Chapter

Novel Treatment Approach against *Candida* spp.: Evaluation of Antifungal and Antibiofilm *In Vitro* Activity of Dendritic Molecules

Natalia Gómez-Casanova, José Luis Copa-Patiño and Irene Heredero-Bermejo

Abstract

Infections caused by the genus *Candida* are a serious threat, especially in the sanitary field. These pathogens are able to generate biofilms, which is one of the main problems because they are difficult to eradicate and are associated with a high mortality rate. These biofilms provide *Candida* species with increased resistance to health care drugs and disinfectants. Currently, the resistance to antifungals is increasing gradually and there are few drugs accepted for clinical use capable of combating them, and, unfortunately, these substances are sometimes toxic at the effective doses required. Therefore, finding new molecules capable of preventing the formation of biofilms or eradicating them once generated is of vital importance. In addition, it is essential to know the appropriate techniques to evaluate a new compound, guaranteeing reliable and precise data. Studies with dendritic systems of cationic nature are recently being carried out, presenting interesting and encouraging results as antimicrobials, against cells cancer cells, surface activating agents, and encapsulation of antibiotic, among others. In this chapter, we will focus on its antifungal capacity, especially its antibiofilm activity against *Candida* spp.

Keywords: *Candida*, treatments, cytotoxicity, biofilms, microscopy, dendrimers, dendrons, dendritic compounds, resazurin

1. Introduction

In recent years, the cases of fungal infections are gradually increasing. These infections range from skin lesions to systemic infections that can lead to the death of the patient. Unfortunately, patients who are immunosuppressed or that suffered from invasive procedures are particularly affected and at risk of taking these kind of infections [1]. One pathogen of particular interest in the clinical field are the species belonging to *Candida* genus. *Candida* spp. are a group of opportunistic pathogens that are usually part of the human microbiota. Under favorable conditions, such as those mentioned above, they can invade tissues and lead to a significant infection

that can result in the death of the patients. There are two common types of *Candida* species: *Candida albicans* and non-albicans *Candida* (NAC). *C. albicans* has been the most frequent species related to fungal infections over the years and, therefore, the most studied. However, studies and infections by other *Candida* species are becoming more frequent, and even also are being isolated more frequently in the clinical environment. Among these NAC species, the most frequent are *C. glabrata, C. tropicalis, or C. parapsilosis* [2]. These pathogens are responsible for the majority of nosocomial fungal infections [3]. The mortality rate associated is around 40%, therefore, these pathogens are highly relevant to hospital environment. In addition, it is important to mention the appearance of new *Candida* species. For example, *C. auris* is associated with mortal candidemia and exhibits multidrug resistance [4].

A relevant virulence factor that some species of the genus *Candida* present is their ability to develop biofilms on diverse surfaces. These biofilms are highly organized communities of cells that are attached to biotic or abiotic surfaces and are surrounded by a self-produced extracellular matrix [5]. This condition is of special clinical relevance because these pathogens can grow and form biofilms in surgical materials, such as catheters or prosthesis. The main problem associated with these biofilms is that in this state, *Candida* cells are much more protected both from environmental conditions, antifungal exposure, and from the immune system. Consequently, treatment failure may occur and, unfortunately, on some occasions, it can cause the death of hospitalized or immunocompromised patients.

The increase in the resistance to current antifungals, their low effect against biofilms, and that they are associated with greater cytotoxicity, make it strictly necessary to find new compounds that may offer new alternatives against these pathogenic yeasts. A new alternative in the search for effective molecules for the treatment of these human pathogens is the use of dendritic systems. Among these systems, there are dendrimers and dendrons, with monodisperse branched structures with a high capacity for multifunctionalization [6, 7]. These structures serve a large number of applications, such as diagnostic agents, drug vectors, drug encapsulation, cancer treatment, antimicrobial treatment, or nucleic acid delivery, among others [8, 9]. Therefore, these molecules can be highly versatile and cost-effective. In addition, they are very interesting not only because they have antimicrobial activity by themselves, but also because they could serve as nanocarriers to increase the bioavailability of antifungal drugs with poor solubility [10, 11].

This chapter will focus on the use of different dendritic compounds as a new antifungal strategy against *Candida s*pp. biofilms. Besides, it will discuss different colorimetric methods for evaluating the biocide activity of these new compounds.

2. *Candida*: Biofilm and stages of biofilm development

Biofilms are extraordinarily complex microbial communities that are adhered to surfaces (biotic or abiotic) and surrounded by a self-produced extracellular matrix, which, among other things, provides them with protection. Biofilm formation of *C. albicans* begins with the adherence of cells to a solid surface, creating a basel layer. This is followed by a phase of cell proliferation and early-stage of filamentation of attached cells. Then, *Candida* cells can invade the surface they have been attached to. The invasion is caused by the secretion of some hydrolytic enzymes, such as hemolysins, proteinases, and phospholipases, being the most studied secreted aspartyl

proteases (SAP) [12]. Afterward, the biofilm matures, and during this phase, we can find both hyphal cells, pseudohyphae cells, and round yeast cells. During the maturation, different extracellular polymeric substances (EPS) are secreted, such as β-1,6-glucan, α-mannan, and β-1,3-glucan, among others. All these substances are part of the extracellular matrix that surrounds the set of cells that constitute the biofilm. Finally, there is the dispersion stage. This stage is particularly important and problematic because it can cause new foci of infection in a patient, worsening the prognosis and can be lethal. The release and dispersal of yeast cells from the biofilm aim to grow in a different location and generate a new biofilm [5, 13].

3. Determination of the viability of a *Candida* biofilm *in vitro*

As it is known, there are different colorimetric methods using redox indicators that allow us to determine the viability of biofilms. These redox indicators are 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl) commonly known as XTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazol named as MTT, or 7-Hydroxy-3H-phenoxazin-3-one 10-oxide commonly known as resazurin, among others. One of the problems associated with the first two viability assays is that they required other substances, such as menadione or phenazine methosulfate [14–17], to evaluate a *Candida* biofilm. On the other hand, another drawback is the use of dimethyl sulfoxide (DMSO) to dissolve the salts in the MTT assay, making it an end-point assay that does not allow to measure viability at different times from the same plate. Performing experiments with dyes, such as MTT, XTT, or similar, would force the researcher to duplicate the number of plates, reagents, and experiments (one plate to achieve the value of absorbance and the other to determine the value of plating on agar plates. These aspects will be carefully explained below). In addition, if there are different time points, more plates will be required. Therefore, the use of these end-point assays leads to a large expenditure of money, time, and resources, tremendously valuable factors in science.

On the other hand, biofilms present among their structure some persister cells, a type of cells that are resistant and do not grow or die in the presence of antifungals, presenting low or no metabolic activity that cannot be fully detected by some colorimetric methods that measure cell viability [18]. For this reason, assays using MTT or XTT could only give us the values obtained after cell metabolic reduction and the absorbance measurement. Therefore, we would be missing tremendously relevant information and we would be giving inaccurate information. However, studies carried out with the resazurin method have shown good efficacy and reliable and complete results [19–22]. Resazurin does not require additives or dissolve salts with DMSO, as is the case with the dyes mentioned above (**Figure 1**).

On the other hand, it is common to find articles that evaluate the efficacy of a compound against biofilms (eukaryotes and prokaryotes) exclusively using crystal violet. This dye stains the biofilm surface that has been generated, however, it lacks value to quantify the viability of the cells that remains viable after *in vitro* treatment. For that reason, the use of crystal violet with the aim of quantifying the biofilm biomass is interesting and advisable. This assay is adequate to determine easily and effectively the amount of biofilm, an essential approach. However, in order to assess the activity of antimicrobials, the ideal is to determine the viability percentage quantitatively using colorimetric assays (resazurin) and qualitatively growing on the agar plates using the "drop plate method" (explained below).

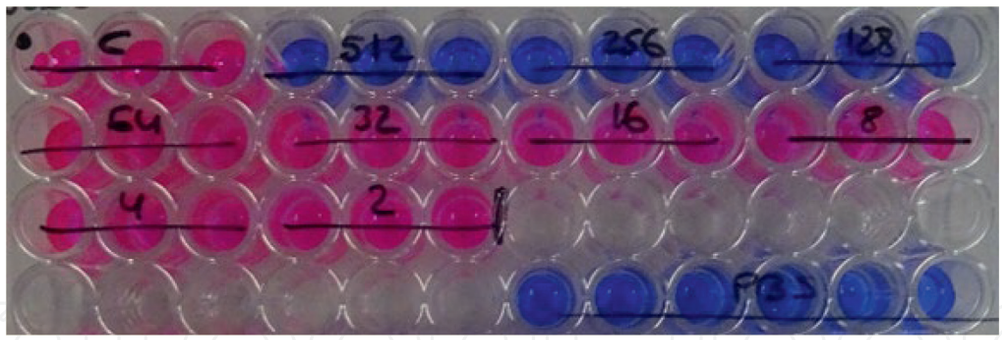

Figure 1.
C. albicans *biofilm treated* in vitro *(c: control, compound concentrations: ranging from 512 to 2 mg/L). Viability determination using resazurin after treatment with an antifungal. Viable cells (active metabolism): pink wells and non-viable cells (low or non-active metabolism): blue wells.*

4. General concepts of biofilm *in vitro* treatment and nomenclature used

The *in vitro* treatment of biofilms can be approached at two stages of their development: 1) treating and inoculating the microorganism at the same time, that is, before biofilm generation, and 2) treating the biofilm after it adheres to a surface and form a mature biofilm. Both procedures give us different results. In the first case, it would indicate the capacity of the compound to prevent the generation of a *Candida* biofilm, while in the second case; it would indicate the ability of the treatment to eliminate a mature and highly organized biofilm with complex functions. In the literature, different types of acronyms can be found to indicate the values obtained in biofilm studies. Usually, it is common to mention MBIC (minimum biofilm inhibitory concentration) and MBEC (minimum biofilm eradication concentration). However, other acronyms that provide more information on the damage caused by the compounds have recently emerged and that will be explained in detail next.

Referring to the different treatments we can differentiate studies [19–22]:

1. *Treatment to evaluate the prevention of biofilm formation*: In these studies, the microplates are washed after treatment, and resazurin is added to each well. After 24 hours, the absorbance is quantified. Subsequently, a homogeneous suspension from each well is grown on an agar medium for 24–48 hours. This will reach to obtain different data:

    - In the first step, we would obtain the value of the minimum biofilm inhibition concentration (MBIC). The minimum concentration inhibits the formation of biofilm structure, although the cells are not dead.

    - In the second step, we would obtain the value of the minimum fungicidal concentration in biofilm (MFCB). The minimum concentration completely avoids the formation of biofilm structure, because the cells are dead.

2. *Treatments to evaluate the eradication of established biofilms:* In this case, the microplates with previously established biofilms are washed after treatment, and resazurin is added to each well. After 24 hours, the absorbance is quantified. Subsequently, a homogeneous suspension from each well is cultured on an agar medium for 24–48 hours. This will reach to obtain different data:

- In the first step, we would obtain the value of the minimum biofilm damaging concentrations (MBDC). The minimum concentration damages cells by disrupting an established biofilm, though they may not all be dead.

- In the second step, we would obtain the value of the minimum biofilm eradicating concentration (MBEC). The minimum concentration completely kills the microbial cells of an established biofilm.

The data obtained by this method and in this way, allow us to consider even the persister cells that are not assessed with other methods due to the inefficiency of the technique. The MFCB and MBEC values allow us to determine 100% cell death if no colonies grow on the agar plates. Therefore, the results obtained with the colorimetric assay, such as resazurin or other dyes, should never be independent of the culture on agar plates to determine these values.

5. Dendritic cationic compounds

The problem associated with the high rate of resistance against commercial antifungals is a real fact and threat in the hospital setting. These resistances are mainly due to the inadequate and massive use of these antimicrobials [23, 24]. Besides and unfortunately, the number of antifungals approved for clinical use is relatively limited, and the required doses are frequently associated with high toxicity [25]. Therefore, it is crucial to find compounds capable of eradicating *Candida* cells, not generating resistances, and not being cytotoxic.

In this regard, cationic dendritic systems are of special interest and are actively studied due to their attractive characteristics and properties. The hydrophobic/hydrophilic balance of these systems is determined by increasing dendritic generation and, in consequence, it determines how they interact with the cell membranes [21, 26]. Among these kinds of molecules, two main types can be differentiated: *dendrimers* and *dendrons*. Both belong to the family of dendritic polymers of controlled synthesis and monodispersity. Dendrimers are globular macromolecules formed by a central nucleus, formed by one or more atoms, from which branches emerge from which other ramifications can grow, creating radial layers called dendritic *generations*. Finally, on the periphery are the terminal functional groups [9, 27]. On the other hand, dendrons are the elementary unit of dendrimers that have a focal point from which branches grow (**Figure 2**).

The main dendritic families selected for studies in the biomedical field are poly (amino amide) (PAMAM), poly(propylamine) (PPI), poly(phosphorhydrazone) (PPH), poly(L-lysine) (PLL), carbosilane, and polyester dendrimers [9]. In this chapter, we will focus on the carbosilane dendritic family. The structure of carbosilane dendrimers is defined by carbon-carbon and carbon-silicon bonds. These molecules exhibit flexible, nonpolar, and thermally stable properties. In addition, they are usually formed by polar groups to favor their solubility.

The use of these dendritic systems has been studied in both gram-negative and gram-positive bacteria [28–31], including planktonic phase and biofilms [20, 22, 32, 33]. Its efficacy has also been evaluated in eukaryotes, such as yeasts (*Candida* spp.) and protozoa. Treatments against amoebae both in the trophozoite and cyst phases have been registered [34–38]. But in addition, its effectiveness against viruses has also been reported. Anionic carbosilane dendrimers have been shown to significantly

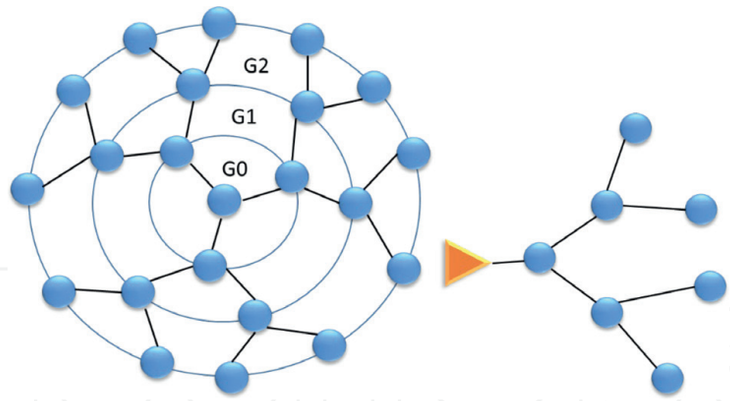

Figure 2.
Schematic representation of a dendrimer (left) and a dendron (right).

inhibit X4-HIV-1 infection [39]. In cell cultures, the effect of polyanionic carbosilane dendrimers has also been seen to prevent infection by the hepatitis C virus [40]. They have even been suggested as a possible strategy against SARS-CoV-2 [41]. Therefore, the use of dendritic systems is highly versatile and with promising results in these areas. On other occasions, dendrimers are associated with antimicrobials to improve their characteristics. For example, interesting results have been observed associating PAMAM dendrimers with amphotericin B to improve its toxicity and solubility [10].

6. Treatment of *Candida* biofilms with cationic dendritic compounds

The use of these dendritic compounds has been reported against both planktonic cells and biofilms. The *in vitro* biofilm studies are more complex, laborious, and critical. Besides, preceding biofilm treatment, the ideal is to test the production of biomass with dyes, such as crystal violet; therefore, verify the optimal conditions for its generation. In fact, it is important because it is not the same to treat a biofilm in formation, a mature biofilm, or an old biofilm.

The cell death caused by these dendritic compounds occurs by the interaction between the charges of the compounds with the cell membranes, destabilizing them. For this reason, the most frequently used dendritic compounds against bacteria and yeasts are the cationic systems because they have greater affinity; therefore, have much better activity again microorganisms. This fact has been achieved by different authors [42].

6.1 *In vitro* treatment with cationic dendrimers

In a study published in 2020, a dendrimer called BDSQ024 (**Figure 3**) was used against biofilms formed and in the formation of the *C. albicans* strain from the Spanish Type Culture Collection (CECT) 1002 and a clinical isolate of *C. albicans* from the Príncipe de Asturias University Hospital (Madrid, Spain) [19]. This highly stable dendrimer was a generation 0 compound with a tetrasiloxane core ([SiO] 4). From its nucleus grows four branches with -NMe_3^+ terminal groups that correspond to the periphery of the molecule. The BDSQ024 dendrimer showed antifungal activity, preventing the formation of *C. albicans* 1002 biofilms at 16–32 mg/L (MBIC) after 48 hours of treatment. In addition, it also presented a promising MBDC value of 16

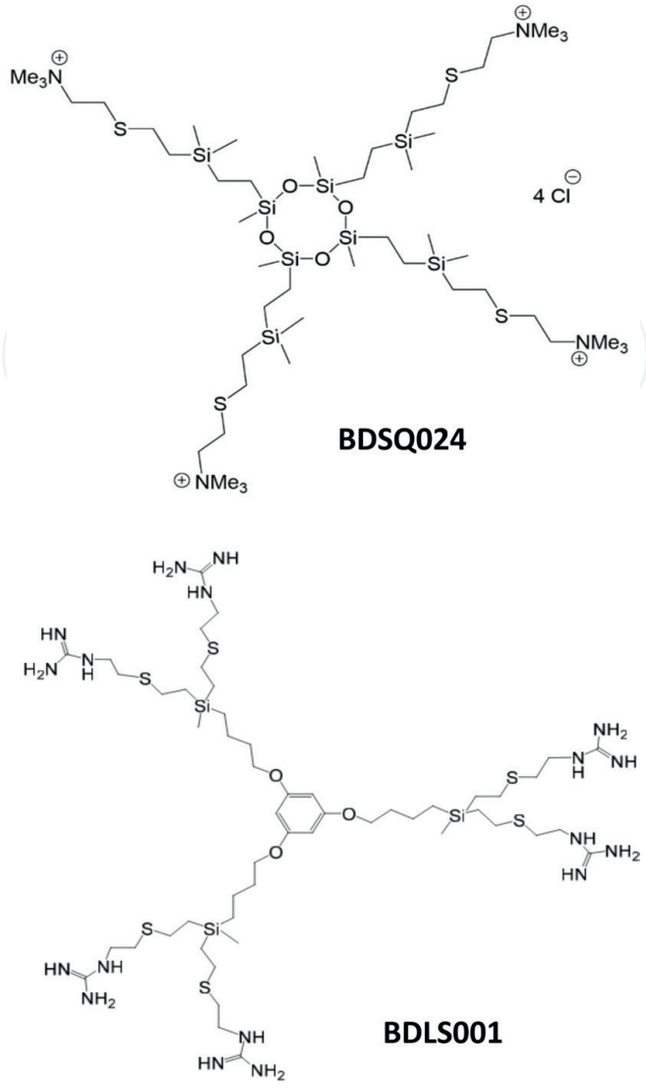

Figure 3.
Schematic structure of BDSQ024 dendrimer and BDLS001. Images have been obtained from the manuscript of [19, 43].

mg/L. However, it was not able to eradicate all the cells of a previously formed biofilm (MBEC). Notably, although the BDSQ024 dendrimer has greater activity against the clinical strain of *C. albicans* (MBIC value of 8 mg/L) than the one obtained against the CECT strain, the results of the MBDC and MBEC values were the same for both strains after 48 hours of treatment. Additionally, it was found that the addition of fresh compound after 48 hours of incubation did not improve its activity after incubating for another 24 hours (72 hours of treatment). In this article, it was also found another compound that was effective in preventing the formation of biofilms of *C. albicans* at 32 mg/L (MBIC). The compound was named BDLS001 and presented $C_6H_3O_3$ in its core and $(NHC(NH)NH_2)_6$ (6 Guanidine) as functional groups (**Figure 3**). The (BDLS001) dendrimer was tested in other studies against planktonic cells of *Escherichia coli*, *Staphylococcus aureus*, a strain of multi-resistant *S. aureus* (MRSA), and *Acanthamoeba polyphaga* trophozoites [43]. The results showed a clear efficacy against these microorganisms, both eukaryotes and prokaryotes. These data show the broad spectrum of this compound. In other studies, the antifungal and antibiofilm activity of oligostyrylbenzenes, poly(phenylene) vinylene dendrimers,

was also evaluated against *C. tropicalis*. Same as observed with other dendrimers, the cationic ones showed better antifungal activity. The compounds had antibiofilm activity, referring to the compound in the article as "compound 2" [42].

6.2 *In vitro* treatment with cationic dendrons

Interesting results of dendrons inhibiting the formation of *C. albicans* biofilms have also been reported in the literature, as is the case with compound YF017 (**Figure 4**) [19]. The dendron presented a maleimide group at the focal point and had positive charges on the surface (4 NMe_2H^+). This compound presented a MBIC and MFCB value of 32 mg/L. In addition, it has been evaluated against *S. aureus* biofilms. The results were somewhat worse than against *Candida*, detecting a MBIC value of 64 mg/L and a MFCB value of 128 mg/L [20]. Some of the dendritic compounds have been synthesized using existing compounds (non-antimicrobials) as molecules to place at focal point positions. In this way, we find molecules, such as carbosilane cationic dendrons with 4-phenylbutyric acid (PBA) located as the focal group. In a reported study, three compounds with PBA focal group were tested against *Candida* [21]. The $ArCO_2G_2(SNMe_3I)_4$ (**Figure 4**) dendron was the most active in preventing

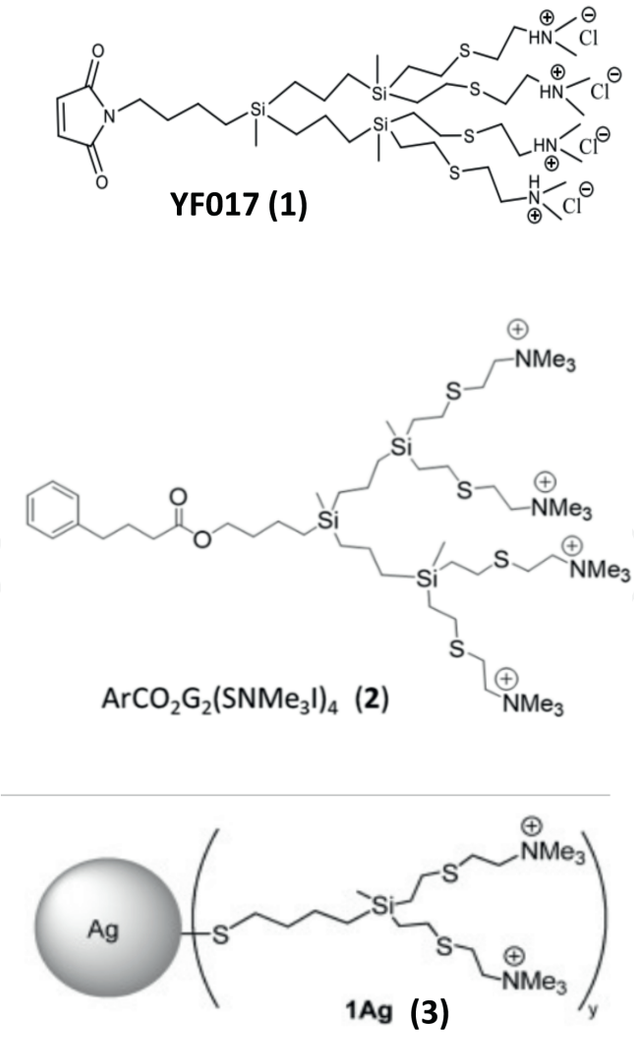

Figure 4.
Schematic structure of YF017 dendron (1) and $ArCO_2G_2(SNMe_3I)_4$ dendron (2), $AgNP(SG_1(S\text{-}NMe_3^+)_2$ (1Ag) (3). Images have been obtained from the manuscript of [20, 21, 28].

the formation of the *C. albicans* biofilm (MBIC value of 16 mg/L) and severely damaged a previously established biofilm (MBDC value of 64 mg/L). The beneficial effects of this compound against a NAC pathogenic species, *C. glabrata*, were also observed (data not shown).

6.3 *In vitro* treatment with dendron functionalized nanoparticles

The use of silver or gold nanoparticles to be functionalized with dendrons is another approach currently under study. The use of silver as an antimicrobial agent is well known. Also, the good activity of silver nitrate against biofilms [21] has been observed. Therefore, the good efficiency that a compound that presents these metals bound to dendritic systems would have to be expected. However, finding the perfect combination is complicated. Currently, dendronized silver nanoparticles (AgNPs), coated with cationic carbosilane dendrons, have been synthesized. Its activity has been tested against bacteria and yeasts. AgNP(SG_1(S-NMe_3^+)$_2$ (1Ag) (**Figure 4**) was highly active against *C. albicans* and *C. glabrata* with an MIC value of 1.8 mg/L in planktonic cells [28]. In another study, functionalized silver nanoparticles were also used: HSG1($SNMe_3^+$)2:HSHPEG (ratio 1:1), HSG2($SNMe_3^+$)4:HSHPEG (ratio 1:1) and HSG3($SNMe_3^+$)4:HSHPEG (ratio 3:1) [19]. None of the three compounds presented a MBIC value lower than 128 mg/L against *C. albicans* biofilm. Other interesting molecules are gold nanoparticles (AuNP), which can be also homofunctionalized with dendrons or heterofunctionalized, for example, with carbosilane cationic dendrons and (polyethylene) glycol (PEG) ligands [44]. Other paper reported a MIC value for *C. albicans* and *C. glabrata* of 47.6 mg/L using AuNP(SG_3(S-NMe_3^+)$_8$) (3Au) [28].

6.4 Synergistic studies between dendritic compounds and other molecules

In the clinical field, the use of antimicrobials in combination (combine therapy) is an interesting alternative, especially to overcome resistances or avoid the generation of resistance, and reduce the concentration of cytotoxic compounds. For this reason, many studies are carried out to find appropriate combinations that allow the reduction of the effective concentration of the drug to be administered. Synergy studies can determine whether there are synergistic, additive, antagonistic, or indifferent effects. The effect is synergistic when the activity of the two compounds together is much greater than when they are administered individually.

Synergistic studies have been carried out between dendritic systems and commercial antifungals, such as amphotericin and caspofungin. Amphotericin's mechanism of action is based on its binding to yeast membrane sterols, affecting membrane permeability and ultimately cell death. On the other hand, caspofungin inhibits the synthesis of beta (1,3)-D-glucan, a component of the yeast wall. In a study of the prevention of biofilm formation of *C. albicans*, three synergistic effects were determined using the dendrimer BDSQ024 (**Figure 3**), and these two mentioned antifungals [19]. In the case of amphotericin, a synergistic combination was achieved using the dendrimer at 8 mg/L and the antifungal at 0.06 mg/L. The results with caspofungin were even better. In this case, two synergistic effects were determined, the first using 8 mg/L of BDSQ024 dendrimer and 0.007 mg/L of caspofungin, and the second case, using 4 mg/L of BDSQ024 dendrimer and 0.03 mg/L of caspofungin. When the previously established biofilms were treated in a dendrimer-antifungal combination, the MBEC value was not reduced, however, the growth on agar plates was notably reduced with respect to the individual treatment.

Other studies reported the use of different substances to treat biofilms of *Candida* spp. In the combined therapy studies using $ArCO_2G_2(SNMe_3I)_4$ (**Figure 3**) and silver nitrate ($AgNO_3$), it was possible to reduce the viability of a biofilm in the formation of *C. albicans* to 11.6% when treated with the combination 8 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 4 mg/L of $AgNO_3$ [21]. In addition, it was also possible to determine a suitable combination capable of eradicating a previously established biofilm using concentrations of 32 mg/L $ArCO_2G_2(SNMe_3I)_4$ and 32 mg/L of $AgNO_3$ (MBEC). In this case, cell destruction was verified by scanning electron microscopy (SEM) (**Figure 5**). SEM approach is an interesting alternative that allows us to corroborate our results and to know the real damage that has been caused and how the compound affected the cell morphology or the cell membrane. Besides it also allows us to evaluate the thickness of the biofilm and the presence of cell dispersion.

Other combinations have been used, for instance, with ethylenediaminetetraacetic acid (EDTA), a chelating agent [21]. Combination therapy reduced the viability of a forming *C. albicans* biofilm to 24% using 8 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 32 mg/L of EDTA. Additionally, satisfactory results were found in the treatment of previously established biofilms. Total biofilm eradication (MBEC) was achieved using 256 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 16 mg/L of EDTA. The viability of a previously established biofilm was even reduced to 42.6% using 32 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 16 mg/L of EDTA.

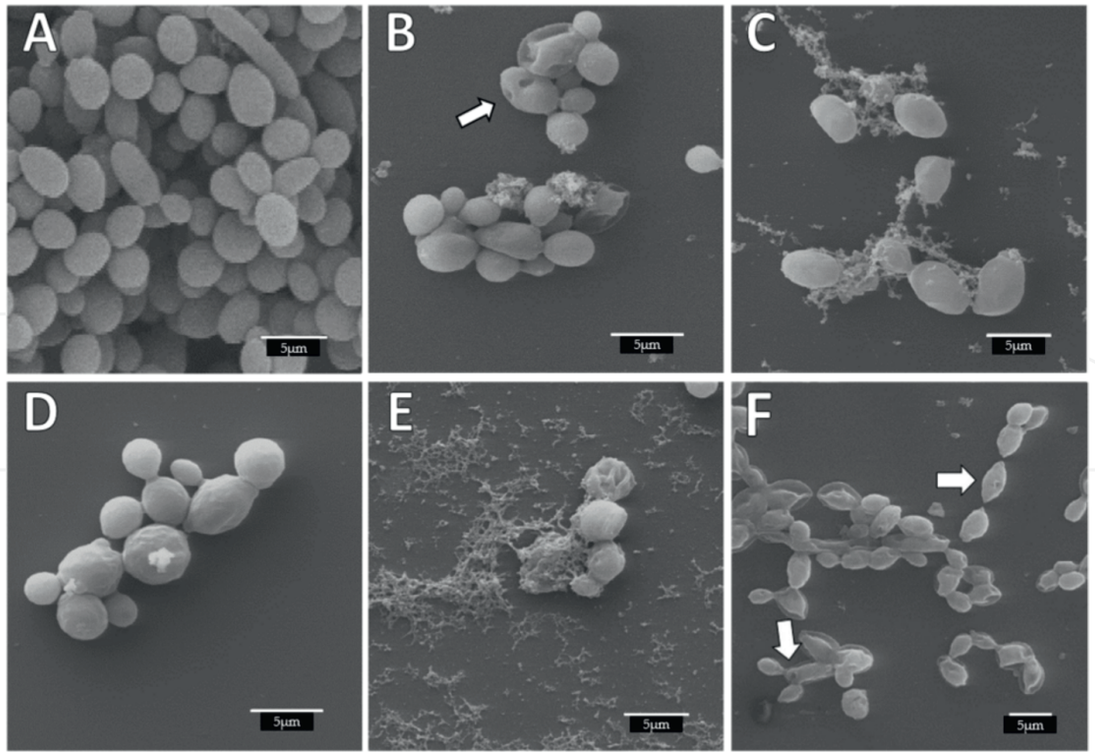

Figure 5.
Evaluation of cell damage of an established biofilm of C. albicans *treated with a dendron, $AgNO_3$ and EDTA observed by scanning electron microscopy (SEM). (A) Untreated; (B) 256 mg/L $ArCO_2G_2(SNMe_3I)_4$ (2) dendron; (C) 32 mg/L $AgNO_3$; (D) 256 mg/L EDTA; (E) 32:32 mg/L combination $ArCO_2G_2(SNMe_3I)_4$ (2):$AgNO_3$; (F) 256:32 mg/L combination $ArCO_2G_2(SNMe_3I)_4$ (2):EDTA White arrows: collapsed cells. SEM images were obtained from a previous study [21].*

7. Cytotoxicity, a key element

Finding new compounds with antifungal activity is a complicated task, especially, finding non-cytotoxic compounds. For example, the commercial antifungal amphotericin B is fungicidal with wide activity, however, it is toxic, affecting the kidney and the central nervous and hematopoietic systems [45]. The cytotoxicity of dendritic systems is often generation dependent, increasing the cytotoxicity with the generation. In this case, it also affects the number of groups on the surface and whether they are anionic, neutral, or cationic compounds. Positively charged dendrimers tend to show greater cytotoxicity [46, 47]. Despite this, it is possible to find references for effective compounds against microorganisms, especially eukaryotes, with a cytotoxic effect below the MIC and/or MBIC values. Furthermore, adding PEG ligands to dendritic systems structures may solve or improve the cytotoxicity problem as these ligands have been shown to improve biocompatibility as well as solubility [22, 44, 46]. Another alternative to reduce cytotoxicity is by using synergistic combinations of different compounds. These combinations will allow treatment of microorganisms at lower concentrations; therefore, the cytotoxicity will also decrease [19].

8. Conclusion

The generation of biofilms of *Candida* species is a major social problem, especially in clinical and hospital settings, and associated with immunocompromised patients. Therefore, finding new compounds capable of preventing the generation of biofilms and/or eliminating the viability of an established biofilm is strictly necessary. The use of new techniques, such as the resazurin colorimetric method, may allow us to evaluate *in vitro* the viability of a biofilm and to eliminate large costs in its process, providing reliability and security for the data obtained. Finally, the dendritic systems present a high activity against *Candida* biofilms. The summarized results are very promising, their high solubility in water and their low cytotoxicity make them a very interesting alternative as antifungals.

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Conflict of interest

The authors declare no conflict of interest.

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