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Influence of bioactive metal fillers on antimicrobial properties of PA12 composites produced by laser-based Powder Bed Fusion of Polymers

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Abstract

Purpose: This study investigated the influence of three types of metallic microfillers, spherical silver and spherical, and dendritic copper, on the ability of polyamide 12 (PA12) to inhibit microorganism growth on the surfaces of samples produced using laser-based powder bed fusion of polymers (PBF-LB/P). The aim of this study was to initially characterize these materials regarding their potential applicability for parts dedicated to use in the hospitals, which surfaces are periodically disinfected using chemical and/or physical measures.

Methods: Composite powders with filler concentrations of 0.5%, 1%, 2%, and 5% by weight were prepared using the mechanical mixing method and processed using PBF-LB/P. Three common hospital pathogens responsible for healthcare-associated infections: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* were tested. Additionally, the safety of the composites was assessed through in vitro tests using human cell lines: keratinocytes and fibroblasts.

Results: The research reveals that addition of copper or silver causes decrease in bacterial colony viability when compared to the material without a filler, but an insignificant effect on antifungal properties. There was no significant impact within the tested range of filler's content on the antibacterial properties. Furthermore, a strong effect of the microfillers on tested material's toxicity is observed.

Conclusions: The addition of metallic microfillers enhances the antibacterial response of polymeric materials processed with PBF-LB/P. Nevertheless, the observed varying levels of cytotoxicity toward eukaryotic cell lines underscore the need for further studies on the analysed materials to unequivocally determine their potential applicability as materials for short-term contact with human skin in a hospital setting.

Keywords: laser-based powder bed fusion of polymers, polyamide 12, cytotoxicity, antimicrobial, composites

1. Introduction

Laser-based Powder Bed Fusion of Polymers (PBF-LB/P) belongs to a broader category of manufacturing methods known as additive technologies (AM). These methods allow fabrication of physical objects from various materials, such as plastics, metal alloys, and ceramics. They utilise physical phenomena, such as gluing, sintering, melting, and polymerization to selectively combine these materials. Material addition is typically performed layer-by-layer, in a cyclic manner. The operation of these devices is guided by digital documentation, which often takes the form of three-dimensional models. The important point

to be stressed is that there has been a notable trend towards using AM to produce high-value, end-user products [38].

The COVID-19 pandemic highlighted that AM is a suitable solution for supplying medical equipment and devices in large quantities [6]. In response to the COVID-19 pandemic, healthcare facilities have significantly intensified their disinfection protocols, also focusing on high-touch surfaces, such as door handles [4]. Recognizing these surfaces as potential vectors for microbial transmission, many hospitals treating COVID-19 patients have implemented rigorous cleaning schedules, often disinfecting these critical touchpoints every few hours. This increased frequency of disinfection represents a proactive approach to minimize the risk of cross-contamination and control the spread of microorganisms within hospital environments. The effectiveness and implications of these enhanced disinfection practices are crucial areas of investigation in the ongoing battle against COVID-19. Therefore, materials with antimicrobial properties provide added value. They should provide antimicrobial protection between time-points (2-4 hours) when disinfection by means of UV radiation and/or chemical agents are performed. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* are prevalent hospital pathogens, responsible for a wide range of infections, that include the lungs, circulatory system, stomach, skin, and soft tissues - including chronic wounds, especially in patients with a compromised immune system [15,20,33,41]. A substantial number of patients with COVID-19 succumb to subsequent complications due to bacteria, such as *Pseudomonas*, *Staphylococcus*, *Klebsiella*, or from *Candida* fungi, leading to complications such as pneumonia [21]. Polymers generally lack intrinsic antimicrobial properties, with some exceptions, the most notable being chitosan [13,19,21,36]. To imbue these properties, modifications to the polymer matrix or the surface of the finished product are required. Several methods can achieve this, including chemical alteration of the polymer, inclusion of organic, or inorganic compounds, and surface modification through coatings or adjustments to its spatial structure [22,24,25]. Given this, the antimicrobial activity of polymers is desired, but limited. Antimicrobial properties of polymer parts can be utilized to reduce the risk of transmission of pathogens with a high potential for complications in immunosuppressed patients.

A relatively new approach is the use of antibacterial materials in additive technologies. The increased availability of antimicrobial materials was particularly evident in feedstock materials dedicated to the material extrusion group [42]. The material portfolio of several manufacturers has expanded to include feedstock polymers in the form of composites with a matrix based on thermoplastics, such as PLA, TPU, or PET-G. For filaments, antimicrobial properties are conferred by bioactive fillers such as titanium, zirconium, or magnesium oxides,

and metallic fillers, such as copper or silver, often on carriers (such as Al_2O_3). In most instances of commercial antimicrobial filaments, a nano-sized filler is used. It is usually combined with a ceramic carrier that facilitates its proper dispersion inside the feedstock material [27]. However, in the less diverse market of materials dedicated to the PBF/P group, powders with antimicrobial properties are not yet commercially available.

Despite robust research activity on composites, to date, only a handful of teams have conducted preliminary studies on methods to produce materials with antimicrobial properties tailored for PBF-LB/P. In two investigations, the approach involved altering the polymer matrix with an inorganic compound [35, 37]. Turner et al. used a commercially available antibacterial filler, B65003 (Biocote, Coventry, England), which is a silver-infused phosphate glass [35]. The employment of fillers with irregular shapes no larger than 40 μm, allowed mechanical mixing to be used as a method for the preparation of composite powders. The study evaluated the influence of a 1 wt% additive on mechanical, and antimicrobial properties, as well as cytotoxicity. The antibacterial efficacy of the B65003 additive is attributed to silver, arising from direct interactions between silver ions released, and cellular components (proteins, membranes, DNA), or indirect damage caused by reactive oxygen species, such as hydrogen peroxide [39,40]. Vilardell et al. also leveraged the antibacterial characteristics of metals, specifically copper microparticles measuring 60 μm in diameter [37]. The PA12-based feedstock material was crafted using a mechanical mixing method with two distinct filler quantities (10 wt% and 20 wt%). Mechanical and thermal properties, surface quality, and internal structure were evaluated. The research did not evaluate the impact of incorporating copper filler on the cytotoxicity of the material. In addition to antimicrobial properties, metal fillers, with a content greater than 5 wt%, can be used in PBF-LB/P as electronic circuit carriers [3], agents that improve thermal conductivity [16], electrical conductivity [25], or the wear behaviour of polymer composites [26].

In this study, we continue the evaluation of PA12 composites with metallic microfillers [11]. The previous publication confirmed that the addition of metallic fillers, up to a weight ratio of 5%, does not significantly affect the mechanical properties and microstructure of parts fabricated using PBF-LB/P. This research examines for the first time the effect of spherical silver, spherical copper, and dendritic copper fillers at low concentrations (0.5 wt%, 1 wt%, 2 wt%, and 5 wt%) on the antimicrobial and cytotoxicity responses of samples additively fabricated from PA12 composites. The objective of the study was to initially assess the applicability of the analysed materials regarding their future performance as hospital door

handles and other surfaces that are periodically sterilised by means of other (chemical and physical) measures.

2. Materials and methods

2.1. Composite powders preparation

The mechanical mixing method was used to prepare composite polymer powders. The mixtures were based on polyamide 12 (PA2201, EOS GmbH, Krailling, Germany) with three different types of metallic microfillers [11]: spherical silver (KGHM Polska Miedź S.A., Lubin, Poland), spherical copper (ECKA Granulate Velden GmbH, Velden, Germany) and dendritic copper (WARCHEM Sp. z o.o., Warsaw, Poland) (Tab. 1). Each filler was mixed with the base material in 4 weight concentrations containing 0.5%, 1%, 2%, and 5% of the additive. To prepare the mixtures, a CapsulCN V-1 device (Capsulcn International Co., Ltd., 51 Kaifayilu, Rui'an, Zehjiang, China) equipped with a V-shaped container was used to mix powder materials. Each powder composition was mixed for 90 minutes at a speed of 20 rpm. The resulting mixtures had a uniform colour without visible agglomerations of the filler. The homogeneity of metallic filler distribution was previously tested and confirmed to be fair or good [11].

Tab. 1. Particle size distribution of a silver (Ag), spherical copper (Cu(S) and dendritic copper (Cu(D)) microfillers as 10-th, 50-th and 90-th centile of particle diameter [10].

Material	d10 [µm]	d50 [µm]	d90 [µm]	(d90-d10) / d50	<10 µm [%]
Ag	36.42	62.31	90.78	0.87	0.00
Cu(S)	16.52	39.41	63.72	1.20	3.59
Cu(D)	10.28	28.00	59.84	1.77	9.44

2.2. Fabrication PBF-LB/P of PA12 composites

A total of 13 different powder compositions (1 neat PA12 and 12 composites) were used to manufacture samples for biological tests. The samples were cylindrical in shape and had dimensions of ø10 mm x 2 mm (Fig. 1a). The sample manufacturing process was carried out using Formiga P110 (EOS GmbH, Krailling, Germany) equipped with a small build chamber with dimensions of 60 mm x 60 mm. Each manufacturing process consisted of 30 cylindrical samples built in horizontal orientation (defined as XYZ in ISO/ASTM5292), as presented in Fig. 1b. The layer thickness of 0.1 mm and standard parameters dedicated to the PA2201 material (EOS scanning style, which corresponds to parameters listed in Tab. 2.) were used. The preheating temperature in the build chamber was set at 171°C and 151°C in the removal chamber. The filler content in the samples was measured in a previous study. Reconstruction

images obtained with computed tomography (XCT) allowed to assess the content of a filler and its distribution in the polymer matrix [11]. The measured content was different from the theoretical ones, which can affect antimicrobial properties and cytotoxicity, which is why the measured content is listed in the Tab. 3. Furthermore, the distribution of the fillers was evaluated, and can be described as good or fair, excluding the Cu(S) series, where the filler particles tend to sediment during the manufacturing process.

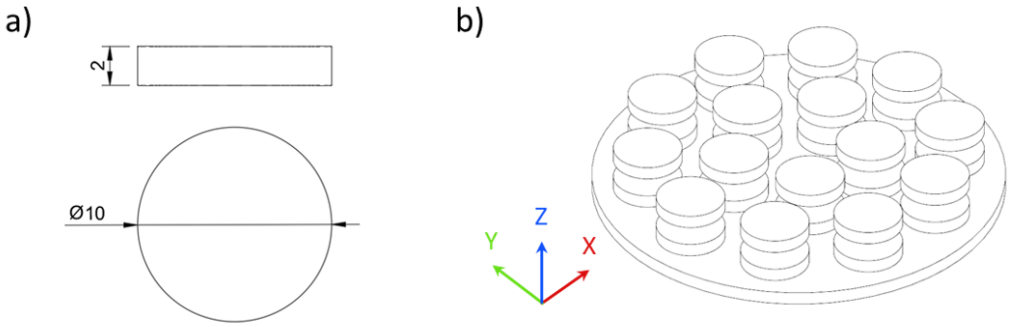

Fig. 1. Drawing of a) cylindrical shaped sample dedicated for microbiological test and b) samples in relation tomachine coordinates of PBF-LB/P device.

Tab. 2. Parameters of the PBF-LB/P process for Formiga P110.

Process parameters	**Contour**	**Hatching**	**Upskin / Downskin**
Laser power [W]	16	21	21/21
Scanning speed [mm/s]	1500	2500	2500/2800
Hatching [mm]	n/a	0.25	0.25/0.25
Additional information	scanning strategy: alternating XY; contour count: 1; upskin/downskin thickness: 0.3 mm		

Tab. 3. Results of measurements of filler content depending on its type and designed content.

		Measured filler content [wt%]		
Filler type		**Ag**	**Cu(S)**	**Cu(D**
Designed content [wt%]	**0.5**	0.34	0.22	0.69
	1	1.46	0.46	0.83
	2	1.81	0.97	3.62
	5	6.29	2.72	7.38

2.3. Antimicrobial properties

The evaluation of antimicrobial properties, as the ability to colonise the surface by microorganisms, was carried out using American Tissue and Cell Culture (ATCC, Manassas, Virginia, USA) strains of *Staphylococcus aureus* (number 6538), *Pseudomonas aeruginosa* (number 15442) and *Candida albicans* (number 10231). Strains of *S. aureus*, *P. aeruginosa, and C. albicans* were seeded in a Muller-Hinton liquid medium with the addition of 5% sheep blood (Argenta, Poznań, Poland). The studies were carried out using methods to assess the level of biofilm formation after an incubation time of 4 hours (to reflect the frequency of disinfection of clinical high-touch surfaces in hospitals):

- based on the non-specific ability of crystal violet to bind to bacterial biomass as detailed in Coffey et al. within [5];
- based on the reduction of colourless tetrazolium chloride to red formazan crystals in the presence of live, metabolically active microorganisms, developed by Grela et al. in the publication [8];

The control surface for each of the methods was commercially available cylindrical samples with dimensions of 10 mm x 1 mm made of polypropylene dedicated to cell cultures. The tests were carried out in 6 repetitions. The viability of the strains defined as 100% was taken according to the growth control sample, designated as C++ (sample without antimicrobial properties). The ability to inhibit the growth of microbial colonies was calculated as viability according to the formula (1):

$$\text{colony viability} = \frac{colony\ count\ on\ the\ analyzed\ surfaces}{colony\ count\ on\ the\ control\ surface} \times 100 \qquad (1)$$

Colony counts for each run were calculated based on absorbance, which was measured at wavelength 570 nm for crystal violet (ChemPur, Piekary Śląskie, Poland) staining or 490 nm for the tetrazolium (Sigma, Saint Louis, Missouri, USA) test using a MultiScan Go Spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA).

2.4. The cytotoxicity tests on cell lines

The cytotoxicity evaluation of the additively processed materials was carried out using two cell lines: HaCaT keratinocyte and L929 fibroblasts American Tissue and Cell Culture (ATCC, Manassas, Virginia, USA). Cell lines, maintained at passage 9, were used for the experiments. Cells were seeded in a 96-well plate with a count of 20,000 cells per well in RPMI-1640 (Biowest, Riverside, Missouri, USA) for fibroblasts or DMEM for keratinocytes culture medium supplemented with 10% bovine serum and antibiotics (penicillin 100 U/ml,

streptomycin 0.1 mg/ml, Biowest, Riverside, Missouri, USA). According to the guidelines contained in the ISO 10993-5:2009 standards, after 48 hours of culture in an incubator at 37°C in an atmosphere of 5% CO2, cells were stimulated with the prepared solutions (RPMI/DMEM-based extracts) for the next 24 hours. The extracts were obtained, following protocol reported by Repetto et al., by immersion of sample's disk (prepared in such a manner that it fits the diameter of well of 24-well plate) with appropriate medium for cell culturing (1 mL) for 24h/37°C [28]. The 8 disks were applied for this purpose, 100 µL of extract from each setting was introduced to the 8 wells containing keratinocytes or fibroblasts cells. The growth control for the experiment consisted of cells that were not treated with extracts, to which fresh medium was applied. The experiment's usability control (positive control) consisted of cells exposed to 70% ethanol for 3 minutes. After a given time, the cell stimulation solutions were removed and then Neutral Red dye (100 µl, 40 µg/ml) was added in cell culture medium according to Repetto et al [28]. Cells prepared this way were incubated for 2 h at 37°C, in an atmosphere of 5% CO_2. After 2 hours, the dye was removed by rinsing with a PBS solution. To prepare the samples for absorbance readings, they were incubated with shaking for 30 minutes in 150 µl of a de-staining solution composed of 70% ethanol, water, and glacial acetic acid mixed in a volume ratio of 50:49:1 (Avantor, Gliwice, Poland). The absorbance was read using a Multiscan Go (Thermo Fischer Scientific, Waltham, MA, USA) spectrophotometer at a wavelength of 540 nm. The survival rate of the untreated cells was taken as a growth control of 100% (C++). The viability of the extracts was determined according to the formula (2):

$$\text{cell viability} = \frac{analyzed\ surfaces\ absorbance}{control\ surface\ absorbance} \times 100 \qquad (2)$$

2.5. Statistical analysis

The results of the biological tests are presented as mean values with standard deviation. The determination of statistical differences was made using one-way analysis of variance (one-way ANOVA) with Tukey's post hoc test (OriginPro, OriginLab Corporation, Northampton, MA, USA). The means were classified into groups (labelled with uppercase letters), the means that do not share a group are considered significantly different at $p < 0.05$. Moreover, statistical differences between samples and growth control (C++) and reference material without a filler (ref.) are labelled (difference with C++ as * and with ref. as #).

3. Results

3.1. Antimicrobial properties

The results of testing the material's ability to inhibit the growth of microbial colonies are presented in Fig. 2. The reference material (ref.), without the addition of a filler, is characterized by a moderate level of antimicrobial properties, depending on the tested microorganism. In addition, the polymer matrix used (PA2201) is characterized by a greater ability to inhibit colonization than the equivalent widely used in industry (PA2200). This is confirmed by the results obtained by Karoluk et al. after an 8 hour incubation in TBS and artificial saliva medium [12]. The composite materials are characterized by higher antibacterial properties (that are not within same group with C++ or ref. samples).

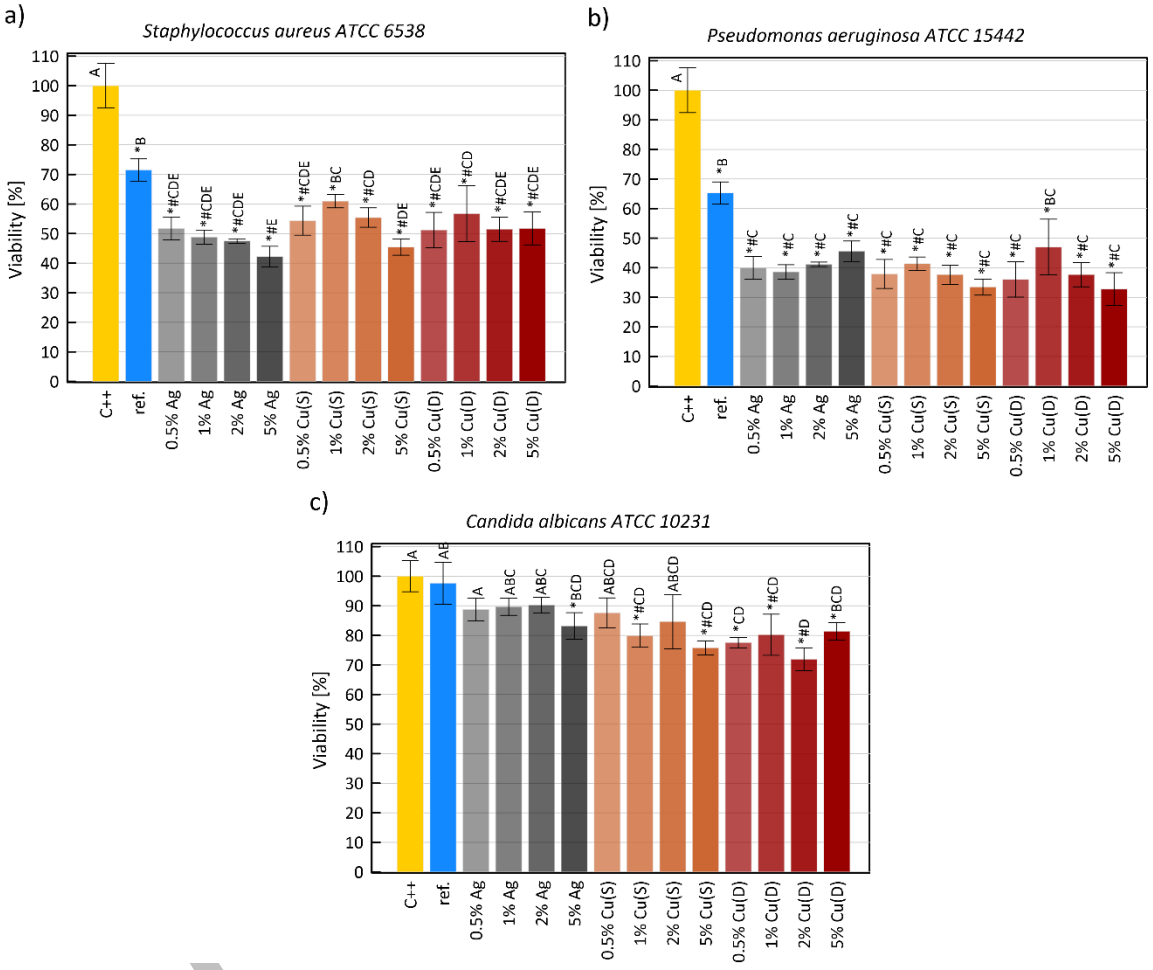

Fig. 2. Colony viability depending on the type and content of filler and type of colony: a) S. aureus ATCC 6538, b) P. aeruginosa ATCC 15442, c) C. albicans ATCC 10231.

3.2. The cytotoxicity tests

The results of the cytotoxicity tests against various cell lines are presented in Fig. 3. According to the ISO 10993-5:2009 standard, only a reduction of colony more than 30% characterises the material as cytotoxic. A result above 100% indicates that the exposure of cells to sample-conditioned medium promoted growth in the tested cells, which means that their number exceeded that of the control system. The cytotoxicity of the examined materials was notably influenced by the specific type of cell tested and both the type, and quantity of the

filler. For the HaCaT keratinocyte line, the reference material (which did not contain filler, ref.), and those with a 0.5% silver content enhanced cell growth beyond the levels observed in the control system. Furthermore, materials supplemented with Cu(S) and those with a 1% Ag content showed minimal cytotoxicity, with viability percentages ranging from 80% to 100%. It should be noted that the muted impact on cell viability, especially compared to Cu(D), could be attributed to significant discrepancies between the intended and actual amounts of Cu(S) filler, as detailed in [11].

Furthermore, a high standard deviation was observed in the Cu(S) series for both 2% and 5%. This deviation arises from the varying local concentrations of the filler content on the tested surface of certain samples. The elevated content on these surfaces led to a decrease in the viability of keratinocytes in specific samples. Other series of composites, specifically 2% and 5% of Ag as well as 1%, 2%, and 5% of Cu(D), are considered toxic for the evaluated cell line. A pronounced cytotoxicity of the tested materials is evident in relation to the L929 fibroblast line. Only the series containing 0.5% silver shows low cytotoxicity. The rest of the composites, including the reference material, exhibit a survival rate below 60%. Thus, according to the standard, they are deemed toxic to the investigated cell line.

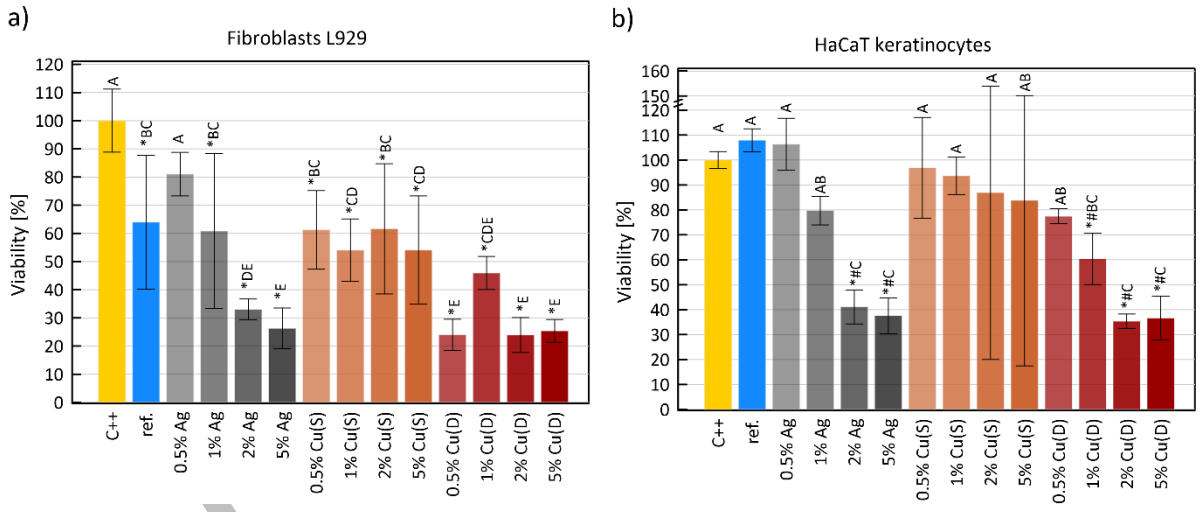

Fig. 3. Cells viability depending on the type and content of filler and cell lines: a) L929 fibroblasts b) HaCaT keratinocytes.

4. Discussion

It is essential to emphasize that, given the intended uses of the material, its cytotoxic effects on human cell lines and antimicrobial activity are of paramount importance. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* are known to inhabit certain areas of the body without causing harm under normal circumstances. However, in the hospital environment, particularly where COVID-19 patients are treated, these organisms can become

highly problematic. Hospitals treating patients with COVID-19 often use invasive devices and procedures that can compromise patient barriers, and the heavy use of antibiotics can disrupt normal microbiota, leading to overgrowth of opportunistic pathogens. Furthermore, the immune systems of COVID-19 patients can be weakened, making them more susceptible to infections. Therefore, the presence and management of these pathogens are of increased concern in COVID-19 units due to the increased risk of secondary hospital-acquired infections [1, 7].

The enhancement in antimicrobial properties of the analysed composites can be observed even at the lowest weight concentrations for each of the fillers. The differences, whether between batches of the additive or the amount used, are minimal, often falling within the margin of statistical error (composite are within groups obtained from Tukey's post hoc test). Materials with Ag fillers exhibit superior performance against Gram-positive *S. aureus* and slightly less efficacy against Gram-negative *P. aeruginosa*. The influence of the filler's shape and size on the systems analysed appears negligible (at confidence level of $p<0.05$). However, when comparing the two types of copper, it is worth noting that the series with the Cu(S) filler showed a content significantly lower than what was intended, which was caused by the specificity of powder deposition during the manufacturing process (Tab. 3) [11]. Both the reference material, and the developed composites possess a modest capacity to inhibit *C. albicans* growth. The addition of Ag or Cu(S) fillers, at concentrations ranging from 0.5% to 2% by weight, had a minimal impact on colony viability (being in A and B Tukey's test groups together with growth control and reference material). Cu(S) and Cu(D) fillers display a more pronounced antifungal effect compared to Ag, with a maximum reduction in viability exceeding 20%. Literature research, particularly a study conducted by Turner et al., also attests to the efficacy of silver-based fillers against *S. aureus* and *P. aeruginosa* [35]. Moreover, in the context of materials supplemented with copper, Vilardell et al.'s publication confirms the filled surface's ability to inhibit *Escherichia coli* growth [37]. However, it is crucial to highlight that the research undertaken by the two teams is somewhat challenging to juxtapose with the findings presented in this study due to varying testing conditions. The most significant discrepancies include the medium used and a longer duration of the test, with colony viability assessed after 24 hours.

Our observations of reduced bacterial counts of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, as well as a decrease in the viability of eukaryotic cells such as fibroblasts and keratinocytes in specific settings, following exposure to copper and silver-containing disks, suggest a broad-spectrum antimicrobial and cytotoxic potential of these

metals. The mechanisms underlying these observations are multi-faceted. Firstly, the release of metal ions from copper and silver is known to be toxic to microorganisms. These ions can disrupt vital cellular functions by binding to cellular proteins, DNA, or RNA, leading to cell damage and death. This action explains the antimicrobial effect observed against both bacteria and fungi. Secondly, copper and silver can induce the production of reactive oxygen species, leading to oxidative stress and cellular damage. This mechanism is likely responsible for the reduction in both microbial and eukaryotic cell numbers, as oxidative stress can affect a wide range of cell types. Thirdly, the integrity of the cell membrane can be compromised by these metal ions, resulting in the leakage of essential cellular components and subsequent cell death. This phenomenon might contribute to the decreased viability of both microbial and eukaryotic cells [8,31]. The differences observed between the copper and silver series are due to the different antimicrobial performance of the filler used. The overall antimicrobial efficacy can depend on factors such as the specific polymer matrix, the release rate of ions, and the interactions at the interface between the polymer and bacteria. To gain a comprehensive understanding of these differences in performance, further extended research, likely involving narrowed series and detailed investigations into the properties of the filler material, is warranted.

In our study, we observed a distinct variance in the sensitivity to silver or copper disks among *C. albicans, P. aeruginosa, and S. aureus*. Notably, *C. albicans* demonstrated less sensitivity compared to the bacterial species. This difference in antimicrobial response can be attributed to several biological and physiological factors inherent to these organisms. Firstly, the cell wall composition of *C. albicans*, a fungus, is markedly different from that of bacteria. The fungal cell walls are typically thicker and composed of a complex matrix, which may impede the penetration and thus the efficacy of metal ions. This barrier could potentially reduce the susceptibility of *C. albicans* to the antimicrobial action of copper and silver. The difference in growth and metabolic rates between fungi and bacteria is also a crucial factor. The slower growth and metabolism of fungi like *C. albicans* could mean that the impact of copper and silver ions is delayed or less apparent compared to the rapid-growing bacteria. The exact reasons for this variance, potentially involving differences in cell wall composition, growth rates, ion uptake mechanisms, and biofilm formation, warrant further investigation. However, such analyses, including in-depth studies on the mechanisms of metal uptake and resistance in *C. albicans*, fall beyond the scope of this current article.

In addition, it can be hypothesized that, despite the inherent structural differences, particularly in terms of cell wall composition and thickness, the applied metals exhibited a pronounced and comparable antimicrobial efficacy against both species - *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). This outcome suggests that the broad-spectrum nature of the metals' antimicrobial action was sufficiently potent to transcend these cellular differences, effectively impacting both types of bacteria. On the contrary, the relative insensitivity of *Candida albicans*, despite its exposure to the same antimicrobial agents, requires further examination. As mentioned above, the fungal cells of *C. albicans* are considerably larger and possess a significantly thicker cell wall compared to the bacterial cells of *P. aeruginosa* and *S. aureus*. It is plausible that these dimensional and structural attributes of *C. albicans* may confer a degree of resistance or reduced susceptibility to the antimicrobial effects of the metals.

Moreover, as previously stated, the difference in antibacterial properties between Cu(S) and Cu(D) series is most probably caused by the Cu(S) content being significantly lower than what was intended (added during the mixing process). This is an important fact that should be considered when choosing antimicrobial microfillers for polymer powders dedicated to PBF-LB processes. Small particles, less than 20 µm, tend to sediment and deposit on the surface of the machine during the process, resulting in a reduction in the final filler content, as well as its poor repeatability of distribution in the polymer matrix that ultimately affects its antimicrobial properties. Therefore, microfillers with large amounts of spherical particles below 20 µm are not recommended. The fact that standard deviations of average antimicrobial effect are high for specific microorganisms tested, certainly warrants further examination. A careful assessment of major variables is essential, including the sample topography, filler distribution, species- and strain-specific responses, as well as the types of tests performed.

The cytotoxicity of the reference material is consistent with the data reported in the literature [12]. The ions released by silver or copper are reactive to human cells [2,17,22], which is expected to cause most of the analysed composites cytotoxic. However, when comparing series supplemented with Ag and Cu(D), where the actual content is relatively similar, the Ag filler shows reduced toxicity. The difference observed can be attributed to several factors, including the chemical nature of the ions, their reactivity and their interactions with biological systems. Determining the origin of differences in the cytotoxicity in following biological systems (PA12 with metallic filler) needs further and more detailed studies. Furthermore, a notable trend is the drop in cytotoxicity for the 0.5% Ag series. Turner et al.

documented a similar trend in the PA12 silver composite following PBF-LB/P (PA2200 with 1.0% antibacterial filler containing silver) [35]. Turner reported that, the viability of the fibroblast colonies was noted to be greater than that of the growth control sample (surpassing 100%). This suggests the absence of cytotoxicity and indicates that the test cells were stimulated to proliferate beyond the levels seen in the control system. This observed decrease in cytotoxicity can be attributed to the activation of cellular survival mechanisms in response to stress factors, provided that they do not exceed the threshold for irreparable damage, as corroborated in the literature [17]. Regarding the copper-infused materials presented by the team led by Vilardell et al., no cytotoxicity evaluations have been performed, therefore, the safety of these developed materials remains unconfirmed [31]. It is worth emphasizing that, for both cell lines, the addition of the Ag filler, compared to Cu(S) and Cu(D), had a less negative impact on their viability, while at the same time, the ability to inhibit the growth of microorganisms was comparable. Noteworthy, the majority, if not all, locally active antimicrobial compounds also exhibit a certain level of cytotoxicity in vitro toward eukaryotic cells. This phenomenon is observed with clinically used antiseptic agents such as, for example, polihexanide, povidone-iodine, and octenidine hydrochloride. This does not exclude the possibility of further testing in animal models and, ultimately, the administration of these agents to patients suffering from infectious diseases. Rather, it provides data on potential contact times and concentrations at which the application of a specific product results in a maximum antimicrobial effect while minimizing the risk to eukaryotic cells [14].

Both silver and copper additives can induce cytotoxicity in human cell lines [2,18,23]. Cytotoxicity has been reported to depend on dose size, particle size, contact time, as well as cell type. The differences observed in the current research confirm previously reported dependencies. Furthermore, depending on the cell type, filler, or filler concentration, there are different threshold limit values that do not show cytotoxicity (Fig. 3). Various threshold values have also been reported [2,8]. Furthermore, in the case of polymer-metal composites, the type of matrix will also affect overall cell viability [34].

The most promising powder composition is represented by a mixture with 0.5% Ag, which is characterized by limited cytotoxicity and increased antibacterial properties. These features are added values that can be particularly important when designing and manufacturing, for example, parts of personal protective equipment or spare parts for medical devices [10, 24]. Antimicrobial properties are especially important in crisis situations, such as the COVID-19 pandemic, and help reduce transmission of microorganisms not only in hospitals, but also in public buildings or public transport.

Despite the promising antimicrobial results, it should be noted that the levels of cytotoxicity observed in the tested eukaryotic cell lines varied between samples and even within results obtained for the same type of sample. This variation might be attributed to the specific production processes of the fillers. However, such results render the data, particularly those concerning the impact of the samples on eukaryotic cells, highly preliminary. Therefore, further research using different in vitro models is necessary before drawing unequivocal conclusions about the true potential of these samples for use on hospital surfaces.

5. Conclusions

An investigation was conducted on the impact of bioactive fillers on the antimicrobial properties of PA12 samples produced using PBF-LB/P. The study included a reference material (neat PA12) and 12 composites formulated with various bioactive metallic fillers. The salient findings include:

• Metallic fillers, at all concentrations examined, enhance the inhibition of bacterial colony growth. Within a 4-hour test duration, there was a 15% to 40% more pronounced reduction in colonies of Gram-positive strains (*S. aureus*) and a 28% to 50% reduction for Gram-negative strains (*P. aeruginosa*).

• Incorporating Ag, Cu(S), and Cu(D) fillers at levels of up to 5% by weight has a modest effect in preventing the growth of the fungal colony. Over a 4-hour test span, a 7.5% to 26% larger decrease was recorded in *C. albicans* colonies relative to the base material.

• Enhancing the filler content from 0.5% to 5% by weight subtly adjusts antimicrobial attributes. The exact change in colony survival varies between 4 and 9 percentage points, depending on the filler type or the bacterial strain.

• Utilizing Ag and Cu(S) fillers at concentrations of 0.5% by weight ensures that the composites remain safe for skin contact. This was affirmed through a study on keratinocyte cell lines, where post-PBF-LB/P processed materials registered a survival rate that exceeded 80%.

Future endeavours will focus on the investigation of antimicrobial material in the context of devising a procedure for its reuse in the PBF-LB/P process. This includes pinpointing the optimal method to reclaim the material post-process and subsequently regenerating it to establish a ready-for-implementation process with consistent parts production repeatability. In addition, expansion of the composition of the material with fillers or additives boasting antifungal properties is preferred to further amplify the inhibition of fungal colony growth.

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