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Sterilization of silicone-based hydrogels for biomedical application using ozone gas: Comparison with conventional techniques

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ABSTRACT

Sterilization of hydrogels is challenging due to their often reported sensitivity to conventional methods involving heat or radiation. Although aseptic manufacturing is a possibility, terminal sterilization is safer in biological terms, leading to a higher overall efficiency, and thus should be used whenever it is possible.

The main goal of this work was to study the applicability of an innovative ozone gas terminal sterilization method for silicone-based hydrogels and compare its efficacy and effects with those of traditional sterilization methods: steam heat and gamma irradiation. Ozone gas sterilization is a method with potential interest since it is reported as a low cost green method, does not leave toxic residues and can be applied to thermosensitive materials.

A hydrogel intended for ophthalmological applications, based on tris(trimethylsiloxy)silyl] propyl methacrylate, was prepared and extensively characterized before and after the sterilization procedures. Alterations regarding transparency, swelling, wettability, ionic permeability, friction coefficient, mechanical properties, topography and morphology and chemical composition were monitored. Efficacy of the ozonation was accessed by performing controlled contaminations and sterility tests. In vitro cytotoxicity testes were also performed.

The results show that ozonation may be applied to sterilize the studied material. A treatment with 8 pulses allowed sterilizing the material with bioburdens ≤ 10^3 CFU/mL, preserving all the studied properties within the required known values for contact lenses materials. However, a higher exposure (10 pulses) led to some degradation of the material and induced mild cytotoxicity. Steam heat sterilization led to an increase of swelling capacity and a decrease of the water contact angle. Regarding gamma irradiation, the increase of irradiation dose led to an increase of the friction coefficient. The higher dose (25 kGy) originated surface degradation and affected the mechanical properties of the hydrogel by inducing a significant increase of the Young's modulus.

Overall, the results show that ozonation may be considered as a valid and promising alternative for the sterilization of silicon-based hydrogels for biomedical applications.

1. Introduction

Hydrogels are cross-linked networks of polymers with the ability to swell water, increasing significantly their original mass [1–4]. These tridimensional structures represent an extremely versatile class of materials: they can be produced based on natural and/or synthetic polymers, with distinct properties that can be useful for a vast range of potential applications in the biomedical field [4]. Nowadays, hydrogels tend to be increasingly more complex and smart systems that can be used in devices comprising more than one special feature and functionality such as mechanical support, extended controlled drug release or stimuli-responsive capabilities [4]. For example, in the ophthalmology field, hydrogels found application in soft contact lenses (SCLs), intraocular lenses, ocular adhesives for wound repair, and more recently as in situ forming hydrogels and drug-eluting systems [1–3,5], playing different functions (e.g. correction of visual problems, protection, hydration maintenance, release of drugs). In particular, there has been a considerable raise of interest in using the well accepted contact lenses as vehicles for controlled drug release systems [6–12].

Whatever the biomedical application, it is important to assure the capability of turning the advances of the materials science in this area into actual products, in the safest and greenest possible way. This can

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bring several challenges [13]: one of the major ones is the sterilization procedure. In fact, medical device related infections are still a major problem in health care: an efficient sterilization of all materials that contact or are placed into the body is crucial to minimize the incidence of these infections [14]. Sterilizing soft biomaterials, such as hydrogels, is particularly challenging for biomedical industry [15], since these materials may be quite sensitive to heat or irradiation. The choice of the most suitable process depends on the nature and characteristics of each specific hydrogel composition, and, in a certain way, on its application.

There are several well-established terminal sterilization methods available (e.g. moist or dry heat, gamma irradiation and gas sterilization such as ethylene oxide). These methods may lead to changes on chemical, physical and mechanical properties of the materials and eventually origin the formation of toxic residues [15–18]. When selecting a sterilization method, economic aspects are also very important to keep in mind, regarding equipment, installations, training, and safety of the personal. Another essential aspect that must be considered is the need of using environmental-friendly technologies. Nowadays, there is the need for new economic, reproducible, greener and safer methods of decontamination and sterilization, that can be applied as a post manufacture final sterilization method, without compromising the materials properties and functionality [15,19].

Ozone gas treatments have emerged as an innovative technology of great potential, especially in food industry and water purification/decontamination [20–23]. Ozone gas is a potential candidate for use as a sterilizing agent of medical devices due to its well-known powerful oxidative action [24–32]. The possibility to alter different process parameters (e.g. time of exposure, gas concentration, humidity) allows to adapt this sterilization method to different types of device and material, bringing advantages over conventional methods, such as steam sterilization and gamma radiation. Since the ozone sterilization process takes place at low temperatures, it can be suitable for sterilizing heat sensitive materials. Recently, it was shown that this method was able to preserve the main characteristics of PLGA nanofibers [33].

Ozone treatment is considered a low cost, safe and green process, with no post-processing toxic residues, other than oxygen [27,33,34]. Personal safety is also ensured since a potential gas leak is easily detected due to the strong pungent characteristic gas odor, minimizing toxic exposure [27,30].

The aim of this work was to determine the possible applicability of an innovative ozone gas sterilization method for silicone-based hydrogels intended for ophthalmological applications as SCLs materials. A hydrogel containing [Tris(trimethylsiloxy)silyl]propyl methacrylate (TRIS) was prepared and used in the study. This monomer is present in the composition of several commercial SCLs materials (e.g. balafilcon A, lotrafilcon A, lotrafilcon B). TRIS is a silicon-containing hydrophobic monomer that allows increasing significantly the oxygen permeability of the SCLs materials. It is usually combined with hydrophilic monomers with polar groups that bring benefits in terms of fluid and ion transport and ensure a high degree of comfort for the wearers. The effect of different treatment conditions was assessed through an extensive characterization protocol carried out before and after sterilization. Alterations in properties such as swelling capacity, transparency, hydrophilicity, chemical structure, friction coefficient, surface topography/morphology and mechanical properties were evaluated. The effectiveness and safety of the new ozone method was assured by performing sterility tests along with preliminary in vitro cytotoxicity assays. For comparative purposes, the effect of two conventional methods (gamma irradiation and steam heat sterilization) was also studied.

2. Materials and methods

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), 2,20-azobis(2-methylpropionitrile) (AIBN), 3-tris(trimethylsilyloxy)silylpropyl 2-methylprop-2-enoate (TRIS) and dichloromethane, were all purchased from Sigma-Aldrich. Poly(vinylpyrrolidone vinylpyrrolidone) (PVP K30, KollidonVR 30) was kindly provided by BASF. N-vinylpyrrolidone (NVP) and sodium chloride (NaCl) were obtained from Merck, carbon tetrachloride (CCl_4) from Riedel-de HaeGen, and dimethyldichlorosilane ($Si(CH_3)_2Cl_2$) from Fluka. Distilled and deionized (DD) water was used for all preparations. Tryptone soya broth (TSB) was from Bacto®. Geobacillus stearothermophilus ATCC 7953 from 3 M Attest and *Bacillus pumillus* from NAMSA STP-06 were used for controlled contamination purposes. NCTC clone 929 (CCIAL 020) cell line from ATCC - CCL-1 was used for cytotoxicity assays, neutral red dye was acquired from National Aniline Division; Eagle medium from Sigma Aldrich.

2.2. Hydrogel preparation

The hydrogel TRIS/NVP/HEMA was prepared according to a previously reported method [8]. Briefly, TRIS (silicone monomer), NVP (hydrophilic additive), HEMA and EGDMA were added to prepare a mixture with concentrations of 0.94 M, 3.58 M, 1.53 M, and 30 mM, respectively. The mixture was then degassed by ultra-sounds (5 min) and bubbled with a gentle stream of nitrogen for 15 min, before the addition of AIBN (initiator) to a final concentration of 10 mM. The final mixture was injected into a mold consisting of two silanized glass plates separated by a teflon spacer. The glasses were previously silanized according to the procedure described by Vasquez et al. [35], which consisted in incubating them in a 2% solution of dimethyldichlorosilane in CCl_4 for 1 h and then rinsing with CH_2Cl_2, and drying with nitrogen. The polymerization reaction was performed at 60 °C for 24 h. The obtained hydrogel sheets were removed from the mold and washed over 5 days, with distilled and deionized water, renewed several times a day, in order to remove unreacted monomers. The hydrated samples (0.30–0.37 mm in thickness) were cut into discs with 10–12 mm of diameter, dried overnight in an oven at 40 °C and kept in a closed recipient until further use.

2.3. Characterization

2.3.1. Transparency

Transparency studies were carried out using a Thermo Scientific – Multiscan Go spectrophotometer. The percentage of the visible light (wavelength range from 400 to 700 nm) transmitted through the hydrated hydrogels was measured. For that, the hydrated samples were placed on the external surface of the quartz cuvette. Measurements were done in triplicate.

2.3.2. Swelling capacity

The swelling capacity (SC):

$$SC = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

was calculated by weighting the hydrogel sample in its dry state (W_0) and when fully hydrated (W_t). The hydration of the hydrogel was done by placing the sample in a tube with DD water (3 mL). Weight measurements were done over time till constant weight was achieved. The samples were carefully blotted with paper before each measurement. The swelling studies were carried out in water at 25 °C, being all tests performed in triplicate.

2.3.3. Ionic permeability

The fully hydrated hydrogel was mounted in a home-made diffusion cell between the donor compartment, filled with 24 mL of NaCl solution (130 mM) and the receiver compartment, containing 36 mL of DD water. The conductivity of the fluid in the receiver chamber was determined as a function of time for a minimum of 10 h, using a conductivimeter (HANNA Instruments H1763100). The conductivity