

Review article

Going below and beyond the surface: Microneedle structure, materials, drugs, fabrication, and applications for wound healing and tissue regeneration



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ABSTRACT

Microneedle, as a novel drug delivery system, has attracted widespread attention due to its non-invasiveness, painless and simple administration, controllable drug delivery, and diverse cargo loading capacity. Although microneedles are initially designed to penetrate stratum corneum of skin for transdermal drug delivery, they, recently, have been used to promote wound healing and regeneration of diverse tissues and organs and the results are promising. Despite there are reviews about microneedles, few of them focus on wound healing and tissue regeneration. Here, we review the recent advances of microneedles in this field. We first give an overview of microneedle system in terms of its potential cargos (e.g., small molecules, macromolecules, nucleic acids, nanoparticles, extracellular vesicle, cells), structural designs (e.g., multidrug structures, adhesive structures), material selection, and drug release mechanisms. Then we briefly summarize different microneedle fabrication methods, including their advantages and limitations. We finally summarize the recent progress of microneedle-assisted wound healing and tissue regeneration (e.g., skin, cardiac, bone, tendon, ocular, vascular, oral, hair, spinal cord, and uterine tissues). We expect that our article would serve as a guideline for readers to design their microneedle systems according to different applications, including material selection, drug selection, and structure design, for achieving better healing and regeneration efficacy.

1. Introduction

Wound management has brought huge financial burdens to the society, and the situation will be further exacerbated with the rise in aging population and numbers of patients with chronic wounds worldwide [1]. With growing demands for wound care, topical wound dressing combined with advanced drug delivery systems have been developed during the past few decades, such as nano/micro carriers, tissue engineering scaffolds, and hydrogels [2]. These strategies have their own advantages and have maximized the drug delivery effect by controlling the release profile and increasing the cargo retention rate. However, due to the barrier effect of the wound clots or scar tissues, obstruction is still

highly prevalent in terms of drug molecule delivery in wound bed. As a result, more effective topical delivery systems are needed for wound healing.

Microneedle, as a novel drug delivery system consisting of an array of microscale needles, has attracted widespread attention due to its non-invasiveness, simple operation, topical and controllable drug delivery, and diverse cargo loading capacity [3]. Although microneedles are initially designed to penetrate the stratum corneum of the skin for transdermal drug delivery, many emerging applications have been revealed in recent years [4], including promotion of wound healing and regeneration of diverse tissues, like skin [5], cardiac [6], bone [7], tendon [8], ocular [9], vascular [10], oral [11], hair [12], spinal cord

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[13], and uterine tissues [14]. Microneedles have unique advantages in the wound healing and tissue regeneration: (1) The microscale needle tips can easily pass through the physical barriers at wound sites, like clots, scars, and exudates, and sustainably release drugs. This could greatly enhance the drug delivery efficiency compared with other wound dressings. Meanwhile, the application process is noninvasive and painless, leading to a much better patient compliance than traditional hypodermic injection. (2) Microneedles can provide mechanical stimulation for wound healing and tissue regeneration by nature. Due to its needle array structure, microneedles could influence the wound mechanically in two ways. One is the tissue penetration which gives a mechanical stimulus inducing the collagen deposition and reorganization [15,16]. The other is that the microneedle array can support the insertion area and change the local stress environment [17]. These are both beneficial to the scar reduction during wound healing. (3) Microneedles may overcome the drug resistance of biofilms in wound bed. They can easily break down the biofilms into planktonic bacteria, overcoming the drug resistance physically [18]. (4) The structure of microneedles allows for loading of a wide range of drugs, including small and large molecules [19,20], nucleic acids [21], nanoparticles [22], extracellular vesicles [12], or even cells [23,24]. (5) When microneedles are made of some responsive materials (e.g., poly (N-isopropylacrylamide)) [25], they can monitor the wound conditions with different biochemical and physical signals such as pH [26], reactive oxygen species (ROS) [27], temperature [28], and proteins [20], enabling the microneedles to deliver drugs on demand according to the wound healing phase.

There have been many reviews discussing microneedles from different aspects, focusing on its fabrication, material selection, structure designs, and applications (e.g., anti-infection, disease treatment and

diagnosis, biosensing, and cosmetic applications) [3,18,29–32]. However, there lacks a comprehensive discussion on the current progress and challenges of using microneedles for wound healing and tissue regeneration. To fill this gap, we give a detailed review focusing on considerations and designs of microneedle-assisted wound healing and regeneration in various organs and tissues. In this review, we first introduce the design of microneedle system in terms of its potential cargos (e.g., small molecules, macromolecules, nucleic acids, nanoparticles, extracellular vesicles, cells), structural designs (e.g., multidrug structures, adhesive structures), material selection, and drug release mechanisms. We then briefly summarize the different fabrication methods of microneedles (e.g., microelectromechanical system (MEMS)-based methods, micromolding, drawing-based methods, 3D-printing-based methods), including their advantages and limitations. Finally, we discuss in detail the current applications of microneedles in wound healing and tissue regeneration (e.g., skin, cardiac, bone, tendon, ocular, vascular, hair, spinal cord, uterine, and oral tissues), along with the current limitations of each application. The latest works in recent two years, which have not been covered in other reviews, are included. Authors' viewpoints and perspectives for this emerging field are also provided. We expect this review will provide a guideline for advanced microneedle system design in terms of wound healing and tissue regeneration, including material selection, drug selection, and structure design, to help researchers improve their microneedle system design for better wound management.

2. Design of microneedles for drug release

Fig. 1 shows typical microneedle structure. The typical length of microneedles ranges from 25 μm to 2000 μm , corresponding to the

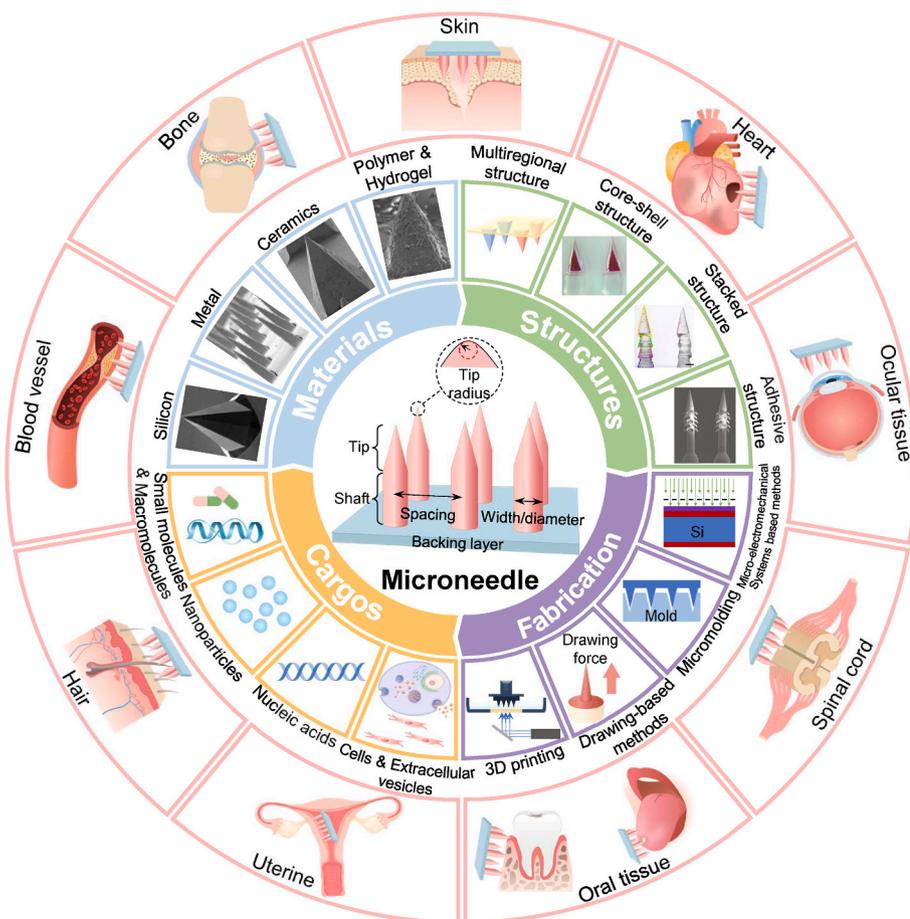


Fig. 1. Schematic diagram for application of microneedles in wound healing and tissue regeneration. Figure of silicon microneedles is reproduced with permission [183]. Copyright 2015, IOP. Figure of metal microneedles is reproduced with permission [65]. Copyright 2004, Springer. Figure of ceramic microneedles is reproduced with permission [103]. Copyright 2018, Elsevier. Figure of polymer & hydrogel microneedles is reproduced with permission [184]. Copyright 2019, ACS. Figure of core-shell structure is reproduced with permission [76]. Copyright 2020, Nature. Figure of stacked structure is reproduced with permission [78]. Copyright 2021, Elsevier. Figure of adhesive structure is reproduced with permission [185]. Copyright 2020, Wiley.

average thickness of epidermis along with stratum corneum (10–15 μm) at about 2000 μm [33]; the typical tip radius ranges from 1 μm to 25 μm for efficient penetration. For successful insertion, the strength and stiffness of the needles should be sufficient to resist the breakage and buckling upon penetration. Needle properties are determined by its material properties, needle geometry, and especially the width of the needle shaft, which usually ranges from 10 μm to 300 μm [34]. General

design considerations (like spacing, tip radius) for microneedles can be found in other reviews [3,35]. Here, we focus on various structure designs, material uses, and drugs embedded in microneedles and the drug release mechanisms of the microneedle systems.

Table 1
Summary of microneedles for wound healing.

Application	Cargos	Cargo type	Materials	Material type	Fabrication method	Ref	
Anti-bacteria	Gentamicin	Antibiotics	Polyethylene glycol-gentamicin sulfate	Polymer	Micromolding	[188]	
	Tetracycline	Antibiotics	Silk fibroin	Polymer	Micromolding	[19]	
	Amphotericin B	Antibiotics	Gantrez 169 BF	Polymer	Micromolding	[189]	
	Miconazole	Antifungal medication	Gantrez 170 BF	Polymer	Micromolding	[38]	
	Green tea extract	Phytochemicals	HA	Polymer	Micromolding	[190]	
	Voriconazole	Antifungal medication	Polyglycolic acid	Polymer	Contact drawing	[191]	
	Methylene blue	Photosensitizer	Gantrez AN-139	Polymer	Micromolding	[192]	
	Itraconazole	Antifungal medication	Polyglycolic acid	Polymer	Contact drawing	[193]	
	Besifloxacin	Antibiotics	PVA/PVP	Polymer	Micromolding	[194]	
	Kanamycin	Antibiotics	Suckerin	Polymer	Micromolding	[195]	
	Clindamycin	Antibiotics	HA/PVA	Polymer	Micromolding	[187]	
	Cephalexin	Antibiotics	CS-porous carbon nanocomposites	Polymer	Micromolding	[196]	
	Amphotericin B	Antibiotics	PVA/PVP	Polymer	Micromolding	[197]	
	Amphotericin B	Antibiotics	eShell 200	Polymer	Micromolding	[198]	
	Nano silver	Nanoparticles	CMC	Cellulose	Micromolding	[199]	
	ZnO nanobushes	Nanoparticles	Polystyrene	Polymer	Micromolding	[200]	
	Amphotericin B	Antibiotics	CS-polyethylenimine copolymer	Polymer	Micromolding	[36]	
	Graphene oxide	Nanoparticles	PVP/CMC/HA	Polymer	Micromolding	[22]	
	Doxycycline	Antibiotics	PLGA nanoparticles/PCL nanoparticles/CS	Polymer	Micromolding	[39]	
		Carvacrol (CAR)-PCL nanoparticles	Phytochemicals	–	–	–	[201]
		Nano silver	Nanoparticles	PEGDA/gelatin/sucrose	Polymer	Micromolding	[202]
		Premna microphylla/centella asiatica	Phytochemicals	PEGDA	Polymer	Micromolding	[203]
		CuO ₂ /TiO ₂	Nanoparticles	HA	Polymer	Micromolding	[132]
	Zinc oxide	Nanoparticles	Sericin	Polymer	Micromolding	[131]	
Anti inflammation	TNF- α specific antibodies	TNF inhibitors	CMC	Cellulose	Micromolding	[204]	
	TNF- α specific antibodies	TNF inhibitors	HA	Polymer	Micromolding	[205]	
	Meloxicam	NSAID	PVP/PCL	Polymer	Micromolding	[41]	
	Alkaloids	Natural medicine	–	–	–	[206]	
	Salicylic acid	NSAID	3-heptyl-1-vinylimidazolium bromide/HEMA/PEGDA	Polymer	Micromolding	[42]	
		Triamcinolone acetonide	Corticosteroid medication	PVP/HA	Polymer	Micromolding	[207]
		Bee venom gel	Natural medicine	–	–	–	[208]
		Paeoniflorin	Natural medicine	–	–	–	[209]
		Methotrexate	Antimetabolites	HA	Polymer	Micromolding	[210]
		Etanercept	TNF inhibitors	HA	Polymer	Micromolding	[211]
	Methotrexate	Antimetabolites	PVP/PVA	Polymer	Micromolding	[212]	
	Betamethasone	Corticosteroid	HA	Polymer	Micromolding	[150]	
Angiogenesis	Cardiac stromal cells	Cells	PVA	Polymer	Micromolding	[6]	
	VEGF	Growth factors	CS	Polymer	Micromolding	[213]	
	iPSC-derived cardiomyocytes/VEGF/IL-10	Cells/growth factors/cytokines	GelMA	Polymer	Micromolding	[63]	
	VEGF	Growth factors	Silk fibroin	Polymer	Micromolding	[20]	
	Adeno-associated virus/VEGF	Gene/growth factors	PVA	Polymer	Micromolding	[48]	
	ADCSs/PDGF-D	Cells/growth factors	Methacrylated HA	Polymer	Micromolding	[128]	
	VEGF	Growth factors	Graphene oxide/PVA	Polymer	Micromolding	[139]	
	Conditioned media	Culture media	HA	Polymer	Micromolding	[158]	
	Basic fibroblast growth factor	Growth factors	GelMA/N-acryloyl glycinamide/PEGMA	Polymer	Micromolding	[154]	
	Human endometrium-derived adventitial cell	Cells	GelMA	Polymer	Micromolding	[14]	
Scar reduction	Triamcinolone	Corticosteroids	HA	Polymer	–	[214]	
	siRNA targeting (TGF- β RI)	siRNA	HA/mesoporous silica	Polymer	Micromolding	[21]	
	Bleomycin	Antitumor Antibiotic	HA	Polymer	Micromolding	[215]	
	Triamcinolone acetonide	Corticosteroids	HA/hydroxypropyl- β -cyclodextrin	Polymer	Micromolding	[216]	
	Shikonin	Herbal medicine	HA	Polymer	Micromolding	[217]	
	Triamcinolone/5-fluorouracil	Corticosteroids	HA/CS/dextran/HP- β -CD	Polymer	Micromolding	[79]	
	Quercetin	Phytochemicals	<i>Bletilla striata</i> polysaccharide	polysaccharide	Micromolding	[134]	

Abbreviation: ADCSs, adipose-derived stem cells; CMC, carboxymethyl cellulose; CS, chitosan; GelMA, gelatin methacryloyl; HA, hyaluronic acid; HEMA, hydroxyethyl methacrylate; HP- β -CD, hydroxypropylbeta-cyclodextrin; IL, interleukin; iPSC, induced pluripotent stem cells; NSAID, nonsteroid anti-inflammatory drugs; PCL, polycaprolactone; PDGF-D, platelet derived growth factor D; PEGDA, poly (ethylene glycol) diacrylate; PLGA, poly (lactic-co-glycolic acid); PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

2.1. Drugs loaded in microneedles

Drug-loaded microneedles used in wound treatment mainly target four different applications, including anti-bacteria, anti-inflammation, angiogenesis promotion, and scar reduction. Table 1 summarizes all the currently used cargos loaded in microneedles for wound healing. The first type of drug is small molecule drug. Typical small molecule drugs include antibiotic and antifungal medications (e.g., amphotericin B [36], tetracycline [37], miconazole [38], clindamycin [27], doxycycline [39]) and anti-inflammation and anti-scarring drugs containing corticosteroids (e.g., triamcinolone acetonide) [40], nonsteroidal anti-inflammatory drugs (NSAIDs, e.g., meloxicam, salicylic acid) [41, 42], and antimetabolites (e.g., methotrexate) [43].

The second type of drug is macromolecule drug, mainly including proteins like growth factors (e.g., vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF)), cytokines (e.g., interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α)), and antibodies (e.g., tumor necrosis factor inhibitor) [44]. Growth factors and cytokines are natural signaling molecules acting between cells, which are important to cell activities like proliferation, migration, immunoregulation, and angiogenesis, playing a central role in coordinating wound healing process. Apart from growth factors and cytokines, tumor necrosis factor (TNF) inhibitor, an antibody drug, is also incorporated into microneedles to treat arthritis and skin disorder [45,46]. It could act on the immune system to inhibit the TNF production, leading to anti-inflammatory effects.

Nucleic acids (i.e. DNA, miRNAs, siRNAs) have also been proved to be effective in treating wound healing by reasons of their interactions with many cellular pathways corresponding to the different phases of wound healing [21,47,48]. In brief, DNA-based therapy aims at delivering exogenous DNA into cells for therapeutic protein expression while miRNA and siRNA both focus on inhibiting translation of messenger RNA (mRNA) specific to the gene of interest. They all show promising therapeutic effects on wound healing including antibiosis, anti-inflammation, angiogenesis, and regulating cell migration [49]. However, nucleic acid-based therapies are limited by the short-term effect and poor delivery efficiency due to the low cellular uptake of exogenous nucleic acids and the harsh extracellular environment (e.g., elimination by immune cells, degradation by enzymes). As a result, effective delivery vehicle and safe delivery route are crucial. Recently, microneedles combined with cationic polymers [50], nanoparticles [21], and viral vectors for nucleic acid delivery show great potential in wound treatment [48], as microneedles could deliver the cargos to wound site directly while the cationic polymers, nanoparticles and viral vectors could carry the nucleic acids to nucleus efficiently.

Another drug type is nanoparticles. When incorporated with microneedles, nanoparticles usually work in two ways: as an antibacterial agent or as a drug carrier. For the former case, the types of antimicrobial nanoparticles would include metallic materials like Au, Ag, ZnO, and CuO [51], carbon-based materials like graphene and carbon nanotube, as well as polymers like quaternary ammonium, chitosan, and some synthetic polymers containing cationic or hydrophobic moieties [52, 53]. For the latter case, different nano- and micro carriers (e.g., poly-D, L-lactide-co-glycolide (PLGA) nanoparticles [54], polylactic-co-glycolic acid (PLGA) nanoparticles [55], calcium phosphate particles [56], gelatin nanoparticles [57,58] have also been combined with microneedles. These nanoparticles are usually embedded within dissolvable microneedles by co-polymerization, solvent evaporation, or as a coating on the surface of solid microneedles.

Another promising therapeutic is extracellular vesicle (EV) which is a type of paracrine factor secreted by cells [59]. EVs including exosomes (30–100 nm in diameter) and micro vesicles (50–1000 nm in diameter), are released from stem/progenitor cells like mesenchymal stem cells (MSCs) (e.g., bone mesenchymal stem cells, adipose-derived stem cells, induced pluripotent stem cell-derived MSCs, and human placenta MSCs) and progenitor cells (e.g., endothelial progenitor cells, and myocardial

progenitor cells). They have shown great potential in wound healing, as they contain a variety of enzymes, nucleic acids (DNA, mRNA, and miRNA), and extracellular matrix (ECM) proteins, which have a synergistic effect on different phases of wound healing [60]. As regulator of intercellular communication, EVs could pass through the cell membrane easily, making it an effective drug compared with naked nucleic acids.

Cell therapy is a traditional and promising strategy for tissue regeneration. MSCs, iPSCs-derived MSCs, adipose-derived stem cells (ADSCs), and endothelial progenitor cells (EPCs) are all suitable choices due to their versatility and proven capability to accelerate wound healing [61]. These cells can be combined with microneedles in three forms: 1. embedded within the microneedles, 2. seeded on the microneedle surface, or 3. directly delivered via hollow microneedles. It is relatively difficult to encapsulate cells into microneedles as fabrication procedures usually involve harsh conditions (e.g., high temperature, chemical solvent, ultraviolet light, vacuum). Additionally, contamination, long-term storage, and cell viability are also significant barriers. Nevertheless, Lee et al. designed a detachable microneedle with a core-shell structure [24]. The outer shells of the needle tips were based on stiff and degradable PLGA while the inner cores were composed of gelatin methacryloyl (GelMA) and MSCs. This design not only ensured the mechanical strength of the microneedles but also maintained the viability of the loaded MSCs. Chang et al. also proposed a cryogenic microneedle for cell delivery and achieved long-term cell storage [23]. Like common cryopreservation, pre-suspended cells with cryogenic medium were poured into a mold for micromolding. After solidification by a stepwise cryogenic procedure, the final cryogenic microneedle was mechanically strong and had excellent cell storage capacity. Cells can also be delivered using microneedles by surface seeding. Tang et al. coated cardiac stromal cells with fibrin gel on the back of the patch [62]. Under this design, these cardiac cells could be migrated through the channels on myocardium created by needles. Similarly, Sun et al. seeded iPSC-derived cardiomyocytes on the patch surface [63]. Induced by the oriented carbon nanotube on the surface, the cardiomyocytes could align in one direction and contract synergistically with heartbeats. The final modality is to deliver cells via hollow microneedles. It works similarly to subcutaneous injection of cell suspension, which is dependent on the transient effects of cells.

2.2. Materials used to fabricate microneedles

At present, different types of materials have been used for microneedle fabrication according to the requirement of different applications, such as metals (e.g., titanium [64], stainless steel [65], tantalum [66]), silicon [67], silicon dioxide [68], ceramics and polymers (e.g., carbohydrates, hydrogels) (Table 1) [69,70]. These materials have different mechanical and degradation properties, which determine their applications. For metals, silicon, and ceramics, their stiffnesses are relatively high (over 10 GPa), and they are nondegradable under normal condition. As a result, they are usually used to open pores on skin for promoting drug permeation, where they are made into hollow microneedles for direct drug solution delivery, or solid microneedles with drugs coated on its surface. Compared with metal, polymers have tunable drug release characteristic by adjusting their concentration, molecular weight, crosslinking density, and charge property. Also, the diverse crosslinking methods and chemical modification techniques enable polymers to mix with different types of drugs into hydrogels, including small molecules, macromolecules, nanoparticles, EVs and nucleic acids. In detail, polymers, like PLGA and polycaprolactone (PCL), possess medium mechanical property (10 MPa–10 GPa) and could sustain *in vivo* for drug release from several weeks to several years. These polymers are suitable for regeneration of cartilages, tendons, and bones, which need longer time for recovery and sufficient mechanical support. Hydrogels, like hyaluronic acid (HA), chitosan, and polyethylene (glycol) diacrylate (PEGDA), have a relatively low mechanical property (usually below 1 MPa) and shorter degradation duration (usually from

several hours to several weeks), allowing for rapid drug release. This fits for applications which need rapid drug release, like anti-infection in early stage of wound healing [71]. Moreover, these hydrogels are suitable for regeneration of organs, like skin, nerves, liver, kidney, which consist of soft ECM. Some of these organs also need faster degradation of biomaterials and drug release in pace with their regeneration [72]. In addition, as an ECM-like material with high water content, hydrogels are suitable for loading water-soluble drugs and proteins, nanoparticles, nucleic acids, EVs and cells. Due to their tunable polymer network density, hydrogels could also be designed for controlled drug release according to the drug size [73].

2.3. Structure of microneedles

Attributed to the current advanced fabrication techniques, especially the micromolding method, diverse cargos could be integrated into a microneedle patch and released in a spatiotemporally controlled way [3, 35]. Different microneedle structures and their fabrication strategies are proposed to precisely control multi-drug distribution in patches, including multiregional structures (Fig. 2A) [74–76], core-shell structures (Fig. 2B), and stacked structures (Fig. 2C) [77–79]. To control the drug release, most researchers would take advantage of the materials' release properties or special structures for programmable release of specific drugs. Fig. 2A demonstrates the multiregional structure and its function by first loading different drugs into different underlying materials, and then integrating them onto different regions of the patch to form multiregional microneedle patch. As these materials have different degradation characteristics, they could release the loaded drugs at different times in a single patch. This strategy of programmable drug release would be suitable for combined therapies of multiple drugs, or for the treatment that requires patients to administer medicine for several times in one day, like diabetes. However, this uneven treatment may not be preferred in some topical wound treatment, where a coordinated drug release would have better efficacy. For core-shell structure (Fig. 2B), core portion and shell portion could be loaded with different drugs so that cargos in shell will firstly be released and then drugs in core will follow, resulting in a sequential drug release. In principle, increasing the number of shell layer allows for more drug types loaded. However, too many layers warrant larger needle size, which may lose

the noninvasive property of microneedles. Therefore, the common layer number is less than three. Another design is the stacked structure (Fig. 2C) which stacks several layers of matrices onto one needle tip. Unlike core-shell structures having a sequential drug release, different layers in the stacked structures start to degrade at the same time, leading to a simultaneous release of multiple drugs. Additionally, this stacked structure (Fig. 2Ci) would better promote the tissue adhesion of the microneedles.

Adhesiveness to the target tissues is another crucial factor in designing the structure of a microneedle. The mechanism of microneedle structural adhesion can be classified into two categories: the swelling structure, and the biomimicking structure. Swelling microneedles (Fig. 2D) rely on the water absorption efficiency of their needle tips (some viable swelling materials options would be hyaluronic acid, GelMA, and polystyrene-block-poly(acrylic acid)) [80–82]. After piercing the skin or other tissues, the hydrogel-based tip will take up body fluid and swell rapidly. Depending on the structure of the needles, it could mechanically interlock with the target tissue. Biomimicking microneedles (Fig. 2E) focus more on effective structures to interlock with the target tissues using structures like backward barbs, and pagoda-like structures [78,83–85]. These structure may not impede the penetration of microneedles during the insertion process, yet they could generate huge withdrawal force when pulled out due to the mechanical interlock of these structures with tissues. The advantage of these mechanical structures is that they could adhere to tissues both in dry and wet environment. The smoothness of the tissue surface will also not influence their adhesion. In addition to the mechanical interlock, octopus-sucker-like structures have also been integrated into microneedles (Fig. 2F) [86]. It could adhere to tissues by creating partial vacuum between suction cups and tissue surface. The advantage of this kind of structure is that they are more suitable for adhesion in wet environment.

2.4. Drug release mechanism

As mentioned in section 2.2, most of the materials used in microneedle fabrication are dissolvable polymers. This allows the loaded drugs to be released mainly in three ways: diffusion, degradation, and triggered release, all of which depends on the drug size and the mesh size

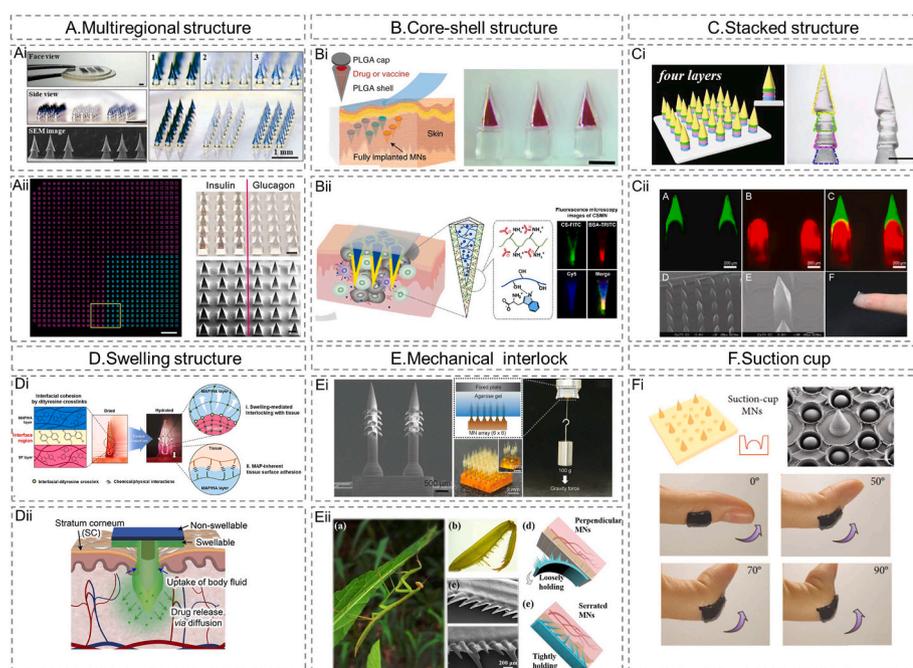


Fig. 2. Structure design of microneedles for wound healing. (A) Typical multiregional microneedles. (Ai) is reproduced with permission [74]. Copyright 2020, AAAS. Scale bar, 1 mm (Aii) is reproduced with permission [75]. Copyright 2020, PNAS. Scale bar of left panel, 2 mm. Scale bar of left panel, 300 μ m. (B) Typical core-shell microneedles. (Bi) is reproduced with permission [76]. Copyright 2020, Nature. Scale bar, 300 μ m (Bii) is reproduced with permission [77]. Copyright 2020, Elsevier. (C) Microneedles with stacked structures. (Ci) is reproduced with permission [78]. Copyright 2021, Elsevier. Scale bar, 350 μ m (Cii) is reproduced with permission [79]. Copyright 2021, Elsevier. Scale bar, 200 μ m. (D) Typical swelling microneedles. (Di) is reproduced with permission [81]. Copyright 2019, Elsevier. (Dii) is reproduced with permission [82]. Copyright 2017, Elsevier. (E) Typical bioinspired structures for mechanical interlock with tissues. (Ei) is reproduced with permission [185]. Copyright 2020, Wiley. (Eii) is reproduced with permission [84]. Copyright 2019, Elsevier. (F) Microneedles with a suction cup structure. Reproduced with permission [86]. Copyright 2020, AAAS. Scale bar, 300 μ m.

of the polymers. Firstly, in the case of diffusion, size of drug molecules is smaller than the mesh size in polymer network so that drugs could smoothly pass through the polymer network. Since mesh size in hydrogel network ranges from 5 nm to 100 nm, which is larger than most macromolecules [73], making diffusion mostly happen in hydrogels. Swelling or deformation-induced mesh enlargement could also lead to the diffusion of drug molecules, even if the drug size is initially bigger than the mesh size. This kind of diffusion is important for hydrogel microneedles as they are usually dehydrated at first for better mechanical property. After insertion, the dehydrated hydrogels will absorb body fluid and swell rapidly, resulting in a rapid release of drugs. This release process occurs over a short period of less than 1 day, which is necessary in some cases. For example, at the beginning of wound healing, an immediate relief, fast anti-infection, and blood coagulation are important for the following healing process, which need a rapid or burst drug release [87]. Secondly, polymers like PLGA and PCL are hydrophobic and have very dense polymer chains. Most drug molecules can hardly pass through their polymer network [88], consequently they could only be released along with the polymer degradation induced by hydrolysis or enzymatic degradation. This process leads to a long-term drug release lasting for several weeks to several months. This sustained release is necessary for regeneration of some tissues like cartilage, tendons, and bones, which need long term recovery. Thirdly, stimulus-responsive release methods, which release drugs upon triggers by specific external factors, like pH, temperature, light, and biomolecules, have become an active research area in fabricating microneedle systems [89,90]. It is preferred for wound management, as wound healing contains four different phases, which need different drugs. In detail, pH in chronic wound is above 7 which is higher than that in healthy skin with pH around 5.5. This alkaline environment supports the bacterial breeding which also facilitates the rise in pH. Then the bacterial infection aggravates the inflammation. Temperature increases and level of many enzymes, like matrix metalloproteinase, human neutrophil elastase, and lysozyme, are elevated. In addition, excessive proinflammatory cytokines (e.g., IL-6, C-reactive protein) and ROS are also produced leading to the wound trapped in the inflammation phase. All these biochemical and physical signals, which mainly focus on infection and inflammation, are important indicators that could be detected by stimulus-responsive materials (e.g., poly(vinyl alcohol), N-isopropylacrylamide) [91]. Different types of antibacterial or anti-inflammatory drugs could then be released upon triggering by these smart materials according to the degree of wound healing which could reduce the side effects.

Table 2
Comparison of different microneedle fabrication methods.

Fabrication methods	Speed	Precision	Cost	Other pros and cons	Ref	
MEMS-based methods	Low	High	High	<ul style="list-style-type: none"> × Difficult to fabricate complicated structures × Basic material limited to silicone and photocurable polymers 	[218, 219]	
Micromolding	Average	High	Low	<ul style="list-style-type: none"> × Difficult to fabricate complicated structures ✓ A large variety of basic material ✓ Easy to load drugs 	[220]	
Drawing-based methods	Mechanical force drawing	Low	Low	Low	<ul style="list-style-type: none"> × Cannot fabricate complicated structures × Basic material limited to thermoplastic materials 	[112]
	Contact drawing	High	Low	Low	<ul style="list-style-type: none"> × Cannot fabricate complicated structures × Viscosity of basic material needs adjustment 	[113]
	Electro-drawing	High	Low	Low	<ul style="list-style-type: none"> × Cannot fabricate complicated structures × Conductivity of basic material needs adjustment 	[116]
3D-printing-based methods	Centrifugal drawing	High	Low	Low	<ul style="list-style-type: none"> × Cannot fabricate complicated structures 	[70]
	Two-photon polymerization (TPP)	Low	High	High	<ul style="list-style-type: none"> × Difficult to fabricate objects with large volume ✓ Easy to fabricate complicated structures 	[85,221]
	Stereolithography (SLA)	Average	Average	Average	<ul style="list-style-type: none"> ✓ Able to fabricate complicated structures 	[222]
	Projection-based printing (PBP)	High	Average	Average	<ul style="list-style-type: none"> ✓ Able to fabricate complicated structures 	[185]
	Fused deposition modeling (FDM)	Average	Low	Low	<ul style="list-style-type: none"> × Cannot fabricate complicated structures × Needs post treatment 	[123]

3. Fabrication of microneedles

Currently, microfabrication techniques including MEMS-based methods, micromolding, drawing-based methods, and 3D-printing-based methods are used to fabricate microneedles [92,93]. The pros and cons of different fabrication approaches are summarized in Table 2.

3.1. MEMS-based methods

Lithography and etching, belonging to MEMS technique, have been used in microneedle fabrication for several decades. Originally, lithography is a process to transfer pre-designed mask patterns onto a film called resist, which is sensitive to specific radiation sources (ultraviolet light, X-ray, electron beam) (Fig. 3A(b)). Among all stimulus, photolithography is the most widely used lithography, activated by UV light and X-ray onto different photosensitive polymers, such as SU-8 [94], poly (methyl methacrylate) (PMMA) [95], KMPR® [96], PEGDA, and chitosan lactate [97,98]. Due to its selective shape-forming property, researchers take advantage of photolithography to fabricate microneedles based on photosensitive polymers without help of etching [94, 95,97]. However, for silicon-based microneedle fabrication, etching is necessary, which originally is a process following lithography to eliminate the non-masked but unwanted parts on silicon substrate in traditional MEMS (Fig. 3A(c)–(e)). Wet etching and dry etching are two major types [99]. Dry etching is usually used to drill holes in hollow microneedles made of metals and to control the height of the needles while wet etching is utilized to polish and sharpen the structures (Fig. 3A) [100–102]. In summary, MEMS-based methods are able to fabricate photosensitive-polymer- and silicon-based microneedles. Common structures, like conical, pyramid-alike, and hollow needles, could be fabricated with high precision. By tuning parameters, needle length and tip angle could also be changed. However, the whole process is expensive and time-consuming. In addition, microneedles with complicated structures (e.g., core-shell, stacked, and adhesive structures) cannot be fabricated by this method.

3.2. Micromolding

Micromolding is the most widely used technique for microneedle fabrication currently (Table 1). Its basic process is shown in Fig. 3B. In brief, a negative mold with needle cavities is used for loading material. After the material is filled and cured, a completed microneedle could be demolded from the negative mold. This method is suitable for processing materials like natural and synthetic polymers, ceramics, hydrogels, etc. [35,103] According to different material properties, various curing or

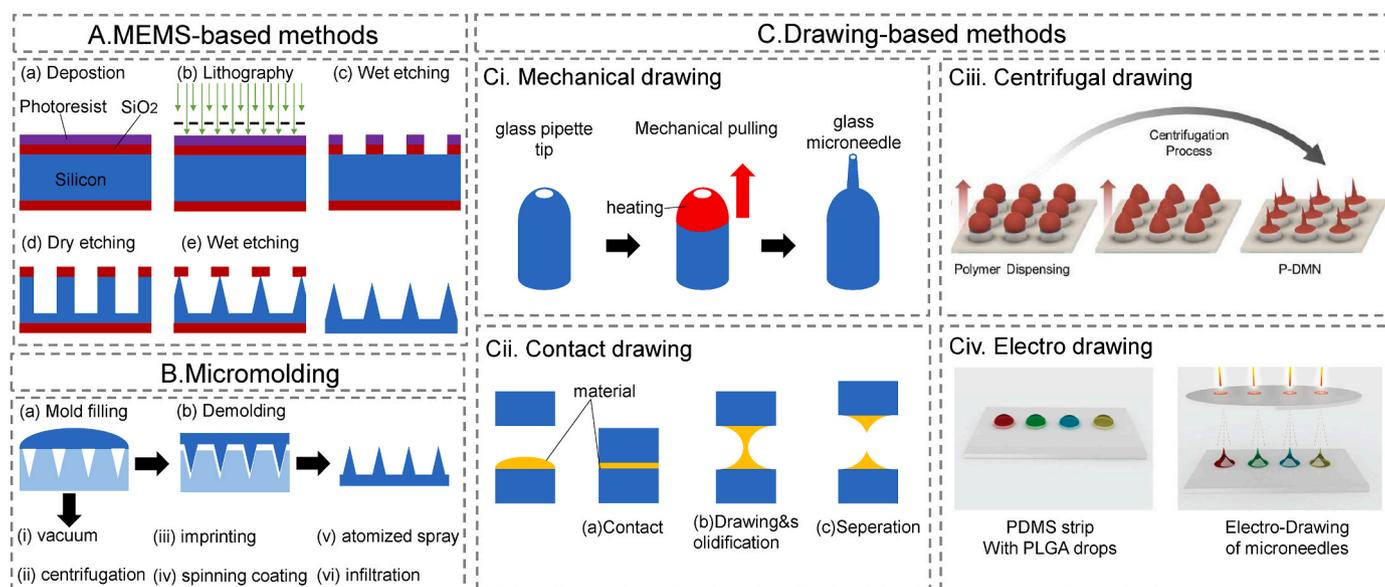


Fig. 3. Fabrication of microneedles. (A) Typical MEMS-based method combining lithography, wet & dry etching. (B) Typical micromolding process, along with six molding filling methods. Principle of (Ci) mechanical drawing, (Cii) contact drawing, (Ciii) centrifugal drawing, reproduced with permission [70]. Copyright 2019, MDPI. (Civ) electro drawing, reproduced with permission [116]. Copyright 2014, Wiley.

shape-forming methods, such as solvent evaporation, photo-crosslinking, chemical crosslinking, melt solidification, sintering, freezing, and freeze-thawing, are also applied [23,69,104–106]. Micromolding possesses many advantages. It is simple, inexpensive, and highly reproducible. In addition, it is easy to form a composite microneedle by adding different materials and cargos into the same mold in sequence. As a result, structures mentioned above, like multiregional, core-shell, stacked, swelling and suction cup structures, can all be achieved by this method, which have a complex release kinetics and functions. Yet, one key issue in micromolding is the mold-filling problem. In microscale, surface tension force plays a dominant role, making it difficult for materials to fill into the mold cavity under the force of gravity. Thus, mold-filling strategies to overcome surface tension are necessary. Researchers have developed several methods, such as vacuum [105], centrifugation, imprinting [4,107], spinning coating [108], atomized spray and infiltration [109,110], to address this mold-filling problem. There are also several limitations in micromolding. Firstly, it is unable to form complex structures, like adhesive structure with barbs and hollow structure. In addition, for some soft and fragile materials like hydrogels, the demolding stress may also cause damage to the final structures. Materials like metal, silicon and glass are also not suitable to be processed by this method.

3.3. Drawing-based methods

Drawing-based methods are a series of techniques that apply different types of drawing forces including mechanical force, adhesive force, electrostatic force, and centrifugal force on the precursor materials to form microneedle-like shapes followed by a specific curing process for microneedle fabrication (Fig. 3C). Mechanical force drawing was derived from the conventional drawn glass micropipette techniques (Fig. 3Ci) [111], and hence it can only produce hollow glass microneedles [112]. Contact drawing methods mainly utilize the adhesive force between the material and the drawing device to form microneedle structures, followed by certain shape-forming processes, like solidification, solvent evaporation and ionic crosslinking (Fig. 3Cii) [113–115]. Electro-drawing method draws the polymer solution droplets by electrohydrodynamic (EHD) force in a non-contact way (Fig. 3Civ) [116]. Finally, centrifugal drawing utilizes centrifugal force to deform and elongate materials into a needle-tip shape (Fig. 3Ciii) [70]. All these

methods merit low expense and mass production. However, they cannot produce sophisticated structures but some common ones like conical or hollow needles, as the drawing force during the fabrication could only be single direction and the addition of other materials is also difficult.

3.4. 3D-printing-based methods

3D printing is a broad concept containing many techniques, including vat photopolymerization, jetting-based printing, extrusion-based printing, and so on. However, as microneedle fabrication requires high precision, till now, only vat photopolymerization based 3D printing has been widely used to create microneedles, including two-photon polymerization (TPP), stereolithography (SLA), projection-based printing (PBP), liquid crystal display (LCD), and continuous liquid interface production (CLIP). Generally, TPP is the only method that could fabricate complicated 3D needles in nanoscale with unique structures [117], such as the rocket-shaped and mosquito-like needles for decreasing the penetration force, bug-inspired structures for rapid drug/vaccine coating, and pyramidal heads for better tissue adhesion [118–120]. Although TPP has high printing resolution, it is limited by low production efficiency, small product volume and high expense. SLA prints in a point-to-point way with resolution about several micrometers while PBP, LCD, and CLIP exploit a layer polymerization method, which can solidify an entire pattern of a layer at one time [121,122]. The approximate resolution of them is 1–2 μm and have a higher production efficiency than TPP and SLA. These vat photopolymerization methods are good at constructing needles with complicated structures, like adhesive structures with barbs, suction cup structures or even any shape if the precision is high enough. However, it is difficult for them to integrate multiple materials into one microneedle patch, as multiple material printing is not easy to achieve. As a result, till now, compound structures with different materials like multiregional, core-shell, and stacked microneedles have not been fabricated by vat photopolymerization. Apart from vat photopolymerization methods, few works also use fused deposition modeling (FDM)-based methods to fabricate microneedles [123,124]. Although FDM methods have limited manufacturing precision, assisted by supporting materials during printing and post treatment after printing [123,124], they could also produce acceptable but basic microneedles.

Many fabrication techniques from other disciplines, like micro

milling [125,126], laser cutting, and laser ablation have also been used as alternatives for microneedle fabrication [65,66]. Compared with the above-mentioned techniques, they have advantage for producing metal microneedles. Although they could produce needle tips with hundreds of micrometers in dimension, they cannot fabricate complicated microneedles due to limited machining precision. Details of some of the alternative fabrication methods of microneedles could be found in other reviews [99,127].

In summary, MEMS-based methods could fabricate microneedles with basic and simple structures based on silicone and some photosensitive polymers; Micromolding is the most widely used method for microneedle fabrication based on most kinds of polymers and ceramics. It is good at producing composite microneedles by adding different materials and cargos in sequence during fabrication. Many complicated structures like multiregional, core-shell, stacked, swelling, and suction cup structures are all achieved by micromolding; Drawing-based methods can only fabricate microneedles with basic conical structure based on glass and some polymers; 3D-printing-based methods are good at forming microneedles with sophisticated structures like adhesive structures with barbs or even arbitrary shapes based on photocrosslinkable polymers. However, it is difficult for them to integrate multiple materials into one microneedle patch.

4. Applications of microneedles in wound healing

As aforementioned, the great versatility, minimal invasiveness, high precision, and effectiveness of microneedles makes it a very useful device in wound management. Numerous studies have shown the applications of microneedles in treating skin, cardiac, bone, ocular, vascular, oral, hair, spinal cord, and uterine tissue as well as wound monitoring, which could greatly assist the wound management and enhance the treatment efficacy. In this section, some of these applications, including materials, structure designs, manufacturing processes, cargos, or drug release methods, treating effects and limitations, will be reviewed to provide the readers some insights regarding the state-of-the-art of microneedles in wound healing.

4.1. Skin regeneration

Skin regeneration is the most typical model in wound management. Most recent studies increasingly applied microneedles in treating skin regeneration due to their unique advantages, like strong penetrability, wound-anchoring ability, and easy handling. In this section, remarkable works about microneedle-assisted skin regeneration in last two years are summarized to provide key reference cases when designing relevant therapeutic strategies.

4.1.1. Acute and chronic wound healing

Acute wounds, caused by trauma and surgery, have a time-dependent and predictable healing process with orderly patterns of tissue repair. Although the epidermis is gone, there are still some dynamic tissue barriers and blood clots weakening the drug delivery to the wound bed. Unlike acute wounds, chronic wounds, usually classified into vascular ulcers, diabetic ulcers, and pressure ulcers, have a harsher environment, and are filled with necrotic tissues, eschar, continuous exudate, ulceration, and biofilms, due to lack of sufficient angiogenesis, excessive hypoxia, high level of proinflammatory cytokines, and infection [2]. As a result, the drug retention is poor, and the topically applied drugs are difficult to reach the wound bed. Different from traditional wound dressings and intravenous injection, microneedles could easily solve this dilemma by bypassing the physical barrier and releasing drugs both in a fast or sustained way, enhancing the therapeutics availability in wound bed. Recent advances in microneedle assisted acute and chronic wound healing are reviewed as follow.

The dynamic tissue barriers and blood clots, which strongly impede the drug diffusion, are critical issues to be addressed. Additionally, low

adhesiveness of most scaffolds also decreases the therapeutic effects of the treatment, as they need to stick to the wound site during the whole healing process. To surmount this problem, KangJu et al. proposed a detachable hybrid microneedle to deliver mesenchymal stem cells (MSCs) for skin regeneration [24]. The system consisted of a PLGA shell, a flexible substrate, and a core portion composed of GelMA hydrogel and MSCs (Fig. 4A). To form this hybrid system, a multistep micromolding strategy was taken to deposit PLGA material and GelMA/MSCs composite into the mold in sequence and crosslinked together. Then a scotch tape, namely the soft substrate, was attached to the crosslinked needle tip and peeled off from the mold to form the final hybrid system. The microneedle system could easily break the barriers in wound site because of the hard PLGA shell and could be detached from the substrate immediately after application. The problem was addressed by its property that allow the needle tips staying in wound site for a long time. Besides, GelMA hydrogel in core portion offered MSCs a great living environment, ensuring over 90% cell viability within 24 h. Although PLGA shell was stiff at first, it would be degraded after insertion for short term and release the cell factors, like vascular endothelial growth factor secreted by MSCs. Also, compared with the *in vivo* results from the untreated group, the treated group decreased the thickness of the fibrotic tissue and increased the quantity of hair follicles. Besides, better re-epithelialization and angiogenesis were also demonstrated in the treated groups. Another recent work by Xu et al. also used living cell loaded microneedles for diabetic ulcer healing (Fig. 4B) [128]. Different from the complicated cell loading process mentioned above, Xu et al. directly mixed adipose-derived stem cells (ADSCs) and platelet derived growth factor D (PDGF-D) with methacrylated hyaluronic acid (HAMA) to form the needle tip with the backing layer made of gelatin, using a basic micromolding method. The PDGF-D could promote the proliferation, differentiation, and migration of ADSCs, maintaining the cell viability in needle tip over 90% for 24 h. To provide a good environment for the ADSCs, the stiffness of the final needle tip was much lower than the normal microneedles. For the *in vivo* test, full thickness wound in type I diabetes rat model was established. Histological and immunostaining results showed that the PDGF-D and ADSC loaded microneedle groups had the thickest granulation tissue, most complete stratum corneum, most organized and dense collagen deposition, and the best neovascularization, indicating that the proposed system was a good cell and drug loading platform and a powerful therapy for wound healing with ductile wound bed.

Another issue about skin regeneration is that different healing stages need different drugs, as the wound pathophysiology of different patients may also differ from each other. This creates a challenging but essential scenario for multidrug delivery and customized therapy. To address this, Derakhshandeh et al. proposed a 3D-printed microneedle with programmable drug delivery capability [124]. The whole system consists of two parts: the microneedle part and the wireless controlling module (Fig. 4C). The microneedle part is fabricated by FDM 3D printing based on a biocompatible resin called Veroclear RGD 810. To hold overhanging structures and print cavities of hollow needles, a gel-like soluble material was used as support material during the printing process. The support material is removed by immersing in NaOH solution after printing. This system could pump and deliver VEGF on demand and with high precision when combined with the controlling module. According to the results, this device delivered VEGF to chronic wound sites successfully, and subsequently improved re-epithelialization, wound closure, and angiogenesis compared to topical injection of VEGF.

In addition to the external controlling drug delivery devices, stimuli-responsive materials are also used for controllable delivery. Zhang et al. developed a detachable microneedle loaded with black phosphorus (BP) and hemoglobin (Hb) for delivering responsive oxygen under the stimulation of near infrared radiation (NIR) to promote wound healing [129]. The whole system was fabricated by micromolding, composed of polyvinyl acetate backing layer and GelMA needle tips loaded with BP and Hb (Fig. 4D). The polyvinyl acetate backing layer could dissolve

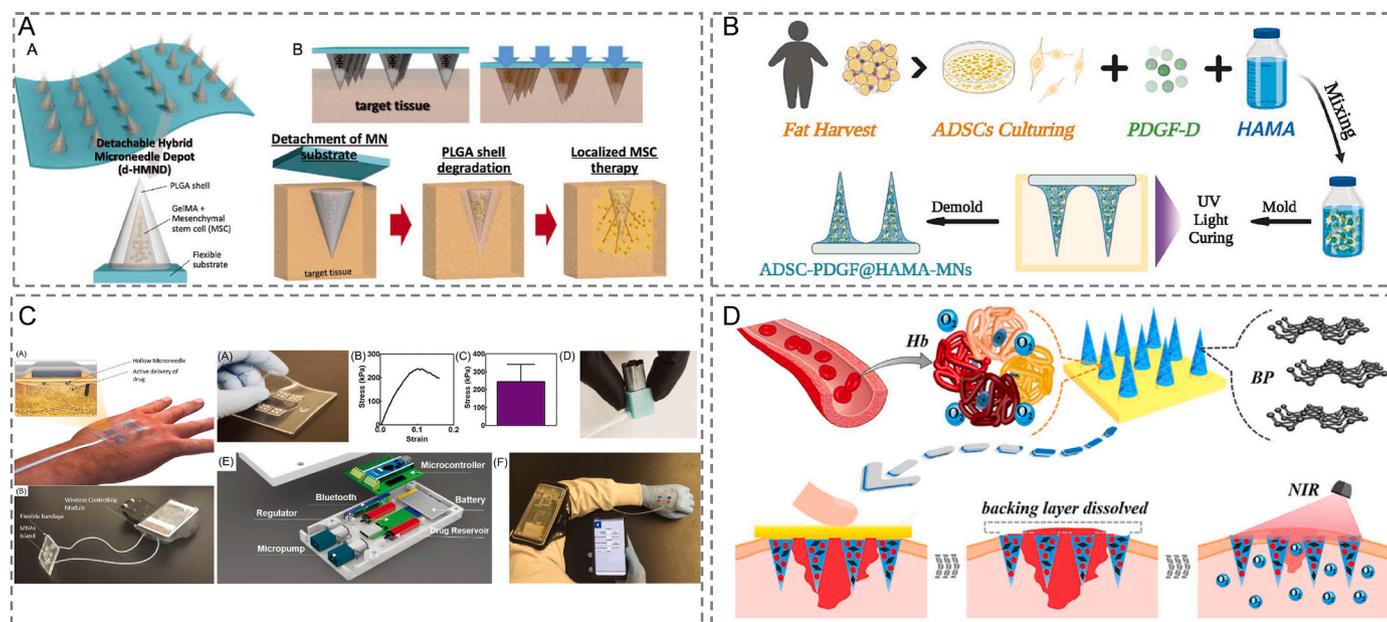


Fig. 4. Application of microneedles in skin regeneration. (A) Schematic diagram of using detachable hybrid microneedle depot (d-HMND) loaded with mesenchymal stem cell for promoting wound healing. Reproduced with permission [24]. Copyright 2020, Wiley. (B) Schematic illustrations of microneedles loaded with ADSCs and PDGF-D for chronic wound healing. Reproduced with permission [128]. Copyright 2022, Wiley. (C) Schematic diagram of using wirelessly controlled smart bandage equipped with microneedles for active delivery of therapeutics. Reproduced with permission [124]. Copyright 2020, Wiley. (D) Schematic illustrations of using near infrared radiation (NIR)-responsive separable microneedles loaded with black phosphorus quantum dots (BP QDs) and oxygen carrying hemoglobin for promoting wound healing. Reproduced with permission [129]. Copyright 2020, ACS.

immediately after insertion, leaving the needle tip in wound site. Due to the photothermal effect of BP, the local wound temperature would increase after NIR irradiation. With the increased temperature, Hb lost its oxygen binding affinity, leading to the responsive oxygen release. Its efficacy was demonstrated in an *in vivo* experiment, in which BP + Hb + NIR group had the fastest speed for skin regeneration. From the Masson's staining and immunohistochemistry staining, BP + Hb + NIR group also showed the best ECM reconstruction, tissue remodeling, and the least inflammation.

In summary, for normal and chronic wounds, microneedles are necessary to improve the drug retention and overcome physical barriers in wound bed. Tissue adhesive or detachable structures are recommended to avoid the patch from falling off due to the motion. For the treatment, multistage drug release pacing with healing process is important, especially for chronic wounds which cannot heal by themselves. A combined therapy with anti-infection and anti-inflammation in early phase and angiogenesis in late phase are necessary. Therefore, special designs, like multiregional, core-shell, and stacked structures, or loading micro- and nanoparticles are recommended leading to multistage drug delivery.

4.1.2. Infected wound healing

Skin infections caused by bacteria, fungi, and viruses are the most common infections. When the number of bacteria in wound bed reaches a threshold ($\sim 1 \times 10^5$), a local wound infection forms and the wound healing will be impeded [130]. The infection may spread into surrounding tissues, causing deeper infection, systemic infection, increased wound size and enhanced drug resistance, which could be life-threatening. However, traditional anti-infectious methods all have shortcomings. For instances, systematic administration of antimicrobial drugs has poor targeting ability and brings severe side effects. Topical cream and ointment with antibiotics could only treat superficial infection and cannot reach deeper tissues. Hypodermic needle injection is painful and invasive. In addition, the drug resistance of biofilm bacteria, which is protected by hydrated extracellular polymeric substances, is much higher than planktonic bacteria [18]. As a result, more researches

apply microneedles for anti-infection, as the microneedles could topically deliver drugs into deeper tissues in a painless way and easily break the protective film of the biofilm bacteria, overcoming the drug resistance of biofilm and extremely enhancing the therapeutic effects. Table 1 summarizes the anti-infectious microneedle systems in recent ten years and most of them have been reported by other reviews [18]. Some latest works are introduced as follow.

For example, Deng et al. proposed a lamprey-teeth-inspired antibacterial microneedle for infected wound healing [131]. This system was made of sericin mixed with antibacterial zinc oxide nanoparticles (ZNPs) by micromolding. As shown in Fig. 5A, the needle structures varied in the same patch. The central needles were shorter and vertically placed while the edge needles were longer with tilt angle inward like lamprey teeth. This structure could provide a dragging force to promote the wound closure. For the *in vivo* test, a mouse wound model was established and confirmed that the lamprey-teeth-inspired structure could accelerate the healing speed with less scar formation and more hair regrowth due to the existing dragging force. An infectious wound model was further established and demonstrated that ZNP-loaded bio-inspired microneedles significantly promoted the healing process and suppressed the infection. In addition to the drug therapies, Liang et al. developed an ultrasound activatable microneedle for anti-infection based on sono-chemodynamic and sono-thermal therapy (Fig. 5B) [132]. This system was made of hyaluronic acid (HA) mixed with $\text{CuO}_2/\text{TiO}_2$ (CT) dispersion by micromolding. With ultrasound activation, CT was decomposed and released Cu^{2+} ions and H_2O_2 , which was internalized by bacteria. Then the H_2O_2 was transformed into toxic ROS radicals, like hydroxyl radical, by the Fenton-like reaction and killed bacteria. On the other hand, the ultrasound could be efficiently absorbed and dissipated by the TiO_2 nanosheets and be transferred into heat, leading to thermal damage of the bacteria. To verify the *in vivo* antibacterial effect of the microneedles, a multidrug-resistant *Staphylococcus aureus* (MRSA) infected murine wound model was constructed. From the *in vivo* results, the CT microneedle with ultrasound group significantly accelerated wound closure and eliminate over 99.9999% MRSA in wound bed within 12 days. Inflammation responses were also

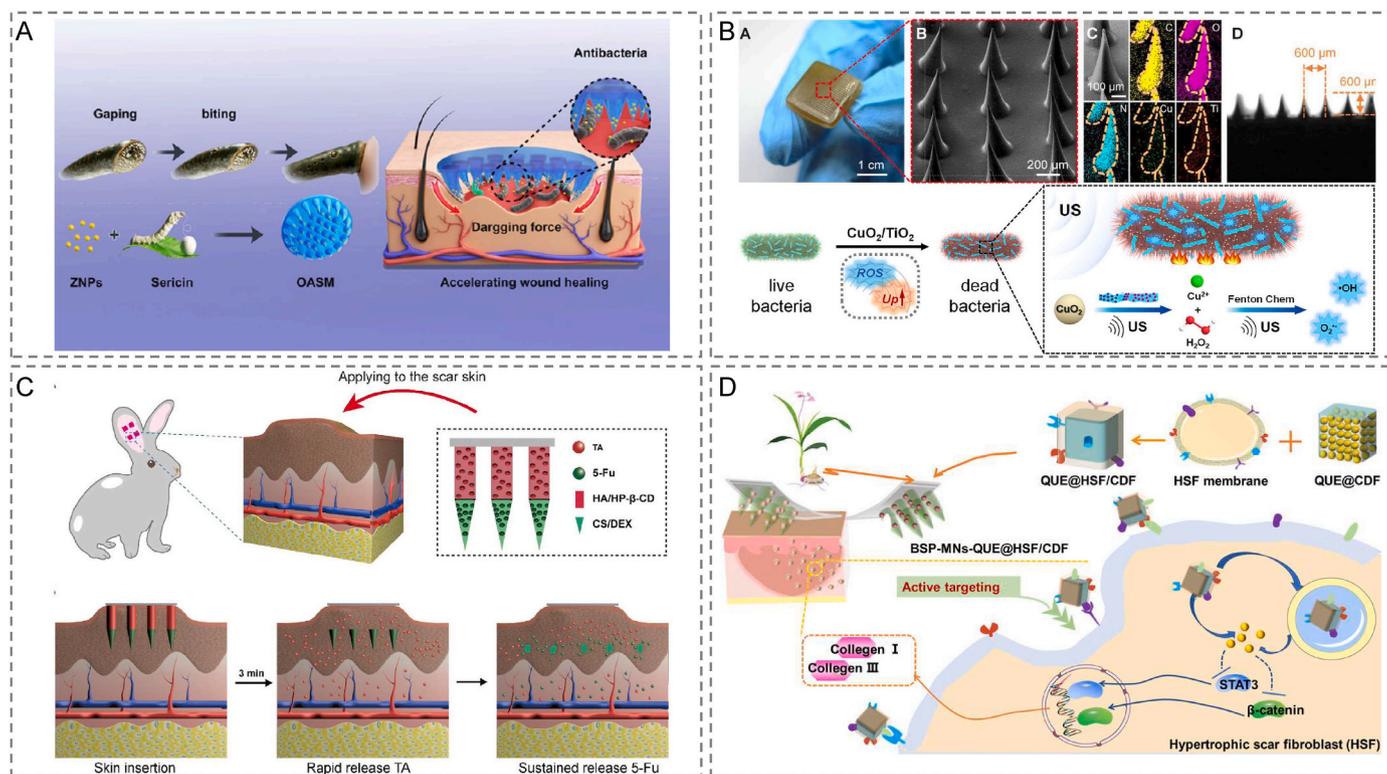


Fig. 5. Application of microneedles in infected wounds and scar wounds. (A) Schematic diagram showing the design and fabrication of bioinspired antibacterial sericin microneedles for infected wound healing. Reproduced with permission [131]. Copyright 2022, ACS. (B) Morphology of the ultrasound activatable microneedles and the illustration of possible antibacterial mechanism of CuO₂/TiO₂. Reproduced with permission [132]. Copyright 2023, Elsevier. (C) Schematic illustration of the system design of the bilayer microneedle with 5-Fu in needle tip and TA in needle shaft. Reproduced with permission [79]. Copyright 2021, Elsevier. (D) Schematic illustration of the working principle of the BSP-MNs-QUE@HSF/CDF. Reproduced with permission [134]. Copyright 2021, ACS.

ameliorated with decreased level of TNF- α and IL-6 in serum. Histological analysis also showed that the CT microneedles with ultrasound could reduce inflammation and had the maximum collagen deposition. In general, the proposed microneedle system had excellent antibacterial activity and could promote collagen alignment in wound tissues.

In summary, the infected wounds are difficult to treat due to depth of infection and drug resistance of biofilm. Microneedle provides an ideal tool to solve both problems by reaching deep tissues and physically breaking biofilm.

4.1.3. Burn wound healing

Burn injury is one of the most serious traumas to skin with high mortality. Although patients survive after burn injury, they still need help to improve the scar formation, which reduce their life quality. There are two kinds of scar caused by burn injury, namely the hypertrophic scar (HS) and keloid scar, and the prevalence after burn injury is very high, usually leading to contractures, neuropathic pain, and surface irregularities [133]. Currently, medical needling, which does a repetitive puncturing on burn scars using a roller equipped with metal microneedle arrays, has been used in clinic and beauty industry. The microneedles penetrate the scar and pierce the blood vessels of the dermis, which stimulate the collagen redeposition and dermal reorganization. The scar collagen will be broken and physiological collagen in a latticed form increased, accompanied by an increase of fibronectin and glycosaminoglycans, leading to a flat skin surface with better elasticity [16]. In addition to metal microneedle rollers, Zhang et al. recently proposed a drug-free microneedle system for HS treatment [17]. The microneedle system was fabricated by micromolding, consisting of silk fibroin (SF) needle and PVA backing layer. The authors hypothesized that the microneedles could regulate the local biomechanics and ultrastructure of tissues by penetrating the dermis and mechanically

interacting with the local tissues. To verify the hypothesis, an *in vivo* test using HS rabbit model was firstly conducted. After 60 days of treatment, the SF microneedles significantly improved the scar appearance and reduced the scar thickness, hardness, and tensile strength. Histological analysis also indicated that SF microneedles reduced the collagen deposition and the number of somatic cells in the scar area. It also downregulated the TGF- β 1 and α -SMA expression verified by the western blot. To further reveal the mechanism and molecular signal transduction pathway, fibroblast-populated collagen lattice system (FPCL), an *in vitro* scar tissue model only containing fibroblasts and collagen, was established and different groups of microneedles were applied on this system. Transcriptome sequencing results showed that the SF microneedles could interrupt the mechanical communication between the cell and the ECM via integrin–focal adhesion kinase (FAK) pathway, leading to fibrosis reduction. In summary, it has been proved that the physical intervention induced by pure microneedles could reduce the mechanical communication in scar tissues and create a low-stress environment, leading to a scar reduction.

In addition to the pure microneedles mentioned above, most of the advanced microneedle systems for HS therapy are based on dissolvable polymers and drug loading. Yang et al. proposed a bilayer dissolvable microneedle loaded with triamcinolone acetonide (TA) and 5-fluorouracil (5-Fu) which are both frequently used for HS treatment [79]. As shown in Fig. 5C, the needle tip was made of chitosan (CS) and dextran (DEX) encapsulated with 5-Fu for sustained release of 5-Fu while the needle shaft was made of HA and hydroxypropyl beta-cyclodextrin (HP- β -CD) integrated with TA for fast release of TA. The backing layer was made of polyvinylpyrrolidone and a multistep micromolding method was used for fabrication. For the *in vivo* test, HS model on rabbit ears was established. After three times of treatment, the scars in TA-5-Fu loaded microneedle group showed diminished redness and smoother

surface compared to other groups. Histological analysis also showed good results in TA-5-Fu loaded microneedle group. The thickness of the epidermis and dermis was significantly reduced, and the fibroblasts restored regular arrangement. Meanwhile, the collagen fibers showed decreased density and regular arrangement, indicating a suppression of abnormal fibroblasts proliferation and collagen fiber deposition. RT-qPCR results also revealed that the expression level of both Col I and TGF- β 1 were downregulated in TA-5-Fu loaded microneedle group, indicating a scar reduction potential. Another work done by Wu et al. paid attention to the targeted drug delivery to hypertrophic scar fibroblasts (HSFs) for HS treatment (Fig. 5D) [134]. They firstly designed a diphenyl carbonate cross-linked cyclodextrin metal organic framework (CDF) and then loaded quercetin (QUE), a promising compound on treating HS, into the CDF to increase the solubilization of QUE. For the targeting ability, HSF membrane was used to encapsulate the QUE loaded CDF (QUE@HSF/CDF) so that the complex could actively target the HSFs. The QUE@HSF/CDF was then mixed with *Bletilla striata* polysaccharide (BSP) solution for the fabrication of the microneedles (BSP-MNs-QUE@HSF/CDF) by micromolding. From the *in vivo* test based on HS-bearing rat model, the BSP-MNs-QUE@HSF/CDF could activate the Wnt/ β -catenin and JAK2/STAT3 pathways and reduce the deposition of Col I and III in HS, showing great therapeutic effect on HS treatment. There are many other works using microneedles to deliver different drugs, like angiotensin II receptor blocker, gallic acid, etc.,

which demonstrated significant progress in using microneedles in HS treatment.

In summary, HS and keloid scar, which form after burn injury and possess higher hardness and thickness than normal tissues, are difficult to treat. This requires microneedles to have enough needle strength and stiffness for scar penetration. Metal or stiff polymer microneedles with or without drugs have both been proved to be effective in treating HS or keloid scar. On the one hand, microneedles could pierce scar tissues and stimulate the collagen reorganization. On the other hand, the microneedle array embedded in scar could regulate local stress environment of tissues, modulating the collagen deposition of fibroblasts for reducing scar formation. Assisted with anti-scar drugs by drug coating or encapsulation, the microneedle treatment efficacy will be enhanced.

Although being effective, microneedle assisted skin regeneration still faces many limitations. For example, wound models established in most articles are simple with mild environment. Although many of them constructed infected or diabetic wound models, most wound models are at its early stage and few of them have a severe infection or ulceration. Additionally, the application sites are mostly limited to the back of rats, which has less motions. Wounds in neck, knee, elbow, or other joints, which have more complicated stress environment with periodic tension force, are seldom investigated. All these problems are critical to the clinical translation of microneedles and there is still a long way to go.

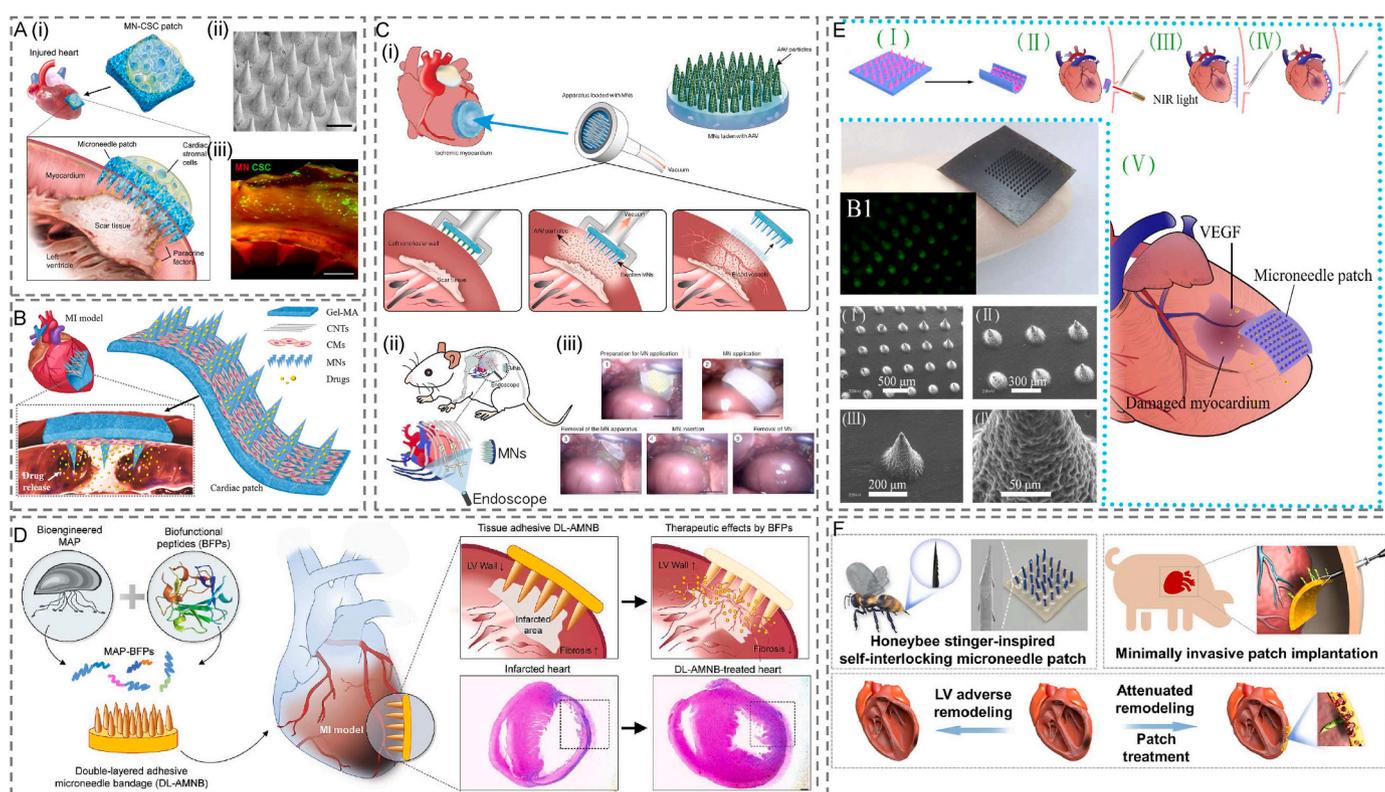


Fig. 6. Application of microneedles in cardiac tissue regeneration. (A) (i) Schematic diagram showing the overall design used to test the therapeutic benefits of cardiac stromal cell (CSC)-loaded microneedles on infarcted heart; (ii) Representative SEM image showing the microneedle morphology; (iii) Representative fluorescence image in the lower right corner indicating that DiO-labeled CSCs (green) were encapsulated in fibrin gel and then integrated onto the top surface of microneedles (red). Reproduced with permission [6]. Copyright 2018, AAAS. Scale bar, 500 μ m. (B) Schematic diagram of using CNTs-integrated microneedles loaded with induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs) for the treatment of an infarcted heart. Reproduced with permission [63]. Copyright 2021, Elsevier. (C) (i) Schematic diagram showing the overall design using adeno-associated virus (AAV) microneedles to deliver genes for treatment of ischemic myocardial disease; (ii) Diagram showing the application of the microneedles to a rat heart; (iii) A series of endoscopic images demonstrating the application of microneedles for delivery of therapeutic agents to a rat heart. Reproduced with permission [48]. Copyright 2020, AAAS. Scale bar, 600 μ m. (D) Schematic overview of double-layered adhesive microneedle bandages (DL-AMNB) including a biofunctionalized peptide (BFP)-fused mussel adhesive proteins (MAP) for myocardial infarction (MI) treatment. Reproduced with permission [138]. Copyright 2021, Elsevier. (E) Schematic diagram showing the working principle of NIR-triggered self-interlocking microneedles for minimally invasive treatment of myocardial ischemia and microneedle morphology. Reproduced with permission [139]. Copyright 2021, ACS. (F) Schematic illustration of the fabrication, working principle of the honeybee stinger-inspired microneedles. Reproduced with permission [140]. Copyright 2022, Elsevier.

4.2. Cardiac tissue regeneration

Cardiovascular disease (CVD) is the leading cause of mortality all over the world [135]. Typical CVDs, like myocardial infarction (MI) and heart failure, are caused by cardiomyocyte death or dysfunction. Many therapeutic agents, including biomaterials, cells, genes, and growth factors, have been applied and showed great effects [136,137]. However, direct injection has many limitations decreasing their efficacy, like low cell retention, rapid clearance under *in vivo* condition, and short half-life of proteins, making assistance from engineered patches warranted. Common cardiac patches are limited by low tissue adhesion, leading to difficulty in delivery of drugs into deeper necrotic cardiac tissues. As a result, microneedles become emerging solutions in constructing novel cardiac patches to solve these problems.

Tang et al. developed a cardiac stromal cell (CSC)-loaded microneedle patch for therapeutic heart regeneration after myocardial infarction [62]. The microneedle part was fabricated from aqueous solution of poly(vinyl alcohol) (PVA) by micromolding (Fig. 6A). After the PVA microneedle was fabricated, CSCs were mixed with fibrin gel and seeded on the back surface of the microneedles. Thanks to the microchannels created by the microneedles between the CSCs and myocardium, paracrine factors secreted by CSCs could be delivered to the cardiac wound site. *In vivo* results showed that CSC-loaded microneedle group could reduce apoptosis and promote angiogenesis in rat MI models. It also increased infarct wall thickness and viable tissue in wound site, indicating great cardiac tissue regeneration. Another study involving loading cardiomyocytes on microneedle was conducted by Sun et al. [63]. The patch is composed of three distinct layers, including GelMA based microneedles, aligned conductive carbon nanotube (CNT) layer, and induced pluripotent stem cell (iPSC)-derived cardiomyocytes layer (Fig. 6B). To prepare the conductive microneedles, a multistep micromolding approach was taken. Aligned CNT was precoated on the mold surface and 30% (w/v) GelMA was added into the mold as basic material. After UV polymerization, the CNT layer was combined with GelMA. They were then peeled off from the mold together, forming the conductive microneedles. Finally, iPSC-derived cardiomyocytes were seeded on the microneedle and grew aligning the CNT. The *in vivo* results well demonstrated that the cardiac patch could adhere to the heart tissue. Implantation of this device encapsulated with cytokine could effectively inhibit left ventricular wall thinning and enhance the cardiac pump function compared with other groups. Both examples demonstrate the potential of utilizing microneedles to deliver cells for cardiac disease treatment.

Despite microneedle-assisted cell therapy being promising and effective, it has some limitations including scarce cell source, complicated procedure, low cell viability during processing, and difficult storage. Another promising method to treat cardiac diseases would be the cardiac gene therapy. Shi et al. proposed an adeno-associated virus (AAV)-VEGF-loaded microneedle (Fig. 6C) [48]. The whole system was also fabricated out of PVA using micromolding. Then by dipping in solution of AAV9 vectors with cytomegalovirus promoters containing the gene sequence for VEGF165 (AAV-VEGF), the PVA microneedle was coated with AAV-VEGF. The surface loading of the agent and swelling property of PVA could enable AAV-VEGF burst-release after application. Compared with conventional direct injection which confines therapeutic agent to the injection site, this microneedle patch could achieve even distribution of agent at the impaired myocardium. In view of *in vivo* heart performance and histological examinations, AAV-VEGF loaded microneedles could significantly enhance heart function, reduce cardiac scar size and infarcted areas, and improve myocardial perfusion in MI rat model compared with direct injection group.

Apart from gene therapy, proteins have also been loaded in microneedles for cardiac regeneration. Lim et al. designed a bio-adhesive microneedle with core-shell structure for MI treatment (Fig. 6D) [138]. It was fabricated by a two-step micromolding method. Firstly, silk fibroin (SF) solution was added into the negative mold to form the shell

part. Then solution of mussel adhesive protein (MAP) decorated with bio-functional peptides (e.g., VEGF, arginylglycylaspartic acid (RGD) peptide) (MAP-BFP) was poured into the mold to form the core part. After air drying, it was peeled off to generate a core-shell microneedle. After insertion into tissues, the dried SF hydrogel, having strong water-absorbing property on the tip, would absorb interstitial fluid and swell rapidly to interlock with tissues, leading to tissue adhesion. Besides, the loaded bio-functional peptides were allowed to diffuse across the enlarged SF polymer network because of the swelling of SF. From the results in rat MI model, the MAP-BFP based microneedle groups significantly delayed the progression of ventricular remodeling and increased the wall thickness, compared with the untreated groups. Deposition of scar tissue was also reduced in the treated groups, indicating the potential of improving cardiac function in the long term. All these good results partially depended on the property of the adhesive microneedles which could deliver the agent to deeper infarcted area and prolong the retention of therapeutic peptides.

Another interesting work done by Fan et al. proposed a VEGF-loaded and NIR triggered unfolding microneedle patch [139]. The microneedle system was fabricated based on a mixture of VEGF, graphene oxide (GO), and PVA. Due to the remarkable photothermal effect of GO, the GO-PVA microneedle system was endowed with shape memory function. With this property, the microneedle implantation can be exerted via a minimally invasive surgery, which only needs to open a small incision on the chest. The microneedle will be folded into small size before surgery. Upon delivery inside the body, the patch would be unfolded by NIR light and then inserted into the heart tissue. By optimizing the ratio of GO to PVA, the recovery rate of microneedles could reach 90% in 10 s. For the *in vivo* test, mouse MI model was used. From the echocardiography results on day 7, the VEGF-loaded microneedles with NIR light stimulation greatly recovered the heart function. Histological and immunofluorescent staining results also showed that the proposed treatment reduced the myocardial fibrosis and increased the number of new blood vessels, indicating a good restoration of the myocardial function. In addition to the works mentioned above, there are also many other recent works using microneedles to load cells, large or small molecules, which have achieved remarkable results in myocardial regeneration.

Although many works discuss about the effect of drug loaded microneedles on cardiac repair, few of them investigate the effects of pure microneedles and its underlying mechanism. Recently, Lu et al. proposed a drug-free microneedle patch with a honeybee stinger-inspired structure for heart tissue adhesion [140]. To fabricate the microneedle system, firstly, the backward barbs were cut on the surface of the polypropylene polymer fiber. Then the fiber with barbs was segmented into barbed microneedles and the barbed needle tips were arrayed in a PDMS mold. Finally, polyurethane solution was poured into the mold and formed the backing layer after the solvent evaporation. The tissue adhesive force of the microneedle patch was tested to be around 0.1 N per needle and the *in vivo* test proved the tissue adhesive ability. In addition, the authors investigated the mechanical support of the microneedles to the infarcted myocardium. Compared with no patch and sutured patch groups, the microneedle group could significantly reduce the tissue stress and strain in the infarcted area. As a result, after implantation into rats with MI, the microneedles could significantly recover the cardiac function and reduce the infarction size from the echocardiography analysis and histological results. RNA sequencing results further revealed that the underlying mechanism of the pure microneedle treatment was involved in ECM organization, response to TGF- β and mechanical stimulus, and glucose metabolic process, indicating that the microneedles could provide mechanical support to the heart and repair the infarcted area through energy metabolism reprogramming.

In summary, the current cardiac microneedle patches have shown its advantages on recovering the myocardial infarction both physically and chemically. However, further improvements are needed in this

application. As heart is an internal organ and thoracotomy is inevitable for the implantation, minimally invasive surgery could be a better choice. This requires microneedles to have a small size or a shape memory property. Meanwhile, a reliable implantation of the microneedle is also difficult, due to the heartbeat and tissue elasticity. Currently, many researchers use a suction apparatus which is effective and harmless. For further application in a minimally invasive surgery, the apparatus may also need modification. Finally, most articles focused on early MI treatment. The current microneedle strategies may need further enhancement for an advanced MI treatment.

4.3. Bone, cartilage, and tendon regeneration

Microneedles have advantages in easy operation and painless treatment, making it increasingly prevalent for treating bone-relevant tissues, including cartilage and tendon. Compared with traditional dressing and oral administration, microneedles could deliver drugs to wound site directly, avoiding the drug degradation. Moreover, microneedle treatment is painless and could release drugs in a long term, which is suitable for the slow bone tissue regeneration. In recent years, many works focusing on microneedle-assisted rheumatoid arthritis (RA) treatment and tendon regeneration have achieved remarkable results and will be discussed in this section [8,141–143].

RA is a systematic autoimmune and inflammatory disease which could lead to cartilage/bone erosion and joint inflammation [144]. Conventional therapies all have limitations. For instance, articular injection requires professional operation and patient compliance, and common drug dressings have poor agent permeation. To solve this problem, Cao et al. proposed to utilize microneedles as carrier to load DEK-targeting aptamer (DTA) for better treatment of collagen-induced arthritis (Fig. 7A) [141]. DEK protein is a critical therapeutic target to RA, as it is important for the formation of neutrophil extracellular traps (NETs) which is highly correlated with inflammatory arthritis. DTA is a typical agent for blocking DEK protein. The microneedle was first produced by micromolding method. The needle tips were composed of methacrylate-modified hyaluronic acid (mHA) and the base layer was made of ethocel. After the microneedle was fabricated, a modified DTA called DTA6, which had higher stability and DEK binding affinity, was coated onto the microneedle surface by drop coating method. The *in vivo* results revealed that DTA6 loaded microneedle group significantly reduced the expression of TNF- α , IL-6, and bone erosion in mouse paws.

Compared with intravenous injection group, the microneedle group showed similar or better efficacy, because of their better agent distribution. In another study conducted by Zhou et al., a triptolide (TP) incorporated liposome (TP-Lipo)-loaded microneedle was used to treat osteoarthritis (OA) [142]. TP is a promising anti-inflammatory drug for OA but with poor solubility and rapid clearance in human body. Therefore, liposome was used to improve the loading quantity in microneedles, retention time in human body, and the agent absorption rate. The whole microneedle system was also constructed by micromolding. The needle tips were made of TP-Lipo and hyaluronic acid mixture while the base layer was made of PVA. After insertion, hyaluronic acid would absorb water and swell rapidly, leading to a sustained release of TP-Lipo (much slower than intra-articular injection). From the *in vivo* pharmacodynamic results, TP-Lipo loaded microneedles significantly reduced swelling of knee joint and expression of inflammatory cytokines, like TNF- α , IL-1 β , IL-6. The micro-CT and histological results further showed that TP-Lipo loaded microneedles significantly reduced cartilage destruction and OA symptoms compared to direct injection. This was because it could prolong drug retention in the injury site. In addition to the above works, there are also many other researches using microneedle-assisted therapies for OA treatment [43,145].

Tendon is a tough and dense fibrous connective tissue which connects muscles to bones. But if tendon is injured, tendon regeneration will take several months to years. Unfortunately, neither surgery nor conservative treatment strategies can provide long-term therapeutic benefits, and frequent oral and injection administration for long-term recovery may bring side effects and pain. To address this, Yao et al. developed a novel microneedle system to load parathyroid hormone (PTH), for tendon regeneration acceleration (Fig. 7B) [8]. Regarding the microneedle system, it was made of PVA which is water swellable but insoluble, supporting the transdermal diffusion of PTH. The cumulative release of PTH demonstrated that the microneedle patches could deliver parathyroid hormone efficiently in the first 4 h. It was also found that the PTH loaded microneedles significantly increased the expression level of collagenase type 1 (COL1A) and decorin (DCN) to promote ECM secretion, and hence better tendon healing in terms of its histology, biomechanics, and gene expression, proving that microneedle assisted tendon treatment is a promising and practical strategy for tendon regeneration. Alternatively, Liu et al. designed a detachable microneedle system to deliver exosomes modified by a nitric oxide nanomotor (EXO/MBA) for Achilles's tendinopathy regeneration (Fig. 7C). Firstly,

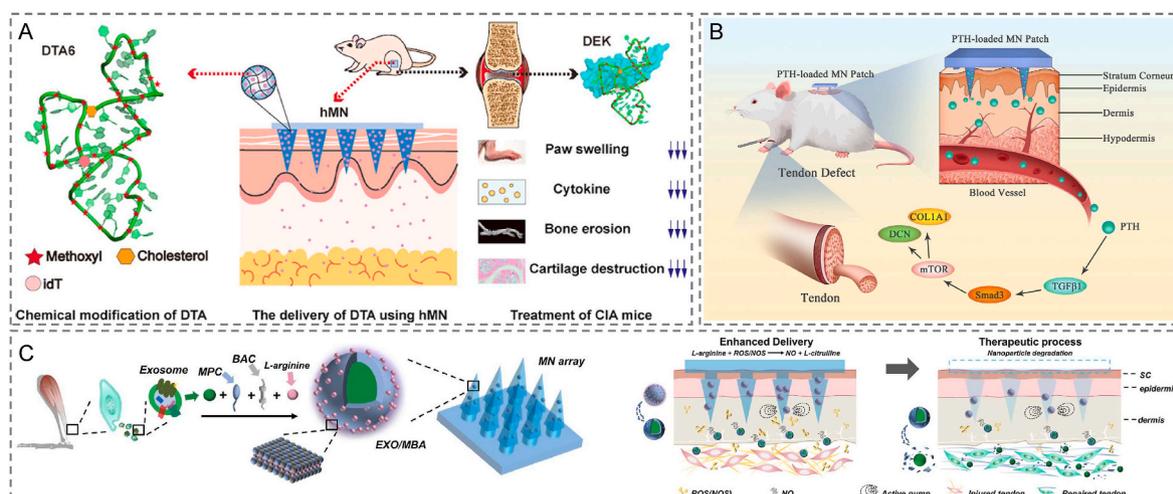


Fig. 7. Application of microneedles for tendon, cartilage, and bone regeneration. (A) Schematic illustration of using DEK-targeting aptamer loaded hydrogel microneedles for attenuating collagen-induced arthritis. Reproduced with permission [141]. Copyright 2021, ACS. (B) Potential mechanism involved in that parathyroid hormone (PTH)-loaded microneedle patches promoted tendon repair. Reproduced with permission [186]. Copyright 2020, Wiley. (C) Schematic illustration showing the manufacturing procedure of exosomes modified by a nitric oxide nanomotor (EXO/MBA)-loaded microneedle and healing process of Achilles tendinopathy after application of EXO/MBA-loaded microneedles. Reproduced with permission [143]. Copyright 2021, ACS.

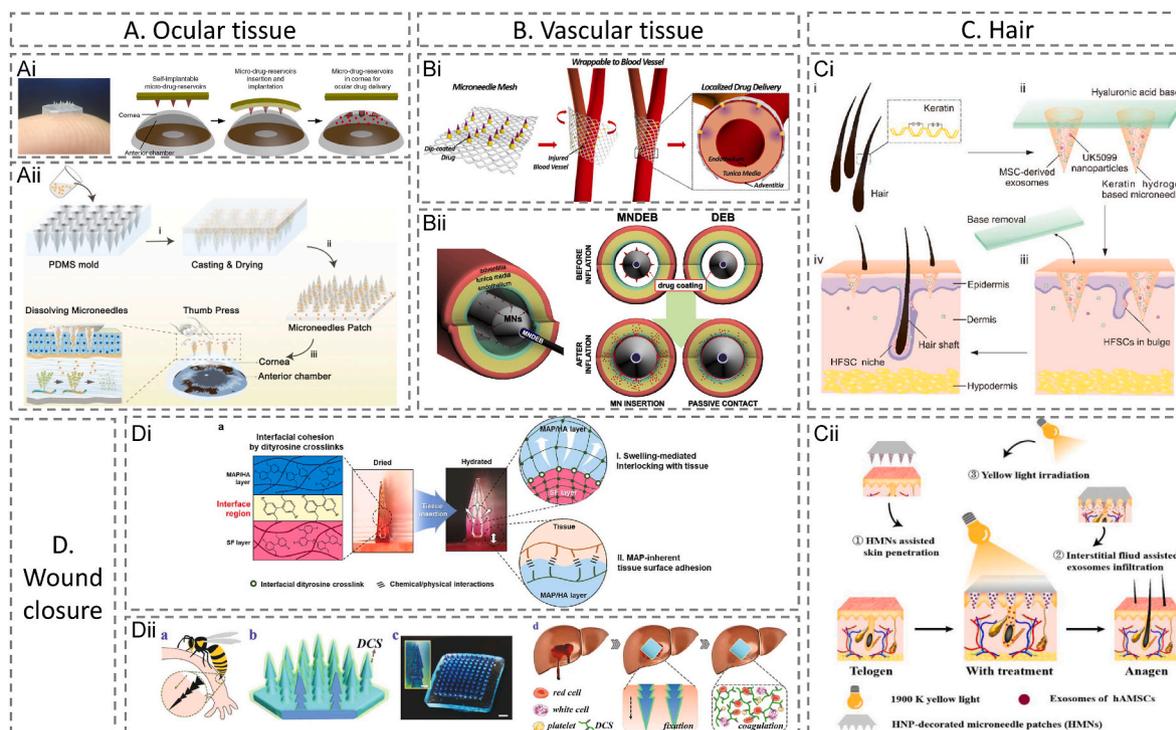


Fig. 8. Applications of microneedles in regeneration of other organs. (A) Application of microneedles in ocular tissue regeneration. (Ai) Illustration of eye-contact patch, which was equipped with an array of self-implantable micro-drug-reservoirs, for ocular drug delivery. Reproduced with permission [9]. Copyright 2018, Nature. (Aii) Preparation and application of fluconazole (FA)-loaded PLA-hyaluronic acid microneedles. Reproduced with permission [147]. Copyright 2022, Wiley. (B) Application of microneedles in vascular tissue regeneration. (Bi) Schematic diagram of wrappable microneedle mesh, perivascular installation of microneedle mesh on blood vessels and drug delivery into vascular tissues. Reproduced with permission [4]. Copyright 2017, Elsevier. (Bii) Illustration of microneedle drug eluting balloon (MNDEB) indicating that the drug coated microneedles onto balloon surface enhance the efficiency of endovascular drug delivery by microneedle insertion compared to conventional DEB. Reproduced with permission [149]. Copyright 2020, Elsevier. (C) Application of microneedles in hair regrowth. (Ci) Schematic diagram of using a detachable microneedle patch loading mesenchymal stem cell-derived exosomes and drugs for hair loss therapy. Reproduced with permission [149]. Copyright 2019, ACS. (Cii) Schematic diagram of using the low-color-temperature yellow (1900 K) light along with exosomes of human amniotic mesenchymal stem cell (hAMSC)-loaded microneedles for promoting hair regrowth. Reproduced with permission [153]. Copyright 2021, Elsevier. (D) Application of microneedles in wound closure. (Di) Illustration of working mechanisms of a hydrogel-forming adhesive microneedle consisting of a mussel adhesive protein (MAP)-based swellable and sticky shell and a silk fibroin (SF)-based non-swellable core. Reproduced with permission [81]. Copyright 2019, Elsevier. (Dii) Design and hemostatic function of the bioinspired pagoda-like microneedle patches. Reproduced with permission [78]. Copyright 2021, Elsevier. Scale bar, 1 mm and 300 μm (inset).

EXO was modified by 2-methacryloyloxyethyl phosphorylcholine (MPC), N,N'-bis(acryloyl) cystamine (BAC), and L-arginine to form EXO/MBA. When released, L-arginine on EXO/MBA would be converted to nitric oxide by NOS or ROS as driving force, like a nanomotor. This could promote the exosomes distribute more evenly across the wound site. The microneedle is made by micromolding with the needle tip composed of PVA, GelMA, and EXO/MBA with its backing layer made of hyaluronic acid. EXO/MBA microneedle group could reduce the Achilles's tendinopathy inflammation and promote tendon cell proliferation *in vivo*. In addition, in the wound site, the expression of Col1a was increased while ECM degradation was decreased. The combination of microneedle and nanomotor design enabled the EXO accumulated at wound site more efficiently.

Most works about microneedle assisted bone-related tissue regeneration focus on RA treatment. In the future, microneedle may also be used as a scaffold or implant for periosteum, cartilage, or bone regeneration.

4.4. Regeneration of other organs and microneedle-assisted wound closure

Microneedles have been exploited for other tissue regeneration, like ocular tissue [9,146–148], vascular tissue [4,10,149], oral tissue [11, 150,151], hair [12,152,153], spinal cord [13], and uterine [14,154, 155]. Many microneedle-assisted wound closure strategies have also been used for hemostasis of internal organs [78,81]. In this section, how

microneedles are used in treating these tissues and its advantages will be discussed.

4.4.1. Ocular tissues

Effective drug delivery into eyes is always challenging due to the barriers in ocular tissue, like corneal epithelium. Conventional therapies have been reported with great limitation. For example, oral administration needs large dose which leads to systemic side effects but still results in low drug concentration in eyes. Intraocular injection using hypodermic needles could cause much pain to the patient and have a risk of infection or even permanent damage to eyes. Other conventional topical drug delivery methods, like eye drops, also suffer from low drug permeation, low bioavailability, and fast clearance of its effect. To overcome this, Than et al. developed a double-layer detachable microneedles for effective ocular drug delivery [9]. The whole system was fabricated by micromolding similar to previous systems. Except, the outer layer of needle tip is made of the mixture of crosslinked methacrylated hyaluronic acid (MeHA) and drug (anti-angiogenic monoclonal antibody (DC101)) while the inner layer was made of the mixture of hyaluronic acid and another drug (diclofenac, an anti-inflammatory compound) (Fig. 8Ai). The microneedle could penetrate across ocular barriers without difficulty. After insertion, DC101 in the outer layer was steadily released and reduced 90% neovascular area in eyes. Following that, diclofenac in the inner layer was released rapidly providing a synergistic therapy for anti-inflammation. In one study, Shi et al. used a

dissolving microneedle for treating fungal keratitis [147]. The whole system was made by micromolding of poly(D,L-lactide) (PLA) and hyaluronic acid mixture loaded with fluconazole (Fig. 8Aii). The microneedles could penetrate epithelial layer without parent irritation in preliminary tests. It could also prolong the drug retention time to 2.5 h in conjunctival sac. Furthermore, the microneedle group presented superior anti-inflammatory effect than conventional eyedrop agents and comparable therapeutic efficacy with intrastromal injection groups *in vivo*. Another issue of intraocular injection is the needle retraction which is dangerous especially when the patients need repeated injection. To address this issue, Lee et al. developed a self-plugging microneedle (SPM) which could avoid needle retraction and seal the scleral puncture after insertion [148]. Different from common microneedle patches, the SPM did not have a backing layer and only had the needle tip which consisted of two parts including the drug releasing component and the scleral plugging component. The drug releasing component was a PLA needle tip which was fabricated by contact drawing method and then coated with rhodamine B (model drug). The scleral plugging component was placed at the bottom end of the needle tip, made of swellable MeHA hydrogel. Once the whole SPM was completely inserted, the MeHA would contact the eyeball and swell to plug the scleral hole. From the *in vivo* results, the SPM could plug the scleral hole successfully and deliver the model drugs into deeper retinal tissues. Histological results indicated that no obvious inflammatory infiltration occurred and the tissue integrity at the insertion site was the same with normal tissue after 7 days of application.

According to the works mentioned above, many dissolvable drug-loaded microneedles have been used for ocular drug delivery and all show promising results. However, the basic materials of the microneedles still need attention, as the metabolic activity of some ocular tissue is weak. Polymers with low molecular weight, low stiffness and fast degradation rate are recommended.

4.4.2. Vascular tissues

Intimal hyperplasia (IH), caused by vascular tissue damage, frequently occurs after some vascular surgeries, such as coronary or peripheral bypass grafting and arteriovascular grafting. It can be alleviated by drugs like paclitaxel and sirolimus. However, effective delivery of these drugs to wound site is challenging. Developing perivascular drug delivery (PDD) devices also have many problems, like controlled drug release, deformation caused by the compression of the device, and targeting ability of drug delivery device. To solve these problems, Lee et al. developed a wrapable microneedle mesh to deliver sirolimus for better IH treatment [4]. The whole system consisted of PLGA needle tips coated with sirolimus and a PLGA surgical mesh as a backing layer (Fig. 8Bi). It was fabricated by transfer molding method involving producing needle tips by micromolding and then transferring the needle tips to the mesh. The drug sirolimus was then coated onto the needle surface by dip coating. The mechanical results revealed that the mesh was as soft as vascular tissues and easy to wrap, which is convenient for surgery. From the *in vivo* results, the microneedle meshes reduced neointimal formation significantly, compared with the groups without microneedles. It also avoided causing any vessel distortion after surgery. Another cardiovascular disease is atherosclerosis, which is usually treated by stent implantation. However, this is always accompanied by side effects like in-stent restenosis and stent thrombosis. Drug eluting balloon (DEB) is a promising intraluminal drug delivery method to replace common vascular stents, but it has low drug delivery efficiency due to short action time. Yet if we are to combine DEB with microneedles (MNDEB) as proposed by Lee et al., it can enhance the efficacy of traditional DEB [149]. In this study, the needle tips were first produced by micromolding based on a UV-curable adhesive called 208-CTH and then transferred to a balloon surface (Fig. 8Bii). The drug paclitaxel (PTX) was then coated onto the needle tip by dip coating. Needle tip demonstrated its ability to be inserted into luminal vasculature successfully *in vivo*. The MNDEB group showed superior drug

delivery efficiency and reduction in atherosclerosis than the DEB group. Another similar work was done by Li et al. recently [10]. Li et al. also integrated an array of microneedle tips on traditional DEB by transferring the needle tips fabricated by micromolding. Instead of direct coating of the drugs onto microneedles, Li et al. loaded the drugs into the needle tip which contained PTX, melanin, lauric acid (LA), polycaprolactone (PCL), and polylactic-co-glycolic acid (PLGA). In addition to the drugs, PCL and PLGA could provide mechanical strength for needle tips while LA with low melting point (~44 °C) was used for NIR controlled needle detachment. With the NIR heating, LA in the needle tips would melt leading to a weakened bond between the needle tip and the base. Thus, a tiny force pulling the balloon catheter could detach the tips, leaving the drug-loaded tips in the vessel tissues for a long-term action. Rabbit atherosclerotic model was used for the *in vivo* test. The ultrasonography, computerized tomography angiography (CTA) and histological results at 6 months demonstrated that the drug-loaded microneedle balloon group had the smallest stenosis area, indicating a significant deceleration of intimal hyperplasia compared with DEB and drug-free microneedle balloon groups.

4.4.3. Oral tissues

Oral inflammatory diseases are most common oral lesions, including oral ulcers, periodontitis, and oral lichen planus. They greatly influence the quality of people's daily life and may even result in oral cancer. Drug delivery to oral tissue is challenging due to bacterial infection, oral saliva flow, and poor drug retention. As a result, the therapeutic effects of traditional methods, like injection, drops, and ointment cream, are compromised. Local injections could cause severe pain and lead to fear and anxiety of patients. Drops and ointment cream are suboptimal choices due to saliva secretion, swallowing, and chewing, leading to low drug retention [151,156].

To solve the dilemma, Zhang et al. proposed a modular microneedle which could deliver multiplex drugs for promoting periodontal tissue regeneration [11]. The dissolvable backing layer of the microneedle was made of gelatin loaded with tetracycline for a burst release. The needle tip was composed of crosslinked GelMA loaded with two kinds of carriers, including biodegradable nanoparticles (NP) and heparin-coated mesoporous silica microparticles (SiMPs). The NPs were also used to carry tetracycline for a sustained release while the heparin-SiMPs were loaded with IL-4 and TGF- β for immunoregulation and tissue regeneration. The *in vivo* results demonstrated the proposed microneedle could effectively inhibit bacterial growth by the two-stage release of tetracycline. qPCR results also indicated that the released IL-4 and TGF- β not only decrease the inflammation but also induce osteogenic activity and collagen matrix synthesis in local periodontal cells. From the 8-week microcomputed tomography results, microneedle treated groups showed a significant decrease in distance between the alveolar bone crest and cemento-enamel junction (CEJ) and increase in alveolar bone area. The recovered bone area was significantly improved compared with other groups, including control and pure microneedle group.

Another recent work done by Guo et al. used dissolvable HA microneedles loading betamethasone for treating oral ulcer [150]. Unlike topical application of ointment, microneedles could easily break the physical barrier of tongue abdomen mucosa and realize a rapid sub-mucosal dissolution of drugs. The whole system was made of HA loading betamethasone via micromolding. To investigate the *in vivo* efficacy of the proposed system, oral ulcer models were used. After 5 days of treatment, the drug loaded microneedle group led to a shrinkage in wound size compared with control, ointment, and pure microneedle group. The histological examinations also indicated that the betamethasone, HA and the microneedle synergistically promoted angiogenesis and suppressed inflammation, leading to a painless and high-efficient oral ulcer treatment.

4.4.4. Hair

Hair is the appendage of skin tissue. Hair loss is a common disorder

resulting from health conditions like aging and medication. Although effective, traditional clinical therapies have some disadvantages. For example, the hair follicle transplantation is a costly, invasive, and painful surgery while application of drug solutions like minoxidil have poor transdermal efficiency. To solve the dilemma, Yang et al. developed an exosome-loaded hydrogel microneedle for promoting hair regrowth. The needle tips were based on hair-derived keratin hydrogel mixed with mesenchymal stem cell (MSC)-derived exosomes and a small molecular drug called UK5099 while the backing layer is made of hyaluronic acid (Fig. 8Ci). In the *in vivo* animal experiments, the microneedles could transport exosomes and drugs efficiently to hair follicle in a sustained way. It induced pigmentation and hair regrowth within only 6 days where the regrown hair demonstrated proper physiological characteristics [12]. In another work inspired by progress of seed germination, Hong et al. developed soluble microneedle patches loaded with exosomes of human amniotic mesenchymal stem cells (hAMSCs) and hair nanoparticles (HNP) composed of keratin and melanin. It is fabricated by micromolding out of MeHA (Fig. 8Cii) [153]. From the animal experiments, this microneedle-assisted therapy could effectively facilitate hair regrowth within 7 days compared with single drug groups or non-microneedle groups without obvious adverse effects or inflammation occurred in both the *in vitro* and *in vivo* studies. In this work, the microneedles also promote the drug delivery efficiency into the deep scalp.

In a recent work, Yan et al. proposed a separable microneedle patch, with needle tip made of PVA and backing layer made of dissolvable HA, for hair regeneration [157]. The needle tips encapsulated chitosan lactate (CL) and exosomes from adipose-derived stem cells (ADSCs-EXO). The *in vitro* results demonstrated the CL could not only release L-lactate to promote cell growth but also avoid potential infection due to its inherent antibacterial properties. The ADSCs-EXO could promote cell proliferation through activating Wnt signaling pathway. The *in vivo* results showed that the microneedle group loaded with CL and ADSCs-EXO presented the best hair re-coverage compared with direct application of Minoxidil or CL and ADSCs-EXO, due to the synergetic effect of microneedles, CL, and ADSCs-EXO. Histological assessment also indicated that CL/ADSCs-EXO-loaded microneedle group had the largest hair bulb diameter and accelerated the telogen–anagen transition of hair follicles to promote hair growth. In summary, this drug-free microneedle system shows better hair regeneration effects than the direct application of Minoxidil or CL and ADSCs-EXO.

Another recent work done by Yuan et al. utilized a conditioned media (CM) loaded microneedle for androgenetic alopecia (AGA) treatment by promoting perifollicular angiogenesis [158]. The whole system was composed of a HA needle tip integrated with CM derived from hypoxia pretreated MSCs and a polyvinyl pyrrolidone (PVP) substrate, fabricated by a multistep micromolding. The *in vitro* data showed that CM collected under hypoxic condition had higher HIF-1 α expression than that under normoxia condition, which could elevate the VEGF expression and greatly improve the tube formation and migration of endothelial cells. Histological and immunostaining results using a mouse AGA model both indicated that a larger proportion of hair follicles entered into the anagen phase. The application of the CM loaded microneedle sped up the activation of hair follicle stem cells by elevating VEGF expression and inducing perifollicular angiogenesis. Interestingly, not only the CM loaded microneedles, but pure microneedles also exhibited great capacity in promoting hair regrowth due to the mechanical stimulation to the neovascular network.

4.4.5. Spinal cord

Spinal cord injury (SCI), damage to the spinal cord that causes temporary or permanent changes in its function, is one of the most serious diseases with high mortality and deformity. The main obstacles of SCI treatment include blood brain barrier (BBB), neuronal loss, inflammation, and glial scar formation, which greatly compromise the effect of traditional therapies. For example, oral administration only has

limited therapeutic effects due to BBB; drug injection has poor drug retention in wound area and may cause secondary trauma; cell transplantation also has poor cell retention and cell viability. To solve these problems, Han et al. utilized a GelMA-based microneedle loaded with 3D-cultured MSC-derived exosome (3D-Exo) for spinal cord regeneration [13]. Microneedles could not only improve the drug delivery efficiency and drug retention time, but also bring limited harm to the tissue, avoiding the secondary trauma. Meanwhile, the exosome is also a good substitution to the cell therapy. Quantitative proteomics and western blot results proved that the proposed 3D-Exo has better capacity in immune regulation and neuronal regulation than the 2D-cultured MSC-derived exosomes. Then, the 3D-Exo was mixed with GelMA hydrogel and used for microneedle fabrication based on the micromolding method, and the 3D-Exo-loaded microneedles were transplanted to rats with SCI. The results showed that the 3D-Exo-loaded microneedle group had the highest Basso, Beattie, and Bresnahan score, indicating the best hindlimb locomotor function recovery. The immunostaining and western blot results showed that the 3D-Exo-loaded microneedle group had the lowest glial fibrillary acidic protein (GFAP) expression, which is related to glial scar formation, and lower inflammation related protein expression. In summary, the proposed microneedle system effectively regulates the immune response, scar formation and promotes the spinal cord regeneration.

Although being effective in the works mentioned above, microneedle implantation into spinal cord is complicated and even dangerous. On the one hand, as spinal cord is surrounded by spine, the microneedle implantation may need complicated surgery to open the spine, which may cause secondary trauma. On the other hand, the basic material used for microneedle fabrication should not be too stiff, as the spinal cord is soft and fragile. The stiff needle tip may harm the spinal cord tissue easily and cause severe complications like paralysis.

4.4.6. Uterine

Intrauterine adhesions (IUAs), caused by endometrial injury, is a common gynecological disease, which may lead to pregnancy loss, hypomenorrhea, amenorrhea and infertility. Many strategies have been developed for the treatment, like hormone therapy, transcervical resection, and intrauterine scaffolds. However, these methods still have limitations, such as high recurrence rates after surgery, biological inertia, risk of dislocation, low drug delivery efficiency and so on [159]. To break through these limitations, several works have used microneedles for uterine regeneration and IUA prevention.

In one study, Zhang et al. proposed a growth factor loaded microneedle system with arrowhead needle tip and anisotropic surface adhesion, fabricated by a multistep micromolding method [154]. The arrowhead needle tip was based on GelMA hydrogel containing basic fibroblast growth factor (bFGF) for tissue anchoring and healing acceleration while the backing layer was composed of two layers where the upper layer was made of N-acryloyl glycinamide (NAGA) hydrogels for cell adhesion and the lower layer was made of poly(ethylene glycol) methacrylate (PEGMA) hydrogels for anti-adhesion. Rat IUA model was established to investigate the effects of microneedles and bFGF on preventing intrauterine adhesion and promoting endometrium regeneration. Hematoxylin-eosin (HE) staining results indicated that the microneedles alone could inhibit the intrauterine adhesions effectively and the bFGF could significantly improve the endometrium recovery. Masson's trichrome staining and CD31 immunostaining results showed that bFGF loaded microneedles had the most regular collagen deposition and neovascularization, indicating the best tissue regeneration. After 6-week treatment using bFGF loaded microneedles, the rats were pregnant successfully and gave birth to healthy baby rats.

Another work done by Li et al. presents a novel microneedle system loaded with human endometrium-derived adventitial cell (En-ADVs) spheroid and lactoferrin (LF) used for uterine regeneration [14]. The system was fabricated by a common micromolding method based on GelMA hydrogel with an array of microwells on the backing layer. The

needle tips were loaded with LF for antibacteria and the microwells were used for the formation and loading of En-ADVs spheroids. The *in vitro* results demonstrated the 3D En-ADVs spheroids exhibited better pluripotency maintenance, proliferation, migration, and angiogenesis than the traditional 2D and 3D cell culture. For the *in vivo* test, a rat Asherman's syndrome model was established and then the cell spheroid-loaded microneedles were transplanted for 90 days. Histological results indicated that cell spheroid-loaded microneedle group showed the best restoration of endometrium thickness and number of glands but greatly alleviated the fibrosis and scar formation compared with the control and pure microneedle groups. Immunostaining results of α -SMA and vWF also proved the best smooth muscle regeneration and neovascularization in cell spheroid-loaded microneedle group. After 90-day transplantation, the cell spheroid-loaded microneedle group also showed significant functional restoration proved by the higher expression of HOXA10, an indicator of endometrial receptivity, and the pregnant rate.

4.4.7. Wound closure

Traditional wound closure devices, such as staples and sutures, are not favorable for many internal organs and can often result in complications such as local ischemia and air/fluid leakage. Microneedles are considered as a new method to effectively anchor internal organ tissues with minimal invasion and better tissue connectivity. In one study, Jeon et al. developed a novel hydrogel-forming adhesive double layer microneedle patches using natural bio-adhesive swellable mussel adhesive protein (MAP) (Fig. 8Di) [81]. This microneedle patch consisted of a MAP-based shell and a non-swellable silk fibroin (SF)-based core. It is proved that this new device demonstrated strong adhesion and good biocompatibility *in vivo*, while achieving superior wound sealing ability for luminal leaks *ex vivo* by promoting sealing and functional healing process, so that it can be utilized to stabilize vascular and gastrointestinal wound healing. Other than increasing the connectivity of wet internal wounds, the tissue regeneration could also be promoted by the hemostasis conditions inside organs. In this case, Zhang et al. developed a pagoda-like multilayer microneedle patch to achieve better hemostasis for internal organs (Fig. 8Dii) [78]. This unique structure was achieved by a multistep micromolding based on poly (ethylene glycol) diacrylate (PEGDA). After the microneedle patch was fabricated, dodecyl-modified chitosan (DCS) was coated on the surface for blood cell accumulation. This multilayer microneedle patch can interlock with a variety of tissue types, including heart, liver, spleen, and kidney. For *in vivo* demonstration of hemostatic capability, the microneedle system was used in the treatment of liver, spleen, and kidney bleeding in rabbit models and showed great efficacy. Compared with medical tapes and glues, this DCS multilayered microneedle could adhere to tissues better even under severe conditions such as massive on-going blood loss. Both examples demonstrate the potential clinical application of microneedle patches in wound closure under harsh conditions and hemostasis maintenance of internal tissues. Inspired by the claw of eagle, Zhang et al. developed a biomimetic microneedle patch for incisional wound healing [160]. This microneedle patch encapsulated liquid metal (LM) and had a claw-like clamping structure that could stick to the skin to assist wound closure without secondary dehiscence. It was found that this device could guide cell migration by utilizing a spatial electric field. The electric patch group also showed its wound closure effect was better than the suture group, with better collagen deposition and least inflammation *in vivo*.

4.5. Wound monitoring

In addition to being a drug carrier, microneedles have also been widely used as transdermal sensors for biochemical signal monitoring and disease diagnosis, due to its unique structure which could penetrate deeper tissue layer and extract critical testing samples like sweat, interstitial fluid, and blood. Large amounts of powerful and intelligent monitoring microneedles have been developed in recent years, although

the microneedle-assisted wound monitoring is still in its early stage [29, 161]. Since many biochemical signals in the wound bed are different from that in the normal tissues or organs, including its pH [162,163], ROS [164], temperature [165], protein [20], enzyme [166], and glucose, it is essential to monitor these factors to determine the specific wound healing phase and the suitable treatment. Furthermore, compared with common wound patches, microneedles offer additional advantages by penetrating into the scar tissues and detecting deeper signals inside the wound bed [25]. In this section, works using microneedles with wound-monitoring or wound-responsive abilities will be summarized.

One characteristic of the healthy skin would be to have a pH around 4 to 6, and which is significantly lower than the pH of 7 in chronic wounds [167]. Based on this principle, Ullah et al. developed a pH-responsive-polymer-coated microneedle system (Fig. 9A) [168]. The microneedle was fabricated by multistep coating. The most inner part is a commercial stainless-steel microneedle. Then the drug loaded porous PLGA was coated on the surface. Finally, a pH-sensitive polymer called Eudragit S100 was coated as the outer shell, which dissolves only at alkaline pH (pH > 7), enabling the smart release of the encapsulated drugs. From the *in vivo* drug release test, the coated microneedle exhibited significantly more drug release in wound pH conditions. Although no further wound healing experiment was conducted, the pH-responsive drug release capacity of the microneedle was minimally demonstrated.

In addition to pH, ROS is another typical signal existing at the wound site. It plays an important role to recruit immune cells at the early stage of wound healing. However, excessive ROS could lead to the destruction of ECM and some growth factors, leading to a vicious cycle of inflammation [2]. To regulate the ROS level of the bacterial wound, Zhang et al. designed a smart microneedle system made of drug-loaded ROS-responsive PVA matrix again using micromolding method (Fig. 9B) [164]. As ROS level in the inflammatory tissue exceeds 500 μ M, the microneedle degrades by cleaving its crosslinking network of PVA and releases the contained antimicrobial drugs (clindamycin), eliminating the wound bacteria (*P. acnes*) both *in vitro* and *in vivo*. Although this study was an acne vulgaris treatment, the same principle could be applied in wound healing of other tissues.

Apart from pH and ROS, some key protein markers can be exploited as detection target, allowing microneedles to become a smart device. Many studies have paid attention to detect inflammation-related proteins, which is crucial to regulate the wound inflammation. In one study, Gao et al. designed an intelligent silk fibroin-based microneedle dressing (i-SMD), which integrated a temperature-responsive and inflammation-responsive N-isopropylacrylamide (NIPAM) hydrogel to promote the wound healing correspondingly (Fig. 9Cii) [20]. Since the volume phase transition temperature of NIPAM hydrogel is around 37 °C, when the wound temperature rose due to the inflammatory reaction, the hydrogel would collapse and release the contained VEGF to promote the healing process; meanwhile, the system could also detect the presence of interleukin-6 (IL-6) and C-reactive protein (CRP), which are essential proteins involved in inflammation phase. To realize this system, the interstitial fluid was first directed by microfluidic channels into the detection area. The biomarkers within the liquid would then bind to specific fluorescence-labeled antibodies, and finally an inverse opal photonic crystal (IO PC) structures were introduced to amplify the bio-signals. From the *in vivo* experiments, more granulation tissues and collagen deposition could be found in the i-SMD group, proving its excellent capacity in promoting wound healing. In another study, Guo et al. designed a similar shark tooth-microneedle which could also detect inflammation-related proteins [169]. The whole system was composed of three parts: the human epidermal growth factor (hEGF)-loaded microneedle part was first fabricated into a sharp tooth shape by micromolding based on inverse opal photonic crystal, then the bio-sensing part for protein detection and MXene flexible electrodes for motion monitoring were integrated onto the microneedle patch

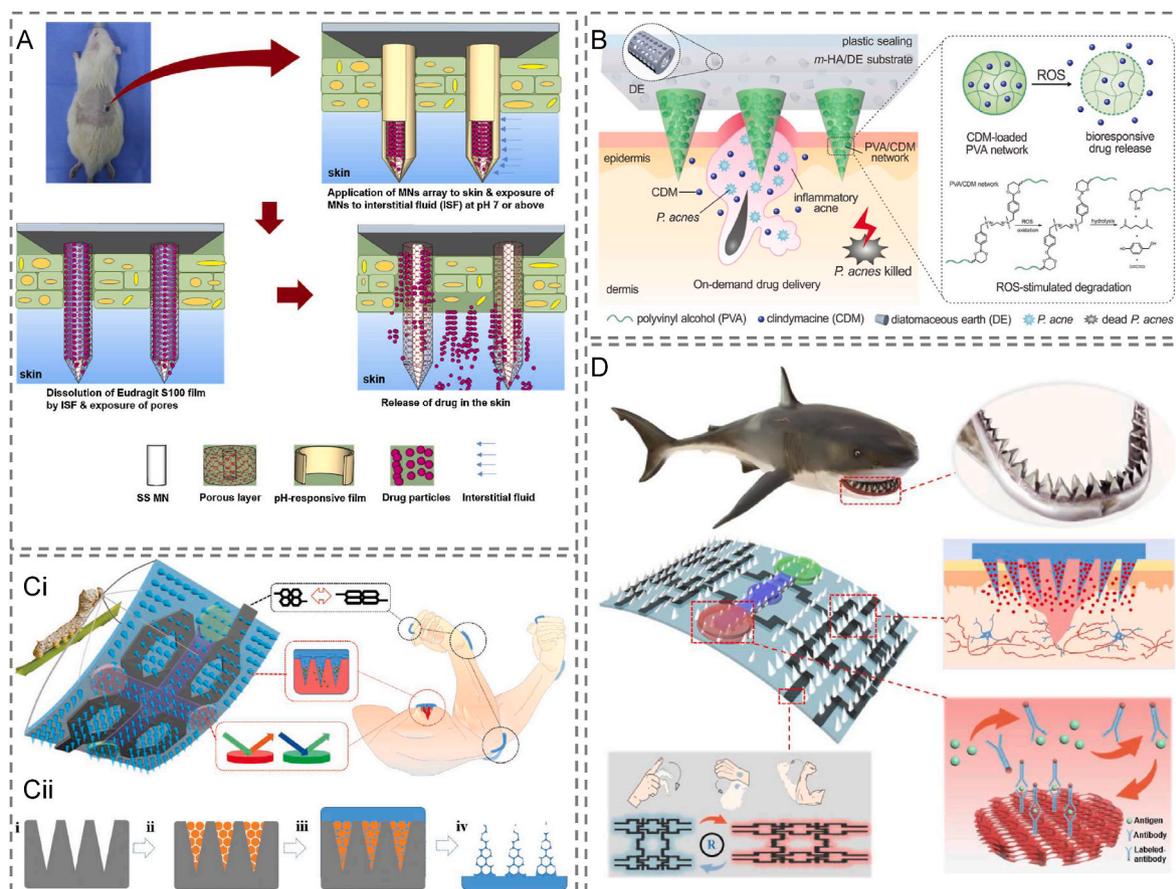


Fig. 9. Wound monitoring. (A) Schematic illustration of the pH-responsive-polymer-coated microneedle system, reproduced with permission [162]. Copyright 2021, Elsevier. (B) Schematic illustration of ROS-responsive PVA microneedles, reproduced with permission [187]. Copyright 2018, Wiley. (Ci) Schematic illustration of the intelligent silk fibroin-based microneedle system. (Cii) Fabrication process of the IO PC structures, reproduced with permission [20]. Copyright 2021, Wiley. (D) Illustration of using shark tooth-inspired microneedles for intelligent wound management including motion sensing, biochemical analysis, and healing, reproduced with permission [169]. Copyright 2021, ACS.

(Fig. 9D). The biosensing part was based on immunofluorescence technique and could successfully detect proteins including IL-6 and CRP. The microneedle system significantly facilitates recovery of the wounds *in vivo*, leading to less inflammation and better regenerated epithelial tissues with dense and organized structures. The shark tooth-inspired structure also improved the adhesion to wounds and prolonged the drug treatment period for sustained releases.

5. Conclusions and future perspectives

In this review, we discuss the current situations using microneedle systems in wound healing and tissue regeneration. Specific design considerations including potential loads (e.g., small molecules, macromolecules, nucleic acids, nanoparticles, extracellular vesicles, cells), structure designs (e.g., multidrug structures, adhesive structures), material selection (e.g., metals, silicon, ceramics, polymers), and drug release mechanism (e.g., diffusion, degradation, triggered release) for microneedles and microfabrication methods have been reviewed comprehensively followed by the current application cases of using microneedles for wound healing and tissue regeneration (e.g., skin, cardiac, bone, tendon, ocular, vascular, oral, hair, spinal cord, and uterine tissues). With all these summaries and analyses, we believe that microneedles have shown its great potential in managing and promoting the healing process of wounds not only in skin, but also in many other tissues.

Although promising, application of microneedles in wound healing remains in its early stage. Here we discuss some requirements and

prospects for further development of microneedles in wound management, including intellectualization, customization, structure optimization, and functional integration. Then the translational impact of the microneedles and their potential development for addressing clinical challenges will also be discussed.

Firstly, intellectualization is an inevitable tendency for future microneedles, covering several areas like drug delivery, biosensing and diagnosis, electrical control, etc. Current research on microneedle-assisted wound management mainly focuses on drug delivery and its final efficacy. A few of them involves simple wound monitoring or smart materials for stimuli-responsive drug delivery. However, most of them are triggered by single factor or only detect single indicator, which cannot reflect the complicated environment in the wound bed and may lead to some unexpected results. For a reliable smart microneedle system, at least 2–3 biochemical factors, involving pH, ROS, protein, enzyme, and glucose, should be taken as diagnostic indicators to give a more accurate evaluation on the wound conditions. After the comprehensive diagnosis by analysis module, the control module will control the pump to deliver specific drugs to microneedles at certain flow rate according to wound conditions. Finally, the whole system will keep monitoring the wound condition and deliver different drugs until the wound is healed. To realize this system, the microneedle array should contain two parts, namely the drug delivery part and the monitoring part. Needles in the drug delivery part should be in hollow structure or made of hydrogel to deliver drugs smoothly while needles in the monitoring part may load biosensing materials (e.g., enzymes, bio-receptors) and conductive materials for transmitting electrical signal

[170]. However, this kind of design, involving different structures and materials in different areas of microneedles, is difficult to fabricate. Till now, few works have fully met all these requirements. Therefore, current fabrication methods need improvement and new techniques should be applied. For example, multi-material 3D printing, which could change feed stocks during printing process, seems a promising technique to fulfill those requirements [171]. Particularly, some works have fabricated arbitrary 3D microscale structures consisting of different materials [172]. It is achievable to use this technique to design more complex and multifunctional microneedle system. In addition to the fabrication problems, foundational studies and standards, such as highly sensitive biosensor design, miniature flexible and biocompatible electronics, remain to be settled. For control and analysis modules, wearable electronic devices with Internet of things (IoT) capacity for data acquisition and analysis and software applications on cellphone for wireless control and data display should be designed. This will also involve writing the control algorithms for close-loop drug delivery.

Secondly, customization is also necessary in the future for precision medicine, as microneedle will face diversity of application site and different patients. In detail, different people may need different drug dose and have different wound areas, structures (e.g., curvature of eyes, diameter of vessels, different organ surfaces and shapes, thickness of epidermis), and conditions (e.g., elasticity difference of tissues at different ages). To address this, 3D printing is critical to personalized customization, as it could print arbitrary structures according to 3D models obtained from patients. However, it will be expensive and time-consuming by only using 3D printing. In some circumstances, a model may be suitable for a group of patients. Then 3D printing assisted micromolding technique could be used for mass production and decreasing expense. The 3D printing will firstly print the master mold of the product according to the patient's 3D model. Subsequently, the master mold will be used to produce the negative mold for further micromolding. Finally, by simply adding, curing, and demolding materials using the negative mold, final products could be fabricated quickly in large quantities.

Furthermore, structure optimization is also important for further development of microneedles for improved adhesion property, larger drug loading capacity, better drug delivery efficiency, and higher integration density. In detail, up to date, researchers have proposed many practical and efficient structures, like serrated structures for adhesion [84], snake fang-inspired structures for more efficient drug delivery [173]. In addition, to increase the drug loading capacity which is an inherent limitation of microneedles due to its small dimension, extending the drug loading space to the microneedle backing layer or introducing external pump and containers for continuous drug supply have been applied [174,175]. Moreover, to meet the requirements for multidrug delivery, multichannel structures and microfluidics-assisted structures are also proposed [176,177]. These structures all show great potentials in improving the performance of microneedles and should be taken into consideration for commercialized microneedle products. However, all of these need the microneedles to have higher integration density in the future, posing a challenge to the current fabrication methods. It involves multi-material fabrication and nano fabrication techniques, which are complicated and yet achieved. A possible solution is to combine multi-material 3D printing with implosion fabrication [178]. Implosion fabrication takes advantage of the swelling of hydrogels so that we could use multi-material 3D printing to fabricate an object with larger scale and then let the object shrink to the desired dimension. It doesn't need to improve the precision of current 3D printing, but also has the potential for micro-/nanofabrication of microneedles. However, for all these designs to be put into practical use, there is still a long way. Future studies should also consider microneedle-assisted treatments for other tissues like brain, nerves, muscles, etc.

Additionally, more functions, like mechanical, electrical, magnetic, and ultrasound stimulation, could also be integrated into microneedle

systems. Mechanical regulation property of microneedles has been investigated and proved to be effective for the wound management, especially for the scar reduction, by stimulating collagen reorganization and changing the local stress environment. The mechanical stimulus effect can be further enhanced when constructing a microneedle system using an elastic and adhesive backing layer. Electrical and magnetic field have also been proved to be effective on inducing cell migration, orientation and changing cell functions, especially for cardiomyocytes and nerve cells which inherently generate electrical signals [179]. Although electricity has been employed in microneedles and shows good results recently, the application method is unpractical: Most works add conductive components to make the whole patch conductive and then two conductive microneedles are used as electrodes and attached to two sides of wound with voltage applied between the two patches [180,181]. This is troublesome as the patients have to carry a power supply all the time. The situation is worse for internal organs and tissues, as the power supply and the placement of microneedles will be a big issue. Wireless power supply or piezoelectrical materials are both good solutions which could achieve the electricity generation in single patch and this can be investigated in the future. Magnetic field integrated microneedles have not been used for regeneration-related application which may be a future direction, as it has been proved to affect cell migration, adhesion which are important to wound healing [182]. Ultrasound has been used with microneedles for sono-thermal and sono-chemodynamic therapy to kill bacteria, as mentioned above [132]. Currently, few works investigate the effect of ultrasound integrated microneedle patch on tissue regeneration, which need further studies in the future.

Finally, the clinical translation of microneedles is very meaningful but still on its way. In most cases, microneedle is a "best of both worlds" solution, which has better drug permeation than common ointment or gel but lower invasiveness and pain than conventional needles. In addition, for external tissue regeneration, it is easy to use without need of professional operation like hypodermic injection. Patients can even use by themselves. As a result, the clinical use of microneedles must bring in huge change to many traditional treatments, especially in the skin tissue, oral tissue, and hair regeneration. Additionally, researchers need to take clinical problems into consideration when designing microneedle systems. For example, microneedles with shape memory property could achieve a minimally invasive surgery for microneedle implantation; incorporation of microneedles with drug eluting balloon could significantly enhance the drug delivery to blood vessels; microneedles with adhesive structures could realize rapid wound closure and drug delivery simultaneously to simplify surgery.

Ethics approval

The manuscript is not related to clinical study. No need for ethics approval and consent.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioactmat.2023.04.003>.

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