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Analysis of the influence of gentamicin sulfate and cooling technique on the mechanical properties of Poly (methyl methacrylate)

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ABSTRACT
Poly (methyl methacrylate) is a polymer widely used in various biomedical applications. In orthopedics, this polymer may be used as a structural reinforcement in fracture, aiding bone regeneration. To avoid fracture contamination, surgeons may add a certain homogeneously amount of antimicrobial to the Poly (methyl methacrylate). To prevent thermal necrosis during polymerization, which can reach up to 100° C, cooling techniques are used, to ensure the cells integrity during the process. Despite the great importance of Poly (methyl methacrylate) as a biomaterial, there is still a lack of information on the mechanical properties of this polymer, especially when there is modifications on Poly (methyl methacrylate) composition and its preparation temperature. This work aims to evaluate the influence of the addition of Gentamicin Sulfate and application of cooling technique on the mechanical behavior of Poly (methyl methacrylate) in three commercial types. For this, the mechanical tests of compression, bending and torsion were performed. With these tests it was found that, despite the reduction of the mechanical resistance of Poly (methyl methacrylate) with the cooling technique and antimicrobial addition, the polymer can still be safely used as bone cement.

Keywords: Antimicrobial, Biomedicine, Mechanical Tests, Orthopedics.

1 INTRODUCTION

Poly(methyl methacrylate) is one of the materials of great importance in the polymer industry, recognized as the most used acrylic commercially available, because it presents high mechanical strength and dimensional stability, with high modulus of elasticity at room temperature, low deformation until there is rupture, in addition to high abrasion resistance (COCCO *et al*., 2009). Poly(methyl methacrylate) is a biomaterial that has been used in orthopedics since 1940 (MENDES, 2006), in applications such as bone cementation, which consists of the use of the polymer to fill the space between the prosthesis and the host bone, conferring greater mechanical resistance to the bone (GIORDANO *et al*., 2007), as well as the manufacture of oral prostheses developed to protect the dental pulp, reestablishment of masticatory functions and oral aesthetics (MAZARO *et al*., 2015). In this context, it is important to select a material with coloration similar to the natural conditions of the oral mucosa. Of course, Poly(methyl methacrylate) is colorless. Depending on the application, it is possible to add biocompatible pigments, making the prosthesis made gain an appearance similar to the natural organ, providing better aesthetics and greater satisfaction for the patient (SHIBAYAMA *et al*., 2016).

It is also possible to use antimicrobial along with the polymer, forming a homogeneous mixture, in order to facilitate healing and prevent the proliferation of bacteria in the region where the prosthesis was applied, conferring greater effectiveness in the treatment (AZI *et al*., 2010).

The polymerization reaction of Poly(methyl methacrylate) is always exothermic. The energy released is approximately 130 cal/g of monomer, and can reach a temperature of up to 100 °C, until the mass of the polymer passes completely to the solid state, with greater rigidity (NELSON *et al*., 1986). When poly(methyl methacrylate) is applied to living beings, greater caution is required, because the exothermic reaction of the polymer can be lethal to various types of cells of organisms, such as gonadal cells, embryonic, blood, cartilaginous, carcinomas, among others (LEESON *et al*., 1993). In this case, it is necessary to adopt cooling techniques to reduce the maximum temperature from the polymerization reaction and avoid this side effect.

Despite the vast use of poly(methyl methacrylate) for the manufacture of prostheses, with association with antimicrobials and cooling techniques, a deeper analysis of changes in the mechanical properties of the material when applying these techniques is necessary. The purpose of this work is to perform compression, bending and torsion tests with specimens of said polymer, from the same manufacturer, marketed in types of commercial nomenclature, according to the color of the product, and that will give rise to Poly (methyl methacrylate), with and without the addition of antimicrobial and temperature decrease of the substance throughout the process, and thus, to verify if these actions significantly alter the mechanical properties of the material.

2 MATERIALS AND METHODS

The preparation of the specimens and the performance of the mechanical tests were done in the Materials Testing Laboratory, linked to the Collegiate of Mechanical Engineering of the Federal University of the São Francisco Valley (UNIVASF), in Juazeiro-BA, at a temperature of 25°C and Relative Humidity of the air equal to 60%, following the guidelines of item 7.1 of the ASTM F451-86 standard, which deals with the standardization of the preparation of acrylic bone cement. The standard specifies that the experiment be carried out at a temperature between 21°C and 25°C and Relative Humidity between 40% and 60%.

For the accomplishment of this work, the self-polymerizable acrylic resin (Polymethylmethacrylate) was used, commercialized in polyethylene containers, with the nomenclature "Vipiflash", manufactured by VIPI Indústria, Comércio, Exportação e importação de produtos odontológicos LTDA. Manufactured by the same company, polymethylmethacrylate was used with commercial nomenclatures "Colorless" (without pigmentation), "Pink" and "Black" (with biocompatible pigmentation, added by the manufacturer itself). We also used the thermopolymerizable acrylic liquid (methylmethacrylate), supplied by the same manufacturer, marketed in a glass container.

The antimicrobial used was Gentamicine Sulfate, supplied by Interlab distributor of scientific products LTDA. For the weighing of the substances added in the preparation of the specimens, the Shimadzu ATX 224 analytical balance was used, in addition to the Instrutemp ITCD-2000 digital chronometer to measure the time of initiation of the exothermic reaction and total time of reestablishment of temperature of the polymer, and to measure the temperature of the specimens, a laser thermometer was used, model MT-320A, from Minipa. The Laboratory also has a hygrometer, used to measure the temperature and relative humidity of the ambient air. Wood molds were used, specific for each specimen format, according to the mechanical test performed, which received a thin layer of Vaseline, to facilitate the removal of the specimens after the polymerization process.

2.1 PREPARATION OF THE SPECIMENS

To prepare the specimens to be used in the mechanical tests, 33.33% of mass of liquid methylmethacrylate and 66.67% of mass of Poly(methyl methacrylate) powder were added, following a ratio of 2:1 of the polymer in relation to the monomer, as recommended by Vipiflash (2022). For this work, specimens were made for the Compression, Flexion and Torsion tests, using the Poly(methyl methacrylate) marketed in the colors: Colorless, Pink and Black.

The cooling technique consists of the preparation of Poly (methyl methacrylate) at a temperature of 25 ° C, applying this in wooden molds with a temperature of 10 ° C still in the pasty phase. After applied in the molds, the polymer is kept in a refrigerated environment, remaining with an approximate temperature of 15 °C, increasing this temperature only with the occurrence of the exothermic reaction from the polymerization, still in a refrigerated environment. The specimens were removed after this reaction, when they again reached a temperature of 15 °C. The dosage of the antimicrobial Gentamicin Sulfate powder was 1g for each 40g of Poly(methyl methacrylate), according to an experiment carried out by Chang *et al*. (2013), DePuy Synthes (2016), and Dunne *et al.* (2008).

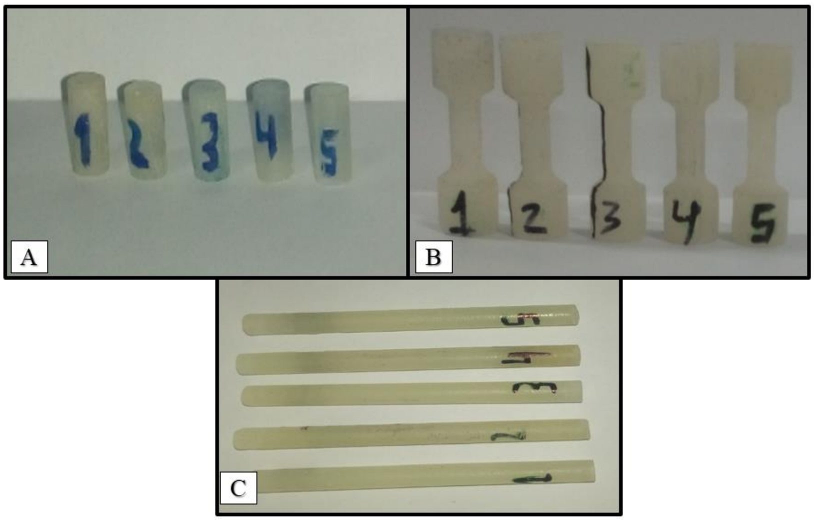
For each mechanical assay used (Compression, Bending and Torsion), five specimens of Poly (methyl methacrylate) were prepared for each classification, as shown in Table 1 and Figure 1, using the three commercial nomenclatures (Colorless, Pink and Black), as well as the application of antimicrobial and use of the cooling technique, as shown in Figure 2, following the guidelines of item E5 of Annex E of ISO 5833:1992, which deals with the standardization of the preparation of acrylic bone cement to be applied in implants for surgery, totaling 180 specimens.

TABLE 1. Types of poly(methyl methacrylate) specimens according to antimicrobial mass and temperature.

Classification of Polymer Specimens	**Gentamicin Sulfate Mass**	**Total polymer mass**	**Mold temperature**	**Temperature of the polymer in the mold**	**Number of specimens**
No cooling/ No antimicrobial	0g	60 g	25°C	25°C	45
With cooling/ No antimicrobial	0 g	60 g	10°C	15°C	45
No cooling/ With antimicrobial	1 g	61 g	25°C	25°C	45
With cooling/ With antimicrobial	1 g	61 g	10°C	15°C	45

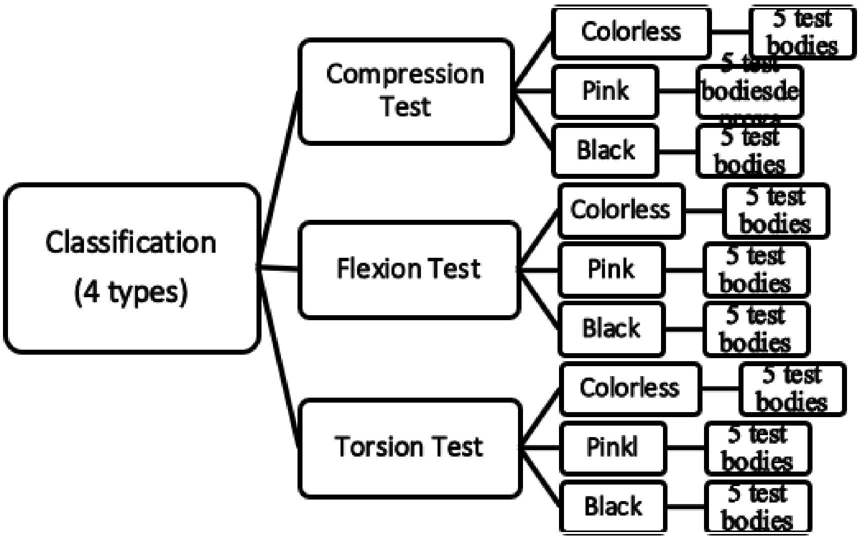
Source: Self-authored (2022).

FIGURE 1. Sample of specimens of Poly(methyl methacrylate) used in the Compression (A), Torsion (B) and Bending (C) tests.



Source: Self-authored (2022).

FIGURE 2. Hierarchical schematization and number of specimens prepared for each classification according to mechanical test and trade name.



Source: Self-authored (2022).

The homogenized polymer, still in the pasty phase, was placed in wooden molds, which have a diameter and length equal to the specimens respective to the mechanical tests. The specimens for the compression tests have a diameter of 6 mm and a length of 12 mm, as specified by item 7.9 of ASTM F451-86 and item E. 4.7 of Annex E of ISO 5833:1992. The specimens for the bending tests have a length of 70 mm and a diameter of 6 mm. The specimens for the torsion tests have a diameter of 6 mm and a length of 12 mm in the central part, a total length of 24 mm and a diameter of 12 mm at the ends, where the mounting brackets of the specimen are installed. The smaller diameter in the center of the specimen allows the rupture to happen first in this region, preventing the fixation on the ends from damaging the specimen for the test.

After at least 60 minutes after the application of the homogeneous mixture of the polymer in the molds without cooling, the specimens were removed from the molds, as suggested by item E 4.5 and E 4.6 of Annex E of ISO 5833:1992, and after 24 hours, they were subjected to final polishing with silicon carbide sandpaper and water, with the aid of a precision square, to ensure perfect alignment between the surfaces of the ends. All the cooling specimens were only removed from the molds after three times the time established for removal of the mold specimens and final polishing defined by ISO 5833:1992, since Santos (2011) mentions that the decrease in the temperature of the acrylic increases the time for polymerization. After completion, the specimens were submitted to mechanical tests.

2.2 EXECUTION OF MECHANICAL TESTS

To perform the mechanical tests, the Universal Assay Machine EMIC DL 10000 was used, according to the classification and hierarchical schematization, shown in table 1 and Figure 2, respectively.

In the Compression Tests, the specimens were subjected to a test speed equal to 20 mm/min, as suggested in item E 4.9 of Annex E of ISO 5833:1992 and item 7.9.2 of ASTM F451-86, using a load cell with a maximum capacity of 5 kN and preload of at least 49 N, as suggested by items E 2.6 of Annex E of ISO 5833:1992 and item 7.8.2.1 of ASTM F451-86, respectively. The specimens were placed between two cylindrical plates, which approach each other due to the movement of the universal machine, keeping the contact surface flat between the specimens.

In the Bending Tests, the specimens were bi-supported in support, with a distance between the ends of 60 mm, and were submitted to a test speed equal to 5 mm/min, using a load cell with a maximum capacity of 5 kN and preload of at least 30 N, meeting the specifications in Annex F of ISO 5833:1992. To perform the Torsion Tests, it was necessary to use the device designed by Cardoso (2016), coupled to the Universal Testing Machine, as specified by the author. The assay was performed with a velocity equal to 0.09 rad/min, according to the assay performed by Berto *et al*. (2012), Load cell with a maximum capacity of 5 kN and preload of at least 0.21N.m.

3 RESULTS

After performing the mechanical tests, the Tesc® software was used for data collection and the Microsoft Office Excel® software to calculate the desired mechanical properties.

With the results obtained, it was possible to obtain the maximum rupture stress and the Modulus of Elasticity in the Compression and Flexion Test, through Equation (1) and Equation (3), respectively, having the results shown in Figure 3, Figure 4, Figure 5 and Figure 6.

$$\sigma=F/A \tag{1}$$

$$\varepsilon=\delta/L \tag{2}$$

$$E=s/e \tag{3}$$

Where:

σ- Rupture Tension (Pa);

F- Force (N);

A- Cross Section Area (m^2);

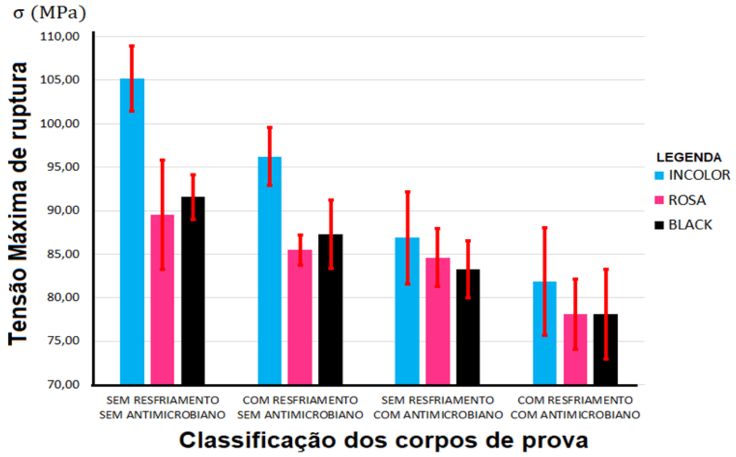
ε- Normal Specific Deformation (dimensionless);

E- Modulus of Elasticity or Young's Modulus (MPa);

δ-Deformation per unit length (m);

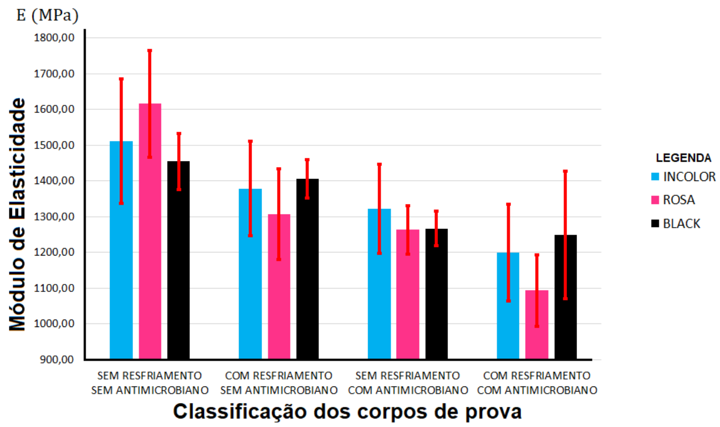
L- Length of specimen (m).

FIGURE 3. Maximum rupture stress of Poly(methyl methacrylate) in Compression Tests.



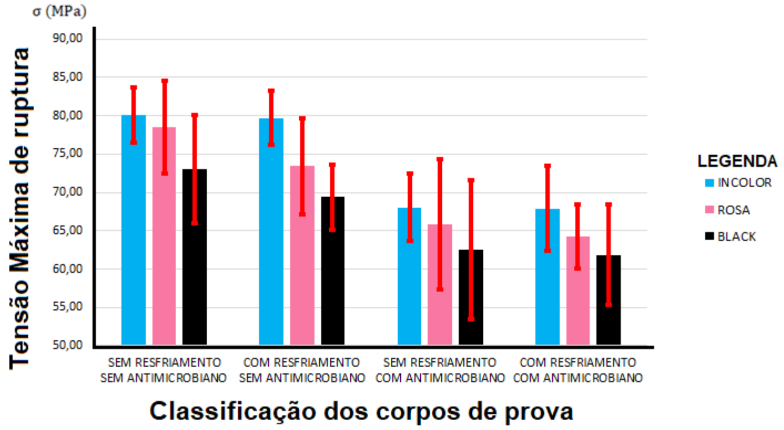
Source: Self-authored (2022).

FIGURE 4. Modulus of Elasticity of Poly(methyl methacrylate) in Compression Tests.



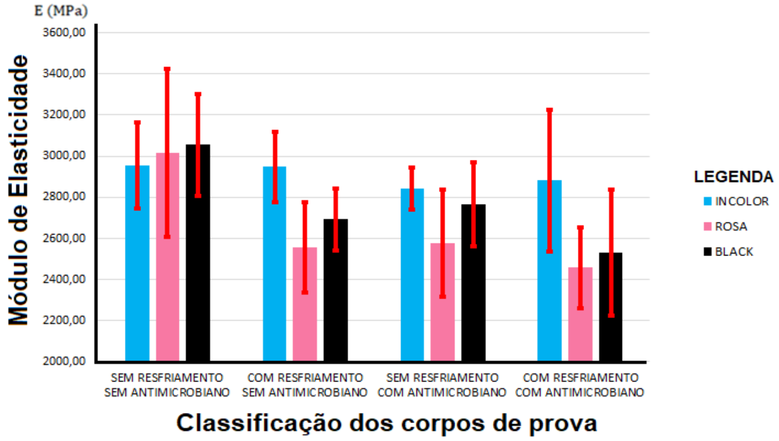
Source: Self-authored (2022).

FIGURE 5. Normal Rupture Stress of Poly(methyl methacrylate) in Flexion Tests.



Source: Self-authored (2022).

FIGURE 6. Modulus of Elasticity of Poly(methyl methacrylate) in Bending Tests.



Source: Self-authored (2022).

With the results obtained in the Torsion Test, it is possible to obtain the Shear Stress by Equation (4) and the Transverse Modulus of Elasticity (Torsional) by Equation (5), respectively, having the results shown in Figure 7 and Figure 8.

$$\tau = (M \times c)/I \quad (4)$$

$$G = t/c \quad (5)$$

Where:

τ- Shear Stress (Pa);

M- Torsor Moment (N.m);

c- External Radius of the Axis (m);

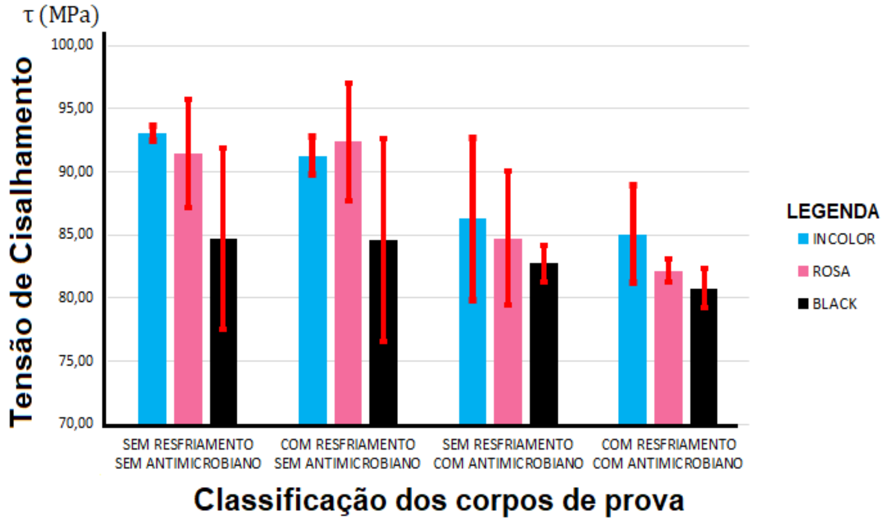
I - Moment of Inertia ().m^4

G- Transverse Modulus of Elasticity (Pa);

τ- Shear Stress (Pa);

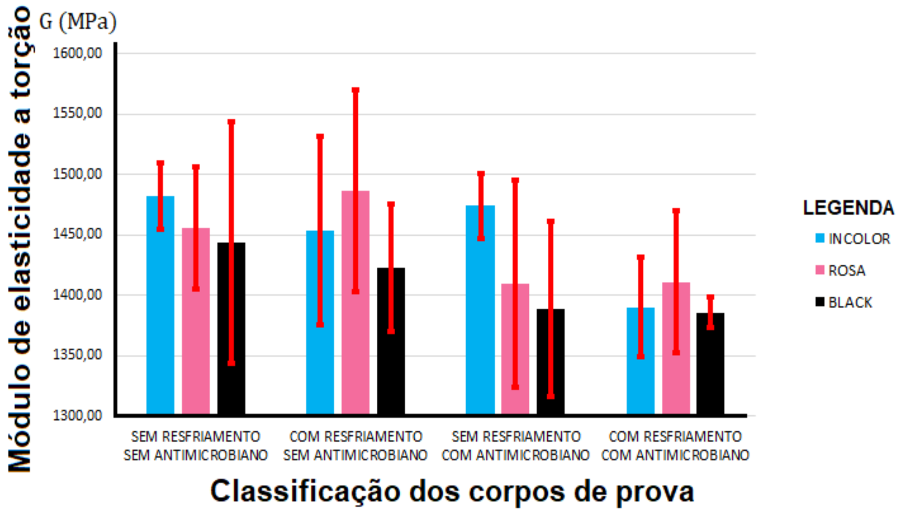
γ – Shear Deformation (rad).

FIGURE 7. Maximum Shear Stress of Poly(methyl methacrylate) in Torsion Tests.



Source: Self-authored (2022).

FIGURE 8. Modulus of Elasticity of Poly(methyl methacrylate) in Torsion Tests.



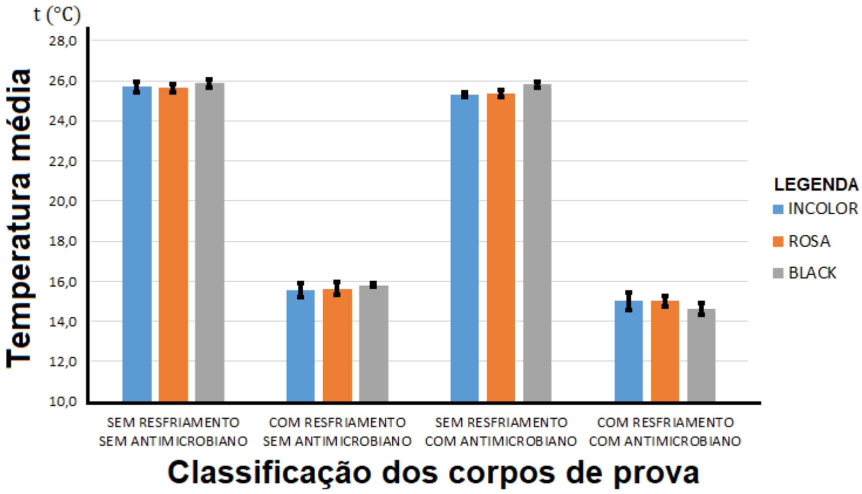
Source: Self-authored (2022).

3.1 TEMPERATURES AND PREPARATION TIME OF POLY(METHYL METHACRYLATE)

In view of the impact that the exothermic polymerization reaction of Poly(methyl methacrylate) can cause cell death (thermal necrosis), the temperatures of the specimens were collected before the beginning of the exothermic reaction and the maximum temperature reached throughout the process, in addition to the time for the beginning of the exothermic reaction and the total time for the specimen to reach the temperature of the beginning of the process. Thus, it was possible to make an analysis of the impact that the cooling technique and the addition of antimicrobial can cause in the preparation time and if the cooling technique is effective to avoid thermal necrosis.

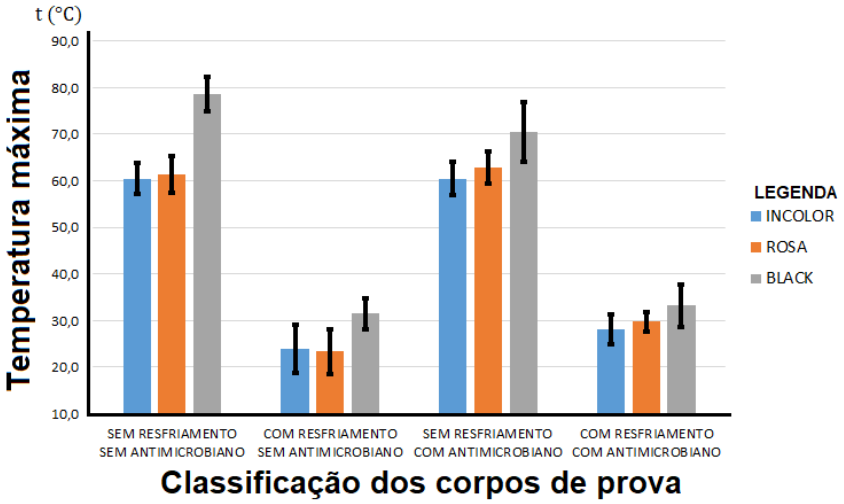
The average temperature of the poly(methyl methacrylate) before the exothermic reaction and the maximum temperature of the polymer reached in the process were measured with the use of a laser thermometer throughout the process, and the results obtained are shown in Figure 9 and Figure 10. During all processes, it is possible to perceive the stability of temperatures before the start of the exothermic reaction, with low standard deviations. However, the same does not happen after the beginning of the exothermic reaction, which causes the temperature to increase precipitously, especially in the specimens without the cooling technique. The highest temperatures, in all situations, were reached by Poly (methyl methacrylate) with commercial nomenclature (coloration) "Black", which reached a temperature higher than 80 °C in some prepared batches. The standard deviation of the time measurements increased precipitously, due to the oscillation in the reading of these temperatures in the different batches.

FIGURE 9. Average temperature of Poly(methyl methacrylate) before the start of the exothermic reaction.



Source: Self-authored (2022).

FIGURE 10. Maximum Temperature of Poly(methyl methacrylate) during the exothermic reaction.

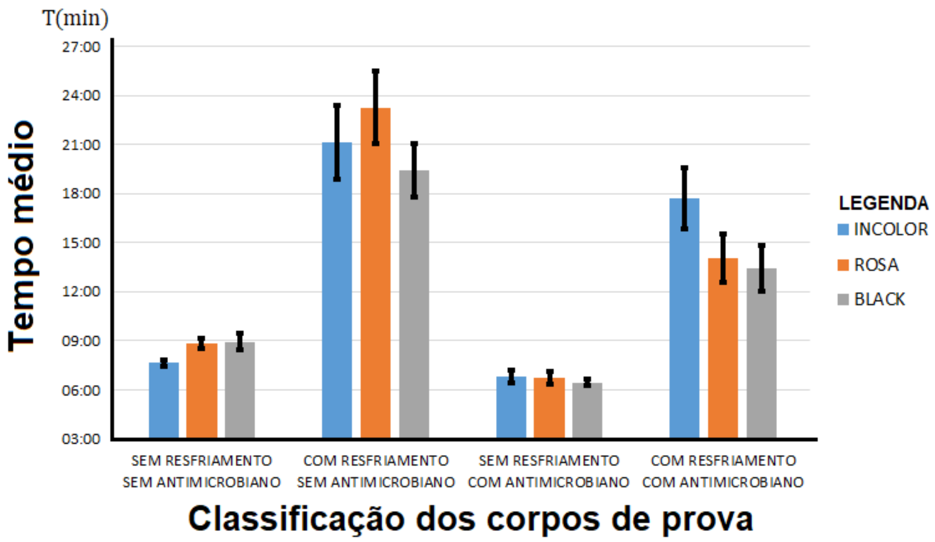


Source: Self-authored (2022).

In all situations, the addition of antimicrobials slightly increased the maximum temperature during the exothermic reaction. However, it was noticed that the cooling technique considerably decreased the maximum temperature reached during the process. Boner *et al*. (2009) states that the minimum temperature for the occurrence of thermal necrosis is 45 °C. In all situations, the Poly(methyl methacrylate) in the commercial nomenclatures (colorless) and "Pink" would be the safest for applications in living beings, considering that in all specimens of both stains did not reach a temperature higher than 35 °C, while there is a greater risk of necrosis when using the Poly(methyl methacrylate) of commercial nomenclature (staining) "Black", which reached higher temperatures during the exothermic reaction, but below 40 °C.

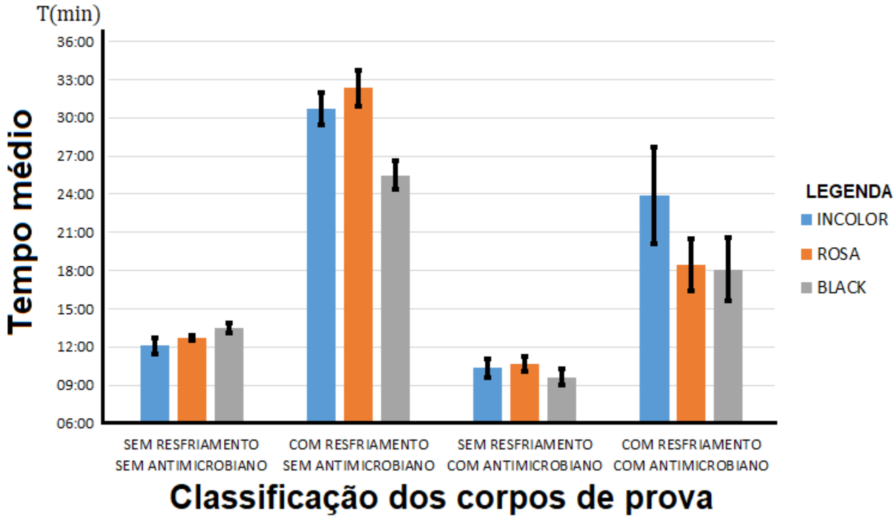
The addition of antimicrobial moderately reduced the time to start the exothermic reaction in Poly(methyl methacrylate) in all situations, especially for the commercial nomenclatures (stains) "Rosa" and "Black". However, the cooling technique influenced much more significantly. The reduction in temperature before the start of the exothermic reaction by 10 °C was responsible for an increase of more than 150% in the time of initiation of the exothermic reaction and the time for the temperature to reduce to the temperature of the beginning of the process soon after the exothermic reaction, as can be seen in Figure 11 and Figure 12, respectively.

FIGURE 11. Time to start the exothermic reaction of Poly(methyl methacrylate).



Source: Self-authored (2022).

FIGURE 12. Total polymerization time of Poly(methyl methacrylate).



Source: Self-authored (2022).

4 CONCLUSIONS

With the accomplishment of this work, it was possible to determine the mechanical properties of Poly(methyl methacrylate) in the Compression, Flexion and Torsion assays, marketed by Vipiflash (2022) in three types of commercial nomenclatures (Colorless, Pink and Black), these being submitted to the technique of cooling and/or addition of antimicrobial.

The cooling technique applied to Poly(methyl methacrylate) proved to be effective to prevent thermal necrosis, since with this technique, the polymer did not exceed the maximum temperature of 40°C for the commercial nomenclature (coloration) "Black" and 35°C for the commercial nomenclatures (stains) "Pink" and "Colorless" during the exothermic polymerization reaction, while the temperature for the onset of thermal necrosis is 45°C, ensuring safety for the application of the polymer in living beings, with greater safety when using Poly (methyl methacrylate) in the commercial nomenclatures (stains) "Pink" or "Colorless".

Despite the effectiveness of the cooling technique in preventing the occurrence of thermal necrosis during the exothermic reaction of Poly(methyl methacrylate), the application of this technique causes a reduction in the resistance to rupture and lower modulus of elasticity of the material in all mechanical tests. This happens because the polymerization reaction, responsible for the formation of the material, is facilitated by the increase in temperature, and the opposite effect is expected when reducing the temperature, which explains the fact that Poly(methyl methacrylate) reduces its mechanical resistance when applying the cooling technique. However, this does not prevent the use of this technique in the polymer in biomedical applications, since the criteria of resistance to rupture and modulus of elasticity meet all the requirements of ISO 5833:1992, which standardizes the requirements for the preparation of bone cements, and cooling is extremely beneficial to the biomedical implant, by preventing thermal necrosis.

The Poly(methyl methacrylate) marketed by Vipiflash (2022) with commercial nomenclatures "Rosa" and "Black" have lower resistance to rupture and lower modulus of elasticity than the colorless polymer (pure), since substances that do not chemically interact with the polymer hinder the formation of macromolecules. However, in terms of mechanical strength, this reduction in the strength of the material does not prevent its use in biomedical applications, since the criteria of resistance to rupture and modulus of elasticity meet all the requirements of ISO 5833:1992, which standardizes the requirements for the preparation of bone cements.

The addition of 1.64% by mass of the antimicrobial Gentamicin Sulfate to Poly(methyl methacrylate) causes a decrease in the maximum resistance to rupture and a decrease in the modulus of elasticity of the material, which is a consequence of the addition of a non-polymer to the material. The antimicrobial does not chemically interact with Poly(methyl methacrylate), and does not modify its crystal structure. Nevertheless, this reduction in the strength of the material does not prevent its use in biomedical applications, since the criteria of resistance to rupture and modulus of elasticity meet the requirements established by the ISO 5833:1992 standard, which standardizes the requirements for the preparation of bone cements.

The drop in ambient temperature from 25 °C to 15 °C was sufficient to increase the time to start the exothermic reaction and total preparation time of Poly (methyl methacrylate) by more than 150%, due to the greater difficulty that the polymer has for the formation of its macromolecule, which is favored with the increase in temperature.

LIABILITY STATEMENT

The authors are solely responsible for this work.

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