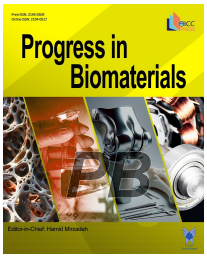

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Silver Nanoparticles (AgNPs)/Gold Nanoparticles (AuNPs) Contained 3D Printed Structures For Tissue Engineering Applications

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Original Research

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Abstract:

In order to solve the lack of organ donors and promote tissue healing, tissue engineering combines biology, engineering, and materials science to develop biological replacements that can maintain, repair, or improve tissue function. Its objective is to use scaffolds, cells, and bioactive materials to generate functional tissues, which may be employed to address organ failures and degenerative disorders. 3D printing technology allows for the development of intricate scaffolds that resemble the extracellular matrix using a variety of biomaterials, such as nanoparticles, bioceramics, and natural and synthetic polymers. Due to their antibacterial and biocompatible qualities, silver and gold nanoparticles are added to these materials to improve wound healing and therapeutic efficacy. Additionally, the special characteristics of gold nanoparticles support medical imaging and sensing applications. By enhancing scaffold performance and infection prevention while encouraging cell proliferation and tissue regeneration, the incorporation of nanoparticles into tissue engineering scaffolds promotes regenerative medicine. In this work, 3D printed structures using AgNPs and AuNPs for applications in tissue engineering and regenerative medicine are reviewed. In order to develop or select materials for 3D printing structures that are effective and advantageous in tissue engineering, the academic and clinical sectors benefit from studying previous investigations in this field. This review offers a thorough analysis of the uses of silver and gold nanoparticles in tissue engineering by integrating a dual emphasis on these particles within different 3D-printing methods. Compared to previous assessments, it also has an organized summary in Table 1, which improves readability and clarity.

Keywords: Tissue engineering and regenerative medicine (TERM); Silver nanoparticles (AgNPs); Gold nanoparticles (AuNPs); 3D printing

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1. Introduction

In order to develop biological replacements that enhance, repair, or preserve tissue function, the interdisciplinary area of tissue engineering incorporates concepts from materials science, engineering, and biology [1]. Tissue engineering is important because it has the potential to transform medicine by solving the lack of organ donors and making it possible to repair damaged tissues [2, 3, 4]. The objective of tissue engineering is to produce functional tissues that can work with the body's natural processes using scaffolds, cells, and bioactive substances [5, 6, 7]. This field has the potential to

be effective in the treatment of a number of diseases, such as degenerative diseases and organ failures [8, 9, 10, 11, 12, 13]. Additionally, it reduces the need for animal models by paving the way for improvements in drug testing and disease modeling [14, 15]. There are various methods to fabricate scaffolds or similar structures for tissue engineering applications; among those, 3D printing is one of the most prominent and frequently used fabrication methods [16, 17, 18, 19].

By allowing the production of intricate and versatile structures that closely resemble the natural extracellular matrix, 3D printing technology is frequently used in the development of tissue engineering structures [20, 21]. The development of biocompatible scaffolds with specific qualities that are necessary to promote tissue regeneration and cell proliferation is made possible by this technology [22, 23, 24, 25, 26]. Applications include the manufacturing of personalized implants [27, 28, 29], the bioprinting of live cells to produce functional tissues [30, 31, 32], and the modeling of organs for surgical and pharmaceutical applications [33, 34, 35]. Depending on the application and the target tissues, various types of biomaterials are employed for 3D printing of tissue engineering structures. Natural and synthetic polymer-based materials [36, 37, 38], bioceramics [39, 40, 41, 42, 43], metal-based nanoparticles [44], hydrogels [45, 46, 47], and composites of two or more types of these materials are often used as feeds of 3D printing devices for the development of 3D printed tissue engineering structures [48, 49, 50, 51, 52]. Silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) are among the agents that have been added to base materials, including bioceramics and polymers, for the synthesis of these composites [53, 54, 55, 56]. The AgNPs may be utilized in many different fields, including electronics [57, 58], analytical chemistry [59], and medicine [60, 61], as well as they are effective for a number of applications, such as biosensing [62, 63, 64, 65, 66, 67, 68, 69], environmental monitoring [70], food safety [71], and regenerative medicine [72, 73]. Physical processes, such as laser ablation [74], chemical reduction utilizing agents like sodium citrate [75, 76], and biological approaches employing plant extracts or bacteria [77, 78], are some of the methods used to synthesize AgNPs. The AgNPs are essential to tissue engineering because of their biocompatibility and antibacterial properties [79, 80, 81]. To lower the risk of infection, improve wound healing, and encourage cell proliferation, they are often used in scaffold and implant coatings [82, 83, 84, 85]. AgNPs may also act as carriers for targeted drug delivery, thereby enhancing therapeutic effectiveness [86, 87]. On the other hand, AuNPs are renowned for their distinct catalytic [88, 89], optical [90, 91], and electrical properties [92, 93]. They are effective in imaging and sensing applications because of their surface plasmon resonance properties, which permit significant light absorption and scattering [94, 95, 96]. Chemical reduction, laser ablation, and biological techniques using bacteria or plant extracts are some of their synthesis techniques [97, 98]. These techniques open up a variety of applications of AuNPs in analytical chemistry [99, 100], biotechnology [101, 102], and medicine [103, 104] by allowing for control over size and form, which affects their behavior and functioning [105]. The AuNPs are also increasingly utilized in tissue engineering structures, especially in developing composites with polymer-based materials, since AuNPs do not form oxides at high temperatures, which is necessary for fused deposition modeling (FDM) 3D printing of polymer-based composites [56, 106, 107]. In addition, the antibacterial properties of AuNPs may aid in preventing implant infections, boosting the effectiveness of several biomedical applications, and increasing overall outcomes in regenerative medicine [108].

The present work is a review of 3D printed structures containing AgNPs and AuNPs for applications in tissue engineering and regenerative medicine (TERM); a schematic of the present review is presented in Scheme 1. The investigation of the literature in this field is helpful for academic and clinical sectors to design or select materials for 3D printing structures that are effective and applicable in tissue engineering. The authors attempted to investigate every single article and retrieved and mentioned their key information in the following sections so that readers can easily follow the subject and obtain the information they need.

2. Materials and methods

The reviewed studies related to sections 3 and 4 were extracted from the Web of Science (WOS) database. The search strategy for section 3 included keywords related to 3D printing, tissue engineering, and silver nanoparticles, as well as their synonyms. In addition, the search strategy related to section 4 contained the keywords relevant to 3D printing, tissue engineering, and gold nanoparticles, along with their synonyms. The inclusion criteria were relevant articles published in the English language and with accessible full-texts. In addition, the exclusion criteria were review papers and articles published in other languages.

3. Silver nanoparticles (AgNPs) contained 3D printed structures for tissue engineering applications

As mentioned in section 1, AgNPs, due to their antibacterial properties, have been used as coatings or in composite forms for 3D printed tissue engineering structures. In this section, recent and significant studies that developed 3D printed structures composed of or coated with AgNPs for regenerative medicine applications are reviewed. Table 1 is the summary presentation of the reviewed investigations in this section.

Using aminated poly-L-lactic acid (EPLA) and nanosilver/zinc-coated black phosphorus (BP@(Zn+Ag)) nanocomposites made by a green *in situ* photodeposition technique, Chen et al. used cryogenic 3D printing to build a multifunctional scaffold. The homogeneous dispersion of nanoparticles was verified by characterization. *In vitro* tests revealed improved cell viability and osteogenic activity in addition to more than 96% antibacterial efficacy against *S. aureus* and *E. coli*. Improved new bone tissue formation was demonstrated *in vivo* in a rat calvarial defect case. Future studies should evaluate these scaffolds'

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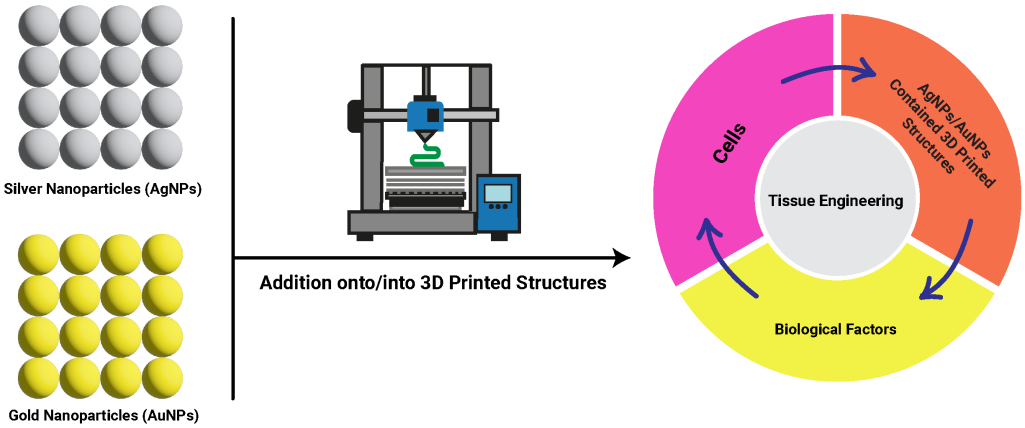
Table 1. Summary of the literature reviewed in the present study, separated by objectives, nanoparticles used, 3D printing techniques, material compositions, results, limitations and challenges, as well as future applications and recommendations.

Objective(s)	Hard/Soft Tissue or Device Applications	Nanoparticles Used	3D Printing Techniques	Material Compositions	Results	Limitations and Challenges	Future Applications and Recommendations	Ref.
Develop conductive supramolecular gels for soft nanoelectronics applications	Soft	AuNPs formed *in situ* from Au(III) solution	Extrusion-based 3D-printing with controlled flow rate and layering	DBS-$CONHNH_2$/AuNP DBS-COOH	DBS-$CONHNH_2$ gels exhibited significantly higher conductivity with AuNPs	Low absolute conductivities; challenges in printing stability and resolution	Potential in soft bioelectronics, tissue engineering, and dynamic materials	[109]
Develop a reliable *in vitro* platform for nanoparticle-modified scaffolds	Not mentioned specifically	Gold and silica nanoparticles for surface modification of scaffolds	—	PLA-AuNPs PLA-Silica Nanoparticles	Enhanced cell viability, mineralization, and protein adsorption observed significantly	*In vivo* testing, reliability, and scalability of synthesis methods remain concerns	High-throughput screening for biocompatibility in tissue engineering applications	[110]
Develop a multifunctional for bone tissue engineering	Hard	AgNPs and zinc nanoparticles (ZnNPs)	Cryogenic 3D printing	Aminated poly-L-lactic acid (EPLA) and BP@(Zn+Ag)	High antibacterial activity and enhanced osteogenic properties	—	Orthopedic implants for bone defect repair	[111]
Investigate Aerosol Jet® printing for 3D microstructuring capabilities	Not mentioned specifically	AgNPs	Continuous jet deposition, layer-by-layer, point-wise printing strategies	AgNPs, PEDOT:PSS, collagen, hydroxyapatite	Successfully printed micropillars, complex structures; varying biocompatibility	High cytotoxicity of AgNPs; process control and repeatability issues	Broadened use in tissue engineering, bioelectronics, and micromanufacturing sectors	[112]
Evaluate the biocompatibility and osteogenesis of 3D printed scaffolds	Hard	AgNPs	Solvent-free 3D printing	PCL combined with 0.5 wt% AgNPs	PCL/AgNPs scaffolds show improved cell attachment and osteogenic differentiation	Limited mechanical properties, no long-term biocompatibility tests conducted	Promising for bone tissue engineering; explore different nanoparticle compositions	[**111**]
Develop a 3D-printed Janus patch for bacteria elimination and wound healing	Soft	AuNPs	Extrusion-based 3D printing for layer-by-layer hydrogel patch fabrication	Top layer: PEGDA with BTO-Au; Bottom layer: GelMA with VEGF	Significant antibacterial activity and enhanced tissue regeneration *in vivo*	—	Explore customization for specific wounds and integrate additional therapeutic agents	[113]
Evaluate nanoparticle toxicity using 3D bioprinted organoid scaffolds	Not mentioned specifically	AgNPs	Customized extrusion-based 3D bioprinter for cell-laden hydrogel scaffolds	Alginate, gelatin, and Matrigel-based hydrogels	Increased cell proliferation, reduced oxidative stress, and enhanced nanoparticle uptake	Complexity in bioprinted multilayer control; limited long-term studies	Advocate for more 3D bioprinting in nanotoxicology and nanomedicine	[114]
Development of antibacterial scaffolds for bone tissue engineering	Hard	AgNPs	DIW 3D printing	AgNP-PLGA	Enhanced mechanical strength, antibacterial activity of scaffolds, and excellent repairing capability	Potential nanoparticle agglomeration and release control issues	Bone tissue engineering Infection prevention in implants *In vivo* animal experiments to further verify the antibacterial function of the scaffolds	[115]
Fabricate self-assembled gel tubes and filaments for tissue engineering	Not mentioned specifically	AuNPs	DIW method for creating stable gel structures efficiently	DBS-$CONHNH_2$ and calcium alginate hybrid gels for enhanced stability	Biocompatibility was demonstrated, with AuNPs promoting stem cell metabolism significantly	Mechanical stability of low molecular weight gelators remains a concern	Potential for advanced tissue engineering, emphasizing optimization of gel formulations	[116]
Develop multifunctional scaffolds for bone tissue engineering and infection control	Hard	AuNPs	FDM using a custom modified extrusion printer	PVA, AuNPs, and ampicillin	Scaffolds demonstrated good biocompatibility, mechanical strength, and antimicrobial properties	Potential issues with protein absorption and cell adhesion in PVA	Explore broader applications in personalized medicine and advanced orthopedic implants	[117]



Scheme 1. Schematic of 3D printed structures containing AgNPs and AuNPs for applications in tissue engineering.

long-term safety and bioactivity for application in bone tissue engineering [111]. In another investigation, Seiti et al. investigated the use of three distinct types of inks based on collagen, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), and AgNPs in Aerosol Jet® printing (AJ®P) for 3D microstructuring. Through the adoption of various printing techniques, including point-wise, layer-by-layer, and continuous jet deposition, they demonstrated that it was possible to produce dense and intricate leaf- or flake-like structures (particularly with the AgNPs-based ink) and lattice units (collagen-based ink), as well as arrays of micropillars with different aspect ratios. More specifically, this work demonstrated that the production of 3D AJ®-printed microstructures is only feasible with a certain set of printing settings and is strongly dependent on the fast-drying phenomenon of the ink co-solvents during the printing process. Collagen and PEDOT:PSS had modest toxicity; however, AgNPs indicated considerable cytotoxicity. Results indicated that AJ®P could produce versatile, biocompatible structures for use in electronics and the biological sciences. In order to increase performance, future studies would concentrate on improving mechanical properties and assessing process parameters [112]. The biocompatibility and osteogenic potential of a polycaprolactone/0.5 wt.% silver nanoparticle (PCL/AgNPs) scaffold developed via solvent-free 3D printing and a green synthesis technique were examined in a work conducted by Mira et al. Using human Wharton's jelly mesenchymal stem cells (hWJMSCs), the biocompatibility and osteogenesis-inducing potential were evaluated. Figure 1 indicates SEM images of scaffolds with and without hWJMSCs. The findings demonstrated that, in comparison to PCL alone, PCL/AgNPs scaffolds markedly increased osteogenic gene expression (RUNX2, COL1A1, and OPN) as well as proliferation, penetration, and cell adhesion. The study's *in vitro* nature was one of its drawbacks, despite being promising. In order to validate these results and evaluate the scaffold's efficacy in a physiological setting, future research should use *in vivo* models [128].

One of the main issues for public health is the toxicity of nanoparticles that are inhaled or absorbed via contact. The toxicity of nanomaterials must be continuously assessed. Little is known regarding the use of 3D bioprinting for nanotoxicology research, despite the fact that it has recently been employed for 3D culture in the context of drug release and tissue regeneration. In order to determine the toxicity of nanoparticles on lung cells using organoid-based scaffolds, Gerbolés et al. investigated the use of 3D bioprinting for nanotoxicology tests (figure 2). They exposed the scaffolds to AgNPs (11 – 14 nm) and latex-fluorescent (40 nm) by printing cell-laden materials. Comparing the 3D environment to 2D cultures, the results demonstrated a considerable increase in cell numbers and viability, as well as a decrease in lipid peroxidation and low cell death during a 21-day period. This method highlights the differences between 2D and 3D cell cultures by enabling a more accurate simulation of nanoparticle exposure. Notwithstanding its potential, limitations include challenges with cell seeding and possible mechanical disruption during printing. According to the results, 3D bioprinting might greatly advance nanotoxicological research and increase the effectiveness and safety of nanomedicines [114].

Among the major and current challenges in the clinical settings are infectious bone defects. Chen et al. addressed the challenge of infectious bone defects by developing antibacterial scaffolds by employing AgNPs/PLGA through direct ink writing (DIW) 3D printing (figure 3). The scaffolds exhibited uniform microstructure, enhanced mechanical strength through the addition of AgNPs, and continuous silver ion release. Characterization through SEM and XRD confirmed the successful deposition of hydroxyapatite (HAP) and even distribution of AgNPs, demonstrating effective antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*. Cytotoxicity assays indicated excellent biocompatibility with mouse embryo osteoblast precursor cells (MC3T3-E1). These findings suggest that AgNPs/PLGA scaffolds hold significant potential for bone tissue engineering, with future *in vivo* studies recommended to further evaluate their antibacterial and bone repair capabilities [115].

Since not all the materials show suitable biocompatibility, one of the most common limitations related to 3D printing in the field of biomedicine is the selection of appropriate

Continued of Table 1.

Objective(s)	Hard/Soft Tissue or Device Applications	Nanoparticles Used	3D Printing Techniques	Material Compositions	Results	Limitations and Challenges	Future Applications and Recommendations	Ref.
Design biocompatible 3D printed scaffolds with antibacterial properties	Not mentioned specifically	AgNPs	FDM	PCL combined with AgNPs	Improved mechanical properties and significant antibacterial activity	Maintaining biocompatibility while integrating antibacterial coatings remains challenging	Potential for tissue engineering applications and further surface modifications	[118]
Develop flockable biodegradable microfibers for biomedical applications	Not mentioned specifically	AgNPs	—	PCL filled with AgNPs and chitosan adhesives	Enhanced cell proliferation, antimicrobial activity, and tissue formation observed	Difficulties in achieving uniform fiber cutting and charge distribution	Potential use in regenerative medicine, prosthetics, and surface coatings	[119]
Develop gelatin-based inks with enhanced stiffness, antibacterial properties, and UV protection	Not mentioned specifically	AgNPs	Extrusion printing technique used for fabricating complex 3D scaffolds	Gelatin, polyacrylamide, and AgNPs in a double-network hydrogel structure	Improved mechanical properties, antibacterial effectiveness, and UV-blocking capabilities	Mechanical properties are still affected by AgNPs concentration; optimization is needed	Customized antimicrobial and UV protective dressings for tissue engineering applications	[120]
Develop core-shell silver self-assemblies for bioapplications and functionalities	Not mentioned specifically	Core-shell nanoparticles, specifically silver micro/nanoclusters and polydopamine	Electron beam melting (EBM)	Silver titanate, polydopamine, titanium substrates, and various organic compounds	Enhanced antimicrobial activity, protein affinity, and robust biofilm resistance demonstrated	Control over morphology is limited; the scalability of synthesis methods is challenging	Promising for implantable devices; explore further in tissue engineering applications	[121]
Investigate the biocompatibility of industrial ABS scaffolds for cell culture	Not mentioned specifically	AuNPs	FDM	Industrial ABS with surface-modified AuNPs	Enhanced cell proliferation and spheroid formation in 3D compared to 2D	Industrial ABS's biocompatibility not well established; further validation is required	Explore additional nanoparticles and optimize scaffold designs for therapeutic applications	[122]
Develop a biomimetic, antibacterial wound dressing to enhance healing	Soft	AgNPs	3D bioprinting	Poly(dimethylsiloxane) infused with silicone oil and AgNPs	iPDMS/AgNPs demonstrated effective antibacterial activity and promoted wound healing	Need for extensive testing under varied clinical conditions	Potential in advanced wound care and personalized medical devices development	[123]
Develop gold nanoparticle-coated scaffolds for enhanced bone tissue engineering	Hard	AuNPs	—	PCL scaffolds coated with polydopamine and AuNPs.	Enhanced osteogenic differentiation and new bone formation observed *in vivo*	Need for controlling nanoparticle size and potential biodistribution issues	Explore cyclic peptide incorporation for improved adhesion and uptake efficiency	[124]
Develop multilayered scaffolds for repairing infectious bone defects	Hard	Nanosilver encapsulated silk fibrin	EBM	Ti6Al4V alloy, silk fibrin, nanosilver, and titanate layers	Enhanced antibacterial activity and improved osteogenic responses observed	Initial cellular stress; long-term efficacy needs further investigation	Customized scaffolds for complex bone defects in clinical settings	[125]
Detect defects in 3D printed materials using AuNPs' properties	Not mentioned specifically	AuNPs	Dual extrusion with functionalized filament	PLA blended with varying concentrations of AuNPs	Defects identified nondestructively; detectable as small as 0.2 mm	Detection limited to specific geometries; requires precise nanoparticle dispersion	Develop multifunctional materials; enhance smart materials for diverse applications	[126]
Develop 3D printed bionic ears integrating biological tissue and electronics	Both soft and hard	AgNPs	Syringe extrusion-based 3D printing	Chondrocyte-seeded alginate hydrogel with AgNPs-infused silicone	Bionic ears demonstrated enhanced auditory sensing and electromagnetic signal reception	Complex anatomical structures and the integration of biological materials remain challenging	Explore other organs; enhance functionalities with diverse nanoscale materials	[127]

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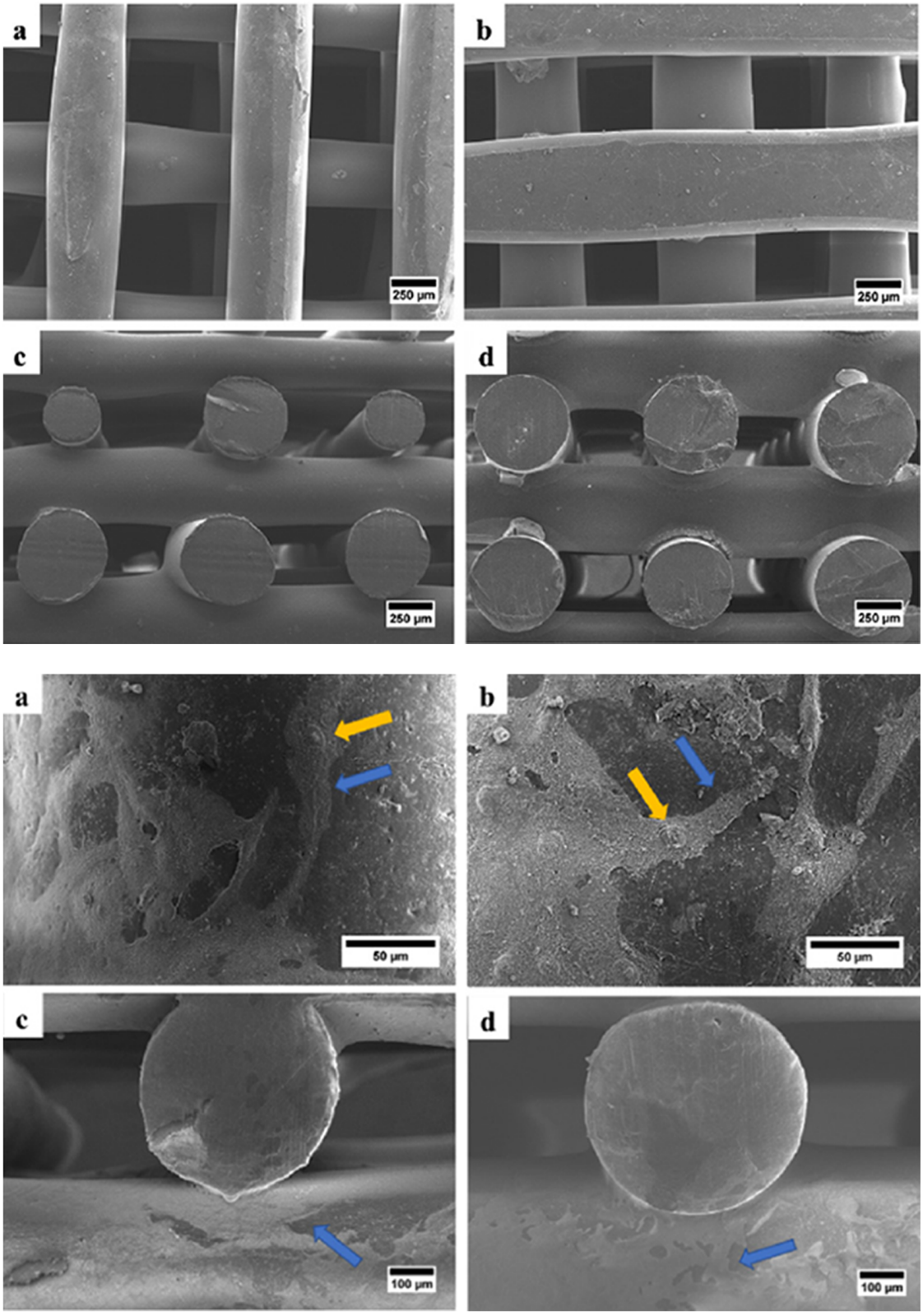

Figure 1. SEM images of scaffolds without (top images) and with (bottom images) hWJMSCs. Labels (a) and (b) in both categories are related to the top views of PCL and PCL/AgNPs, respectively. In addition, labels (c) and (d) in both categories are related to side views of PCL and PCL/AgNPs, in respective order. Reprinted from [128] under the CC BY 3.0 license. Copyright (2023) Royal Society of Chemistry.

materials for this purpose. Ninan et al. explored a plasma nanoengineering approach to enhance the properties of 3D-printed polycaprolactone (PCL) scaffolds for tissue engineering. Using FDM, scaffolds were fabricated without solvents, followed by plasma deposition to modify their surfaces and immobilize AgNPs. The scaffolds maintained

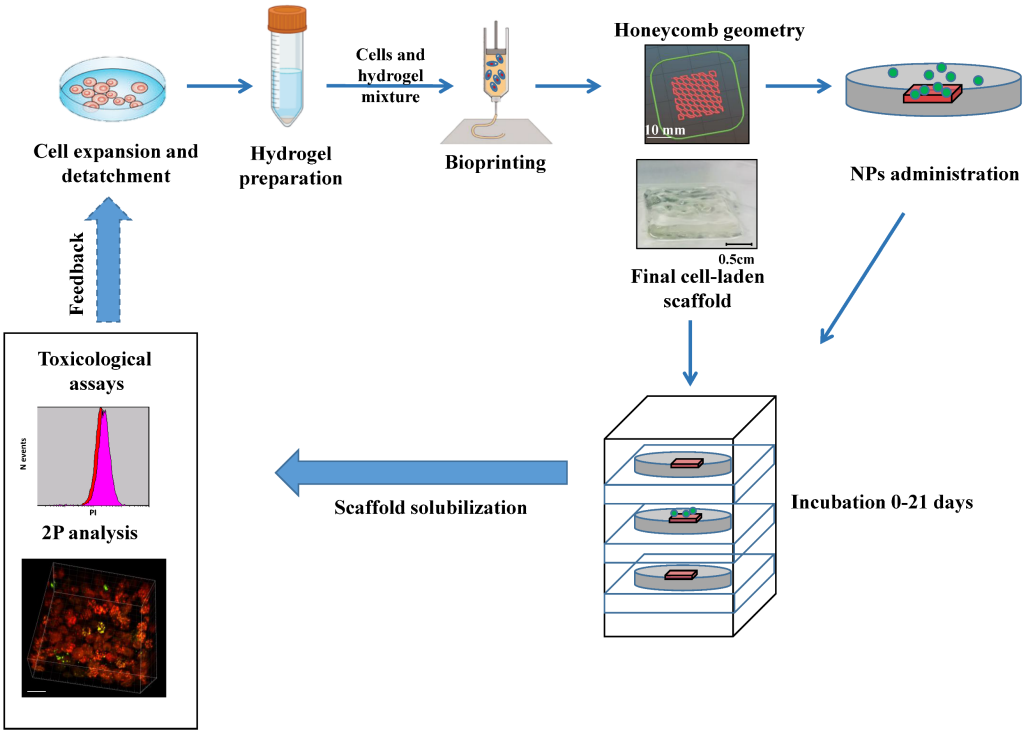

Figure 2. Schematic of the experimental protocols used in [114]. In this figure, NPs denote polydispersity colloidal AgNPs or carboxyl fluorescent nanosphere, and 2P implies two-photon microscopy. Reprinted from [114] under the CC BY 4.0 license. Copyright (2023) MDPI.

their porosity and mechanical integrity, exhibiting improved mechanical properties and complete inhibition of pathogen growth. *In vitro* studies showed good biocompatibility and a reduction in pro-inflammatory cytokines in macrophage cultures, essential for wound healing. Moreover, *in vivo* tests in Sprague Dawley rats revealed enhanced angiogenesis and no foreign body reactions for scaffolds functionalized with AgNPs for 6 hours, highlighting the potential for developing biomaterials with improved biological properties. Designing biomaterial structures with improved biological characteristics may be made more sustainable with the help of the 3D printing technique, which this study presents. The results of this investigation provide novel surface engineering options that may be used in the field to improve the characteristics of biomaterial structures [118]. Using Coulombic driving forces, conductive microfibers are propelled toward an adhesive-coated substrate by electrostatic flocking, a textile engineering approach that leaves a forest of aligned fibers. This approach is confined to microfibers that can transmit and accumulate charges from charging surfaces to microfibers, while being a simple method of creating anisotropy along a surface. McCarthy et al. introduced a novel method for electrostatic flocking using insulative polymer microfibers by incorporating conductive fillers based on percolation theory, enabling charge accumulation for flocking. The AgNPs were added to PCL microfibers, which were then flocked onto various substrates. The scaffolds exhibited antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and showed favorable *in vitro* cell responses and new tissue formation *in vivo*. Limitations included challenges in achieving uniform fiber cutting and charge distribution. Future research should focus on optimizing these processes and exploring broader applications in biomedical and other engineering fields [119].

Furthermore, Liu et al. developed a polyacrylamide/gelatin/silver nanoparticle (PAAm-Gelatin-AgNPs) ink to enhance the mechanical properties of gelatin-based hydrogels used in bioprinting for tissue engineering. By creating a double network with physically cross-linked gelatin and covalently cross-linked PAAm, the ink demonstrated improved tensile and compression strength. The AgNPs provided antibacterial properties and UV protection, making the ink suitable for applications like UV protective dressings. The shear-thinning property of the ink enabled the successful printing of complex 3D scaffolds. This innovative ink offers a promising material for artificial tissues and biomedical applications, although further exploration of its long-term effects and biocompatibility is recommended [120]. Although direct growth of core-shell nanoparticles (CSNs) from a solid substrate is still challenging, CSNs offer several interesting features for advanced device applications. Jia et al. developed a mussel-inspired method for synthesizing CSNs composed of nanosilver and polydopamine (nAg/PD) directly on solid substrates. Silver titanate was hydrothermally grown on titanium, reacting with dopamine to form nAg/PD CSNs. The resulting substrates exhibited enhanced protein affinity, radical scavenging, and effective antibacterial activity against *Staphylococcus aureus*, promoting osteoblastic cell survival in co-culture. The adaptability of this approach was demonstrated through modifications to 3D-printed scaffolds, which exhibited photothermal properties. Future research should explore the hierarchical structures

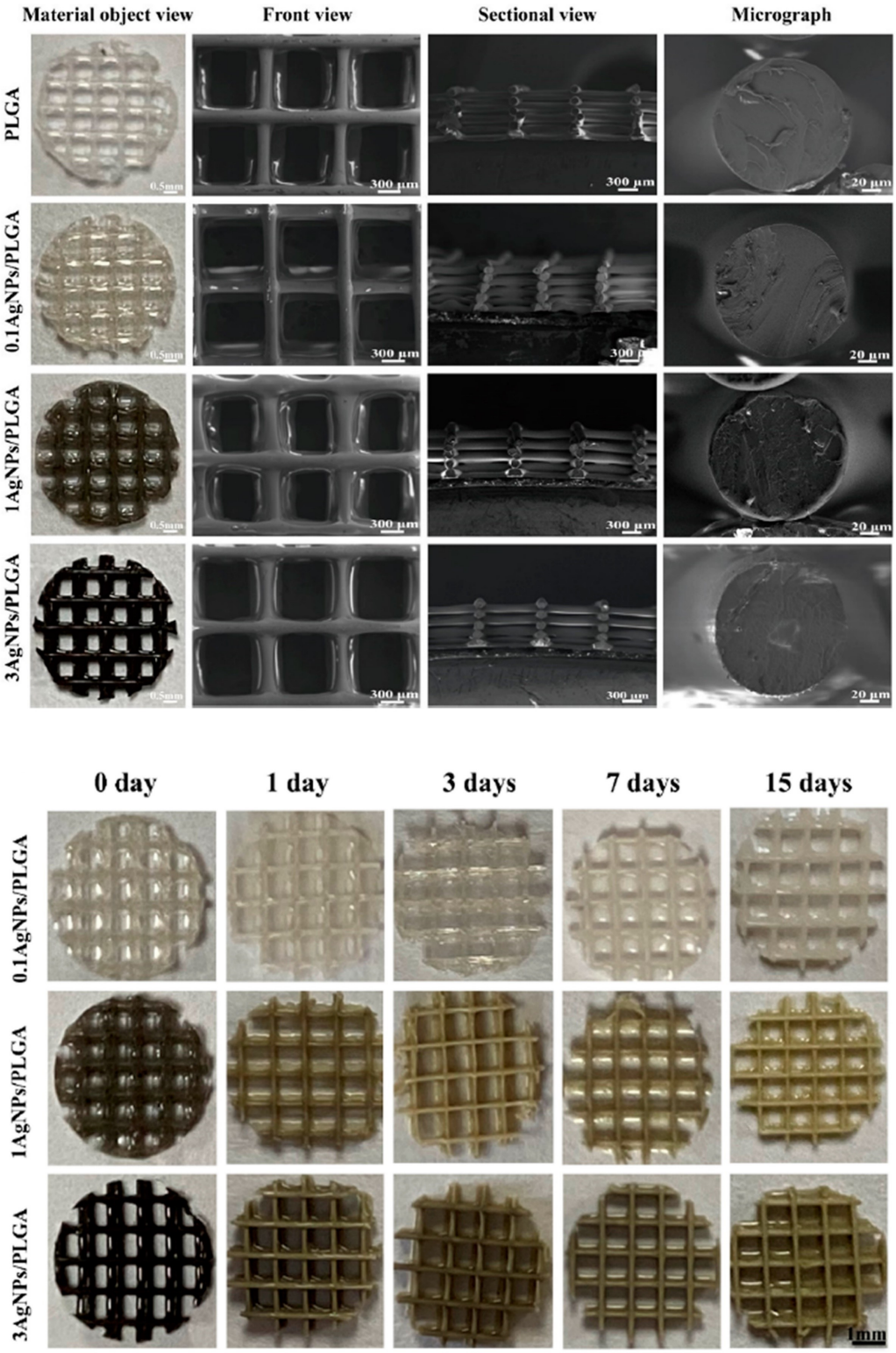

Figure 3. Photographs and SEM images of scaffolds with different AgNPs content shown in different views (top images). Photographs of scaffolds with different AgNPs content before and after immersing in 50 mL of deionized water. Reprinted from [115] under the CC BY 4.0 license. Copyright (2023) MDPI.

and chemical versatility of these materials for broader applications in biotechnology and medicine [121].

In addition, Shi et al. developed a biomimetic, environmentally-friendly wound dressing using oil-infused polydimethylsiloxane (iPDMS) integrated with antibacterial AgNPs. The 3D-printed iPDMS/AgNPs exhibited antifouling properties, prevented blood staining, and effectively targeted drug-resistant bacteria like *Staphylococcus aureus* and *Escherichia coli*. Optimized rheological parameters were established for printing, and SEM images confirmed a uniform mesh structure. *In vitro* assays showed no cytotoxicity, while *in vivo* tests

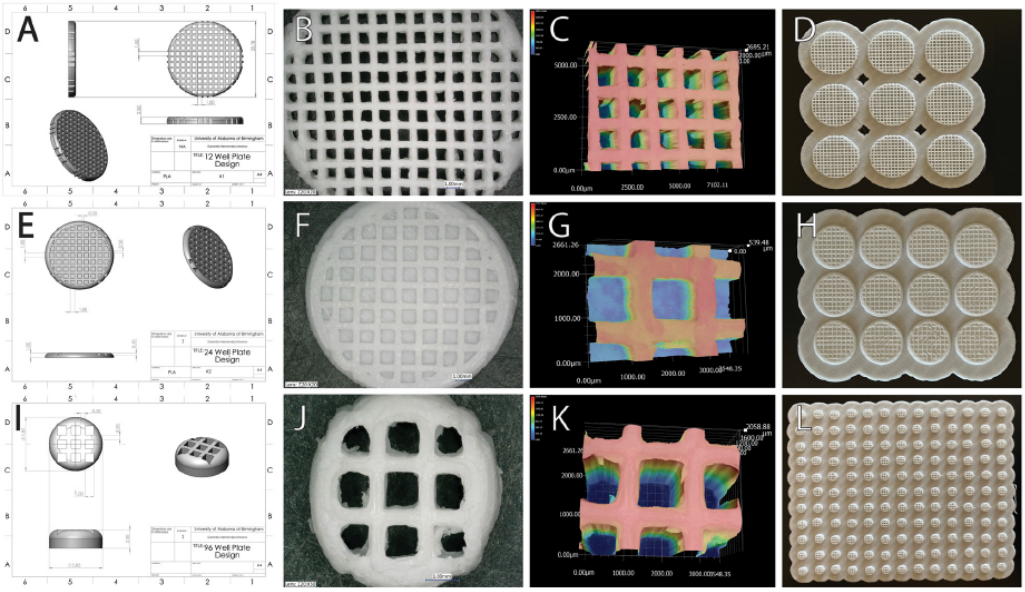

Figure 4. Scaffolds designed, analyzed, and fabricated in [110]. (A, E, I) CAD designs, (B, F, J) top view of scaffolds, (C, G, K) Keyence analysis, (D, H, L) top views of final products of 3D printing wafers. All images, from top to bottom, are related to 12-well, 24-well, and 96-well plates, respectively. Reprinted from [110] under the CC BY-NC 3.0 license. Copyright (2024) Royal Society of Chemistry.

indicated accelerated wound healing through enhanced epithelialization and granulation tissue formation. These findings suggest strong potential for iPDMS/AgNPs as an advanced wound dressing. Future studies should assess the long-term efficacy of these dressings [123]. In another study, Jia et al. developed 3D porous titanium scaffolds with osteogenic and antibacterial properties to tackle infectious bone defects. Using metallic powder 3D printing, the scaffolds were enhanced with a titanate layer and nanosilver-encapsulated silk fibrin multilayers. The scaffolds demonstrated improved hydrophilicity, sustained antibacterial activity against *Staphylococcus aureus*, and effective prevention of biofilm formation. Additionally, they promoted osteoblastic proliferation and mineralization. The design flexibility and cost-effectiveness of these scaffolds made them promising for complex bone defect treatment.

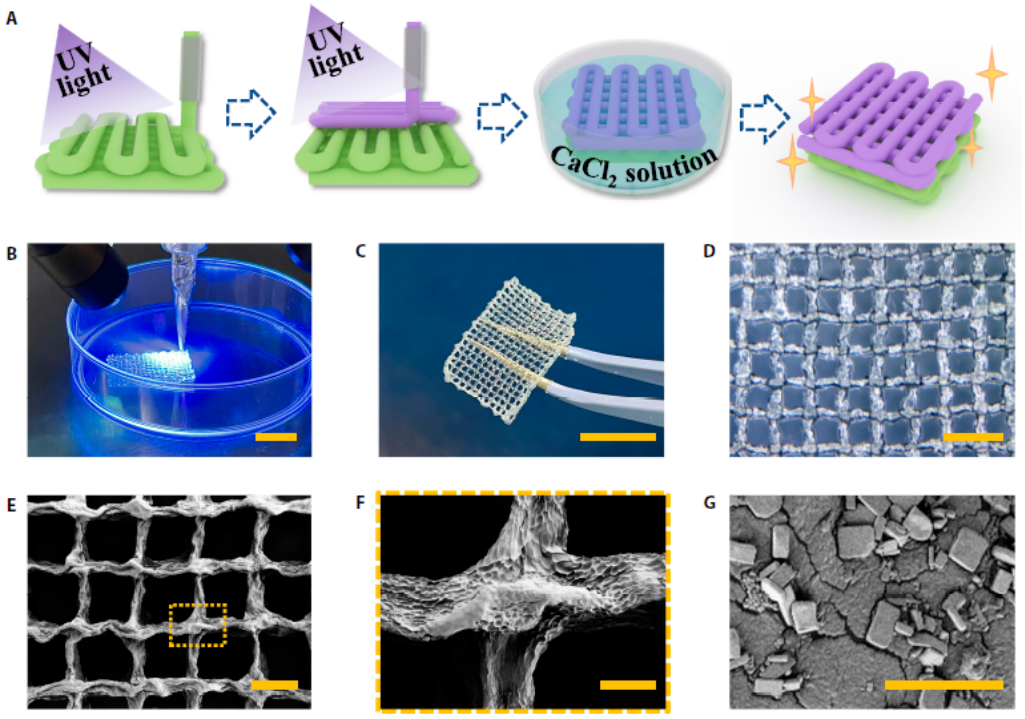

Figure 5. (A) Fabrication method of 3D-printed Janus hydrogel patch, (B) 3D printing process, (C, D) digital and optical images of the 3D printed scaffold, (E, F, G) magnified images of BTO-Au nanocomposites decorated scaffolds in the scale bars of 1 mm, 200 μm, 20 μm, respectively. Reprinted from [113] under the CC BY 4.0 license. Copyright (2023) Science Partner Journals.

Future studies should optimize fabrication techniques and assess *in vivo* performance to ensure safety and efficacy [125]. Mannoor et al. introduced a novel approach to creating bionic organs by 3D printing biological tissues integrated with electronic components. The researchers developed a bionic ear using a cell-seeded hydrogel matrix and a conducting polymer infused with AgNPs. This design allowed for the cultivation of cartilage tissue around an inductive coil antenna, enabling the ear to receive radio frequency signals. The bionic ear demonstrated enhanced auditory sensing capabilities and could process stereo audio. This work highlighted the potential for seamless integration of biological and electronic functionalities, paving the way for the future development of hybrid tissues and organs using various nanoscale materials [127].

4. Gold nanoparticles (AuNPs) contained 3D printed structures for tissue engineering applications

As mentioned in sections 1, due to their high thermal stability and appropriate antibacterial properties, AuNPs are interesting options to be employed in tissue engineering-related structures. In this section, recent and significant studies that developed 3D printed structures composed of or coated with AuNPs for regenerative medicine applications are reviewed. Table 2 is the summary presentation of the reviewed investigations in this section.

Vadukoote et al. investigated the wet-spinning technique to 3D-print gels from low-molecular-weight gelators (LMWGs) based on the 2,4-dibenzylidenesorbitol (DBS) scaffold, aiming to develop materials with differential conductivity. Gel stripes made from DBS-$CONHNH_2$, which could incorporate Au(III) to form AuNPs through *in situ* reduction, were compared to less conductive DBS-COOH. The incorporation of AuNPs enhanced the conductivity of DBS-$CONHNH_2$ by up to 12.5-fold. Despite promising results, the absolute conductivity remained low (approx. 100 μS/cm). Future research should focus on improving conductivity for applications in soft nanoelectronics and tissue engineering [109]. Moreover, Hernandez-Moreno et al. introduced a novel *in vitro* platform for characterizing nanoparticle surface-modified 3D-printed PLA scaffolds, as shown in figure 4, focusing on their bioactivity and biocompatibility. Gold and silica nanoparticles were deposited on scaffolds using plasma electroless reduction and dusty plasma methods. The results indicated that AuNP modification enhanced cell viability, mineralization, and protein adsorption compared to control 3D PLA scaffolds. However, this study did not definitively establish the osteogenic potential. Future research should explore gene expression and stem cell differentiation to further assess the scaffolds' osteogenic capabilities and improve their application in tissue engineering [110].

In another study, Huang et al. presented a novel 3D-printed Janus piezoelectric hydrogel patch designed for enhanced wound healing and bacterial infection management. As demonstrated in figure 5, the patch consisted of a top layer made from poly(ethylene glycol) diacrylate hydrogel, which contained AuNP-decorated tetragonal barium titanate (BTO) for ultrasound-triggered release of reactive oxygen species (ROS), and a bottom layer of methacrylate gelatin that released growth factors to promote tissue regeneration. Moreover, *in vivo* tests demonstrated effective infection elimination and improved tissue repair in infected mouse wounds. The hydrogel's design allowed for targeted ROS release and supported cell proliferation, making it a promising tool for personalized wound management. Future studies could explore its application in various clinical settings [113].

Piras et al. explored the fabrication of self-assembled tubular and filamentous structures using low molecular weight gelator dibenzylidenesorbitol-based $CONHNH_2$ combined with polymer gelator calcium alginate. The methods included the development of core-shell gel structures and gel filaments via wet spinning. The AuNPs were generated *in situ* upon exposure to $AuCl_3$, enhancing stem cell metabolism and demonstrating biocompatibility. In addition, DBS-$CONHNH_2$ gel filaments were successfully 3D-printed using a syringe pump and drawing robot. The constructs demonstrated remarkable stability in water for over eight days, outperforming previous low molecular weight gelators. This stability, along with high shape fidelity, suggests potential applications in tissue engineering and cell culture. There is a need for further *in vitro* studies on cell growth and osteogenesis, and future work is suggested to investigate the impact of gel shaping on cell behavior [116].

In another investigation, Topsakal et al. focused on developing 3D scaffolds for bone tissue engineering using AuNPs and ampicillin (AMP) to enhance biocompatibility and antimicrobial properties. The scaffolds, made from polyvinyl alcohol (PVA) composites (PVA, PVA/AuNP, PVA/AMP, and PVA/AuNP/AMP), were produced through advanced 3D printing techniques. Characterization via SEM and FTIR confirmed suitable pore sizes for bone regeneration and no modifications upon mixing. *In vitro* assays demonstrated that the PVA/AuNP/AMP scaffolds are biocompatible, osteo-inductive, and antimicrobial. These findings suggest potential for clinical applications in treating orthopedic infections while promoting bone formation. Future studies should explore clinical translation and effectiveness in real-world scenarios [117].

Furthermore, Ulrich N'deh investigated the use of low-cost industrial acrylonitrile butadiene styrene (ABS) filament for 3D scaffolds to enhance cell growth, spheroid formation, and drug screening. Two-layer square scaffolds were printed with varying infill densities, with the 40% infill density scaffold (IA3D) demonstrating optimal conditions for cell proliferation, porosity, and micropore uniformity. Gold nanoparticle-coated scaffolds (GIA3D) significantly improved biocompatibility, increasing spheroid formation in HepG2 cancer cells by 1.3-fold compared to IA3D and 38-fold compared to 2D cultures. GIA3D also enhanced drug resistance in HepG2 cells, suggesting potential for *in vitro* studies and drug screening. Future research should explore further modifications and applications [122]. Lee et al. explored the design of scaffolds coated with AuNPs on a polydopamine (PDA) layer applied to 3D-printed PCL scaffolds, aimed at enhancing bone tissue engineering. The re-

sults indicated that the PDA coating significantly improved AuNPs growth, promoting osteogenic activity in human adipose-derived stem cells (hADSCs). Moreover, *in vivo* tests indicated excellent new bone formation. This innovative approach offered promising applications for regenerative therapies in the orthopedic and dental fields. Future work will focus on incorporating cyclic peptides with the arginine-glycine aspartate sequence to enhance AuNPs uptake and cell adhesion, setting the stage for further research [124]. In another work, Brubaker et al. explored the integration of AuNPs into 3D printing to enhance defect detection in printed parts. By embedding AuNPs in a poly(lactic acid) (PLA) matrix, researchers developed a functionalized filament that allowed for the nondestructive identification of material defects, such as missing print layers, with a sensitivity of 0.2 mm. The absorbance of the AuNPs provided a linear relationship with the number of print layers, enabling precise localization of defects. This stable optical sensing method suggested promising applications for smart materials in various fields, paving the way for future developments in nanofunctionalized 3D printing technologies [126].
Table 1 is the summary of the reviewed articles in the present work, which have employed AgNPs and/or AuNPs contained structured fabricated via 3D printing for tissue engineering applications.

5. Discussion and future directions

Nowadays, researchers are looking for new methods to treat diseases and injuries related to various tissue problems, such as musculoskeletal [129, 130], cardiovascular [131], liver failure [132, 133], wounds and burns [134, 135, 136], etc. The TERM is among the methods that have received attention in recent years in the field of treating tissue injuries and related disorders. Among the main pillars of TERM are supportive structures that improve cell growth and proliferation. 3D printing is among the frequently used techniques for the fabrication of these structures [137]. In order to produce these structures with 3D printing, the selection of biomaterials is one of the most important pillars. Recently, AgNPs and AuNPs have been among the frequently employed materials that have been employed as coatings and composites due to various properties, such as chemical stability, temperature stability, antibacterial properties [117, 138, 139, 140, 141], etc. The present review was an extensive investigation of AgNPs/AuNPs 3D printed structures for applications in TERM.
According to the results of the reviewed studies, with AuNPs, DBS-$CONHNH_2$ gels showed noticeably increased conductivity, improving protein adsorption, mineralization, and cell survival [109]. In addition to strong antibacterial activity and *in vivo* tissue regeneration, PCL/AgNPs scaffolds demonstrated enhanced cell adhesion and osteogenic differentiation [128]. AuNPs greatly increased stem cell metabolism, and scaffolds indicated improved mechanical strength, antibacterial activity, and outstanding healing capacity [116, 117]. Along with improved biofilm resistance and enhanced UV-blocking properties, there was also an increase in tissue development, cell proliferation, and antibacterial activity when using AgNPs and AuNPs [120, 121]. Moreover, *in vivo*, iPDMS/AgNPs successfully promoted wound healing and the production of new bone [123]. The potential of tissue engineering, soft bioelectronics, and dynamic materials for a range of applications is highlighted in future recommendations. For tissue engineering, high-throughput biocompatibility screening is recommended, especially for orthopedic implants intended to repair bone defects [110]. While incorporating therapeutic chemicals and promoting 3D bioprinting might promote nanotoxicology and nanomedicine, investigating tailored nanoparticle compositions could improve bone tissue engineering [128, 114]. *In vivo* animal research is required to confirm the scaffolds' antibacterial properties in infection prevention [115]. Together with multipurpose materials, custom antimicrobial and UV protection dressings should be developed [120]. Other recommendations call for the development of customized scaffolds for intricate bone abnormalities [125] as well as the investigation of cyclic peptide inclusion for increased scaffold effectiveness [124]. For comprehensive developments in regenerative medicine and prosthetics, it is also recommended to extend research to other organs and improve functionality using a variety of nanoscale materials [127]. In recent years, machine learning (ML) and artificial intelligence (AI) have been extensively used for data analysis, optimization, prediction, etc., in various fields [142, 143, 144, 145, 146]. Since a step forward has been taken in the use of AI and ML in TERM [147, 148], it is recommended that researchers pay special attention to this issue in their future studies. These technologies offer real-time monitoring of implanted scaffolds, enhance drug delivery by adjusting release profiles, and allow personalized therapies based on patient data. Furthermore, therapists can select the best treatment plans and scaffolds with the use of AI-driven decision support systems. The combination of AI and ML has the potential to significantly improve TERM, resulting in more specialized and efficient regenerative treatments.

6. Conclusion

The investigation of TERM has shown notable progress by combining 3D printing with innovative biomaterials, including AuNPs and AgNPs. These materials show promise in resolving a variety of tissue-related problems by improving cell proliferation, antibacterial qualities, and general tissue regeneration. Future investigations should concentrate on customized nanoparticle compositions, high-throughput biocompatibility screening, and *in vivo* tests to confirm scaffold efficacy. Furthermore, TERM's integration of ML and AI offers chances for better data optimization and analysis. Ongoing advancements in biomaterials and techniques demonstrate potential for improving regenerative medicine therapies.

Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Assistant, Quillbot, and Grammarly to rephrase and edit the text grammatically and in terms of typos. After using these tools/services, the authors reviewed and edited the content as needed and take full responsibility for the content of the

publication.

Authors contributions

All authors contributed equally to the conception, design, execution, and writing of this work. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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