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Effectiveness in Sterilization of Objects Produced by 3D Printing with Polylactic Acid Material: Comparison Between Autoclave and Ethylene Oxide Methods

Eficácia na esterilização de objetos produzidos pela impressão 3D com material ácido polilático: Comparação entre os métodos autoclave e óxido de etileno

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Abstract

Objective Due to the popularity of 3D technology, surgeons can create specific surgical guides and sterilize them in their institutions. The aim of the present study is to compare the efficacy of the autoclave and ethylene oxide (EO) sterilization methods for objects produced by 3D printing with polylactic acid (PLA) material.

Methods Forty cubic-shaped objects were printed with PLA material. Twenty were solid and 20 were hollow (printed with little internal filling). Twenty objects (10 solid and 10 hollow) were sterilized in autoclave, forming Group 1. The others (10 solid and 10 hollow) were sterilized in EO, composing Group 2. After sterilization, they were stored and referred to culture. Hollow objects of both groups were broken during sowing, communicating the dead space with the culture medium. The results obtained were statistically analyzed (Fisher exact test and residue analysis).

Results In group 1 (autoclave), there was bacterial growth in 50% of solid objects and in 30% of hollow objects. In group 2 (EO), growth occurred in 20% of hollow objects, with no bacterial growth in solid objects (100% of negative samples). The bacteria isolated in the positive cases was non-coagulase-producing *Staphylococcus* Gram positive.

Conclusions Sterilization by both autoclave and EO was not effective for hollow printed objects. Solid objects sterilized by autoclave did not demonstrate 100% of negative samples and were not safe in the present assay. Complete absence of

Keywords
- sterilization
- printing, three-dimensional
- ethylene oxide
- biodegradable plastics

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contamination occurred only with solid objects sterilized by EO, which is the combination recommended by the authors.

Resumo

Objetivo Devido à popularidade da tecnologia 3D, cirurgiões podem criar guias cirúrgicos específicos e esterilizá-los nas suas instituições. O objetivo do presente estudo é comparar a eficácia dos métodos de esterilização por autoclave e óxido de etileno (OE) de objetos produzidos pela impressão 3D com material ácido polilático (PLA, na sigla em inglês).

Métodos Quarenta objetos em formato cúbico foram impressos com material de PLA. Vinte eram sólidos e 20 eram ocos (impressos com pouco enchimento interno). Vinte objetos (10 sólidos e 10 ocos) foram esterilizados em autoclave, formando o Grupo 1. Os demais (10 sólidos e 10 ocos) foram esterilizados em OE, compondo o Grupo 2. Após a esterilização, os objetos foram armazenados e encaminhados para cultura. Objetos ocos de ambos os grupos foram quebrados durante a semeadura, comunicando o espaço morto com o meio de cultura. Os resultados obtidos foram analisados estatisticamente (teste exato de Fisher e análise de resíduo).

Resultados No grupo 1 (autoclave) houve crescimento bacteriano em 50% dos objetos sólidos e em 30% dos objetos ocos. No grupo 2 (OE) o crescimento ocorreu em 20% dos objetos ocos, com ausência de crescimento bacteriano nos objetos sólidos (100% de amostras negativas). A bactéria isolada nos casos positivos foi *Staphylococcus* Gram positivo não produtor de coagulase.

Conclusões A esterilização tanto em autoclave quanto pelo OE não foi eficaz para objetos impressos no formato oco. Objetos sólidos esterilizados em autoclave não demonstraram 100% de amostras negativas, não sendo seguro no presente ensaio. Ausência completa de contaminação ocorreu apenas com objetos sólidos esterilizados pelo OE, sendo a combinação recomendada pelos autores.

Palavras-chave
- esterilização
- impressão tridimensional
- óxido de etileno
- plásticos biodegradáveis

Introduction

The use of three-dimensional (3D) technology for the printing of objects by additive manufacturing (AM) or 3D printing (prototyping) has been growing exponentially in the health area (orthopedics, bucomaxilofacial surgery, neurosurgery, and cardiac surgery, among others).[1] It can be applied for educational purposes (printing of anatomical parts, for example), surgical planning, creation of customized implants, orthotics, and external fixers and surgical reparators.[2–5] Specifically in the orthopedic area, surgeons and patients have benefited from this technology in the creation of surgical guides and in the prior planning for the intraoperative use of printed parts, guiding the correct position during osteotomies, bone perforations, and placement of various types of implant materials (Kirschner wires, drills and screws, etc.), reducing surgical time and improving accuracy.[6–10] With the popularization and greater accessibility of home 3D printers, surgeons have planned and created their guides in a homemade mode, sterilizing them in their institutions for use during surgery, discarding them after their application. The most used materials in mold prototyping are plastic filaments in polylactic acid (PLA) or acrylonitrile butadiene styrene (ABS) polymer, due to their cost-effectiveness and handling, but both still have difficulties for sterilization, mainly because they are thermosensitive. Some countries have rules for the specific processing of these types of 3D printed materials, but we have not found them in our environment so far.[11] The objective of the present work is to compare the efficacy and reliability of the autoclave and ethylene oxide (EO) methods for sterilization of objects printed in PLA, enabling their safe use in surgeries.

Material and Methods

Objects were designed in 3D format, creating standard STL files for prototyping (stereolithography), using the computer-assisted design (CAD) software Rhinoceros, version 5.5.4, licensed. After their creation, the files were prepared for 3D printing with the software Simplify3D, EULA, version 4.0.0, licensed, and were forwarded to printing on PLA plastic material. The printer used was the home model (desktop) Minibot 120. In the printing process, different percentages of object filling (infill) were chosen, creating totally solid ("massive") models or with empty space inside (hollow with "dead space"). (►**Figure 1**) Thus, 20 objects were printed in square format (1 cm^2), named solids (S), and 20 in rectangular ($5.0 \times 2.0 \times 0.5$cm), hollow objects, named "nonsolid" (NS). Two study groups were separated, the first

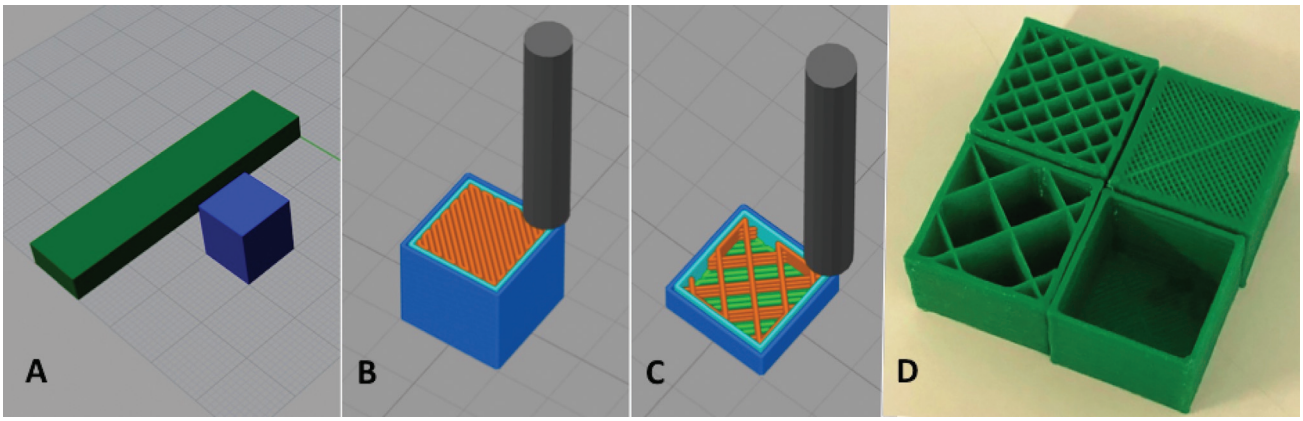

Fig. 1 Computer images demonstrating the creation of study objects with 3D technology. A) square and rectangle drawing in CAD software; B) preparation of the object for 3D printing with internal filling (infill) of 100%; C) preparation of the object with partial internal filling (hollow); D) photography showing printed objects with different filling percentages. Source: authors' file.

with 10 objects type S and 10 type NS (G1), and the second (G2) in the same way, totaling 2 groups with 20 objects each. Objects from G1 were sent for sterilization by the steam method with autoclave (Sercon model), being processed by the "fast cycle" method at 121°, preventing the melting of the part. Objects from G2 were sterilized by the EO method ("cold") in a specialized center contracted by the institution. Each object was sterilized and packed separately in a standardized manner with double plastic protection, keeping it sterile and stored in an appropriate environment for 1 week (►**Figure 2**). In the 2nd week, the objects were referred to culture in the microbiology laboratory of the institution. The procedures were performed by a specialized professional, duly attired, with the samples manipulated in a standard environment (laminar flow chapel for the protection of products handled inside, avoiding external contamination), after sterilization of the flow with 70% alcohol and with continuously lit fire. All samples from groups G1 and G2 were placed in sterile vials with Brian Heart Infusion (BHI) broth, which is an enrichment medium used in the recovery of fastidious or nonfastidious microorganisms, including aerobic and anaerobic bacteria and fungi) and maintained for 48 hours in an oven (34° to 37°C). At this stage, the NS type objects of the 2 groups were broken immediately before being introduced into the BHI culture medium, communicating the internal space ("dead space") with the exterior in order to also analyze the effectiveness in sterilization inside the hollow parts. For this reason, NS-type objects were printed in rectangular format, making them easier to break. (►**Figure 3**) After 48 hours, the samples were sowed in Blood Agar-MacConkey (using a rich base that provides growth conditions for most microorganisms) and in MacConkey Agar (a culture medium intended for the growth of Gram-negative bacteria and indication of lactose fermentation). After sowing, the cultures were kept in a greenhouse for 24 hours (for bacterial growth and subsequent reading) and the samples in broth were returned to the greenhouse (34° to 37°C) for incubation for another 15 days. After this period, they were sowed again in the same way, being submitted to a new reading. The collected data were analyzed with the aid of IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA) software and of the Fisher exact test, followed by residue analysis when statistical significance was observed.

Fig. 2 Photograph showing storage mode of objects printed in double plastic after sterilization. Source: authors' file.

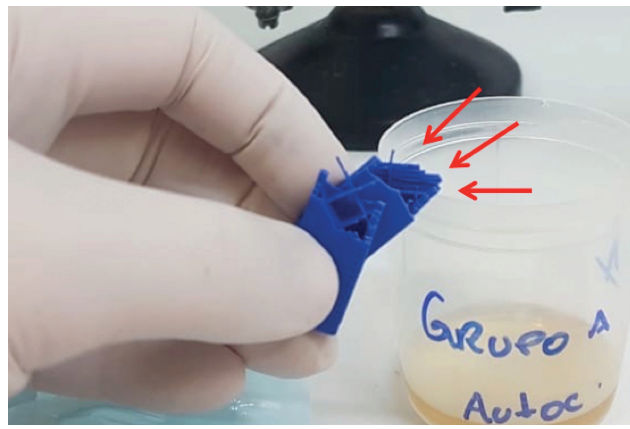

Fig. 3 Photography demonstrating detail in the process of sowing hollow objects (NS), which were broken immediately before placement in Brian Heart Infusion (BHI) broth. Source: authors' file.

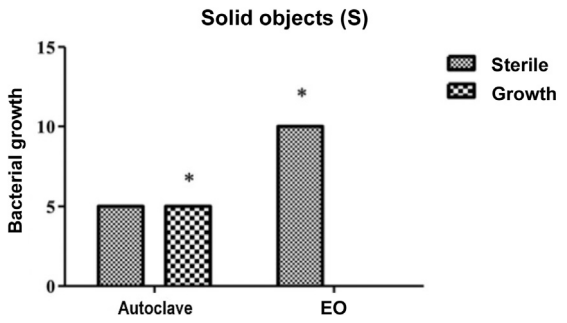

Fig. 4 Graphic demonstration of the statistical analysis comparing positive (growth) and negative (sterile) results after reading the crop samples with solid pieces (S). Abbreviation: OE, ethylene oxide; * Statistically significant value after residue analysis. Source: research data.

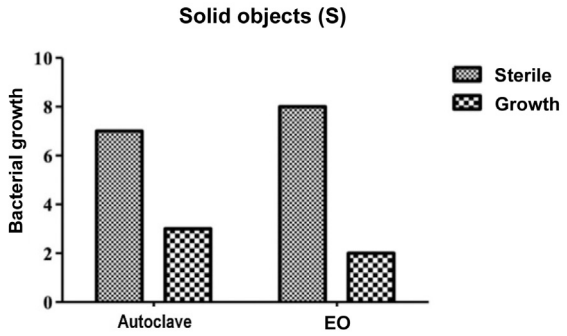

Fig. 5 Graphic demonstration of the statistical analysis comparing positive (growth) and negative (sterile) results after reading the samples of cultures with hollow pieces (NS). Abbreviation: OE:, ethylene oxide. Source: research data.

Results

The results after 48 hours and 15 days of incubation were similar. In group G1 (sterilized in autoclave), there was bacterial growth in 50% of the samples of S objects (50% negative) and in 30% of NS objects (70% negative). In group G2 (sterilized in EO), there was no growth in 100% of the samples of S objects, but growth was observed in 20% of the NS objects (80% negative). These data, including the statistical calculations performed, are shown in ►**Table 1** and in ►**Figures 4** and **5**. The bacteria isolated in all cases of contamination was non-coagulase-producing *Staphylococcus* Gram positive.

Discussion

The use of 3D technology in medicine has grown rapidly, benefiting several areas with its application, including orthopedics,[2] which is demonstrated by the growing number of publications on the subject. In a systematic review, Tack et al.[1] initially collected 7,482 papers for analysis. Among these, 60% were studies with applications of printed surgical guides or surgical planning. Despite the ease in manufacturing domestically these objects, the type of material and its sterilization remain the greatest difficulties. Among the available materials, PLA is the most used synthetic because it is biocompatible, nonpolluting (biodegradable and from renewable resources), low-cost, and is easy to handle, being also the material of preference by the authors.[12,13] For medical use, its main disadvantage is being thermosensitive, with the beginning of its melting occurring from 120°C, which can cause deformation in the part during the processes of steam sterilization and high temperature (autoclave), making its use unfeasible.[14] Since autoclave is the most accessible sterilization option available in most hospitals, it can be used by being programmed to run in "fast cycle" mode as an alternative for thermosensitive objects, subjecting the material to 121°C for a shorter period. This has demonstrated effective preservation of the original PLA.[12,15,16] The alternative method viable in our environment for "cold" sterilization of thermosensitive materials is EO.[17–20] Other "cold" methods, such as plasma gas and gamma rays, among others, are also effective, but are costly and may become unfeasible in some institutions. In a recent systematic review, Davila et al. concluded that the most universally used methods for this type of material are EO and gamma rays. Other methods, such as hydrogen peroxide/plasma gas, peracetic acid, and ozone have been explored as alternatives, but there is no defined standardization yet.[21] Materials more resistant to autoclave, such as the

Table 1 Results of bacterial growth in samples distributed by group (G1 and G2) and by type of objects (solid and hollow)

	Objects, n (%)		
	Autoclave	EO	p-value†
	n = 10	n = 10	
Solid parts (S)			
Negative	5 (50.0)	10 (100.0)[b]	0.033
Positive	5 (50.0)[b]	0 (0.0)	
Hollow parts (NS)			
Negative	7 (70.0)	8 (80.0)	0.999
Positive	3 (30.0)	2 (20.0)	

Abbreviatio ns: EO, ethylene oxide; Negative, samples without bacterial growth; NS, hollow parts; Positive, samples with bacterial growth; S, solid parts.
†obtained after applying the Fisher exact test; [b] statistically significant value after residue analysis. Source: research data.

resin used in the dental environment, also require more expensive printers and raw material. Regulatory mechanisms standardize the use of autoclave and EO in the processing of the most common surgical materials, but this has not yet been clearly established for the objects obtained with 3D printing in our environment. For materials considered thermosensitive (punch batteries, endoscope plastic parts, etc.), EO remains the most recommended to prevent possible melting.[14,20] A concern in our study was regarding the efficacy in complete sterilization, including the internal space created in rectangular parts (NS), differentiating from the efficacy observed in solid parts (S). Printing with partial internal filling (% *infill*) is common in household printings because the process is faster and more economical by using less raw material. Neches et al.[22] and Skelley et al.[23] demonstrated efficient sterilization of PLA printed objects automatically by the high temperature generated for the melting of the material during the printing of the objects, including the interior of the parts ($\sim$ 200°C), requiring no further processing. Aguardo-Maestro et al.[24] compared autoclave, OE, and plasma gas methods in the sterilization of hollow printed objects after inoculating a bacteria suspension inside them, finding efficacy only in the first two methods. The plasma gas method was recommended by the authors only for objects without internal space (solids).[24] Our results demonstrated failures in the efficacy of the sterilization of hollow parts (NS) both by autoclave (G1) and by EO (G2), with bacterial growth in 30 and in 20% of the samples, respectively, suggesting that the "dead space" was not properly sterilized by neither method. Autoclave sterilization was also not proven safe by the "fast cycle" method, with contamination observed, in addition to the 30% of contamination observed in NS type parts and to the 50% of contamination observed solid parts (S). Therefore, we do not recommend autoclave for PLA sterilization. The Type S parts sterilized by EO were the only ones that did not have bacterial growth. The use of EO, in addition to being effective in this type of printing (S), has the advantage of not deforming PLA due to the the risk of its melting because it is a "cold" method. Therefore, we recommend, for objects printed with PLA material, full-fill printing (100% *infill*) and sterilization in EO as an alternative to autoclave. As limitations of the present study, we can include the nonblinding and nonrandomization of objects, the possibility of contamination during preparation and sowing, the absence of a control group and of a comparison with other types of material. The small number of samples decreases the statistical relevance of our results, but does not invalidate it, since the sample test performed prior to the application of the statistical test showed a confidence of 95%, with a sampling error of 5% (or 0.05). Thus, future studies are necessary to define the most effective method for the sterilization of these objects, standardization, and control by regulatory mechanisms.

Conclusion

Sterilization by both autoclave and OE was not effective for hollow printed objects. Solid objects (printed with 100% internal filling) sterilized by autoclave did not demonstrate 100% of negative samples and were not safe in the present assay. Complete absence of contamination occurred only with solid objects sterilized by EO, with this being the combination recommended by the authors.

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Conflict of Interests
The authors have no conflict of interests to declare.

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