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Microbial load analysis in silicone gel breast implants

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ABSTRACT

Silicone breast implants consist of biomaterials widely used in breast reconstitution surgeries or in mammary augmentation for esthetic reasons. A preliminary stage of the implant production process is vulcanization, which consists of heating the implant to 165±5°C for approximately 9 hours. The aim of this work was to evaluate the bioburden of silicone breast implants prior to the vulcanization process and the decline in bioburden due to this process, and to confirm the sterility of the gel contained in the membrane. Breast implant production stages were evaluated by microbial counting in different steps, according to the USP 32 methodology. To evaluation of decrease in microbial load, spores strips were introduced inside the implant, and after vulcanization cycles the strips were removed from the implant. The strips were transferred to tubes containing TSB, followed by incubation for 7 days at 30-35°C. The results obtained showed that the level of microbial contamination of gel implants is relatively low, and that vulcanization allowed for the inactivation of up to 10^8 spores. This study led us to the conclusion that vulcanization leaded to sterility of the gel inside the product. Thus, the final sterilizing process contributed to an increase in the Sterility Assurance Level.1.

Keywords: Silicone. Breast implant. Sterilization. Dry heat. Vulcanization. Bioburden.

INTRODUCTION

The human body is vulnerable because its tissues and organs are subjected to illnesses and injuries that can cause pain, loss of function, movement restrictions, or even incapacity. In many cases, the treatment of situations like these involves the removal of the affected tissue or organ and its replacement by a graft of living tissue or an artificial analogue - a biomaterial.

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One current definition characterizes biomaterials as "materials (synthetic or natural; solid or, sometimes, liquid) used in medical devices or in contact with biological systems" (Ratner et al., 2004), whereas biomaterials are classically defined as "part of a system that treats, increases or replaces any tissue, organ or function of the body" (Helmus & Tweden, 1995). The various types of biomaterials include metals, composites, ceramics, glass and polymers, with silicone biomaterials being included in the latter category. Silicones are synthetic polymers: semi-organic compounds with chemical structures based on alternate units of silicon and oxygen. Starting from silica, several reaction stages occur before the formation of siloxanes (Guidoin et al., 1973). After polymerization, depending on the size of the chains formed and the ratio of branched and linear siloxanes, the silicone obtained can assume fluid, gel or elastomeric forms.

Silicone biomaterials present unique properties as high chemical stability, a large electric resistance, biological compatibility, high thermal resistance, resistance to adverse climate and temperature conditions, low superficial tension and good lubricity. It can assume varied shapes. Theses characteristics makes the silicone usefull to many aplications. In the field of health, silicone is employed in medical, pharmaceutical and cosmetic techniques, including aesthetic and repairing plastic surgeries, ophthalmology, tissue reconstruction, oral formulations and protective creams, among others. The breast is one of a number of tissues and structures that can be substituted with silicone implants in augmentation or reconstruction surgeries, although the use of silicone is controversial due to adverse effects.

Infection is one potential risk, and is the main cause of morbidity after breast implantation and of complications in 2·0 to 2·5% of the surgeries. The origin of infection in women with implants has been difficult to determine, but research indicates the possibility of contaminated implants, the surgery itself or the surgical environment and the patient's skin or mammary ducts. Several reports even suggest that the site of implantation is vulnerable to infection when bacteria infect another location migrate through the bloodstream (Pittet et al., 2005).

In order to guarantee the safety of the products being implanted, agencies after interaction with university,

research institute and producers, determines measured and control mechanisms. The measures and mechanisms are accomplished essentially for the specifications of quality of the productive process, the product and its distribution, with the verification of the good manufacturing and control practice (Brasil, 1977, 2001). Thus, standardized and validated procedures must be part of the manufacture and control of biomaterials. Silicone gel breast implants are part of this group, and great attention is devoted to the sterilization processes to which they are submitted, since, in the dependence of material´s nature, they can endenger the chemical structure of polymer, being able to influence in the biocompatibility.

Studies (Azevedo, 2004; Lucas et al., 2003) have been carried out to characterize the differences between these processes. Those that proved to be adequate and efficient involved the use of dry heat in silicone gel implants with smooth and textured surfaces; ethylene oxide in silicone gel implants with smooth and textured surfaces and surfaces with a polyurethane covering; and gamma irradiation of implants previously filled with saline solution. It was found that smooth-surface implants sterilized by ethylene oxide and submitted to intentional stress via a "Bleed Test" presented a spread gel mass value greater than those of implants submitted to dry heat sterilization (Azevedo, 2004). The dry heat sterilization method is advantageous for this reason, as well as for the fact that it does not generate toxic residues, which can occur in sterilization using ethylene oxide (Lucas et al., 2003).

Microbial inactivation using dry heat occurs by means of an oxidative mechanism in constituent cells, with more drastic conditions being generated than with moist heat (Russell et al., 1999). Sterilization occurs due to an increase in temperature -180°C to 300°C; at the lower value, only sterilization is achieved, while despyrogenicity occurs at the higher temperature (Pinto et al., 2003) - with heat irradiation distributed in the burden as uniformly as possible. The typical biological indicator used is *Bacillus atrophaeus* ATCC 9372 spores, previously known as *Bacillus subtilis* var *niger* ATCC 9372. The biological indicator must be placed at a position of fairly difficult access for the sterilizing agent, thus simulating the worst situation with regard to the load items to be sterilized (ABNT NBR ISO 11138-1, 2004).

Validation of a sterilizing process consists in verifying the efficacy of the process, with last stage including a performance qualification and an evaluation of the lethality toward the biological indicators. The overkill method, the combined method (biological indicator/ bioburden) or the absolute bioburden method are among the validation options available. The latter requires strict quantitative and resistance control of the bioburden. The combined method takes into account both the bioburden control (less strict) and the biological indicator. The overkill method takes advantage of the high resistance of the biological indicator, showing a lethality rate of 10^6 combined with an assurance of sterility security (SAL) of 10^{-6}. This is the half-cycle principle, in which a population of 10^6 of a resistant indicator is inactivated during the validation cycle, which represents the exposition period, and the cycle length is then doubled during routine operation. The overkill method is widely employed in the sterilization of thermostable materials. However, it can result in adverse effects when used for long sterilization periods (Agalloco, 2007).

In the case of sterilizing of silicone breast implants by means of dry heat, the chosen condition for this work was to submit the material to a temperature of 121°C for 36 to 40 hours in order to inactivate the biological indicator (USP 32, 2009). The use of a high temperature occasionally promotes the hydrolysis and/or fusion of the polymer matrix, which can influence implant biocompatibility (Park & Lakes, 2007). This fact justifies evaluation of the manufacturing steps to assess the advantages and disadvantages with respect to the bioburden and sterility specifications of the product, with the goal of correlating them to critical quality parameters.

Vulcanization, one of the production process stages, requires a heating temperature of 165 ± 5°C to be maintained for approximately 9 hours in order to achieve the desired chemical characteristics of the product and promote the ideal physical properties (Williams, 2000), as well as biocompatibility and sterility. These thermal conditions have an interesting effect on the reduction of the product bioburden, with advantages stemming from the fact that they both confer a higher sterility assurance level (SAL) and promote sterility with a lower exposure time to high temperatures, thus reducing any adverse effects on the product.

Therefore, this work evaluated breast implant production stages using microbial counting of different phases in order to evaluate critical aspects in a possible contamination due to productive process, as well as to observe the reduction in bioburden as a result of vulcanization. Taking into account the fact that planning a sterilization process with a defined probability of survivors depends on knowledge of the initial microorganism population in the product (bioburden) and on the inactivation kinetics of the bioburden when exposed to the lethal treatment, it is essential to evaluate vulcanization and its influence on the sterilization process conditions.

MATERIALS AND METHODS

Sample

Microbial counting was carried out by means of sampling of the raw material components A (Poliol) and B (Isocyanate) prior to the generation of silicone gel and after mixture of the components A and B. The implant capsules (or membranes) were also sampled after thermal treatment of these components (pre-vulcanization) after a considerable storage time in order to evaluate the level of contamination of the internal part of the membrane.

The implants (gel-filled membranes) were also sampled and evaluated prior to and after being submitted to vulcanization.

Table 1 shows the amounts of material used and sample replications for each condition case.

For evaluation of the decrease in microbial load during vulcanization, three cycles of vulcanization were carried out, with analysis of 20 silicone implant units for each cycle. Each unit contained spores strips, "Graded

Biological Indicators" with loads of 10^4, 10^5, 10^6, 10^7 and 10^8 microbial spores of *Bacillus atrophaeus* ATCC 9372. The strips were introduced into the gel inside the implant. After vulcanization, they were removed from the implant and placed, one by one, into a plastic bag for final analysis. Thus, for each cycle of vulcanization 100 spores strips were used, for a total of 300 strips after the three cycles.

Table 1: Amounts of material used and sample replications, packaging material employed and storage time, in the case of the membranes.

Material			Packaging / Storage Times*	Sampling	Replication
Membrane			Plastic bags / less than 1 month*	Unit	3
			Plastic bags / 6 months*	Unit	3
			Plastic bags / 10 months*	Unit	3
Silicone Gel	Part A (Poliol)	Batch 1	Pot of 80mL	50g	3
		2	Pot of 80mL	50g	3
		3	Pot of 80mL	50g	3
	Part B (Isocyanate)	Batch 1	Pot of 80mL	50g	3
		2	Pot of 80mL	50g	3
		3	Pot of 80mL	50g	3
Mixture (Parts A+B)		Batch 1	Pot of 80mL	50g	3
		2	Pot of 80mL	50g	3
		3	Pot of 80mL	50g	3
Full textured membranes of 700mL with silicone gel - before vulcanization (Implants not vulcanized)		Batch 1	Plastic bags	Unit	3
		2	Plastic bags	Unit	3
		3	Plastic bags	Unit	3
Full textured membranes of 700mL with silicone gel - after vulcanization (Implants vulcanized)		Batch 1	Plastic bags	Unit	3
		2	Plastic bags	Unit	3
		3	Plastic bags	Unit	3
Total of Samples					54

METHOD

The bioburden was determined in the raw material, in the polymer component mixture, in the internal parts of membranes and in the implants before and after vulcanization.

The bioburden on the internal part of the membrane was determinate by introducing about 500 mL of sterile physiological solution with 0.1% Tween 80 inside the membranes, followed by agitation for about 30 minutes. Samples of 100 mL of the physiological solution from the previous step after the extration were filtered through membranes with a 0.45-µm pore size, with three replications for each sample, and submitted to microbial counting according to the USP 32 methodology.

For the silicone gel components (Part A and Part B), the mixture (Part A + B) and the implants before and after vulcanization, samples of 10 g were taken for microbial counting according to the USP 32 methodology. This method has previously been validated to guarantee reliable results.

Decreases in microbial load were evaluated by aseptically transferring strips previously submitted to vulcanization cycles to tubes containing TSB (Tryptic Soy Broth), followed by incubation for 7 days at 30-35°C. The tubes were evaluated daily for the presence of microbial growth. Prior to submission to the vulcanization challenge (10^4 to 10^8 microbial spores), the strips were evaluated to determine the number of spores using the sowing depth method, according to the USP 32 methodology.

RESULTS

Quantification of the levels of microbial load throughout production was an important component of the study because it allowed for evaluation of how the different steps of the manufacturing process contribute to increases or reductions in the bioburden of the product. Evaluation of the efficacy of vulcanization as a method to reduce bioburden was done with the goal of to contribute to reduction of the time of sterlization, thus increasing the practicality and reliability of the process, and consequently the stability of the product. As a final step, it was important to verify whether the temperature and time of the vulcanization process were effective in reducing the microbial load, as well as the number of logarithmic cycles required, using microorganisms that were resistant to the process, standardized and inoculated in their in the form of spores and in exponentially growing loads to the cellulosic supports (graded biological indicators).

The results of microbial counting for each of the steps mentioned are shown in Table 2. Those referring to the strips prior to submission to challenge (10^4 to 10^8 microbial spores) in Table 3. Those derived from evaluation of decreases in microbial load show that there was no growth verified in all tubes of the three cycles.

Table 2: Results of microbial counting in the mentioned steps.

Material			Samples	Aerobic Bacteria	Anaerobic Bacteria	Mold and Yeast
Membrane	Less than 1 month		1	ND	ND	07
			2	07	ND	ND
			3	14	20	ND
	6 months		1	ND	07	ND
			2	ND	27	ND
			3	07	40	07
	10 months		1	ND	54	ND
			2	ND	74	ND
			3	ND	34	ND
Silicone Gel	Part A (Poliol)					
		Batch 1	1	ND	ND	ND
			2	ND	ND	ND
			3	ND	ND	ND
		Batch 2	1	ND	ND	ND
			2	ND	ND	ND
			3	ND	ND	ND
		Batch 3	1	ND	ND	ND
			2	ND	ND	ND
			3	ND	ND	ND
	Part B (Isocyanate)					
		Batch 1	1	ND	ND	ND
			2	ND	ND	ND
			3	ND	ND	ND
		Batch 2	1	ND	ND	ND
			2	ND	ND	ND
			3	ND	ND	ND
		Batch 3	1	ND	ND	ND
			2	ND	ND	ND
			3	ND	ND	ND

Material		Samples	Aerobic Bacteria	Anaerobic Bacteria	Mold and Yeast
Mixture (Parts A + B)					
	Batch 1	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
	Batch 2	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
	Batch 3	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
Full textured membranes of 700mL with silicone gel - before vulcanization (Implants not vulcanized)					
	Batch 1	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
	Batch 2	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
	Batch 3	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
Full textured membranes of 700mL with silicone gel - after vulcanization (Implants vulcanized)					
	Batch 1	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
	Batch 2	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
	Batch 3	1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND

Obs.: ND – Not Detected (< 10 CFU/g)
ND – Not Detected (< 1 CFU/unit) – for referring results to the membrane
UFC – Colony-forming unit

Table 3: Results of the evaluation of the strips before submission to challenge (10^4 to 10^8 microbial spores) as for the number of spores by sowing depth method. Manufacturer of the Strips: Raven Biological Laboratories Inc.

Control Strips	Spores/strip
10^4	1,2 X 10^4
10^5	6,3 X 10^5
10^6	1,2 X 10^6
10^7	1,1 X 10^7
10^8	3,3 X 10^7
Control	1,6 X 10^6

The results obtained show that the process under study is effective in controlling the bioburden, with low microbial contamination in each of the production stages. The results obtained reveal that the heating due to vulcanization, which is applied during the production of silicone mammary implants to generate the desired product characteristics, also sterilizes the insides of the implants (gel) and inactivates up to 10^8 spores present inside the studied products.

DISCUSSION

One important parameter in the development and validation of a sterilization process is the D-value, which can be defined as the time required to reduce the initial microbial population by 1 $\log_{10}$, or 90%. After determining the D-value for the sterilization of a particular product through the use of a heating method under specific conditions, it is then possible to estimate the time necessary to reach a sterility assurance level (SAL) of 10^{-6} (Paulson, 1995). In this case, dry heat was the sterilization method chosen. A preliminary evaluation determined the amount of time needed for a sterility assurance level of 10^6. The half-cycle method was then used to calculate a period of 36 hours as being the time necessary for a sterility assurance level of 10^{12}.

The required Sterility Assurance Level (SAL) for mammary implants is 10^{-6} according to ISO 14607:2002 - *Implants for surgery – specific requirements for mammary implants* – in item 9, which is about sterilization, reports to ISO 14630:1997 *Non-active surgical implants – General requirements*. It indicates that additional care is required to effectively assure the safe use of the product.

Despite the fact that ABNT NBR ISO 11138-1 Norm dictates the placement of the biological indicator at a position of fairly difficult access for the sterilizing agent, thus mimicking a worst-case scenario, when we take the absence of contaminants inside the gel into account, the concept of a worst-case scenario is not linked to the physical issue of limited access due to poor heat transmission in silicone, but to the incidence of superficial contaminants. At any rate, the localization of the biological indicator called for by the Norm was complied with in this study.

The use of graded biological indicators makes it possible to monitor the influence of exposure time on the sterilizing agent, with microbial death being assessed in terms of the growth or lack of growth of the indicator during the considered time. If we take into account the fact that the microbial death is essentially logarithmic, and the death rate is constant under specific and constant conditions of sterilization, the initial number of spores used in the biological indicator allows for determination of the necessary exposure time to achieve the negative exponential value desired, usually 10^{-6} (Kereluk & Gaughran, 1977).

The results obtained show that the process under study is effective in controlling the bioburden, with low levels of microbial contamination observed during all production stages. The raw material used in the production of implants is incompatible with microbial life, which contributes to the low contamination levels. However, proliferation of certain microorganisms present on the surface of the material is possible, depending on their type and on the level of relative humidity. Some types of microorganisms require a minimum level of nutrients (organic matter) for growth. Water activity coupled with storage time is sufficient for their development. Membranes stored for long periods (10 months) showed higher levels of contamination, which is a sign that this stage of production requires more control despite the fact that the membranes are protected from the environment (in plastic bags), stored under adequate temperature and humidity conditions and will later be submitted to sterilization. It is important to mention that the values of microbial load found in the membranes may be related to the fact that this stage of the productive process suffers from significant levels of manipulation, with the hands of operators coming into contact with the membranes known to be a significant

source of contamination. During normal activities, the loss of skin scales occurs at a rate of about 10^4 per minute. The contaminants carried by these scales are non-pathogenic micrococci, diphtheroids and staphylococci, but they can also include *Staphylococcus aureus* as part of their normal flora. Others, like *Salmonella* and *Escherichia coli*, although not constituents of the resident flora, can occasionally be associated with it, depending on the hygienic habits of the operators (Pinto et al., 2003). Despite the fact that these microorganisms are not highly resistant to the sterilization process, their presence must be considered. It is known that the presence of Gram-negative bacteria can result in the production of endotoxins, which is not permissible in implants. Therefore, the absence of endotoxins must be confirmed through analysis.

The results obtained reveal that the heating process of vulcanization, which is applied in the production of silicone mammary implants in order to obtain the desired product characteristics, also sterilizes the insides of the implants (gel), and was able to inactivate up to 10^8 spores present inside of the product studied. This fact justifies further studies on new sterilization conditions using dry heat with reduced exposure times because the interiors of the implants are already sterile following vulcanization. Thus, great attention must be paid to the procedures used for sterility assurance with regard to the external part of the product, and they must be validated under specific manufacturing conditions.

The results showed that vulcanization is effective not only in the reduction of the microbial load, but also for ensuring the sterility of the gel inside the product. Thus, the final sterilization process contributed greatly to a higher Sterility Assurance Level (SAL), which is of interest if we take into account the tendency toward the adoption of parametric release and the concept of a combined validation bioburden/biological indicator rather than overkill. Evaluation of validation by the combined method could offer the advantage of reducing exposure times to high temperatures, thus improving the physico-chemical and functional characteristics of the implant.

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RESUMO

Análise da carga microbiana de implantes mamários de silicone

Os implantes mamários de silicone constituem-se em biomateriais que têm sido amplamente utilizados em cirurgias para reconstituição da mama ou para o aumento do tamanho da mama por motivos estéticos. Uma etapa preliminar do processo produtivo do implante é a vulcanização, que consiste no aquecimento do implante a 165±5°C por aproximadamente 9 horas. O objetivo deste estudo foi avaliar a carga microbiana dos implantes mamários de silicone antes do processo de vulcanização, o decaimento da carga microbiana neste processo e confirmar a esterilidade do gel contido internamente à membrana. Os estágios do processo produtivo dos implantes mamários foram avaliados pela contagem microbiana em diferentes etapas, de acordo com a metodologia da USP 32. Para avaliação do decaimento da carga microbiana, tiras de esporos foram introduzidas no interior do implante e após os ciclos de vulcanização foram retiradas do implante. As tiras foram transferidas para tubos contendo TSB, seguidos pela incubação por 7 dias a 30-35ºC. Os resultados obtidos mostraram que o nível de contaminação microbiana dos implantes gelatinosos é relativamente baixo e que a vulcanização possibilitou a inativação de até 10^8 esporos. Este estudo nos leva à conclusão que a vulcanização levou à esterilidade do gel interno ao produto. Desta forma, o processo esterilizante final contribuiu para um aumento no Nível de Garantia de Esterilidade.1.

Palavras-chave: Silicone. Implante mamário. Esterilização. Calor seco. Vulcanização. Biocarga.

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