

Review

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Review

Greener Approaches to Combat Biofilm's Antimicrobial Resistance On 3D Printed Materials: A Systematic Review

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Highlights

- All surfaces are subject to contamination and Biofilm formation.
- Recently, 3D printing has emerged as an excellent technology to produce satisfactory, affordable, and bioactive materials.
- The most prevalent and recent studies analyzing biofilm development on 3D printed surfaces as well as the adopted microorganisms, materials and 3D printing technologies are presented and categorized.
- The review's key contribution is its thorough discussion of greener strategies to manufacture antimicrobial/antibiofilm 3D printed interfaces.
- With an emphasis on the medical, environmental, agri-food, and other relevant disciplines, this review covered research from a wide range of fields.

Abstract: In recent years, Additive Manufacturing (AM), commonly referred to as 3D printing, has garnered the attention of the scientific community due to its capacity to transform ordinary and traditional items into customized materials at an affordable cost, through various AM processes. Antimicrobial/antibiofilm 3D printed materials are one of the most trending research topics, owing to the growing concerns over the emergence of complex microbial structures called "biofilms" on various surfaces. The review provides an overview of the evolution of additive manufacturing (AM) technologies and their various derivatives, along with a brief description of their materials and applications. It also introduces how biofilms can represent an advantageous lifestyle for microbial populations. The primary objective of this research was to conduct a systematic review on the development of planctonic or biofilm forms of microorganisms on 3D printed materials. The article summarizes commonly studied microorganisms on these materials and presents their 3D printing process, materials, as well as the fields covered by each of the analyzed papers. To the best of our knowledge, this is the first all-inclusive systematic review that amalgamated research conducted in diverse fields to assess the development of biofilms on surfaces produced through three-dimensional printing. Most notably, this review presents a comprehensive account of sustainable approaches for producing antimicrobial materials through 3D printing. Additionally, we assess their advancements in various fields such as medicine, environment, agri-food, and other relevant sectors. The findings of our literature review can be used to recommend appropriate microorganisms, 3D printing materials, and technologies for academic and industrial research purposes focusing on the development of microbial biofilms on 3D printed surfaces. Furthermore, it highlights the potential of environmentally-friendly modified AM technologies to combat biofilms in clinical and non-clinical areas. Our goal with this review is to help readers gain a better understanding of fundamental concepts, inspire new researchers, and provide valuable insights for future empirical studies focused on eradicating biofilms from 3D printed materials.

Keywords: 3D printing; additive manufacturing; 3D printed materials; biofilm; antimicrobial activity

1. Introduction

In a world that is undergoing continual change, human civilization has an increased desire to research and explore new technologies that are superior in both their application and overall process. As a matter of fact, developing antimicrobial materials thanks to technological advancements could be of a great interest in many fields, and can greatly aid in coming up with sustainable solutions in the fight against some of the society's biggest threats, such as antibiotic resistance and emerging novel pathogens. And with the researcher's focus shifting more toward multifunctional antimicrobial materials, modern technologies have recently increased their efforts to boost the effectiveness of such materials, notably 3D printing technologies. By definition, and according to the ISO/ASTM (International Organization for Standardization/American Society for Testing and Materials standards), 3D printing is described as "the act of combining materials typically layer by layer to produce objects using a 3D model data [1]. This technology involves an overlapping of materials in order to produce components with intricate shapes promptly by precisely accumulating materials based on a computer-aided design (CAD) model or computed tomography (CT) scan under computer control [2]. Additive manufacturing is reportedly a fast growing technology, applied in a broad range of disciplines, including tissue engineering, regenerative medicine, aerospace engineering, and even food industry and construction fields [3]. As stated by Campbell and Ivanova [4] AM is now increasingly considered as a revolutionary innovation that provides a different concept for engineering design and production, with substantial impacts on economics, geopolitics, ecology, intellectual property, and safety. In their assessment on printable and multipurpose polymer composites, Bekas *et al.*, (2019) mentioned that additive manufacturing can provide a number of benefits, including minimal material consumption, reduced waste, customization flexibility, and geometric sophistication, which explains the urgent demand to create antifouling materials using 3D-printing processes [5]. To produce such materials, multiple studies followed for instance simple methods of coating polymer filaments with nanoparticles, fibers, or metal flakes readily accessible [6]. However, papers dedicated to studying biofilm adhesion on 3D printed interfaces or understanding the development of antibacterial qualities of 3D-printed materials aren't that many. As far as we know, a comprehensive exploration of the techniques utilized to produce 3D printed surfaces with antimicrobial/antibiofilm properties has not been conducted yet, emphasizing the novelty of the present work.

One of the most prevalent 3D printing technologies are those based on the extrusion of materials, especially bio-plotting or more commonly fused deposition modeling (FDM) [7], thanks to their ability to produce components using a variety of biocompatible or biodegradable materials, and in certain circumstances even the printing of living cells or bacteria can be successfully achieved [8]. Besides fused deposition modeling (FDM) techniques, the rest of AM technologies can be broadly classified into the following types based on their printing principles: directly ink writing (DIW), photocuring (SLA, DLP), laminated object manufacturing (LOM), laser sintering and laser melting (SLS, SLM), photopolymer jetting (Ployjet), and binder jetting (3DP). These 3D printing technologies provide a variety of pricing, performance, and material alternatives. When it comes to 3D printing materials, polymers, metals and ceramics are by far the most widely used materials, as well as hybrids, composites, or functionally graded materials (FGM)[9].

The number of research papers incorporating 3D printing has risen significantly in recent years. In the year 2013, the number of articles identified in Web of Science using (WOS) the search phrases "3D printing" or "additive manufacturing" has skyrocketed by thousands each year, reaching a total of 10,000 in 2016. Since, 3D-printing is increasingly being integrated in many areas, namely biomedicine, which explains the pressing demand for antimicrobial 3DP materials, because of which smart materials, nanomaterials, functional materials, biomaterials, composites and many others have been developed and

highly investigated for 3D printability in order for them to acquire products complexity, multi-performance ability [10], and more importantly antimicrobial activity and overall efficiency.

The possibility of pathogen contamination, especially through the development of biofilms, poses a serious threat to 3D printed materials. Biofilms are renowned for their naturally occurring resistance to antibiotics, which can be 10 to 1000 times higher than that of planktonic cells [11]. This makes it difficult to manage biofilm contamination, particularly in therapeutic settings [12]. Statistically, biofilm production is involved in up to 80% of microbial infections, increasing dramatically the occurrence rates and morbidity [13]. Which calls for urgent solutions to inhibit the adhesion of these communities on all surfaces, including three-dimensionally printed ones [14].

To overcome the shortcomings of antimicrobial remedies, researchers have been working on various strategies these past few years to develop surfaces made of modified materials that are antimicrobial by nature. This antimicrobial activity can be achieved by either killing microorganisms as soon as they adhere to the surface or by preventing the formation of microbial colonies. By producing materials that are microbe-repellent, microbe-killing, anti-adhesive, or biocide-releasing, antimicrobial surfaces can be successfully generated. Coating, vapour deposition, sol-gel, plasma deposition, laser-mediated technique, electrochemical method, etc, are often used to generate such surfaces [17]. Moreover, recently 3D printing has been employed for this purpose by directly incorporating the antimicrobial substance during the printing process, in order to provide either a microbiostatic or microbicidal activity to the desired material. Small molecules, macromolecules, polymers, ceramics, metals, and nanocomposites exhibiting microbicidal properties against bacteria, fungus, and viruses are the ones referred to as antimicrobial substances [18]. In this context, all of the reviewed research utilized a precise 3D printing technology in accordance with a single or multiple antimicrobial. The materials used in these studies were precisely defined to create 3D printed surfaces with microbicidal properties, specifically for use in fields such as healthcare, food, or environmental sectors.

Throughout the years leading up to 2022, and based on WOS results, there was a significant rise in studies published on the formation of biofilms on 3D printed materials, with particularly substantial increases in the last 3–4 years (Figure 1).

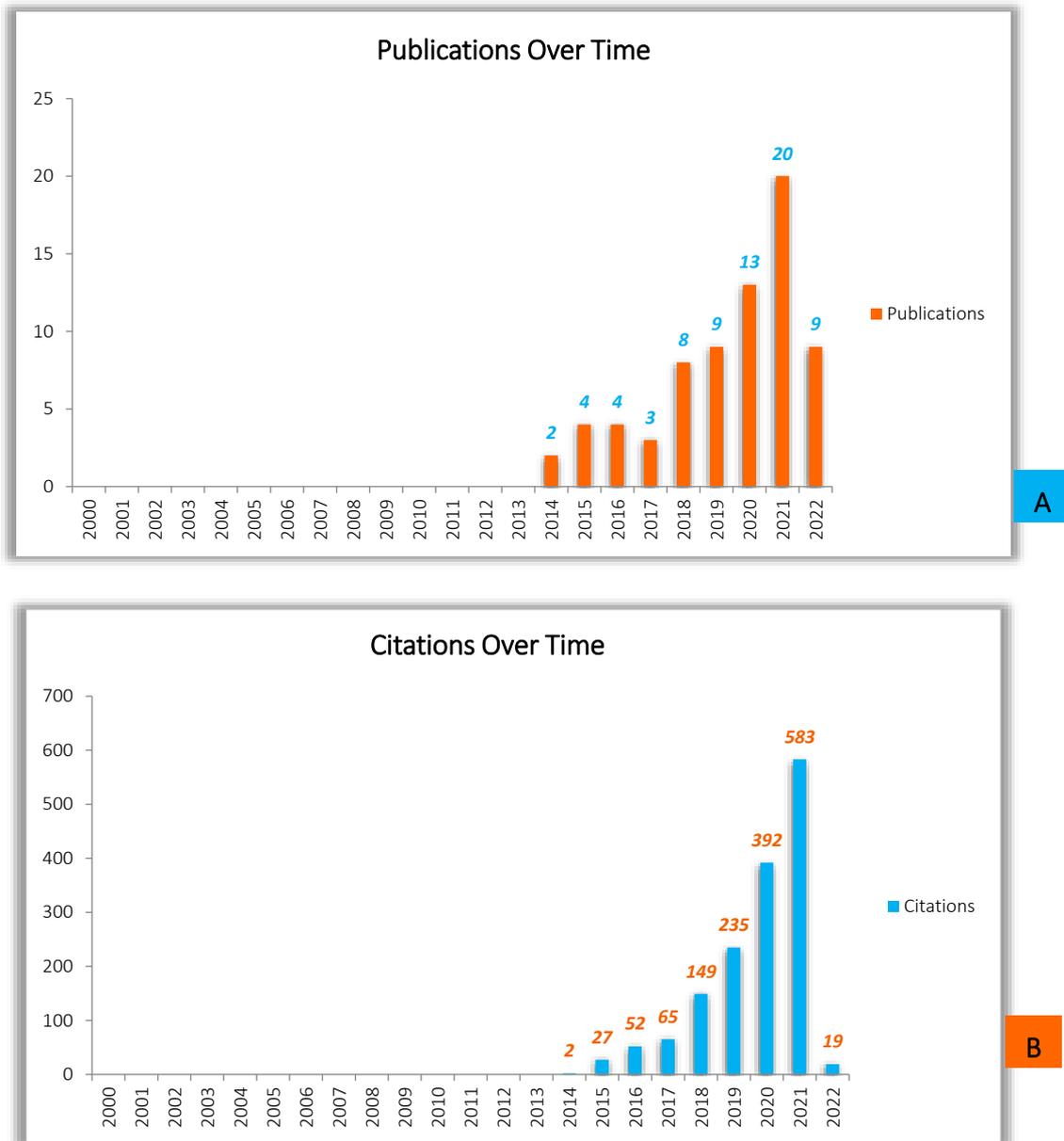


Figure 1. Quantitative analysis of the scientific literature on Biofilm formation on 3D printed materials (Source: ISI WEB OF SCIENCE, MAY 2022). **A** – Shows the distribution of publications by year of release for papers written and produced throughout a ten-year period (2012–2022). The overall pattern of Biofilm development on 3D printed materials-related article publication demonstrates that the most productive year was 2021, with twenty ($n=20$) documents submitted, preceded by thirteen ($n=13$) articles reported in 2020, nine ($n=9$) published articles in 2019, and the same number of papers was also published in 2022. Nevertheless, it's worthy to note that the 2022 statistic represents articles made over a five-month timeframe, and therefore the amount of literature is likely to rise by the end of the year. The study of biofilm growth on 3D printed objects is increasingly gaining popularity. In 2017, however, there was a slight decrease in the number of publications. This illustrates that, while the total number of publications in this field is expanding, research interest is always fluctuating. **B** – This figure also represents the average number of citations each year. Although the number of citations is constantly increasing, the expansion is not as steady.

Nonetheless, the integration of 3D printing in biotechnology and antimicrobial bioengineering fields is still relatively uncommon. Most reviews primarily emphasize 3D printing as a groundbreaking

technique and describe its applications in a specific field, such as medical, agri-food, or environmental. Several other reviews solely focus on advancements in 3D printing for producing internal and external devices and equipment designed to combat biofouling problems, with its potential uses mainly in healthcare, food industries, or other areas.

This review article provides an in-depth overview on the development of 3D printing as a pervasive technology, all while detailing the wide range of processes it offers, and also lists the variety of materials that are suitable for additive manufacturing, along with their various potential uses. Further, the susceptibility of 3D printed materials to contamination was similarly covered. Based on the reviewed research articles we were able to collect, classify and quantify each Biofilm forming microorganism inside a well defined application category, to the best of our knowledge such analysis appears to be the first of its kind. Most significantly, and in a manner unparalleled by any other paper, our review highlighted the current understanding of 3DP material biofouling, all while delivering an extensive list of green strategies used in recent research articles in order to inhibit and prevent the formation of biofilm on 3D printed surfaces, not only in clinical settings but also in every other field of research, All of this was done while precisely detailing different AM technologies used, and by providing a brief description of the adopted antimicrobial approaches, the effectiveness of our study's demonstration of the antibacterial and anti-adhesive procedures adds to its originality and authenticity.

2. Methods: Search Strategy & Data Sources

A comprehensive literature search was conducted starting February 14th, 2022, using Web of Science, Scopus, and Science Direct databases. On May 30th 2022, the data collecting process came to an end. The following keywords were used in the title or the summary to find articles of interest: "3D printing" OR "additive manufacturing," AND "biofilm" OR "bacteria", AND "3D printed Materials," AND "3D printing technologies." The search was restricted to papers written in English. Data that did not report on a microbiologic investigation of 3D printed materials particularly or a research on their antimicrobial characteristics was omitted. Some of the relevant publications referenced in the chosen articles were also included. The search findings were logically selected and neatly summarized, then represented through matrices or illustrated in the form of descriptive graphics. In this case, Mendeley was selected to serve as the reference manager.

The extraction of data from the preselected articles was done with the following details in mind: **(i)** 3D printing technology description, printable materials and their potential applications, **(ii)** microorganisms, factors behind their adhesion to surfaces and their ability to form biofilms on 3D printed interfaces, **(iii)** investigations on the development of biofilms on 3D printed materials, **(iv)** recent antimicrobial greener strategies tested on surfaces obtained by AM technologies.

3. 3D Printing /Additive Manufacturing

3.1. 3D Printing Technologies

Over the years, there have been several improvements and advancements in 3D printing technologies. In the field of additive manufacturing, achieving the desired shape of a product involves a layer-by-layer deposition of coatings during the printing process [19]. AM processes could be categorized into seven classes based on ASTM Standards. These technologies could be liquid, solid, or powder-based [20]. In a typical AM procedure many characteristics should be taken in consideration since they highly effect the process, including manufacturing speed, resolution, quality, affordability, build volume, interface finish, and product strength (Table 1). Nevertheless, printing speed and resolution have been the most important parameters for each of the 3D printing technologies [21].

In general, and based on the information presently analyzed the major widely commercialized three-dimensional printing technologies are : **(i)** fused filament fabrication (FFF), also known as fused deposition modeling (FDM); **(ii)** selective layer/laser sintering (SLS); **(iii)** stereolithography (SLA); **(iv)** digital light processing (DLP); **(v)** polyjet / inkjet 3D printing; and **(vi)** electronic beam melting (EBM)[23]. Table 1 provides a more detailed description of some of these prominent strategies.

3.2. 3D Printing Materials

3D printing or additive manufacturing is the process that uses computer-aided design (CAD) to create fully functional, three-dimensional items through a layering method utilizing a variety of materials [19], namely polymers, metals ceramics, as well as hybrids, composites, and functionally graded materials (FGM) [9]. 3D printable materials usually have several specific attributes well-suited for the application in order to achieve design goals. The most important characteristics cited by the reviewed papers are: **i)** tensile strength, **ii)** elongation, **iii)** hardness, **iv)** thermal stability, **v)** biocompatibility, **vi)** environmentally friendly and **vii)** low cost. Overall, in Table 2 a summary of the distinctive properties of some 3D printing materials, along with the industries and application domains that each of the material categories covers.

Table 1. Summary of 3D printing technologies, their principles, the corresponding materials, as well as their advantages and disadvantages, Based on .

FAMILY	TECHNIQUE	PRINCIPALS/ PROCESS	MATERIAL CONDITION	ACTIVATION SOURCE	TYPICALLY USED MATERIALS	ADVANTAGES	INCONVENIENCES
MOLTEN MATERIALS DEPOSITION	FDM (Fused Deposition Modelling)	Extrusion through a preheated nozzle, and deposition in thin layers that bind and fully solidify by cooling on the substrate	Filaments	Heat	-Ceramics -Edible materials, -Thermoplastics.	-Good resistance, -Low cost, -Multi-material capability, -Production of complex 3D structures.	-Clogging of the nozzle, -High roughness of the printed objects, -Layer by layer appearance, -Poor surface quality.
	FFF (Fused Filament Fabrication)						
PHOTO-POLYMERIZATION	SLA (Stéréo- lithography)	Photosensitive liquid polymer exposed to laser (mainly UV) or free radicals solidifies through photopolymerization	Thermoset liquids	UV, LED	-Photocurable resin, -Photopolymers, - Thermoplastic polymers.	- High printing resolution, - Precise geometries, -Reproducibility, - Smooth surface finish.	-High cost, -Limitation of materials, -Relatively slow printing process, -Requires post-processing, -Release of toxic fumes during printing.

	DLP (Digital light processing)	The polymer exposed to light projections (mainly UV) emitted by a digital projector solidifies by photopolymerization.	Soft materials	UV, LED	<ul style="list-style-type: none"> - Resins, - Waxes. 	<ul style="list-style-type: none"> -Cost effective, - High precision, - Reduced time compared to SLA, -Simultaneous printing of several compact objects with less detail. 	<ul style="list-style-type: none"> - Limited range of materials, -Need for adapted systems (ventilation ...), -SLA generally provides higher resolution and better surface finish than DLP technology, - Thickness limit,
MATERIAL JETTING	3D InkJet	The drops of photopolymer deposited on the working platform are exposed to UV light and solidified by light curing.	Inks	UV	<ul style="list-style-type: none"> - Gypsum, -Photo-polymers, - Polymers, - Waxes. 	<ul style="list-style-type: none"> - High level of precision and complexity, - Possibility of using several materials. 	<ul style="list-style-type: none"> - Expensive materials and printers, - Fixed resolution, - Long processing time, - Need for a material support.

	Poly /Multijet	Printing layer by layer, projection of microdroplets and photopolymerization with UV light	Inks	UV	- Polymer resins.	<ul style="list-style-type: none"> - Advanced inkjet technology, - No post-processing, - Printing of objects combining several materials and colors, - Relatively low cost and printing time, - Smooth finish. 	<ul style="list-style-type: none"> - Highly sensitive to sun and temperature, - Slow process, - Weak finished products.
POWDER BINDING	SLS (Selective laser sintering)	Using a highly powered CO2-laser beam to sinter the powder particles, another powder coating is then added and smoothed using a recoater.	Powder	Heat	<ul style="list-style-type: none"> -Ceramics, -Metals, - Polymers (especially polyamides and derivatives). 	<ul style="list-style-type: none"> - Ability to build articulated parts with various characteristics, - High level of complexity, <ul style="list-style-type: none"> - Good resistance, - Wide range of materials, - No need for support. 	<ul style="list-style-type: none"> - Accuracy limited to the fineness of the powder, - Rough and slightly granular finish, - Limited material range, <ul style="list-style-type: none"> - Powdery surface, - Requires post-processing, - High cost

	3DP (Agglomeration of powder bonding)	Application of little colored glue droplets in various sizes to powdery layers until the desired effect is achieved.	Powder	Chemical	-All materials supplied in powder form are used.	-Ambient processing environment, -Easy removal of carrier powder, -Low cost, -Multi-material capability, -Low installation cost.	-Binder contamination, - Binder jet clogging, -Limited volume constructed, -Poor surface quality, -Poor porosity of the final product.
	DMLS (Direct Metal Laser Sintering)	A laser to deposit and fuse a metallic powder is used allowing for a layer-by-layer printing.	Powder	Heat	- Metals	- Complex geometries, - Dense components usage, - High construction speeds - Large objects production, - Remarkable objects strength, -Possibility of combining materials.	-High cost, -Less complex and detailed objects, - Mandatory polishing step, - Use of X-rays.

Table 2. Characteristics of popular 3D printing materials, as well as their applications, Inspired by and information supplied by the following websites ; 3Dnatives, 3D printing industry, Formlabs and Sculpteo.

Family	Materials	Properties	Applications/Industries
<u>Plastics</u>	ABS	Solid and resistant	Medical devices, Automotive, Aerospace.
	Nylon	Good chemical resistance, high fatigue resistance and high impact resistance	The supply of high fatigue strength parts in the aerospace and automotive industries, such as antenna covers, custom production tools, friction inserts and pressure fits, appears to be of good quality and efficient in this material.
	PC	High tensile and flexural strength	Perfect for aerospace and automotive molding and blow molding, functional prototypes, tools and assembly.
	PET PETG	Relatively hard and light, good impact resistance and firmer than ABS.	The manufacture of parts that must be both strong and flexible.
	PLA	Good tensile strength and surface quality	Suitable for mock-ups and prototypes for the home and office that involve visually pleasing and environmentally friendly elements.
	PP	Abrasion resistance and stress absorption. Good balance between stiffness and flexibility.	Mainly used in packaging activities, production of electrical items and equipment, automotive sector and household appliances manufacturing.
	PVA	Biodegradable and easily soluble, and allows quick cleaning of 3D printed structures.	Mainly used as a support material for printing PLA and/or ABS products.
	Resin	High resolution, smooth and delicate surface components with strong chemical bonding between layers and short build time.	Progressively developed for mass production. Resin 3D printing has a bright future, ranging from jewelry to construction projects to medical uses.
	Cobalt-chrome	Biocompatible, very high hardness, corrosion resistance, high strength and high ductility.	Cobalt chromium objects can be used in the fields of health and dental research, as well as in high-temperature areas such as jet engines.
	Precious metals	Good ductility, inalterability and low mechanical resistance.	The additive manufacturing of precious metals is intended for the jewelry and dental sectors, as well as for various applications in industrial environments.

<u>Metals</u>	Stainless steel	High wear resistance, corrosion resistance, high hardness and ductility.	Stainless steel components are used in the automotive sector, manufacturing industry, marine industry, medical technology and machine building.
	Titanium	Corrosion resistance, biocompatibility, low thermal expansion, high strength and low density.	Clinical technology, aviation, automotive, marine, jewelry and design are just some of the uses for titanium products.
<u>Ceramics</u>	UV curable monomers	Thermal tolerance, toughness and mechanical performance are all excellent. Ceramic 3D printing allows the production of functional objects with high precision and technical ceramic qualities.	Ceramic 3D printing has a wide range of applications, including construction, tableware, automotive, aerospace, telecommunications and electronics.
<u>Composite materials</u>	Possibility to create composites using computer models and then produce parts with optimized technical properties using 3D printing.	Less heavy, but also stronger and more rigid, and resistant to climate change and chemical exposure. With a longer life expectancy. There is also flexibility of shape: the material is much softer, making it easier to produce certain shapes.	Sensors, Fracture resistant composites and 3D piezoelectric polymers.
<u>Smart materials</u>	Shape memory polymers	Delicate, adaptable and constantly changing. Capable of changing their physical characteristics (shape, color, elasticity...) or even having an effect on their environment when exposed to changes in temperature, pH, mechanical stress, light or electric field.	Actuator, Sensor, Jewellery, Gripper.

3.3. 3D Printing Materials and Technologies Commonly Employed Based on the Reviewed Literature

The following section offers an overview of the research articles that were reviewed. Tables 3 and 4 were created to simplify the analysis of the data by summarizing the AM techniques, materials (both before and after the application of antimicrobial strategies), microorganisms, and fields of study that were mentioned in the literature that was reviewed.

3.3.1. Reviewed 3D Printing Technologies

Molten polymer deposition (n=29) was the most prevalent AM method in the reviewed publications, more specifically, (n=4) for FFF and (n=25) for FDM, followed by SLA (n=8), DLP (n=6), SLS (n=4), SLM (n=4), 3D InkJet (n=4), and DIW (n=2) (Tables 3 and 4). A few articles did not specify the technology utilized, whereas (n=2) papers mentioned using SLA and SLS for the printing of the same materials (Figure 2).

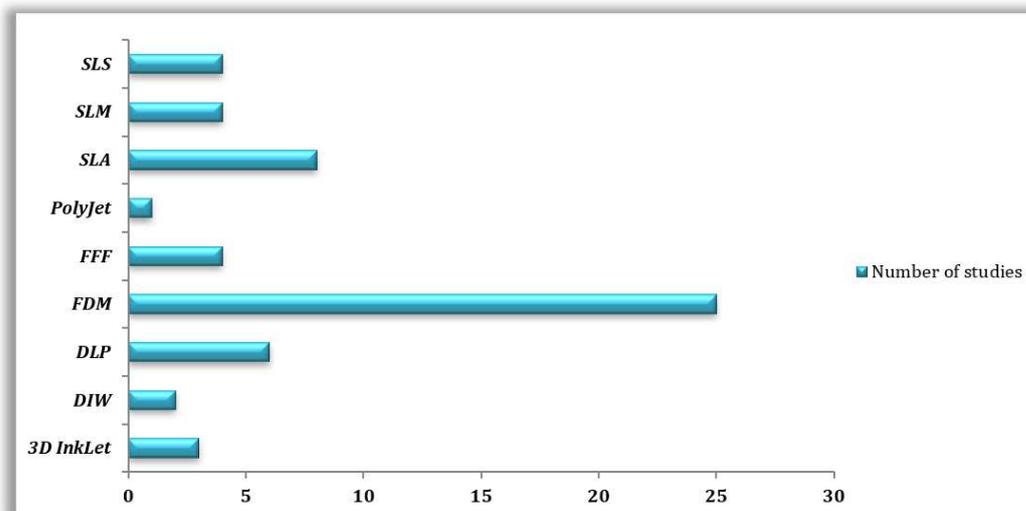


Figure 2. Types of 3D printing technologies used in the reviewed studies.

3.3.2. Reviewed 3D Printed Materials

All in all, and according to our research the most utilized 3D printing material is PLA and its variations, with (n=19) out of (n=50) articles dedicated to this popular thermoplastic. To be more precise, (n=6) papers tested PLA material in its normal state, furthermore in (n=4) out of (n=6) cases FDM/FFF was the printing technology adopted by the researchers, whereas the (n=2) left papers printed PLA using SLA/SLS techniques. The other (n=10) articles tested PLA material in its composite form, (n=9) articles treated PLA using FDM/FFF processes, while only (n=1) paper utilized SLA technology. Lastly, (n=3) works combined the study of PLA in its normal as well as in its composite forms, in which the printing was realized with FDM/FFF techniques. Second place was taken by Resin materials with a total of (n=9) studies, four of which used DLP printing technology (n=4), followed by SLA (n=3) and finally FDM (n=2). Another exploited material is called PMMA, with a total of six articles (n=6), half of them studied normal PMMA material (n=3) using SLA (n=2) and InkJetting (n=1) technologies. On the other hand PMMA composites (n=3) were obtained either with FDM (n=1), SLA (n=1) or InkJetting (n=1) approaches. SLS technology was best suited with materials such as polyamide (n=3) and Nylon (n=1). PCL (n=2) and PDMS (n=1) were both printed using three different manufacturing techniques; FDM, SLA and PolyJetting. Many other materials were studied, including PET (n=2), PVA (n=2), PEGDA (n=2), ABS (n=2), PEEK (n=1), PNIPAM (n=1), Poly-TCDMDA (n=1) and Poly-EGDPEA (n=1) (Figure 3).

Metals were also highly studied especially in their pure form (n=5), in this case titanium (n=2), cobalt-chrome (n=1), copper (n=1) and stainless steel (n=1) were tested, employing techniques such as SLM (n=2), SLS (n=1) and FDM (n=1). Only 3 studies focused on metal composites utilizing different manufacturing techniques, which are FDM (n=1), SLA (n=1) and SLM (n=1) (Figure 4).

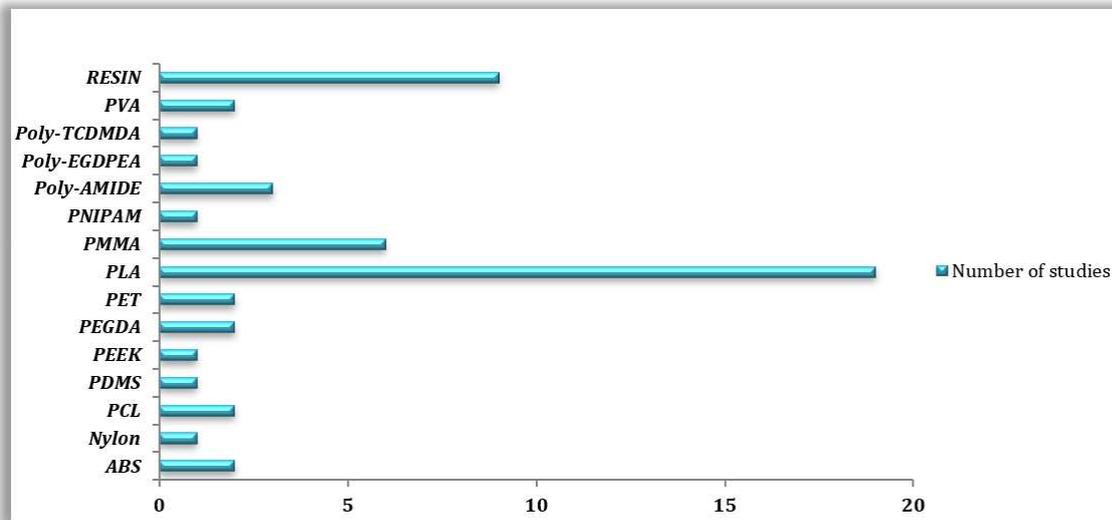


Figure 3. Types of 3D printed Polymers used in the reviewed studies.

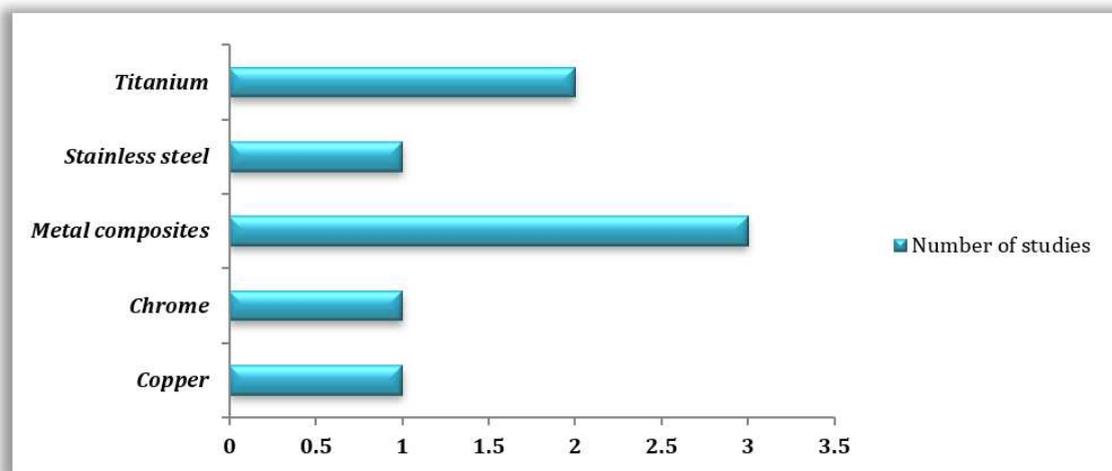


Figure 4. Types of 3D printed metals used in the reviewed studies.

FDM/FFF was by far the most prevalent AM technology according to this investigation, with PLA and resin as the most popular materials. PLA is highly selected due to its biodegradability low cost, non-toxic, adaptable, strong, resistant to corrosion, and longer lifespan. Compared to ABS and polyamides, the low melting point of PLA is one of its biggest advantages when 3D printing [48]. Yet, its low temperature resistance and brittleness, limits its use in comparison to ABS [49]. Its biodegradability, on the contrary, makes it a stronger candidate for use in a variety of biological applications, including bone fixation, drug delivery microspheres, and biomedical engineering [50], All of the above explains why PLA material was the subject of the majority of analyzed studies in the medical field (n=19).

Table 3. Microorganisms often investigated on different 3D printing materials, along with their corresponding 3D printing technologies.

3D PRINTING TECHNOLOGY	3D PRINTING MATERIAL	MICROORGANISM STUDIED	REFERENCES	
FDM	PLA	<i>Escherichia coli</i>	[51]	
		N/A	[52]	
		<i>Staphylococcus aureus</i>	[53]	
		<i>Staphylococcus epidermidis</i>		
		<i>Escherichia coli</i>	[54]	
		<i>Pseudomonas aeruginosa</i>		
		<i>Listeria monocytogenes</i>		
		PLA 3D850	N/A	[55]
			N/A	[56]
	PLA resin	N/A	[56]	
		<i>Staphylococcus aureus</i>	[57]	
	PLA resin	<i>Staphylococcus aureus</i>	[58]	
		<i>Escherichia coli</i>		
	DMHB resin	<i>Escherichia coli</i>	[59]	
		<i>Bacillus subtilis</i>		
	PET	<i>Escherichia coli</i>	[59]	
		<i>Bacillus subtilis</i>		
	PCL	<i>Marine Flora</i>	[60]	
	PVA	<i>Mycobacterium abscessus</i>	[61]	
		<i>Mycobacterium bovis</i>		
		<i>Mycobacterium smegmatis</i>		
	PDMS	<i>Marine Flora</i>	[60]	
	PEEK	<i>Escherichia coli</i>	[62]	
<i>Staphylococcus aureus</i>				
SEBS	N/A	[52]		
Metal (Cu)	<i>Escherichia coli</i>	[58]		
	<i>Staphylococcus aureus</i>			
SLA	PLA Giahntarm	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	[63]	

	Plactive™	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	
	PCL	<i>Marine Flora</i>	[60]
	PDMS	<i>Marine Flora</i>	
	ABS (VisiJet®)	<i>Marine Flora</i>	
	VeroClear™ (Similar to PMMA)	<i>Marine Flora</i>	
	Elastic resin	N/A	[64]
	Acrylic resin	<i>Buccal Flora</i>	[65]
	Bisacrylic resin		
	Resin	<i>Escherichia coli</i>	[66]
		<i>Bacillus cereus</i>	
	PNIPAM Hydrogel	<i>Escherichia coli</i>	[67]
PMMA	<i>Candida albicans</i>	[68]	
SLM	Titanium alloys	<i>Staphylococcus aureus</i>	[69]
		<i>Staphylococcus epidermidis</i>	
		<i>Streptococcus mutans</i>	
	Titanium Ti6Al4V	<i>Staphylococcus aureus</i>	[70]
			[71]
	Stainless steel	<i>Staphylococcus pseudintermedius</i>	[70]
		<i>Staphylococcus aureus</i>	
Cobalt-Chrome	<i>Staphylococcus pseudintermedius</i>		
SLS	Acrylic resin	<i>Buccal Flora</i>	[72]
	Bisacrylic resin		
	Stainless Steel Alloys	<i>Escherichia coli</i> <i>Bacillus cereus</i>	[66]
	PLA	<i>Escherichia coli</i> <i>Bacillus cereus</i>	
	Polyamide	<i>Escherichia coli</i> <i>Bacillus cereus</i>	
	Nylon	<i>Escherichia coli</i> <i>Bacillus cereus</i>	

	Polyamide12	<i>Saccharomyces cerevisiae</i>	[73]
DLP	PEGDA 575	<i>Marine Bacteria</i>	[74]
	Resin (PMMA based)	<i>Streptococcus mutans</i>	[75]
	Flexible resin	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	[76]
	Hard ENG resin	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	
	PEGDA	N/A	[77]
	PEGDMA	N/A	
	InkJet	Poly-TCDMDA	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>
Poly-EGDPEA		<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	
PET		<i>Staphylococcus aureus</i>	[79]
		<i>Pseudomonas aeruginosa</i>	
PMMA	<i>Staphylococcus aureus</i>	[80]	
PolyJet	PCL	<i>Marine Flora</i>	[60]
	PDMS	<i>Marine Flora</i>	
	ABS (VisiJet®)	<i>Marine Flora</i>	
	VeroClear™ (Similar to PMMA)	<i>Marine Flora</i>	

Table 4. Microorganisms frequently studied on antimicrobial 3D printed materials developed using greener techniques, plus the appropriate 3D printing technology.

3D PRINTING TECHNOLOGY	3D PRINTED MATERIAL	STUDIED MICROORGANISMS	REFERENCES
FDM	PLA (+Antimicrobials)	<i>Escherichia coli</i>	[11]
		<i>Staphylococcus aureus</i>	
		<i>Pseudomonas aeruginosa</i>	
	PLA (+Graphene)	<i>Pseudomonas aeruginosa</i>	[81]
	PLA COS, (+ COS+ ZnHNTs + Ag)	<i>Staphylococcus aureus</i>	[53]
		<i>Staphylococcus epidermidis</i>	
	PLA (+AcAc)	<i>Staphylococcus aureus</i>	[54]
<i>Pseudomonas aeruginosa</i>			

	PLA (+Ag)	<i>Escherichia coli</i>	[82]
		<i>Staphylococcus aureus</i>	
		<i>Pseudomonas aeruginosa</i>	
	PLA (+Ag NW)	<i>Staphylococcus aureus</i>	[83]
		<i>Escherichia coli</i>	
	PLA (+Col)	<i>Staphylococcus aureus</i>	[84]
	PLA (+MH)		
	PLA (+cHA)		
	PLA (+NF)	<i>Staphylococcus aureus</i>	[85]
	PLA (+HA)		
	PCL (+ASA)	<i>Staphylococcus aureus</i>	[86]
	PMMA (+ATB)	<i>Escherichia coli</i>	[87]
PLGA/HA (+HACC)	<i>Staphylococcus aureus</i>	[88]	
Metal (Cu + PLA resin)	<i>Escherichia coli</i>	[58]	
	<i>Staphylococcus aureus</i>		
Metal (Polished Bronze + PLA resin)	<i>Escherichia coli</i>	[58]	
	<i>Staphylococcus aureus</i>		
FFF	ABS (+AgNPs)	<i>Acinetobacter baumannii</i>	[89]
		<i>Escherichia coli</i>	
		<i>Pseudomonas aeruginosa</i>	
		<i>Staphylococcus aureus</i>	
		<i>Candida albicans</i>	
	PLA (+AcAc)	<i>Pseudomonas aeruginosa</i>	[90]
		<i>Staphylococcus aureus</i>	
PLA (+TEOS)	<i>Listeria monocytogenes</i>		
PLA (+Graphene)	N/A	[56]	
PLA (+Lignin)	<i>Staphylococcus aureus</i>	[57]	
SLA	PMMA (+Nitrides)	<i>Staphylococcus epidermidis</i>	[91]
		<i>Escherichia coli</i>	
	Elastic resin (+Hydrochloride Lidocaine)	N/A	[64]
	PNIPAM (+CNF)	<i>Escherichia coli</i>	[67]
	Nanomodified Alumina	<i>Listeria monocytogenes</i>	[92]
<i>Staphylococcus aureus</i>			
<i>Staphylococcus epidermidis</i>			

		<i>Escherichia coli</i>	
	PLA (+NF)	<i>Staphylococcus aureus</i>	[14]
SLM	Titanium (+HACC)	<i>Staphylococcus aureus</i>	[93]
SLS	Polyamide 12 (+ 1%B65003)	<i>Staphylococcus aureus</i>	[94]
		<i>Pseudomonas aeruginosa</i>	
	Polyamide 12 (+UV stabilizer)	<i>Saccharomyces cerevisiae</i>	[73]
DLP	GGMMA (+LNP TM +AgNP)	<i>Escherichia coli</i>	[95]
		<i>Staphylococcus aureus</i>	
	Resin (+ QAC)	<i>Escherichia coli</i>	[96]
Resin (+SH-QAC)	<i>Staphylococcus epidermidis</i>		
DIW	Ceramic (+3Y-TZP)	<i>Escherichia coli</i>	[97]
		<i>Streptococcus salivarius</i>	
	MG-PVA	<i>Escherichia coli</i>	[98]
	MG(+LEV)-PVA(+VAN)	<i>Staphylococcus aureus</i>	
G(+RIF)MG(+LEV) PVA(+VAN)			
Inkjet	PMMA (+MPC)	<i>Staphylococcus aureus</i>	[99]
		<i>Streptococcus mutans</i>	
		<i>Klebsiella oxytoca</i>	
		<i>Klebsiella pneumonia</i>	
	PMMA (+SB)	<i>Staphylococcus aureus</i>	
		<i>Streptococcus mutans</i>	
		<i>Klebsiella oxytoca</i>	
		<i>Klebsiella pneumonia</i>	
	Plastic (+Gel +ATB)	<i>Escherichia coli</i>	[100]

3.4. Microorganisms Involved in Biofilm Formation on 3D Printed Materials

Bacteria are present in the environment in two forms: planktonic and sessile, both of which have existed on earth since the manifestation of the earliest microbial species [101]. In their planktonic form, microorganisms are isolated in suspension in a liquid medium. In sessile form they are associated in a

complex structure called Biofilm [102]. Biofilm formation is quite advantageous and beneficial for almost all microorganisms, especially bacteria on any biotic or abiotic surface, including 3D printed materials. Biofilm formation can be divided into five stages: (i) initial attachment (ii) reversible adhesion (iii) irreversible adhesion (iv) maturation and (v) dispersion as illustrated in Figure 5.

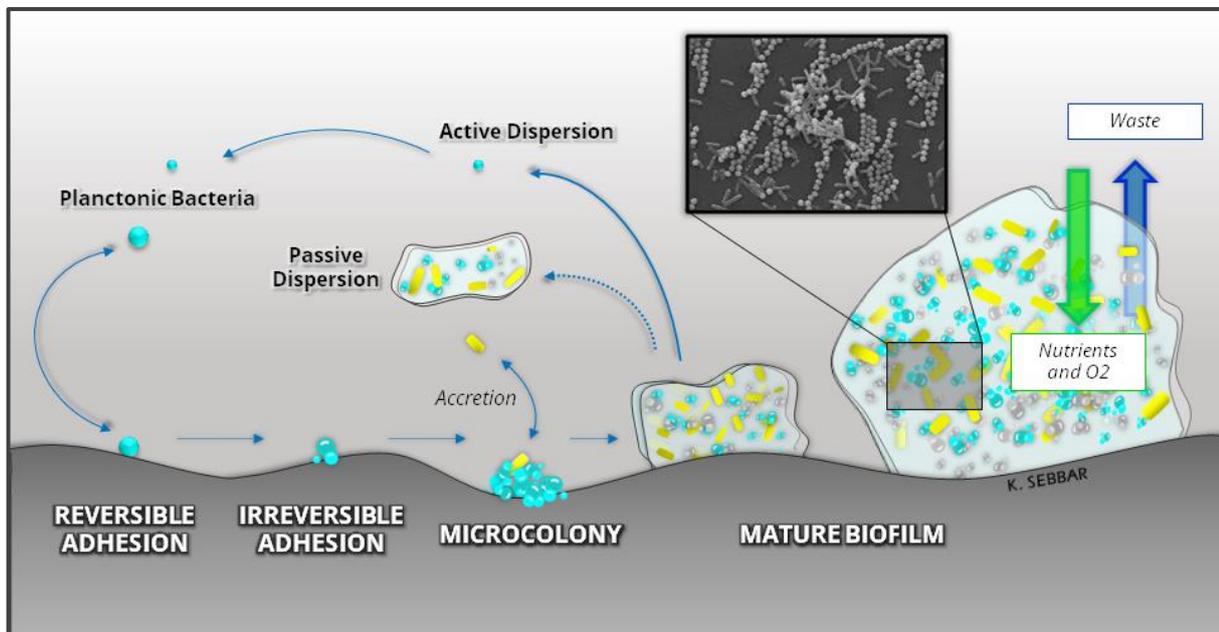


Figure 5. Development and structure of a bacterial Biofilm.

Therefore, according to data obtained from scientific literature regarding the biofouling of 3D printed surfaces, and also according to our collected data on the antibacterial properties of such materials, Special attention was paid to *Staphylococcus aureus* (n=29), *Escherichia coli* (n=20) and *Pseudomonas aeruginosa* (n=11) being the top three most commonly studied bacterial species on 3D printed surfaces, in both forms whether as planktonic cells or as biofilms (Table 5) and (Figure 6).

E. coli is the gram-negative rod-shaped bacterium best known for its biofilm-forming capabilities. *E. coli* can secrete toxins, polysaccharides, and biofilms, making their eradication and treatment quite difficult. In spite of that, *Staphylococcus aureus* was found to be the model studied bacteria (n=29), Because of its well-known involvement in various diseases linked to biofilm formation on different type of surfaces [105]. Other bacteria such as, *Staphylococcus epidermidis* (n=5), *Listeria monocytogenes* (n=3), *Bacillus cereus* (n=1), *B. subtilis* (n=1), *Klebsiella oxytoca* (n=1), *K. pneumonia* (n=1), *Mycobacterium smegmatis*, (n=1), *M. abscessus* (n=1), *M. bovis* (n=1), *Acinetobacter baumannii* (n=1) and *Streptococcus mutans* (n=3), *S. salivarius* (n=1) as well as fungi species such as, *Saccharomyces cerevisiae* (n=2) and *Candida albicans* (n=2) have been the subject of other studies conducted on the biofouling of 3D manufactured materials. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* are frequently studied as they are among the most prevalent bacteria found on human skin and hair [106].

Table 5. Microorganisms often studied on 3D printed materials, their fields of use, and the number of studies dedicated to each microorganism collected from the examined publications.

Studied microorganism	Field of study	Number of studies
<i>Acinetobacter baumannii</i>	Medical	1
<i>Bacillus cereus</i>	Others	1
<i>Bacillus subtilis</i>	Food industry	1

<i>Buccal flora</i>	Medical	1
<i>Candida albicans</i>	Others	2
<i>Escherichia coli</i>	Medical, Food industry, Environment, Biotechnology, Others	20
<i>Klebsiella oxytoca</i>	Medical	1
<i>Klebsiella pneumoniae</i>	Medical	1
<i>Listeria monocytogenes</i>	Food industry	3
<i>Marine Bacteria</i>	Environment	1
<i>Marine Flora</i>	Environment	1
<i>Mycobacterium abscessus</i>	Medical	1
<i>Mycobacterium bovis</i>	Medical	1
<i>Mycobacterium smegmatis</i>	Medical	1
<i>Pseudomonas aeruginosa</i>	Medical, Food industry, Environment, Others	11
<i>Saccharomyces cerevisiae</i>	Others	2
<i>Staphylococcus aureus</i>	Medical, Food industry, Environment, Others	29
<i>Staphylococcus epidermidis</i>	Medical, Food industry	5
<i>Staphylococcus pseudintermedius</i>	Medical	1
<i>Streptococcus mutans</i>	Medical	3
<i>Streptococcus salivarius</i>	Medical	1

Furthermore, they are responsible for many diseases, such as hospital acquired pneumonia, nosocomial bloodstream infections, diarrheal infections, meningitis, wound infections, and septicemia . Through our research we were also able to notice that most of the microorganisms studied belong to the medical field, where (n=14) out of the (n=20) species most commonly investigated for their formation on 3D printed surfaces, were stains isolated in clinical environments or studied on some specific medical devices (Table 5)and(Figure 6). This predominance could be simply explained by the fact that bacterial contamination, biofilm infections, in particular, are extremely challenging to treat in a clinical setting since the germs that reside within are significantly more resistant to antibiotics and disinfectants [13], which calls for more studies and solution searching in this field.

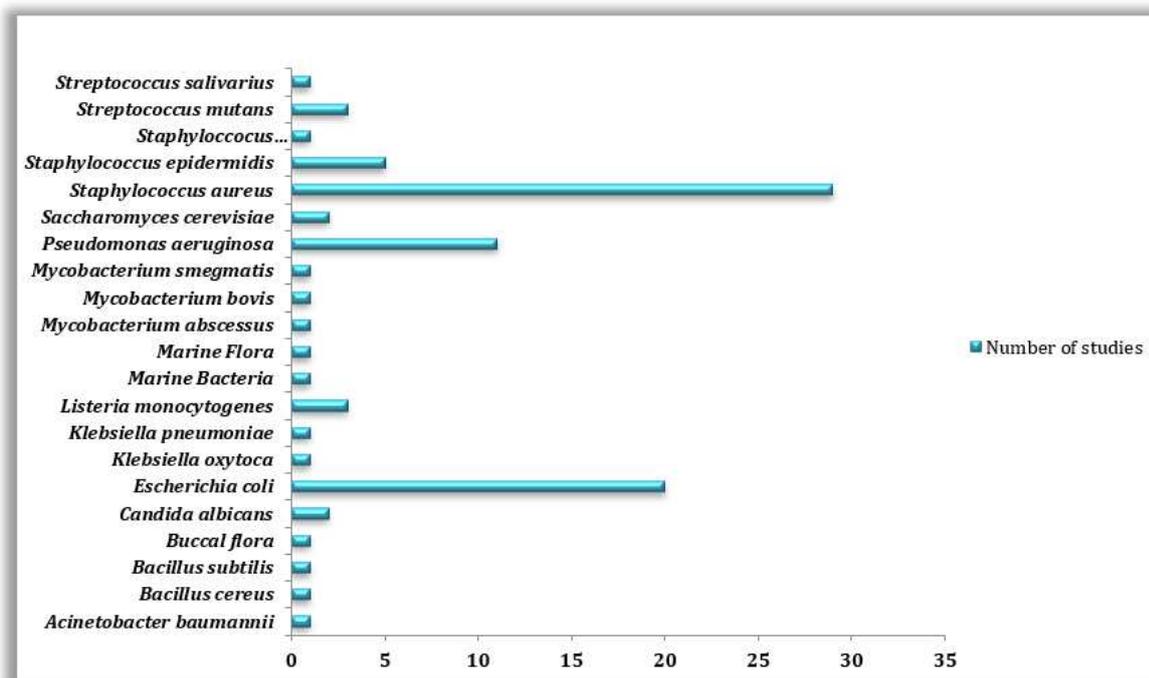


Figure 6. Types of microorganisms used in the reviewed studies.

4. Antimicrobial Approaches Against the Microbial Proliferation or the Formation of Biofilms Adopted by Recent Publications

This section provides a summary of the recently reviewed articles. Table 6 summarizes some existing natural or synthetic control approaches against the proliferation of microorganisms and surface biofouling of AM manufactured surfaces.

First of all, it's worth mentioning that (n=37) out of the (n=50) investigated papers were articles that addressed the biofouling problem in 3D printed objects, in which a variety of green approaches in order to avoid microbial attacks on such surfaces were introduced and evaluated. Once more, we noticed a predominance of medical applications when it comes to this type of research with a total of (n=28) articles out of (n=37). Then Agri-food domain with only two studies (n=2), the same number of studies belonged to the environmental field (n=2), whereas the last five articles involved a variety of fields (n=5). The data presented in Table 6 indicates that PLA was the most extensively researched antimicrobial material produced using 3D printing, with (n=11) studies conducted on it. In the upcoming sections, we will delve into the various treatments and modifications that have been applied to this material, and the same goes for the rest of the materials. Additionally, the most commonly studied microorganism was *Staphylococcus aureus*, with (n=28) studies conducted on this well-known bacterium frequently used as a model species due to its involvement in numerous infections biofilm related [105]. Furthermore, the examinations that were carried out concluded that the integrity of papers adopted one out of the four major approaches: (i) antimicrobial 3D printed materials (n=2), (ii) printing process modification (n=5), (iii) surface coatings (n=19), (iv) 3D printed composites with antimicrobial properties (n=8) and rarely (v) combined / hurdle therapy (n=3) (Table 6).

Table 6. Summary of antimicrobial approaches used against the proliferation of microorganisms and surface biofouling of AM manufactured surfaces.

	1) Antibiofilm coatings through atmospheric pressure plasma for 3D printed surgical instruments	PLA (+AcAc)	<i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i>	Acrylic acid (AcAc) coatings applied by plasma polymerization were deposited on 3D printed polylactic acid (PLA) Petri dishes. AcAc coatings with less number of plasma passes were more effective, and showed up to a 50% relative biofilm reduction compared to the untreated plates.	[54]
	2) Engineering a multifunctional 3D-printed PLA-collagen/minocycline nanoHydroxyapatite scaffold with combined antimicrobial and osteogenic effects for bone regeneration	PLA (+Col) PLA (+Col+MH) PLA (+Col+MH+cHA)	<i>Staphylococcus aureus</i>	3D printed poly (lactic acid) (PLA) scaffolds with bioinspired surface coatings had the ability to reduce bacterial biofilm formation. PLA 3D printed scaffolds were further multifunctionalized with collagen (Col), minocycline (MH) and bioinspired citrate-hydroxyapatite nanoparticles (cHA).	[84]

	<p>3) Antibacterial efficacy of quaternized chitosan coating on 3D printed titanium cage in rat intervertebral disc space</p>	<p>Ti (+HACC)</p>	<p><i>Staphylococcus aureus</i></p>	<p>Mesh-like titanium (Ti) cages that anatomically fit into the discs were fabricated by 3D printing. Additionally, an antibacterial coating was applied with quaternized chitosan (Ti-HACC). All of the in vitro tests showed that Ti-HACC cages have antibacterial properties. Implanting Ti-HACC cages in vivo instead of normal Ti cages, the amount of bacteria in the removed cages decreased significantly.</p>	<p>[93]</p>
	<p>4) AgNPs-decorated 3D printed PEEK implant for infection control and bone repair</p>	<p>PEEK (+AgNPs)</p>	<p><i>Escherichia coli</i> & <i>Staphylococcus aureus</i></p>	<p>In this study, they developed a novel Ag-decorated 3D printed PEEK via catecholamine chemistry, where silver nanoparticles (AgNPs) were evenly anchored on the surface. The Ag-decorated 3D PEEK scaffolds displayed significant antibacterial and antibiofilm effects towards Gram-negative and Gram-positive bacteria.</p>	<p>[62]</p>

	<p>5) Studies on the cytocompatibility, mechanical and antimicrobial properties of 3D printed poly(methyl methacrylate) beads.</p>	<p>PMMA (+GEN) PMMA (+TOB) PMMA (+NF)</p>	<p><i>Escherichia coli</i> & <i>Staphylococcus aureus</i></p>	<p>Gentamicin sulfate, tobramycin, and nitrofurantoin were doped into PMMA and antibiotic-doped 3D printed beads, disks, and filaments were successfully printed. Growth inhibition assays demonstrated the efficacy of antibiotic-loaded PMMA 3D printed constructs in inhibiting bacterial growth.</p>	<p>[109]</p>
	<p>6) Explorative study on the antibacterial effects of 3D-printed PMMA/nitrides composites</p>	<p>PMMA (+Si) PMMA (+Zr) PMMA (+Hf) PMMA (+Al)</p>	<p><i>Escherichia coli</i> & <i>Staphylococcus epidermidis</i>.</p>	<p>This study suggests that PMMA/nitride coatings can improve the antibacterial properties of PMMA implants. The application of nitride-PMMA composite coatings on 3D printed parts increased their resistance to bacteria colonization. Four different nitrides were tested: silicon, zirconium, hafnium and aluminum.</p>	<p>[91]</p>

	<p>7) Dual-functional 3D-printed composite scaffold for inhibiting bacterial infection and promoting bone regeneration in infected bone defect models</p>	<p>PLGA (+HA+HACC)</p>	<p><i>Staphylococcus aureus</i></p>	<p>In this study a HACC-grafted 3D-printed PLGA/HA porous scaffold endowed with a dual antibacterial and osteogenic functionality was manufactured. PLGA/HA/HACC composite scaffold exhibited enhanced anti-infection and bone repairing capability in two different infected bone defect models.</p>	<p>[88]</p>
	<p>8) Polymer infiltrated ceramic networks with biocompatible adhesive and 3D-printed highly porous scaffolds Polymer infiltrated ceramic networks with biocompatible adhesive and 3D-printed highly porous scaffolds</p>	<p>Ceramic (+3Y-TZP)</p>	<p><i>Escherichia coli & Streptococcus salivarius</i></p>	<p>The novel porous zirconia scaffolds prepared using (PICN) and 3D-printing technologies by the deposition of (3Y-TZP) and Pluronic® hydrogel ceramic paste. The scaffolds exhibit antimicrobial properties similar to that of 3Y-TZP, as has been demonstrated by the adhesion and proliferation tests with <i>E. coli</i> and <i>S. salivarius</i> bacteria.</p>	<p>[97]</p>

	<p>9) Controlled-release of free bacteriophage nanoparticles from 3D-plotted hydrogel fibrous structure as potential antibacterial wound dressing</p>	<p>Hydrogel (+HZJ phage)</p>	<p><i>Escherichia coli</i></p>	<p>Phage-embedded hydrogel fibers were used to create porous wound dressing material using three-dimensional (3D) printing. This antibacterial dressing was capable of slowly releasing lytic phages and effectively suppressing bacterial growth for up to 24 h was produced in this study. This model represents an attractive means to reduce use of antibiotics and other additives in conventional dressings.</p>	<p>[110]</p>
	<p>10) Ink-jet 3D printing as a strategy for developing bespoke non-eluting Biofilm resistant medical devices</p>	<p>Poly-TCDMDA Poly-EGDPEA</p>	<p><i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i></p>	<p>Bespoke devices were manufactured through ink-jetting using bacterial biofilm inhibiting formulations without the need for eluting antibiotics or coatings. The 3D printed poly-TCDMDA and poly-EGDPEA were selected on the basis of their in vitro bacterial biofilm inhibitory properties. <i>P.aeruginosa</i> biofilm formation on poly-TCDMDA was reduced by ~99%</p>	<p>[78]</p>

				when compared with medical grade silicone.	
11) 3D scaffold with effective multidrug sequential release against bacteria biofilm	MG-PVA MG(+LEV)-PVA(+VAN) G(+RIF)MG(+LEV)PVA(+VAN)	<i>Escherichia coli & Staphylococcus aureus</i>		Hierarchical 3D multidrug scaffolds based on nanocomposite bioceramic and polyvinyl alcohol (PVA) prepared by rapid prototyping with an external coating of gelatin-glutaraldehyde (Gel-Glu) have been fabricated. These 3D scaffolds contain three antimicrobial agents (rifampin, levofloxacin and vancomycin). This combined therapy is able to destroy Gram-positive and Gram-negative bacteria biofilms as well as inhibit the bacteria growth.	[98]
12) Towards fabrication of 3D printed medical devices to prevent biofilm formation	PLA (+NF)	<i>Staphylococcus aureus</i>		In this study the incorporation of the antibiotic nitrofurantoin (NF) in the polymer carrier material and 3D printing of a model structure resulted in an inhibition of biofilm colonization.	[14]

	<p>13) 3D printed bioceramics for dual antibiotic delivery to treat implant-associated bone infection</p>	<p>CPS (+RIF+VAN)</p>	<p><i>Staphylococcus aureus</i></p>	<p>Rifampin- and vancomycin-laden calcium phosphate scaffolds (CPS) were fabricated by (3D) printing to treat an implant-associated <i>S. aureus</i> bone infection. All vancomycin- and rifampin-laden CPS treatments significantly reduced the bacterial burden compared with vancomycin-laden PMMA.</p>	<p>[80]</p>
	<p>14) Antimicrobial Thiol-ene-acrylate Photosensitive Resins for DLP 3D Printing</p>	<p>Resin (+QAC) Resin (+SH-QAC)</p>	<p><i>Escherichia coli</i> & <i>Staphylococcus aureus</i></p>	<p>In this contribution, a thiol-ene-acrylate ternary system was chosen as the antibacterial 3D printing matrix resin. Two quaternary ammonium salt-type antibacterial agents (QAC and SH-QAC) were designed and prepared to achieve contact antibacterial effect. Both antibacterial photo-sensitive resins have been successfully applied in DLP technology to fabricate tooth model with high precision.</p>	<p>[96]</p>

15) Durable Oral Biofilm Resistance of 3D-Printed Dental Base Polymers Containing Zwitterionic Materials	PMMA (+ MPC) PMMA (+ SB)	<i>Klebsiella oxytoca</i> , <i>Klebsiella pneumonia</i> , <i>Staphylococcus aureus</i> & <i>Streptococcus mutans</i>	This study indicates that the addition of MPC or SB into PMMA results in durable oral salivary Biofilm inhibition, with the maintenance of physical and mechanical properties.	[99]
16) Anti-biofilm multi drug-loaded 3D printed hearing aids	Flexibale resin (+Cipro+FA) Hard resin (+Cipro+FA)	<i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i>	Two polymer resins for 3DP, "ENG hard" and "Flexible" loaded with two antibiotics, ciprofloxacin and fluocinolone acetone. All multi-drug-loaded devices exhibited a hydrophilic surface, excellent blood compatibility and anti-biofilm activity against <i>P. aeruginosa</i> and <i>S. aureus</i> .	[76]
17) Use of 3D Printing for the Development of Biodegradable Antiplatelet Materials for Cardiovascular Applications	PCL (+10% ASA +1% RIF)	<i>Staphylococcus aureus</i>	PCL and ASA were used to prepare biodegradable antithrombotic vascular grafts using an extrusion-based 3D printing technique. Moreover, RIF was combined with ASA and PCL to obtain antimicrobial vascular grafts. These materials were capable of inhibiting the growth of <i>S. aureus</i> .	[86]

	<p>18) Rational design of additively manufactured Ti6Al4V implants to control <i>Staphylococcus aureus</i> biofilm formation</p>	<p>Titanium alloy Ti-6Al-4 V</p>	<p><i>Staphylococcus aureus</i></p>	<p>In order to modify the surface topography of metallic implants for directed <i>Staphylococcus aureus</i> biofilm restriction, lowering the angle during SLM printing gave metallic surfaces lower roughness, lower hydrophobicity, higher surface energy, and fewer partially melted metal particles without altering the bulk surface chemistry, which directly correlated with significantly lower biofilm coverage and an associated reduction in microbial biomass.</p>	<p>[71]</p>
	<p>19) Antioxidant PLA Composites Containing Lignin for 3D Printing Applications: A Potential Material for Healthcare Applications</p>	<p>PLA (+LIG+TC)</p>	<p><i>Staphylococcus aureus</i></p>	<p>3D printed meshes were prepared using PLA/LIG composite materials. These meshes can provide mechanical protection to the wound while providing antioxidant activity. Soluble patches containing drugs can be applied to the surface of the mesh. The drug can diffuse through the mesh pores to the</p>	<p>[57]</p>

			wound. In the present work, they used TC an antibiotic compound which showed a significant reduction in bacterial adherence.	
20) Three Dimensional Printed Polylactic Acid (PLA) Surgical Retractors with Sonochemically Immobilized Silver Nanoparticles: The Next Generation of Low-Cost Antimicrobial Surgery Equipment	PLA (+AgNPs)	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i>	A surgical retractor was created using a commercial polylactic acid (PLA) thermoplastic filament and a simple and scalable sonochemical deposition method to create a thin layer of silver (Ag) nanoparticles (NPs). <i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>E. coli</i> , bacteria viability were all reduced when the PLA retractor was coated with Ag NPs (PLA@Ag).	[82]
21) Manual polishing of 3D printed metals produced by laser powder bed fusion reduces biofilm formation	Titanium alloy Ti6Al4V Stainless steel 316L Cobalt chromium alloy CoCr	<i>Staphylococcus aureus</i> & <i>Streptococcus pyogenes</i>	This study suggests that metallic implants produced by laser powder bed fusion should be polished since the polishing of 3D printed titanium alloy, stainless steel, or cobalt chromium alloy disks has significantly reduced biofilm growth on these surfaces.	[70]

	22) Bacterial Biofilm Growth on 3D-Printed Materials	LAB-made or commercially available PLA (+Antimicrobials)	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , & <i>Staphylococcus aureus</i>	Biofilm formation depends on some of the polymer's antibacterial activities. They compared their tested materials with commercially available antimicrobial PLA polymers. The greatest antimicrobial and antibiofilm activity were observed in the case of BRS PLA polymer. According to the manufacturer, this polymer contains about 40% metal.	[11]
	23) Three-Dimensional Printing of Drug-Eluting Implants: Preparation of an Antimicrobial Polylactide Feedstock Material	PLA (+NF) PLA (+HA)	<i>Staphylococcus aureus</i>	Nitrofurantoin (NF) and hydroxyapatite (HA) were successfully mixed and extruded with up to 30% drug load with and without addition of 5% HA in polylactide strands, which were subsequently 3D-printed into model disc geometries. Disks with 30% drug loading were able to prevent surface-associated and planktonic growth of <i>S. aureus</i> over a period of 7 days.	[85]

24) Digital light processing (DLP) 3D-fabricated antimicrobial hydrogel with a sustainable resin of methacrylated woody polysaccharides and hybrid silver-lignin nanospheres	Resin GGMMA (+LNP +AgNPs)	<i>Escherichia coli</i> & <i>Staphylococcus aureus</i>	A bio-based antimicrobial resin was developed for DLP printing engaging GGMMA as a photocrosslinkable polymeric matrix and the nanocomposite lignin nanoparticles that are surface-embedded with silver nanoparticles (LNP@Ags) as a high-performance antimicrobial reagent. The GGMMA/LNP@Ag hydrogel also possesses high antimicrobial activity due to the bactericidal ability of Ag ⁺ that was leached out of the hydrogel in a sustained manner.	[95]	
25) Changes in tribological and antibacterial properties of poly(methyl methacrylate)-based 3D-printed intra-	PMMA based resin (+ 0,1% ND)	<i>Streptococcus mutans</i>	The present study aimed to evaluate the role of nanodiamonds (NDs). Using a solution-based mixing technique, 0.1 wt% ND was incorporated into the PMMA, and	[75]	

	oral appliances by incorporating nanodiamonds			specimens were 3D-printed for tribological and bacterial analysis. The addition of 0.1 wt% ND in the PMMA-based resin for 3D printing resulted in significant resistance to <i>S. mutans</i> .	
26)	3D printed antimicrobial PLA constructs functionalised with zinc-coated halloysite nanotubes-Ag-chitosan oligosaccharide lactate	PLA (+ ZnHNTs-Ag-COS)	<i>Staphylococcus aureus</i>	3D printed polylactic acid (PLA) constructs were alkali-treated to increase hydrophilicity and functionalized using a suspension of Zinc/HNTs-Ag-Chitosan Oligosaccharide Lactate (ZnHNTs-Ag-COS). Antibacterial evaluation confirmed the anti-biofouling potential of the PLA constructs (which was a function of the Ag content in the material).	[53]
27)	Exploiting Generative Design for 3D Printing of Bacterial Biofilm Resistant Composite Devices	Ink A & Ink B	<i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i>	This work has demonstrated the manufacture of MM-IJ3DP printed devices that are personalisable through generative design guided co-deposition of inks to create functional composites that are both	[79]

				resistant to bacterial biofilm formation and achieve a specific deformation profile.	
	28) Antimicrobial Activity of 3D-Printed Acrylonitrile Butadiene Styrene (ABS) Polymer-Coated with Silver Nanoparticles	ABS (+AgNPs)	<i>Acinetobacter baumannii</i> , <i>Candida albicans</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i>	This work describes a unique approach for attaching a layer of AgNPs to 3D-printed polymer acrylonitrile butadiene styrene (ABS) plastic using acetone. For all examined bacterial species, AgNP-coated ABS (AgNP-ABS) indicated considerable eradication of live bacteria after 4 hours, and for the tested fungal stain, it was within 19 hours.	[89]
	1) Reduction of biofilm formation on 3D printing materials treated with essential oils major compounds	PET (+CR or TML) DMHB resin(+CR or TML)	<i>Bacillus subtilis</i> & <i>Escherichia coli</i>	This study aimed to investigate the effect of thymol and carvacrol on the physicochemical characteristics of DMHB resin and PET using the contact angle method. Finally it was recommended to incorporate the studied major compounds into the composition of PET and resin materials in order to use them in the food industry.	[59]

	2) Atmospheric pressure cold plasma anti-biofilm coatings for 3D printed food tools	PLA (+AcAc) PLA (+TEOS)	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> . & <i>Pseudomonas aeruginosa</i> ,	Plasma-polymerized acrylic acid (AcAc) and tetraethyl orthosilicate (TEOS) coatings were used to minimize biofilm development on 3D printed PLA materials. The reduction in bacterial adhesion and biofilm development might be explained by chemical (hydration layer formation) and morphological (distance between peaks) changes induced by plasma-polymerized treatments.	[90]
	1) Single-Step 3D Printing of Silver-Patterned Polymeric Devices for Bacteria Proliferation Control	Resin (+ AgNPs)	<i>Environmental microorganisms</i>	An acrylate resin containing silver nitrate (AgNO ₃) as a silver precursor is employed to generate silver nanoparticles (AgNPs). The fabricated silver-patterned devices exhibit different surface features that might be exploited in systems working in a marine environment to control Biofilm proliferation.	[74]

	2) Bacterial Biofilm Formation on Nano-Copper Added PLA Suited for 3D Printed Face Masks	Plactive™	<i>Escherichia coli</i> , <i>Pseudomonas aeruginos</i> & <i>Staphylococcus aureus</i>	In this study they analyzed a 3D printing material as a substitute for single-use face masks: Plactive™. Compared to unblended PLA (Giantarm™), Plactive™ PLA material has showed antimicrobial activities against Gram positive <i>S. aureus</i> but not for Gram negative <i>P. aeruginosa</i> and <i>E. coli</i> .	[63]
	1) 3D-Printable Materials for Microbial Liquid Culture	"Flexible", "ClearV2" & "TangoPlus"	<i>Escherichia coli</i>	Mass spectrometry was used to identify leached chemicals that inhibited bacterial growth. The FormLabs, "Flexible" and "ClearV2," and the Stratasys "TangoPlus" materials inhibited growth to varying degrees.	[111]
	2) 3D printed antibacterial silver nanowire/poly lactide nanocomposites	PLA (+AgNW)	<i>Escherichia coli</i> & <i>Staphylococcus aureus</i>	Antibacterial 3D printed nanocomposites : Silver nanowire (Ag NW) loaded polylactide (PLA) nanocomposites were investigated. The Ag NW loaded PLA nanocomposites show bactericidal activity against <i>E. coli</i> and <i>S. aureus</i> ,	[83]

			which are the most common bacteria types living in public areas.	
3) Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small is small enough?	Nanoporous Alumina	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> & <i>Staphylococcus epidermidis</i>	Anodic nanoporous surfaces, the approach exploits anodisation to create alumina surfaces with cylindrical nanopores with diameters ranging from 15 to 100 nm. This method have effectively minimised bacterial attachment or biofilm formation by all the microorganisms tested.	[92]
4) Use of silver-based additives for the development of antibacterial functionality in Laser Sintered polyamide 12 parts	Polyamide 12 (+ 1% B65003 silver phosphate glass)	<i>Pseudomonas aeruginosa</i> & <i>Staphylococcus aureus</i>	A commercially available antimicrobial additive (Biocote® B65003) was combined with a widely used Laser Sintering powder (polyamide 12, EOS PA2200) to create an antimicrobial material suitable for a range of potential uses. The composite material was able to reduce numbers of planktonic bacteria in its surroundings and	[94]

				numbers of biofilm bacteria attached to the surface.	
	5) Printing accuracy, mechanical properties, surface characteristics, and microbial adhesion of 3D-printed resins with various printing orientations	PMMA (with a modified printing orientation)	<i>Candida albicans</i>	The goal was to see how printing orientation can affect 3D-printed denture base resin microbiological response. Denture base polymethyl methacrylate (PMMA) was used to print samples in three different printing orientations (0, 45, and 90 degrees). <i>C. albicans</i> response was assessed, With statistical significance, specimens printed at 90°<45°<0° orientation degrees included a greater percentage of <i>C. albicans</i> .	[68]

Due to their inherent antibacterial qualities, recently synthetic polymers are without a doubt one of the most employed materials in this context without the need for eluting antibiotics or coatings (n=2). For instance, Walsh *et al.*, (2016) have presented a method for assessing the microbial liquid culture potential of ten 3D-printable polymeric materials. They found six 3D-printable materials that created tubes suited for dealing with aqueous liquids, yet during a Mass spectrometry analysis used to identify leached chemicals that inhibited bacterial growth, only three materials; the FormLabs, "Flexibile" and "ClearV2," and the Stratasys "TangoPlus" materials were able to inhibit bacterial growth to varying degrees [111]. In a similar study, where both surface coating and antibiotic elution are not necessary, He *et al.*, (2022) created bespoke devices employing ink-jetting and bacterial biofilm inhibitory compositions. The ability of the 3D printed poly-TCDMDA and poly-EGDPEA to inhibit bacterial biofilms in vitro was assessed. The growth of *Pseudomonas aeruginosa* biofilms on poly-TCDMDA was 99% less than that on medical grade silicone [78]. On another note surface modification of 3D printed parameters (n=5), represent an interesting approach more and more adopted in recent studies. A study led by Sarker *et al.*, (2019) in order to modify the surface topography of metallic implants for directed *Staphylococcus aureus* biofilm restriction, they proved that reducing the angle during SLM printing resulted in metallic surfaces with lower roughness, relatively low hydrophobicity, increased surface energy, and fewer partially melted metal particles without changing the bulk surface chemistry, which was directly correlated with considerably lower biofilm adhesion and microbial biomass minimization [71]. In this context, an illustrative strategy of this approach was also reported by Shim *et al.*, (2019) where they described that printing orientation affects the microbiological reaction of 3D-printed denture base material PMMA, a denture foundation, was utilized to print samples in three distinct printing orientations (0°, 45°, and 90°). The reaction of *Candida albicans* was studied. It was found that the specimens printed at 90° < 45° < 0° orientation degrees had a higher proportion of *C. albicans*. More interestingly and contrasting to other methods, where the generation of AgNPs is usually carried out at a post-treatment stage, this work introduced a substitute strategy to carry out photopolymerization of the resin and photogeneration of AgNPs within the same impression process [68]. With the same goal, which is surface treatment, Er-rahmani *et al.*, (2022) have used the contact angle technique to assess the influence of thymol and carvacrol on the physicochemical features of DMHB resin and PET. A considerable change in the physicochemical properties of both surfaces was observed following treatment. Finally, it was suggested that the key molecules analyzed could be included into the composition of PET and resin materials for future uses in the food industry [59]. The results demonstrated in a study by González Flores *and al.*, (2022), were attained by arbitrarily changing the printing settings during the printing procedure, which allowed for the selective photogeneration of silicon nanoparticles while still in the stage of 3D light printing. Finally, they showed that these 3D-printed items with silver patterns have shown antibacterial efficacy against environmental germs, including maritime environment in order to regulate biofilm growth proliferation [74].

An additional procedure for the manufacturing of antimicrobial AM products involves applying surface coatings using multiple antimicrobial agents. Many illustrative applications of this technique were recently reported (n=19), especially those involving silver nanoparticles coatings (n=4). One of the most recent publications in this context was realized by Wang *et al.*, (2022) in which a bio-based antimicrobial resin was developed for DLP printing, engaging GGMMA as a polymeric matrix and the nanocomposite lignin nanoparticles surface-embedded with silver nanoparticles (LNP@Ags) as a high-performance antimicrobial reagent. The antibacterial activity of the GGMMA(+LNP +Ag) hydrogel is enhanced by the bactericidal capacity of Ag⁺, which was seeped out of the hydrogel continuously over time [95]. Another form of coatings calls for antibiotics (ATBs), natural compounds and other chemical substances application (n=15). Furthermore, Shen *et al.*, (2021) were able to achieve porous wound dressing material with phage-embedded hydrogel fibers. This antibacterial dressing was created with the capability to slowly but surely release lytic phages (HZJ phage) and efficiently inhibit bacterial growth of *Escherichia coli* for up to 24 hours. This concept appears to be a viable option for reducing the reliance on antibiotics and other substances in conventional coatings [110]. Additionally, as demonstrated by Muro-Fraguas *et al.*, (2020) organic compounds can also serve as

another form of coating. Acrylic acid (AcAc) coatings obtained by plasma polymerization were deposited on 3D printed polylactic acid (PLA) Petri plates to prevent the formation of biofilms and to avoid serious infections. When compared to untreated plates, AcAc coatings with fewer plasma passes were more effective, showing up to a 50% reduction in relative biofilm [54].

Last but not least, antimicrobial 3D printed devices have also been developed exploiting composites. Since composite manufacturing constitute a relatively novel approach, this explains the limited work done in this area of 3D printing [113]. Based on our findings, the development of antimicrobial composites was the focus of eight reviewed papers (n=8). In general, metals like zinc, aluminum, or silver, among others, ceramics [97], chitosan [88], and many more have all been used to generate some of the antimicrobial composites. According to a study realized by Marin and al. (2021) PMMA / nitrides composites were able to increase the antibacterial characteristics of PMMA implants. The use of nitride-PMMA composite coatings on 3D printed items made them more resistant against bacterial colonization. Silicon, zirconium, hafnium, and aluminum were the four nitrides studied. When compared to controls, all composite materials demonstrated antibacterial properties, with hafnium nitride being the most effective against *E. coli* and aluminum nitride being the most effective against *S. epidermidis* [91]. He *et al.*, (2021) have shown how to make personalized MM-IJ3DP printed devices using generative design guided ink co-deposition to generate functional composites that are resistant to bacterial biofilm development and have a customized deformation profile. When compared to regularly used silicone rubbers, the bacterial biofilm coverage of the resultant composites is reduced by up to 75%, plus no bioactives were required or necessary [115].

Finally, combined therapy involves the fusion of a number of strategies to achieve maximized level of antimicrobial activity. In this review (n=3) investigated studies combined two or more of the previously discussed solutions. Garcia-Alvarez and colleagues did put this theory to the test adopting rapid prototyping to create hierarchical 3D multidrug scaffolds based on nanocomposite bioceramic and polyvinyl alcohol (PVA) with an exterior coating using gelatin-glutaraldehyde (Gel-Glu). Three antibacterial agents were included in these AM scaffolds (RIF, LEV, and VAN). This combination treatment was able to kill and limit the growth of both Gram-positive and Gram-negative bacteria biofilms [98]. Humayun *et al.*, (2020) reported that a zinc/HNTs-ag-chitosan oligosaccharide lactate solution was employed to functionalize the 3D printed polylactic acid (PLA) structures after they were alkali treated to increase their hydrophilicity (ZnHNTs-Ag-COS). The resultant PLA construction's anti-biofouling ability was validated by antibacterial testing utilizing *Staphylococcus aureus* cultures during the agar diffusion technique [53]. Lastly and according to a study conducted in 2019 by Dominguez-Robles *et al.*, PLA/LIG composite materials were used to create 3D printed meshes. One of this study's major objectives was to combine an antibiotic (TC) with lignin (LIG), which has been shown to have significant antibacterial action against Gram-positive and Gram-negative bacteria. As a result, *S. aureus* adherence to the materials was effectively reduced in the materials containing 2% (w/w) of TC (tetracycline). However, LIG did not exhibit any antibacterial action in this instance. As a result, the amount of germs adhered to the 3D printed material's surface can be highly reduced employing PLA/LIG/TC composites [57].

5. Conclusion

Numerous review articles have been published on additive manufacturing covering a range of topics related to 3D printed materials and the potential risks of contamination associated with them. Some of these reviews even discussed methods for manufacturing antimicrobial 3DP surfaces. However, most of these articles tend to provide a broad overview of AM processes and their applications in a particular field, or they focus on only one aspect mentioned above without addressing the other.

As the authors of this article, our goal was to demonstrate that while there are promising developments in the use of antimicrobial 3D printed objects, it is crucial to critically and realistically assess the current state of research in various fields such as medicine, environment, and food industries. To achieve this, we gathered information on antimicrobial approaches that have been tested on 3D printed materials, analyzed the AM technologies that have been utilized thus far,

identified suitable materials, and highlighted the germs that are typically targeted across all fields that have been studied to date. The aim of this work is to showcase the efficacy of greener methods in inhibiting biofilm formation on 3D printed surfaces, we also believe that in the near future, through such in-depth investigations we can promote the safe incorporation of antimicrobial / antibiofilm 3D printed surfaces into our day to day practices.

Based on our observations, planktonic microorganisms were the most widely studied form of microbes on 3D printed materials, followed by biofilms, and only a few studies have investigated both forms concurrently. It was also noted that the medical field has been the most active in researching microbial infections due to the increasing incidence rate. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were the top three most commonly tested bacteria on 3D printed surfaces, mainly due to their involvement in the development of multi-resistant biofilms responsible for the majority of incurable infection cases. Furthermore, we observed that FDM technology and PLA materials, whether pure or modified, were the most extensively researched as they provide a durable and cost-effective approach for developing user-specific products.

Our findings indicate that the majority of antimicrobial approaches studied on 3D printed materials employed either like a "before printing" approach, which involved incorporating the antimicrobial substance with the 3D printing material, or as an "after printing" approach, which involved applying a variety of surface treatments or a simple modification of 3D printing parameters. In some cases, a "combination of multiple strategies" was also used simultaneously.

This review article draws its conclusions and observations from the search conducted on Web of Science, Scopus, and Science-Direct. Although the number of published experimental articles is limited, the existing literature still provides a strong groundwork for future research. Exploring 3D printing and antimicrobial processes further may uncover new insights that could broaden the scope of this review and its applicability to various fields of study.

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List of Abbreviations

3DP	Three Dimensional Printing
3Y-TZP	Yttrium-stabilized tetragonal zirconia polycrystal
AcAc	Acrylic acid
AgNP	Silver nanoparticle
AgNW	Silver nanowire
Al	Aluminum
AM	Additive Manufacturing
ASA	Acetylsalicylic acid
ATB	Antibiotic
Cipro	Ciprofloxacin
Col	Collagen
CPS	Calcium phosphate scaffolds
CUR	Curcumin
DLP	Digital Light Processing
EBM	Electron Beam Melting
EPS	Extracellular Polymeric Substances

FA	Fluocinolone Acetonide
FDM	Fused Deposition Modelling
Gel-Glu	Gelatin-glutaraldehyde
Gen	Gentamicin
GGMMA	Methacrylated O-acetyl-galactoglucomannan
HA	Hydroxyapatite
HACC	Quaternized chitosan
Hf	Hafnium
HZJ	Bacteriophage
LIG	Lignin
LNP	Lignin nanoparticle
MPC	2-methacryloyloxyethyl phosphorylcholine
NDs (A-ND)	Amine-functionalized
NDs (ND)	Non-functionalized
NF ou NIT	Nitrofurantoin
PBF	Powder Bed Fusion
PEEK	Polyetheretherketone
PICN	Polymer-infiltrated ceramic network
PLGA	Polylactide-co-glycolide
Poly-EGDPEA	Polyethylene glycol dicyclopentenyl ether acrylate
Poly-TCDMDA	Pol-mers contained monomer D, tricyclodecane-dimethanol diacrylate
RIF	Rifampin/ Rifampicin
SB	Sulfobetaine methacrylate
Si	Silicon
SLA	Stereolithography
SLM	Selective Laser Melting
SLS	Selective Laser Sintering
TEOS	Tetraethyl orthosilicate
Ti	Titanium
TOB	Tobramycin
VAN	Vancomycin
WOS	Web of science
Zr	Zirconium

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