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Atomic Force Microscopy analyses of Polycaprolactone/Alendronate systems intended to produce biomaterials

Análise de Microscopia de Força Atômica de sistemas Policaprolactona/Alendronato destinados à produção de biomateriais

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ABSTRACT

The development of biomaterials such as scaffolds, films, membranes and others has attracted attention. Polymeric materials play an essential role in producing biomaterials for bone tissue regeneration, however, selecting materials within this context is still a significant challenge, especially for osteoporotic bones. Composite powders based on polycaprolactone (PCL) and sodium alendronate bisphosphonate (ALE) were recently applied to biomaterials development by solvent casting, compression molding, additive manufacturing, and other techniques. ALE is widely used in osteoporosis treatment and presents some side effects when administered orally or intravenously. Combining ALE with polymers to produce controlled drug-release systems is a promising alternative for mitigating these side effects. Hence, this work aims to evaluate, by atomic force microscopy, the morphology of films obtained using the system in powder form (PCL/ALE). Thus, films were produced by solvent casting using chloroform and PCL_ALE (0.2 g of ALE: 1.5 g of PCL). Compression molding was also applied to have films using PCL/ALE (2.0 MPa; 100 °C). The films were characterized using SEM and AFM analysis. The AFM analysis showed that the technique affected the films' morphology and may be an important tool for selecting appropriate production methods for future applications in bone regeneration.

KEYWORDS: Osteoporosis, Bisphosphonate, PCL/sodium alendronate.

RESUMO

Os materiais poliméricos desempenham um papel essencial na produção de biomateriais para a regeneração do tecido ósseo, onde a seleção de materiais ainda é um grande desafio, principalmente para tecidos osteoporóticos. Pós compostos de policaprolactona (PCL) e bisfosfonato de alendronato de sódio (ALE) foram aplicados ao desenvolvimento de biomateriais por solvente *casting*, moldagem por compressão, manufatura aditiva e outras técnicas. O ALE é amplamente utilizada no tratamento da osteoporose e apresenta efeitos colaterais quando administrada por via oral ou endovenosa. Combinar ALE com polímeros para produzir sistemas de liberação controlada de fármacos é uma alternativa promissora para mitigar esses efeitos colaterais. Assim, este trabalho visa avaliar, por microscopia de força atômica, a morfologia de filmes obtidos pelo sistema na forma de pó (PCL/ALE). Desta forma, os filmes foram produzidos por *solvent casting* usando clorofórmio e PCL_ALE (0,2 g de ALE: 1,5 g de PCL). A moldagem por compressão também foi aplicada para obter filmes usando PCL/ALE (2,0 MPa; 100 °C). Os filmes foram caracterizados usando análises de SEM e AFM. A análise AFM mostrou que a técnica afetou a morfologia dos filmes e pode ser uma ferramenta importante para selecionar métodos de produção adequados para futuras aplicações em regeneração óssea.

PALAVRAS-CHAVE: Osteoporose, Bifosfonatos, PCL/alendronato de sódio.

INTRODUCTION

Osteoporosis is characterized by the destruction of bone microarchitecture, loss of mass, decrease in bone strength, and increase in the risk of fractures[1]. The number of people that suffer from osteoporosis increases as the population ages. In Brazil, the Unified Health System's expenses on treatment and assistance are high, and this disease affects around ten million people[2].

Most osteoporosis treatment involves using bisphosphonates such as sodium alendronate (ALE)[3]. Bisphosphonates inhibit osteoclast activity. However, the use of ALE can cause collateral effects in this drug's oral and intravenous administration. Among them, it is possible to mention: irritation of the esophagus, fevers, ocular inflammation, and mandibular osteonecrosis[4]. The application of drug delivery systems can be an excellent alternative to reduce these effects. Local drug delivery can improve the patient's quality of life since the treatment with a bisphosphonate is usually long[4,5].

Despite the evolution in developing new materials, there are still no clinically approved and specifically adapted biomaterials for applications in osteoporotic bones. Polymeric materials play an essential role in biomaterial production for bone tissue regeneration. Thus, combining the appropriate polymer with drugs is essential to obtain a proper drug delivery system.

In recent years, Polycaprolactone (PCL), a biocompatible polymer, has been widely studied. It is because this polymer can be molded by various techniques for the production of biomaterials, such as electrospinning, solvent casting, compression molding, and also in additive manufacturing techniques, including the selective laser sintering (SLS) method[6,7].

It is commonly found in literature materials molded in different forms. However, finding pre-defined compositions applied to biomaterial product is not. Silva et al. (2021)[8] produced PCL/Alendronate systems to produce biomaterials (PCL_ALE_s). The authors prepared biomaterials in the form of films. The developed system (PCL_ALE_s) is so interesting since that can be used as a supplier for different techniques, such as the additive manufacturing technique of SLS, compression molding, and solvent casting, potentially after that assist in the treatment of osteoporosis, promoting the local release.

A scanning electron microscope was applied to investigate the surface of the biomaterials (in the form of a film) prepared using (PCL_ALE_s). However, according to the authors, identifying PCL in films was difficult. The present work aims to complement the study of Silva et al. (2021)[8] to understand better film morphology using Atomic Force Microscopy (AFM).

AFM technique represents a handy tool for investigating surfaces. One of the advantages of using AFM is that this technique yields direct spatial mapping of surfaces with a nanometre resolution[9]. AFM was used by Posodowska et al. (2015)[10] to evaluate an injectable nanoparticle-loaded hydrogel system for the local delivery of sodium alendronate. Moreover, AFM was also applied by Iles et al. (2021)[11] that studied alendronate sodium-polymeric nanoparticles.

In the present work, AFM images of topography and contrast phase showed that the ALE availability on the surface of the film produced using PCL_ALE_s depends on the applied technique, even though the starting material is the same.

MATERIALS AND METHODS

PCL (Capa 6500, average molecular weight of 50,000 g/mol) was kindly donated by Perstorp, and ALE trihydrate was supplied by Farmácia Alternativa (Polydrug Laboratories Pvt. Ltd.). Acetone was obtained from Merck (ACS, ISO, Reag. PhEur Merck). Chloroform purchased by (VETEC, Ltda.).

PCL/Alendronate systems intended to produce biomaterials

The PCL/Alendronate systems (PCL_ALE_s) consist of bisphosphonate particles coated with PCL. PCL_ALE_s. This system obtention was previously described in Silva et al. (2021)[8] and involves the preparation of PCL solution in acetone (20% w/v) under magnetic stirring of 900 rpm. The solution is dripping to ALE particles, previously frozen at -5 °C. Based on Silva et al. (2021)[8], the proportion used was 1.5g of ALE: 1 mL of PCL solution. The mixture of ALE and PCL was kept at room temperature for 24 h to evaporate the solvent and obtain the coated powder.

Production of biomaterials using PCL/Alendronate systems

The films were produced by solvent casting and compression modeling as described in Silva et al. (2021)[8], using the PCL/Alendronate systems (PCL_ALE_s), produced as suppliers for the production of biomaterials.

Films production by compression modeling

The compression molding occurred at 100 °C for 90 s and 2MPa in a press (Starmaker SM-15, 200 W of Power). The films were named according to the applied technique. Thus, CM_PCL_ALE are films produced by compression molding.

Films production by solvent casting

In the solvent casting technique, 5ml of acetone solubilized 1g of the coated PCL_ALE_s.

After the powder solubilization, under magnetic stirring of 900 rpm for 60 min, the solution was kept at room temperature for 24h for solvent evaporation. The sample was named as SC_PCL_ALE.

Atomic Force Microscopy (AFM)

AFM analyses were performed to evaluate the PCL_ALE_s, and the obtained products were carried out in Atomic Force Microscopy (JPK Instruments, model Nanowizard). The images were obtained in intermittent contact using a Micromasch NSCT-20 cantilever with a nominal spring constant of 7.4 N/m. The surface roughness of the samples was also determined using this equipment and was expressed as square surface roughness (SQR).

Scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS)

SEM and EDS evaluated the morphology and composition of the sample. For SEM analyses, all samples were covered with a thin layer of gold by vacuum spraying (25 mA for 4 minutes) and observed on the SEM with an acceleration voltage of 20 kV. It used two electron microscope detectors, an Everhart-Thornley detector (ETD) for secondary electrons and a back-scatter detector (BSED). EDS analysis was also carried out to investigate the chemical composition of the films[8].

RESULTS AND DISCUSSION

PCL/Alendronate systems (PCL_ALE_s) characterization

The system PCL_ALE_s can be defined as ALE particles coated with PCL (see scheme in Fig. 1a). Since the procedure of PCL_ALE_s production involves the application of PCL solution to coat ALE particles. Figure 1 shows SEM images of PCL_ALE_s. As stated in Fig. 1, the formation of PCL films around the ALE particles occurred. However, it is possible to observe isolated particles and regions containing agglomerates (Fig. 1b - see highlighted area).

The PCL_ALE_s were analyzed by AFM (Fig. 1c and d). The topography images show particles of different sizes, as observed in SEM analyses (Fig. 1 a). In a phase AFM image (Fig. 1d), it is possible to see a well-individualized particle. Posadowska et al. (2015)[10] evaluated nanoparticles containing ALE and also observed a rounded structure similar to this present in Fig. 1d.

Figure 1c did not present phase contrast. The phase contrast occurs due to the presence of different materials on the surface. In Fig. 1d, the lack of phase contrast indicates that PCL coats particles.

The PCL_ALE_s present characteristics of both PCL and ALE. It is because the PCL_ALE_s production did not significantly change the properties of PCL and ALE[8]. The PCL_ALE_s was thermally characterized by Silva et al. (2021)[8]. The results revealed that PCL presented an endothermic transition (melting) around 63.23°C. The ALE exhibits two endothermic peaks at approximately 125 and 130 °C related to the dehydration process. The PCL_ALE_s thermal behavior is essential to producing biomaterials by compression molding and other techniques based on PCL melting.

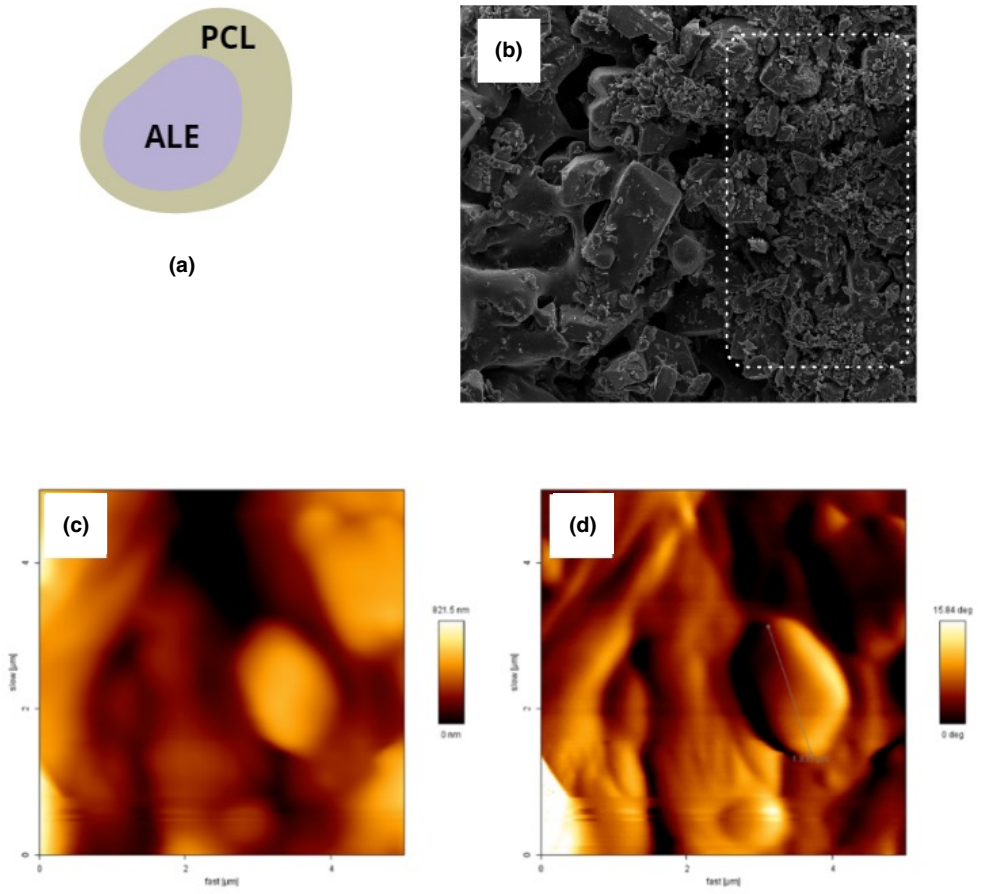

Figure 1: Schematic representation of PCL_ALE_s particle (a); SEM images of PCL_ALE_s (b).300 X.; AFM topography image of PCL_ALE_s (c) and AFM phase image of PCL_ALE_s (d).

Source: Elaborated by the authors.

Production of biomaterials using PCL/Alendronate systems

Various methods can be applied to have biomaterials. Different chemical-physical principles are applied in sample preparation. For example, a solvent is solvent-casting a polymer in the solvent-casting technique. In compression molding, film formation is related to the thermal behavior of the polymer, which melts under pressure. The present work produced films by compression molding at 100 °C. At the temperature used, only PCL melts.

Table 1 shows the EDS analysis results. The drug ALE presents the element phosphorus in this composition. Thus, phosphorus identification indicates ALE presence. EDS is a technique that allows the investigation of surface composition. Comparing the EDS results obtained for CM_PCL_ALE and SC_PCL_ALE, it is possible to state that the amount of phosphorus on the surface of the SC_PCL_ALE is more significant than in the CM_PCL_ALE.

Table 1: Elements quantification by Energy-dispersive X-ray spectroscopy (EDS).

Element	SC_PCL_ALE (Wt%)	CM_PCL_ALE (Wt%)
C K	37.47	55.24
N K	9.26	0
O K	4.23	-
Na k	-	0.23
P K	49.05	11.30
k k	-	30.23

Source: Elaborated by the authors.

Figure 2 shows SEM images of SC_PCL_ALE. It is possible to state that an irregular surface was formed. Similar SEM images were obtained by Tarafder and Bose (2014)[6]. Figures 2b and c show AFM images of SC_PCL_ALE. Topography images (Figs. 2b and c) suggest the formation of agglomerates. Figure 2d presented phase contrast indicating regions, probably, without PCL. However, EDS (Table 1) analysis shows the presence of both materials on the surface.

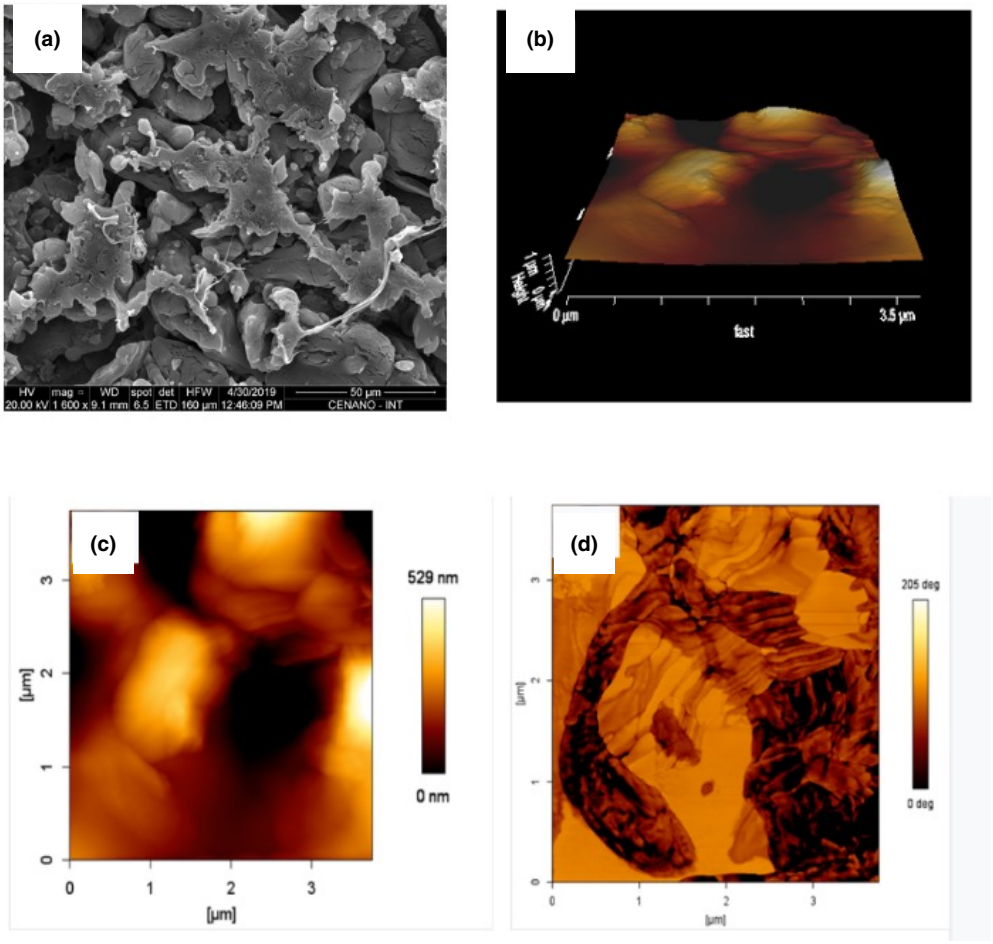

Figure 2: SEM images of SC_PCL_ALE (a); AFM topography image of SC_PCL_ALE (b) and (c) and AFM phase image of SC_PCL_ALE (d).
Source: Elaborated by the authors.

Figure 3 shows the SEM and AFM images of CM_PCL_ALE. SEM images show an irregular surface. AFM topography images showed a surface different from that observed for the SC_PCL_ALE. However, no phase contrast was observed in Fig. 3d. The structures observed in Fig. 3b is related to ALE particles coated with PCL. The analysis of topographic images of SC_PCL_ALE (Fig. 2) and CM_PCL_ALE. SEM (Fig. 3) shows different structures. Thus, root means square roughness (RMS). RMS of SC_PCL_ALE surface was 529.9 nm, and the CM_PCL_ALE was 706 nm. This difference in surface roughness results from the methodology applied.

Silva et al. (2021)[8] studied the ALE delivery by films produced by compression molding and solvent casting (the same applied in this work). The *in vitro* release was made in a potassium phosphate buffer (pH 7.4) at 37 °C to simulate the patient's physiological conditions. Although the proportion of polymer and drug is the same in SC_PCL_ALE and CM_ PCL_ALE, the ALE release rate was not the same.

The release of drugs from polymeric matrices involves transfer processes of mass, and different types of these phenomena can be observed. As an example, the diffusion of fluid in the system, the diffusion of the drug out of the system, the dissolution of the drug, polymer swelling, polymer chain relaxation, matrix erosion, initial polymeric, and osmotic effects, among other phenomena[8]. To better understand the drug release behavior and the phenomena involved in ALE delivery process, the experimental data were adjusted by models generally used in different studies: zero-order, first-order, Hixson-Crowell, Higuchi, and Korsmeyer-Peppas. The Higuchi model, which is based on the Fick law, fitted the SC_PCL_ALE delivery data.

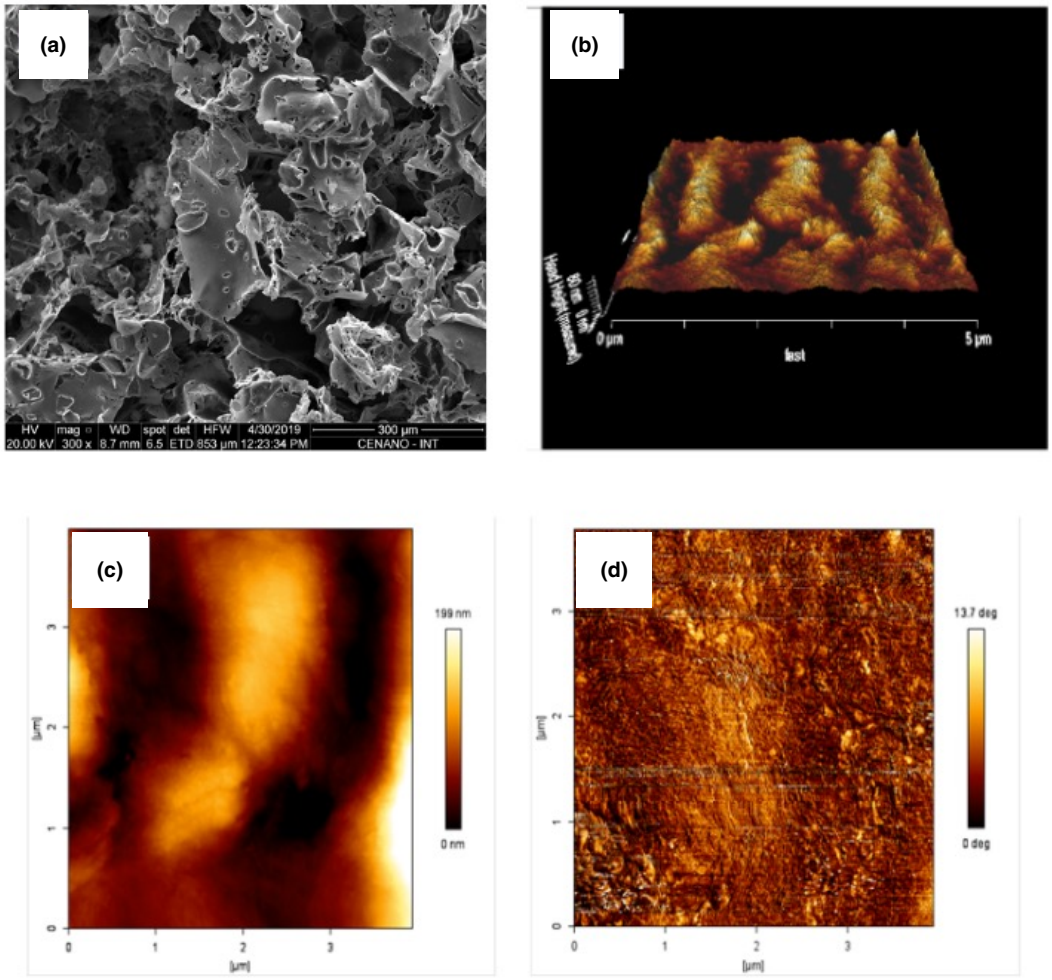

Figure 3: SEM images of CM_PCL_ALE (a); AFM topography image of CM_PCL_ALE (b) and (c) and AFM phase image of CM_PCL_ALE (d).
Source: Elaborated by the authors.

The Higuchi model, which Takeru Higuchi developed in 1961, is based on Fick's law. The general expression of the Higuchi model can be seen in Eq. 1[12].

$$f_t = K_H t^{\frac{1}{2}} \quad (1)$$

with K_H being the Higuchi dissolution constant.

In the present work, AFM analyses showed that the film produced by solvent casting presented better uniformity and less roughness, which may have contributed to a more uniform release, and, consequently, fit the Higuchi model. The cited models did not adjust to the ALE release from CM_ PCL_ALE. It can be associated with irregularities on this surface, as seen in Fig. 3b. However, at the end of 3 days, the film produced by compression molding released more ALE than solvent casting[8]. It is probably due to the drug distribution, morphology, and roughness, as shown by AFM, SEM, and EDS analysis. Indeed, a rougher surface provides a greater contact area, and it can contribute a long way to the increase of ALE release.

CONCLUSION

AFM analyses showed that morphology was strongly affected by methodology. AFM analyses allowed a better investigation of film surface morphology. The topographic contrast reveals that technique affected film

morphology, and the roughness values were 529.9 +/- 11 and 706 +/-13, respectively, for films produced by solvent casting and compression molding. Films produced by compression molding were rougher than those produced by solvent casting. AFM phase contrast and EDS analyses suggest differences among the surface ALE concentration in films. ALE tends to concentrate more on the surface of solvent-casting films. AFM images of topography and contrast phase and EDS showed that the ALE availability on the surface of the film produced using PCL_ALE_s depends on the applied technique, even though the starting material is the same. The AFM analysis helped to elucidate the ALE release mechanism reported in the literature for films produced using the PCL_ALEs as starting material due to the nanoscale surface characterization, and may also be an important tool for understanding the best production method for biomaterials that will be used in future applications for bone regeneration.

AUTHORS' CONTRIBUTION

Conceptualization: Mendonça RH, Silva CER and Silva TG; **Funding acquisition:** Mendonça RH, Silva CER, Silva TG and Garcia YA; **Research:** Mendonça RH, Silva CER, Silva TG and Garcia YA; **Methodology:** Mendonça RH, Silva CER, Silva TG and Garcia YA; **Resources:** Mendonça RH, Silva CER, Silva TG and Garcia YA; **Supervision:** Mendonça RH; **Writing - Preparation of original draft:** Mendonça RH, Silva CER, Silva TG and Garcia YA; **Writing - Proofreading and editing:** Mendonça RH, Silva CER, Silva TG and Garcia YA.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study

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