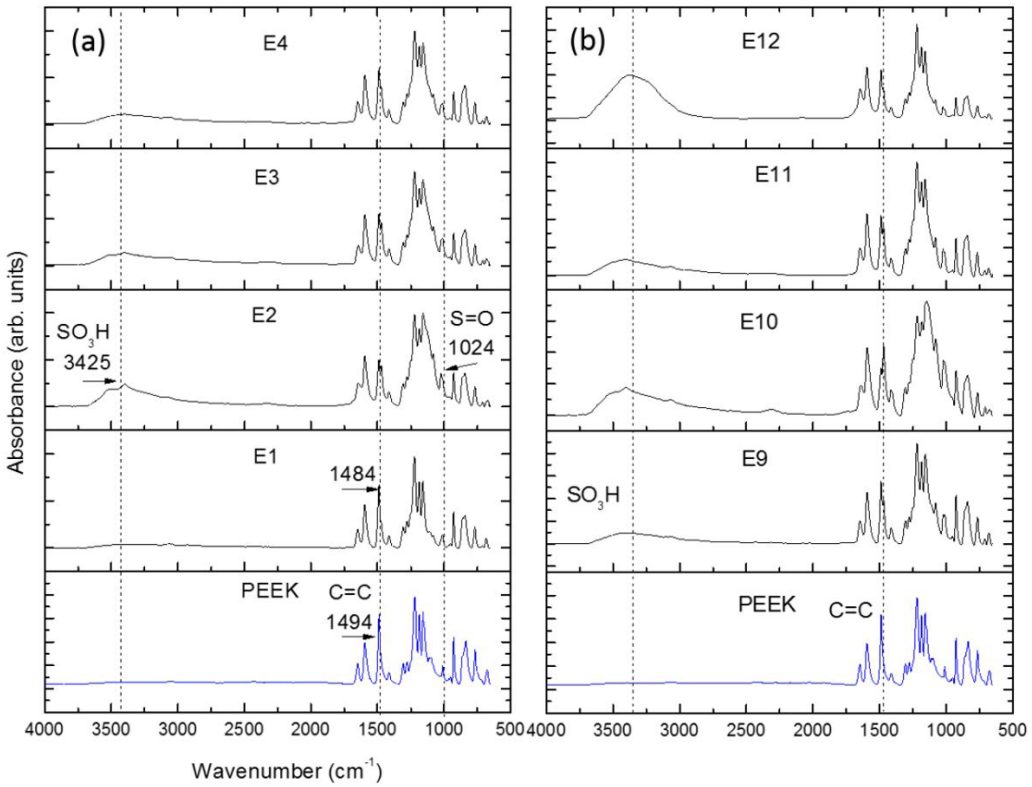

Figure 1. FTIR spectra of (a) PEEK, E1 to E4 samples; (b) PEEK, E9 to E12 duplicates.

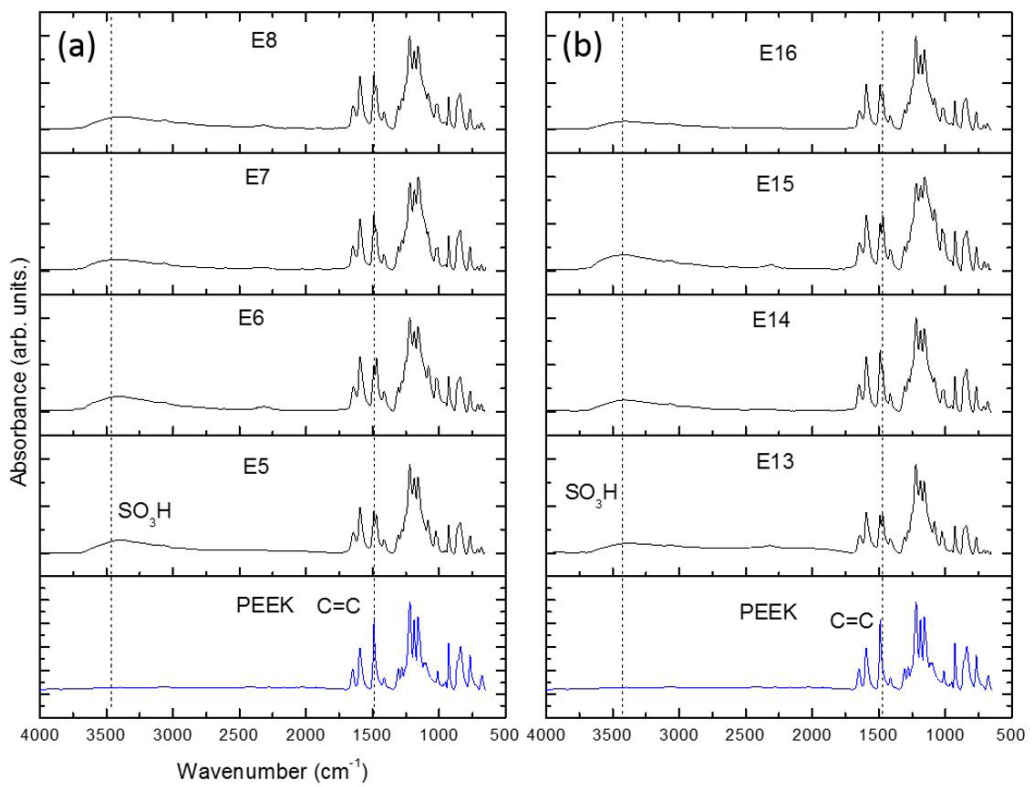

Figure 2. FTIR spectra of (a) PEEK, E5 to E8 samples; (b) PEEK, E13 to E16 duplicates.

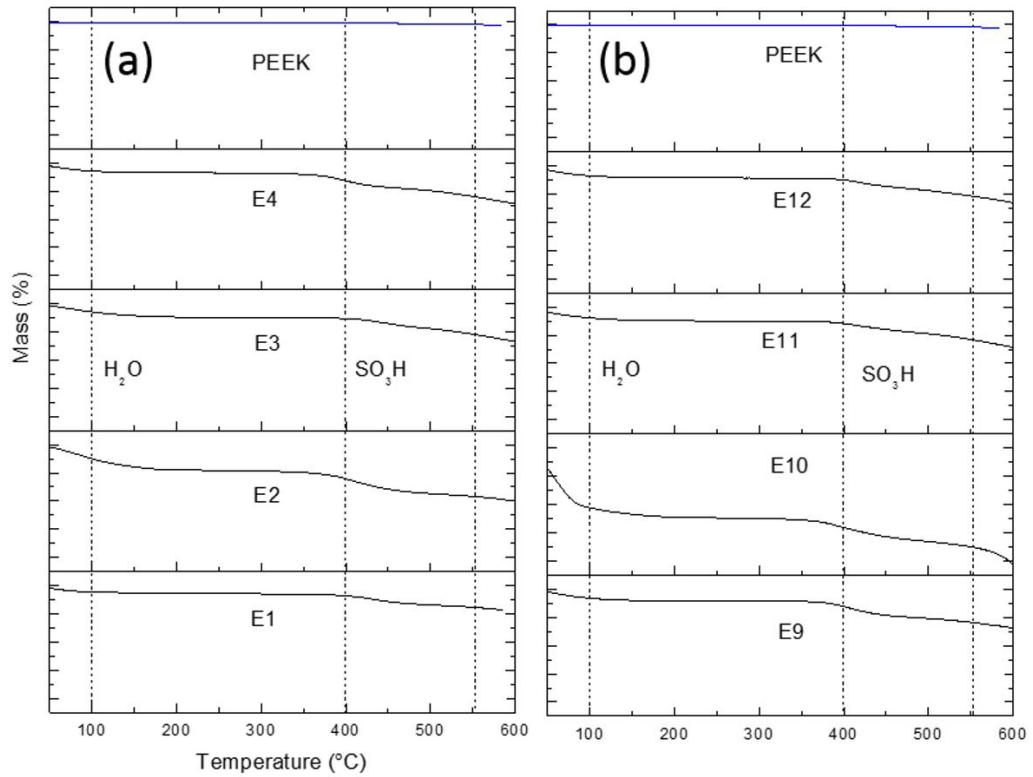

Figure 3. TGA curves of (a) PEEK, E1 to E4 samples; (b) PEEK, E9 to E12 duplicates.

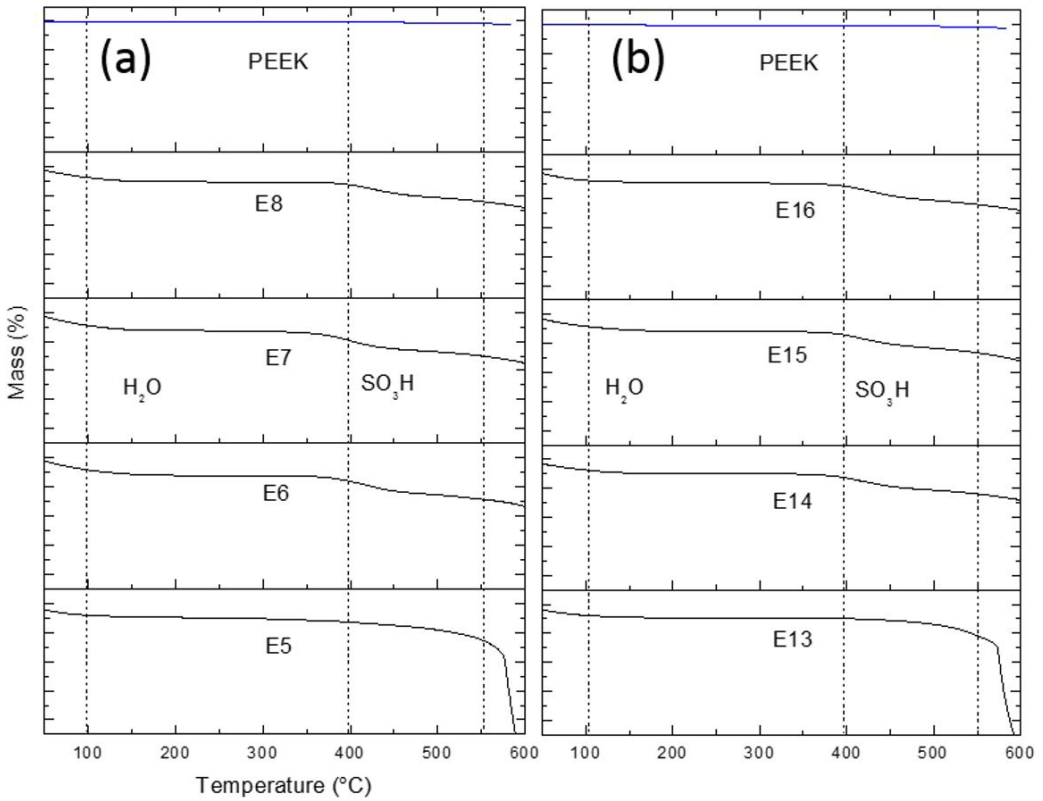

Figure 4. TGA Curves of (a) PEEK, E5 to E8 samples; (b) PEEK, E13 to E16 duplicates.

Some samples had their best combination impaired, such as E1 and E14, all of them presented a higher %DS and a contact angle inconsistent, it was observed probably by acid residues. In future studies, it is important to detail the influence of acid residues.

Table 5 shows the Minitab optimization analysis. Considering the adjustments and confidence intervals, the best combination of input parameters to obtain the desired outputs were a freeze-drying time of 24 h, sulfonation time of 3 h, and HA addition time of 1.5 h. Samples E7 and E15 had these combinations. Almasi et al.[13], Kalambettu and Dharmalingam[6], and Zhou and Lee[18] conducted a similar study with another combination. This is a novel research.

3.4 Cell viability

Considering the optimum result of E7/E15, the cell viability of the membrane was analyzed for use in biomaterials. Figure 5 shows the membrane cytotoxicity according to BS EN ISO 10993-5:2009—Tests for *in vitro* cytotoxicity[9]. Cell viability is specified when calculated values are above 70%.

Cytocompatibility results of optimum samples toward the fibroblast-like L929 cells show an average cell viability of 86%, which is not much higher than the specification (70%). This percentage is probably due to extensive agglomeration of the HA filler particles in SPEEK/HA membrane. Cells are known to be very sensitive to surface energy and chemistry[13]. Besides, it should be considered the adverse conditions to sulfonated PEEK at laboratory as well as the acid residues. In this way, the average cell viability shown satisfactory and contributes to their application as a biomaterial. But when it was studied these applications, it is important to detail the influence of acid residues.

3.5 Cell adhesion

Cell adhesion analysis was performed through SEM with a gold-metallized surface and results are shown in Figure 6. Figure 6a shows the structure of a SPEEK/HA membrane without cell adhesion. Cell adhesion can be observed in Figure 6b, where the SPEEK/HA membranes show a layer of coating, which is in agreement with the literature[5,6]. This confirms the possibility of their use as a biomaterial.

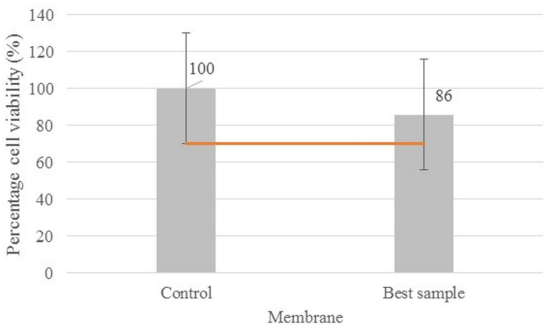

Figure 5. Cell viability of L929 cell lines by MTT method.

Table 4. DOE results correlating samples and output results.

Samples	%DS	Contact angle (degrees)	% Mass loss at 350 °C
E1	83.96	32.25	11.51
E2	54.04	28.53	16.86
E3	62.88	29.51	7.77
E4	67.93	23.92	10.30
E5	49.96	28.41	-----
E6	61.60	31.97	12.64
E7	70.13	25.14	12.95
E8	66.12	24.71	10.71
E9	68.64	22.23	11.62
E10	48.70	27.64	15.68
E11	48.40	18.00	8.21
E12	47.83	26.05	7.05
E13	41.81	32.19	-----
E14	75.21	24.55	11.17
E15	61.78	24.03	10.93
E16	54.20	31.23	11.50

Table 5. Minitab optimization analysis.

Solution	Freeze-drying time	Sulfonation time	HA addition time	%DS	Contact angle	% Mass loss	Compound desirability
1	-1	1	1	70.66	24.63	10.94	0.62
Output	Adjustment	Standard adjustment error	Confidence bounds 95%				
%DS	70.66	8.03	(52.15; 89.18)				
Contact angle	24.63	2.37	(19.17; 30.09)				
%Mass loss	10.94	3.04	(3.93; 17.95)				

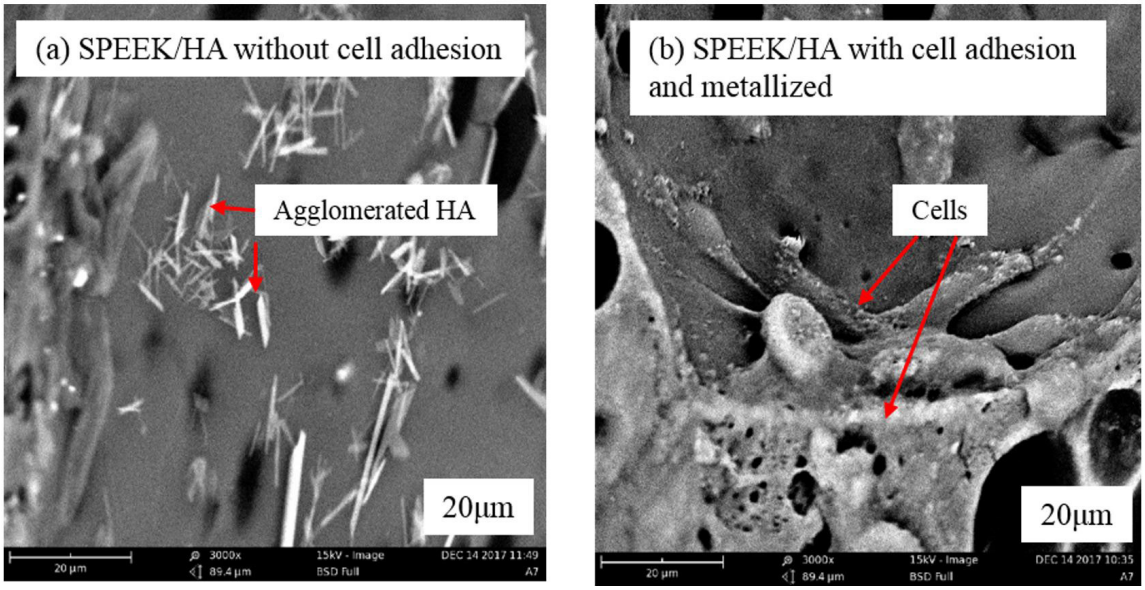

Figure 6. SEM micrographs of membranes (sample E7) (a) SPEEK/HA before cell adhesion; (b) SPEEK/HA after cell adhesion and gold metallization.

4. Conclusions

This study reported a process evaluation of SPEEK/HA membrane application as biomaterials. The chemical deposition technique used did not involve high temperatures, and the sulfonated structure improved wettability, cell adhesion, and growth. This technique of surface treatment is relatively cheap and easy to perform. Hence, this method can be adopted to modify PEEK membranes for better application as biomaterials.

5. Acknowledgements

This work was supported by Coordination for the Improvement of Higher Level Personnel – CAPES.

6. References

1. Wang, M., Tang, S. J., McGrady, L. M., & Rao, R. D. (2013). Biomechanical comparison of supplemental posterior fixations for two-level anterior lumbar interbody fusion. *Journal of Engineering in Medicine*, *227*(3), 245-250. http://dx.doi.org/10.1177/0954411912465057. PMid:23662340.
2. O'Reilly, E. B., Barnett, S., Madden, C., Welch, B., Mickey, B., & Rozen, S. (2015). Computed-tomography modeled polyetheretherketone (PEEK) implants in revision cranioplasty. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, *68*(3), 329-338. http://dx.doi.org/10.1016/j.bjps.2014.11.001. PMid:25541423.
3. Siddiq, A. R., & Kennedy, A. R. (2015). Porous polyetheretherketone (PEEK) manufactured by a novel powder route using near-spherical salt bead porogens: characterisation and mechanical properties. *Materials Science and Engineering C*, *47*(1), 180-188. http://dx.doi.org/10.1016/j.msec.2014.11.044. PMid:25492187.
4. Aguiar, K. R., Batalha, G. P., Peixoto, M., Ramos, A., & Pezzin, S. H. (2012). Produção de membranas híbridas zirconizadas de SPEEK/Copolissilsesquioxano para aplicação em células a combustível do tipo PEM. *Polímeros: Ciência e Tecnologia*, *22*(5), 453-459. http://dx.doi.org/10.1590/S0104-14282012005000060.
5. Zhao, Y., Wong, H. M., Wang, W., Li, P., Xu, Z., Chong, E. Y., Yan, C. H., Yeung, K. W., & Chu, P. K. (2013). Cytocompatibility, osseointegration, and bioactivity of three-dimensional porous and nanostructured network on polyetheretherketone. *Biomaterials*, *34*(37), 9264-9277. http://dx.doi.org/10.1016/j.biomaterials.2013.08.071. PMid:24041423.
6. Kalambettu, A., & Dharmalingam, S. (2014). Fabrication and *in vitro* evaluation of sulphonated polyetheretherketone/nano-hydroxyapatite composites as bone graft materials. *Materials Chemistry and Physics*, *147*(1-2), 168-177. http://dx.doi.org/10.1016/j.matchemphys.2014.04.024.
7. Montero, J. F., Tajiri, H. A., Barra, G. M., Fredel, M. C., Benfatti, C. A., Magini, R. S., Pimenta, A. L., & Souza, J. C. (2017). Biofilm behavior on sulfonated polyetheretherketone (sPEEK). *Materials Science and Engineering C*, *70*(1), 456-460. http://dx.doi.org/10.1016/j.msec.2016.09.017. PMid:27770916.
8. Kurtz, S. M., & Devine, J. N. (2007). *Applications of polyetheretherketone in trauma, arthroscopy, and cranial defect repair*. In S. Lovald & S. M. Kurtz (Eds.), PEEK biomaterials handbook, (pp. 243-260). New York: Springer .
9. International Organization for Standardization – ISO. (2009). *BS EN ISO 10993-5: biological evaluation of medical devices: tests for in vitro cytotoxicity*. Genebra: ISO.
10. Conceição, T. F., Bertolino, J. R., Barra, G. M., Mireski, S. L., Joussef, A. C., & Pires, A. T. (2008). Preparation and characterization of polyetheretherketone derivatives. *Journal of the Brazilian Chemical Society*, *19*(1), 111-116. http://dx.doi.org/10.1590/S0103-50532008000100016.
11. Jiang, R., Kunz, H. R., & Fenton, J. M. (2005). Investigation of membrane property and fuel cell behavior with sulfonated polyetheretherketone electrolyte: temperature and relative humidity effects. *Journal of Power Sources*, *150*(4), 120-128. http://dx.doi.org/10.1016/j.jpowsour.2005.03.180.
12. Xing, P., Robertson, G. P., Guiver, M. D., Mikhailenko, S. D., Wang, K., & Kaliaguine, S. (2004). Synthesis and characterization of sulfonated polyetheretherketone for proton

exchange membranes. *Journal of Membrane Science*, *229*(1-2), 95-106. http://dx.doi.org/10.1016/j.memsci.2003.09.019.

13. Almasi, D., Izman, S., Assadian, M., Ghanbari, M., & Abdul Kadir, M. R. (2014). Crystalline HA coating on PEEK via chemical deposition. *Applied Surface Science*, *314*(30), 1034-1040. http://dx.doi.org/10.1016/j.apsusc.2014.06.074.
14. Jaafar, J., Ismail, A., & Mustafa, A. (2007). Physicochemical study of polyetheretherketone electrolyte membranes sulfonated with mixtures of fuming sulfuric acid and sulfuric acid for direct methanol fuel cell application. *Materials Science and Engineering A*, *460*(15), 475-484. http://dx.doi.org/10.1016/j.msea.2007.02.095.
15. Janaki, K., Elamathi, S., & Sangeetha, D. (2005). Development and characterization of polymer ceramic composites for orthopedic applications. *Artificial Organs*, *22*(3), 169-178. Retrieved in 2018, February 12, from http://medind.nic.in/taa/t09/i3/taat09i3p169.pdf
16. Zaidi, S. J., Mikhailenko, S. D., Robertson, G., Guiver, M., & Kaliaguine, S. (2000). Proton conducting composite membranes from polyetheretherketone and heteropolyacids for fuel cell applications. *Journal of Membrane Science*, *173*(1), 17-34. http://dx.doi.org/10.1016/S0376-7388(00)00345-8.
17. Mahjoubi, H., Buck, E., Manimunda, P., Farivar, R., Chromik, R., Murshed, M., & Cerruti, M. (2017). Surface phosphonation enhances hydroxyapatite coating adhesion on polyetheretherketone and its osseointegration potential. *Acta Biomaterialia*, *47*(1), 149-158. http://dx.doi.org/10.1016/j.actbio.2016.10.004. PMid:27717913.
18. Zhou, H., & Lee, J. (2011). Nanoscale hydroxyapatite particles for bone tissue engineering. *Acta Biomaterialia*, *7*(7), 2769-2781. http://dx.doi.org/10.1016/j.actbio.2011.03.019. PMid:21440094.

Received: Feb. 12, 2018
Revised: July 19, 2018
Accepted: Sept. 12, 2018