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Enzymatic detergents in the reprocessing of health products

Detergentes enzimáticos no reprocessamento de produtos para a saúde

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ABSTRACT

Introduction: Enzymatic detergents are widely used in the cleaning of the reprocessing of medical devices (MD), such as endoscopes, surgical instruments and dental-hospital materials. **Objective:** To identify researches that approach the effectiveness of these detergents in the removal of dirt present in MD, aiming to answer questions regarding their effective action. **Method:** The integrative review method, which allows the analysis of scientific research in a broad and systematic way, was used, favoring the characterization and dissemination of the knowledge produced. We used the following electronic databases: Scientific Electronic Library Online (SciELO), National Library of Medicine (PubMed), Scopus and Web of Science. Articles published from 2002 to 2017 were included, using the following descriptors: enzymatic and detergents and cleaning. **Results:** After the analytical reading of the 148 articles found, 113 were excluded (repetition [48] was the main cause of exclusion) and 35 were selected. **Conclusions:** It was verified the diversity of MD used. It was also verified that depending on their complexity, MD influence on the final results of the analysis. Most of the experimental research specifically on the action of enzymatic detergents (71% - 20/28) emphasize their effectiveness in the removal of microbial and/or biofilm and other soils. Therefore, they are indicated for the reprocessing of MD, such as laparoscope, laryngoscope, endoscope and endodontic instruments. Endodontic instruments have an advantage over other MD, since they do not have internal surfaces that cannot be reached.

KEYWORDS: Detergents; Enzymes; Efficacy; Hospital Infection; Health Surveillance

RESUMO

Introdução: Os detergentes enzimáticos são amplamente utilizados, na etapa de limpeza do reprocessamento de produtos para a saúde (PPS), como endoscópios, instrumentos cirúrgicos e materiais odonto-hospitalares. **Objetivo:** Identificar pesquisas que abordem a eficácia desses detergentes na remoção da sujidade presente em PPS e visem responder a questionamentos em relação a sua ação efetiva. **Método:** Foi utilizado o método de revisão integrativa que permite a análise de pesquisas científicas de forma ampla e sistemática, favorecendo a caracterização e a divulgação do conhecimento produzido. Foram utilizados os bancos de dados eletrônicos *Scientific Electronic Library Online* (SciELO), *National Library of Medicine* (PubMed), Scopus e *Web of Science*. Foram incluídos os artigos publicados no período de 2002 a 2017, tendo como descritores *Enzymatic and Detergents and Cleaning*. **Resultados:** Dos 148 artigos encontrados, 113 foram excluídos a partir da leitura analítica dos textos, sendo a principal causa de exclusão, os artigos repetidos (48). Foram selecionados para o presente estudo 35 artigos. **Conclusões:** Verificou-se a diversidade de PPS empregados que, dependendo da sua complexidade, influenciam nos resultados finais da análise. Em sua maioria (71% - 20/28), as pesquisas de caráter experimental sobre ação dos detergentes enzimáticos enfatizam sua eficácia na remoção de carga microbiana e/ou biofilme e outras sujidades. Portanto, são indicados para o reprocessamento de PPS, como os laparoscópicos, laringoscópios, endoscópios e instrumentos endodônticos. Este último apresenta vantagem em relação aos demais PPS, uma vez que não têm superfícies internas que não possam ser alcançadas.

PALAVRAS-CHAVE: Detergentes; Enzimas; Eficácia; Infecção Hospitalar; Vigilância Sanitária

INTRODUCTION

Medical devices (MD) subject to reprocessing are manufactured from raw materials and structural conformation, which enable repeated cleaning, preparation and disinfection or sterilization actions, until they lose their efficacy and functionality. These products include, for example: endoscopes, surgical instruments and dental-hospital materials. MD reprocessing involves a set of actions related to pre-cleaning, receiving, cleaning, drying, integrity and function evaluation, preparation, disinfection or sterilization, storage and distribution for the consumer units[1].

These products are widely consumed across the world. In Brazil, a study carried out in São Paulo verified the amount of MD consumed in the Sterilization Center of a high-complexity hospital. From August 2016 to April 2017, the monthly average was 48,777 items, and the main consumer units were the Surgical Center (42%) and the Intensive Care Unit (40%)[2].

MD can become reservoirs or sources of microorganisms as a result of inadequate reprocessing practices and thus cause hospital infection in exposed patients[3]. Studies have revealed the presence of microorganisms of clinical importance in endoscopes after reprocessing. Chiu et al.[4] have shown, in a prospective, five-year study in Thailand, that the number of positive cultures found in the biopsy channel was 13.6% (57/420), which was considered significant. More than 68.4% of the microorganisms identified were Gram negative non-fermenting glucose bacteria, often associated with a variety of infections, mainly in immunocompromised patients.

In France, 13 cases of patients infected or colonized by Carbapenem-producing *Klebisiella pneumoniae* (KPC) were found. Among these cases, seven were contaminated during the use of the same duodenoscope, which had previously been used by a patient identified as a source case, and the other cases occurred because of patient-patient transmission. Failure to reprocess the duodenoscope has been suggested as a possible cause of infections[5].

In Brazil, epidemic outbreaks of infections after video-assisted procedures have been reported in Rio de Janeiro and other states. Irregularities have been detected in the reprocessing of medical devices where inadequate manual cleaning and possible resistance of the microorganism causing the infection to the disinfectant used may have enabled outbreaks of rapidly growing mycobacteria[6]. Southworth[7] and Dancer et al.[8] described infection outbreaks related to failure at some stage of MD reprocessing, especially in the cleaning step.

The Brazilian National Agency of Health Surveillance (Anvisa) is the regulatory agency for these products in Brazil. It regulates their registration and notification prior to their marketing, observing and controlling quality criteria to ensure efficacy and safety[9]. The enzymatic detergent for hospital use is regulated by the Resolution of the Collegiate Board of Directors (RDC) n. 55, of November 27, 2012, of Anvisa. This regulation determines that enzymatic detergents are considered risk II and are subject to registration with Anvisa[10]. One of the requirements for registration is the report of enzymatic activity, including the determination of proteolytic activity and amylolytic activity, when it is declared.

Cleaning means to remove dirt from objects, normally using water with detergents or enzymatic products[11]. This stage is decisive for the effectiveness of the process. In addition to a surfactant, enzymatic detergents for cleaning MD contain at least one hydrolytic enzyme of the subclass of the proteases, and they may also contain other enzymes, like amylase and lipase, which, through their enzymatic activity, catalyze a degrading reaction of specific substrates and thereby remove clinical dirt[1]. The enzymes included in formulations of detergents for restricted use in healthcare establishments can dissolve organic residues and thus sanitize the MD and unblock channels with residues and coagulated substances[12].

The organic matter present in the instruments after use, if not properly removed, interferes with the disinfection process in two ways: it reacts with the chemical agent to prevent its action or protects the microorganism from the action of the product[11]. Two main types of risks are associated with the reuse of MD: transmission of infectious microorganisms and change of product performance after reprocessing, which may result in damage and a safety problem for patients and healthcare professionals[3]. Considering the importance of the cleaning agents for the reprocessing of MD to be effective, concerns arise regarding the efficacy of the enzymatic detergents available in the market. In this way, we aimed to identify studies that addressed the quality of enzymatic detergents in relation to their efficacy in the reprocessing of MD, in the scope of their use in healthcare facilities, in order to answer questions about the effective action of these detergents.

METHOD

The method used in this study was the integrative review of the literature on the use of enzymatic detergents in the reprocessing of medical devices. This type of study enables the analysis of scientific research in a broad and systematic manner, favoring the characterization and dissemination of the knowledge produced[13]. The guiding question was defined as: are the enzymatic detergents used in the reprocessing of medical devices effective in removing dirt?

The following inclusion criteria were determined: papers in the English or Portuguese languages that addressed the use of enzymatic detergents in non-experimental studies (example: evaluation of reprocessing difficulties, application of enzymatic detergent evaluation instruments, situation assessment) and in experimental studies involving the evaluation of the efficacy of enzymatic detergents in MD reprocessing.

The present research encompassed papers published from 2002 to 2017. A survey was conducted on the following

databases, available electronically: Scientific Electronic Library Online (SciELO), National Library of Medicine (PubMed), Scopus and Web of Science. Using search terms *Enzymatic and Detergents and Cleaning*. We excluded the papers that, although they approached the subject of enzymatic detergents, did not present neither experimental nor non-experimental studies regarding the use of these detergents in MD reprocessing.

The selection of the papers was done first after the analysis of titles and abstracts. In cases where the titles and abstracts were not enough to determine this first selection, we searched for the complete publications. In relation to the duplicity of papers found in the different databases, the documents originally identified in each of them were ordered by title, excluding those that appeared more than once. The prevailing source of information was that where the paper appeared for the first time, in the following order: SciELO, PubMed, Scopus and Web of Science.

To analyze the papers, the following categorization was used as systematization: type of study, type of MD studied, types of analyses performed. The reasons for the exclusion of the papers and their respective quantitative data are shown in Table 1.

For the most part, the excluded documents were research studies on the use of enzymatic detergent involving other products, like textiles, cotton treatment, bone cleaning, dairy industry, membrane cleaning, greasy soil and hard surfaces.

In the evaluation, we verified: percentage of publications by database, year of publication, origin of journals and content of the papers (experimental and/or non-experimental studies).

RESULTS

We found 148 papers in the electronic search. Of this total, 113 were excluded (Table 1) after the analytical reading of the texts, and 35 documents were selected. The distribution according to the database is shown in Figure 1. The highest percentage of papers (74%) was found in the PubMed database.

According to the origin of the journals in which the papers were published (Figure 2), the United States (40%) and England (34%) stand out. In Brazil, there were four studies done within the analyzed period.

The evaluation of the publication years showed the highest number of publications in 2004 and 2006 (Figure 3).

Of the papers included in the present investigation, 89% were concerned with experimental studies involving different methods

Table 1. Reasons for exclusion and number of papers on detergent efficacy.

Reason for exclusion	N. of papers excluded
Survey on infection prevention practices	1
Use of enzymatic detergents on living surfaces	2
Studies on the evaluation of ED residues found in MD after cleaning	7
Studies involving products other than MD	29
Studies on enzymatic synthesis, characterization and purification	15
Repeated studies	48
Studies on label information	2
Cleaning studies involving one type of enzyme	2
About ingredient development of detergent formulation	3
Paper without access to full text	4

MD: Medical devices, ED; Enzymatic detergent.

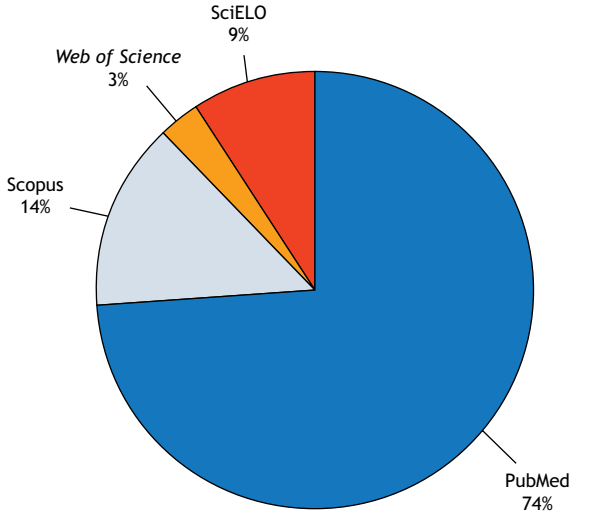

Figure 1. Publications on the efficacy of enzymatic detergents according to the databases searched.

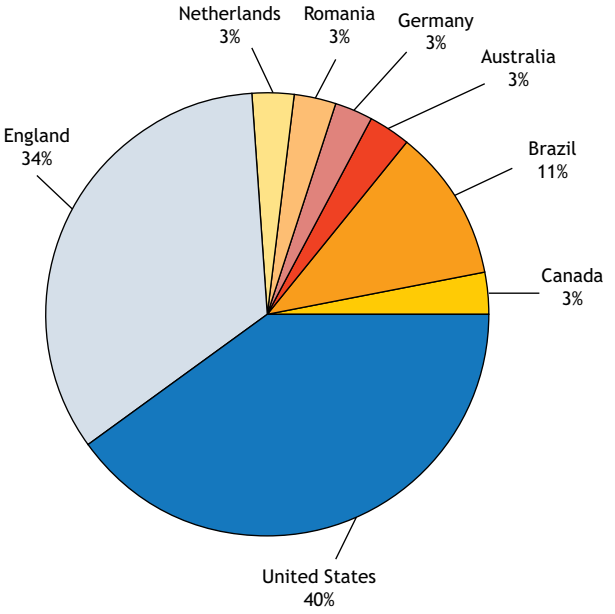

Figure 2. Publications on the efficacy of enzymatic detergents by nation of origin.

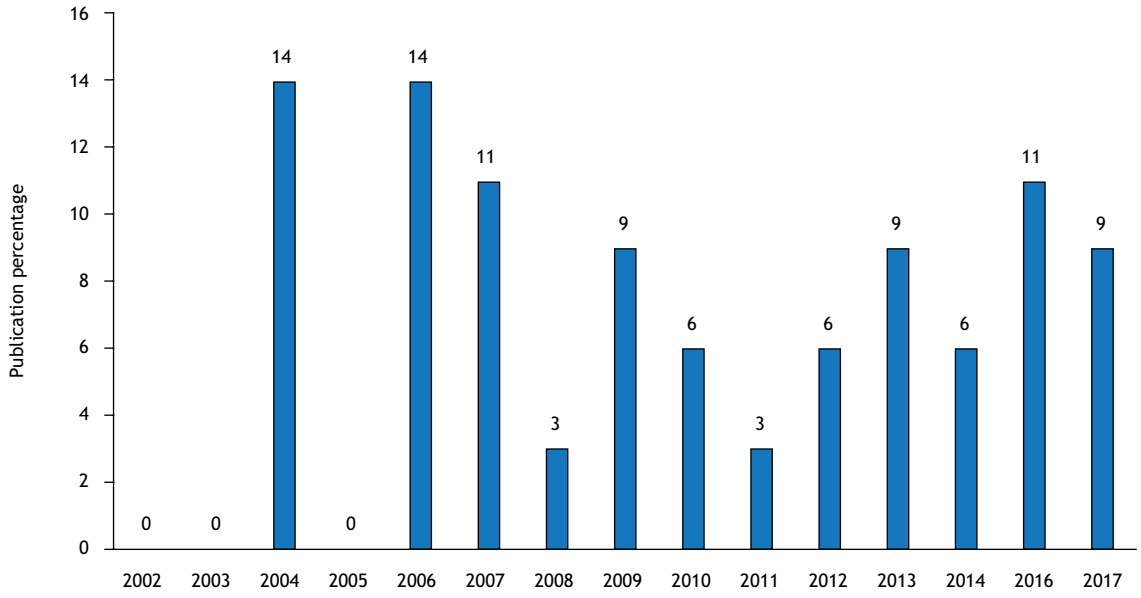

Figure 3. Publications on the efficacy of enzymatic detergents from 2002 to 2017

Table 2. Distribution of papers with experimental studies on the efficacy of enzymatic detergents.

Study Type - Removal of microbial load and/or biofilm involving the use of enzymatic detergents	
Result	Reference
Effective	14, 15+, 16, 17+, 18, 19, 20, 21, 22, 23,24, 25+26+, 27
Not effective	28, 29
Study Type - Removal of residues* involving the use of enzymatic detergents	
Result	Reference
Effective	17+, 25+26+, 30, 31, 32, 33, 34, 35
Not effective	36

* Residues include organic matter like protein, blood, hemoglobin.
\+ Paper fit into more than one type of study.

and types of dirt (biofilm and residues like blood and proteins) and comparisons between the efficacy of enzymatic detergents versus non-enzymatic detergents (31/35) and only 11% were from non-experimental studies.

Twenty experimental papers related to efficacy concluded that enzymatic detergents are in fact active to remove microbial load and/or biofilms and residues. Four papers fit into more than one type of study (papers 15, 17, 25 and 26) (Table 2).

In three publications the enzymatic detergents used were not considered effective. Da Costa Luciano et al.[28] evaluated four enzymatic detergents for hospital use to remove *Enterococcus faecalis* and *Pseudomonas aeruginosa* biofilm, against which none of the detergents tested produced a reduction higher than 1 $\log_{10}$ of colony forming units (CFU)/cm^2, according to the criterion adopted by the authors. The evaluation was done by counting viable microorganisms, quantification of proteins and carbohydrates and scanning electron microscopy.

Another investigation by the same group of researchers also using biofilm of *E. faecalis* and *P. aeruginosa* was performed. Four detergents (two enzymatic and two non-enzymatic) were tested alone or in combination with orthophthalaldehyde, glutaraldehyde or hydrogen peroxide. The results showed that none of the detergents alone was able to remove the biofilm or reduce the bacterial level[29]. The step of pre-immersion in enzymatic detergent prior to ultrasonic cleaning was evaluated in endodontic devices by Aasim et al.[36]. The authors performed experiments in cleaning processes with and without enzymatic detergents, concluding that there is no benefit in the use of these detergents because no significant difference was found with the inclusion of pre-immersion.

Among the papers selected, five compared the efficacy of enzymatic and non-enzymatic detergents for the removal of biofilm (Table 3). In four studies, both enzymatic and non-enzymatic detergents showed an effect on biofilm removal, but non-enzymatic detergents had a superior effect[37,38,39,40]. However, no significant difference was observed when the contact time of three, five and seven minutes for the action of the enzymatic detergents on endoscopes was studied[37]. On the other hand, in the study by Alfa et al.[15], residues and microorganisms like *E. faecalis* and *P. aeruginosa* were found after the use of a non-enzymatic detergent (Table 3).

Three publications concerning the experimental evaluation of cleaning indicators in automated machines were analyzed, and the indicators of thermostable Adenylate Kinases (tAK) and Pinnacle Monitor for Automated Enzymatic Cleaning Procedures

Table 3. Distribution of papers with comparative studies on the efficacy of enzymatic and non-enzymatic detergents.

Type of Study - Comparison of the use of ED and NED detergents in the removal of microbial and/or biofilm load	
Result	Reference
More effective NED	37, 38, 39, 40
More effective ED	15+
Type of Study - Comparison of the use of ED and NED in the removal of residues*	
Result	Reference
More effective NED	41

*Residues include organic matter like protein, blood, hemoglobin.
+ Paper fit into more than one type of study.
ED: Enzymatic detergent; NED: Non-enzymatic detergent.

Table 4. Experimental studies on the evaluation of cleaning indicators in automated machines.

Reference	Indicator evaluated	Result
42	*thermostable Adenylate Kinases (tAK)* (enzyme indicator)	tAK - suitable indicator to discriminate the performance of different automated cleaning processes
43	*Pinnacle Monitor for Automated Enzymatic Cleaning Processes* (PNCL) WC (wash-check) TOSI®	PNCL - more sensitive to changes in cleaning cycle parameters (changes in temperature, washing time and level of active enzymatic detergent) followed by WC and TOSI®
44	*Sonocheck* (ultrasonic energy level monitor)	The Sonocheck indicator can be used to measure the mechanical action or the main functional capacity of an ultrasonic bath, but not its cleaning performance

WC: *Wash-Checks*; TOSI: *Test Object Surgical Instrument*.

(PNCL) were considered adequate. The latter is considered more sensitive to changes in cleaning cycle parameters when compared to other indicators like Wash-Checks (WC) and Test Object Surgical Instrument (TOSI®) (Table 4).

The present study also included four non-experimental studies that evaluated parameters like protocols, MD reprocessing workflow, risk involved in the cleaning step and consequent safety implications of the disinfection and sterilization process. Among them, a study done in Brazil, in endoscopy services, showed failures in several reprocessing stages that may affect the quality of the process[45]. The degree of difficulty in cleaning also affects the quality of the sterilization process[46] in the same proportion, and a careful evaluation of the quality and efficiency of the enzymatic detergents prior to their acquisition is required. The efficiency of the process was also related to the appropriate time of immersion of the MD in the enzymatic solution and the number of enzymes of an enzymatic detergent was also found to be essential[47]. A study carried out in Romania, in Equipment Processing Centers of university hospitals and private or public outpatient units, showed that, in general, disinfection and sterilization of endoscopes and accessories were performed adequately, based on pre-established protocols, and approximately 76% of the units used detergent solutions of the enzymatic type[48].

DISCUSSION

The use of enzymatic detergents in MD reprocessing has grown in recent years due to the fact that cleaning with enzymatic agents, which act at a neutral pH, appears to be less harmful to the MD than alkaline cleaning agents[25].

Most of the papers we analyzed deal with experimental studies, which focus on the assessment of the efficacy of enzymatic detergents for the elimination of microbial biofilms. Biofilms can form in channels of equipment contaminated with organic matter, like digestive and gastrointestinal juices, mucus, food bits, blood and other bacteria. Preventing biofilm formation is critical, because not all reprocessing methods can safely dispose of viable bacteria within the biofilm matrix[15], and this difficulty may still vary in relation to the type of MD involved. Therefore, according to Vickery et al.[39], small failures in the reprocessing protocol can result in material buildup, for example, within endoscopes, and to promote the development of the biofilm. In addition, MD that cannot be fully disassembled for complete cleaning are susceptible to the presence of biofilms. Bacteria and fungi present in the MD can protect themselves from external factors and multiply in biofilms, posing a high risk of cross-infection[14].

The studies included in this research have shown several evaluations of the efficacy of enzymatic detergents. Zühlsdorf et al.[25] analyzed ten different cleaning processes and concluded that biological load reduction was the most sensitive and important parameter to be considered in the study when the efficacy of cleaning and reprocessing processes of flexible endoscopes was evaluated[25].

For Stiefel et al.[17], the incorporation of appropriate enzymes significantly improves endoscope cleaning performance. In that study, the researchers found a 95% removal of *Staphylococcus aureus* and 90% of the biofilms of *P. aeruginosa* in a 96-well plate system. Another study done in Brazil, where single-use laparoscopic instruments (SULI) were cleaned with ultrasonic washer and enzymatic detergent, after contamination with bacterial spores and sheep blood, presented 100% negative results for the recovery of contaminating microorganisms[14].

A study on multi-species biofilm of *Flavimonas oryzihabitans*, *Lactobacillus brevis*, *Euconostoc mesenteroides* and *Saccharomyces cerevisiae* with enzymatic detergent, composed of various enzymes, has shown a significant reduction in the number of viable microbial cells. These detergents may help to remove biofilm by degrading the layers of exopolysaccharides to which the microorganisms are incorporated[27]. The type of detergent, the ideal concentration and the time of action are important factors in the removal of microorganisms. Thus, in the study by Augustin et al.[24], enzymatic detergents were more powerful against bacterial biofilms after 30 min of

incubation. When the efficacy of bacterial removal of gastroscopes by enzymatic detergent was compared to the efficacy of chlorhexidine-based products, both showed a reduction in microbial load, with the presence of *Pseudomonas* species in both products[22].

In relation to other MD, the efficacy of enzymatic detergents was shown to be effective in the cleaning of rigid nasal endoscopes after *in vitro* inoculation with *S. aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*[18]; and in implantable cardiac electronic devices, with reduction of biological load[26]. For Alvarado et al.[20], the use of a sterile, high-quality disposable polyurethane sheath in a nasopharyngoscope during a clinical examination, combined with enzymatic detergent cleaning and disinfection with 70% ethanol, can be a reliable instrument to be used in patients. Although the enzymatic treatment does not provide the required "immediately after use" decontamination for highly soiled MD, its use represented an effective cleaning protocol for the reduction of biological load[21]. Another study pointed to the survival of microorganisms only when the non-enzymatic detergent was used, emphasizing the importance of the detergent used in the cleaning step of the reprocessing of endoscopes[15] and other MD that may be subject to reprocesssing.

In contrast, a study done with a biofilm model of *E. faecalis* and *P. aeruginosa* found that none of the detergents used in the study alone could remove the biofilm or reduce the bacterial level[29]. In this sense, if the biofilm builds up in the flexible channels of the endoscope during repeated rounds of reprocessing, neither the detergent nor the high level disinfectant will provide the expected level of bacterial removal or killing[28]. This controversy over the studies on the expected efficacy of biofilm removal in practice[49] can be warranted by the use of inadequate parameters in the development of the cleaning agent and may lead to an overestimation of its performance due to the relevance of the microorganisms used, conditions of biofilm formation, or biofilm removal reading[17].

According to Vickery et al.[38], bacteria residing in biofilms are difficult to culture, therefore, a "negative" culture in such circumstances does not necessarily mean the absence of live bacteria capable of causing infection *in vivo*. Another issue is the specificity of each enzyme and the unknown nature of the biofilm, which makes it difficult to obtain a mixture of enzymes that can universally treat all the biofilms found in a hospital environment[27].

As for the studies related to the removal of other types of dirt, like residual clinical dirt, protein and hemoglobin, several suggest the efficacy of enzymatic detergents in the removal of these residues from MD[17,26,30,31,33,35].

In the study by Stiefel et al.[17], artificial blood removal was confirmed through the use of cleaning indicators, thus certifying the efficient removal of residues with the use of enzymatic detergents. In this sense, a standardized protocol developed by Crawford et al.[26], which involved the use of an enzymatic detergent to clean and subsequently sterilize implantable cardiac electronic devices also proved to be effective in terms of sterility, significant reduction of protein and residual hemoglobin, and compliance with the biocompatibility standards for reusable medical devices.

In the literature, effective dirt removal processes have been reported for endodontic instruments[31,33,34], which are advantageous over other MD, since they do not have inner surfaces that cannot be reached[34]. On the other hand, for Aasim et al.[36] there is no benefit in relation to the pre-immersion in enzymatic detergents for endodontic devices prior to ultrasonic cleaning.

In this sense, a study that evaluated the performance of 15 products, including enzymatic and non-enzymatic detergents, has shown that most enzymatic detergents are effective to remove biofilms of *Escherichia coli*, as well as some non-enzymatic products, indicating that the statement that enzymatic detergents are better than non-enzymatic detergents should be considered with caution[23]. In the studies selected in this work in which these detergents are compared, a superior effect of the non-enzymatic detergent for biofilm removal has been shown[37,38,39,40]. In relation to the removal of other types of dirt in the study conducted by Walker et al.[41], a non-enzymatic general purpose cleaner achieved maximal removal of blood.

As for non-experimental studies, they highlight the need to establish protocols to ensure the quality of MD reprocessing and the prevention of cross-contamination[45]. Failures in the reprocessing steps, difficulties in cleaning due to the characteristics of the equipment, need to evaluate the quality of the enzymatic detergents used and time of exposure were the critical elements found by these investigations. The immersion time suggested by the manufacturers of the products analyzed in this study was 2 to 5 minutes[47].

The main difficulty found in the present research was the definition of the categories to be studied, to enable a qualitative-quantitative analysis. In some situations, the same paper fit into two separate categories.

CONCLUSIONS

In scientific literature it is clearly recognized that careful cleaning is of paramount importance for effective MD reprocessing. Therefore, this review allowed us to identify different studies on the efficacy of enzymatic detergents in the reprocessing of this type of device. For the most part (71% – 20/28), experimental studies on the action of enzymatic detergents have emphasized their efficacy in the removal of microbial and/or biofilm and other types of dirt. Therefore, they are indicated for the reprocessing of MD like laparoscopes, endoscopes and endodontic instruments. Moreover, the latter have an advantage over other MD, since these instruments do not have internal surfaces that cannot be reached. Regarding the parameters used in the study, the immersion time depends on what is determined by the manufacturer.

There is a clear need to establish protocols and standardize cleaning processes, considering the particularities of each healthcare provider and, thus, ensure that the MD are adequately reprocessed so as not to pose risks to the patients' health.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.