REVIEW ARTICLE

3D bioprinting for auricular reconstruction: A review and future perspectives

Anna Onderková, and Deepak M. Kalaskar*

University College London, Royal Free Hospital, Rowland Hill Street, London NW3 2PF, UK

(This article belongs to the *Special Issue: Advances in bioprinting technology*)

Abstract

Congenital abnormalities or acquired trauma to the auricle can result in a need for ear reconstruction and negatively impact a person's quality of life. Autografting, alloplastic implants, and prostheses are available to treat these issues, but each requires multiple surgical stages and has limitations and complications. Three-dimensional (3D) bioprinting promises to allow the creation of living, patient-specific ear substitutes that could reduce operative morbidity. In this review, we evaluate the current state of 3D bioprinting methods through a systematic search and review of 27 studies, aiming to examine this emerging technology within the context of existing reconstructive options. The included studies were all non-randomized experimental studies, except for a single pilot clinical trial. Most of these studies involved both *in vitro* and *in vivo* experiments demonstrating the potential of 3D bioprinting to create functional and anatomically accurate engineered cartilaginous frameworks for surgical implantation. Various ways of optimizing printing were identified, from choosing the most suitable material and cell type for the construct to addressing scaffold deformation and shrinkage issues. 3D printing has the potential to revolutionize reconstructive ear surgery by creating functional and aesthetically pleasing auricles. While more research into printing parameters, bioinks, cell types, and materials could optimize results, the next step is to conduct long-term *in vivo* clinical trials in humans.

Keywords: 3D bioprinting; Auricular Reconstruction; Tissue Engineering; Bioinks; Patient-specific Implants; Cartilaginous Frameworks

*Corresponding authors:
Anna Onderková
(annaonderkova@doctors.org.uk)

Deepak M. Kalaskar
(d.kalaskar@ucl.ac.uk)
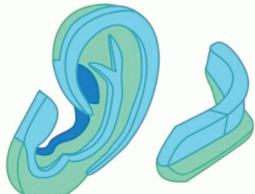
1. Introduction

The external ear can be congenitally missing (anotia) or malformed (microtia) or may sustain acquired damage in the form of trauma, burns, or after skin cancer excision[1,2]. The absence of an external ear can impact a patient's quality of life through impaired conductive hearing[3], loss of symmetry, difficulty wearing glasses or hearing aids, and the combined psychosocial and developmental consequences of all of these factors, particularly in children[1,4]. The auricle has significant aesthetic value[5], and its absence can be seriously stigmatizing in adults[3,5]. Conversely, following ear reconstruction, 74% of adults and 91% of children report improved self-confidence and social interactions[6].

Table 1. An overview of chief differences between landmark surgical methods in auricular autografting (adapted from Dr. Nagata's original sketches[13])

Method		Tanzer (1959)	Brent (1980)	Nagata (1992)
Costal cartilage frame				
Total number of operations		6	4	2
Operation time	1st stage	1 h—Lobule transposition	3 h—Lobule transposition	8 h—Costal cartilage graft
	2nd stage	3 h—Costal cartilage graft	1 h—Costal cartilage graft	8 h—Ear projection
	3rd stage	2 h—Tagus construction	2 h—Tagus construction	
	4th stage	2 h—Separating the ear	2 h—Separating the ear	
	5th stage	1 h—Temporary tunnel		
	6th stage	2 h—Closing the tunnel		
Number of costal cartilages harvested		3	3	4 for 1st stage (ribs 6–9) 2 for 2nd stage (ribs 4 and 5)
Chest wall deformity		Occur	Occur	Do not occur because the perichondrium is left in place and refilled using diced cartilage remnants from cutting before closing. Thus, the cartilage regenerates over time.
Resorption risk		High due to insufficient blood supply	High due to insufficient blood supply	No resorption due to good blood supply
Wire sutures used		5	5	85 needed for 1st stage 20 needed for 2nd stage

1.1. Current reconstructive surgeon's toolbox

Current options for treating auricular deformities or absence include cartilage autografting, alloplastic polyethylene implants such as Medpor, and prostheses[3,7].

Most (91.3%) surgeons prefer autografting over alloplasty[7], partly because autografted cartilage has a lower risk of being extruded through the skin or of soft-tissue necrosis, whereas Medpor is susceptible to minor trauma and secondary infections, dehiscence, and implant extrusion[8]. Medpor implants typically require coverage with a temporoparietal fascial flap to prevent such complications[3]. Even then, results are suboptimal, as manufacturing limitations mean implants are not customized, and aesthetic results are a compromise at best[9]. Furthermore, the material is inherently stiff[10] and easily triggers immune reactions[11].

The alternative route is that of prosthetics, which have evolved considerably in recent years in terms of cosmetic results. Prostheses can be divided into those applied with a silicon adhesive and the surgically osseointegrated. This is particularly valuable in patients with inadequate available loco-regional skin, such as in burns or in cases where autologous reconstruction was unsuccessful. They are generally a last resort or reserved for patients who prefer minimally invasive treatment[5].

1.2. Current surgical techniques in autografting

The main reconstructive techniques in sculpting rib cartilage into an autografted auricle are those by Brent, Tazner, and Nagata, with notable variations like that by Firmin to improve and adjust results[3,12]. While this complex reconstruction requires specific surgical and artistic skills—thus limiting the field to a small number of experienced surgeons—the final result is still often less than perfect[3]. This is because the reconstruction is far from simple, regardless of technique.

The operations require substantial rib cartilage resection (see Table 1). Thus, the initiation of ear reconstruction must often be delayed until the child is of an adequate age to have enough usable cartilage. Furthermore, the procedure involving the chest wall as a donor site can
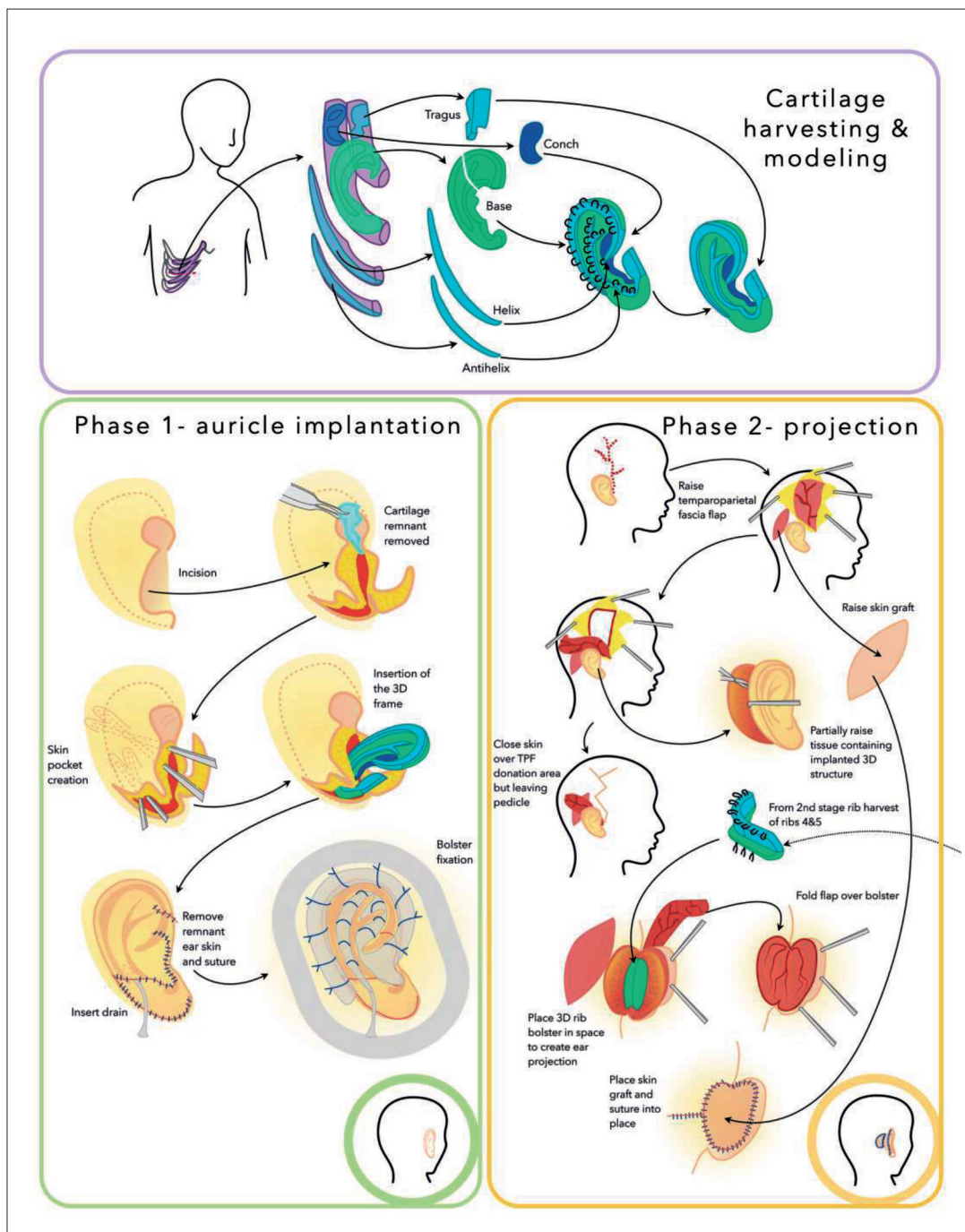
Figure 1. Nagata's technique (artwork inspired by Dr. Nagata's original sketches[13]). Abbreviation: TPF, temporoparietal fascia flap.

lead to significant morbidity, with potential complications ranging from severe postoperative pain to life-threatening conditions such as infections and pneumothorax[4,10].

Lengthy operative time is another drawback; at least two-staged surgeries are required. For example, in the Nagata technique[13] (see Figure 1), harvested and reshaped cartilage is introduced under the skin in the first stage. In the second stage, the ear projection is refined, thus not only improving aesthetic results but also limiting potential strain on overlying skin[4].

1.3. How 3D printing could revolutionize the field over the next decade

One of the main challenges in auricular reconstruction is the lack of suitable donor tissue. Three-dimensional (3D) printing

technology is able to produce biocompatible scaffolds, which can then be seeded with cells, or molds in which cartilage can be cultivated into the desired shape. Several time-consuming, multi-staged surgical steps (particularly rib harvesting and shaping) could be bypassed[3,4].

Before engineering a substitute, the structure of the auricle should be considered. At the most basic level, the structure is skin-covered cartilage with associated vasculature. The complex structure has distinct 3D parts such as the helix, antihelix, concha, tragus, and lobule. It is primarily composed of elastic cartilage covered by skin, with a thin layer of connective tissue, the perichondrium, in between. Auricular cartilage comprises proteoglycans, type II collagen, and an elastin network. Elastic fibers allow the ear to undergo extensive deformation, while glycosaminoglycans (GAG) confer compressibility[9]. Both properties, along with the complex shape, are important for the auricle's functionality and, unfortunately, from a reconstructive perspective, are quite distinct from most other cartilages found in the human body[10].

Cartilage is an avascular and aneural tissue, and thus has a poor intrinsic self-repair capacity[14]. However, from a tissue engineering perspective, this avascularity is advantageous because if new elastic cartilage can be grown or printed *in vitro*, a functioning vasculature does not have to be generated with it. Vessels around the engineered ear should be able to provide essential substances to chondrocytes through diffusion[15].

3D printing, as an additive manufacturing technique, fabricates physical constructs from digital models, layer by layer. This technology potentially facilitates the creation of patient-specific, anatomically complex scaffolds. However, limitations include time intensity for complex structures, potential discrepancies between the mechanical properties of printed materials and native tissues, and resolution constraints that may impact the replication of intricate auricular structures. Despite these challenges, 3D printing presents a unique advantage in customization compared to traditional fabrication methods[3,4,16].

At least theoretically, 3D-printed auricles promise relative ease of implantation, anatomic accuracy and compatibility, and thus, excellent aesthetic results.

2. Methodology

This comprehensive literature review sought to investigate the potential role of 3D printing in creating implantable constructs for reconstructive auricular surgery and how far it is from being routinely implemented in clinical practice.

In order to answer this, a broad systematic search in the PubMed, Cochrane, and Web of Science databases using the search terms ((3D *print*) OR (additive manufacturing)) AND ((auricle OR pinna OR ear) reconstruction) was conducted. The date of the last search was 22 October 2022, but the search was not otherwise time-limited. The identified papers were then imported into Covidence software by Cochrane to conduct the review and follow the PRISMA standard. This process identified 202 original studies. After duplicates were removed, the titles and abstracts of 141 studies were screened. Seventy-three studies were identified and underwent full-text review. Of these, 27 studies were finally included (see Figure 2 for workflow and inclusion and exclusion criteria).

3D printing technologies (see Figure 3) can aid and enhance all of the existing reconstructive options. However, in this review, focus was placed on methods that could directly enhance surgical reconstruction by creating new implantable tissue-engineered personalized auricles for patients. Thus, additional studies on, for example, surgeons using costal cartilage models to practice autografting[17], to create templates and guides along which to cut[18], or to plan the placement of bone-anchored prosthetic devices[19] were excluded.

3. Results

In terms of design, all of the studies included (see Table 2) were non-randomized experimental studies, except for the single landmark pilot clinical trial in human subjects by Zhou *et al.*[20]. While the majority (*n* = 15) of the studies involved both *in vitro* and *in vivo* experiments (including the human study), some were purely *in vitro* (*n* = 6) and some purely *in vivo* (*n* = 6) animal studies.

Several animal models were used, with the majority being in rodents (*n* = 16) but also in rabbits (*n* = 2), sheep (*n* = 1), pigs (*n* = 1), and goats (*n* = 1). It should be noted that the species into which the scaffold was implanted did not always match the donor tissue for the scaffold. For example, miniature pigs' cartilage was inserted into scaffolds that were eventually implanted into mice[11]. Several rodent model studies noted that since rodents' skin is different to human skin (lacking sweat glands, proportionally thinner, containing an additional muscle layer, and healing by withering), they may not be the ideal animal model[3]. On the other hand, sheep have similar fascial characteristics to humans[21]. However, even then, the physiology differs; thus, all studies noted that the goal was to establish human trials.

3.1. Direct versus indirect printing

Five of the 27 included studies utilized indirect 3D printing, in which a negative mold of the desired auricular shape is printed, meaning that the accurate and
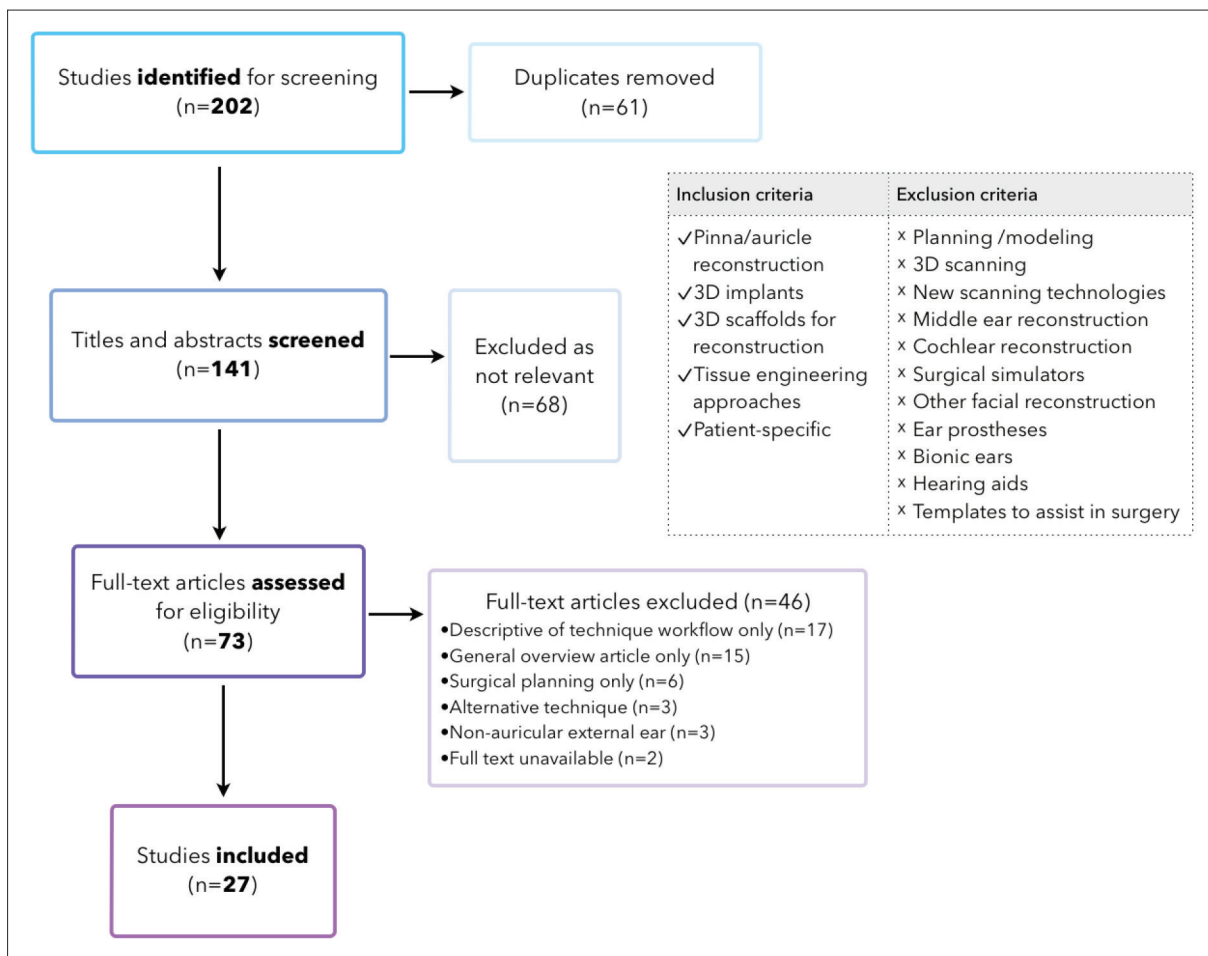
Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of article selection process, along with inclusion and exclusion criteria.

personalized shape can be utilized without being limited to a specific medium. The mold can thus be printed in any non-biocompatible material, such as a resin[20,22], a fused deposition modeling (FDM)-printed meshed co-polymer like butenediolvinylalcohol (BVOH)[23] or a powder via selective laser sintering[24]. Alternatively, the ear shape can be printed in polycaprolactone (PCL) and then used to create a set of silicon casting molds[11]. The mold can then be used to cast or grow the final auricular scaffold, which can then be cell-seeded[25], or alternative materials like diced cartilage and platelet-rich plasma may be used instead[24].

Conversely, the majority (81.5%, $n = 22$) of the studies utilized direct printing. This was mostly extrusion-based printing, in which a material is extruded through a nozzle onto a print bed, and the scaffold is built up layer by layer[9]. In some cases, the scaffold was printed alone ($n = 4$). Most of the time, it was printed first and then seeded with cells ($n = 13$)[26]. However, a large proportion of the studies

($n = 10$) were focused on true bioprinting, in which the cells were already suspended in a hydrogel, and this bioink and the scaffold were printed together[27].

Several of the studies introduced specific modifications to their bioprinting approach, such as incorporating multiple printer heads within a "tissue building system" that facilitated the simultaneous printing of various materials. This allowed Lee *et al.* (2014), for example, to create an ear-shaped structure with two cell types in hydrogels and polyethylene glycol (PEG) scaffold to be printed together[26]. Similarly, other mechanical alterations, like creating and maintaining a negative pressure environment, helped to promote cartilage growth and maturation[25].

Some studies overcame the pliable nature of some bioinks by using photocurable elements to harden the scaffold either during or after printing. For example, Xia *et al.* (2018) created a photo-crosslinkable gelatin containing the chondrocytes for printing and thus adapted
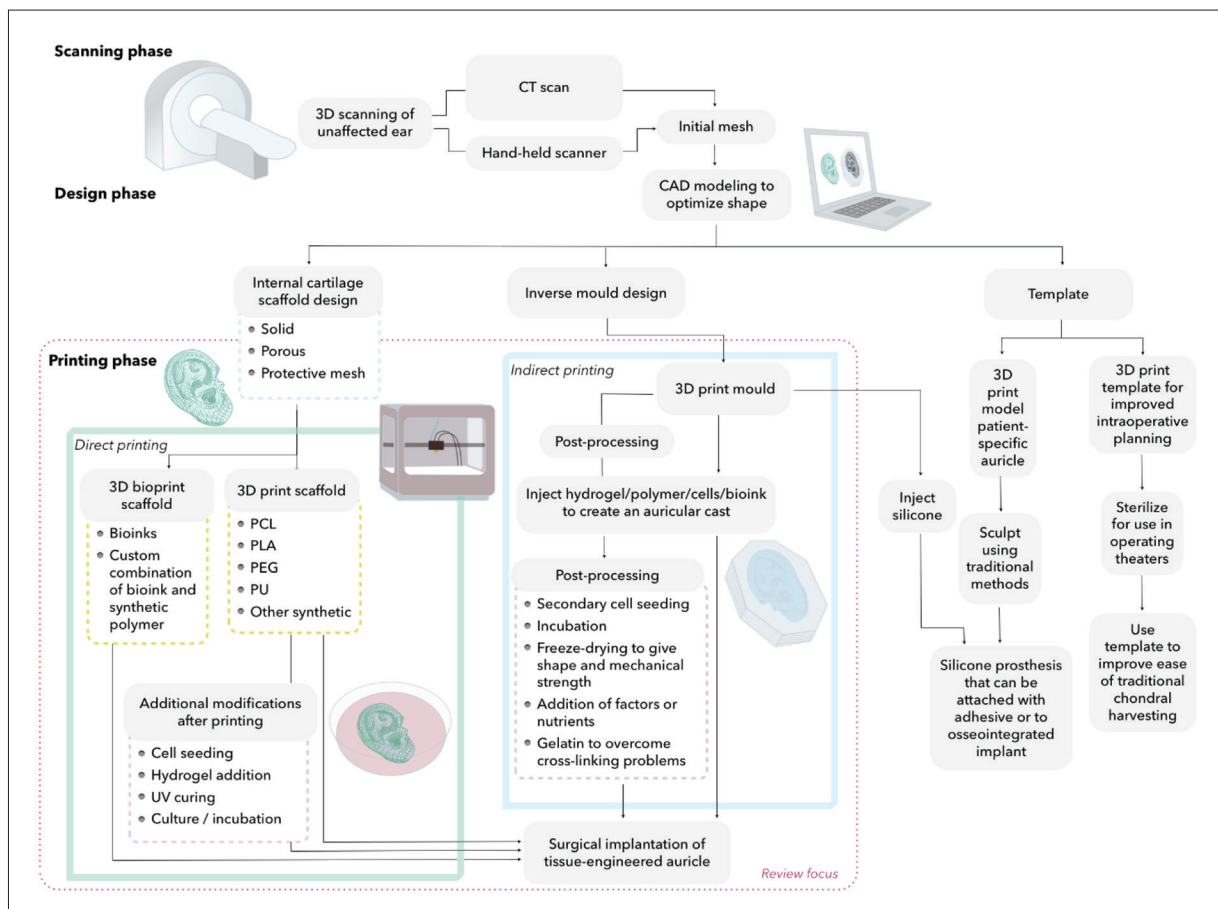
Figure 3. A summary of the ways in which 3D technology is being used to aid auricular reconstruction. Abbreviations: CT, computed tomography; PCL, polycaprolactone; PEG, polyethylene glycol; PLA, polylactic acid; PU, polyurethane; UV, ultraviolet.

their printer to cure their printed scaffold with blue light while new layers were deposited[14]. Similarly, Jia *et al.* (2022) used a multi-nozzle extrusion system that allowed the printing of cell-laden bioink and PCL alternately, and their methacrylate-enhanced bioink was subsequently light-cured into a sturdier shape[11]. Interestingly, Visscher *et al.* (2021) found that UV curing after printing improved construct stiffness to maintain the desired anatomical shape and suggested that this altered starting gel stiffness had an effect on cellular behavior and may have promoted cellular activity and maturation of the chondrocytes[28].

In the studies reviewed, a variety of 3D printing techniques were employed. Extrusion-based printing was the most commonly used in approximately 18.5% of studies, followed by digital light processing (DLP) bioprinting (7.4%). Fused deposition modeling, laser sintering, multi-head tissue/organ building system, and molds printed by selective laser sintering (SLS) were each used in about 3.7% of studies. The remaining studies utilized other or unspecified techniques. For a more detailed breakdown, refer to Table 2.

3.2. Cell selection

Selection of the right cell type is critical for implant success. Between 100 and 250 million chondrogenically potent cells that can form well-organized tissue are rich in GAG, collagens, and elastin and are needed for auricle formation[29]. In the studies reviewed, chondrocytes were the most commonly used cell type for auricle reconstruction (33.3%), reflecting their critical role in forming well-organized tissue rich in GAG, collagens, and elastin. However, chondrocytes have limited ability to expand and tend to dedifferentiate into fibroblasts, producing a fibrous extracellular matrix (ECM) with poor mechanical properties[23]. Mesenchymal stromal cells, used in about 14.8% of studies, on the other hand, are easily expandable but tend to undergo hypertrophy and differentiate toward the osteogenic lineage. Microtia-derived auricular chondrocytes (mACs) and cartilage progenitor cells can also be used[29] (and were employed in about 11.1% of cases) but are less effective at forming cartilage than normal auricular chondrocytes; thus, other cell types must be incorporated into the construct[23]. Notably, about 25.9%

of the studies used alternative cell sources, such as human adipose-derived stem cells (ASCs), novel human auricular cartilage progenitor cells (AuCPCs), bovine auricular chondrocytes, and tonsil-derived mesenchymal stem cells. Finally, in 14.9% of the studies, no specific cell type was mentioned.

Additionally, the nutrient supply the growing cells possess affects the quality of the generated tissue. Despite cartilage typically being largely avascular, additional strategies have been suggested, such as creating perfusion microchannels to allow better nutrient diffusion[30] or engineering myoglobin complexes on membranes to improve cell survival and tissue development[29].

An additional difficulty identified was achieving a good distribution of chondrocytes and uniformity of ECM with conventional cell seeding techniques, leading to insufficient mechanical stability and, in turn, macroscopic deformation. For this reason, the printing material chosen must not easily trigger aseptic inflammation so that the formation of ECM is not restricted[11]. The optimum material would have properties that would stimulate cartilage formation. For example, materials with low stiffness (<2–10 kPa) were found to promote cartilage formation, whereas scaffolds with high stiffness (>10 kPa) did not and consequently failed to grow cartilage well[15].

3.3. Material comparison
Synthetic polymers, such as PCL, polylactic acid (PLA), PEG, and polyurethane (PU), have been the focus of 3D printing material selection for cartilage regeneration due to their biocompatibility, mechanical properties, and degradation characteristics. PCL, the most frequently printed material in this review, has been used in the biomedical field in implants and sutures for over 70 years[31], and is biocompatible with good bioresorbility and mechanical stiffness[9], making it a good choice for surgical reconstruction as it can be safely and gradually absorbed over 4 years without causing adverse reactions[20], while supporting the structure as the cellular components around it mature[11]. PLA is biocompatible, affordable, and also degrades slowly, retaining its integrity throughout elastic cartilage maturation[10]. PEG is biocompatible and dissolves, without adverse effects on cell viability, within 40 min of being submerged in an aqueous environment, making it suitable as a sacrificial material for when short-term support is required for printing[27]. PU is strong, versatile, and resilient, and while its biodegradability is a point of contention[32], Kim *et al.* (2019) compared porous and non-porous structures printed using PU and found that the porous variety encouraged cell proliferation[30]. Finally, bioinks, composed of a mixture of cells, growth factors, and other biocompatible substances suspended in a gel or hydrogel matrix, provide a supportive environment and allow direct printing of structure that more closely mimics the natural anatomy of the ear[11].

A variety of hydrogels were used as scaffolds for auricular cartilage tissue engineering in these studies. The most commonly used hydrogel was a combination of gelatin and alginate, which was utilized in several studies[8,9,27,28,32]. This hydrogel was primarily used for 3D bioprinting of auricular cartilage, although the specific properties of the hydrogel were not detailed in these studies. Another study by Zopf *et al.* (2018) used a hyaluronic acid/collagen hydrogel for seeding primary porcine auricular chondrocytes onto 3D-printed scaffolds[54]. However, the properties of the hydrogel were not explicitly mentioned[51]. In a study by Jia *et al.* (2020), an ACM/gelatin hydrogel was used. The mechanical properties showed an opposite trend to pore size and porosity, and the degradation rate enhanced with increasing acellular cartilage matrix (ACM) proportion. The hydrogel demonstrated excellent biocompatibility, with a cell seeding efficiency of more than 90%[25]. It should be noted that the specific properties of the hydrogels were not always detailed in the studies, and as such, a comprehensive comparison of the hydrogels used is not possible based on the available information.

3.4. Time *in vivo* and evaluation successful outcomes
One of the most significant drawbacks of all the *in vivo* animal studies in evaluating optimum cell type and material selection was the limited time the scaffolds spent *in vivo* (see Figure 4). Overall, this was an average of 110 days. The bioink studies were the shortest (mean = 53.25 days). Conversely, the longest-running animal study was that by Yin *et al.* (2020), which lasted 365 days and in which a composite scaffold composed of polyglycolic acid (PGA)/PLA and a PCL core was seeded and implanted in mice[22].

The longest-running study in this review is the ongoing human pilot trial, which was at the 2.5-year mark at publication. This landmark study had several limitations, such as a small sample size of only five patients. However, it showed it is possible to design, print, and integrate patient-specific auricles in human subjects. Notably, the patients underwent 12 weeks of tissue expansion before the reconstruction and subsequently two scar revisions were performed at 6 and 18 months, allowing for tissue biopsies of the scaffold at the same time, which showed good cartilage formation. Good aesthetic results were only achieved after 9 months, once postoperative inflammation subsided[20].

All studies attempted to evaluate the resulting printed structure's properties objectively. Histopathology, electron microscopy, ultrasound scans, micro-computed

Table 2. Overview of reviewed studies on 3D bioprinting for auricular reconstruction

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Chung et al. (2020)[9]	To assess the feasibility of using a hybrid printing approach to fabricate a scaffold for the outer ear of clinically relevant size. Attempted to address the distinct regions in the auricular cartilage by varying the pattern design (as opposed to hybrid scaffolds printed with one specific mechanical modulus across the entire construct).	*In vitro*	N/A	Direct printing	Extrusion	Cells in bioink + scaffold printed together	Resembling pinna; other shape
Lee et al. (2014)[27]	To develop a non-toxic method for producing inverse pyramidal and bowl-shaped structures that are optimized for auricle printing.	*In vitro*	N/A	Direct printing	Multi-head tissue/ organ building system	Scaffold printed first and then seeded with cells	Resembling pinna; other shape
Otto et al. (2021)[29]	To investigate the usability of human AuCPCs in auricle 3D printing.	*In vitro*	N/A	Direct printing	Fused deposition modeling (extrusion)	Cells in bioink + scaffold printed together	Resembling pinna; other shape

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
PCL	GelMA-HAMA cell-supportive bioink	Photocuring of the printed bioink was using a 400 nm UV source at a focal distance of 5.0 cm and an intensity of 15–30%	Histopathology	• The design of a print affects its stiffness and flexibility significantly. • Scaffolds printed with a 400 µm nozzle tip had the lowest compressive modulus but were similar in stiffness to native auricular cartilage and had the fastest printing time. • Increasing the nozzle diameter decreases the compressive modulus of PCL scaffolds, while increasing strand spacing and using orientations of 0/45° leads to more flexible structures. • Scaffolds can serve as a temporary support for host tissue integration and chondrocyte differentiation. A scaffold with similar properties before implantation could improve its handling and shape retention after implantation. • Cells remained viable for up to 7 days after printing. • The presence of a surrounding hydrogel during the printing process helps protect cells from shear forces at the nozzle tip, leading to good cell viability during the printing process.	• Further improvements could be developed to generate smoother intersections for each region • Incorporate a gradual decrease or increase of the strand spacing of each part at the junction
PCL & PEG	Human ASCs exposed to chondrogenic induction medium and adipogenic induction medium	The cell-printed structures were incubated a week at 37°C in a humidified atmosphere containing 5% CO_2. The sacrificial PEG layer was dissolved.	Mechanical testing; electron microscopy	• The sacrificial layer technique allowed for the construction of complex structures of any shape. • The PEG sacrificial component did not impact cell viability or proliferation and could be easily dissolved in water or cell culture media within 40 minutes. • Hydrogels may provide a better environment for chondrocyte proliferation, but printed adipocytes had a lower proliferation rate. • Chondrocytes and adipocytes had similar proliferation rates when printed separately, and chondrogenesis and adipogenesis occurred effectively when the two cell types were co-printed and co-cultured. • An ear-shaped structure containing both chondrocytes and adipocytes not only maintained its shape, but also regenerated both auricular cartilage and earlobe fat.	A system for incubating printed cells will need to be developed and attached to the printer in order to keep the cells alive during the printing of large structures such as an ear, as the viability of the printed cells may be compromised during this process.
PCL	Novel human AuCPCs	Cultured *in vitro* in chondrogenic media for 30 days	Histopathology; micro-CT scan; mechanical testing	• Extrusion printing does not negatively impact cell viability, metabolic activity, or the production of GAGs. • The extrusion of AuCPCs through a microvalve system did not harm cell viability (which remained at 8% over 10 days), metabolic activity (which was not different between cast and printed cells), or GAG production (which occurred over 28 days *in vitro*). • PCL scaffolds with various strand spacings support GAG production.	Short-term study; no *in vivo* testing of this method yet

(*Continued*)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Visscher et al. (2016)[50]	To create a rapid production pathway and mechanically stable scaffold structures with an optimal biochemical ECM environment for generating and maintaining proper ear cartilage.	*In vitro*	N/A	Direct printing	Extrusion	Scaffold only	Other shape
Visscher et al. (2018) [51]	To design and 3D-print an easily-assembled cartilage implant for auricular reconstruction.	*In vitro*	N/A	Direct printing	Extrusion – 3D printing a two-part PCL mold in the shape of human auricular cartilage and injecting this construct with a mixture of alginate and chondrocytes before culturing the final construct *in vitro*.	Cells in bioink + scaffold printed together; scaffold printed first and then seeded with cells	Resembling pinna

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
				• PCL scaffolds maintained good shape retention both immediately after printing and after 30 days of *in vitro* culture. • The hybrid ear-shaped construct produced cartilage-specific components in abundance. • 400 µm and 800 µm scaffolds are relatively stiff and inflexible, while 1000 µm and 1200 µm scaffolds allow for more bending when manipulated by hand. However, using wider strand spacings also reduces control over the fine architecture of the structure.	
PCL	Goat mesenchymal stem cells, chondrocytes and perichondrocytes.	Printing is paused half-way to insert a collagen I/III scaffold into the PCL cage. Then after printing, hydrogel is added on top of that.	Histopathology; mechanical testing	• A novel cage construct consisting of a combined hydrogel and collagen I/III scaffold within a 3D-printed synthetic PCL cage can prevent *in vitro* scaffold contraction. • Six PCL cage constructs can be produced and printed in 2 h with good printing accuracy and minimal oozing effects. • Cell-seeded hydrogels showed significant contraction *in vitro*, reducing to 15–48% of their original volume after 28 days of culture depending on the cell type. • Histological evidence of GAG deposition was present in all scaffolds and internal scaffolds. • Chondrocyte- and chondrocytes perichondrocyte (CP)-seeded scaffolds produced the most collagen, while adipose-derived stem cells and chondrocytes had lower GAG production than other cell types. • The Young's modulus of all scaffolds and internal scaffolds increased by 50% after 28 days of *in vitro* culture compared to 14 days of culture.	No *in vivo* testing of this method yet
PCL	Chondrocytes from Dutch milk goats	Molds were disinfected with 70% ethanol for 1 h, washed with sterile PBS and dried in a sterile incubator. All parts were coated with sterile gelatin to block the pores and create a sealed mold. Once placed in proliferation medium at 37°C, the gelatin liquefied and left the pores, allowing for nutrient and oxygen exchange through the mold pores.	Histopathology; Mechanical testing	• It was found that a porous synthetic outer layer with high mechanical strength was needed to withstand forces during *in vivo* tissue maturation. • An inner "natural" core made of a biomimetic environment for cartilage tissue formation was an effective way to achieve biointegration. • Beads cultured in chondrogenic medium had higher levels of GAGs and type II collagen deposition.	Long-term *in vivo* experiments are required to test preclinical applicability

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Visscher et al. (2021) [28]	To develop a hydrogel-based bioink that recreates the complex cartilage microenvironment.	*In vitro*	N/A	Direct printing	Extrusion	Cells in bioink + scaffold printed together; scaffold only	Resembling pinna; other shape
Bhamare *et al.* (2021) [26]	To synthesize pinna from printable bioink. Ear pinna bioink was prepared from xenogenic goat cartilage by adding polymers and optimized for 3D printing.	*In vitro*; *in vivo* animal	Rat	Direct printing	Extrusion	Cells in bioink + scaffold printed together	Resembling pinna
Dong *et al.* (2021) [10]	To determine whether 3D-printed, biocompatible scaffolds would "protect" maturing hydrogel constructs from contraction and topography distortion.	*In vitro*; *in vivo* animal	Rat	Direct printing	Extrusion	Scaffold printed first and then seeded with cells	Other shape

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/ integration	Findings	Limitations and suggested improvements
Bioink	Porcine ear cartilage	UV crosslinking after printing	Histopathology; mechanical testing; other such as mass spectrometry proteome analysis	• A photo-crosslinkable cartilage-derived ECM-based bioink was successfully developed for auricular cartilage reconstruction and supported the activity and maturation of chondrocytes in bioprinted constructs. • Decellularized cartilage-derived ECM can be used for cell-based 3D bioprinting. • The inclusion of gelatin, HA, and glycerol improved printability and initial structural integrity, while UV polymerization increased stiffness. • The stiffness of the gel may affect cell behavior.	*In vitro* only • Large protein heterogeneity observed between samples in the mass spectrometric analyses, which makes it difficult to draw a specific conclusion on effects of protein abundance on growth
Bioink	Goat cartilage used in bioink	Ethylene oxide used to sterilize scaffold before implantation. It was trimmed into pieces (10 mm in width), and rinsed with saline containing antibiotic solution.	Histopathology; ultrasound scan; micro-CT scan; mechanical testing	• The ear had biodegradable properties despite containing polymers. • Mechanical strength of 3D-printed pinna showed similar results like normal ear pinna. • Pinna was biocompatible (*in ovo* and *in vivo*) having newly developed chondrocytes, elastin fibers, progenitor cells of ECM after transplantation. • Within 30 days, the transplanted 3D-printed pinna regenerated chondrocytes, GAG, elastin fibers, collagen, and retained its ECM. • The occurrence of angiogenesis after grafting showed that the 3D-printed ear pinna was accepted by the rat ear and had non-toxic properties similar to those of a native ear pinna.	Small study in animals only
PLA	Bovine auricular chondrocytes	Chondrocytes in collagen hydrogels were added to the printed constructs. Cell-loaded constructs were cultured in DMEM overnight before implantation.	Histopathology	• Scaffolds were fabricated using injection molding to protect auricular cartilage constructs from external compression and intrinsic contractile forces, resulting in significant reduction of contraction and preservation of complex topography. • Injection molding allows for the creation of a more fully interconnected porous network and is particularly effective for quickly producing homogenous volumes with high accuracy. • Mechanically attaching a cell-seeded collagen construct to an external structure helps maintain shape retention during extended culture *in vitro*. • Larger (12-mm wide) porous PLA discs featuring a ridge on the surface were designed to simulate the shape of the helical rim and compensate for anticipated contraction, blunting, and distortion. After 3 months *in vivo*, the helical rim feature was better preserved in the injection molded (SInj) and scaffolded (S) groups compared to the naked (N) group, which lost the rim feature and became a flat disc of cartilage. • The presence of an external PLA scaffold did not hinder the formation of healthy cartilage within the constructs.	Using bovine cells is a necessary step toward eventually using human cells to produce clinically translatable results. Using a rodent model is limiting because the loose nature of rodent skin does not accurately replicate the compressive forces experienced by scaffolds under human auricular or scalp skin.

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Jang *et al.* (2020) [8]	To discover what happens when ASCs are co-cultured with chondrocytes in a 3D hybrid scaffold of PCL.	*In vitro*; *in vivo* animal	Rat	Direct printing	Extrusion	Scaffold printed first and then seeded with cells	Resembling pinna
Jia *et al.* (2022) [11]	To evaluate the effectiveness of a new approach for creating biological auricular equivalents using a biomimetic microporous photo-crosslinkable cartilage-derived ECM with precise shapes and a bioactive bioink based on ACMMA, GelMA, PEO, and PCL through the use of multi-nozzle 3D bioprinting technology.	*In vitro*; *in vivo* animal	Mice	Direct printing	Multi-nozzle extrusion by alternately printing type 1 (cell-laden bioink) and type 2 (PCL)	Cells in bioink + scaffold printed together	Resembling pinna; other shape
Kim *et al.* (2019)[30]	To investigate whether customized 3D-printed PU scaffolds with adequate microstructure provide biomechanical properties suitable for reconstruction of congenital ear defects.	*In vitro*; *in vivo* animal	Mice	Direct printing	Extrusion	Scaffold printed first and then seeded with cells	Resembling pinna

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
PCL	Human ASCs and rabbit articular chondrocytes	Incubation in culture media at 37°C with 5% CO_2	Histopathology; electron microscopy	• The proposed hybrid structure exhibited improved mechanical properties due to the PCL framework and demonstrated reasonable chondrogenesis *in vitro* and *in vivo*. • The compressive modulus of the alginate/PCL hybrid scaffold was found to be around 2.64 ± 0.34 MPa, similar to that of native auricular tissue. • Results suggest that chondrocytes should be added to the scaffold when it is implanted in the subcutaneous area without any surrounding cartilage, as it is difficult to regenerate cartilage using MSCs alone without chondrocytes or cartilage remnants.	Unclear sample size
Bioink—A microporous photo-crosslinkable bioactive bioink based on cartilage-derived ECM with the assistance of GelMA and PEO.	Bama miniature pigs's auricular cartilage	Two groups of cell-laden constructs were immersed in a culture medium for 24 h to dissolve PEO to form porous structures	Histopathology; micro-CT scan	• By using multi-nozzle 3D bioprinting technology to control the distribution of chondrocyte-laden bioink and PCL, microporous auricular equivalents with precise shapes and satisfactory mechanical strength were successfully fabricated. • Mature auricular cartilage tissue with high morphological accuracy, good elasticity, numerous cartilage lacunae, and cartilage-specific ECM deposition was successfully regenerated in nude mice. • The inclusion of PCL significantly improved the shape fidelity of auricular equivalents. The modulus of regenerated auricular cartilage without PCL support was over 65% of native cartilage, while that of regenerated auricular cartilage with PCL support was approximately 2.6 times greater than native cartilage. • Although the PCL occupied space, it did not affect the formation of mature cartilage tissue in the bioink area while providing sufficient strength and stiffness support.	The feasibility of the technology in large animal models is still yet to be optimized and verified by further experiments.
PU	Tonsil-derived MSC (for biocompatibility testing only)	None	Histopathology; micro-CT scan; mechanical testing; electron microscopy	• 3D-printed, implantable ear scaffolds made of PU are biomimetic, biocompatible, easily fabricated, and flexible. • The PPU scaffold used in this study was designed to have a specific microstructure in terms of pore size and uniform pore lattice architecture, which encouraged the ingrowth of surrounding tissue. In comparison, the Medpor scaffold had an irregular microstructure (highly variable pore size and distribution of pores) that did not allow for close tissue interactions. • Neovascularization was only observed in PPU scaffolds, indicating that a pore size of 200 mm may be critical for promoting tissue ingrowth after auricular reconstruction.	Further studies are needed to clarify the long-term behavior of implanted, regular-sized, 3D-printed PPU scaffolds in terms of shape and elasticity.

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Mukherjee *et al.* (2021) [21]	To assess the degradation behavior and tissue compatibility of hybrid scaffolds (PCL-hydrogel) compared to single material PCL scaffolds *in vitro* and *in vivo*. The study wanted to understand the biological reaction to printed scaffolds (independent of stem cells) in an immunocompetent host.	*In vitro*; *in vivo* animal	Sheep (similar fascial anatomy)	Direct printing	Extrusion	Cells in bioink + scaffold printed together; scaffold only	Other shape
Tang *et al.* (2021) [15]	To explore the use of 3D printing to fabricate bioactive artificial auricular cartilage using chondrocyte-laden GelMA and PLA for auricle reconstruction.	*In vitro*; *in vivo* animal	Mice	Direct printing	Fused deposition modeling (extrusion)	Scaffold printed first and then seeded with cells	Resembling pinna

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
PCL	N/A	None	Histopathology; ultrasound scan; micro-CT scan; electron microscopy	• Both porous 3D-printed PCL and hybrid scaffolds showed similar and homogenous degradation *in vitro*. *In vivo*, they exhibited minimal irritation or inflammation in surrounding tissue over a 6-month period in an immunocompetent animal model that closely resembles human soft tissue biology, although the host response varied between animals. • The hybrid scaffolds had a higher percentage mass loss than control scaffolds due to the presence of degrading hydrogels that contributed to a higher initial weight. However, the degradation profile was dominated by PCL in both hybrid and PCL-only scaffolds. • SEM showed that degradation occurred from the outer surface inward for each strand. • *In vivo*, the scaffolds were well tolerated for the duration of the experiment, with serial ultrasound and CT scans showing minimal reaction in surrounding subcutaneous tissue over 6 months. Ex vivo, the scaffolds displayed localized hyperemia with peripheral pallor and pseudo-capsule formation, consistent with a localized inflammatory response, indicating good biocompatible properties with no macroscopic differences between test and control samples. • Control specimens, when 3D-reconstructed, had less tissue integration compared to all test samples in both sheep, regardless of PCL configuration. This may be attributed to the presence of the hydrogel.	The inclusion of GelMA-HAMA with the PCL demonstrated better tissue ingrowth. However, its impact on cellular differentiation can only be evaluated in future experiments embedded with cells.
PLA	Rabbit ear chondrocytes; grafts were also taken from mice	XX	Histopathology; mechanical testing; electron microscopy	• The successful induction of auricular chondrogenesis *in vivo* was demonstrated using a photosensitive GelMA hydrogel to allow chondrocytes to bind to a customized auricular scaffold. • A biologic auricle with a PLA material as the inner core for support was constructed. This not only provides mechanical support for cartilage regeneration for morphological maintenance *in vitro* and *in vivo*, but also allows the ester bonds of PLA to be slowly hydrolyzed, providing sufficient time for the engineered cartilage to mature and acquire mechanical properties while gradually replacing the degrading PLA scaffold.	Short-term study and thus long-term ability to withstand immune response was not tested.

(*Continued*)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Xia *et al.* (2018)[14]	To establish novel scaffold-fabricated strategies for native polymers and provide a novel natural 3D scaffold with satisfactory outer shape, pore structure, mechanical strength, degradation rate, and weak immunogenicity for cartilage regeneration.	*In vitro*; *in vivo* animal	Mice + goats	Direct printing	Pneumatic extrusion-based bioprinter with a 405 nm blue light.	Scaffold printed first and then seeded with cells	Resembling pinna; other shape
Xie *et al.* (2022) [53]	To present an ECM compound bioink derived from cartilage microtissues and its use in cartilage regeneration, specifically the auricle	*In vitro*; *in vivo* animal	Mice	Direct printing	DLP bioprinting	Cells in bioink + scaffold printed together	Resembling pinna; other shape

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
Photo-curable hydrogel (meth-acrylic anhydride + gelatinous + hyaluronic acid)	Goat auricular cartilage-derived chondrocytes	After 3D printing, the scaffolds were frozen at –80°C for 4 h and lyophilized for 48 h. The scaffolds were then sterilized with ethylene oxide for subsequent use.	Histopathology; mechanical testing; electron microscopy	• Photo-crosslinkable gelatin and HA can be fabricated as a porous scaffold with a precise outer shape, good internal pore structure, high mechanical strength, and good degradation rate, through photocuring 3D printing and lyophilization. • The scaffolds combined with chondrocytes successfully regenerated mature cartilage with typical lacunae structure and cartilage-specific ECM both *in vitro* and *in vivo*. • Chondrocytes were able to adhere to, survive within, and proliferate effectively in the scaffolds. • *In vitro*, cartilage-like tissue was successfully regenerated within 2 weeks, which was faster than the 4–8 weeks it took to regenerate cartilage using polyglycolic acid/polyglycolic acid(PLA/PGA) scaffolds. • In immunocompetent large animals, the 2-week *in vitro*-engineered cartilage successfully regenerated stable mature cartilage with no obvious inflammatory reaction observed, despite the presence of abundant residual scaffold. This suggests that 2 weeks of *in vitro* culture is optimal for the current scaffolds to permit autologous *in vivo* cartilage regeneration in future clinical applications, which could greatly decrease associated patient treatment costs and waiting times.	Mechanical strength of the scaffolds warrants further enhancement, and the feasibility of regenerating precisely shaped cartilage needs to be further explored.
Bioink	Porcine chondrocytes	The printed auricular constructs were placed in a complete culture medium for 20 days.	Histopathology; mechanical testing; electron microscopy	• It was showed that microtia chondrocytes extracted from residual ear tissue can be used to create auricular cartilage for clinical use, as they had chondrongenic, osteogenic and adipogenic differentiation potential. • Chondrocytes and stem cells were combined with a hydrogel to create a bioink. This bioink was then used with DLP bioprinting to create auricular constructs that had high elasticity, high printing accuracy, and low swelling ratio. • Compared with extrusion bioprinting, DLP is highly accurate and may cause less mechanical damage to cells • The GelMA+chondrocytes group was more prone to internal cell death due to a lack of nutrition, while the cells in the GelMA+microtissues group fared better, as the cells could perform intercellular connections and secret more bioactive substances • After *in vitro* culture, a large amount of ECM was deposited, and mature cartilage was observed to regenerate after subcutaneous implantation in mice for 12 weeks.	• Small print size • Repeating the process of freezing and thawing the sample and trying using supercritical CO_2 as a disinfectant may improve the decellularization method, as the current method using ethanol and peracetic acid caused a significant loss of GAG content

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Zopf *et al.* (2014) [52]	To determine the potential of an integrated, image-based CAD and 3D printing approach to engineer scaffolds for head and neck cartilaginous reconstruction for auricular and nasal reconstruction.	*In vitro; in vivo* animal	Pigs	Direct printing	Laser sintering	Scaffold printed first and then seeded with cells	Resembling pinna; other shape
Zopf *et al.* (2018) [54]	To determine the effect of auricular scaffold microarchitecture on chondrogenic potential in an *in vivo* animal model.	*In vitro; in vivo* animal	Athymic rodents	Direct printing	Laser sintering powder 3D printer	Scaffold printed first and then seeded with cells	Resembling pinna
Apelgren *et al.* (2018) [3]	To evaluate if an integrated 3D bioprinted cartilage construct has the capacity to serve as a bed for a full-thickness skin graft.	*In vivo* animal	Rat	Direct printing	Extrusion 3D bioprinter using nano-fibrillated cellulose/alginate bioink	Cells in bioink + scaffold printed together	Other shape

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
PCL	Chondrocytes were isolated from harvested porcine auricular cartilage	Chondrocytes were seeded into the auricular PCL scaffolds using a type I collagen gel. The cell suspension was pipetted into the PCL scaffolds, and the constructs were placed in an incubator (37°C, 5% CO_2) for 30 minutes for gelation to occur.	Histopathology	• A novel image-based CAD/CAM 3D printing process in the production of bioresorbable PCL scaffolds with a defined porous architecture for cartilaginous frameworks was introduced. The technique utilized 3D printing for auricular and nasal scaffold production and control of the pore architecture. • The surgical implantation of scaffolds was performed with ease, and scaffold porosity allowed for the versatility and ease of suture placement. The PCL material maintained excellent foundational support and appearance when implanted in subcutaneous tissue. • The PCL scaffolds all demonstrated histologically native-appearing cartilage growth. However, the scaffolds had not reached complete confluent cartilage coverage after two months *in vitro*.	Implantation into the pigs was only to demonstrate appearance, so long-term *in vivo* performance was not studied.
PCL	Porcine auricular cartilage chondrocytes	Chondrocytes seeded into the auricular PCL scaffolds using a type I collagen/HA composite gel. Seeded constructs were cultured in sterile, dynamic conditions with incubation at 37°C, 5% CO_2. After 4 weeks of *in vitro* culture, they were implanted into rodents.	Histopathology	• Auricular constructs with two micropore architectures were rapidly manufactured with high-fidelity anatomic appearance. • Subcutaneous implantation of the scaffolds resulted in excellent external appearance of both anterior and posterior auricular surfaces. • Comparing two different microporous scaffold architectures demonstrated the importance of scaffold design in optimizing auricular cartilage growth. • Results suggest that a defined arrangement of spherical pores yielded more robust auricular tissue growth. • The creation of spherical micropores within the scaffold architecture appears to impart greater chondrogenicity to the bioscaffold.	• Short time *in vivo* (4 weeks) • No mechanical testing of scaffold • Study size unstated
Bioink	Human bone marrow–derived mesenchymal stem cells & human nasal chondrocytes	After printing, constructs were crosslinked with 100 mM $CaCl_2$ for 5 minutes in 37°C. The constructs were washed with a balanced salt solution. The printed constructs were then immediately implanted subcutaneously into the mice.	Histopathology	• 3D-bioprinted cartilage that has been allowed to integrate *in vivo* is a sufficient base for a full-thickness skin graft. • Transplanted skin survived subcutaneously in all the 20 surviving animals. • No necrosis was observed. There were no macroscopic signs of complications. • However, lymphocytic infiltration was observed as a sign of inflammatory activity. • The majority of the chondrocytes did not survive (speculated to be due to diffusion limit).	• Since rodents heal by withering, they may not be the ideal model for studying the human healing process in all aspects. • Moreover, rodents' skin lacks apocrine sweat glands and is proportionally much thinner than humans' and has an additional muscle layer that makes the comparisons difficult. • Short study time. The long-term outcome of shape stability, elastic features, and tissue integrity are crucial factors that have to be addressed in further studies.

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Brennan *et al.* (2021) [4]	To evaluate the design and initial performance of the auricular scaffold in a preclinical animal model study. Our hypothesis was that the two-stage approach would limit the overlying soft tissue strain and thus result in lower rates of soft tissue ulceration, necrosis, and related complications compared to the single-stage reconstruction.	*In vivo* animal	Rats	Direct printing	Laser sintering PCL with a mixture of 4% hydroxyapatite (Plasma Biotal) where the HA serves primarily as a flowing agent for powder spreading during the laser sintering process	Scaffold only	Resembling pinna
Chang *et al.* (2020) [47]	To analyze the use of highly translatable 3D–printed auricular scaffolds with and without novel cartilage tissue inserts in a rodent model.	*In vivo* animal	Rat	Direct printing	Extrusion	Scaffold printed first and then seeded with cells	Resembling pinna

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/ integration	Findings		Limitations and suggested improvements
PCL	N/A	Sterilized by low-temperature ethylene oxide gas sterilization prior to implantation	Histopathology; micro-CT scan; mechanical testing	• A 3D-printed bioscaffold was developed for auricular reconstruction with a realistic anatomic appearance that meets or exceeds the best outcomes of rib cartilage grafting • The shape of the construct was durable and did not experience contraction or distortion over the course of the experiment. • The scaffold demonstrated robust tissue ingrowth and angiogenesis, suggesting it could support the growth of cartilaginous tissue. • The technique resulted in unparalleled ear appearance *in vivo*. • Ulceration was a problem in all the rats, regardless of single or two staging. • Minor superficial ulcers occurred most commonly at the lateral (86% of animals) and superior (29% of animals) helix of the scaffold. • Promisingly, there were high rates of improvement in ulceration over time with only a quarter of models demonstrating worsening in ulceration over the course of experimentation. Bolstered by findings of robust angiogenesis, the authors speculated that the improvement in wound healing was secondary to the angiogenesis and transformation of a bare PCL scaffold to an integrated, vascularized tissue implant. • The porous architecture design may improve outcomes by reducing skin complications and allowing increased tissue and vascular ingrowth to enhance reconstruction and accelerate healing of skin ulceration.		H&E was used to assess for vascularization. Quantification of angiogenesis has inherent challenges based off how the histology captures the blood vessels and the lack of specificity of H&E to stain vessels. So other stains should be used. Additional work is necessary to assess the potential to cellularize the scaffold with the ultimate goal of gradual replacement with a native cartilage matrix.
PCL	Porcine punch biopsies inserted into scaffolds to facilitate chondrocyte seeding	All scaffolds were sterilized with ethylene oxide Prior to implantation. Half of the sample was cartilage-seeded, with the other unseeded.	Histopathology; micro-CT scan	• Vascularization was observed in both unseeded and seeded scaffolds, indicating that non-autologous tissue implants do not inhibit vessel formation. • This technique is readily clinically translatable as it eliminates *in vitro* requirements and accompanying regulatory burden. • The 3D-printed auricles with and without cartilage inserts were structurally similar to human ears and measurements remained consistent. • There was no significant retraction or degradation of scaffolds during the experiment, suggesting resistance to contractile forces and sustained feasibility of such implants. • Micro-CT imaging showed infiltration of soft tissue into the pores of hybrid scaffolds. • Healing of ulceration was seen in both conditions, with smaller rates of scaffold-induced ulceration in cartilage-seeded scaffolds.		No statistically significant difference was observed between cartilage-seeded and unseeded scaffolds in any category of ulceration. This may be due to the small sample size or the methods used to measure ulceration. The use of additional immunohistochemistry might clarify whether chondrocytes are present beyond the limits of the cartilage punch biopsy and from which tissue they arise.

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Dong *et al.* (2022) [48]	To determine the minimum fraction of human auricular chondrocyte required to form healthy elastic cartilage when co-cultured with human MSCs.	*In vivo* animal	Rat	Direct printing	Extrusion (FDM)	Scaffold only	Other shape
Park *et al.* (2017) [49]	To apply 3D cell printing to fabricate a tissue-engineered graft, and evaluate its effects on cartilage reconstruction.	*In vivo* animal	Rabbit	Direct printing	Multi-head tissue/organ building system (extrusion)	Cells in bioink + scaffold printed together; scaffold printed first and then seeded with cells	Other shape
Jia *et al.* (2020) [25]	To create and test a proper scaffold created with ACM with precise human-ear-shape and necessary mechanical strength that will elicit a low inflammatory reaction.	*In vitro*; *in vivo* animal	Mice	Indirect printing	Cast-molding and freeze-drying mixture	Scaffold printed first and then seeded with cells	Resembling pinna; other shape

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/ integration	Findings	Limitations and suggested improvements
PLA	Bone marrow-derived mesenchymal stem cells	Cells were cultured, passaged, and then slowly injected into the scaffolds and gelled at 37°C for 1 h. Cell-loaded constructs were then cultured overnight before implantation.	Histopathology; micro-CT scan	• A combination of MSCs and chondrocytes was used to create cartilage that demonstrated consistent mechanical function, even when the ratio of MSCs to chondrocytes was high. • When chondrocytes made up only 10% of the initial cell population, the resulting tissue had characteristics similar to native elastic cartilage in terms of both biomechanics and biochemistry. • Co-implantation of a small number of chondrocytes with MSCs in a type I collagen matrix resulted in the production of cartilage that was indistinguishable from native auricular cartilage after 6 months *in vivo*. • It is not yet clear if the more efficient cartilage formation observed in this study is due to the differentiation of MSCs toward a chondrogenic lineage, a trophic effect of the MSCs, or a combination of both, but this is the subject of ongoing research. • The use of a small number of chondrocytes could be an important step toward the clinical translation of auricular tissue engineering due to the limited availability of auricular cartilage donor tissue.	The implantation of the constructs in nude rats, which manifest different skin characteristics from humans (e.g. looseness of rodent skin does not simulate the high pressure caused by implantation under the tight scalp), as well as significant differences in immunocompetency.
PCL	Primary chondrocytes from the New Zealand white rabbit	Incubated at 37°C for 1 h. Fabricated CSHS was crosslinked using $CaCl_2$. Washed with PBS thrice. Then chondrocytes were seeded onto the CSHS with the same cell density with CSS.	Histopathology; mechanical testing; electron microscopy	• A multi-head tissue/organ building system can successfully be used to 3D-print a cell-printed structure (CPS) using layers of alginate bio-ink encapsulating chondrocytes and PCL. • The CPS was found to have a higher efficiency of cellular settlement, improved survival and function of chondrocytes *in vitro* compared to a cell-seeded scaffold (CSS). • When implanted in a rabbit ear with a cartilage defect, the CPS led to complete cartilage regeneration after 3 months, while the CSS and autologous cartilage only led to incomplete healing. • These results suggest that 3D printing synthetic polymer scaffolds with hydrogel materials and cells can be a viable alternative to using autologous cartilage for auricular reconstruction.	Small sample
Other: ACM/ gelatin-PCL scaffold	Goat chondrocyte seeding	A cell suspension was seeded into each scaffold. This was followed by 24 h of incubation	Histopathology; electron microscopy	• The scaffold showed excellent biocompatibility and successfully regenerated human-ear-shaped cartilage that retained a satisfactory shape, good elasticity, abundant lacuna structure, and cartilage-specific ECM deposition. • Cell seeding efficiency in both ACM/gelatin and gelatin scaffolds was more than 90%, which was significantly superior than that of PGA/PLA scaffolds.	Important unknowns remain, including how best to optimize scaffold preparation, evaluation of scaffold biosafety, and the feasibility of human-ear-shaped cartilage regeneration in large animals.

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Landau *et al.* (2021) [23]	To develop a clinical-grade, 3D-printed biodegradable auricle scaffold that formed stable, custom-made neocartilage implants.	*In vitro*; *in vivo* animal	Mice	Indirect printing	BVOH mold printing and subsequent seeding	Scaffold printed first and then seeded with cells	Resembling pinna; other shape
Yin *et al.* (2020) [22]	To assess the feasibility and *in vivo* long-term fate of elastic cartilage regenerated with an accurate human-ear shape using PCL inner support-strengthened biodegradable scaffold and expanded MCs to identify factors that may result in discrepant clinical outcomes.	*In vitro*; *in vivo* animal	Mice	Indirect printing	Resin 3D-printer to create a set of ear-shaped negative molds. A pair of PGA/PLA layers (150 mg PGA fibers each) and a PCL layer were then separately pre-shaped by the molds.	Scaffold printed first and then seeded with cells	Resembling pinna; other shape

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/ integration	Findings	Limitations and suggested improvements
				• Chondrocytes grew well on the scaffold with significant proliferation over time and very few dead cells were observed at any of the time points. • By 14 days, the chondrocytes had spread over the entire scaffold. • Histological analysis performed at 6 weeks showed that the ear-shaped constructs had preliminarily formed cartilage-like tissue with typical lacuna structure and strong positive cartilage ECM staining. • However, the regenerated tissue showed a hybrid structure, and some fibrous tissue could be observed among the regenerated cartilage regions. • At 12 weeks, the constructs formed mature cartilage-like tissue with abundant lacuna structure and more homogenous cartilage ECM distribution, despite little fibrous tissue could still be observed in small areas.	
PCL	Chondrocytes and MSCs	Mold was freeze-dried and washed three times with water, PBS and then with a growth medium to create a hydrophilic environment for cell seeding.	Histopathology; micro-CT scan; mechanical testing; electron microscopy	• 3D printing was used to create a PCL ear-shaped cartilage for transplants for microtia patients. • The method can also be modified for use in cases of complete lack of auricular cartilage, such as anotia and trauma, by integrating costal cartilage cells. • The process is fast and simple for engineering a medical-grade auricle. • *In vitro* assessments showed a significant advantage for the use of monoculture auricular cartilage cells, but no significant differences were observed in the constructs of various cell types after implantation into mice. • No significant differences were observed in lacuna development or collagen-2, elastin, GAG, and collagen expression between the two constructs, regardless of cultivation time.	Small scale
A pair of PGA/ PLA layers and a PCL layer were separately pre-shaped by the molds.	Microtia chondrocytes	Cells seeded and examined for cell adhesion 24 h after incubation	Histopathology; mechanical testing	• All the cartilages generated by different patients' cells were highly consistent in both qualitative and quantitative data, indicating high repeatability and stability of the construction technology of cartilages. Besides, PCL inner support had no inhibitory effect on cartilage regeneration, maturation, tissue integration, mechanical strength, and shape maintenance even after the complete degradation of PCL. • The implanted auricle framework could survive for a long time and successfully regenerate mature and continuous cartilage with an accurate human-ear shape. • After 12 weeks of *in vitro* culture and 12 months of implantation *in vivo*, a porcelain white ear-shaped cartilage with a shape similarity to the original mold of 93.02% was formed.	Large animal models are needed

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape
Liao *et al.* (2019) [24]	To reconstruct the auricle using a porous, hollow, 3D-printed mold and autologous diced cartilage mixed with PRP	*In vivo* animal	Rabbit	Indirect printing	Molds printed by SLS	Scaffold only	Resembling pinna
Zhou *et al.* (2018) [20]	To clinically apply tissue-engineered and 3D-printed ear-shaped cartilage to human subjects for the first time.	*In vitro*; *in vivo* human trial	N/A	Indirect printing	A resin model was generated through 3D printing. This resin ear model was used to cast a pair of negative molds.	Scaffold printed first and then seeded with cells	Resembling pinna

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements
				• The PCL support was completely covered by the regenerated cartilage with satisfactory integration. • Tissue-engineered cartilage shows typical phenotype of mature elastic cartilage similar to microtia ear tissue with typical lacuna structures and strong positive staining of safranin O, type II collagen, and Verhoeff-van Gieson (EVG).	
Other: PGLA membrane	Diced cartilage graft	PRP was mixed with diced cartilage and sodium citrate, and this paste was inserted into the printed porous scaffold	Histopathology; mechanical testing	• The porous, hollow, poly-amide auricular mold prepared by 3D printing and packed with diced cartilages and PRP graft showed appropriate biomechanical properties and maintained it shape, with good chondrocyte viability and the production of a cartilaginous extracellular matrix • It was found that mixing the cartilage with a PRP improved the viability of the diced cartilage when wrapped in a PLGA membrane. • At 4 months, the diced cartilage pieces within the mold had fused, and the gross appearance was similar in shape to an auricle. • No complications (e.g., hematoma, seroma, or infection) were observed at the implantation site during the postoperative period. • The results of histological staining showed that the diced cartilages after shaping retained viable chondrocytes and a complete ECM, with positive staining for GAGs, collagen fibers, and elastic fibers, which determine the biomechanical properties of the cartilage.	The synthetic, non-biodegradable poly-amide induced a cystic-like reaction around the implant. It is unclear whether this translates in the clinical setting to the formation of a seroma or a chronic granuloma/foreign body reaction. Another limitation of this work is that we did not explore the possibility of using other biodegradable materials, such as PLGA for the hollow auricle mold.
PGA, PLA, and PCL were processed into the ear scaffold	Isolated microtia chondrocytes	Expanded microtia cartilage cells were evenly dropped onto the PGA/PLA layer of the scaffold, followed by 5-h incubation at 37°C, 5% CO_2 The construct was then cultured in chondrogenic medium for 12 weeks.	Histopathology; mechanical testing; electron microscopy; other: photography and visual inspection of final clinical result at 1, 2, 3, 6, 9, 12, 18, 24, and 30 months post-surgery to record swelling, inflammation signs, and shape recovery. MRI (1.5T) to trace cartilage regeneration and PCL core degradation	• It is possible to successfully design, fabricate, and re-generate patient-specific external ears (in human subjects). • 12 weeks of tissue expansion were used to create a large enough skin flap. A facial flap was used to cover the back and the helix rim of the engineered ear graft, which not only provided sufficient blood supply, but also protected the graft from extrusion. • Subsequent surgeries (scar revision) were conducted for removing the pedicle of skin flap at 6 months and repairing scar at 18 months, which allowed for tissue biopsies of the implanted ear framework. • After two weeks, the initial postoperative edema slowly reduced, and the shape of the reconstructed ear, as well as the color of the covered skin, gradually recovered. Within 6 months post-implantation, only the basic ear contour was observed, while key auricular structures, such as helix, triangular fossa, anti-helix, and cavum conchae, became gradually distinct after 9 months.	• Small sample of only 5 patients • Longest follow-up of only 2.5 years (other cases 2–18 months): unknown result after complete degradation of PCL scaffold (takes 2–4 years) • Only pilot study: further work necessary to translate this into routine clinical practices • Optimization and standardization in scaffold fabrication, cell expansion, and *in vitro* cartilage engineering, surgical procedures are still required. • Single center

(Continued)

Table 2. *Continued*

Study	Aim of study	Study setting	Animal model (if any)	Study focus	3D printing technique	Components	Printed shape

Abbreviations: ACM, ; ACMMA, methacrylate-modified acellular cartilage matrix; ASCs, adipose-derived stem cells; AuCPCs, auricular cartilage progenitor cells; CAD, computer-aided design; CAM, computer-aided manufacturing; CPS, cell-printed structure; CSHS, cell-seeded hybrid scaffold; CSS, cell-seeded scaffold; CT, computed tomography; DLP, digital light processing; DMEM, Dulbecco's Modified Eagle Medium; ECM, extracellular matrix; FDM, fused deposition modeling; GAG, glycosaminoglycan; GelMA, gelatin methacrylate; H&E, hematoxylin and eosin staining; HA, hyaluronic acid; HAMA, hyaluronic acid methacrylate; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; PBS, phosphate-buffered saline; PCL, polycaprolactone; PEG, polyethylene glycol; PEO, poly(ethylene oxide); PGA, polyglycolic acid; PGLA, poly(lactic-co-glycolic acid); PLA, polylactic acid; PPU, perforated polyurethane; PRP, platelet-rich plasma; PU, polyurethane; SEM, scanning electron microscopy; SLS, selective laser sintering; UV, ultraviolet.
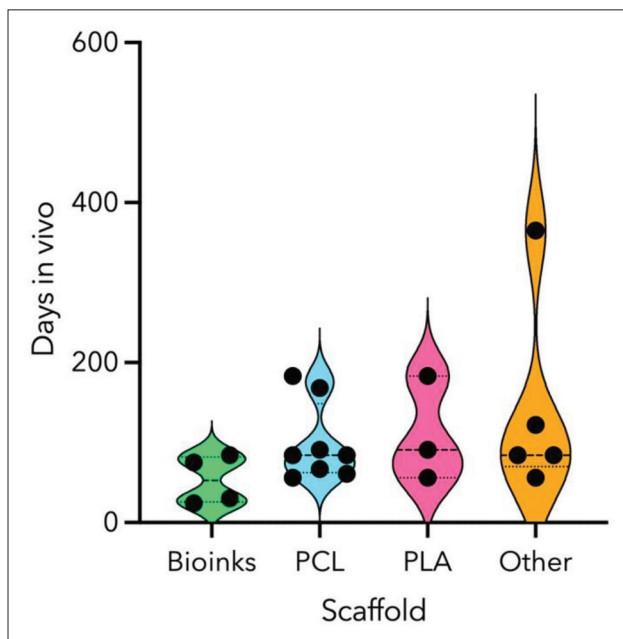

Figure 4. Time *in vivo* per scaffold type in experimental animal studies. Abbreviations: PCL, polycaprolactone; PLA, polylactic acid.

tomography (CT) scanning, and mechanical testing were all used to assess the resulting tissue-engineered auricles. However, given the wide range of printing methods, variable time *in vivo*, and heterogeneous use of outcome measures in the included studies, meaningful meta-analysis comparing scaffold materials and cartilage formation was not possible.

4. Discussion

4.1. Overcoming current obstacles to clinical translation

Auricular reconstruction is a challenging endeavor, partly due to the complex 3D geometry of the auricle[4], so unique to each individual that the pinna has been proposed as a forensic identifier[5].

This review demonstrates that 3D printing has the potential to have a significant impact on this relatively niche but complex area of reconstructive surgery by enhancing current surgical reconstructive options, and before clinical translation can truly occur, this technology requires optimization in multiple areas and large-scale clinical trials.

The printed auricle's desired anatomically detailed shape should be ensured for a lifetime, even after postoperative inflammatory processes have attempted to ravage the scaffold. Prevention of topographical blunting and volume loss requires both excellent cellular performance and abundant cartilage matrix, organized to mimic native elastic cartilage's histological and biomechanical properties. A certain degree of scaffold shrinkage is expected due to extrinsic compressive forces exerted by the overlying soft tissue, myocontractile

Printed material	Cell nature/type	Notable post-printing modifications	Assessment of success/integration	Findings	Limitations and suggested improvements

				• At 12 months, the reconstructed auricle presented high stiffness and low flexibility, whereas at 24 months, an obvious improvement in inflexibility with more distinct structures were achieved.
				• Among the total five cases, four cases showed obvious cartilage formation after 6 months post-implantation (one case was lost to follow-up).
				• MRI conformed a significant portion of PCL has degraded (complete degradation of PCL *in vivo* normally requires 2–4 years). Biopsied samples revealed formation of mature *in vivo* cartilage at 6 months and 18 months post-operatively.
					• Multiple additional surgical steps incorporated:
					  • Tissue expanded preoperatively for 3 months (psychosocial impact)
					  • Split-thickness skin graft from groin was required
					  • Scar revision surgeries were required at 6 and 18 months

scarring, and intrinsic contractile forces as the scaffold matures[10]. Therefore, a fine balance must be struck between creating an auricle that is strong enough to maintain its shape and yet pliable enough to mimic true elastic cartilage and not cause ulceration, which happens when the mechanical stiffness of the skin is low in relation to the construct[33]. This means that the structure needs to either be synthetic with perfect mechanical properties and completely inert and free from biodegradation or that it needs to be partially biological and, over time, integrate into the patient's body. Several of the studies in this review attempted to solve this conundrum by combining bioinks and multiple materials or through specific post-printing modifications. However, all studies agree on the necessity of longer-term *in vivo* research. This is crucial to verify that the bioprinted constructs will maintain their form and functionality over time, without issues such as deformation or collapse[29].

Since more than 1 in 500 people is affected by an external ear deformity per year, large-scale production would be desirable. However, currently available bioprinting technologies are not ready for mass production, and concerns have been raised that the costs associated with cell harvest and expansion prior to construct fabrication are currently prohibitive to routine clinical use[34]. Arguably, this cost could be offset in saved operating time, not to mention the reduced morbidity (and thus cost) associated with not needing to perform rib cartilage grafting. In fact, Brennan

et al. (2021) found that the implantation of a bioscaffold can be performed in under 25 min, as the surgical techniques involved are theoretically much simpler[4].

Additionally, regulatory approval is essential for 3D bioprinting applications in auricular reconstruction. In the United States, personalized 3D-printed medical devices are regulated under the medical device category or with custom device exemptions. The United States Food and Drug Administration (FDA) provides guidelines for 3D-printed materials to ensure product quality, efficacy, and classification for regulations. In the European Union, the regulation of 3D-printed medical devices has evolved. Previously, 3D-printed medical device products followed legislation like AIMDD 90/385/EE, MDD 93/42/EEC, and IVDMDD 98/79/EC, with medical devices classified based on patient contact duration, degree of invasiveness, and implantation/contact location in the human body[34,35]. However, the current regulation, known as the Medical Device Regulation (MDR 2017/745), has replaced these directives, providing a more comprehensive and stringent framework for the approval and post-market surveillance of medical devices[36]. Moreover, 3D-bioprinted tissues do not directly fall into existing regulatory categories. They are considered "bio-objects," which fall in between the existing categories of living and non-living matter, thus requiring new regulations/laws for clinical trials and commercialization. In this context, the European Regulation No. 1394/2007 and Directive 2001/83/EC,

which govern advanced therapeutic medicinal products, may provide some guidance[37,38].

Despite the progress in regulatory approval for 3D-printed medical devices, such as the FDA-approved AXIOM 20 3D printer and iFuse-3D implant, clear regulatory pathways for 3D-bioprinted tissues and their clinical applications are still needed[39].

4.2. Adjuvant considerations
From a surgical standpoint, this emerging technology should be examined holistically, including adjuvant techniques and factors that may impact outcomes.

4.2.1. Additional cover
In some trauma or burns cases, when more skin cover is necessary and cannot be sourced locally, a radial forearm pre-laminated flap may be a good source of additional skin, with an auricular scaffold implanted in the forearm before the main reconstruction[2]. Alternatively, good outcomes are achievable with skin grafts on top of 3D-printed scaffolds[3]. As with allografts, a temporoparietal fascial flap may be used with scaffolds to help mitigate soft tissue complications[4]. However, this carries additional local morbidity and prolongs operating time, so ultimately, a flap is not needed in the reconstruction at all.

4.2.2. Hearing restoration
Conductive hearing loss is present in 85% of microtia case[35]; thus, canalplasty is usually required to obtain a patent external auditory canal (EAC). The timing of the canaloplasty is linked to planned auricular reconstruction. It is typically done after it so an unoperated and unscarred field is preserved for auricle placement, and the delay avoids the need to center the framework over a drilled-out canal[40].

3D printing is also being developed for EAC reconstruction[41], and so far, techniques like using drug-releasing implants have been incorporated to prevent postoperative restenosis[42]. Tympanic membranes are also being printed[43], as are ossicles[44]. However, these efforts appear to be at a more fledgling stage than auricular reconstruction, probably because of the more complex functional requirements. Many techniques and materials used for auricular reconstruction may eventually be transferrable to other reconstructions, such as the EAC and vice versa.

5. Conclusion and future outlook
The auricle is uniquely histologically suitable for 3D printing, but a plethora of challenges remain. The fact that the majority of the existing evidence in this field is thus far only foundational and that this review identified only one level IV study demonstrates that this is very much an evolving field. However, it is a field that holds much promise, and the emergence of human trials internationally attests to this. Notably, phase 1 and 2 human trials are currently underway in the United States and are due to be completed by February 2028[45,46]. This demonstrates that this particular innovation has the potential to be adopted into clinical practice soon. However, a number of regulatory and practical concerns still need to be overcome. There is tremendous translational potential, and even if it may not necessarily become a routine component of microtia reconstruction overnight, auricle bioprinting may pave the way for 3D printing in other reconstructive efforts in plastic surgery over the next decade.

Conflict of interest
None.

Author contributions
Conceptualization: Anna Onderková
Supervision: Deepak M. Kalaskar
Visualization: Anna Onderková
Writing – original draft: Anna Onderková
Writing – review & editing: Anna Onderková, Deepak M. Kalaskar

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data
Not applicable.

References
1. Humphries S, Joshi A, Webb WR, *et al.*, 2021. Auricular reconstruction: Where are we now? A critical literature review. *Eur Arch Oto-Rhino-Laryngol*, 279(2): 541–56.

   https://10.1007/s00405-021-06903-5

2. Rendón-Medina MA, Hanson-Viana E, Arias-Salazar L, *et al.*, 2022, Auricular total reconstruction with radial

forearm prelaminated flap assisted by 3D surface imaging and 3D printing. *Plast Reconstr Surg - Glob Open*, 10: e4580.

https://10.1097/gox.0000000000004580

3. Apelgren P, Amoroso M, Säljö K, *et al.*, 2018, Skin grafting on 3D bioprinted cartilage constructs in vivo. *Plast Reconstr Surg - Glob Open*, 6: e1930.

https://10.1097/gox.0000000000001930

4. Brennan JR, Cornett A, Chang B, *et al.*, 2021, Preclinical assessment of clinically streamlined, 3d-printed, biocompatible single- and two-stage tissue scaffolds for ear reconstruction. *J Biomed Mater Res Part B*, 109(3): 394–400.

https://10.1002/jbm.b.34707

5. Jovic TH, Stewart K, Kon M, *et al.*, 2020, Auricular reconstruction: A sociocultural, surgical and scientific perspective. *J Plast Reconstr Aesthet Surg*, 73(8): 1424–1433.

https://10.1016/j.bjps.2020.03.025

6. Horlock N, Vögelin E, Bradbury ET, *et al.*, 2005, Psychosocial outcome of patients after ear reconstruction. *Ann Plast Surg*, 54(5): 517–524.

https://10.1097/01.sap.0000155284.96308.32

7. Ross MT, Cruz R, Hutchinson C, *et al.*, 2018, Aesthetic reconstruction of microtia: A review of current techniques and new 3D printing approaches. *Virtual Phys Prototyp*, 13(2): 117–130.

https://10.1080/17452759.2018.1430246

8. Jang CH, Koo YW, Kim GH, 2020, ASC/chondrocyte-laden alginate hydrogel/PCL hybrid scaffold fabricated using 3D printing for auricle regeneration. *Carbohyd Polym*, 248: 116776.

https://10.1016/j.carbpol.2020.116776

9. Chung JH, Kade JC, Jeiranikhameneh A, *et al.*, 2020, 3D hybrid printing platform for auricular cartilage reconstruction. *Biomed Phys Eng Express*, 6: 035003.

https://10.1088/2057-1976/ab54a7

10. Dong X, Premaratne ID, Bernstein JL, *et al.*, 2021, Three-dimensional-printed external scaffolds mitigate loss of volume and topography in engineered elastic cartilage constructs. *Cartilage*, 13(1).

https://10.1177/19476035211049556

11. Jia L, Hua Y, Zeng J, *et al.*, 2022, Bioprinting and regeneration of auricular cartilage using a bioactive bioink based on microporous photocrosslinkable acellular cartilage matrix. *Bioact Mater*, 16: 66–81.

https://10.1016/j.bioactmat.2022.02.032

12. Justicz N, Dusseldorp JR, Shaye D, 2017, Firmin technique for microtia reconstruction. *Oper Tech Otolaryngol Head Neck Surg*, 28(2): 90–96.

https://10.1016/j.otot.2017.03.005

13. Nagata S, Maruyama S, 2022, Auricular reconstruction Nagata method. *Nagata Microtia and Reconstructive Plastic Surgery Clinic.*

https://www.nagata-microtia.com/method.html (accessed November 26, 2022).

14. Xia H, Zhao D, Zhu H, *et al.*, 2018, Lyophilized scaffolds fabricated from 3D-printed photocurable natural hydrogel for Cartilage Regeneration. *ACS Appl Mater Interfaces*, 10(35): 31704–31715.

https://10.1021/acsami.8b10926

15. Tang P, Song P, Peng Z, *et al.*, 2021, Chondrocyte-laden Gelma hydrogel combined with 3D printed PLA scaffolds for auricle regeneration. *Mater Sci Eng C*, 130: 112423.

https://10.1016/j.msec.2021.112423

16. Roopavath UK, Kalaskar DM, 2022, Introduction to three-dimensional printing in medicine, in 3D Printing in Medicine. *Woodhead Publishing*, Cambridge 1–27.

https://10.1016/B978-0-323-89831-7.00008-0

17. Berens AM, Newman S, Bhrany AD, *et al.*, 2016, Computer-aided design and 3D printing to produce a costal cartilage model for simulation of auricular reconstruction. *Otolaryngol Head Neck Surg*, 155(2): 356–359.

https://10.1177/0194599816639586

18. Hong CJ, Giannopoulos AA, Hong BY, *et al.*, 2019, Clinical applications of three-dimensional printing in otolaryngology–head and neck surgery: A systematic review. *The Laryngoscope*, 129(8): 2045–2052.

https://10.1002/lary.27831

19. Beckers O, Coppey E, Mommaerts MY, 2021, Computer-aided design and manufacturing construction of a pilot guide for a bone-anchored epithesis to replace an absent pinna. *Int J Oral Maxillofac Surg*, 50(7): 815–819.

https://10.1016/j.ijom.2020.10.006

20. Zhou G, Jiang H, Yin Z, *et al.*, 2018, In vitro regeneration of patient-specific ear-shaped cartilage and its first clinical application for auricular reconstruction. *EBioMedicine*, 28(2): 287–302.

https://10.1016/j.ebiom.2018.01.011

21. Mukherjee P, Chung J, Cheng K, *et al.*, 2021, In vitro and in vivo study of PCL-hydrogel scaffold to advance bioprinting translation in microtia reconstruction. *J Craniofac Surg*, 32(5): 1931–1936.

https://10.1097/scs.0000000000007173

22. Yin Z, Li D, Liu Y, *et al.*, 2020, Regeneration of elastic cartilage with accurate human-ear shape based on PCL strengthened biodegradable scaffold and expanded microtia chondrocytes. *Appl Mater Today*, 20: 100724.

https://10.1016/j.apmt.2020.100724

23. Landau S, Szklanny AA, Machour M, *et al.*, 2021, Human-engineered auricular reconstruction (hear) by 3D-printed molding with human-derived auricular and costal chondrocytes and adipose-derived mesenchymal stem cells. *Biofabrication*, 14(1): 015010.

https://10.1088/1758-5090/ac3b91

24. Liao J, Chen Y, Chen J, *et al.*, 2019, Auricle shaping using 3D printing and autologous diced cartilage. *The Laryngoscope*, 129(12): 2467–2474.

https://10.1002/lary.27752

25. Jia L, Zhang Y, Yao L, *et al.*, 2020, Regeneration of human-ear-shaped cartilage with acellular cartilage matrix-based biomimetic scaffolds. *Appl Mater Today*, 20: 100639.

https://10.1016/j.apmt.2020.100639

26. Bhamare N, Tardalkar K, Parulekar P, *et al.*, 2021, 3D printing of human ear pinna using cartilage specific ink. *Biomed Mater*, 16(5): 055008.

https://10.1088/1748-605x/ac15b0

27. Lee J-S, Hong JM, Jung JW, *et al.*, 2014, 3D printing of composite tissue with complex shape applied to ear regeneration. *Biofabrication*, 6(2): 024103.

https://10.1088/1758-5082/6/2/024103

28. Visscher DO, Lee H, van Zuijlen PPM, *et al.*, 2021, A photo-crosslinkable cartilage-derived extracellular matrix bioink for auricular cartilage tissue engineering. *Acta Biomater*, 121(1): 193–203.

https://10.1016/j.actbio.2020.11.029

29. Otto IA, Capendale PE, Garcia JP, *et al.*, 2021, Biofabrication of a shape-stable auricular structure for the reconstruction of Ear Deformities. *Mater Today Bio*, 9: 100094.

https://10.1016/j.mtbio.2021.100094

30. Kim HY, Jung SY, Lee SJ, *et al.*, 2019, Fabrication and characterization of 3D-printed elastic auricular scaffolds: A pilot study. *The Laryngoscope*, 129(2): 351–357.

https://10.1002/lary.27344

31. Christen M-O, Vercesi F, 2020, Polycaprolactone: How a well-known and futuristic polymer has become an innovative collagen-stimulator in esthetics. *Clin Cosmet Investig Dermatol*, 13: 31–48.

https://10.2147/ccid.s229054

32. Zhang X, Battiston KG, McBane JE, *et al.*, 2016, Design of biodegradable polyurethanes and the interactions of the polymers and their degradation by-products within in vitro and in vivo environments. *Adv Polyureth Biomater*, 75–114.

https://10.1016/b978-0-08-100614-6.00003-2

33. Reighard CL, Hollister SJ, Zopf DA, 2018, Auricular reconstruction from rib to 3D printing. *J 3D Print Med*, 2(1): 35–41.

https://10.2217/3dp-2017-0017

34. Di Gesù R, Acharya AP, Jacobs I, *et al.*, 2019, 3D printing for tissue engineering in otolaryngology. *Connect Tissue Res*, 61(2): 117–136.

https://10.1080/03008207.2019.1663837

35. Sekar MP, Budharaju H, Zennifer A, *et al.*, 2021, Current standards and ethical landscape of engineered tissues—3D bioprinting perspective. *J Tissue Eng*, 12(1): 204173142110276.

https://10.1177/20417314211027677

36. European Parliament, Council of the European Union. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. Official Journal of the European Union. 2017 [cited 2023 Jun 7].

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02017R0745-20200424

37. European Parliament, Council of the European Union. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. Official Journal of the European Union. 2007 [cited 2023 Jun 7].

https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32007R1394

38. European Parliament, Council of the European Union. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official Journal of the European Communities. 2001 [cited 2023 Jun 7].

https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001L0086:EN:HTML

39. Ali K, Mohan K, Liu Y-C, 2017, Otologic and audiology concerns of microtia repair. *Semin Plast Surg*, 31(2): 127–133.

https://10.1055/s-0037-1603957

40. Lipan M, Eshraghi A, 2011, Otologic and audiology aspects of microtia repair. *Semin Plast Surg*, 25(4): 273–278.

https://10.1055/s-0031-1288919

41. Mota C, Milazzo M, Panetta D, *et al.*, 2018, 3D fiber deposited polymeric scaffolds for external auditory canal wall. *J Mater Sci: Mater Med*, 29(10).

https://10.1007/s10856-018-6071-3

42. Matin-Mann F, Gao Z, Schwieger J, *et al.*, 2022, Individualized, additively manufactured drug-releasing external ear canal implant for prevention of postoperative restenosis: Development, in vitro testing, and proof of concept in an individual curative trial. *Pharmaceutics*, 14(6): 1242.

https://10.3390/pharmaceutics14061242

43. Kozin ED, Black NL, Cheng JT, *et al.*, 2016, Design, fabrication, and in vitro testing of novel three-dimensionally printed tympanic membrane grafts. *Hear Res*, 340: 191–203.

https://10.1016/j.heares.2016.03.005

44. Hirsch JD, Vincent RL, Eisenman DJ, 2017, Surgical reconstruction of the ossicular chain with custom 3D printed ossicular prosthesis. *3D Print Med*, 3(15).

https://10.1186/s41205-017-0015-2

45. 3DBio Therapeutics, 2022, Aurinovo for auricular reconstruction. ClinicalTrialsgov.

https://clinicaltrials.gov/ct2/show/NCT04399239 (accessed December 7, 2022).

46. Everett H, 2022, 3DBio conducts successful human ear reconstruction with 3D bioprinted Aurinovo implant. 3D Printing Industry.

https://3dprintingindustry.com/news/3dbio-conducts-successful-human-ear-reconstruction-with-3d-bioprinted-aurinovo-implant-210201/ (accessed December 7, 2022).

47. Chang B, Cornett A, Nourmohammadi Z, *et al.*, 2020, Hybrid three-dimensional–printed ear tissue scaffold with autologous cartilage mitigates soft tissue complications. *The Laryngoscope*, 131(E5): 1008–1015.

https://10.1002/lary.29114

48. Dong X, Askinas C, Kim J, *et al.*, 2022, Efficient engineering of human auricular cartilage through mesenchymal stem cell chaperoning. *J Tissue Eng Regen Med*, 16(6): 825–835.

https://10.1002/term.3332

49. Park JY, Choi Y-J, Shim J-H, *et al.*, 2017, Development of a 3D cell printed structure as an alternative to autologs cartilage for auricular reconstruction. *J Biomed Mater Res Part B Appl Biomater*, 105(4): 1016–1028.

https://10.1002/jbm.b.33639

50. Visscher DO, Bos EJ, Peeters M, *et al.*, 2016, Cartilage tissue engineering: Preventing tissue scaffold contraction using a 3D-printed polymeric cage. *Tissue Eng Part C: Methods*, 22(6): 573–584.

https://10.1089/ten.tec.2016.0073

51. Visscher DO, Gleadall A, Buskermolen JK, *et al.*, 2018, Design and fabrication of a hybrid alginate hydrogel/poly(ε-caprolactone) mold for auricular cartilage reconstruction. *J Biomed Mater Res Part B: Appl Biomater*, 107(6): 1711–1721.

https://10.1002/jbm.b.34264

52. Zopf DA, Mitsak AG, Flanagan CL, *et al.*, 2014, Computer aided–designed, 3-dimensionally printed porous tissue bioscaffolds for craniofacial soft tissue reconstruction. *Otolaryngol–Head Neck Surg*, 152(1): 57–62.

https://10.1177/0194599814552065

53. Xie X, Wu S, Mou S, *et al.*, 2022, Microtissue-based bioink as a chondrocyte microshelter for DLP bioprinting. *Adv Healthc Mater*, 11(1): 2201877.

https://10.1002/adhm.202201877

54. Zopf DA, Flanagan CL, Mitsak AG, *et al.*, 2018, Pore architecture effects on chondrogenic potential of patient-specific 3-dimensionally printed porous tissue bioscaffolds for auricular tissue engineering. *Int J Pediatr Otorhinolaryngol*, 114: 170–174.

https://10.1016/j.ijporl.2018.07.033