Improving bioactivity of PEEK composite polymer for bone application

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Abstract
Polyether ether ketone (PEEK) is a promising biomaterial to replace traditional metals or ceramic components for biomedical mimic the bone tissue. However, PEEK has many challenges to feed into 3D printing fused deposition melting (FDM) machine due to its high melting temperature and high viscosity, the main weakness to use in medical application is bioactivity and cell attachment. In this research, biomaterials such as calcium hydroxyapatite (cHAp) and reduced graphene oxide (rGO) is used to combine with PEEK to make a composite material to improve interfaces biocompatibility. The FDM method is applied to print PEEK/rGO/cHAp lattice structures. The effect of nanoparticle on the biocompatibility of the surface newly manufactured composite (PEEK/cHAp/rGO) are thoroughly investigated. Surface treatment with the addition of rGO and cHAp are used to accelerate the biological activity of recombinant surface coating with improved mechanical and biological behaviour for bone grafting. The PEEK/rGO/cHAp composite characteristics are investigated by X-ray diffraction (XRD), differential scanning calorimetry (DSC), and mechanical tests. The result is shown that the composite with the highest ratio of rGO of 5% has more biocompatibility and mechanical strength. The Young modulus and bulk modulus of PEEK increase exponentially with the increase of rGO/cHAp from 3.85 GPa to 54.27 GPa, 25% of 5 wt% addition of rGO. Furthermore, PEEK/cHAp induces apatite formation after several days of immersion in body fluids simulator Dulbecco's Modified Eagle Medium (DMEM) which contains the ions in body fluid and nutrient agar solution (NAS) to determine their biological viability. The In vivo experimental results are shown that cell aggregation and biological activity in the proposed composite (PEEK/rGO/cHAp) is higher than pure PEEK. The one tested with NAS shows more cell growth compared to DMEM.

Keywords: PEEK; cHAp; rGO; composite; 3D printing; bone implant

1. Introduction
The biocompatible materials have significant advantages to expose the human body [1-3]. There is no report in the literature of the significant damage during contact of biocompatible structures with the human body. One of the primary benefits of biomaterials is to fabricate bone scaffolds. The biomaterial used remains connected with the bone structure, usually under mechanical stress, to assist in regeneration bone growth. Thus, when biomaterials products come into contact with the human body, they are called implants [4-6]. The artificial implants requires considering different material properties, such as biocompatibility, and acceptable biological response. Mechanical characteristics often have to be dependent on automatic charges. The thermal properties must not be separated significantly between the thermal expansion and the human body to avoid painful patient dimension changes [7-9].

Metallic materials, particularly steel, titanium alloys, cobalt chrome alloys have excellent mechanical properties under mechanical loading conditions. However, some complications have been observed using these
materials because their elasticity and bone modulus differ significantly, which results in an effect known as anti-stress [10-12]. The elasticity module is higher than the bone tissue. Thus, the pressure passes to the implant, the adjacent bone mechanical load is reduced, and resulting in reduced bone mineral density and osteoporosis. Therefore, polymer materials with physical characteristics such as density and thermal properties, expansion coefficients, thermal and mechanical conductivity similar to the host tissue becomes increasingly attractive alternative to conventional metal [13-15].

The use of polymeric materials in implants began in the 1970s with ultra-high molecular weight polyethene (UHMWPE). This polymer has excellent resistance to abrasion and therefore, it is appropriate biomaterial chose for joint prostheses, mainly acetabular that in some parts subject to sliding wear. Since the mechanical load in bone tissue is very high, UHMWPE cannot be used in bone scaffold. The prosthesis’s femur is mainly fabricated by metal components that capable of supporting high mechanical loads even under conditions of strength. Studies on the use of PEEK to fabricate bone scaffolds has started in the 1980s [19-22]. The researchers believe that apart form bioactivity and cell attachment, this polymer reports excellent biomedical compliant.

This research focuses on the effect cHAp and rGO surface treatment on the PEEK mechanical behaviour in the short and long-term in the bone implant. This study considers the main phases of experimental growth, such as assessing conditions for processing used in the manufactured scaffolding inlay and the PEEK surface covering using rGO and cHAp surface techniques. Different lattice cell structures were designed to simulate bone structures and evaluate process parameters under mechanical tests. Experiments and simulations of tensile and compression tests of polymeric composites and the effects of PEEK coating processes on coatings and the efficiency and mechanical properties of cell culture. Additional research on surface coating study of thermal vibration impact on crystalline morphology of PEEK resins was performed on the DMA flexion and scanning electron microscope (SEM).

2. Method and materials

The schematic of process is illustrated in Fig. 1. This figure is shown the system setup for simulation, cell growth scaffold, printing FDM scaffold, mechanical testing, coating with medium like DMEM, and microwavable nutrient agar of scaffolds.
Manufacturing limitations and recommended pore size for hard tissue engineering applications were decisive factors in the configurations’ choice. Metallic scaffolds usually have levels of porosity ranging from 20% to 80% in the literature. The 3D virtual model is converted into a stereo model (STL) to be interpreted by the additive manufacturing device, FDM machine, responsible for creating scaffolds that effectively simulates interest anatomy. In composite scaffold the rich-polymer phase becomes the matrix and the poor-polymer phase in subsequent processes. The final structure becomes porous as the solvent evaporates [33-35].

The appropriate geometry of the final complex object is generated under computer control. Table 1 illustrates the working parameters for FDM machine. The extruder temperature oscillates between 350°C to 420°C. The feeding rate is fixed into 45 mm/s. The wire diameter is 0.4 mm, and the plate thickness is 0.2 mm. The PEEK filler was reprocessed from granules to filament.

Table 1: The FDM printer working parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Technical specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nozzle diameter</td>
<td>0.4 mm</td>
</tr>
<tr>
<td>Bed width</td>
<td>210 mm</td>
</tr>
<tr>
<td>Layer thickness</td>
<td>0.2 mm</td>
</tr>
<tr>
<td>Printing speed</td>
<td>45 mm/s</td>
</tr>
<tr>
<td>Raster angle</td>
<td>Longest edge</td>
</tr>
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<td>Ambient temperature</td>
<td>30 °C</td>
</tr>
<tr>
<td>Chamber Temperature</td>
<td>90 °C</td>
</tr>
<tr>
<td>Build Plate temperature</td>
<td>110-160 °C</td>
</tr>
<tr>
<td>Nozzle temperature</td>
<td>350-410 °C</td>
</tr>
</tbody>
</table>

2.1. Experimental method

Three porous models were generated using the different lattice unit-cell as illustrated in Fig. 2 in Creo Parametric software. The models were then converted to stereolithography (stl) format for manufacturing. The outer surface of a different lattice can be approximated by [35-37]:

\[ f(x, y, z) = x + a \cos(2\pi x) \cos(2\pi y) \cos(2\pi z) \]  \( (1) \)
While the model could be generated from an equation-based modeller, we have opted for a more conventional method: a single unit-cell was first generated and repeated in three dimensions to obtain full-size cylinders. The apparent density of the sample after sintering was determined using Archimedes’ principle. The model was weighed dry (m) before being weighed into the saturated water sample. The model was left in boiling water for 15 minutes to allow air to be expelled through the opening. On the other hand, the follicles hair is replaced with water is weighed into the submerged sample. The total porosity of the scaffold is obtained according to Equation (2). The relative density can be calculated by dividing the thickness of the material of theoretical appearance, Equation where \( \rho_{th} = 1310 \text{ kg/m}^3 \) is the theoretical density of PEEK., \( R. \) is the apparent density obtained by the Kimedes method [37-39]. [identified the rest of parameters here]

\[
\rho_{app} = \rho_{lq} \left( \frac{m - m}{m} \right) \\
\text{porosity (%) } = \left( 1 - \frac{\rho_{app}}{\rho_{th}} \right) \times 100 \\
\text{Relative density } = \left( \frac{\rho_{app}}{\rho_{th}} \right) \times 100
\]

(2)

2.2. Mechanical testing

The specimens mechanical properties were examined through uniaxial compression tests using an Instron 5584 device equipped with a video extensometer. Compression testing was performed using a fixed cross-head speed of 0.001 mm/s at room temperature according to the standard DIN 50106. Four samples for each structure were used in the experimental investigations. The direction of the reduction in sample was parallel to the SLM building direction.

2.3. Materials

2.4.1 Graphene oxide preparation

 Compared to monolayer graphene, which is still considered a new material, Brodie tried to determine the graphite atomic weight by oxidising it. In contrast, graphene oxide in graphite oxide layers has been known for more than 150 years. Ingestion of nitric acid [39-41] changed the Brodi method, which tried to accelerate graphite oxide production using less aggressive conditions such as the mixture of sodium nitrate concentrated sulfuric acid potassium permanganate, C/O ratio of the two processes were similar. Still, both suggested reactive functions of oxygen to the original material. The structure of rGO was a controversial issue in terms of its existence. The distribution of functional oxygen groups and non-stoichiometric atomic elements [41-43]. The oxygen groups presence has a significant effect on the mechanical and electrochemical properties of rGO compared to graphene. The use of these properties dramatically facilitates the dispersion of rGO in water and different solvents, allowing the preparation of the nanocomposite polymer and scaling processes for the mass production of rGO. On the other hand, the covalent function group oxygen in rGO produces structural defects. This will significantly affect properties such as conductivity and will limit the use of rGO in conductors’ materials.

rGO listed here was obtained by chemically reducing single-layer graphene oxide (GO). In contrast to almost-insulative rGO shows good electrical conductivity. Its high dissolubility in different solvents makes it easy to process a solution to produce conductive nanocomposites. The listed item is rGO solid powder used to prepare conductive rGO dispersions by dissolving it in N, N-Dimethylformamide Solvents with ultrasonication.
Dimethylformamide (DMF) is a clear, colourless, hygroscopic liquid with a slight amine odour. The solvent properties of DMF are desirable because of the high dielectric constant, the solvents aprotic nature, its wide liquid range, and low volatility. A black appearance rGO has been developed explicitly by chemical reduction which, with ultrasound, can be dissolved in water of ~0.24 mg/ml, organic resolvents of ~0.8 mg/ml as DMF, and NMP of ~0.6 mg/ml. Brunauer, Emmett and Teller (BET) specific surface area of 425.0 - 489.4 m²/g commonly used to evaluate and generate gas adsorption data a particular surface area. This method is referenced by several standard organisations such as ISO, USP and ASTM. The sheet size has a several hundred nanometers to several micrometres in the XY plane, see AFM figure at an atomic ratio greater than 3.5 [44-46]. According to the manufacturer and directly supplying physical bio models from biomaterials perfectly suited to virtual models, the team also provided. Osseointegration and biomechanical properties develop implant panels with the widest variety of geometries, measurements and spatial distribution [46-48]. Direct implant technology can be moved to a potential integrated health system for personalised implants for surgical preparation and training.

3. Lattices of 3D nanostructure

In FDM the component is constructed layer after layer so that structures that cannot be built using conventional subtraction techniques such as gravure are created. Composite materials are required to build ultrafine 3D structures for the bone implant with 3D gratings with nanometers long and wide, too small to be seen with the naked eye. These materials have unusual, often surprising properties and after being compressed, they create extremely light biocomposites which return to their original shape [29-32]. 3D printing was used for PEEK pore with improved capability and less weight. We have created a new 3D printed bone structure method by creating a macro reticular structure with regulated porosity. The Figure 2 reflects a comparison of four lattice pores with porous architectures, which contribute to greater body liquid inflow capabilities for the load applied to each cell inside the composite structure of PEEK.
The pore size, $\alpha$ is calculated based on the cell geometric parameters, as defined by the Equation (2) and $\alpha$ for cubic design; (3).

$$\alpha = \zeta - d$$

For the diagonal design: $$\alpha = \sqrt{2} \cdot \left(\frac{\zeta}{2} - d\right)$$

And for pyramidal design, Eqs. (3) and (4) were applied.

$$\alpha_1 = \frac{\sqrt{17}}{4} \cdot \zeta - d$$\hspace{1cm} (4)

$$\alpha_2 = \frac{\zeta}{2} - d$$\hspace{1cm} (5)

3.1. SEM analysis

The experiments were performed using TA Instruments DSC Q2000, digitised differential calorimetry, and modulated DSC. The digitSurf device Mountain 8 Premium software is also used to obtain accurate results. Tensile
tests were performed on PEEK samples coated and uncoated with rGO and cHAp according to ASTM D638 [51-53]. Tensile test for the PEEK sample was cancelled after the model was broken. This was not found in the bend test, as the model was deformed without breaking. Therefore, the coating process effect on the tensile, elasticity and fracture toughness properties can be analysed using static tensile tests.

3.2. Cell culture with DMEM

Cells are a culture with Dulbecco’s Modified Eagle Medium (DMEM), low Glucose 5 pack (Hyclone, Thermo, USA), 10% Fetal Bovine Serum, 1% Penicillin/Streptomycin (15140-122, Life Technologies Co., Carlsbad), and 1% GlutaMAX (Life Technologies Co., Paisley, UK) in a sterile 75 cm3 cell culture flask (Costar, Corning, Tewksbury, MA, USA) add 0.5 L deionised water, and then it is heated to dissolve the powder in water and then steamed at 121 °C for 15-20 minutes. The solution is then refreshed to warm, antibiotics are added, and sterile Petri dishes are delivered to the mixture. Cells were stored in an atmosphere containing 5% CO2 at 37 °C in a culture incubator. The DMEM was renewed twice a week. When cells appear, Fusion Trypsin (GIBCO, Paisley, UK). For 24 hours, the sample area was 3 cm2/ml, humidified to DMEM cells at 37°C, with an extracted volume. The cells were simultaneously pre-mixed 24 hours. The L929 was made up of 30,000 cell/cm2 in 200 μL of DMEM core in one 96 well plates. Each cell extracted the medium, and a separate 150 μL sample replaced the second day (Fig. 3).
3.3. Cell culture with NAS

Nutrient Agar Solution (NAS) 125 ml from 3-Chemical Nutrient Agar Solution is the quickest and forms of Culture Medium. It contains everything necessary for most experiments with no need to add other ingredients or biological fluid for cell culture medium. It was prepared in the microwave bottle in about 60 seconds which can also be done with hot water bath. A standard Petri Dish takes between 10 ml to 20 ml spread thick of Nutrient Agar solution and the printed scaffold gently placed on it. The bottle was supplied with complete detailed instruction on microwaving the bottle, getting your cultures and growing the cell. We also have an option in the drop-down box to provide one pack of 20 sterile inoculation loops. Also, two other options of 1 pk Petri Dishes of total 6 Dishes & 12 loops make complete project or activity set with the Nutrient Agar for culture of Petri Dishes and Inoculating Loops. The Petri Dishes are sealed in polythene and irradiated with UV to eliminate microorganisms. The Nutrient Agar Solution 125 ml is not classified as dangerous according to regulation (EC) 1272/2008 [CLP]. The solution contains a ‘Lab-Lemco’ powder Yeast extract Peptone Sodium chloride Agar [56-58].

4. Results and discussion

When analysing the particles’ morphology on a plate with a magnification of more than 10 m, it was theoretically verified that the spherical particles have tip fusion maintaining a stable centre. The findings appear in Figure 4 particle morphology sample SEM. Several mechanical specimens were produced following the method to examine the effect of mechanical strength on the coating layer. Considering the samples surfaces, there was a slight break in the core with small plastic deformation in the surface area. The model with 90% tensile strength showed that the layer maintained good adhesion to the PEEK substrate even with a highly constant deformation without showing signs of detachment. This can be seen in the comparison of Figure 4 (a-f).
Fig. 4. Characterise the microstructure elemental mapping for (a) C with 53.9 wt%; (b) O with 26.8%; (c) P with 5.1 wt%; (d) Ca with 13.4 wt%; (e) Mg with 0.5 wt%; (f) SEM of HAP in 100 µm

4.1. Macrostructural observations

The actual density of the model is reported in Table 2. Good reproducibility in the weight of the stem and pore diameter in existence is evident in samples produced by FDM, respectively. However, a significant difference in density was found compared to the first version of CAD: the higher the density, the greater the tolerance in the materials actual thickness. At the lowest Octet-Truss level of porosity according to Table 2, a maximum difference of approximately 30% was observed, similar to other studies that report differences in porosity between samples produced with CAD models [58-60] as is commonly known. A loose powder gets stuck in the internal pores and entirely unfused powder particles that settle on the outer surface also, the smaller the pores, the smaller the entrapment of particles within the structure. Flaccidity, which will be discussed in more detail in this study, played a vital role in these observed inaccuracies. The column and pore sizes shown in Figure 5 and Table 2 provide additional information about the geometric properties of the samples produced. It was found that the actual dimensions of the pin were more extensive than those of the part, as previously observed with interest created by FDM [60-62], resulting in pores that were consistently smaller than in the original CAD model. The powder adhesion at the beginning of this phenomenon can be seen clearly in Figures 4c and f. This excess material, shown in red, is responsible for the wrong density.

Table 2 is shown the model design configuration of porous PEEK/rGO/cHAp samples, showing Truncated octahedron more porous solid volume 754.598 mm$^3$ of different lattice structure of 2×2×2 of 0.4 mm cell diameter
### Table 2: Volumes and porosities of different lattice structures

<table>
<thead>
<tr>
<th>Samples</th>
<th>Volume (mm$^3$)</th>
<th>Porosity (%)</th>
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</thead>
<tbody>
<tr>
<td>Octet-Truss</td>
<td>313.534</td>
<td>58.45</td>
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<tr>
<td>Truncated octahedron</td>
<td>143.588</td>
<td>80.97</td>
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<tr>
<td>Octahedron-cross</td>
<td>191.185</td>
<td>74.66</td>
</tr>
<tr>
<td>BCC- octahedron</td>
<td>286.198</td>
<td>62.07</td>
</tr>
</tbody>
</table>

#### Fig 5. The maximum shear modulus of lattice structures for pure PEEK and PEEK/rGO/cHAp in 1wt%, 3 wt%, and 5wt%, red region shows the highest stress area after applied load

### 4.1. Lattices structure

The cells maintained a complex three-dimensional network structure after forty cycles, demonstrating mechanical stability. Therefore, bones have a high capacity for the same weight or size, insufficient pressure, and essential body fluids management applications. This revolutionary approach is particularly relevant for applications in the medical device and cell phone industries. This works in combination with biomedical cell instruments requiring small fabrics. The research would also support microdevices and non-biological cells. And in a larger size due to the light, high-capacity batteries printed in this way, implants can also use this technology.
<table>
<thead>
<tr>
<th>Strain Value</th>
<th>FCC-Octahedron</th>
<th>Truncated octahedron</th>
<th>BCC-Octahedron</th>
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<tr>
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<td><img src="image" alt="BCC-Octahedron" /></td>
<td><img src="image" alt="Octahedron-cross" /></td>
</tr>
</tbody>
</table>

Fig. 6. Finite element analysis of the lattice structures showing the strain behaviours.

Figure 6 shows data for the quadruple zoom factor, and 180 grad SEM smoothing produces spatial 0.116 mm$^{-1}$ and 658 GL2, respectively, for Day 3 and 0.111 mm$^{-1}$ and Day 7 in the first 0.111 mm$^{-1}$ and 729 GL2. The key spatial frequencies of 0.0144 mm$^{-1}$, 0.013 mm$^{-1}$, and 0.0124 mm$^{-1}$, for the first, third and seventh days, respectively. Fig. 7 PEEK composite 3D-printed grid structure used to compare the measurement of a young module in-plane to the strengths of cells snap-fit, according to the relative density.
Figure 7 also shows information for the 3-point fixed injection crack zone created by PEEK test sample before and after the stress assessment. Figure 7(b) shows the pure PEEK molecular weight degradation in vitro for the PEEK/cHAp compound tested to increase the normal stress-strain. It was extensively clarified, and the effect of complexity on subsequent nanoparticle size analysis is shown in Figure 7 (c). It analyses the sensitive fraction to the PEEK nanoparticles brightness scale and the light fraction of the number of the particles. Pick up the box. The study scale and (d) the microstructural failure analysis (Figure 7d) summarised all results showed a constant increase with the number of days.

The surface layers of the phase of coating have gradually increased by 2% and relatively 10%. The sample crystallinity effect was significantly higher than the rGO and cHAp crystalline model with a surface layer shock only. No interference from potential particles of rGO and cHAp was seen in the samples given the analysis methods. There has also been reduced the need for a thermogravimetric test. Different relevant experiments for an
examination of the mechanical comportements of the other specimens were carried out. The trial provided a study of PEEK and composite tensile properties. The sample stress curve was presented in Fig 7 (a).

Three samples were created to guarantee the reproducibility of the results (Fig. 4) and compared with the elastic modulus property under thermal shock conditions with the most significant source of change due to the change. Subtle transition textures after coating the sample, the analysis is performed using test parameters previously predetermined in the model after coating cHAp with PEEK. Two or three evaluations were carried out, and the median curve was often illustrated. From ceramics to organic compounds, printing is difficult, mainly if structures less than 50 microns are built, approximately half a human hair width. A nano-scale measurement laser is used to extract fluids from a given location to a material containing only two photons or light particles. The energy provided to solidify the liquid polymer is necessary. But PEEK cannot react to light as the polymer resin used to build nano-scale structures, not enough to melt metals. There is a chemical reaction that occurs when light reacts with polymers. This hardens and forms specific cHAp in PEEK. Organic bonding solutions and material-bound molecules are used to form polymer-based resins. Instead, a printable metal was used as a scaffold in an experiment that describes organic molecules to create a fluid that resembles a bone implant. The structures designed in computer software were then created by compression of the liquid with a photon laser. Lasers build stronger chemical bonds and make them structural components between organic molecules.
Fig 8 Compressive test of the different lattice structures comparing each of the composites of stress-strain graph of (a) PEEK (b) 1 wt%, (c) 3 wt%, (d) 5 wt%

Thus, the cHAp coating process is shown to have a thermal influence on PEEK, which increases surface crystallisation and, as a result, an elastic response in the regime. The amount of frost stress in the polymer is enriched by the glass. The cHAp layer serves as an anchor to stop higher relaxation levels when the glass transition temperature is reached. The tan value of the rGO and cHAp coated sample is significantly lower than the heat shock alone. The result observed for elastic modulus also confirms this, because only for heat shock samples, followed by PEEK/rGO/cHAp non-mechanical. After bending, the top E in the viscoelastic regimen is reported. The static collection indicates the minimum value for this property. This behaviour demonstrated the effect of a higher crystallinity obtained for samples exposed to heat, shock and treatment with cHAp. DMA tests on coated samples and cHAp coated pieces after 106 mechanical cycles showed different behaviour than models under the same conditions without mechanical stress. This phenomenon can be seen quantitatively. Tables of three reference temperatures for parameters E and tan are shown in Figure 9 (a-f), respectively.
Fig. 9. Tensile test result (a) strain with time, (b) strain to displacement, (c) stress against strain, (d) maximum stress vs. strain, (e) max principal strain vs. max principal stress, (f) max shear stress vs. max shear strain.
principal elastic strain to displacement, (e) max principal stress against max principal strain, and (f) max shear elastic stress and max shear elastic strain

<table>
<thead>
<tr>
<th>PEEK (wt%)</th>
<th>rGO (wt%)</th>
<th>cHA (wt%)</th>
<th>Young Modulus (E) GPa</th>
<th>Shear Modulus (G) GPa</th>
<th>Poisson Ratio (v)</th>
<th>Density (ρ) kg/m³</th>
<th>Bulk Modulus (GPa)</th>
<th>Relative Density</th>
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<td>30.0</td>
<td>15.6565</td>
<td>6.8553</td>
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<td>73.0</td>
<td>2.0</td>
<td>25.0</td>
<td>25.3105</td>
<td>11.4358</td>
<td>0.10663</td>
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<td>0.73512907</td>
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<td>3.0</td>
<td>20.0</td>
<td>34.9645</td>
<td>16.0173</td>
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<td>0.77240566</td>
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<td>81.0</td>
<td>4.0</td>
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<td>44.6185</td>
<td>20.5988</td>
<td>0.08304</td>
<td>1610</td>
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<td>54.2725</td>
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<td>0.077684</td>
<td>1524</td>
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<td>0.85958005</td>
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</table>

4.2. *In-vitro* cytotoxicity results

As shown in Fig. 8, the accumulation of cells was observed in the surface grooves, resulting from the deposition patterns combined manufacturing process. Incorporated and forming a cluster, the PEEK/rGO/cHAp compound cells were dispersed. In the PEEK/rGO/cHAp composite, more prominent actin filaments were observed that adjacent bond cells. In the PEEK/cHAp compound, the cell nuclei were denser than the PEEK surfaces cell nuclei. The alkaline phosphatase activity qualitative and quantitative results are shown in Figs10-11. Fig. 10 (a-b) represent a cell attached to sample scaffold surfaces after using DMEM culturing medium for days, of 50 µm of PEEK after over 24 hours and 50 µm of PEEK/rGO/cHAp composite scaffold after over 24 hours. Fig 10(c-d) shows a 50 µm of PEEK after 3days of 50 µm magnification of cell deposition of PEEK/rGO/cHAp. Figure 10(e-f) represents more spreading of cell alkaline phosphatase activity after seven days of PEEK culturing and a better life cell attachment on the PEEK/rGO/cHAp composite scaffold. The Live/dead staining of cells attached to FDM 3D-printed PEEK composite sample surfaces after culturing with Nutrient Agar Solution is represented in fig 11. Fig. 11 (a-c) shows a 50 µm of PEEK for 24 hours, 50 µm of PEEK/rGO/cHAp for 24 hours with a 10 µm of PEEK/rGO/cHAp showing the live-cell growing. Figure 11 (d-f) show more spreading and alkaline phosphatase activity of cells in 3rd day in 50 µm PEEK, 50 µm of PEEK/rGO/cHAp 3rd day and cell growing more PEEK/rGO/cHAp at 20 µm. Fig.11(g-i) results from 50 µm of PEEK cell spreading with little dead cell in the 7th day and PEEK/rGO/cHAp cell growing circulating with small dead cell in the 7th day. The dead cell spread rapidly on the PEEK cell on the 14th day and PEEK/rGO/cHAp with a dead cell on the 14th day, as shown in Fig 11(j-i).
Fig. 10 Cells attached to sample scaffold surfaces after using DMEM culturing medium for days, showing: (a) 50µm of PEEK after over 24 hours, (b) 50µm of PEEK/rGO/cHAp composite scaffold after over 24 hours, (c) 50µm of PEEK after 3 days, (d) the corresponding magnification of 50 µm of cell deposition of PEEK/rGO/cHAp, (e) more spreading of cell alkaline phosphatase activity after seven days of PEEK culturing, (f) A better life cell attachment on the PEEK/rGO/cHAp composite scaffold.
Fig. 11. Live/dead staining of cells attached to FDM 3D-printed PEEK composite sample surfaces after culturing with Nutrient Agar Solution, showing: (a) 50µm of PEEK for 24hour, (b) 50µm of PEEK/rGO/cHAp for 24hour, (c) 10 µm of PEEK/rGO/cHAp showing the live-cell growing (d) more spreading and alkaline phosphatase activity of cells in 3rd day in 50µm PEEK (e) 50µm of PEEK/rGO/cHAp 3rd day(f) Call growing more on PEEK/rGO/cHAp at 20µm (g) 50µm of PEEK cell spreading with little dead cell in the 7th day (h-i) PEEK/rGO/cHAp cell growing spreading with small dead cell in the 7th day (j) PEEK cell spreading with more over at the 14th day (k-l) PEEK/rGO/cHAp with a dead cell at the 14th day

However, Strength age on characteristic young modules was significantly distinguished, and consequently, the tanning effect was managed. The value obtained for young modulus decreased and, thus, the skin tone. Replace δ
with the reduced sample. A more robust answer during the coating process was present on the surface to be subjected to thermal shock. The cyclical mechanical stress of coagulation [63,64] can be due to tanning reduction. This is heated by the coating process, as seen in the SEM picture of the polymer. On a frozen, cracked surface in the samples with cHAp effectively coated, there was an increase in young modulus indicating a more sterile behaviour. This behaviour demonstrated that the rGO and cHAp particles were separated from the polymeric substrate and resulted from deformation caused by mechanical cycling.

This study did not describe the final evaluation of the surface coating process for rGO/cHAp-coated PEEK mechanical tiredness durability. This is because, even at high levels of stress, such as the 85% flow point of the sample tested, no three PEEK processing conditions were realistic due to the sampling separation for up to one million test cycles. Due to strength of PEEK-coated samples, the same low-pressure release rate only for thermal shock examples, residual stress is higher and is seen as a manufactured form. PEEK processing with cHAp contributes slightly to the mechanical performance of PEEK under strength testing conditions. Therefore, it can lead to faster incorporation into bone tissue engineering, such as implant surgery.

5. Conclusions

In conclusion, PEEK and its biocomposites PEEK/rGO/cHAp were manufactured using the FDM technique using a different composite ratio of 1 wt%, 3 wt% and 5 wt% of rGO as a reference point. The composite scaffold biological activities have been improved by adding rGO and cHAp nanoparticles to the PEEK matrix. The tensile properties and elastic modulus of the PEEK/rGO/cHAp composites with different cHAp contents, of 30 wt%, 20 wt% and 10 wt% to rGO 1wt, 3wt% and 5wt% respectively were evaluated. The 5wt% rGO exhibited more mechanical strength of highest young modulus of elasticity at 54.2725 GPa compare to the control sample of PEEK which has 3.85 Gpa. In-vitro assays showed that the PEEK/rGO/cHAp had better adhesion, spread, and alkaline phosphatase activity than pure PEEK. The PEEK/rGO/cHAp composite induced apatite formation in the DMEM and NAS medium in 14 days. In-vivo results showed that the osseointegration activity of the PEEK/rGO/cHAp composite was higher than that of PEEK, showing more NAS cell growth than DMEM. All these results confirmed that rGO and cHAp significantly improved the biological activity of PEEK and its osteogenesis.

The comparative evaluation of the different lattice structure mimicking the scaffold bone structure was designed and simulated. The rGO and cHAp coated PEEK samples mechanical strength values and the tensile and compressive stress analyses identified the interfacial deterioration. The key influence on the stress of strength can be explained by the increase of surface crystallinity and an accumulation of residual thermal stress generated by the PEEK/rGO/cHAp composite. The lattice structure of the BCC-Octahedron shows more mechanical strength compared to another lattice sample that mimics bone structure in porosity and strength. The most considerable reduction in the rGO/cHAp-coated PEEK rate is due to the coat mechanical force interfacial adhesion degradation. The stress test showed that the shock of the surface coating decreases the composite deformation of PEEK/rGO/cHAp significantly.

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