

## **Development of a 3D polyetheretherketone structure that mimics the cranial bone morphology for use in cranioplasty**

**Desenvolvimento de estrutura 3D de polietereetercetona semelhante a morfologia do osso craniano para uso em cranioplastia**

**Desarrollo de una estructura 3D de polietereetercetona similar a la morfología ósea craneal para su aplicación en la craneoplastia**

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## **Abstract**

Cranioencephalic traumatism (TBI) is a common situation in trauma hospitals and has become responsible for high rates of mortality worldwide. When the victim of TBI is affected by injuries to the skullcap with a need for grafting, problems regarding the availability of suitable and affordable materials eventually happen. In this study, a 3D structure of Polyetheretherketone (PEEK) that mimics the cranial bone morphology for use in cranioplasty was developed. Samples of different formulations, in the form of round bars, were obtained through uniaxial compression, and porosity was controlled by the salt leaching technique. Then, the specimens were characterized in terms of pore morphology and distribution, surface roughness, compression resistance and cytotoxicity. Results exhibited high

levels of similarity of the 3D structures of PEEK to the natural human bone, which indicates the effectiveness of the proposed method in mimicking the morphology of the compact/porous/compact system of the skullcap (diploe).

**Keywords:** 3D structure, Polyetheretherketone; Cranial bone; Salt leaching technique.

### Resumo

O traumatismo cranioencefálico (TCE) é uma situação comum em hospitais de trauma e tem se tornado responsável por altas taxas de mortalidade em todo o mundo. Quando a vítima de TCE é acometida por lesões na calota craniana com necessidade de enxerto, eventualmente surgem problemas de disponibilidade de materiais adequados e acessíveis. Neste estudo, foi desenvolvida uma estrutura 3D de Polieterecetona (PEEK) que mimetiza a morfologia do osso craniano para uso em cranioplastia. Amostras de diferentes formulações, na forma de pastilhas redondas, foram obtidas por compressão uniaxial, e a porosidade foi controlada pela técnica de lixiviação de sal. Em seguida, os espécimes foram caracterizados quanto à morfologia e distribuição dos poros, rugosidade superficial, resistência à compressão e citotoxicidade. Os resultados exibiram altos níveis de similaridade das estruturas 3D de PEEK com o osso humano natural, o que indica a eficácia do método proposto em mimetizar a morfologia do sistema compacto/poroso/compacto da calota craniana (diploe).

**Palavras-chave:** Estrutura 3D; Polieterecetona; Osso craniano; Técnica de lixiviação de sal.

### Resumen

El traumatismo craneoencefálico (LCT) es una situación común en los hospitales de traumatología y se ha convertido en responsable de las altas tasas de mortalidad en todo el mundo. Cuando la víctima de TBI se ve afectada por lesiones en el casco con la necesidad de un injerto, eventualmente surgen problemas con respecto a la disponibilidad de materiales adecuados y asequibles. En este estudio, se desarrolló una estructura 3D de polieterecetona que imita la morfología del hueso craneal para su uso en craneoplastia. Se obtuvieron muestras de diferentes formulaciones, en forma de barras redondas, mediante compresión uniaxial y se controló la porosidad mediante la técnica de lixiviación de sales. Luego, las muestras se caracterizaron en términos de morfología y distribución de los poros, rugosidad superficial, resistencia a la compresión y citotoxicidad. Los resultados mostraron altos niveles de similitud de las muestras con el hueso humano natural, lo que indica la efectividad del método propuesto para imitar la morfología del sistema compacto/poroso/compacto del casco (diploe).

**Palabras clave:** Estructura 3D; Polieterecetona; Hueso craneal; Técnica de lixiviación de sal.

## 1. Introduction

The brain is one of the most important and complex organs of the body. It is responsible for regulating all body functions, interpret information of the outside world and incorporate our personalities. Despite being protected by the bones of the skull, the brain is still vulnerable to some pathologies as head trauma, congenital disorder and tumors, which can affect negatively people's lives (Unterberg et al., 2004).

A common type of cranial trauma is the traumatism brain injury (TBI), an unexpected damage to the brain caused by a strong impact and a leading cause of death among adults (Bavissety et al., 2008; Reznik et al, 2016; Wong & Langley, 2016), that compresses the brain tissue against the bone and can lead to hemorrhages or edemas. People affected by TBI show a higher likelihood of death and decreased ability to perform daily life activities when grown old (Roozenbeek, Mas & Menon, 2013; Stocchetti et al, 2012).

Reports suggest that at least 7.7 million people who have experienced a TBI have disabilities and no clear decrease in this injury-related mortality or improvement of overall outcome has been observed over the past two decades. In the past, the majority of the materials used for cranial bone and brain regeneration exhibited serious disadvantages and complications (Tagliaferri et al, 2006; Teasdale & Jennett, 1974).

Metallic prosthesis and autografts using a rib, for instance, conferred a significant challenge for functional replacement in cranioplasty since the brain has poor regenerative capabilities (Harris et al, 2014; Shah, Jung & Skirboll, 2014; Stichel & Müller, 1998). Given the background, researchers have focused on developing appropriate materials for cranioplasty. One of them is the polyetheretherketone (PEEK), a semi-crystalline polymer with excellent thermal, mechanical and radiation resistance properties (Panayotovi et al, 2016). Additionally, it has an elastic modulus similar to the values of the bone (3-5 GPa), which makes it an excellent material to be used as a biomedical implant (Punchak et al., 2017).

Although there is much scientific research regarding PEEK, specially prosthesis using computer designs for cranioplasties, these materials still require further investigations. A previous study analyzed different 3D PEEK printing studies and concluded that the use of PEEK resulted in a complication rate of 15.3%, being the most common issue infection, hematoma and implant exposure (Alonso-Rodriguez et al., 2015), . However, a recent study found a decrease in complication rate on post-operative PEEK cranioplasties compared to autologous grafts (Gelardino et al., 2015).

Fully functional 3D printed polymer products, however, still don't show proper mechanical properties, besides being still used as conceptual prototypes rather than functional components (Hou, Grijpma & Feijen, 2003). For that reason, researchers have now been trying to develop advanced biocompatible materials that can mimic the cranial bone tissue (Rentsch et al., 2014).

One of the easiest techniques used to create a 3D porous structure is the particle leaching. It consists of selectively leaching one compound present in the sample, such as NaCl salt or organic compounds, to produce porous (Reignier & Huneault, 2006). The salt particles can be incorporated into the polymer in solution or molded together during melting by techniques as compression, extrusion or injection. The mechanism of leaching NaCl crystals is mainly due to the solubilization of porogenic agents in water, through a mechanical drag (Hou, Grijpma & Feijen, 2003).

Although the cost of producing polymeric materials for cranioplasties via 3D printing is becoming increasingly attractive commercially, it is still expensive for small-sized standard implants, as the ones used in cranioplasties (Ventola, 2014). Based on the above considerations, we proposed a PEEK-based porous structure obtained by the leaching technique to improve the biocompatibility and mechanical properties required to cranial implants. The samples obtained exhibited a fibrous structure, similar to the cranial bone.

## 2. Methodology

The following materials and reagents were used for the development of this work: Polyetheretherketone (PEEK) Grade 702 (particle size varied from 10-50 $\mu$ m) – Victrex, Sodium Chloride (NaCl) – Nuclear and L929 fibroblasts cell line – Banco de Células do Rio de Janeiro.

Initially, NaCl crystals were ground using a mortar and pestle. Following that, an amount of 637 particles of the salt were analyzed by optical microscopy 100X magnification, to obtain a proper particle size distribution of the NaCl powder. The particle size distribution was measured using a Hirox Optical Microscope and Image J. Since the particles of the salt were not spherical, Feret Diameter was used as a parameter to obtain the size of each particle.

Round bar samples were produced by two methods, adapted from Santos et al (2017). The first, entitled Porous PEEK (PEP), consists of attaching layers of PEEK and NaCl in the mold before compression. In this case, the bottom of the mold was filled with 3.5g of the salt powder (this was done to make it easier to extract the sample from the mold, after compression), followed by 1.5 g of PEEK, a mix of 1.5g of PEEK and 1.5g of NaCl in the middle, then 1.5g of PEEK on the top. The second method, called PEEK (PE), was prepared by adding 3.5g of the NaCl in the bottom of the mold, then 6 g of PEEK. Both compositions were uniaxially compressed to a pressure of 9 metric tons. After compression, samples were heated at 380°C for 10 min with a muffle furnace and cooled at room temperature. The Porous PEEK samples were submitted to the leaching process. On this procedure, the specimens were immersed in distilled water; this solution was replaced twice a day (this was done to guarantee the total dissolution of the salt in the water). After each immersion, the samples were dried in a convection oven at 110°C for 12h then weighed. This process was repeated until they reached the final weight of ~9,5g, and took 5-6 days. Finally, the specimens were polished for 1min (sandpaper 320 grit) to remove any remaining NaCl powder attached to the surfaces.

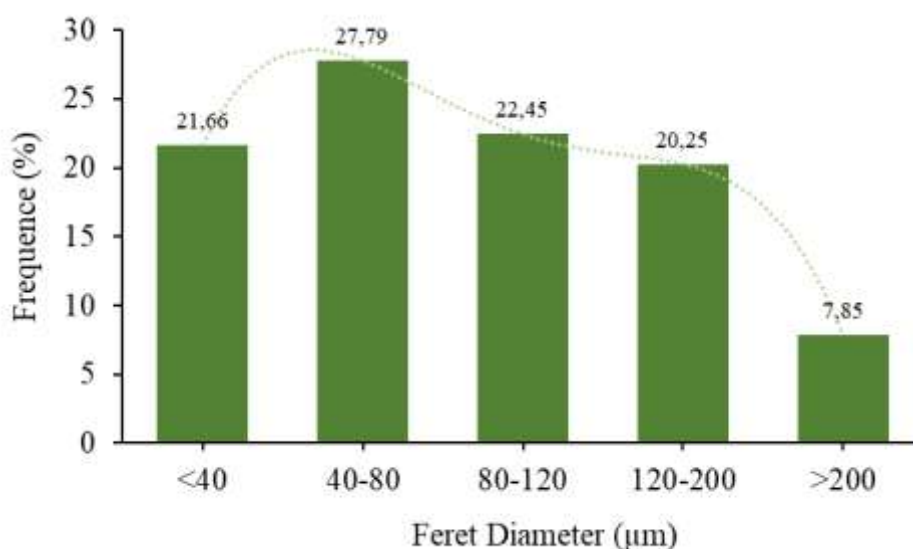
Optical microscopy was used to evaluate the morphology and porous distribution of samples. The analysis was performed with a Hirox Optical Microscope 40X magnification, and analysed using Image J. Atomic force microscope (NEXT Solver, NT-MDT Co.) was used to study the surface roughness of each sample. Operating in contact mode, at a temperature of 22°C and 45% humidity, contact angle analysis was performed with a standard goniometer, using distilled water and an infusion pump rate of 10 mm/h (sessile drop method). The contact angle was measured using a CCD camera. For each sample, five measurements were taken. Unconfined uniaxial compression tests were performed on samples with Instron mechanical testing system (Instron Model 3366). The specimens were pulled in tension using a 15kN load cell with a strain of 30% and a speed of 1 mm/min. Load and displacement data were recorded with the Instron BlueHill 2.3 software and converted to stress and strain values. In vitro cytotoxicity of PEEK was evaluated using MTT cell availability evaluation test according to ISO 10993-5: 2009, with direct contact between material substrate and cells. The cell line used was L929 fibroblasts. The Grubbs' test was performed for outliers. The calculation of the standard deviation was performed by Graph Pad Prism 6. The spectrophotometer used to read the viability of the cells was Victor X3 device, Perkin Elmer.

The analyzes were carried out at the Northeast Biomaterials Evaluation and Development Laboratory - CERTBIO, an accredited laboratory by the ABNT ISSO / IEC 17025: 2005 Standard, CRL 0799 for Chemical and Biological Tests.

### 3. Results and Discussion

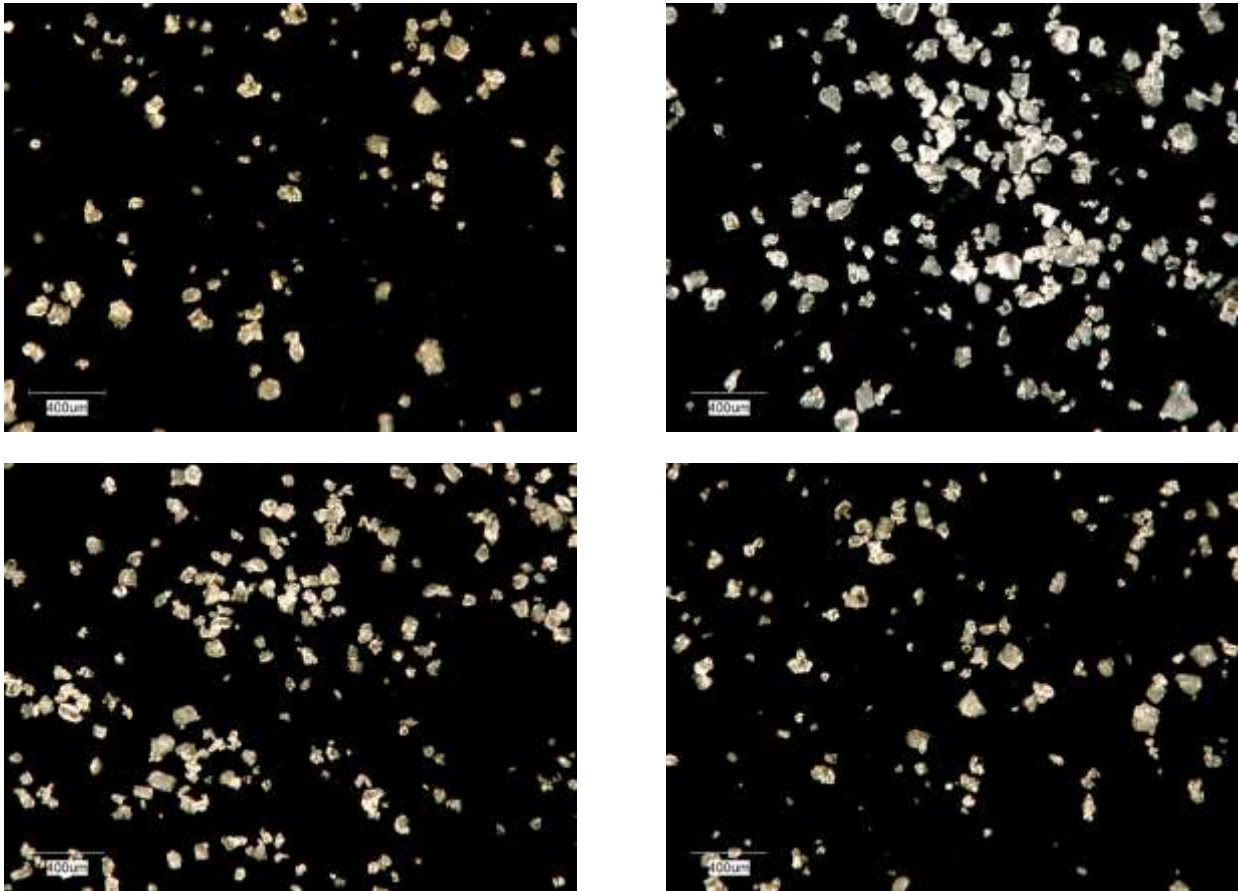
Figure 1 presents the particle size distribution of the NaCl particles. As seen the NaCl particles show irregular shapes and Feret Diameter with a range from 40,5 to 693  $\mu\text{m}$ , and average size of  $97,061 \pm 81,462$ . The particle size distribution can be described as bimodal; the first peak represents particles which vary from 40-80 $\mu\text{m}$ , and the second peak, the particles from 120-200 $\mu\text{m}$  of size. This irregular character, related to the shape and size of the particles, can be best visualized in Figure 2.

**Figure 1.** Particle size distribution of the NaCl particles.



Source: Authors.

**Figure 2.** NaCl crystals used in the leaching technique after grounding.



Source: Authors.

Figure 3 shows the side view of the PE sample, the side and cross-sectional view of the PEP sample. The PEEK samples presented no signs of visible fracture or deformities; also, the cross-section of the porous polymer exhibits an increase of porosity number in the middle with a decreasing number at the extremities.

**Figure 3.** (a) Side view of the PE sample, (b) side view of the PEP sample and (c) cross view of the PEP samples.



Source: Authors.

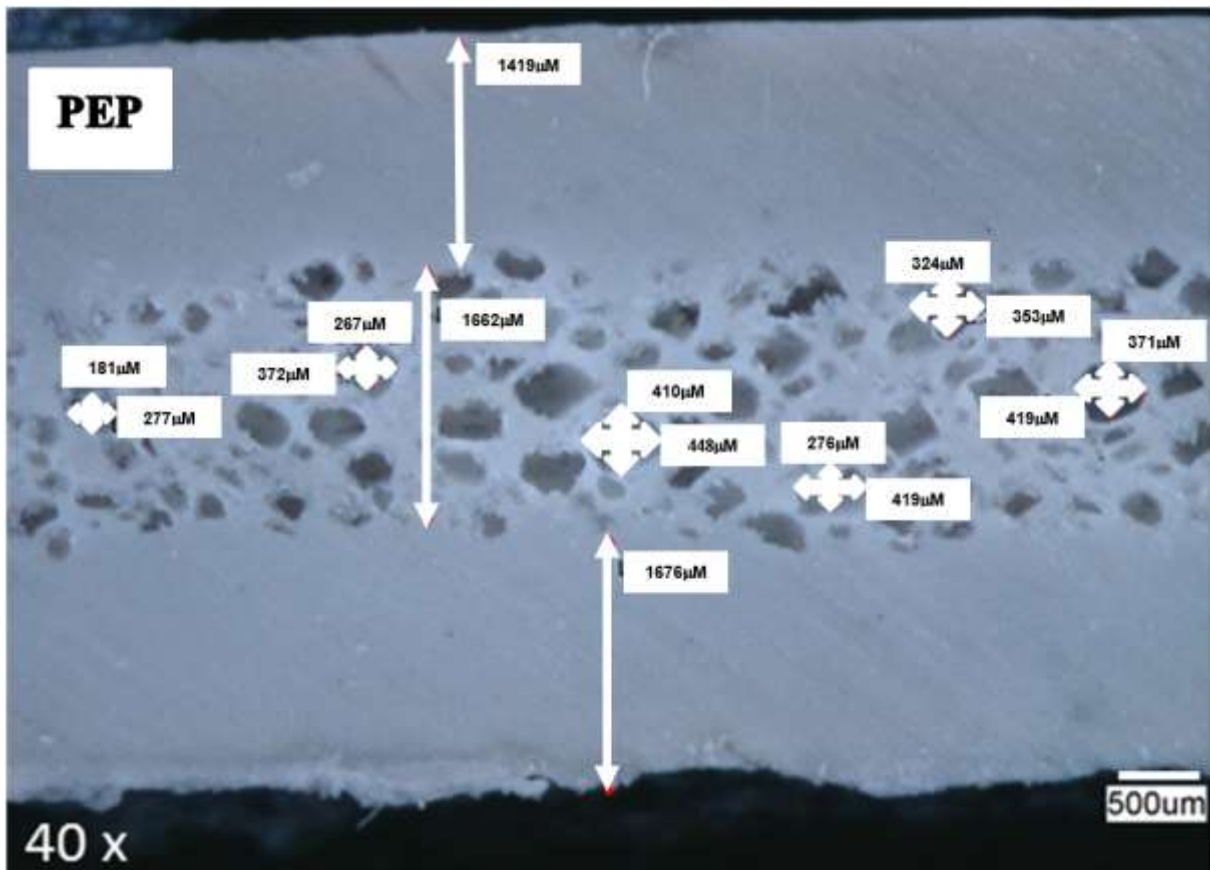
The cross-section of PEP (Figure 3C) revealed variation in pore size and shape distribution. This is due to the different grains sizes of salt crystals leached away by immersion in water (leading to a formation of a porous structure).

Figure 4 shows the optical microscopy of the PEP sample cross section. The porous exhibited predominantly a



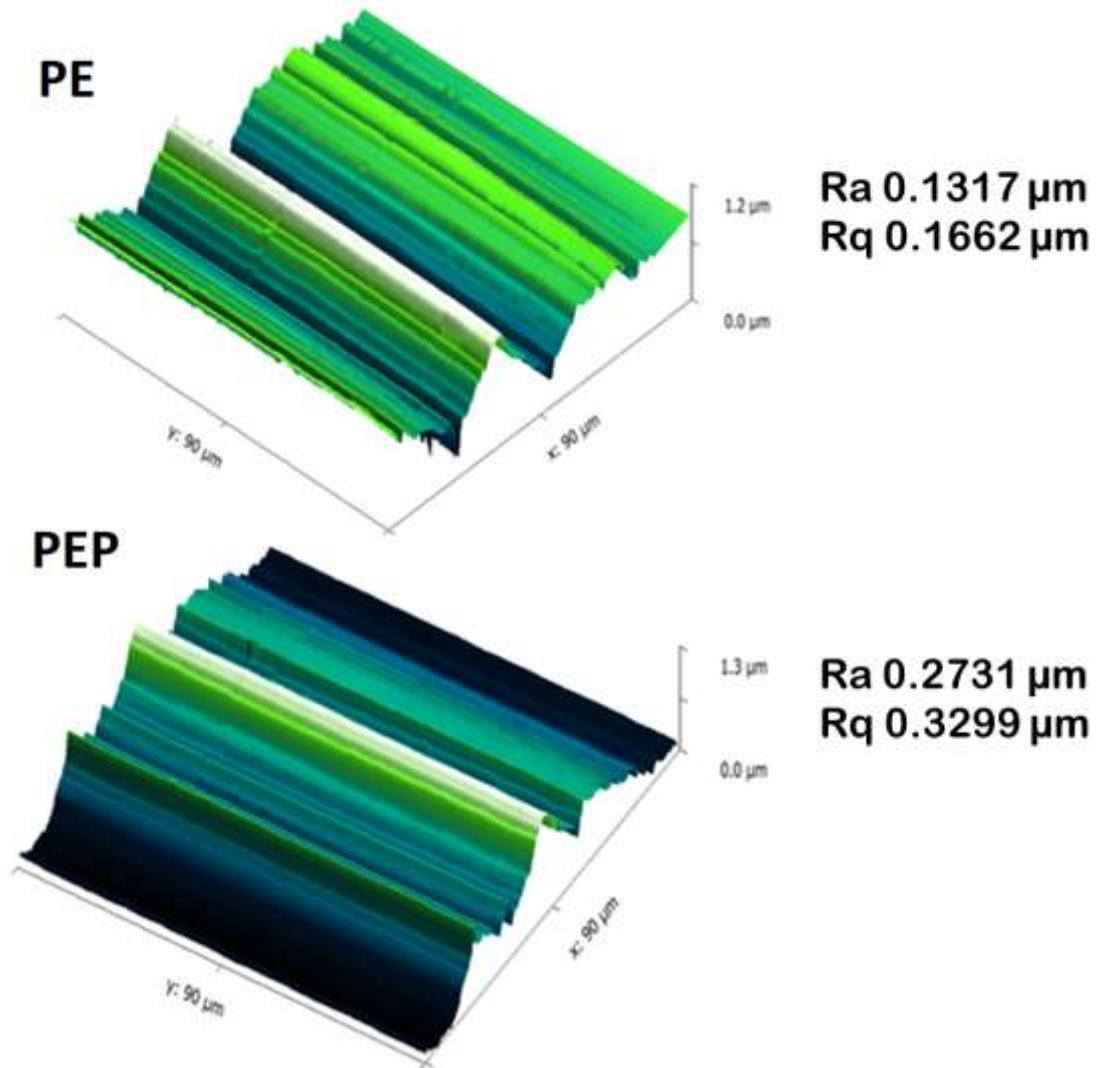
spherical-like shape distributed through the PEEK matrix, varying from 0,115 to 0,2479mm<sup>2</sup> with average size of  $0,5247 \pm 2503 \text{ mm}^2$ . The dense layer showed a mean thickness of 1546 $\mu\text{m}$ , while the porous layer measured 1662 $\mu\text{m}$ . This porous layer may be related to the diploe, which normally presents a variation depending on the specific bone of the skull (though in general it tends to be thicker than the compact bone layer) (Lynnerup, Astrup & Sejrsen, 2005). The results show a slightly increased thickness similar to standard human cranial bones; although, the final thickness can be modified in the production of the samples by varying the amounts of PEEK and NaCl raw powders.

**Figure 4.** Optical microscopy of the cross section of PEP samples.



Source: Authors.

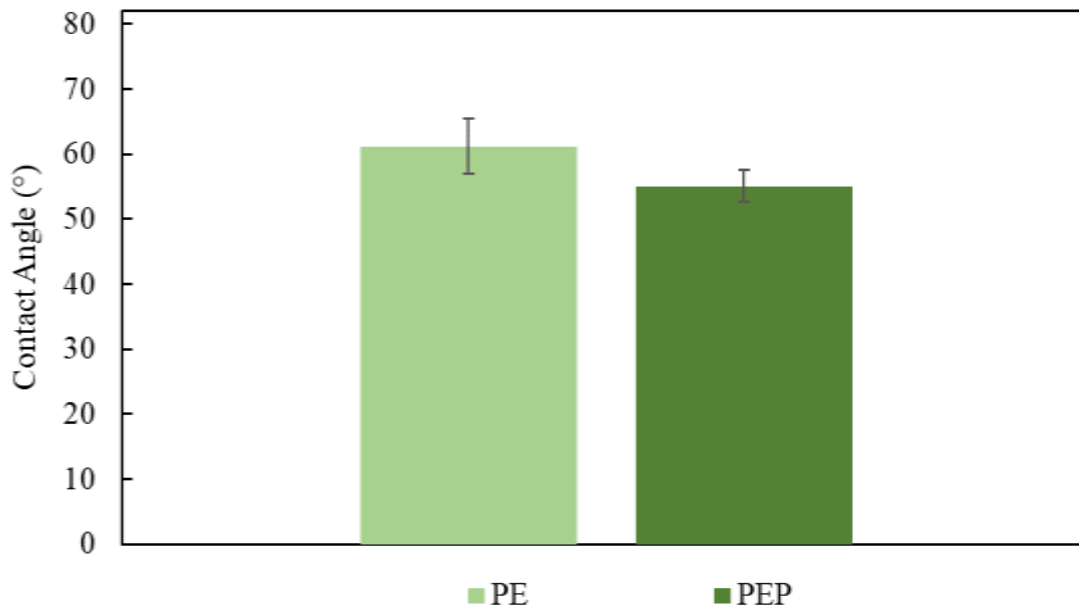
**Figure 5.** AFM images of PE and PEP samples. Roughness average ( $R_a$ ) and Root mean square roughness ( $R_q$ ) of the samples are displayed on the right.



Source: Authors.

The micrographs presented different values of average ( $R_a$ ) and quadratic ( $R_q$ ) roughness for PEP and PE specimens ( $R_a$  and  $R_q$ ) (Figure 5). The surface roughness was higher in PEP samples than in the PEs. So, it's possible to assume that the leaching process promoted an increase of roughness on PEP. That is an important parameter since it can affect the overall cell viability of these polymer surfaces (Lampin et al., 1997). However, the overall roughness of the studied samples was within the range of a smooth surface ( $<1 \mu\text{m}$ ).

**Figure 6.** Wettability of PE and PEP specimens.



Source: Authors.

According to the results shown in Figure 6, the samples presented values of contact angle smaller than  $90^\circ$ , with a mean inferior to  $65^\circ$ , which indicates hydrophilicity (Lampin et al., 1997; Law, 2014). This result was satisfactory, since the implant of PEEK will be subsequently in contact with the human body. With values below  $90^\circ$ , the wettability interaction is large since the liquid attaches and spread towards the hydrophilic surface. The wettability of PEP samples exhibited values  $\cong 10^\circ$  lower than the PE ones. It is important to obtain a hydrophilic surface for biomaterials since cell adhesion is ruled by wettability, surface functional groups, density and type of cell (Alves et al., 2010). It seems to exist a maximum attachment of cells reached in contact angle values from  $40^\circ$  to  $60^\circ$  (Arima & Iwata, 2007; Dowling et al., 2011). The results obtained in the PEP specimens indicate a good cell proliferation.

Uniaxial compression tests (Table 1) revealed that due to its compactness, PE specimens lead to a higher elastic modulus, since they are more compact. The PEP samples had the lowest elastic modulus due to the porosity obtained by the leaching technique. It is noted that the compressive modulus of porous scaffolds relies on the structural properties such as porosity, pore size and autologous structure, which is reported in other studies (Gilardino et al., 2015; Olah et al., 2006; Yin, Qian & Zhang, 2016). This is a great result since PEP specimens showed mechanical properties similar to the cranial bone.

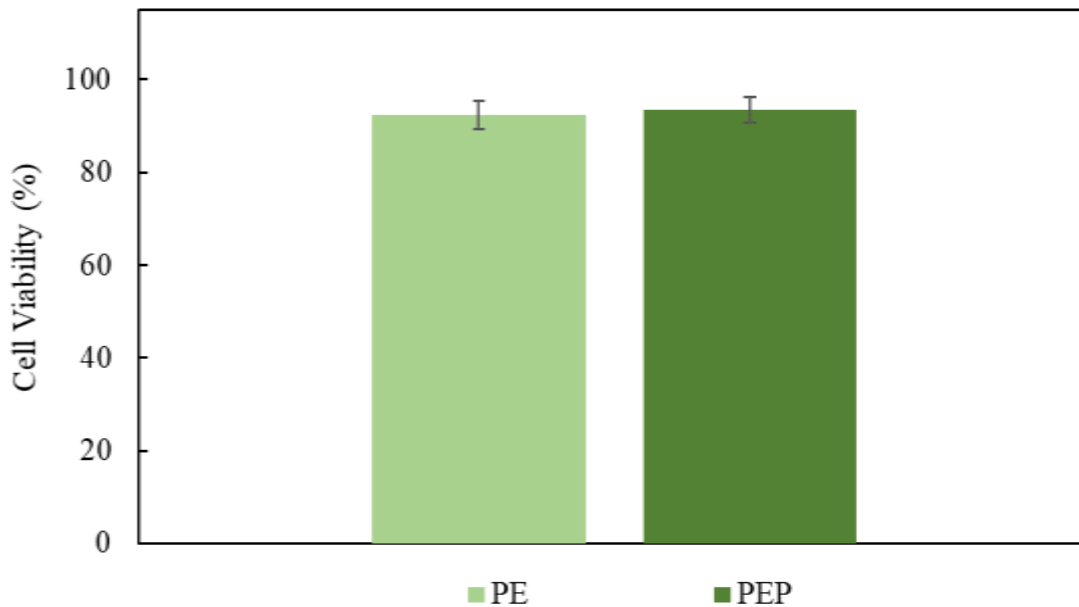


**Table 1.** Elastic modulus and compressive strength of PE and PEP samples.

Samples	Elastic Modulus (MPa)	Compressive Strength (MPa)
PE	110,17	15,204
PEP	22,81	4,576

Source: Authors.

**Figure 7.** Viability of L929 cells exposed to PE and PEP samples.



Source: Authors.

The cytotoxicity results of the samples with their respective standard deviations are shown in Figure 7. Comparing the percentage of cytotoxicity with the minimum value determined by the ISO standard 10993-5: 2009, which is 70%, it is possible to affirm that all samples are feasible since their viability percentage is above 90%. Also, it was found that the values of the viability of PEP samples were increased but are not significantly different from the PEs. Similar reports confirm the viability of PEEK for the application in tissue engineering, obtaining similar results from the ones reported in this work (90% of viability) (Li et al., 2016). Based on the ISO 10993-5, it is possible to confirm that the material studied is non-toxic and cells can proliferate on its surface.

#### 4. Conclusion

In this work, a method to produce a porous PEEK polymer with morphology and mechanical properties similar to the calvaria bone was proposed. The NaCl crystals used presented irregular shapes and Feret Diameter with a range from 40,5 to 693 $\mu$ m. The Porous PEEK samples exhibited a porous polymer structure, with three-layers; the porous layer was thicker than

the compact dense layers. Relatively higher surface roughness and lower contact angle values were observed to the Porous Peek samples, although the overall roughness of those specimens was within the range of a smooth surface.

The porous peek samples exhibited a decrease in the elastic modulus and compressive strength compared to the Peek samples, making them even more similar to the cranial bone. In addition, no signs of toxicity were detected.

In order to better understand the application of the PEEK samples to the proposal described in this work, for future work a pre-clinical study of the PEEK samples must be carried out to evaluate their effectiveness. Overall, we believe this polymer can be used in cranioplasties as a substitute for conventional polymers.

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