Influence of wt. % and Specific Area of HRGO Nanoplatelets Addition on Physical, Thermal, Mechanical Behaviour and Processing Evolution of PMMA Bone Cement for Orthopaedic and Trauma Surgery

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SUMMARY/ABSTRACT
Bone cement is frequently used in different arthroplasty surgical procedures. However, its manufacturing process reaches high temperatures that can produce necrosis in the patient’s surrounding tissues. This article analyses the effect of the addition of different percentages of graphene (0, 10, 0,50 and 1,00 %) and surface densities (150, 300, 500 and 750 m2/g) on the physical, mechanical, and thermal properties of commercial PMMA-based bone cement and its processing. It is concluded that a lower sintering temperature would be reached with this addition, making it more suitable for human use at the same time as it reduces its adverse effects. In contrast, the materials’ density does not show significant changes, which indicates that the addition of HRGO does not significantly increase its porosity. Lastly, the mechanical properties are reduced by almost 20 %. Nevertheless, these properties are high enough so that these new materials can still fulfill their structural function.

Abbreviations: PMMA: Polymethylmethacrylate; HRGO: Highly Reduced Graphene Oxide Layers; DSC: Differential Scanning Calorimetry; FESEM: Field Emission Scanning Electron Microscopy

Keywords: PMMA Bone Cement; Graphene; Mechanical; Physical; Processing; Microstructural Behaviour; Orthopaedic and Trauma Surgery

INTRODUCTION
Bone cement is a polymeric material commonly used as a structural adhesive to fix replacement prostheses when the bone is damaged1. Given that the implantation of prostheses has become an increasingly common practice throughout the world, both due to the rise and ageing of the population and the higher incidence of obesity2 and other pathologies such as osteoarthritis (whose global prevalence is 47 %3), rheumatoid arthritis, arthritis prosthetics and others, the design of new optimised structural adhesives is an essential objective in traumatology and orthopaedics.

However, the limitation of biocompatibility and toxicology significantly reduces the variety of materials that can be used as adhesives (bone cement) for prostheses fixation. These materials are sintered directly in the operating room from two phases: a solid one, in powder form, composed mainly of polymethylmethacrylate microspheres (PMMA), benzoyl peroxide (BPO) as initiating agent and barium sulphate (BaSO4) as radiopacifier to allow it to be detected on control radiographs; and a liquid phase, mainly composed of methyl-methacrylate (MMA), the monomer that reacts to form PMMA in a radical reaction, breaking the carbon-carbon double bond to form saturated carbon bonds, N, N-dimethyl-P-toluidine (DmpT) as a reaction accelerator and hydroquinone as a stabiliser or inhibitor of the polymerisation reaction.

After polymerisation of the bone cement, which usually takes a few minutes, residual MMA always remains, between 2-6 %. However, this amount decreases with time, stabilising after approximately one year to around 1-2 % of residual MMA (Klaus-Dieter, 2013) REFERENCIA NUMERICA. This is because, with increasing viscosity of the bone cement, the mobility of the monomer decreases exponentially. This residue, together with small PMMA particles released from the bone cement during the service life, activate an inflammatory cascade, where after the rupture of the endothelium and vasoconstriction of the area, potentially necrotic ischemia of chemical origin occurs.

Osteoclasts, the only cells capable of resorbing bone4 through highly specialised structures5, can respond to a wide variety of cytokines produced by cells of the innate and adaptive immune systems6,7,8. Particular subclasses of circulating monocytes and dendritic cells, as well as progenitor cells of the monocyte-macrophage line resident in the bone marrow, have the ability to transform into osteoclasts if they are subjected to specific inflammatory signals. Furthermore, the production and activation of T lymphocytes cause a rise in osteoclastogenesis and bone resorption since their interaction with the RANKL-RANK system increases osteoclast survival, delaying their apoptosis and making it possible for several cycles of osteoclastic activity9. All these inflammatory processes can lead to the loss of bone tissue, causing aseptic loosening, which accounts for approximately two-thirds of revisions.

Graphene is an allotropic phase of carbon, ideally being a monolayer (two-dimensional material by definition) and has some exceptional properties: a large specific surface 2630 m2/g, an elasticity modulus of 1 TPa, a tensile strength of 130 GPa, great flexibility, excellent thermal conductivity amongst others and is, furthermore, nominally biocompatible10,11,12. Therefore, adding graphene to commercial bone cement could improve the mechanical properties of the compound (https://www.researchgate.net/publication/343560583_Acrylic_Bone_Cements_Modified_with_Graphene_Oxide_Mechanical_Physical_and_Antibacterial_Properties). Additionally, the percentage of polymerisation from MMA to PMMA could be increased since graphene could inhibit the initiators, reducing the number of active polymerisation points, leading to slower but more efficient polymerisations in PMMA. In particular, highly reduced graphene oxide (HRGO) nanoplates are a derivative of graphene that is not entirely reduced and whose edges are highly reactive. Hydroxyl groups are formed on these limits, among other possible compounds, therefore acting as effective scavengers for polymerisation accelerators.

On the other hand, the MMA polymerisation reaction is highly exothermic, releasing 52-57 kJ / mol of MMA, resulting in a heat release of 1.4-1.7 108 J/m3. Consequently, the bone cement is heated to high temperatures, between 70 - 120 °C. Thermal necrosis in tissues has been shown to occur at temperatures above 50 °C if it exceeds one minute and 45 °C if it exceeds 30 minutes13. The minimum critical point of temperature to delay the death of osteocysts is around 47 °C, causing bone resorption, subsequent replacement, and medium and long-term disturbances in the anchorage.
of the implants.\textsuperscript{14, 15, 16, 17, 18} Acrylic bone cement is in intimate contact with bone, and this exposure to high temperature can cause thermal necrosis in adjacent tissues \cite{Gross et al., 1995}. This can happen both due to direct cell damage with protein denaturation or coagulation alterations that cause a lack of irrigation of the adjacent bone tissue, compromising the success of the intervention and healthcare costs. On the other hand, necrotic debris and tissues provide favourable conditions for bacterial growth and eventually lead to abscess formation.

The structure and vascularity of bone play an essential role in the response of bone tissue to heat. Cancellous bone dissipates heat faster and has a greater capacity for regeneration than compact (cortical) bone, as it has a better supply of blood vessels \cite{Haider ET AL., 1993}. With the addition of graphene, which could delay the polymerisation in a controlled way, a lower temperature rise, and a longer workability time could be achieved by the surgeon.

Regarding mechanical properties, bone cement works well below its glass transition temperature, $T_g$, which implies a brittle-elastic behaviour. Analysed from the point of view of geometric stability over time, the fact that the bone cement works below $T_g$ is optimal, as creep processes are negligible during the useful life of the bone cement, estimated at fifteen years. The lower value of the elasticity modulus of the bone cement facilitates a more homogeneous and even distribution of stress on the bone, thus avoiding stress concentrations that may damage it.

Another property that could optimise the addition of graphene would be the toughness or energy of fracture. The propagation of cracks in the matrix could be impeded or diverted by the presence of graphene. This is because, locally, it would be more favourable to surround the nanoparticle than to fracture it. As the path of the crack increases and becomes more protracted and more tortuous, the energy necessary for the crack to grow and propagate increases\textsuperscript{19, 20, 21}.

Within this context, this article analyses the effect of the addition of different percentages of graphene (0.10, 0.50 and 1.00 %) and several surface densities (150, 300, 500 and 750 $\text{m}^2/\text{g}$) on the physical properties, mechanical, thermal, and processing of commercial PMMA-based bone cement.

**MANUFACTURE OF NEW MATERIALS**

In this study, a commercial bone cement widely used in traumatology was taken as reference material. DePuy Ibérica S.L. supplied the starting excipients with CMW-1 Radiopaque reference, and the composition of these is shown in Table 1. The precursors are presented in two phases: i) solid phase, with polymethylmethacrylate (PMMA) as the main bone cement compound, benzoyl peroxide (BPO) as initiating agent and barium sulphate (BaSO$_4$) as radio-opacifying agent; ii) liquid phase, where there is methyl methacrylate (MMA) as the monomer, N, N-Dimethyl-p-toluidine (DmpT) as accelerator and hydroquinone as stabiliser or inhibitor of the polymerisation reaction.

| SOLID PHASE | LIQUID PHASE |
|-------------|--------------|
| **Compound** | **Proportion** | **Function** | **Compound** | **Proportion** | **Function** |
| Polymethylmethacrylate (PMMA) | 88.85 wt. % | Polymer | Methyl methacrylate (MMA) | 99.18 wt. % | Monomer |
| Benzoyl peroxide (BPO) | 2.05 wt. % | Initiating agent | N, N-Dimethyl-p-toluidine (DmpT) | $\leq 0.82$ wt. % | Accelerator |
| Barium sulphate (BaSO$_4$) | 9.1 wt. % | Radio-opacifying agent | Hydroquinone | 25 ppm | Stabilizer |

To analyse the effect of graphene on the behaviour of the reference bone cement, four types of highly reduced graphene (HRGO) with different values of the specific area ($\text{m}^2/\text{g}$) and proportions were used and supplied by XG Sciences. The characteristic values for these materials are shown in Table 2.

| Graphene | Specific area ($\text{m}^2/\text{g}$) | Atomic oxygen concentration (%) | Mean diameter ($\mu$m) | Density ($\text{g/cm}^3$) |
|----------|----------------------------------|---------------------------------|-----------------------|------------------------|
| HRGO 120 | 120-150                          | $< 1$                           | 15                    | 0.03 – 0.10            |
| HRGO 300 | 300                              | 5                               | $> 2$                 | 0.20 – 0.40            |
| HRGO 500 | 500                              | 7                               | $> 2$                 | 0.20 – 0.40            |
| HRGO 750 | 750                              | 10                              | $> 2$                 | 0.20 – 0.40            |

Its low oxygen content characterises HRGO. However, the greater the surface area, the more difficult it is to reduce the oxygen content due to the high reactivity of the graphene nanoplatelet edge. For this reason, although in Table 2, the oxygen content increases with the specific area, they all correspond to the same HRGO manufacturing procedure.

For the preparation of PMMA compounds with HRGO, a high-resolution Mettler-Toledo balance (up to 10$^{-6}$ g) was used so that the percentage of each component could be determined with very high precision. To achieve a good dispersion of the graphene in the PMMA, once the solid phase of the precursor was mixed with the graphene, this combination was stirred in an ultrasound bath at 4.000 Hz for five minutes. After that, the liquid phase was added, and the exothermic reaction started. Subsequently, the entire mixture was spread on a 100 mm x 100 mm stainless steel mould whose support bases were coated with Teflon to avoid adhesions of the bone cement to the metal, making it easier to separate and avoid introducing undesirable mechanical deformations in the material. The amount of material deposited was calculated so that the resulting plate had a thickness of about 2.4 to 3.0 mm, like the thickness of the bone cement layer used in trauma operating rooms to fix the prosthesis to the bone.

Next, as shown in Figure 1, employing an electromechanical mechanical testing machine (Instron 5866, GB), a pressure of 50 kPa has been exerted perpendicular to the mould surfaces to simulate the pressures at which bone cement is subjected to through a finger or scalpel in operating rooms\textsuperscript{22} and to homogenise the manufacturing conditions of the material\textsuperscript{23, 24, 25, 26}.  

\textsuperscript{14} Gross et al., 1995. 
\textsuperscript{15} Haider ET AL., 1993. 
\textsuperscript{16} ibid. 
\textsuperscript{17} ibid. 
\textsuperscript{18} ibid. 
\textsuperscript{19} ibid. 
\textsuperscript{20} ibid. 
\textsuperscript{21} ibid. 
\textsuperscript{22} ibid. 
\textsuperscript{23} ibid. 
\textsuperscript{24} ibid. 
\textsuperscript{25} ibid. 
\textsuperscript{26} ibid.  

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**Table 1.** Composition of DePuy Ibérica CMW 1 bone cement - Radio-opaque.

**Table 2.** Composition of XG Sciences Highly Reduced Graphene Oxide (HRGO)
Although it was initially suggested to maintain the charge for 20 min to follow the preparation times indicated by the manufacturer, it was observed that the graphene content influences the polymerisation time and, in some cases, it was necessary to maintain the charge for more than 24 h. until the end of the polymer synthesis. Once the desired state in the bone cement was reached, the load was removed, the bone cement detached from the mould, and the material stored in hermetically sealed PET zip-bags at room temperature for one year before testing. Table 3 shows the specific composition and nomenclature used for each of the nine new manufactured materials.

Table 3. Nomenclature of bone cement compositions with HRGO manufactured in the form of 100x100x5 mm³ plates.

| HRGO weight percentage (wt. %) | Surface area of the graphene used (m²/g) |
|--------------------------------|----------------------------------------|
|                                | 120                                    |
| 0,1                            | G120-0.1                               |
| 0,5                            | G300-0.1                               |
| 1,0                            | G120-1.0                               |

Each plate of material in Table 3 was successfully machined in a precision mechanical workshop to obtain specimens with the appropriate shape and dimensions for each test. These details will be shown in the next section.

**EXPERIMENTAL METHODS**

**Physical characterisation**

The density of each material was determined using the Archimedeans immersion method in distilled water at a controlled temperature, using a Mettler-Toledo balance with an LC-P Density unit attached to measure the masses. The nominal dimensions of the test pieces were 26.8 x 3.0 x 2.4 mm. Six tests were carried out for each composition, in which one of them used five test tubes from different areas to identify possible inhomogeneities in the developed material.

**Thermal characterisation**

Differential Scanning Calorimetry (DSC) made it possible to determine the glass transition temperature (Tg) and the amount of residual monomer of the new materials. For this, a Mettler Toledo DSC822e machine was used, with STARe Software, with a heating ramp of 10 °C/min between 20 and 200 °C on 6 mg samples. To achieve cooling at the same speed, liquid nitrogen was used as a refrigerant.

**Mechanical characterisation**

**Tensile tests**

To determine the tensile strength (σ) and the modulus of elasticity (E) of the new materials, simple tensile tests were carried out in an electromechanical testing machine with a ± 1 kN load cell (with resolution lower than ± 0.1 N) and with an LVDT type extensometer with a path of ± 1 mm (with resolution lower than ± 1 µm). The tests were carried out in displacement control with a speed of 100 µm/min and an initial preload of 5,0 N. This preload is intended to achieve the perfect alignment of the specimen and ball joints of the loading device and thus avoid possible twisting or rotations on the sample, which would lead to inaccurate results.

The specimens for these tests were machined in the shape of a dog bone with nominal dimensions of 10 mm testing length, 30 mm total length (L), and 5 mm width at the heads inside the jaws, 25 mm centre shaft (L0), 3 mm width (a) in the central shaft, and 2,4 mm thickness (b). For each material composition, the materials indicated in Table 3 were tested. To calculate the value of the maximum tensile strength (σt), the following formulas were used:

\[
\sigma_t = \frac{F_m}{a \cdot b}
\]

where \(F_m\) is the maximum force obtained during the tensile test, \(a\) is the thickness of the zone of the specimen with a reduced section, and \(b\) is the width of the specimen.

**Fracture Toughness**

In this case, the same mechanical testing instruments were used, but three-point bending tests were performed on specimens notched with 0,13 mm diameter diamond wire to determine fracture toughness (ktc). The tests were carried out with the same measurement system that has been mentioned in the previous point. In this case, the specimens had a preload of 1 N and were tested at a speed of 100 µm/min in displacement control. This time, the experimental device consisted of a system of alumina rollers, 5 mm in diameter, with a span between supports of 20 cm.

The parallelepiped specimens’ nominal dimensions were: length 26,8 mm; width 3,0 mm; edge 2,4 mm. As in the previous case, before each test, the dimensions of each specimen were measured with a calliper with a resolution of ± 0,01 mm. The nominal length of the notches was 1,1 mm and was determined with a NIKON Profile Projector V-12B, with a resolution of ± 0,001 mm.
Finally, to calculate the fracture toughness from the maximum load and the dimensions of the specimens\textsuperscript{37}, the equation that appears below was used:

\[
K_{IC} = F\left(\frac{a}{h}\right) \sigma (\pi a)^{1/2}
\]

\[
F\left(\frac{a}{h}\right) = 1.106 - 1.552 \frac{a}{h} + 7.71 \left(\frac{a}{h}\right)^2 - 13.53 \left(\frac{a}{h}\right)^3 + 14.23 \left(\frac{a}{h}\right)^4
\]

\[
\sigma = \frac{6 M}{h b^2} \quad M = F L \frac{a}{4}
\]

where \(F(a/b)\) is a geometric dependence factor, \(\sigma\) is the breaking stress, \(a\) is the length of the notch, \(b\) is the width of the sample, \(h\) is the edge of the sample, \(L\) is the distance between supports, \(M\) is the maximum moment generated from the forces and \(F\) is the maximum breaking force.

**Microstructural and fractographical analysis**

For the analysis of microstructures and the fracture surfaces of the studied materials, a Zeiss optical microscope (Germany) and a very high-resolution field emission scanning electron microscope (Auriga series, Zeiss, Germany) were used. To study the samples in the scanning electron microscope, the test tubes were fixed to a copper support with silver paint and were made conductive by deposition of a nanometric layer (about 26 nm) of carbon in a LEICA EM ACE600 metallizer (Switzerland).

**RESULTS AND DISCUSSION**

Given the high number of experimental results obtained and the interrelationship between them, this section will show the results for each of the characterisations carried out, followed by a brief discussion of these. Finally, from the microstructural and fractographic analysis of the materials, an integrative discussion will be made of the macroscopical results obtained concerning the deformation and fracture micromechanisms observed at the microscale. It is intended to establish a correlation between microstructure and properties that allows the optimisation of future materials.

On the other hand, it must be considered that in all the physical and mechanical characterisation that will be shown below, each experimental point corresponds to at least six measurements, and the error interval is the mean square error. In the thermal characterisation, only one measurement could be made for each experimental point.

**Physical characterisation**

Table 4 shows the variation of the density, \(\rho\), of each material, depending on the percentage and area of the added HRGO. Each displayed result shows the mean value and root mean square error of at least six. The error obtained in determining the density was, in almost all cases, less than 0.7 %. This has made it possible to detect minimal variations in density with the addition of HRGO.

Since the density of the graphene used is much lower than that of PMMA, it was expected that the density of the composite material would be reduced. Although the graphene addition rates only reach 1 %, using the rule of mixtures, the expected reduction due to this effect should be less than this percentage. On the other hand, the possibility of HGRO platelets acting as pore generation points suggests that this can also reduce the final density. In Table 4, an increase in the surface density of graphene tends to reduce the volumetric density of the compound, which would confirm the hypothesis that the greater the surface area of the HGRO, the more likely it is that nucleation of pores will occur around the platelets. The only exception is for G750-0.1, whose density is much lower than expected, and the relative root mean square error of the measurements is as high as 5 %. This result indicates that, probably, there was a problem during the synthesis of the material that resulted in more porosity than expected.

The reduction in density due to the weight percentage of graphene is so slight that it is within the measurements' error limits. Again, the most remarkable singularity is found in G750-0.1, which leads us to think that perhaps there was some small error during the processing of this material, which contributed to reducing the density in a more significant proportion than expected with the rest of the results.

Table 4. Materials' density versus the percentage and surface area of the added HRGO. Each result shows the mean value and root mean square error of at least six measurements.

| HRGO weight percentage (wt. %) | 120 | 300 | 500 | 750 |
|-------------------------------|-----|-----|-----|-----|
| 0,0 | 1,244 ± 0,002 | 1,240 ± 0,002 | 1,182 ± 0,007 |
| 0,1 | 1,240 ± 0,004 | 1,240 ± 0,002 | – | 1,182 ± 0,007 |
| 0,5 | – | 1,229 ± 0,009 | – | – |
| 1,0 | 1,241 ± 0,003 | 1,236 ± 0,003 | 1,230 ± 0,006 | 1,234 ± 0,004 |

**Thermal characterisation**

Table 5 shows the evolution of the glass transition temperature (\(T_g\)) depending on the percentage and surface area of the added HRGO. As it can be seen, especially in the case of \(T_g\), this parameter is not affected by the presence of HGRO, so it can be considered independent of the presence of HRGO, at least in the proportions in which it was used and long ageing times.

Table 5. Glass transition temperature (\(T_g\)) versus the percentage and surface area of the added HRGO.

| HRGO weight percentage (wt. %) | 120 | 300 | 500 | 750 |
|-------------------------------|-----|-----|-----|-----|
| 0,1 | 70.2 | 69.8 | – | 70.2 |
| 0,5 | – | 68.0 | – | – |
| 1,0 | 68.3 | 69.3 | 70.8 | 70.3 |
Something very different happens with the residual monomer, as can be seen in Table 7. A maximum of residual monomer is obtained with 300 m²/g and 0.5 wt. %, indicated in Figure 2. This maximum is in the middle of the studied values, and we can see a clear trend with a central maximum and a decrease in residual monomer by increasing or decreasing the wt. % or the specific area of HRGO. This leads us to think that two mechanisms facilitate MMA polymerisation for long ageing times. Graphene, especially HRGO, is known to act as an inhibitor of the radical initiator of the radical polymerisation of MMA, as it has a more significant amount of available double bonds compared to GO. Considering this, we can understand that by increasing the HRGO edge, either by increasing the wt. % or the specific area, there is more significant inhibition of the radicals initiating the reaction. Therefore, it is more unlikely that the MMA encounters a radical to polymerise and obtaining a higher residual MMA. Our results demonstrate this in the range of 0.1 to 0.5 wt. % and 120 to 300 m²/g, but this does not explain that when there is a high HRGO, 750 m²/g and 1.0 wt. %, the residual MMA decreases in the long term. For this exciting result that we obtained, we must consider the ease of MMA to diffuse within the polymer itself, this being simpler the fewer polymeric chains there are in the polymer' microstructure. The HRGO edge acts as an inhibitor of nucleation points, and the higher the HRGO and the more significant specific area, the greater the reactive border that inhibits the radicals. If a large part of these radicals were inhibited, we would have reached a situation where there would have been fewer polymer chains, allowing them to slip easily, allowing the MMA to reach one of the reactive points. Reducing the amount of residual monomer is relevant from a clinical perspective, as the potential toxicity caused by MMA is well known (incluir esta referencia: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3177373/).

In addition, the inhibition of radicals induces a slower polymerisation, thus reducing the rate of energy release and the escalation in temperature and increasing the curing time, something observed in previous studies by the group28. This effect would allow controlling, depending on the percentage and density of HRGO added, the clinical needs of curing time and local temperature production. It is interesting as it would make it possible to limit thermal damage to the surrounding tissues during surgery and to increase the working time of the material according to surgical needs.

Figure 2. Residual monomer (MMA) versus specific area and percentage (blue = 0.1 %, orange = 0.5 %, and grey = 1.0 %) by weight of the HRGO.

Table 7. Percentage of residual MMA in the materials, after one year, versus the percentage and surface area of the added HRGO.

| HRGO weight percentage (wt. %) | Surface area of graphene used (m²/g) | Residual MMA (%) |
|-------------------------------|-------------------------------------|------------------|
| 0,1                           | 120                                 | 0,67             |
|                               | 300                                 | 1,04             |
|                               | 500                                 | –                |
|                               | 750                                 | 0,92             |
| 0,5                           | 120                                 | –                |
|                               | 300                                 | 2,05             |
|                               | 500                                 | –                |
|                               | 750                                 | –                |
| 1,0                           | 120                                 | 1,05             |
|                               | 300                                 | 1,67             |
|                               | 500                                 | 0,50             |
|                               | 750                                 | 0,26             |

Mechanical characterisation
From the simple tensile tests, the stress-strain curves of these materials were obtained, which in all cases showed a macroscopically elastic and brittle behaviour until breaking. So that, it was possible to determine the tensile strength, $\sigma_t$, and the modulus of elasticity, $E$, which are shown in Tables 8 and 9, respectively. On the other hand, from the mechanical bending tests at three points on notched specimens, the fracture toughness, $K_{IC}$, was determined, which is shown in Table 10.

Table 8. Tensile strength, $\sigma_t$, of the material versus the percentage and surface area of the added HRGO. Each displayed result shows the mean value and root mean square error of at least six measurements.

| HRGO weight percentage (wt. %) | Surface area of the graphene used (m²/g) | $\sigma_t$ (MPa) |
|-------------------------------|------------------------------------------|-----------------|
| 0,0                           | 120                                      | 38 ± 2          |
|                               | 300                                      | 36 ± 4          |
|                               | 500                                      | –               |
|                               | 750                                      | 37 ± 4          |
| 0,1                           | 120                                      | 39 ± 7          |
|                               | 300                                      | 36 ± 4          |
|                               | 500                                      | –               |
|                               | 750                                      | –               |
| 0,5                           | 120                                      | –               |
|                               | 300                                      | 31 ± 5          |
|                               | 500                                      | –               |
|                               | 750                                      | –               |
| 1,0                           | 120                                      | 30 ± 5          |
|                               | 300                                      | 29 ± 3          |
|                               | 500                                      | 29 ± 5          |
|                               | 750                                      | 20 ± 1          |
Table 9. Modulus of elasticity, E, of the material versus the percentage and surface area of the added HRGO. Each displayed result shows the mean value and root mean square error of at least six measurements.

| Surface area of the graphene used (m²/g) | HRGO weight percentage (wt. %) | 120   | 300   | 500   | 750   |
|----------------------------------------|-------------------------------|-------|-------|-------|-------|
| 0,0                                    | 3.2 ± 0.02                    |       |       |       |       |
| 0,1                                    | 3.2 ± 0.6                     | 3.2 ± 0.2 | –     | 3.0 ± 0.2 |
| 0,5                                    | –                             | 2.8 ± 0.3 | –     | –     |
| 1,0                                    | 3.1 ± 0.1                     | 2.8 ± 0.3 | 2.3 ± 0.2 | 2.2 ± 0.3 |

Table 10. Fracture toughness, KIC, of the material versus the percentage and surface area of the added HRGO. Each displayed result shows the mean value and root mean square error of at least six measurements.

| Surface area of the graphene used (m²/g) | HRGO weight percentage (wt. %) | 120   | 300   | 500   | 750   |
|----------------------------------------|-------------------------------|-------|-------|-------|-------|
| 0,0                                    | 1.28 ± 0.02                   |       |       |       |       |
| 0,1                                    | 1.40 ± 0.02                   | 1.20 ± 0.05 | –     | 1.15 ± 0.05 |
| 0,5                                    | –                             | 1.12 ± 0.02 | –     | –     |
| 1,0                                    | 1.08 ± 0.03                   | 0.99 ± 0.04 | 1.06 ± 0.05 | 0.94 ± 0.06 |

In all these experimental results, the incorporation of HRGO, both in percentage and surface density, degrade all the analysed mechanical properties. HRGO platelets degrade the material, probably due to a decrease in its internal cohesion around them. It would justify that the resistance capacity of the compound is reduced very significantly, even in tiny percentages, for the three analysed parameters. This phenomenon could become an inconvenience for the clinical application of these materials. It is well known that fracture toughness is essential for fatigue life. However, it is essential to understand how it affects the different factors of graphene, such as the specific area, which has never been analysed until now. We have shown that it plays a critical role in the final properties of the cement. On the other hand, the fact that the mechanical properties of the bone cement are inferior to those of the bone and the prosthesis guarantees that, in case of overload, the breakage is produced by this element, which is the most easily replaceable.

Microstructural and fractographic characterisation

The microstructural and fractographic analysis of the studied materials showed that, in some cases, large pores had been trapped inside the material, as can be seen in Fig. 3. In the same image, it can be appreciated that the fracture surface is relatively flat. The former may explain the abnormally low-density values of G750-0.1, while the latter is compatible with the brittle elastic behaviour observed macroscopically for these materials. Nevertheless, Figures 4 and 5 show the typical porosity distribution, usually micrometric pores or smaller.

Figure 3. SEM image of the tensile fracture surface of G120-0.1. A giant air bubble can be seen trapped within the bone cement.
Figures 4 and 5. Microstructure of G120-0.1. The barium sulphate radiopacifier appears as a uniformly dispersed white contrast in the PMMA matrix.

During detailed analysis of the fracture surfaces, poor adhesion of HRGO to bone cement (Figures 6a-6b and 7a-7b) and the existence of unwanted agglomerates of HRGO (Figures 8) were observed. Both factors lead to the creation of cracks and defects around the HRGO platelets.

A more significant number of cracks increases the likelihood of any of them reaching the critical crack size and propagating catastrophically under less stress than the expected critical value. For example, Figure 8 shows a HRGO lamella utterly parallel to the fracture surface. The gap between the bone cement and that HRGO lamella, or between HRGO lamellae, could have been a place where crack propagation started in this case. Consequently, both factors indicate that the presence of HRGO in the PMMA matrix justifies the macroscopic degradation of the mechanical behaviour of the composite observed in the previous section.

Figures 6a y 6b. G120-0.1 fracture surface. The arrows indicate dislocations between the bone cement and the HRGO due to poor adhesion between the two.
CONCLUSIONS

From the experimental observations shown above and their discussion, the following conclusions can be succinctly established:

1. After one year of natural ageing, density and glass transition temperature are not affected by the addition of different percentages and surface densities of HGRO.
2. The amount of residual MMA varies with the amount of HRGO and its specific area. This phenomenon is related to reducing the polymerisation rate in the presence of HRGO and the MMA diffusivity inside the PMMA. It would allow controlling the curing time and the local temperature increases, which would help limit the thermal damage caused to surrounding tissues during surgery and increase the working time of the material if necessary.
3. Due to the addition of different percentages and surface densities of HGRO, the analysed mechanical properties suffer an intense degradation, exceeding 40%.
4. To improve the mechanical behaviour of these compounds, it is essential to find micromechanisms that increase the cohesion between HRGO and the PMMA matrix, like the silanization of graphene.
5. Looking ahead, it would be interesting to extend this study to lower concentrations of HGRO, consider the influence of HGRO on the bacterial colonisation of cement, analyse the evolution of the physical, thermal, and mechanical behaviour of bone cement with graphene after its controlled immersion in physiological serum, and study the influence on antibiotic release when added to bone cement.

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Intersante y sorprendente!!!!