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Inhibition of fungal growth by silicones modified with cationic biocides

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ABSTRACT

Filamentous fungi are present everywhere in our environment and can grow on almost any substance in presence of moisture. Protecting the indoor environment against microbial growth remains a complex issue especially in sanitary applications or where protection against moulds is critical. Silicone samples containing 2% or 5% (w/w) of the cationic biocides polyhexamethylene guanidine dodecylbenzenesulfonate (PHMG-DBS), 1-octyl-3-methylimidazolium tetrafluoroborate (OMIM-BF₄) or 1-dodecyl-3-methylimidazolium tetrafluoroborate (DMIM-BF₄) were tested against a series of fungal species implicated in indoor fungal contamination. Our investigations were conducted according to the international standard ISO 16869 *i.e.* with a carbon-poor Nutrient-Salt-Agar (NSA) culture medium, and also with a carbon-rich Peptone-Dextrose-Agar (PDA) culture medium composed of pancreatic peptone, glucose and agar-agar as the spore suspension agar overlay. The experimental protocol using the PDA medium proved to be the most efficient procedure for discriminating, in a week only, the control analysis and those using modified silicones. Our results demonstrate that silicones containing 5% of polymeric biocide PHMG-DBS present higher antifungal activity on the filamentous fungi than long-chain imidazolium ionic liquids-containing silicones. The possible mechanisms of antifungal action of cationic biocides are discussed. The efficacy of PHMG-DBS demonstrates that such modified-silicones can prevent microbial growth and silicone deterioration by microorganisms in areas with high humidity levels such as kitchens and bathrooms as well as hospitals and microbiology laboratories.

1. Introduction

Silicones, or polyorganosiloxanes, are known for decades to be very high performance materials with an exceptional range of chemical and physical properties including very low toxicity, excellent biocompatibility, physiological inertness, high transparency, hydrophobicity, elasticity, low surface energy, and excellent dielectric properties [1]. Silicones have longer lifespan than other rubbers; they can resist harsh weather, moisture, corrosion, sunlight, pollution and chemical attacks. Their low chain-to-chain interactions lead to elastomers with very low modulus and higher tolerance to permanent stresses. Moreover, silicones have excellent heat and weathering resistance, with an operating temperature ranging from - 50 °C to 250 °C [1–4]. So, for all these reasons they have been used in thousands of products in many industrial domains, including health care, household products, paints and coatings, aerospace, electronics, transportation, restoration and construction [5–7]. For example, products made with silicones have played a key role in the advancements of telecommunications and micro- and

macro-electronics as protective coatings for highly sensitive circuits, microprocessors and semiconductors. Silicones are probably one of the most extensively studied materials and after assessing their safety, they have been largely used in the food-processing industry, incorporated for example into packages and containers to preserve food. In medical applications, medical-grade silicones are often incorporated into medical devices when they need to be flexible and portable. Silicones are ideal for external prostheses and artificial joints because they offer padding and provide maximum comfort to patients. Moreover, due to their excellent biocompatibility, silicones are used as medical tubes, artificial skin and part of artificial hearts and electronic prostheses [4,6,8].

Similarly, in the indoor environment, silicone pervades under many different forms such as in resin paint emulsions, bearing lubricants, pipe connectors, kitchen and bathroom silicone sealants, electronics and a variety of other supports. Likewise, it is becoming more and more common to find silicone products in every home as gaskets used in engine parts and refrigerators, or toys, computer keyboards, telephone

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keypads, earplugs, ear cups *etc.* Nevertheless, number of studies have reported the deterioration of such polymer supports following fungal growth [9–13]. Fungi are more resilient than bacteria to environmental stresses [14]; they produce spores that easily disperse in the air and under favorable conditions, in particular where excessive humidity and organic matter are present for an extended period of time, fungal spores can germinate, leading to the degradation of supporting materials by growing hyphae. Growth and colonization of microorganisms on the material surface can then trigger an epidemiologic risky situation that would be even more serious if a person stays in this confined space. Despite recommendations to reduce moist and aerate more, fungal contamination of the indoor environment remains a complex issue leading to specific and non-specific health problems for people with atopy, chronic pulmonary disease or defective innate immunity [15–19]. Indeed, there is now an increasing body of evidence that a significant number of health problems such as asthma, allergic sinusitis or rhinosinusitis, allergic bronchopulmonary mycoses, hypersensitivity pneumonitis and chronic respiratory infections of fungal origin results from the domestic exposure of receptive patients to fungal allergens and airborne mold spores. Medical indoor environment counselors have been recruited in the past decade to conduct environmental assessments at home of the patients when requested by pulmonologists, allergologists or general practitioners, and to propose, when required, appropriate control measures aiming to reduce the indoor exposure to fungal cells or components [20].

Protecting the indoor environment against microbial growth requires a multitude of proactive measures; one of them can be the introduction of biocides into silicone preparations, especially in sanitary applications or where protection against moulds is critical. In the last few decades, various efficient biocide solutions for silicones have been reported such as organic antibiotics [21], the cationic biocide chlorhexidine [22] and the natural antimicrobial agent Ag⁺ loaded zeolite [23–26]. The bulk modification of liquid silicone formulations with biocides followed by curing the compositions has been described as the most efficient approach from a practical point of view. However, it should be taken into consideration that silicone material network formation includes chemical reaction *in situ*, whereas antimicrobial additives may have reactive functional groups. Thus, the antimicrobial additive can impact cure speed, as well as the physicochemical properties of silicone rubber [27]. Silver ion-containing zeolite is considered as the most promising antimicrobial additive for silicone rubber owing to its broad range of activity against bacteria and moulds. Due to excellent thermal stability and chemical inertness, it can be easily dispersed in organopolysiloxane and does not affect the cured silicone rubber composition [23–26]. The ionic silver incorporated into silicone elastomers imparts their strong positive surface charge with a high affinity for negatively charged groups of microbial cells; this facilitates the contact with microbial cells and the subsequent alteration of the structure of biological molecules, resulting in the inhibition of microbial growth [28,29]. However, it should be noted that the zeolite addition to silicone rubber may significantly decrease its hydrophobicity, and the subsequent increase in water uptake initiates degradation of silicone material [24]. Moreover, silver-based biocides still have a relatively high price.

Synthetic cationic biocides based on long-chain imidazolium ionic liquids (ILs) or guanidinium-based cationic polymers may constitute valuable and cheap alternatives to common inorganic antimicrobial

agents. These compounds present numerous advantages compared to other common antimicrobial agents, including silver- and copper-based compounds. Previous studies showed that they exhibit a broader spectrum of antimicrobial activity including gram-positive and gram-negative bacteria [30–36] and fungi [37–40], and an anti-biofilm activity against a large panel of microorganisms [41,42]. The antimicrobial activity of cationic biocides relies on electrostatic interactions with the negatively charged surface of microorganisms, or hydrophobic interactions with the phospholipid bilayer of fungal cells and the subsequent penetration of biocides into the plasma membrane, which leads to an increase in membrane permeability and leakage of cytosolic components [42,43]. As for the polymeric biocide polyhexamethylene guanidine (PHMG), its antifungal activity involves pores formation within the plasma membrane of fungal cells allowing the cytosol to ooze out [40]. More importantly, compared to inorganic antimicrobials, PHMG and ILs exhibit a major advantage in that their physicochemical properties (water solubility, melting point, thermal stability) can easily be modified using appropriate counter-ions [44–48]. Due to their low melting points, they have much better dispersibility in silicone elastomers that is crucial for the preparation of homogeneous materials. Thus, long-chain 1-alkyl-3-methylimidazolium halides having high water solubility can be transformed into water immiscible ILs by anion metathesis with tetrafluoroboric acid or its sodium salt [47,48]. The anion exchange between water soluble polymeric biocide PHMG chloride and sodium salts of dodecylbenzenesulfonic acid or 2-naphthalenesulfonic acid allowed obtain PHMG compounds having limited water solubility [49,50]. Recent studies have shown that poorly water soluble long-chain ILs, as well as PHMG salts, may be successfully applied as efficient antimicrobial additives to various polymers and protective coatings [44–52]. In fact, polyamide 12 (PA-12) films modified with 2% (w/v) dodecylbenzene sulfonate-conjugated PHMG (PHMG-DBS) showed high activity against the dermatophyte *Trichophyton mentagrophytes* [44]. In addition, when incorporated in silicone rubbers at 2–5% (w/v) final concentration, the water immiscible 1-alkyl-3-methylimidazolium tetrafluoroborate ILs, as well as PHMG-DBS, were found to impart pronounced and lasting antimicrobial properties against the major bacterial and yeasts species causing medical device-related nosocomial infections [52].

In the present study, our aim was to evaluate the efficacy of commercial polydimethylsiloxane modified with cationic biocides polyhexamethylene guanidine dodecylbenzenesulfonate (PHMG-DBS), 1-octyl-3-methylimidazolium tetrafluoroborate (OMIM-BF₄) or 1-dodecyl-3-methylimidazolium tetrafluoroborate (DMIM-BF₄) in preventing growth of filamentous fungi implicated in indoor fungal contamination.

2. Materials and methods

2.1. Synthesis of cationic biocides

Synthesis of the tetrafluoroborate salts of 1-octyl-3-methylimidazolium (OMIM-BF₄) and 1-dodecyl-3-methylimidazolium (DMIM-BF₄) was performed as previously described [48,53], using the following chemicals: 1-methylimidazole (99%), 1-bromooctane (99%), 1-bromododecane (97%), tetrafluoroboric acid (48% in H₂O), methylene chloride (99.8%), ethyl acetate (99.5%) and hexane (95%) from Sigma-Aldrich (Fig. 1).

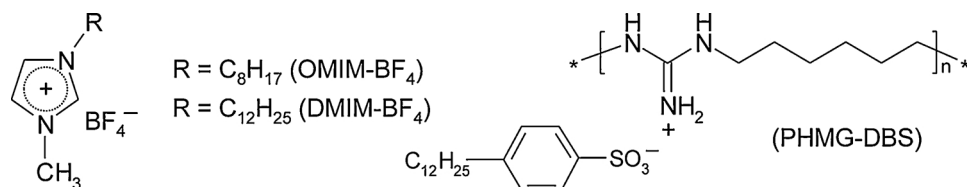


Fig. 1. Imidazolium and guanidinium-based biocides.

Hexamethylenediamine (98%) and sodium dodecylbenzenesulfonate (technical grade) from Sigma-Aldrich, guanidine hydrochloride (98%) from Applichem (Germany), and ethanol (95%) from Fluka (Switzerland) were used for synthesis of the polymeric biocide poly-hexamethylene guanidine dodecylbenzenesulfonate (PHMG-DBS) (Fig. 1) [44,49].

NMR spectra of the synthesized cationic biocides are presented in Supplemental file (Fig. S1).

2.2. Preparation of biocide-containing silicone films

Silicone films were prepared from the two-component room temperature vulcanized silicone rubber “Formasil” (Silol, Ukraine) which consists of semi-transparent liquid paste of uncured polydimethylsiloxane and ethyl silicate as curing agent. Briefly, semi-transparent silicone films containing biocides (thickness ~ 0.5 mm) were obtained by the addition of 2 or 5% (w/w) OMIM-BF₄, DMIM-BF₄ or PHMG-DBS and 5% (w/w) curing agent to the liquid paste, followed by pouring the obtained mixtures onto 94-mm diameter polystyrene Petri dishes which were incubated for 24 h at room temperature. Control samples of neat silicone elastomer were obtained by mixing the curing agent and the liquid paste without any biocide. Freshly prepared silicone samples were cut to 20-mm-diameter disks and samples did not receive any cleaning prior testing.

2.3. Fungal strains used in this study

This study was conducted using reference strains of different mold species selected among the most common filamentous fungi in the environment, based on recommendations of the international protocol ISO 16869 as well as on some previous indoor pollution-related studies [13,54,55]. The filamentous fungi *Alternaria alternata* strain IHEM 18586, *Aspergillus niger* IHEM 5296, *Byssoschlamys spectabilis* (formerly *Paecilomyces variotii*) CBS 628.66, *Cladosporium sphaerospermum* IHEM 18883, *Penicillium chrysogenum* IHEM 20859, *Stachybotrys chartarum* IHEM 20352, *Talaromyces pinophilus* IHEM 5847, and *Trichoderma reesei* IHEM 5651 were obtained from the Institute of Hygiene and Epidemiology-Myecology section (IHEM) culture collection at Sciensano (Brussels, Belgium) or the Centraalbureau voor Schimmelcultures (CBS) culture collection at the Westerdijk Institute (Utrecht, The Netherlands).

2.4. Experimental design

All strains were subcultured on malt-extract agar (MEA) for 14 days at 25 °C. Spore suspensions (10^7 to 10^8 spores/ml) were prepared according to the international standard protocol ISO 16869 except that they were filtered on 100 µm-pore size sterile nylon filters for *A. alternata* and *C. sphaerospermum*, or using 30 µm sterile filters for the other fungal species.

The antifungal activity of modified silicones was investigated using the nutrient salt agar (NSA) culture setting as described in the protocol ISO 16869, or using a slight modification of this protocol corresponding to the peptone dextrose agar (PDA) culture setting. The two culture settings (Fig. 2) were prepared as follows: a primary layer of NSA (20 ml), described in the protocol ISO 16869 and composed of iron, zinc and magnesium sulphate heptahydrate, sodium chloride, manganese sulphate monohydrate, monopotassium phosphate, disodium phosphate dihydrate, and ammonium nitrate was topped with a silicone disk and finally covered with a thin spore suspension agar overlay (16 ml, corresponding to 1–2 mm thickness). Spore suspension agars were prepared by incorporating the fungal spores to either the carbon-poor culture medium NSA described in the standard protocol ISO 16869 or the carbon-rich culture medium Peptone-Dextrose-Agar (PDA) composed of 0.5% (w/v) pancreatic peptone, 1% (w/v) glucose, 0.025% (w/v) chloramphenicol and 1.5% (w/v) agar-agar.

The NSA and PDA culture systems were incubated at 25 °C for three weeks or one week, respectively. NSA cultures then were graded from zero to two according to the rate of fungal growth over the silicone. Grade 0 meant the absence of any visible fungal growth on top and in close proximity to the silicone material, grade 1 was attributed when hyphal growth only could be seen while grade 2 was given when sporulation was also detected. PDA cultures, on the other hand, were assessed by the diameter of growth inhibition zones. Inhibition zones with a diameter greater than 20 mm overpassed the silicones surface area while inhibition zones with a diameter of less than 20 mm were localized on top of the silicone or within its perimeter.

All experiments were performed in triplicates. Diameters of inhibition zones were analyzed on GraphPad prism (version 5.04) using two-way analysis of variance (ANOVA) with Benferroni post-tests.

2.5. Characterization of mechanical and surface properties

Tensile properties (tensile strength and elongation at break) of the films were tested at room temperature using the P-50 universal tensile testing machine (Milaform, Russia). The films were cut in the dimensions of 35 mm × 5 mm × 0.02 mm and the deformation rate was fixed to 10 mm/min. The average values (with standard deviation) were obtained from three distinct measurements.

The contact angle measurements were performed on a custom-built goniometer furnished with optical accessories from Natch SA (Dijon, France). Three drops of deionized water (3 µL) with a resistivity higher than 18 Ω/cm were used to characterize the wettability of each surface. The static contact angle (with an accuracy of approximately ± 2°) was obtained by averaging the angle on both sides of the droplet.

The surface topography of the silicones-based films was obtained with a 3D white light confocal interference microscope (Sensofar PLu Neox, Sensofar Tech, Spain) equipped with a EPI 20 X (NA 0.45) objective. The measurement output is a 3D profile of the surface, with a field of view of 850 µm × 709 µm and an image size of 1232 × 1028 pixels (i.e. lateral resolution of 0.7 µm). Image processing and morphology analysis were performed with the Digital Surf's Mountains Map Premium (version 7.4 8737, Besançon, France) software.

3. Results and discussion

3.1. Mechanical and surface properties of modified silicone samples

Modified silicones containing antimicrobial additives were prepared by incorporating into the bulk resin 2% or 5% (w/v) PHMG-DBS, OMIM-BF₄ or DMIM-BF₄. Incorporation of these additives did not modify the mechanical characteristics of the material, even at the concentration of 5%. As illustrated in Fig. 3a and b, the tensile strength values remained unchanged (from 2.9 ± 0.1 to 3.2 ± 0.15 MPa) and the elongation at break (from 175 ± 16 to $210 \pm 10\%$) was typically the orders of magnitude reported by the manufacturer's for sealant applications. The last fact probably indicates chemical inertness of cationic biocides to the curing agent that did not prevent the formation of cold cured silicone elastomer. Likewise, addition of these antimicrobials affected only slightly the hydrophobic properties since the values obtained by contact angle measurements varied from $108 \pm 2^\circ$ to $111 \pm 2^\circ$ for modified silicones compared to $100 \pm 2^\circ$ for bare silicones (Fig. 3c). On the other hand, the surface roughness varied from 0.06 µm for the bare silicones to 1.2 µm for the silicones with PHMG-DBS (Fig. S2), showing that incorporation of the biocides can clearly modify the surface topography.

One of the main advantages of silicone sealants is their high heat resistance, with working temperatures ranging from - 50 to 250 °C [1,6]. Thus, the thermal stability of Polydimethylsiloxane (PDMS) samples modified with cationic biocides has been evaluated. According to data from thermogravimetric analysis, the thermal decomposition points (weight loss 5%) of the cationic biocides have similar values:

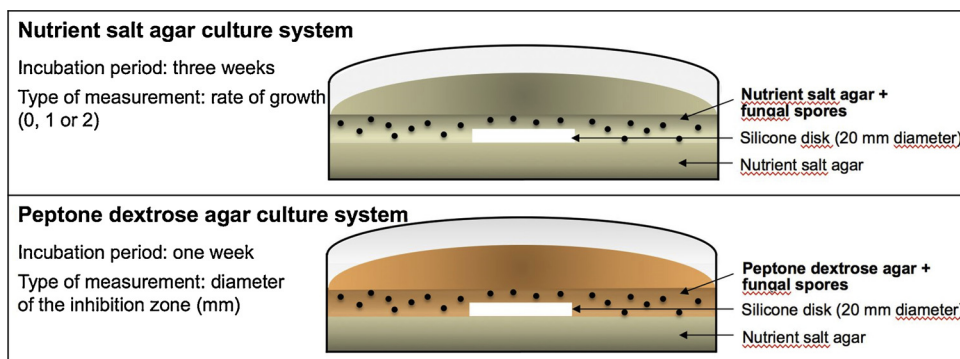


Fig. 2. Fungal cell culture systems. Various filamentous fungi were cultured using a Nutrient salt agar (NSA) culture system according to the international protocol ISO 16,869 where a silicone disk (20 mm in diameter) was maintained between two layers of nutrient salt agar and the rate of growth of fungi, incorporated into the upper layer, was recorded after three weeks of incubation. By contrast, in the peptone dextrose agar (PDA) culture system, the upper layer was replaced by a nutrient rich medium, peptone dextrose agar, in which fungi were seeded and after one week of incubation, the diameter of the growth inhibition zones around the silicone disk was measured.

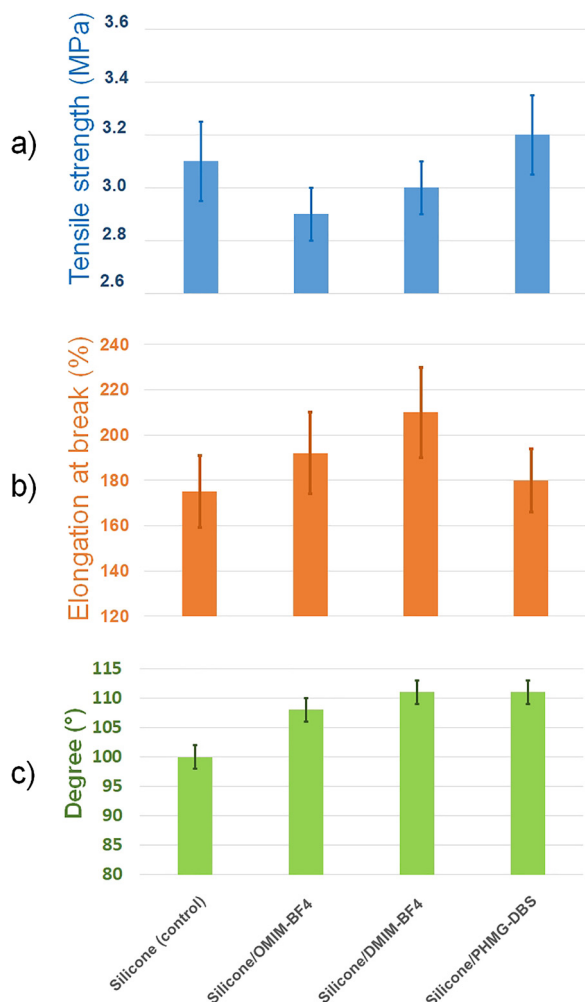


Fig. 3. Physical properties of modified silicones containing 5% OMIM-BF₄, DMIM-BF₄ or PHMG-DBS compared to bare silicone: a) tensile strength, b) elongation at break and c) static contact angle.

343, 325 and 330 °C for OMIM-BF₄, DMIM-BF₄ and PHMG-DBS, respectively. The neat PDMS is thermally stable to at least 350 °C, whereas polymer sample containing 5% of polymeric biocide PHMG-DBS begins to decompose at 308 °C (Fig. 4). Silicone samples modified with imidazolium ionic liquids showed an even more significant decrease in thermal stability, having thermal decomposition point of 254 °C (5% DMIM-BF₄) and 258 °C (5% DMIM-BF₄) (Figs. 5 and 6).

Overall, the obtained data indicate satisfactory thermal properties of modified silicone materials for common industrial applications. However, such pronounced negative impact of ionic compounds on the

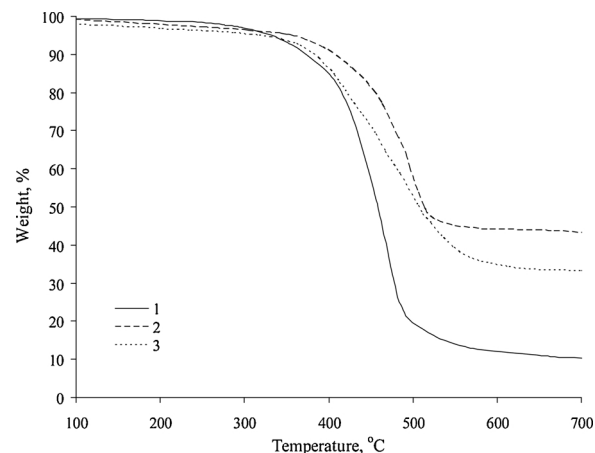


Fig. 4. TGA curves of PHMG-DBS (1), neat silicone (2) and silicone containing 5% of PHMG-DBS (3).

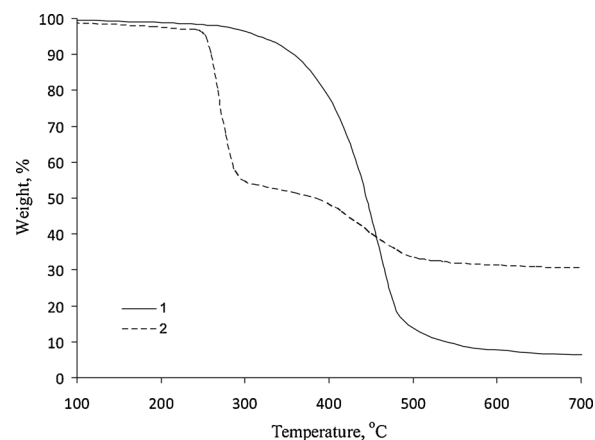


Fig. 5. TGA curves of OMIM-BF₄ (1) and silicone containing 5% of OMIM-BF₄ (2).

thermal stability of PDMS still remains unclear and needs further investigations.

3.2. Study of the experimental protocol

In a previous study [52], we have cultured yeast and bacteria in suspension on top of the silicone samples, then quantified microbial growth by a standard plate count method. Unfortunately, this technique is not suitable here for multicellular microorganisms. Other techniques could have been tested, such as cell viability tests using colorimetric, luminometric or fluorescent reagents, but these techniques only measure a mass of growth without providing any other information

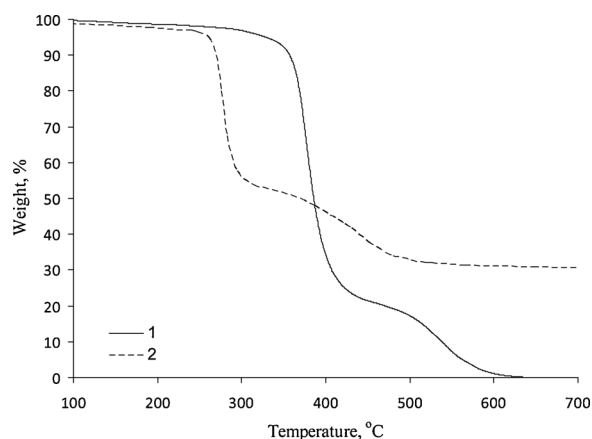


Fig. 6. TGA curves of DMIM-BF₄ (1) and silicone containing 5% of DMIM-BF₄ (2).

concerning the stage of fungal growth. Moreover, a specimen is usually in contact with a defined number of spores in suspension over long periods of time, and in such case, suspensions of low thickness would be subject to dryness. The ISO 16869 international standard protocol was therefore a good alternative because in the form of a thin gel film covering the silicone, this creates a moist environment that promotes both growth in contact with the silicone and evaluation of fungal development.

So, two culture systems were used in this study. The NSA system was prepared in accordance with the ISO 16869 protocol, and the PDA system which differed only by the composition of the overlay agar and the duration of incubation, was developed in an attempt to simulate surfaces with organic deposits and humidity promoting fungal growth.

During preliminary studies (results not shown), a third culture system was also tested, in which the spore suspension was spread on top of the NSA culture medium instead of being incorporated into the NSA or PDA overlay preparation and then the silicone was deposited on top of the spore layer. Despite the good fungal growth after a one-week-incubation period, this last system was abandoned since no difference could be observed between biocide-containing silicones and the controls, perhaps in relation with the high resistance of tested silicones to leaching [52]. This last culture system also highlights the importance of a humid cover on top of the silicones, provided in the form of a thin NSA or PDA gel (> 96% water) covering the silicones, in order to facilitate fungal growth in contact with the silicone samples. Our investigations showed that the PDA culture illustrated in Fig. 7, with the silicone embedded between two layers, was the most efficient procedure to discriminate between control and modified silicones. The NSA culture system came second because of the carbon-poor overlay which barely supported fungal growth, thus making the assessment of fungal growth less evident.

Our two culture systems both have advantages. In fact, silicone biodeterioration can result from primary or secondary microbial growth, which means that the ingredients of the silicone preparation itself can serve as nutrients for microbes (primary microbial contamination), or that the accumulation of organic materials deposited on the silicone, such as soap, skin flakes, and other organic deposits, would constitute the nutritive source for microbial growth (secondary microbial contamination). In both cases, microbial contamination of silicone rubber surfaces leads to microbial biofilm formation, staining, odors, and eventually degradation of the material. An ideal silicone formulation should be able to prevent both primary and secondary microbial contaminations.

3.3. Antifungal properties of prepared silicone samples

In this study, the PDA culture system with a silicone embedded

between two layers simulated a secondary microbial contamination setting. It was chosen for testing all silicone samples with all selected fungal species. The carbon-rich PDA overlay favored germination of fungal spores and allowed a clear demonstration of the inhibitory effect of silicone modifications as can be seen in Fig. 7. Clear areas of growth inhibition could be observed. In some cases, for example *A. niger* and *P. chrysogenum*, hyphal growth and sporulation were not equally inhibited by the biocide contained in tested silicones. In these cases, reported diameters of inhibition zones represented the smallest diameter, corresponding to the onset of hyphal growth. The PDA culture setting showed significant differences in the diameters of growth inhibition zones between silicones containing 5% PHMG-DBS (Fig. 7C) and control silicones (Fig. 7A, $P < 0.001$) with all tested fungi, and these differences remained significant for all fungal species except for *A. alternata* with a lower concentration of PHMG-DBS of 2% (Table 1).

Silicones containing 2% or 5% DMIM-BF₄ led to growth inhibition zones of 7.1 to 11.1 mm on average, respectively. This was not significantly different from those obtained with control silicones which led to inhibition zones varying in diameter from 0 up till 30 mm depending on the fungal species. Finally, silicones containing 2% or 5% OMIM-BF₄ showed nearly no growth inhibition across all tested fungi.

Based on these results, silicone containing 5% PHMG-DBS was chosen for testing with the second culture setting, the NSA culture system according to the international standard ISO 16869. This culture system simulates a primary microbial contamination setting in which the carbon-poor NSA overlay provides a humid environment in contact with the tested silicone. The aim here was to show whether fungi were capable of sustaining their growth by deriving nutrition from the silicone support. Fungal growth in this setting was much less evident even in control samples (silicone without any biocide incorporated). Results were graded from 0 to 2 according to ISO 16869 (Table 2).

Grade 2 was attributed whenever sporulation was perceived (darker areas and/or granular aspect) since conidiation is considered an advanced state of fungal growth, grade 1 was attributed in the case of hyphal growth only and grade 0 in the absence of fungal growth on top of the silicone area. Tests were performed in triplicates and the eight fungal species were tested. Apart from *A. alternata* which showed grade 1–2 growth in test silicones and grade 2 growth in the controls, the fungal growth on top of silicone samples was graded 0 with 5% PHMG-DBS - containing silicones for all the other fungi tested whereas control silicones showed grade 1 or grade 2 growth depending on the fungi. We can say here that in a humid setting, fungi were barely able to grow in the NSA system (carbon poor, three-week culture) compared to the PDA system (carbon rich, one-week culture). Yet in spite of this low growth rate in NSA cultures, we could still observe a clear difference between control silicones and PHMG-DBS - containing silicones. Incorporation of PHMG-DBS (5%) into silicones prevents the microbial contamination even in a humid carbon-poor environment.

3.4. Mechanisms of antifungal action

It should be noted that a few data have been reported concerning the mechanism of antifungal action of cationic biocides. For imidazolium ILs, a two-step inhibitory mechanism has been proposed, similar to that for current azole antifungals [38,39,56]. The first step includes crossing the fungal cell membrane by ILs. Long chain ILs possess similar amphiphilic structures as the phospholipid bilayer of fungal cell membrane that ensures easy penetration of compound to the hydrophobic regions. In the second step they act through the inhibition of the enzyme lanosterol 14 α -demethylase, a cytochrome P-450 enzyme that catalyses the demethylation of lanosterol. This leads to depletion of the plasma membrane in ergosterol that is essential for fungal growth [38,39].

The antifungal activity of 1-alkyl-3-methylimidazolium tetrafluoroborate ILs has been studied by standard disc-diffusion method against test-cultures *Candida albicans* M 885 ATCC 10231 and clinical

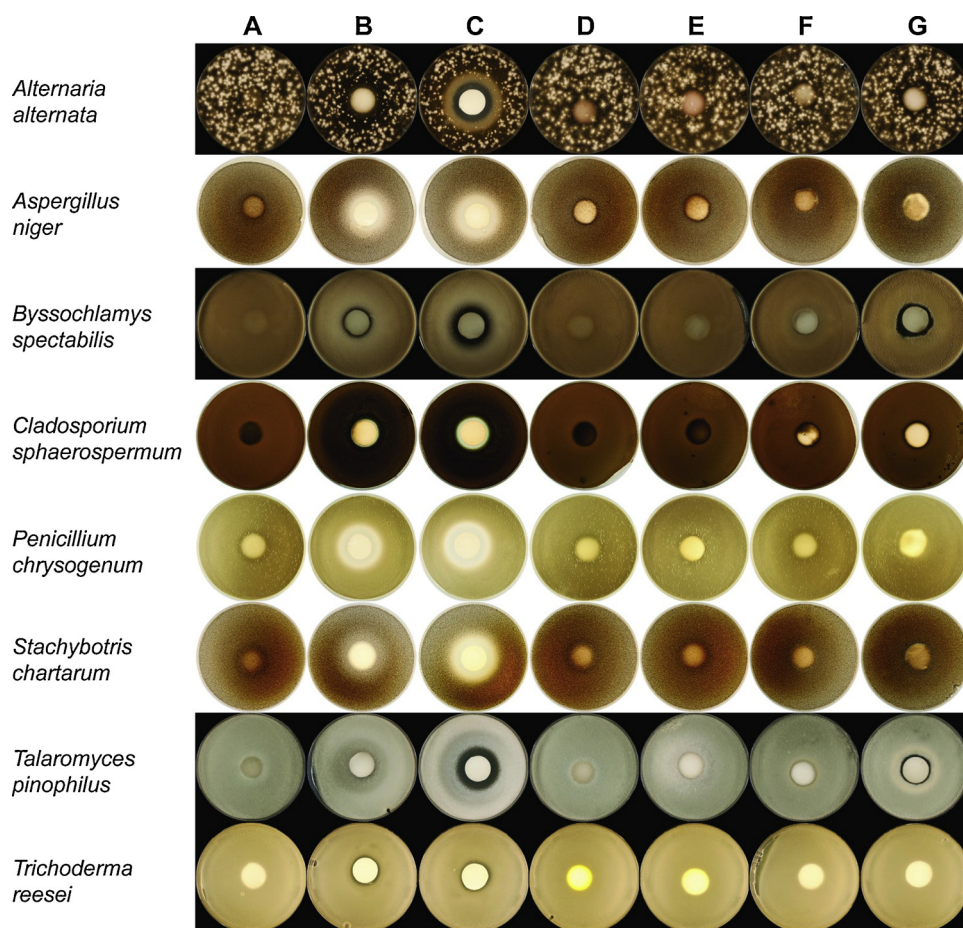


Fig. 7. Fungal growth inhibition in the peptone dextrose agar culture system. The fungal species (top to bottom) *Alternaria alternata*, *Aspergillus niger*, *Byssoschlamys spectabilis*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Stachybotrys chartarum*, *Talaromyces pinophilus*, and *Trichoderma reesei* plated in PDA culture systems with control silicones (A), or with silicones containing 2% (B) or 5% (C) PHMG-DBS, 2% (D) or 5% (E) OMIM-BF₄, or 2% (F) or 5% (G) DMIM-BF₄.

Table 1
Diameter of growth inhibition zones (in mm).

Species and strain	Control	PHMG - DBS 2%	PHMG - DBS 5%	OMIM - BF ₄ 2%	OMIM - BF ₄ 5%	DMIM - BF ₄ 2%	DMIM - BF ₄ 5%
<i>Alternaria alternata</i>	0.0	6.7 ± 11.5	24.7 ± 3.8	0.0	0.0	13.3 ± 11.5	1.7 ± 2.9
<i>Aspergillus niger</i>	0.0	20.0	20.0	0.0	0.0	2.7 ± 4.6	6.7 ± 5.5
<i>Byssoschlamys spectabilis</i>	0.0	20.0	24.7 ± 0.6	0.0	0.0	20.0	29.3 ± 0.6
<i>Cladosporium sphaerospermum</i>	0.0	22 ± 1.0	25.7 ± 0.6	0.0	0.0	3.3 ± 5.8	13.3 ± 11.5
<i>Penicillium chrysogenum</i>	0.0	24 ± 1	27 ± 1.7	0.0	1.0	0.0	11.7 ± 2.1
<i>Stachybotrys chartarum</i>	0.0	14 ± 12.3	25.7 ± 0.6	0.0	0.0	0.0	2.3 ± 4.0
<i>Talaromyces pinophilus</i>	0.0	20.0	28 ± 2.6	0.0	6.7 ± 11.5	17.3 ± 2.1	24 ± 1.7
<i>Trichoderma reesei</i>	0.0	22.3 ± 0.6	21.7 ± 1.5	0.0	0.0	0.0	0.0
Average (mm)	0	18.6	24.7	0	1	7.1	11.1

Table 2
Rate of fungal growth.

Species and strain	Control*	PHMG- DBS 5%*
<i>Alternaria alternata</i>	2 - 2 - 2	1 - 1 - 2
<i>Aspergillus niger</i>	1 - 1 - 1	0 - 0 - 0
<i>Byssoschlamys spectabilis</i>	0 - 1 - 1	0 - 0 - 0
<i>Cladosporium sphaerospermum</i>	2 - 2 - 2	0 - 0 - 0
<i>Penicillium chrysogenum</i>	1 - 1 - 1	0 - 0 - 0
<i>Stachybotrys chartarum</i>	2 - 2 - 2	0 - 0 - 0
<i>Talaromyces pinophilus</i>	1 - 1 - 1	0 - 0 - 0
<i>Trichoderma reesei</i>	2 - 2 - 2	0 - 0 - 0
Average	1.5	0.2

* Results, from 0 to 2, are shown in triplicates, with grade 0 corresponding to the absence of any visible fungal growth on top and in close proximity from the silicone material, grade 1 corresponding to hyphal growth, and grade 2 indicating that sporulation was also detected.

isolates of *Candida albicans*, *Candida glabrata* and *Candida krusei* [57]. Both OMIM-BF₄ and DMIM-BF₄ ionic liquids showed excellent antifungal activity, however, the more hydrophobic DMIM-BF₄ was also more active.

Water soluble polymeric biocide PHMG hydrochloride (PHMG-Cl) was found to have more efficient antifungal activity *in vitro* than amphotericin B, which is a commonly used antifungal drug. PHMG is suggested to adsorb to and penetrate the cell membrane, essentially composed of glucans, mannans and chitin, and to attack the lipid membrane [40]. For instance, the antifungal mechanism assay, using *C. albicans* as a model, shows that PHMG-Cl exerts its antifungal activity by forming pores in the plasma membrane, inducing membrane depolarization with K⁺ leakage which leads to cell shrinkage and ultimately cell death [40]. Thus, despite the very limited water solubility of PHMG-DBS [49], one may speculate a similar mechanism of antifungal activity. Previously reported tests on live or dead fungal cells suggested that sterile-surface polymer, which do not release biocides into the

medium, may also cause microbial membrane damage by contact-active mechanism [44,49,58–60]. Being localized into silicone matrix, the hydrophobic salt PHMG-DBS probably acts similarly to biocides chemically attached to the solid surfaces or introduced into polymer backbone, killing the fungal cells mainly at the polymer/fungal cell interface.

Overall, despite different mechanisms of antifungal activity between imidazolium-based ILs and polymeric biocide PHMG-DBS, one can suppose that the efficiency of silicone films modified with the latter is caused by the higher hydrophobicity of PHMG-DBS. Such property allows better penetration of PHMG-DBS into the hydrophobic fungal membrane core. Indeed, previously reported data have confirmed extremely high extraction resistance of PHMG-DBS from silicone film into water, whereas OMIM-BF₄ and DMIM-BF₄ showed leaching ratio values of 8.5% and 6.4% after 18 days, respectively [52].

4. Conclusion

Silicone samples containing 5% of hydrophobic polymeric biocide PHMG-DBS showed a significant activity against a series of fungal species, thus providing a very promising and effective alternative in the field of antifungal applications. These results strengthen our previous results showing an antimicrobial activity of modified films against bacteria and yeasts [52], and thus demonstrates an activity strong enough to prevent microbial growth and silicone deterioration by microorganisms in areas with high humidity levels such as kitchens and bathrooms as well as hospitals and microbiology laboratories. Further investigations are in progress for more specific applications related to the control of the indoor air quality in residential and professional buildings.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.mtcomm.2019.100716>.

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