Hydrogel-Forming Microneedles

Subjects: Cell Biology

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Controlled drug delivery in the oral cavity poses challenges such as bacterial contamination, saliva dilution, and inactivation by salivary enzymes upon ingestion. Microneedles offer a location-specific, minimally invasive, and retentive approach. Hydrogel-forming microneedles (HFMs) have emerged for dental diagnostics and therapeutics. HFMs penetrate the stratum corneum, undergo swelling upon contact, secure attachment, and enable sustained transdermal or transmucosal drug delivery. Commonly employed polymers such as polyvinyl alcohol (PVA) and polyvinyl pyrrolidone are crosslinked with tartaric acid or its derivatives while incorporating therapeutic agents. Microneedle patches provide suture-free and painless drug delivery to keratinized or non-keratinized mucosa, facilitating site-specific treatment and patient compliance.

drug administration routes | drug delivery systems | drug implants | hydrogels

1. Introduction

“Microneedles” refer to tiny, needle-like structures that are typically less than 1 mm long and used for transdermal delivery of drugs or other therapeutic agents [1]. By penetrating the outer layer of the skin, therapeutic agent(s) can be delivered efficiently and effectively to the tissues beneath and/or to the systemic circulation in a controlled fashion, which is predetermined by the microneedle (MN) patch design, designated anatomical location of application, and material properties of all agents involved.

MNs can be shaped in a variety of ways: array formation or individual needles, while the needles can be formed by a variety of natural/synthetic materials: glass, hydrogels, metals, polymers, silicone, and zeolite. These devices are widespread across various medical fields, especially for applications where minimization of discomfort or pain is desirable, including dermatology, diabetes management, and vaccination [2][3]. In recent years, significant progress has been made in developing microneedles as a promising approach for transdermal drug delivery, such as solid MN, coated MN, hollow MN, and hydrogel MN (**Figure 1**).

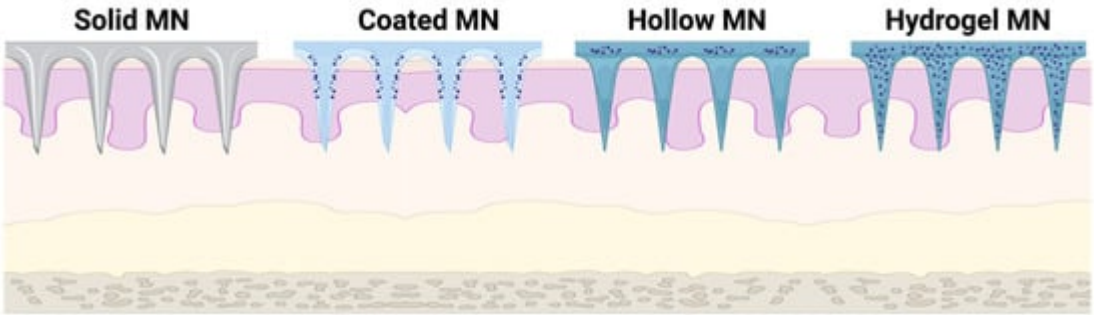

Figure 1. Microneedle (MN) types for transdermal drug delivery. Left to right: Solid MN—solid shaft with sharp tips for skin penetration and drug delivery by dissolution, diffusion, and surface coating; Coated MN—solid base with surface coating for controlled release of encapsulated drug/active ingredients; Hollow MN—needles with a hollow core for fluid injection or collection; Hydrogel MN—biocompatible needles that dissolve upon insertion, releasing loaded drugs or vaccines. The figure was created with BioRender.com.

Hydrogel-forming microneedles (HFMs) exhibit sufficient mechanical strength to penetrate the skin on the application while readily absorbing tissue fluid after implantation into the epithelium [4]. This results in the swelling of the polymer and subsequently forming continuous, unimpeded hydrogel microchannels, which open diffusion portals for drug delivery, thereby allowing the release of the therapeutic agent and the anticipated therapeutic actions in the body [5].

Concerning base/structural ingredients, HFMs could be classified as natural [6] or synthetic hydrogel MNs [7] with structural components from natural (e.g., hyaluronic acid (HA), gelatin, etc.) or synthetic polymers (e.g., polyvinyl alcohol, sodium polyacrylate, methacrylic acid polymers, etc.), respectively. Certain HFMs such as HA hydrogels and Gelatin methacryloyl (GelMA) hydrogels have received more attention due to their decent biocompatibility, degradability, and non-toxic nature, making HFMs one of the most commonly used/advocated local drug delivery protocols in biomedical applications.

Hydrogel MNs are prepared by inducing the polymerization of agents such as acrylamide, N-isopropylacrylamide, or methacrylated HA in the hydrogel, followed by injecting the fluid mixture into an MN mold, left to cure, then de-mold followed by preservation through drying [1]. Gelation of hydrogels can be achieved by a variety of mechanisms [8], including physical cross-linking of polymer chains, electrostatic interactions, and covalent chemical cross-linking, targeting the formation of a hydrophilic polymer network that (i) can absorb water and swell, (ii) has a porous structure that encapsulates the active agent such as the drug to be delivered, (iii) presents in a solid state and therefore minimizes drug loss during transport, and (iv) delivers the drug to a predetermined anatomical location or particular tissue type so as to enhance the therapeutic efficacy of the agent involved [9]. Hydrogel polymerization is often carefully physically and/or chemically modulated for specific controlled drug release protocols in designated therapeutic applications [10].

Compared with traditional drug delivery routes (e.g., oral administration, intra-muscular/-venous injections), transdermal delivery, which mainly involves placing an MN patch on the skin, puncturing the skin stratum corneum, and delivering the drug to the bloodstream through the skin tissue, is a delivery protocol of great interest because of the advantages of self-administration, on-demand drug delivery, good patient compliance, and avoidance of first-pass effect in the liver and/or drug degradation in the gastrointestinal tract [11]. HFM has a wide range of applications, from medicine to cosmetics to biotechnology. HFM has been utilized in the medical field to deliver transdermal drugs, administer vaccinations [3], monitor glucose levels [12], and treat wounds [13]. Microencapsulated cell delivery using HFM [14] has gained increasing interest in recent years. Through this innovative approach, therapeutic cells are encapsulated within hydrogel matrices and delivered through the network of microneedles. Regenerative medicine, tissue engineering, and cell-based therapies are now possible.

2. Materials Used for Hydrogel-Forming Microneedles: Fabrication and Characteristics

Since MNs act by penetrating the protective human skin/mucosal layer, the material must be biocompatible, i.e., not immunogenic nor foreign body reaction prone. In most cases, hydrogels are biocompatible and can be applied without causing any harm/discomfort to the host. Many of the polymers used for HFMs were previously researched and extensively trialed for use in medicine—particularly with the essential properties of biocompatibility and biodegradability (**Table 1**).

Table 1. Materials used for hydrogel-forming microneedles.

Material	Description	Applications
Natural Polymers		
Carbohydrate-based	Polysaccharides with biocompatibility and bioactive properties	- Chitosan or chitosan derivatives: Biocompatible, biodegradable, antimicrobial properties. Used in transmucosal vaccine delivery and oral anesthetic patches. - Hyaluronic acid: Highly biocompatible, water-retention ability. Used in transdermal drug delivery and pain-free dental treatments. - Sodium alginate: Biocompatible, non-toxic, widely available. Used in hemostatic needles, medical dressings, and bone tissue engineering. - Pullulan: Dissolving microneedles with potential for transdermal drug delivery. - Combined carbohydrate-based microneedles: Synergistic effects of chitosan and pullulan, improved mechanical strength and drug loading capacity.
Protein-based	Gelatin and silk fibroin with biocompatibility and controllable degradation	- Water-soluble silk fibroin: Biocompatible, adjustable drug release. Used in sustained-release drug systems and tissue engineering. -

Material	Description	Applications
		Gelatin: Derived from collagen, used in capsules, ointments, and tissue engineering for bone regeneration.
Synthetic Polymers		
Polyvinyl pyrrolidone	Synthetic polymer with good mechanical properties and biocompatibility	- Used in drug delivery systems, such as insulin-loaded microneedles.
Poly (ethylene glycol)	Synthetic polymer with high water solubility and biocompatibility	- Used in hydrogel-forming microneedles for drug delivery.
Poly (methyl vinyl ether-co-maleic acid)	Synthetic polymer with mucoadhesive properties and biocompatibility	- Used in mucoadhesive microneedles for transmucosal drug delivery.
Poly (acrylic acid)	Synthetic polymer with pH-responsive properties and biocompatibility	- Used in pH-responsive hydrogel-forming microneedles for drug delivery.

Biocompatible materials are used to fabricate MNs made of natural polymers. This type of drug delivery system uses MNs that form hydrogels. In carbohydrate-based MNs, natural polysaccharides such as chitosan, cellulose, and starch are used to create the hydrogel matrix. Protein-based MNs natural polymers could be composed of gelatin or silk fibroin as the matrix-forming component. These types of MNs are biocompatible, biodegradable, and non-toxic. Biodegradability, biocompatibility, and swelling capacity are among the properties of these MNs. In the field of drug delivery, wound healing, and transdermal vaccination, natural polymer-fabricated hydrogel-forming MNs have shown promising results.

Natural and synthetic polymer hydrogels differ in their characteristics and applications. Natural polymer hydrogels, derived from biogenic sources such as proteins or polysaccharides, exhibit inherent biocompatibility and biodegradability. These hydrogels typically possess a high water content and can faithfully replicate the extracellular matrix, providing an appropriate microenvironment for cellular growth and tissue regeneration [15]. Synthetic polymer hydrogels offer adjustable and stable matrices for drug delivery, tissue engineering, and biomedical applications. However, synthetic polymer hydrogels may necessitate additional modifications to enhance biocompatibility [16]. In summary, while natural polymer hydrogels excel in biocompatibility and mimicking the native tissue environment, synthetic polymer hydrogels provide multifunctionality and precise control over material properties, making them widely applicable in biomaterials [17]. The choice between natural and synthetic polymer hydrogels depends on the specific requirements of the desired application, balancing factors such as biocompatibility, mechanical performance, and control over functionality.

2.1.1. Carbohydrate-Based Microneedles

Carbohydrates are among the oldest MN base materials [18]. Carbohydrates provide a range of functional groups that allow tunable properties and designated performance to be designed and developed. In living beings, polysaccharides support structural components, energy storage, lubrication, and inter-cellular signal transmission. The use of natural polysaccharides for pharmaceutical applications has become commonplace due to recent discoveries regarding the novel role that biopolymers play in medicine and pharmacy. One crucial aspect of polysaccharides is their biocompatibility, making them suitable for various medical applications without causing harmful effects on the human body.

In addition to biocompatibility, specific polysaccharides have been found to possess bioactive properties, such as the ability to inhibit cancer cells [19]. For example, polysaccharide-based pectin inhibits cancer cells by inducing apoptosis [20], as do extracts from *Grateloupia longifolia*, *Gracilaria lemaneiformis*, and other plants [21]. Therefore, natural polysaccharides with bioactive properties are critical biomaterials for healthcare management.

Chitosan or Chitosan Derivatives

The alkaline glucosamine and *N*-acetyl glucosamine copolymer chitosan (CS) is derived from chitin, which originates from lower life forms such as certain algae, fungi, invertebrates, insects, or crustaceans. This material's superior biocompatibility, biodegradability, and antimicrobial properties make it an essential component of food and biomedical engineering [22]. CS has also been widely used in medicine, cosmetics, and water treatment applications due to its enhanced water solubility and antibacterial activity, which are attributed to the superior interaction between carboxymethyl groups and water and its inherent biocompatibility.

Chitosan hydrogel MNs have also been explored for transmucosal vaccine delivery, e.g., via the mouth or nose portal [23]. Bobbala and Hook delivered an antigen in chitosan hydrogel MNs to the oral mucosa in a rat model and elicited a robust immune response, suggesting the feasibility of HFM oral vaccine delivery [24].

Concerning oral/dental applications, the use of dental pulp stem cell-derived exosomes (DPSC-Exos) was explored [25], targeting the treatment of periodontitis, which is an oral disease condition characterized by a high proportion of proinflammatory macrophages as a result of immune responses to periodontopathogenic bacteria. DPSC-Exos was capable of suppressing periodontal inflammation and modulating immune response due to miR-1246 present in DPSC-Exos, a therapeutic potential in treating periodontitis based on a Gene Ontology term enrichment analysis. The research team demonstrated that incorporating DPSC-Exos into chitosan hydrogel (DPSC-Exos/CS) accelerates healing in mice periodontitis models, which changed the proinflammatory macrophage phenotypes to anti-inflammatory. Further research is on the way for the therapeutic agent carried by chitosan hydrogel [25].

Hyaluronic Acid

Hyaluronic acid (HA) is a glycosaminoglycan found as a natural constituent in epithelia, dermis, and connective tissues, including dental pulp and periodontal connective tissue. The skin contains approximately 50% of the total body HA. The human body degrades about one-third of its total HA daily and replaces the same amount with newly synthesized HA. During physiological conditions, HA is converted to its more hydrophilic sodium salt. It can hold

1000 times its own water weight because HA contains numerous hydroxyl groups. HA water retention ability enables many homeostatic functions as well as support and maintenance of diverse physiological processes in the human body. HA MNs are highly biocompatible, resistant to deformation, and are a common ingredient in skin care products [26].

Moreover, HA MNs are strong enough to penetrate the skin, readily dissolve, and release drug/active ingredients within a predetermined short period. Furthermore, no requirement of heating or organic solvents during HA MN fabrication enhances the preservation and stability of heat- and/or chemical-liable agents/drugs, such as insulin. Gill and Prausnitz [27], and Liu et al. [28] were among the first groups to pioneer the fabrication of insulin-loaded HA MNs. Liu et al. demonstrated that the novel insulin-loaded HA MNs exhibited self-dissolving properties and appeared safe. Hygroscopy, stability, drug release profiles, and dissolution characteristics of the insulin-loaded HA MNs were also characterized via a diabetic rat model by the same group [28].

Oral anesthetic injections without surface anesthesia can increase pain and anxiety during dental therapy. An HA MN patch with MN tips containing fast-dissolving lidocaine hydrochloride (LDC) was developed to address this problem. In the isolated porcine oral mucosa, LDC-containing HA MN patches were able to puncture the stratum corneum at a depth of approximately 0.28 mm. The fast-dissolving LDC on the HA MN patch enables anesthesia within 3 min at the tentative local anesthesia injection site. The adhesive MN patch was proposed to process the potential to aid transmucosal delivery of anesthetics, further enhancing the delivery of pain-free dental treatments. [29].

Sodium Alginate

Sodium alginate (SA) is a linear polysaccharide derived from brown algae. It is composed of poly-(1,4-diaminobenzoic acid) and nanoparticles of 1,4-L-glucuronide in different proportions, and its aqueous solution is highly viscous. As a result of the high number of carboxyl groups and hydroxyl groups in the molecular structure, SA has a high degree of chemical activity and can rapidly form a hydrogel containing a three-dimensional mesh structure, and it is non-toxic and odorless and exhibits outstanding biocompatibility and environmental friendliness. Safe dental application of alginate, e.g., dental impression materials, could be dated back to the 1950s [30]. SA and its derivatives are also readily available, inexpensive, simple, and renewable [31].

Blood loss is a common complication following trauma and surgery that can cause serious harm to the body [32]. Alginate is an excellent hemostatic polymer-based biomaterial as it is biocompatible, biodegradable, non-toxic, quickly gelled, and widely available [33]. In the medical field, SA hydrogels have been widely used, including injectable hydrogels [34], hemostatic needles [35], medical dressings [36], etc.

A North American group developed an injectable, biodegradable scaffold based on alginate microbeads for in vitro bone tissue engineering using periodontal ligament and gingival mesenchymal stem cells [37][38]. The stem cells remained viable in the laboratory and could differentiate into osteogenic and adipogenic tissues. Furthermore, the degradation behavior and swelling kinetics of the scaffold were characterized. Therefore, alginate has proven to be

a promising non-toxic scaffold for stem cells, providing a good strategy for engineering bone tissue [39]. Additionally, a microneedle-mediated drug delivery system containing sodium alginate for immunochemotherapy has been developed. In glioma-bearing mice, microneedles loaded with lipopolysaccharide (LPS) and doxorubicin (DOX) demonstrated excellent efficacy in promoting immune response and inhibiting tumor growth [40].

Pullulan

Pullulan (PL) is an α-(1→6)-linked (1→4)-α-d-tri-glucosides polysaccharide/glucan or maltotriose, which is a carbohydrate biopolymer produced by *Aureobasidium pullulans*. The application of pullulan-based MN has been advocated since 2020 [41][42]. The authors present the first dissolving microneedle (DMN) system using PL. A variety of concentrations of PL gels were tested for viscosity and film formation, and then MNs were created using the appropriate concentration of PL gels. PL DMN was loaded with model molecules and proteins/peptides, and their stability was assessed using circular dichroism. Ex vivo studies were conducted using Franz diffusion cells to determine the permeation of Flu-Na and FITC-BSA-loaded PL-DMN into porcine skin. The findings suggest that PL DMNs can be effective for transdermal drug delivery [43].

In another study, transdermal insulin delivery was achieved using pullulan MN patches. MNs penetrated skin up to 0.38 mm depth and dissolved within two hours, releasing up to 87% of insulin. Storing insulin-loaded MNs at 4–40 °C for four weeks is possible without losing their structure. Additionally, PL MNs were non-cytotoxic, indicating they are suitable for skin application. A non-invasive treatment option for insulin could be made possible by PL MNs based on these findings [44].

Cellulose

In recent years, cellulose, a versatile and biocompatible material, has been garnering attention for its potential use in manufacturing microneedles for transdermal drug delivery [45]. Two studies have examined the use of cellulose-based microneedles and their unique properties. The one-step created semi-dissolving microneedles comprised a water-soluble needle layer and a backing layer containing 2,2,6,6-tetramethylpiperidine-1-oxyl-oxidized bacterial cellulose nanofibers. That material was used as drug reservoirs for delivering more significant quantities of drugs to the skin [46]. Another study examined the inclusion of cellulose nanofibers in dissolving microneedle arrays. Including cellulose nanofibers increased needle stiffness and decreased dissolving and transdermal delivery rates [47].

The cellulose-based microneedle is capable of delivering drugs transdermally. By leveraging the unique properties of cellulose, these microneedles improve drug loading capacity, enhance mechanical properties, and provide controlled drug release, which opens up new possibilities for advancing transdermal drug delivery systems in diverse applications, including oral disease management.

Combined Carbohydrate-Based Microneedles

Wei et al. [48] used CS and PL as raw materials to prepare HFMs. The chitosan-based MNs they developed showed good swelling and water retention properties and biocompatibility. The application of these hydrogel-based MNs as vehicles for drug delivery was then evaluated through a skin insertion study and a drug loading/release study. The mechanical properties of the MNs allowed easy insertion into newborn porcine skin with observed rapid release of drugs [48]. Combining these two polymers may produce synergistic effects, such as improved mechanical strength, swelling behavior, and drug loading capacity [49]. Due to the swelling and water retention properties of CS and the film-forming properties of pullulan, it may be possible to deliver and release medications more effectively. Moreover, the biocompatibility of these materials may reduce the possibility of adverse reactions or tissue damage.

2.1.2. Protein-Based Microneedles

HFMs made from protein-based natural polymers are promising drug delivery systems that use biocompatible materials [50]. Proteins such as gelatin and silk fibroin are matrix-forming materials to manufacture MNs. They demonstrated significant potential in delivering transdermal drugs, vaccinations, and tissue engineering.

Water-Soluble Silk Fibroin

Silk fibroin (SF) is derived from silkworm silk after sericin degradation or degumming as a natural polymer. It comprises 18 amino acids forming a natural structural protein without physiological activity. SF is widely used in preparing sustained-release drug systems because of its biocompatibility, controllable degradation, and adjustable drug release [51][52]. The SF-based drug-sustained release systems can encapsulate and stabilize various small molecules, proteins, and large biomolecules such as DNA, facilitating controlled and prolonged content release [53]. In recent years, much attention has been given to using SF hydrogels in tissue engineering and drug delivery. It was reported that SF hydrogels prepared by various physical or chemical treatments, coupled with other biomaterials, provide a variety of drug release patterns, hence showing promise in the fields of cartilage tissue regeneration, wound repair, anti-cancer, and anti-infection therapies [54].

In an ectopic root canal transplantation model, silk fibroin scaffolds, not in MN format, though containing basic fibroblast growth factor (bFGF), were evaluated for pulp regeneration with DPSCs [55]. It was found that DPSCs seeded in the scaffold survived and displayed cytoplasmic elongation for at least four weeks in culture. Incorporating bFGF into tooth fragments and scaffolds increased the viability of DPSCs. This bFGF-incorporated scaffold generated pulp-like tissue consisting of transplanted and host-derived cells and displayed good vascularity, matrix deposition, and the formation of dentin-like tissue. The results of this study indicate that silk fibroin scaffolds incorporating bFGF are a promising candidate for future treatments in regenerative endodontics [56]. While some studies have explored the use of SF MNs for delivering other types of stem cells for tissue regeneration and wound healing applications, the specific combination of DPSCs and SF MNs in dentistry is yet to be explored.

Gelatin

Gelatin is a jelly-like substance derived from animals and composed of peptides and proteins released by partial hydrolysis of collagen, which are typically obtained from skin, bones, and connective tissues. The hydrolysis

process breaks some bonds between and within the component proteins. Several gelatin chemical characteristics are similar to those of its parent collagen. In general, pig skin and cattle bones are used to manufacture photographic and pharmaceutical-grade gelatin. In terms of its composition, gelatin falls under the category of hydrogels. Various products use gelatin, including capsules, cosmetics, ointments, and foods. Gelatin MNs can be traced to as early as 2013 [57].

Gelatin has been shown to have osteogenic potential, which is often used as a tissue engineering scaffold for bone regeneration [58]. The efficacy of bFGF-gelatin hydrogel complex, not in MN presentation, was evaluated on bone regeneration around dental implants. A total of 24 titanium implants were placed into the mandibles of 4 beagle dogs, and different amounts of bFGF were applied to fill the bone defect sites. A minimum amount of bone regeneration was observed in the groups with 0 and 0.1 mg of bFGF after eight weeks, whereas new bone formation was observed in the groups with 1, 10, and 100 mg of bFGF and autogenous bone. The results suggest that bFGF-gelatin hydrogel complexes with an optimal amount of bFGF can be used to augment bone around implants [59]. Again, the specific combination of bFGF in gelatin hydrogel MNs aiding oral bone tissue healing is yet to be explored further.

2.1.3. Mixed Carbohydrate-Protein Microneedles

One of the first gelatin MNs was a transdermal insulin delivery patch made from starch and gelatin developed by Ling and coworkers (2013). The MNs readily penetrate the test animals' skin upon application and dissolve in five minutes. The effects of insulin-loaded MNs on diabetic rats were similar to those produced by subcutaneous injections, and the hormone-loaded MNs were stable after a month of storage [57].

In a study by Jana and coworkers [60], modified sodium carboxymethyl cellulose (CMC) and gelatin were used to fabricate DMN patches. The authors took advantage of the CMC-gelatin mechanical strength while the MN vehicle itself appeared able to prevent insulin degradation, thus potentially maintaining the shelf-life of this essential hormone.

2.2. Synthetic Polymer Fabricated Hydrogel-Forming Microneedles

Hydrogel MNs made from synthetic polymers can be designed to dissolve or swell in response to moisture or heat, allowing for the controlled release of drugs or other substances into the body. The mechanical properties of synthetic polymer fabricated MNs could be more substantial and usually more consistent than that of natural polymer fabricated MNs, thus improving their effectiveness and reliability. Additionally, synthetic polymers can be customized to acquire specific properties, including tunable degradation rates enabling more versatility for drug delivery.

2.2.1. Gelatin Methacryloyl

Gelatin methacryloyl (GelMA) is a light-cure hydrogel developed by Van den Bulcke and coworkers [61]. The biocompatibility and controlled molding of GelMA led to the extensive application of the agent in the biomedical

field shortly after its development and commercialization. By reacting methacrylic anhydride (MA) with amino groups, the double-bond amide groups would be grafted onto gelatin chains, then cross-linking of the latter would be facilitated through amide chemical reaction induced by UV activation of photoinitiators [62] (**Figure 2**). Since GelMA also exhibits good biodegradability and moldability, it has attracted considerable research interest. The unique characteristics of GelMA hydrogel and its simplicity of preparation made it a good MN candidate for wound healing dressings, drug delivery, biosensing, and tissue regeneration in a wide range of biomedical applications [63]. It was reported that GelMA could be used to prepare MN arrays with acceptable release profiles suitable for the delivery of water-soluble drugs [64]. GelMA hydrogel MNs have numerous applications in the medical field, such as transdermal insulin delivery, wound healing promotion, tissue regeneration, and biosensing. GelMA-based microneedles have been explored in wound healing, which possess adhesive properties and can be loaded with growth factors or other therapeutic agents or cells to promote wound healing processes. Incorporating GelMA microneedles into a wound site allows a controlled release of bioactive molecules, leading to accelerated tissue regeneration and improved healing outcomes [55][65][66]. Despite this, GelMA hydrogel MNs hold great potential for dental and oral applications due to their biocompatibility, biodegradability, and tunable mechanical properties, particularly local anesthesia, periodontal disease treatment, and/or controlled drug delivery.

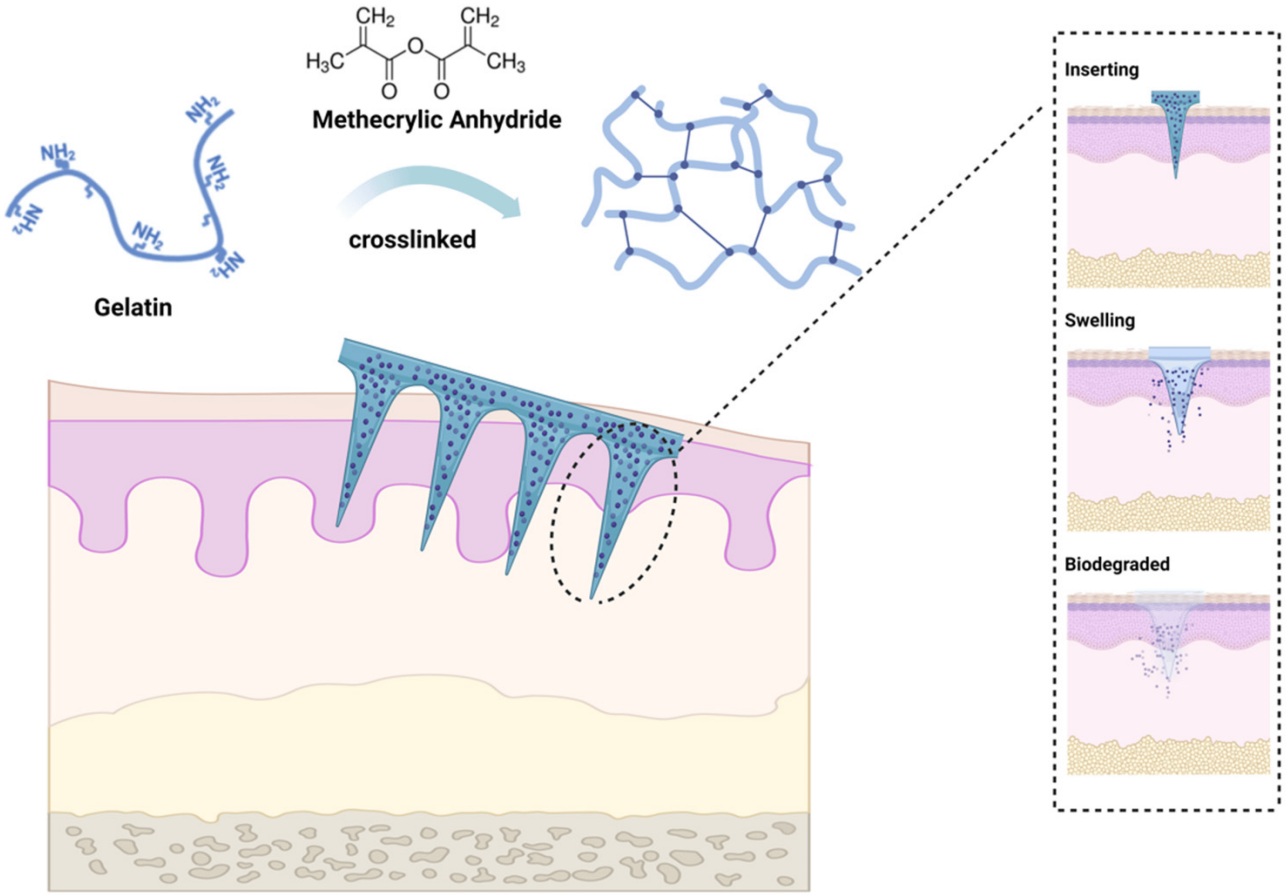

Figure 2. Gelatin methacryloyl (GelMA) microneedle application. GelMA is a cross-linked hydrogel material prepared by grafting methacrylic anhydride (MA) onto gelatin. GelMA MN exhibits sufficient mechanical toughness to penetrate the skin upon insertion, followed by swelling, degradation, and drug release, leaving no residue within the skin tissue. The figure was created with BioRender.com.

2.2.2. Methacrylate-Based Hyaluronic Acid

Methacrylate-based hyaluronic acid (HAMA) is obtained by chemically modifying the natural polysaccharide HA with MA [66]. This composite hydrogel has a continuous three-dimensional network structure, good swelling properties, mechanical properties, and drug loading capacity and is highly stable in a simulated human physiological microenvironment (pH 7.4) [67]. Xu et al. [68] developed a HAMA MN patch that carries platelet-derived growth factor D (PDGF-D) and human adipose-derived stem cells (ADSCs) for the management of diabetic ulcers. According to their diabetic mouse model, the MN patch delivered ADSCs and PDGF-D appeared to promote angiogenesis and wound healing.

At the dental front, a small molecule from the thiadiazolidinone family, NP928, was designed to be directly delivered into damaged teeth via a MAHA injection. Upon being cured by dental blue light securing in situ delivery, NP928 was observed to release from MAHA, which promoted the production of reparative dentine through upregulating Wnt/β-catenin activity in pulp stem cells [67]. Along such lines, the HAMA hydrogel-based MNs format may enhance another therapeutic agent's delivery pathway in repairing damaged dental tissues.

2.2.3. Polyvinyl Alcohol

Polyvinyl alcohol (PVA) is a water-soluble polar polymer with excellent biocompatibility, biodegradability, inherent, non-toxic, and mechanical properties. It can be chemically or physically cross-linked to form a hydrogel, and due to its wound-healing properties via transforming growth factor beta (TGF-β) upregulation [69], PVA hydrogels are also widely used as a matrix material for wound dressings [70].

Stratum corneum poses a formidable barrier to effectively delivering large and/or charged macromolecules such as small interfering RNA (siRNA). The latter would be helpful in skin disorders management [71]. Despite the effectiveness of intradermal siRNA injections, the procedure is painful. MN arrays may be an alternate and effective way for nucleic acid delivery, including siRNAs in a less painful manner. To enable penetration of the skin barrier, a loadable, PVA-based dissolvable protrusion array device (PAD) was developed. A PAD-mediated siRNA delivery effectively silenced a transgenic reporter mouse model in the skin [72].

2.3. Combined Natural and Synthetic Polymer-Fabricated Hydrogel-Forming Microneedles

A study reported that PVA and chitosan (PVA/CS) nanofiber scaffolds are excellent regenerative endodontics models [73]. Using these nanofibers and ciprofloxacin and IDR-1002, a multifunctional scaffold with anti-biofilm and anti-inflammatory properties was created. It was demonstrated that tooth fragments filled with these nanofibers produced pulp-like tissue in vivo. In dentistry, this type of scaffold, in MN/MN patch presentation, could aid regeneration beyond the pulp revascularization [74].

3. Fabrication of Hydrogel-Forming Microneedles

Hydrogel MNs can be prepared in a variety of ways, varying with the hydrogel material(s) and cross-linking mechanism [75][76] (**Table 2**). As mentioned in the previous section, the materials used for the preparation include natural polymers and synthetic polymers, which can be physically cross-linked, electrostatically interacted, or chemically cross-linked to form a hydrogel with the needle patch formation, as described in this section.

Table 2. Fabrication methods for hydrogel-forming microneedles as an example of polymeric microneedle (MN) production.

Method	Description	Advantages	Disadvantages
Micro-molding method	Fabrication using a micro-molding process to create MN structures made of hydrogel materials.	• Precise control over MN dimensions • Easy integration of drug encapsulation • High reproducibility	• Limited scalability • Time-consuming process • Difficulty in complex MN designs
Casting	Casting hydrogel material into MN molds	• Simple and cost-effective fabrication • Suitable for various hydrogel materials • Scalable production	• Limited control over MN geometry • Potential for air bubble formation • Difficulty in achieving uniformity
Electrospinning	Electrospinning hydrogel solution into MN structures	• High flexibility in MN design • Fine control over MN size and shape • Enhanced mechanical strength	• Complex and expensive equipment setup • Limited drug loading capacity • Difficulties in drug release control

Method	Description	Advantages	Disadvantages
3D printed hydrogel-filled microneedle array	3D printing of hydrogel-filled MN arrays [77]	• Precise control over MN geometry • Ability to incorporate multiple drugs • Customizable drug release profiles • Potential for personalized medicine applications	• Limited drug loading capacity • Limited scalability • Limited mechanical strength

brication, ticulously mers are hydrogel formulation is carefully poured or injected into the meticulously fabricated mold cavities without air bubbles by judiciously applying vacuum or centrifugation. Next, the hydrogel is crosslinked chemically or physically to ensure it is uniform and structurally robust. In addition to dehydration, sterilization and surface modifications to enhance drug loading and release properties are some post-fabrication treatments that are meticulously handled after fabrication to enhance mechanical strength [78].

Currently, very few oral/dental MNs are available on the market. Many methods have been explored to produce MNs for dental use, including micro-molding, casting, electrospinning, and 3D-printed hydrogel-filled MN arrays. In recent years, these methods have proven to be promising for developing MNs for dental applications, such as localized delivery of drugs and vaccines into the oral cavity. However, more research is needed to explore the full potential of MNs in dentistry.

3.1. Micro-Molding Method

Micro-molding hydrogel MNs preparation is one standard method applied [79]. Typically, polydimethylsiloxane (PDMS) casts are used, which are cast around a solid master template and then cured at 70 °C for two hours. A negative micro-mold can be produced from the master template for HFM fabrication. Due to the reusable nature of the micro-mold, multiple HFM arrays could be produced easily and quickly, which is particularly advantageous for optimizing parameters. A two-step fabrication is a common technique for fabricating MNs for drug delivery, with active ingredients concentrated at the MN tips (**Figure 3**).

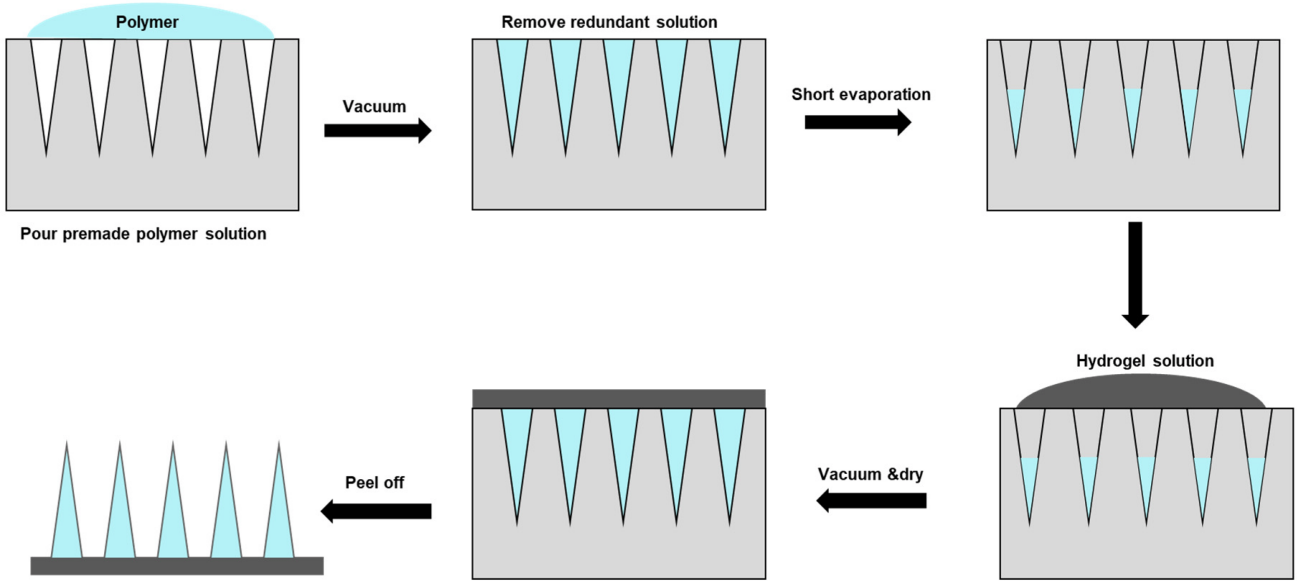

Figure 3. Hydrogel microneedle patch fabrication through a two-step method. First, the polymer solution with active ingredients was covered and filled in the polydimethylsiloxane (PDMS) female mold under a vacuum. Next, the redundant solution was removed to ensure each cavity was filled with the same volume of drug solution. The solution was then let to evaporate. Hydrogel solution was then poured into the mold under a vacuum, and the sample was allowed to cure/solidify and dry in a sealed desiccator overnight, followed by demolding, packaging, and storing.

3.2. Casting

Casting is another standard method of fabricating HFM. By using photolithography or a microfabrication technique, a master mold with the desired MN shape is created. Hydrogel material solution is then poured into the mold, typically composed of a mixture of water-soluble monomers/catalysts and the active ingredient(s). To enable solidification, the aqueous mixture is then allowed to crosslink, typically through UV exposure, heat, and/or a chemical initiator. The MNs can then be removed from the mold and stored until use [80].

The casting method enables the manufacture of MNs with complex shapes and dimensions that can be tailored to specific applications [81]. Additionally, this method allows for incorporating bioactive agents such as drugs, peptides, or growth factors into the hydrogel solution, which can be released upon MN insertion into the skin [80]. The casting method is relatively simple, cost-effective, and scalable, making it an attractive option for producing hydrogel MNs for various biomedical applications.

3.3. Electrospinning

Electrospinning involves drawing a biodegradable polymer solution, e.g., PVA, into a fine jet using a high-voltage electric field. The solvent evaporates as the jet travels toward a collector, and the polymer solidifies into a fibrous mat. HFM can be created by coating fibers with a hydrogel material, such as polyvinylpyrrolidone [82] or HA.

Parameters for electrospinning, e.g., voltage, flow rate, and distance between the needles and the collector, can be controlled to produce MNs of various shapes and sizes [83].

MNs produced by electrospinning have a high aspect ratio, which means their length exceeds their width, allowing them to penetrate the skin more efficiently and effectively [84]. Moreover, the electrospun polymer, by its nature, is capable of releasing the hydrogel-loaded drugs or active agents in a controlled manner because of the ability of manufacturers to control the diameter and length of the MNs and porous structure by adjusting the electrospinning parameters [85]. However, electrospinning is among the more complex processes and requires specialized equipment.

3.4. Three-Dimensional-Printed Hydrogel-Filled Microneedle Array

It remains uncommon that existing hydrogel-filled MN arrays are designed to deliver different therapeutic agents to diverse tissue compartments at particular vicinity and to reach different depths of the targeted tissue mass. To fulfill this, Barnum and coworkers [86] developed an MN array system composed of a 3D-printed resin-based rigid outer layer attached to a drug-washed hydrogel. By fabricating MNs of varying compositions and lengths in a single patch, the same or different drug(s) can be delivered to various depths within the target tissue mass, perhaps with varying dosing protocols. The composition of the hydrogel and the shape of the needles can be adjusted in addition to the spatial distribution of the drug(s) involved. The delivery of vascular endothelial growth factor using a hydrogel-filled MN array was pioneered as a proof-of-concept approach [86].

Informed by various imaging techniques, the hydrogel-filled, 3D-printed, UV-polymerized resin-based, custom-made MN array of different needle densities, shapes, and lengths can be designed to acquire various forms and mechanical properties to ensure successful drug and biologics delivery. In line with the abovementioned concept, polyethylene glycol diacrylate hydrogel was developed, which retains properties for therapeutic drug delivery and skin penetration efficacy. In that study, an array of 100 MNs was successfully printed that release drugs in response to delivery site stimuli/characteristics such as temperature and pH [87].

References

1. Donnelly, R.F.; Raj Singh, T.R.; Woolfson, A.D. Microneedle-based drug delivery systems: Microfabrication, drug delivery, and safety. Drug Deliv. 2010, 17, 187–207.
2. Yang, J.; Liu, X.; Fu, Y.; Song, Y. Recent advances of microneedles for biomedical applications: Drug delivery and beyond. Acta Pharm. Sin. B 2019, 9, 469–483.
3. Kim, Y.C.; Park, J.H.; Prausnitz, M.R. Microneedles for drug and vaccine delivery. Adv. Drug Deliv. Rev. 2012, 64, 1547–1568.
4. Peng, K.; Vora, L.K.; Domínguez-Robles, J.; Naser, Y.A.; Li, M.; Larrañeta, E.; Donnelly, R.F. Hydrogel-forming microneedles for rapid and efficient skin deposition of controlled release tip-

implants. Mater. Sci. Eng. C Mater. Biol. Appl. 2021, 127, 112226.

5. Donnelly, R.F.; Morrow, D.I.; McCrudden, M.T.; Alkilani, A.Z.; Vicente-Pérez, E.M.; O'Mahony, C.; González-Vázquez, P.; McCarron, P.A.; Woolfson, A.D. Hydrogel-forming and dissolving microneedles for enhanced delivery of photosensitizers and precursors. Photochem. Photobiol. 2014, 90, 641–647.
6. Damiri, F.; Kommineni, N.; Ebhodaghe, S.O.; Bulusu, R.; Jyothi, V.G.S.S.; Sayed, A.A.; Awaji, A.A.; Germoush, M.O.; Al-malky, H.S.; Nasrullah, M.Z.; et al. Microneedle-based natural polysaccharide for drug delivery systems (DDS): Progress and challenges. Pharmaceuticals 2022, 15, 190.
7. Turner, J.G.; White, L.R.; Estrela, P.; Leese, H.S. Hydrogel-forming microneedles: Current advancements and future trends. Macromol. Biosci. 2021, 21, e2000307.
8. Nele, V.; Wojciechowski, J.P.; Armstrong, J.P.; Stevens, M.M. Tailoring gelation mechanisms for advanced hydrogel applications. Adv. Funct. Mater. 2020, 30, 2002759.
9. Elisseeff, J. Structure starts to gel. Nat. Mate. 2008, 7, 271–273.
10. Jamaledin, R.; Makvandi, P.; Yiu, C.K.Y.; Agarwal, T.; Vecchione, R.; Sun, W.; Maiti, T.K.; Tay, F.R.; Netti, P.A. Engineered microneedle patches for controlled release of active compounds: Recent advances in release rrofile tuning. Adv. Ther. 2020, 3, 2000171.
11. Alkilani, A.Z.; McCrudden, M.T.; Donnelly, R.F. Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. Pharmaceutics 2015, 7, 438–470.
12. Caffarel-Salvador, E.; Brady, A.J.; Eltayib, E.; Meng, T.; Alonso-Vicente, A.; Gonzalez-Vazquez, P.; Torrisi, B.M.; Vicente-Perez, E.M.; Mooney, K.; Jones, D.S.; et al. Hydrogel-forming microneedle arrays allow detection of drugs and glucose in vivo: Potential for use in diagnosis and therapeutic drug monitoring. PLoS ONE 2015, 10, e0145644.
13. Jeon, E.Y.; Lee, J.; Kim, B.J.; Joo, K.I.; Kim, K.H.; Lim, G.; Cha, H.J. Bio-inspired swellable hydrogel-forming double-layered adhesive microneedle protein patch for regenerative internal/external surgical closure. Biomaterials 2019, 222, 119439.
14. Farias, C.; Lyman, R.; Hemingway, C.; Chau, H.; Mahacek, A.; Bouzos, E.; Mobed-Miremadi, M. Three-dimensional (3D) printed microneedles for microencapsulated cell extrusion. Bioengineering 2018, 5, 59.
15. Caliari, S.R.; Burdick, J.A. A practical guide to hydrogels for cell culture. Nat. Methods 2016, 13, 405–414.
16. Kumar, A.C.; Erothu, H. Synthetic polymer hydrogels. In Biomedical Applications of Polymeric Materials and Composites; Francis, R., Kumar, D.S., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ,

USA, 2016; Chapter 6; pp. 141–162.

17. Bao, Z.; Xian, C.; Yuan, Q.; Liu, G.; Wu, J. Natural polymer-based hydrogels with enhanced mechanical performances: Preparation, structure, and property. Adv. Healthc. Mater. 2019, 8, 1900670.
18. Donnelly, R.F.; Morrow, D.I.; Singh, T.R.; Migalska, K.; McCarron, P.A.; O'Mahony, C.; Woolfson, A.D. Processing difficulties and instability of carbohydrate microneedle arrays. Drug Dev. Ind. Pharm. 2009, 35, 1242–1254.
19. Guo, R.; Chen, M.; Ding, Y.; Yang, P.; Wang, M.; Zhang, H.; He, Y.; Ma, H. Polysaccharides as potential anti-tumor biomacromolecules -A review. Front. Nutr. 2022, 9, 838179.
20. Bian, Y.; Zeng, H.; Tao, H.; Huang, L.; Du, Z.; Wang, J.; Ding, K. A pectin-like polysaccharide from Polygala tenuifolia inhibits pancreatic cancer cell growth in vitro and in vivo by inducing apoptosis and suppressing autophagy. Int. J. Biol. Macromol. 2020, 162, 107–115.
21. Zong, A.; Cao, H.; Wang, F. Anticancer polysaccharides from natural resources: A review of recent research. Carbohydr. Polym. 2012, 90, 1395–1410.
22. Xie, Y.; Xu, B.; Gao, Y. Controlled transdermal delivery of model drug compounds by MEMS microneedle array. Nanomedicine 2005, 1, 184–190.
23. Creighton, R.L.; Woodrow, K.A. Microneedle-mediated vaccine delivery to the oral mucosa. Adv. Healthc. Mater. 2019, 8, e1801180.
24. Bobbala, S.; Hook, S. Is there an optimal formulation and delivery strategy for subunit vaccines? Pharm. Res. 2016, 33, 2078–2097.
25. Shen, Z.; Kuang, S.; Zhang, Y.; Yang, M.; Qin, W.; Shi, X.; Lin, Z. Chitosan hydrogel incorporated with dental pulp stem cell-derived exosomes alleviates periodontitis in mice via a macrophage-dependent mechanism. Bioact. Mater. 2020, 5, 1113–1126.
26. McCrudden, M.T.; McAlister, E.; Courtenay, A.J.; González-Vázquez, P.; Singh, T.R.; Donnelly, R.F. Microneedle applications in improving skin appearance. Exp. Dermatol. 2015, 24, 561–566.
27. Gill, H.S.; Prausnitz, M.R. Coating formulations for microneedles. Pharm. Res. 2007, 24, 1369–1380.
28. Liu, S.; Jin, M.-n.; Quan, Y.-s.; Kamiyama, F.; Katsumi, H.; Sakane, T.; Yamamoto, A. The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. J. Control. Release 2012, 161, 933–941.
29. Zhu, T.; Yu, X.; Yi, X.; Guo, X.; Li, L.; Hao, Y.; Wang, W. Lidocaine-loaded hyaluronic acid adhesive microneedle patch for oral mucosal topical anesthesia. Pharmaceutics 2022, 14, 686.

30. Rubel, B.S. Impression materials: A comparative review of impression materials most commonly used in restorative dentistry. Dent. Clin. N. Am. 2007, 51, 629–642.
31. Demir, Y.K.; Akan, Z.; Kerimoglu, O. Characterization of polymeric microneedle arrays for transdermal drug delivery. PLoS ONE 2013, 8, e77289.
32. Rossaint, R.; Bouillon, B.; Cerny, V.; Coats, T.J.; Duranteau, J.; Fernández-Mondéjar, E.; Hunt, B.J.; Komadina, R.; Nardi, G.; Neugebauer, E.; et al. Management of bleeding following major trauma: An updated European guideline. Crit. Care 2010, 14, R52.
33. Xie, Y.; Gao, P.; He, F.; Zhang, C. Application of alginate-based hydrogels in hemostasis. Gels 2022, 8, 109.
34. Zhai, Z.; Xu, K.; Mei, L.; Wu, C.; Liu, J.; Liu, Z.; Wan, L.; Zhong, W. Co-assembled supramolecular hydrogels of cell adhesive peptide and alginate for rapid hemostasis and efficacious wound healing. Soft Matter 2019, 15, 8603–8610.
35. Xu, Y.; Wang, Z.; Wei, S.; Sun, P.; Bai, H. Hydrogel-coated needles prevent puncture site bleeding in arteriovenous fistula and arteriovenous grafts in rats. Biomed. Pharmacother. 2021, 143, 112113.
36. Zhang, X.; Huang, C.; Zhao, Y.; Jin, X. Preparation and characterization of nanoparticle reinforced alginate fibers with high porosity for potential wound dressing application. RSC Adv. 2017, 7, 39349–39358.
37. Ansari, S.; Diniz, I.M.; Chen, C.; Sarrion, P.; Tamayol, A.; Wu, B.M.; Moshaverinia, A. Human periodontal ligament- and gingiva-derived mesenchymal stem cells promote nerve regeneration when encapsulated in alginate/hyaluronic acid 3D scaffold. Adv. Healthc. Mater. 2017, 6, 1700670.
38. Moshaverinia, A.; Chen, C.; Akiyama, K.; Xu, X.; Chee, W.W.; Schricker, S.R.; Shi, S. Encapsulated dental-derived mesenchymal stem cells in an injectable and biodegradable scaffold for applications in bone tissue engineering. J. Biomed. Mater. Res. A 2013, 101, 3285–3294.
39. Moshaverinia, A.; Chen, C.; Akiyama, K.; Ansari, S.; Xu, X.; Chee, W.W.; Schricker, S.R.; Shi, S. Alginate hydrogel as a promising scaffold for dental-derived stem cells: An in vitro study. J. Mater. Sci. Mater. Med. 2012, 23, 3041–3051.
40. Darge, H.F.; Lee, C.-Y.; Lai, J.-Y.; Lin, S.-Z.; Harn, H.-J.; Chen, Y.-S.; Tsai, H.-C. Separable double-layered microneedle-based transdermal codelivery of DOX and LPS for synergistic immunochemotherapy of a subcutaneous glioma tumor. Chem. Eng. J. 2022, 433, 134062.
41. Tian, Y.; Lee, J.; Bhide, Y.C.; van der Maaden, K.; Huckriede, A.L.; Jiskoot, W.; Frijlink, H.W.; Hinrichs, W.L.; Bouwstra, J.A. Intradermal administration of influenza vaccine with trehalose and pullulan-based dissolving microneedle arrays. J. Pharm. Sci. 2022, 111, 1070–1080.

42. Fonseca, D.F.S.; Costa, P.C.; Almeida, I.F.; Dias-Pereira, P.; Correia-Sá, I.; Bastos, V.; Oliveira, H.; Duarte-Araújo, M.; Morato, M.; Vilela, C.; et al. Pullulan microneedle patches for the efficient transdermal administration of insulin envisioning diabetes treatment. Carbohydr. Polym. 2020, 241, 116314.
43. Vora, L.K.; Courtenay, A.J.; Tekko, I.A.; Larrañeta, E.; Donnelly, R.F. Pullulan-based dissolving microneedle arrays for enhanced transdermal delivery of small and large biomolecules. Int. J. Biol. Macromol. 2020, 146, 290–298.
44. Chang, C.; Zhang, L. Cellulose-based hydrogels: Present status and application prospects. Carbohydr. polym. 2011, 84, 40–53.
45. Song, J.E.; Jun, S.H.; Park, S.G.; Kang, N.G. A semi-dissolving microneedle patch incorporating TEMPO-oxidized bacterial cellulose nanofibers for enhanced transdermal delivery. Polymers 2020, 12, 1873.
46. Kim, J.A.; Park, S.C.; Lee, S.-J.; Kim, J.-C. Cellulose nanofiber-reinforced dissolving microneedles for transdermal delivery of a water-soluble compound. Cellulose 2022, 29, 9881–9897.
47. Wei, H.; Liu, S.; Tong, Z.; Chen, T.; Yang, M.; Guo, Y.; Sun, H.; Wu, Y.; Chu, Y.; Fan, L. Hydrogel-based microneedles of chitosan derivatives for drug delivery. React. Funct. Polym. 2022, 172, 105200.
48. Younas, A.; Dong, Z.; Hou, Z.; Asad, M.; Li, M.; Zhang, N. A chitosan/fucoidan nanoparticle-loaded pullulan microneedle patch for differential drug release to promote wound healing. Carbohydr. Polym. 2023, 306, 120593.
49. Shabnoor, N.S.; Hema Bindu, A.; Patil, A.G.; Aishwarya, S.; More, S.S.; Khan, K.; Padyana, S.; Madhavi, J.; Yadav, A.N.; Ravish, H.; et al. Peptide and protein-based hydrogels for the encapsulation of bioactive compounds and tissue engineering applications. In Protein-Based Biopolymers. From Source to Biomedical Applications; Kalia, S., Sharma, S., Eds.; Woodhead Publishing: Sawston, UK, 2023; Chapter 13; pp. 301–331.
50. Mukherjee, S.; Krishnan, A.; Athira, R.K.; Kasoju, N.; Sah, M.K. Silk fibroin and silk sericin in skin tissue engineering and wound healing: Retrospect and prospects. In Natural Polymers in Wound Healing and Repair; Sah, M.K., Kasoju, N., Mano, J.F., Eds.; Elsevier: Amsterdam, The Netherlands, 2022.
51. Tsioris, K.; Raja, W.K.; Pritchard, E.M.; Panilaitis, B.; Kaplan, D.L.; Omenetto, F.G. Fabrication of silk microneedles for controlled-release drug delivery. Adv. Funct. Mater. 2012, 22, 330–335.
52. Tomeh, M.A.; Hadianamrei, R.; Zhao, X. Silk fibroin as a functional biomaterial for drug and gene delivery. Pharmaceutics 2019, 11, 494.

53. Kundu, B.; Rajkhowa, R.; Kundu, S.C.; Wang, X. Silk fibroin biomaterials for tissue regenerations. Adv. Drug Deliv. Rev. 2013, 65, 457–470.

54. Osathanon, T.; Nowwarote, N.; Pavasant, P. Basic fibroblast growth factor inhibits mineralization but induces neuronal differentiation by human dental pulp stem cells through a FGFR and PLCγ signaling pathway. J. Cell. Biochem. 2011, 112, 1807–1816.

55. Yang, J.-W.; Zhang, Y.-F.; Sun, Z.-Y.; Song, G.-T.; Chen, Z. Dental pulp tissue engineering with bFGF-incorporated silk fibroin scaffolds. J. Biomater. Appl. 2015, 30, 221–229.

56. Ling, M.H.; Chen, M.C. Dissolving polymer microneedle patches for rapid and efficient transdermal delivery of insulin to diabetic rats. Acta Biomater. 2013, 9, 8952–8961.

57. Kim, A.Y.; Kim, Y.; Lee, S.H.; Yoon, Y.; Kim, W.H.; Kweon, O.K. Effect of gelatin on osteogenic cell sheet formation using canine adipose-derived mesenchymal stem cells. Cell Transplant. 2017, 26, 115–123.

58. Hayashi, K.; Kubo, T.; Doi, K.; Tabata, Y.; Akagawa, Y. Development of new drug delivery system for implant bone augmentation using a basic fibroblast growth factor-gelatin hydrogel complex. Dent. Mater. J. 2007, 26, 170–177.

59. Jana, B.A.; Osmani, R.A.; Jaiswal, S.; Banerjee, R.; Karri, V.V.S.R.; Wadhwani, A. Fabrication of carboxymethylcellulose-gelatin dissolving microneedle patch for pain-free, efficient, and controlled transdermal delivery of insulin. J. Pharm. Innov. 2022; online ahead of print.

60. Van Den Bulcke, A.I.; Bogdanov, B.; De Rooze, N.; Schacht, E.H.; Cornelissen, M.; Berghmans, H. Structural and rheological properties of methacrylamide modified gelatin hydrogels. Biomacromolecules 2000, 1, 31–38.

61. Sun, M.; Sun, X.; Wang, Z.; Guo, S.; Yu, G.; Yang, H. Synthesis and properties of gelatin methacryloyl (GelMA) hydrogels and their recent applications in load-bearing tissue. Polymers 2018, 10, 1290.

62. Piao, Y.; You, H.; Xu, T.; Bei, H.-P.; Piwko, I.Z.; Kwan, Y.Y.; Zhao, X. Biomedical applications of gelatin methacryloyl hydrogels. Eng. Regen. 2021, 2, 47–56.

63. Luo, Z.; Sun, W.; Fang, J.; Lee, K.; Li, S.; Gu, Z.; Dokmeci, M.R.; Khademhosseini, A. Biodegradable gelatin methacryloyl microneedles for transdermal drug delivery. Adv. Healthc. Mater. 2019, 8, e1801054.

64. Yuan, M.; Liu, K.; Jiang, T.; Li, S.; Chen, J.; Wu, Z.; Li, W.; Tan, R.; Wei, W.; Yang, X.; et al. GelMA/PEGDA microneedles patch loaded with HUVECs-derived exosomes and tazarotene promote diabetic wound healing. J. Nanobiotechnol. 2022, 20, 147.

65. Chang, W.-C.; Tai, A.-Z.; Tsai, N.-Y.; Li, Y.-C.E. An injectable hybrid gelatin methacryloyl (GelMA)/phenyl isothiocyanate-modified gelatin (gel-phe) bioadhesive for oral/dental hemostasis

applications. Polymers 2021, 13, 2386.

66. Bencherif, S.A.; Srinivasan, A.; Horkay, F.; Hollinger, J.O.; Matyjaszewski, K.; Washburn, N.R. Influence of the degree of methacrylation on hyaluronic acid hydrogels properties. Biomaterials 2008, 29, 1739–1749.
67. Alaohali, A.; Salzlechner, C.; Zaugg, L.K.; Suzano, F.; Martinez, A.; Gentleman, E.; Sharpe, P.T. GSK3 inhibitor-induced dentinogenesis using a hydrogel. J. Dent. Res. 2021, 101, 46–53.
68. Xu, Y.; Wu, X.; Zhang, X.; Zu, Y.; Tan, Q.; Zhao, Y. Living microneedle patch with adipose-derived stem cells embedding for diabetic ulcer healing. Adv. Funct. Mater. 2023, 33, 2209986.
69. Liu, Y.; Vrana, N.E.; Cahill, P.A.; McGuinness, G.B. Physically crosslinked composite hydrogels of PVA with natural macromolecules: Structure, mechanical properties, and endothelial cell compatibility. J. Biomed. Mater. Res. B Appl. Biomater. 2009, 90, 492–502.
70. Park, K.R.; Nho, Y.C. Synthesis of PVA/PVP hydrogels having two-layer by radiation and their physical properties. Radiat. Phys. Chem. 2003, 67, 361–365.
71. Leachman, S.A.; Hickerson, R.P.; Schwartz, M.E.; Bullough, E.E.; Hutcherson, S.L.; Boucher, K.M.; Hansen, C.D.; Eliason, M.J.; Srivatsa, G.S.; Kornbrust, D.J. First-in-human mutation-targeted siRNA phase Ib trial of an inherited skin disorder. Mol. Ther. 2010, 18, 442–446.
72. Gonzalez-Gonzalez, E.; Speaker, T.J.; Hickerson, R.P.; Spitler, R.; Flores, M.A.; Leake, D.; Contag, C.H.; Kaspar, R.L. Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD). Mol. Ther. 2010, 18, 1667–1674.
73. Gonçalves da Costa Sousa, M.; Conceição de Almeida, G.; Martins Mota, D.C.; Andrade da Costa, R.; Dias, S.C.; Limberger, S.N.; Ko, F.; Lin, L.T.; Haney, E.F.; Etayash, H.; et al. Antibiofilm and immunomodulatory resorbable nanofibrous filing for dental pulp regenerative procedures. Bioact. Mater. 2022, 16, 173–186.
74. Sugiaman, V.K.; Jeffrey; Naliani, S.; Pranata, N.; Djuanda, R.; Saputri, R.I. Polymeric scaffolds used in dental pulp regeneration by tissue engineering approach. Polymers 2023, 15, 1082.
75. Pan, X.; Li, Y.; Pang, W.; Xue, Y.; Wang, Z.; Jiang, C.; Shen, C.; Liu, Q.; Liu, L. Preparation, characterisation and comparison of glabridin-loaded hydrogel-forming microneedles by chemical and physical cross-linking. Int. J. Pharm. 2022, 617, 121612.
76. Kolahdoozan, M.; Rahimi, T.; Taghizadeh, A.; Aghaei, H. Preparation of new hydrogels by visible light cross-linking of dextran methacrylate and poly (ethylene glycol)-maleic acid copolymer. Int. J. Biol. Macromol. 2023, 227, 1221–1233.
77. Dardano, P.; Caliò, A.; Di Palma, V.; Bevilacqua, M.F.; Di Matteo, A.; De Stefano, L. A Photolithographic approach to polymeric microneedles array fabrication. Materials 2015, 8, 8661–

8673.

78. Lynch, S.; Liu, C.; Morgan, N.; Xiao, X.; Gomella, A.; Mazilu, D.; Bennett, E.; Assoufid, L.; De Carlo, F.; Wen, H. Fabrication of 200 nm period centimeter area hard x-ray absorption gratings by multilayer deposition. J. Micromech. Microeng. 2012, 22, 105007.
79. Eivazzadeh-Keihan, R.; Noruzi, E.B.; Mehrban, S.F.; Aliabadi, H.A.M.; Karimi, M.; Mohammadi, A.; Maleki, A.; Mahdavi, M.; Larijani, B.; Shalan, A.E. The latest advances in biomedical applications of chitosan hydrogel as a powerful natural structure with eye-catching biological properties. J. Mater. Sci. 2022, 57, 3855–3891.
80. Gholami, S.; Mohebi, M.-M.; Hajizadeh-Saffar, E.; Ghanian, M.-H.; Zarkesh, I.; Baharvand, H. Fabrication of microporous inorganic microneedles by centrifugal casting method for transdermal extraction and delivery. Int. J. Pharm. 2019, 558, 299–310.
81. Krieger, K.J.; Bertollo, N.; Dangol, M.; Sheridan, J.T.; Lowery, M.M.; O'Cearbhaill, E.D. Simple and customizable method for fabrication of high-aspect ratio microneedle molds using low-cost 3D printing. Microsyst. Nanoeng. 2019, 5, 42.
82. Qiang, N.; Liu, Z.; Lu, M.; Yang, Y.; Liao, F.; Feng, Y.; Liu, G.; Qiu, S. Preparation and properties of polyvinylpyrrolidone/Sodium Carboxymethyl pellulose poluble microneedles. Materials 2023, 16, 3417.
83. Yang, H.; Kim, S.; Huh, I.; Kim, S.; Lahiji, S.F.; Kim, M.; Jung, H. Rapid implantation of dissolving microneedles on an electrospun pillar array. Biomaterials 2015, 64, 70–77.
84. Li, W.J.; Laurencin, C.T.; Caterson, E.J.; Tuan, R.S.; Ko, F.K. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. J. Biomed. Mater. Res. 2002, 60, 613–621.
85. Zhang, Y.; Su, B.; Venugopal, J.; Ramakrishna, S.; Lim, C. Biomimetic and bioactive nanofibrous scaffolds from electrospun composite nanofibers. Int. J. Nanomed. 2007, 2, 623–638.
86. Barnum, L.; Quint, J.; Derakhshandeh, H.; Samandari, M.; Aghabaglou, F.; Farzin, A.; Abbasi, L.; Bencherif, S.; Memic, A.; Mostafalu, P.; et al. 3D-printed hydrogel-filled microneedle arrays. Adv. Healthc. Mater. 2021, 10, 2001922.
87. Kundu, A.; Arnett, P.; Bagde, A.; Azim, N.; Kouagou, E.; Singh, M.; Rajaraman, S. DLP 3D printed "intelligent" microneedle array (iμNA) for stimuli responsive release of drugs and its in vitro and ex vivo characterization. J. Microelectromech. Syst. 2020, 29, 685–691.