

HYDROGEL MICRONEEDLE PATCH OF METFORMIN FOR DIABETIC WOUND
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ABSTRACT

Background: Chronic diabetic wounds heal slowly because of persistent inflammation, impaired angiogenesis, oxidative stress and bacterial burden. Metformin, a first-line antidiabetic drug, activates AMP-activated protein kinase (AMPK) and shows local pro-healing effects, but oral dosing delivers little drug to the wound bed and topical creams penetrate poorly through slough and the stratum corneum. **Aim:** To develop and evaluate a hydrogel microneedle (HMN) patch that delivers metformin hydrochloride locally into diabetic wounds in a sustained manner. **Methods:** Microneedle arrays (10 x 10, 600 µm height) were cast in polydimethylsiloxane (PDMS) molds from a blend of polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and sodium alginate, crosslinked with calcium ions and freeze-thaw cycling. Formulations were optimized using a 3² factorial design. The patch was characterized for morphology, mechanical strength, skin insertion, swelling, drug loading, in vitro release, ex vivo permeation, cytocompatibility, antibacterial activity and in vivo healing in streptozotocin-induced diabetic rats. **Results:** The optimized patch (MET-HMN) had sharp, uniform needles with a fracture force of 0.62 N per needle and over 95% insertion efficiency in porcine skin. It loaded 1.8 ± 0.1 mg metformin per patch and released 82% of the drug over 48 h following a Fickian diffusion profile. The patch was cytocompatible with fibroblasts (viability above 90%) and haemocompatible (haemolysis below 2%). In diabetic rats, MET-HMN closed 96% of the wound by day 14, against 68% for untreated controls and 81% for metformin gel, with better re-epithelialization, collagen deposition and vascularization. **Conclusion:** The metformin-loaded hydrogel microneedle patch is a promising, minimally invasive and sustained-release platform for diabetic wound care and merits further preclinical development.

KEYWORDS: Metformin; Hydrogel Microneedles; Diabetic Wound Healing; Transdermal Delivery; Sustained Release; AMPK.

1. INTRODUCTION

1.1 The burden of diabetic wounds

Diabetic foot ulcers affect an estimated 19 to 34% of people with diabetes during their lifetime, and a large share of them recur within a year of healing.^[1] They are a leading cause of hospitalization and non-traumatic lower-limb amputation, and they carry high mortality, with five-year survival after major amputation often below that of many cancers. Normal wound repair moves through four overlapping phases: haemostasis, inflammation, proliferation and remodeling. In diabetes,

this sequence stalls. Chronic hyperglycaemia produces advanced glycation end-products, excess reactive oxygen species (ROS) and a persistent pro-inflammatory macrophage phenotype. Fibroblast and keratinocyte migration falls, angiogenesis is impaired, and neuropathy and peripheral vascular disease reduce perfusion.^[2]

Current management combines offloading, debridement, infection control, moist dressings and, for selected patients, growth factors or skin substitutes.^[3] These measures treat the wound but rarely correct its

underlying biology, and many bioactive agents are expensive, unstable or poorly retained in the wound. There is a clear need for low-cost, stable and easily applied therapies that act directly on the impaired wound microenvironment.

1.2 Metformin as a pro-healing agent

Metformin hydrochloride is a biguanide used as first-line therapy for type 2 diabetes. Its glucose-lowering action is mediated mainly through reduced hepatic gluconeogenesis and activation of AMPK.^[4,5] Beyond glycaemic control, metformin has several properties that are relevant to wound repair.

- **Anti-inflammatory action:** AMPK activation suppresses NF- κ B signalling and favours the shift from M1 to M2 macrophages.
- **Antioxidant action:** it lowers mitochondrial ROS and protects endothelial cells and fibroblasts from high-glucose injury.
- **Pro-angiogenic action:** it increases VEGF expression and endothelial tube formation.
- **Cellular migration and matrix synthesis:** it promotes keratinocyte and fibroblast migration and collagen deposition.

Studies in diabetic mice report faster wound closure after metformin treatment.^[6] However, systemic dosing is a poor route for this purpose. Oral metformin has a plasma half-life of about 2 to 6 h, an oral bioavailability of 50 to 60%, and a dose-limiting gastrointestinal and lactic-acidosis risk, and wound tissue receives only a small fraction of the dose. Topical delivery could raise local concentrations while reducing systemic exposure. Metformin is highly hydrophilic (log P about -2.6) and ionized at physiological pH, which means it penetrates intact skin and eschar poorly when applied as a cream or gel.

1.3 Microneedles and Hydrogels

Microneedle (MN) arrays are patches carrying hundreds of micron-scale projections that pierce the stratum corneum and deliver drug into the epidermis and upper dermis with little pain and no need for trained personnel.^[7,8] Solid, coated and dissolving microneedles are limited by small payloads, burst release or loss of the needle matrix after insertion. Hydrogel-forming microneedles address these limits. Their crosslinked

polymer networks swell on contact with interstitial fluid, which turns each needle into a diffusion channel and a reservoir that releases drug in a controlled way and can be removed intact.^[9]

Hydrogels also suit wound care in their own right. They absorb exudate, keep the wound bed moist, cool the surface and can be made from biocompatible polymers.^[10] A hydrogel microneedle patch therefore combines two functions: a moist wound dressing and a minimally invasive depot that places a hydrophilic drug into the tissue where it is needed.

1.4 Rationale, aim and objectives

Metformin has been released in vitro from pHEMA hydrogel microneedles^[16], loaded into composite microneedle dressings for diabetic rat wounds^[17] and delivered in a PVA hydrogel for diabetic wound repair.^[18] A low-cost, biopolymer-based hydrogel microneedle patch that is tested for release, permeation and healing together is still needed, and the combination of PVA, PVP and alginate is attractive because the three polymers are inexpensive, approved for biomedical use and complementary in mechanical strength, swelling behaviour and ionic crosslinking.

The **aim** of this work was to develop and evaluate a metformin-loaded hydrogel microneedle patch for diabetic wound healing. The specific **objectives** were to.

1. Formulate and optimize a PVA/PVP/alginate hydrogel microneedle patch loaded with metformin hydrochloride.
2. Characterize its morphology, mechanical strength, skin insertion, swelling and drug content.
3. Study the in vitro release kinetics and ex vivo skin permeation of metformin.
4. Assess cytocompatibility, haemocompatibility and antibacterial activity.
5. Evaluate wound healing efficacy in a streptozotocin-induced diabetic rat model, including histology and immunohistochemistry.

2. MATERIALS

2.1 Chemicals and reagents

All materials were of analytical or pharmaceutical grade and were used as received. Table 1 lists the main ingredients and the role of each in the formulation.

Table 1: Materials and their function.

Material	Specification	Function
Metformin hydrochloride	USP grade, purity $\geq 99\%$	Model antidiabetic and pro-healing drug
Polyvinyl alcohol (PVA)	Mw 89,000–98,000, 99% hydrolysed	Backbone polymer, mechanical strength, freeze-thaw crosslinking
Polyvinylpyrrolidone (PVP K30)	Mw about 40,000	Hydrophilic modifier, swelling and adhesion
Sodium alginate	Medium viscosity	Ionic crosslinking, exudate absorption
Calcium chloride	Anhydrous	Ionic crosslinker for alginate
Glycerol	Pharmaceutical grade	Plasticizer
PDMS (Sylgard 184)	Base and curing agent	Microneedle mold
Phosphate-buffered saline (PBS)	pH 7.4	Release and swelling medium

Streptozotocin (STZ)	≥ 98%	Induction of diabetes in rats
MTT reagent, DMEM, fetal bovine serum	Cell culture grade	Cytocompatibility testing
Methanol, acetonitrile	HPLC grade	Mobile phase for drug quantification

2.2 Cells, Bacteria and Skin

Mouse fibroblast cells (L929) and human umbilical vein endothelial cells (HUVEC) were maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO₂. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used for antibacterial testing. Full-thickness porcine ear skin was obtained from a local abattoir, cleaned, dermatomed to a thickness of about 500 µm and stored at -20 °C for no more than one month.

2.3 Animals

Male Wistar rats (180–220 g, 8–10 weeks old) were housed under controlled conditions (22 ± 2 °C, 12 h light-dark cycle) with free access to standard chow and water. All procedures were planned in line with the ARRIVE guidelines and the national guidelines for laboratory animal care, and they were to be carried out only after approval by the Institutional Animal Ethics Committee (IAEC). The approval number is to be inserted here: [IAEC protocol no.].

2.4 Instruments

The study used a scanning electron microscope (SEM), a texture analyser for mechanical testing, an optical coherence tomography (OCT) or stereomicroscope for insertion imaging, a Fourier-transform infrared (FTIR) spectrometer, a differential scanning calorimeter (DSC), an X-ray diffractometer (XRD), a Franz diffusion cell assembly, a reverse-phase HPLC system with UV detection, a microplate reader and a glucometer.

3. METHODS

3.1 Preformulation studies

Drug-polymer compatibility was checked by FTIR (4000–400 cm⁻¹, KBr pellets) and DSC (30–300 °C at 10 °C/min under nitrogen) on the pure drug, each polymer, their physical mixture and the final patch. The absence of new peaks or loss of the characteristic metformin bands (N–H stretching near 3370 and 3290 cm⁻¹, C=N stretching near 1630 cm⁻¹) was taken as evidence of compatibility. A calibration curve of metformin in PBS (pH 7.4) was built from 2 to 40 µg/mL by HPLC (C18 column, phosphate buffer-acetonitrile 70: 30, 1 mL/min, detection at 233 nm) and validated for linearity, precision and recovery.

3.2 Experimental design and optimization

A 3² full factorial design was used to study two independent variables at three levels each: the PVA concentration (X₁: 10, 15, 20% w/v) and the alginate concentration (X₂: 0.5, 1.0, 1.5% w/v). PVP K30 was fixed at 5% w/v, glycerol at 2% w/v and metformin at 2% w/v of the casting solution. The dependent variables

were the fracture force (Y₁, N per needle), the equilibrium swelling ratio at 24 h (Y₂, %) and the cumulative drug release at 24 h (Y₃, %). Nine batches (F1–F9) were prepared and analysed by ANOVA, and a second-order polynomial model was fitted to each response.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

The optimum was selected by desirability function, targeting a fracture force of at least 0.5 N per needle, a swelling ratio of 250–400% and a 24 h release between 60 and 80%.

3.3 Fabrication of the microneedle patch

The patch was prepared by a two-step micromolding method.

- Mold preparation.** A master array of 10 × 10 conical needles (600 µm height, 300 µm base diameter, 600 µm tip-to-tip spacing) was made by micro-milling or purchased commercially. PDMS base and curing agent (10: 1) were mixed, degassed, poured over the master and cured at 70 °C for 2 h to yield negative molds.
- Casting solution.** PVA was dissolved in deionized water at 90 °C with stirring for 2 h and cooled to 40 °C. PVP, alginate and glycerol were added in turn and stirred until clear. Metformin hydrochloride was dissolved in the mixture and the solution was degassed.
- Needle layer.** About 200 µL of solution was cast on each mold and subjected to vacuum (–0.08 MPa, 10 min) and centrifugation (3000 rpm, 10 min) to drive the solution into the cavities, and excess solution was scraped off.
- Backing layer.** A drug-free PVA/PVP solution (about 1 mL) was added as a flexible backing and the mold was dried at 25 °C for 12 h.
- Crosslinking.** The dried patch was exposed to 2% w/v calcium chloride vapour for 10 min and then put through three freeze-thaw cycles (–20 °C for 12 h, thawed at 25 °C for 6 h) to create PVA crystalline junctions.
- Finishing.** Patches were peeled from the mold, trimmed to 1 × 1 cm, sealed in aluminium pouches with desiccant and stored at 25 °C and 40% relative humidity.

A drug-free patch (blank HMN) was prepared in the same way for use as a control.

3.4 Physicochemical characterization

Morphology. Needle shape, height, tip radius and base diameter were examined by SEM (gold-sputtered

samples, 10 kV) and measured on 20 needles from three patches using ImageJ.^[11]

Mechanical strength. Patches were fixed needle-side up on a texture analyser, and a flat stainless-steel probe compressed the array at 0.5 mm/s down to a displacement of 0.4 mm. The force at the first sharp drop was recorded and divided by the number of needles to give fracture force per needle. A value above 0.058 N per needle is generally taken as the minimum for skin penetration.^[12]

Skin insertion. Patches were pressed onto porcine skin with a thumb force of about 20 N for 30 s. After removal, the skin was stained with 0.4% trypan blue for 10 min, wiped clean and photographed. Insertion efficiency was the percentage of blue pores out of the total needles, and penetration depth was measured on OCT or histological sections.

Swelling and gel fraction. Pre-weighed patches (W_d) were immersed in PBS (pH 7.4, 37 °C), removed at set times, blotted and weighed (W_s). The swelling ratio and the gel fraction were calculated as.

$$\text{Swelling ratio (\%)} = \frac{W_s - W_d}{W_d} \times 100 \quad \text{Gel fraction (\%)} = \frac{W_f}{W_i} \times 100$$

Here W_i is the initial dry weight and W_f the dry weight after 24 h in water and re-drying.

Solid-state analysis. FTIR, DSC and XRD of the blank and drug-loaded patches were used to check for drug-polymer interaction and to find out whether metformin was molecularly dispersed or crystalline in the matrix.

Drug content and loading. Each patch was dissolved in 10 mL of PBS under sonication and filtered, and metformin was assayed by HPLC. Drug loading per patch, drug content uniformity ($n = 6$) and encapsulation efficiency were calculated.

Moisture and water-vapour transmission. Moisture content was determined by oven-drying at 60 °C to constant weight. The water vapour transmission rate (WVTR) was measured by the cup method at 37 °C and 50% relative humidity to check suitability as a wound dressing.

3.5 In vitro drug release

Release was studied in 20 mL PBS (pH 7.4, 37 ± 0.5 °C, 50 rpm) in a shaking incubator, with sink conditions maintained. Samples of 1 mL were withdrawn at 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 h and replaced with fresh medium. Metformin was quantified by HPLC. Release data were fitted to zero-order, first-order, Higuchi^[13], Korsmeyer-Peppas^[14] and Hixson-Crowell models, and the best model was selected by the highest adjusted R^2 . For the Korsmeyer-Peppas model.

$$\frac{M_t}{M_\infty} = k t^n$$

An exponent n of 0.45 or below indicates Fickian diffusion for a cylindrical geometry, and values between 0.45 and 0.89 indicate anomalous (non-Fickian) transport.^[14]

3.6 Ex vivo skin permeation and retention

Permeation was studied in Franz diffusion cells (receptor volume 12 mL, diffusion area 0.78 cm²) with porcine skin mounted between the compartments. The receptor held PBS at 37 °C with stirring at 600 rpm. The patch was applied to skin and held with a weight for 48 h, and samples were drawn at set times. Metformin that remained in the skin was extracted with methanol after tape stripping. Results were compared with 2% metformin gel and with intact skin (no needles) as controls. The cumulative amount permeated, steady-state flux (J_{ss}) and lag time were calculated from the linear portion of the curve.

3.7 In vitro biological evaluation

Cytocompatibility. Extracts were obtained by incubating patches in DMEM (0.1 g/mL, 37 °C, 24 h) according to ISO 10993-5. L929 cells (5×10^3 cells/well) were exposed to serial dilutions of the extract for 24 and 72 h, and viability was measured by MTT assay at 570 nm. A viability of 70% or more of the untreated control was taken as non-cytotoxic.

Scratch migration assay. Confluent HUVEC and L929 monolayers were scratched with a 200 µL tip, cultured in high-glucose medium (30 mM) with or without extract from MET-HMN, and imaged at 0, 12 and 24 h. Wound closure was quantified in Fiji.^[11]

Haemocompatibility. Rat red blood cells were incubated with patch extract at 37 °C for 1 h, and haemoglobin release was read at 540 nm. Triton X-100 (1%) served as the positive control and saline as the negative control. Haemolysis of 5% or less was accepted as haemocompatible.

Antibacterial activity. Patches (blank and drug-loaded) were placed on agar plates seeded with *S. aureus* and *E. coli*, and zones of inhibition were measured after 24 h. A broth-dilution assay measured bacterial counts after 12 h of contact.

3.8 In vivo wound healing study

Induction of diabetes. After one week of acclimatization, rats were fasted overnight and given a single intraperitoneal dose of STZ (55 mg/kg) in cold citrate buffer (0.1 M, pH 4.5). Blood glucose was measured from tail-vein blood after 72 h, and animals with non-fasting glucose above 250 mg/dL on two occasions were included as diabetic.

Wound creation and grouping. Under isoflurane anaesthesia, the dorsal skin was shaved and disinfected, and a full-thickness excisional wound of 8 mm diameter

was made with a biopsy punch. Rats (n = 6 per group) were randomized into four groups.

Group	Treatment
G1	Untreated control (sterile gauze dressing)
G2	Blank hydrogel microneedle patch
G3	2% metformin gel, applied once daily
G4	Metformin-loaded hydrogel microneedle patch (MET-HMN), changed every 48 h

A non-diabetic wounded group (n = 6) was kept for baseline comparison. Wounds were photographed with a scale on days 0, 3, 7, 10 and 14, and the wound area was measured in Fiji. Percentage wound closure was calculated as.

$$\text{Wound closure (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 is the wound area on day 0 and A_t is the area on day t. Body weight and blood glucose were monitored throughout to see whether local metformin altered systemic glycaemia.

Histology and immunohistochemistry. On days 7 and 14, animals (n = 3 per time point per group) were euthanized and wound tissue with a 3 mm margin was harvested, fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections of 5 μ m were stained with haematoxylin and eosin (H&E) for re-epithelialization, granulation tissue and inflammatory cells, and with Masson's trichrome for collagen. Immunohistochemistry targeted CD31 (angiogenesis), α -SMA (myofibroblasts), TNF- α and IL-6 (inflammation) and phospho-AMPK. Staining was scored by two blinded observers.

3.9 Stability

Patches in sealed aluminium pouches were kept at 25 ± 2 °C / 60% RH and 40 ± 2 °C / 75% RH for 3 months, following ICH Q1A guidance. Appearance, mechanical strength, drug content and release at 24 h were checked at 0, 1 and 3 months.

3.10 Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Two groups were compared with Student's t-test, and several groups with one-way or two-way ANOVA followed by Tukey's post hoc test. Non-normal data were analysed with the Kruskal-Wallis test. A p value below 0.05 was considered significant. Analysis was carried out in GraphPad Prism and design analysis in Design-Expert.

4. RESULTS

All values in this section are illustrative placeholders. Replace them with your experimental measurements.

4.1 Compatibility and optimization

FTIR spectra of the physical mixture and of the final patch kept the characteristic metformin bands (N–H near 3370 and 3290 cm^{-1} , C=N near 1630 cm^{-1}), with only a broadening of the O–H band consistent with hydrogen bonding to PVA and alginate. In DSC, the sharp melting endotherm of metformin at about 232 °C was absent in the patch, and XRD showed no crystalline drug peaks. Together these results suggest that metformin was molecularly dispersed in the matrix and was not chemically altered.

Table 2 gives the 3² factorial batches and their responses. Fracture force rose with both PVA and alginate (higher crosslink density), whereas swelling and 24 h release fell. The polynomial models were significant ($p < 0.01$) with adjusted R^2 of 0.96 for fracture force, 0.94 for swelling and 0.95 for release, and lack of fit was not significant. PVA had the larger effect on every response.

Table 2: Factorial design batches and responses (mean, n = 3).

Batch	PVA (% w/v)	Alginate (%w/v)	Fracture force Y_1 (N/needle)	Swelling at 24 h Y_2 (%)	Release at 24 h Y_3 (%)
F1	10	0.5	0.31	520	88
F2	10	1.0	0.38	450	81
F3	10	1.5	0.45	390	74
F4	15	0.5	0.48	410	76
F5	15	1.0	0.62	340	62
F6	15	1.5	0.71	295	55
F7	20	0.5	0.69	330	61
F8	20	1.0	0.83	260	49
F9	20	1.5	0.94	215	41

Desirability analysis picked 15% PVA and 1.0% alginate (batch F5, desirability 0.91) as the optimum, and this

composition was used for all later studies as MET-HMN. Batches with 10% PVA were too soft, and batches with 20% PVA released too little drug for a 48 h dressing.

4.2 Morphology and mechanical properties

SEM showed 100 well-formed conical needles per patch, with a height of $592 \pm 14 \mu\text{m}$, a base diameter of $297 \pm 9 \mu\text{m}$ and a tip radius below $10 \mu\text{m}$. The needle surface was smooth and the backing layer was continuous, with no air bubbles or broken tips. Fracture force was $0.62 \pm 0.04 \text{ N}$ per needle, about ten times the 0.058 N needed for skin insertion, and the needles kept their shape after compression to 0.4 mm . The patch weighed $74 \pm 3 \text{ mg}$, was 1.1 mm thick and bent through 90° without cracking, which suggests that it can follow wound contours.

4.3 Skin insertion

Trypan blue staining of porcine skin showed $96.3 \pm 2.1\%$ of the needles had formed microchannels. OCT and histology placed the penetration depth at $410 \pm 35 \mu\text{m}$, or about 70% of the needle length, which is typical because skin deforms under the needle. In healthy skin, the

microchannels closed within about 6 h after the patch was removed, so the barrier recovers quickly.

4.4 Swelling, gel fraction, drug loading and dressing properties

The patch swelled rapidly in PBS, reaching $210 \pm 12\%$ in 1 h and an equilibrium of $340 \pm 18\%$ at 24 h, and the needles became soft and porous but stayed intact and could be peeled off whole. Gel fraction was $86 \pm 3\%$, which confirms a stable network. Drug loading was $1.8 \pm 0.1 \text{ mg}$ per patch, content uniformity was $98.6 \pm 1.9\%$ of the label claim ($n = 6$) and encapsulation efficiency was $91 \pm 3\%$. WVTR was $2,380 \pm 140 \text{ g/m}^2/\text{day}$, inside the $2,000\text{--}2,500 \text{ g/m}^2/\text{day}$ range reported as ideal to keep a wound moist without maceration, and moisture content was $7.4 \pm 0.5\%$.

4.5 In vitro drug release

Release was biphasic, with an initial phase from the needle tips and surface (24% in 2 h) followed by a slower, sustained phase as drug diffused through the swollen network. The cumulative release reached $62 \pm 3\%$ at 24 h and $82 \pm 4\%$ at 48 h (Table 3).

Table 3: Cumulative release of metformin from MET-HMN in PBS (pH 7.4, 37 °C; mean $\pm$ SD, $n = 3$).

Time (h)	0.5	1	2	4	8	12	24	36	48
Release (%)	9 ± 1	15 ± 2	24 ± 2	35 ± 3	47 ± 3	55 ± 3	62 ± 3	73 ± 4	82 ± 4

Model fitting gave the best correlation for the Higuchi model (adjusted $R^2 = 0.987$), followed by Korsmeyer-Peppas ($R^2 = 0.982$), first order (0.941) and zero order (0.864). The release exponent n was 0.43, so release followed Fickian diffusion through the hydrogel.

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Figure 1. In vitro release of metformin from MET-HMN in PBS (pH 7.4, 37 °C). Release is rapid in the first 2 h and then slows, as expected for diffusion-controlled release.

4.6 Ex vivo permeation and skin retention

MET-HMN gave a much higher and faster permeation than metformin gel (Table 4). The patch cut the lag time from 4.2 h to under 1 h, raised the steady-state flux 6.8-fold and left more drug in the skin, which is useful for a wound that needs a local depot.

Table 4: Ex vivo permeation of metformin through porcine skin (mean $\pm$ SD, $n = 4$).

Parameter	MET-HMN	2% metformin gel
Cumulative permeation at 48 h ($\mu\text{g}/\text{cm}^2$)	$1,480 \pm 95$	210 ± 28
Steady-state flux, J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	31.2 ± 2.6	4.6 ± 0.8
Lag time (h)	0.8 ± 0.2	4.2 ± 0.7
Skin retention at 48 h ($\mu\text{g}/\text{cm}^2$)	312 ± 27	95 ± 14
Enhancement ratio (flux vs gel)	6.8	1.0

4.7 Cytocompatibility, cell migration and haemocompatibility

Cell viability remained $96 \pm 3\%$ at 24 h and $92 \pm 4\%$ at 72 h in undiluted MET-HMN extract, well above the 70% limit. In the scratch assay done in high-glucose medium, 24 h closure was $38 \pm 5\%$ in the control, $41 \pm 5\%$ with blank HMN extract and $71 \pm 6\%$ with MET-HMN extract ($p < 0.001$), which shows that the effect came from metformin and not from the polymer. Haemolysis was $1.6 \pm 0.4\%$, below the 5% limit.

4.8 Antibacterial activity

The blank patch had no inhibition zone. MET-HMN produced modest zones of $8.2 \pm 0.6 \text{ mm}$ against *S. aureus* and $6.9 \pm 0.5 \text{ mm}$ against *E. coli* beyond the patch edge, and reduced broth counts by about 1.2 log CFU/mL after 12 h. This action is weak and complements, but does not replace, an antibiotic where infection is present.

4.9 In vivo wound healing

STZ-treated rats had a mean blood glucose of $412 \pm 38 \text{ mg/dL}$ at the start of the experiment, against 104 ± 9

mg/dL in non-diabetic animals. Wound closure was fastest with MET-HMN at every time point (Table 5). By day 14, MET-HMN wounds were $96 \pm 2\%$ closed, close to the $98 \pm 1\%$ of non-diabetic rats and significantly better than metformin gel ($81 \pm 4\%$, $p < 0.01$) and untreated diabetic controls ($68 \pm 5\%$, $p < 0.001$). The blank patch gave a small gain ($74 \pm 4\%$), probably from

its moist dressing effect. Blood glucose at day 14 did not differ between treated and untreated diabetic groups (405 ± 41 vs 418 ± 36 mg/dL, $p > 0.05$), so the benefit was local rather than systemic. Body weight and general behaviour were normal in all groups, with no sign of erythema, oedema or infection at the application site.

Table 5: Wound closure (%) in diabetic rats (mean $\pm$ SD, n = 6).

Group	Day 3	Day 7	Day 10	Day 14
G1 Untreated control	12 ± 3	31 ± 5	49 ± 6	68 ± 5
G2 Blank HMN	16 ± 3	38 ± 5	56 ± 5	74 ± 4
G3 Metformin gel	18 ± 4	47 ± 5	66 ± 5	81 ± 4
G4 MET-HMN	24 ± 4	58 ± 5	80 ± 4	96 ± 2
Non-diabetic reference	25 ± 3	60 ± 4	82 ± 4	98 ± 1

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Figure 2. Wound closure over 14 days in diabetic rats. Only MET-HMN approaches the healing rate of non-diabetic animals; the gel and blank patch fall between it and the untreated control.

4.10 Histology and immunohistochemistry

On day 14, H&E sections of MET-HMN wounds showed an almost complete, thick epidermis, mature granulation tissue, many new capillaries and few inflammatory cells.

Untreated wounds had an incomplete epithelium, a dense inflammatory infiltrate and loose granulation tissue. Masson's trichrome revealed thick, well-aligned collagen in MET-HMN wounds and thin, disorganized collagen in controls. Immunohistochemistry (Table 6) showed more CD31-positive vessels and phospho-AMPK in MET-HMN wounds and less TNF- α and IL-6, which agrees with the proposed AMPK-mediated mechanism.

Table 6: Day 14 histological and immunohistochemical scores (mean $\pm$ SD, n = 3).

Parameter	G1 Control	G2 Blank HMN	G3 Metformin gel	G4 MET-HMN
Re-epithelialization (%)	52 ± 6	58 ± 5	74 ± 6	93 ± 4
Collagen area (%)	24 ± 3	27 ± 4	38 ± 4	56 ± 5
CD31-positive vessels per field	5.8 ± 1.1	6.4 ± 1.2	9.7 ± 1.4	15.2 ± 1.8
TNF- α positive area (%)	31 ± 4	28 ± 4	19 ± 3	9 ± 2
IL-6 positive area (%)	29 ± 4	27 ± 3	18 ± 3	8 ± 2
Phospho-AMPK score (0–3)	0.6 ± 0.3	0.7 ± 0.3	1.4 ± 0.4	2.3 ± 0.3

4.11 Stability

After 3 months at 40 °C and 75% RH, MET-HMN kept its appearance and flexibility, with a drug content of $97.4 \pm 1.5\%$ of the initial value, a fracture force of 0.58 ± 0.05 N per needle and a 24 h release of $60 \pm 3\%$. None of these changed significantly from baseline ($p > 0.05$), and the patch showed no sign of discolouration or crystallization.

5. DISCUSSION

This study set out to build a metformin-loaded hydrogel microneedle patch that is strong enough to pierce skin, soft enough to act as a dressing and able to release a hydrophilic drug steadily for two days. The optimized MET-HMN met these aims and, in a diabetic rat model, closed wounds faster and more completely than a conventional metformin gel.

5.1 Formulation and mechanical performance

The factorial design showed the central trade-off in hydrogel microneedles: a denser network gives stronger needles but swells less and releases more slowly. PVA supplied the crystalline junctions created by freeze-thaw cycling, while calcium-crosslinked alginate added ionic

bridges and absorbed exudate, and PVP improved hydrophilicity and adhesion. The optimum (15% PVA, 1.0% alginate) sat in the middle of the design space, where fracture force was about ten times the insertion threshold^[12] and the swelling ratio still allowed a diffusion-controlled release. This margin of safety matters because wounds are uneven and the skin around ulcers is often thickened or callused in diabetic patients, so a patch needs more strength than normal skin would demand.

The insertion depth of about 70% of needle length is in line with reports for other microneedle arrays and reflects skin deformation. For a wound application the depth is adequate, since the target cells (keratinocytes, fibroblasts, endothelial cells and macrophages) are located in the epidermis and upper dermis. Deeper penetration is neither needed nor desirable in neuropathic skin.

5.2 Drug release and permeation

The release profile fitted the Higuchi and Korsmeyer-Peppas models, with an exponent below 0.45, so diffusion of metformin through the swollen network

controlled release.^[13–15] The early burst (24% in 2 h) comes from drug close to the needle surface and is helpful because it brings local concentrations quickly into the therapeutic range, while the following phase gives the sustained supply that a once-every-two-days dressing needs. A dose of about 1.8 mg per patch is small compared with the 500–2,000 mg daily oral dose of metformin, so the systemic exposure from even several patches is expected to be low. This agrees with the unchanged blood glucose in treated rats.

The six-fold gain in flux over gel is the main advantage of the microneedle design. A small, charged, hydrophilic molecule such as metformin cannot partition into the stratum corneum or cross eschar, so gel formulations rely on slow passage through damaged tissue. By creating aqueous channels, the needles remove this barrier and place the drug directly in the viable tissue, as shown by the higher skin retention. The same mechanism has been exploited for other hydrophilic drugs in hydrogel-forming systems.^[9]

5.3 Biological effects and mechanism

The scratch assay in high-glucose medium indicates that metformin restores the migration of fibroblasts and

endothelial cells that is lost under hyperglycaemic stress. In vivo, the faster closure, more complete re-epithelialization and denser collagen are consistent with this action. The immunohistochemical pattern points to the mechanism that has been proposed for metformin: AMPK activation^[5], with lower TNF- α and IL-6 (less NF- κ B-driven inflammation), more CD31-positive vessels (more angiogenesis) and greater matrix deposition. These readouts are correlative, and proof of an AMPK-dependent effect would need an AMPK inhibitor such as compound C or AMPK knockout animals.

The small gain from the blank patch shows that the hydrogel itself helps by keeping the wound moist and absorbing exudate, and the larger gain from MET-HMN shows that drug delivery adds a separate effect. The patch also performed better than the same drug applied as a daily gel, even though the gel was given more often, which supports the idea that delivery into the tissue matters as much as the drug.

5.4 Comparison with other approaches

Table 7 sets the patch against the options that are commonly used for diabetic wounds.

Table 7: MET-HMN compared with other wound-care approaches.

Approach	Strengths	Weaknesses
Standard moist dressings	Cheap, easy to use, absorb exudate	No bioactive effect
Topical growth factors	Directly stimulate repair	High cost, unstable, short tissue retention
Oral or injected metformin	Established systemic safety record	Little reaches the wound, systemic side effects
Metformin gel or cream	Simple, cheap	Poor penetration, frequent application
MET-HMN (this work)	Painless depot delivery, sustained release over 48 h, dressing and drug in one, low drug cost	Needs scale-up, large-animal and clinical validation

Compared with growth factor therapies, metformin is a small molecule that is stable at room temperature, inexpensive and already approved, which makes it attractive for low-resource settings, where diabetic foot disease is most severe. The patch could also be combined with antibacterial or antioxidant agents, and the weak antibacterial effect seen here suggests that metformin alone should not be relied on in infected wounds.

5.5 Translational considerations

Three practical points will shape the clinical use of this patch. First, the patch must be applied to a debrided wound bed, because thick slough or necrotic tissue would block needle penetration. Second, microneedle application on neuropathic and ischaemic feet needs clear criteria on perfusion and on infection. Third, manufacturing must be reproducible: micromolding is scalable by roll-to-roll or injection-molding methods, and metformin and the polymers are low in cost, which should keep the production cost moderate.

6. Limitations and Future Work

The study has several limitations that should be kept in mind.

- **Animal model.** Rodent wounds heal partly by contraction, whereas human wounds heal mainly by re-epithelialization and granulation. Splinted wounds or porcine models would be a better match for human healing.
- **Wound type and duration.** The 14-day excisional wound is an acute model in a diabetic host and does not fully reproduce the chronic, infected, ischaemic ulcer seen in patients.
- **Group sizes.** Six animals per group (three per histology time point) limit statistical power, and the histology readouts are semi-quantitative.
- **Mechanism.** AMPK involvement was inferred from phospho-AMPK staining and not tested with inhibitors or knockout models.
- **Pharmacokinetics.** Metformin concentration in wound tissue and in plasma over time was not measured, so systemic safety is inferred from glucose and body weight only.

- **Skin model.** Porcine ear skin differs from ulcerated human skin, and permeation through wound tissue was not studied directly.
- **Single dose and single design.** Only one drug loading and one needle geometry were tested.

Future work should include dose-ranging, tissue and plasma pharmacokinetics, tests in infected and ischaemic wound models, large-animal studies, sterilization (for example gamma or ethylene oxide) and its effect on the polymers, long-term stability studies, a full biocompatibility package under ISO 10993 and scale-up. Smart variants, such as patches that release drug in response to glucose, pH or ROS or that carry an antibiotic or growth factor in addition to metformin, are further directions.

7. CONCLUSION

A hydrogel microneedle patch of metformin, made from PVA, PVP and alginate and optimized by factorial design, was developed and evaluated for diabetic wound care. The patch had sharp, mechanically strong needles that pierced skin reliably, swelled to a moist dressing, loaded 1.8 mg of drug per patch and released it by Fickian diffusion over 48 h. It raised ex vivo drug flux 6.8-fold over a conventional gel, was cytocompatible and haemocompatible, and in diabetic rats healed wounds faster and more completely than untreated, blank-patch and metformin-gel groups, with better re-epithelialization, collagen deposition and angiogenesis and lower inflammatory markers, and without changing systemic glucose. These findings support the metformin hydrogel microneedle patch as a low-cost, painless and sustained-release platform for diabetic wound healing, and they justify further preclinical and translational studies.

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