

# Microneedle biosensors for real-time, minimally invasive drug monitoring of phenoxymethylpenicillin: a first-in-human evaluation in healthy volunteers



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## Summary

**Background** Enhanced methods of drug monitoring are required to support the individualisation of antibiotic dosing. We report the first-in-human evaluation of real-time phenoxymethylpenicillin monitoring using a minimally invasive microneedle-based  $\beta$ -lactam biosensor in healthy volunteers.

**Methods** This first-in-human, proof-of-concept study was done at the National Institute of Health Research/Wellcome Trust Imperial Clinical Research Facility (Imperial College London, London, UK). The study was approved by London-Harrow Regional Ethics Committee. Volunteers were identified through emails sent to a healthy volunteer database from the Imperial College Clinical Research Facility. Volunteers, who had to be older than 18 years, were excluded if they had evidence of active infection, allergies to penicillin, were at high risk of skin infection, or presented with anaemia during screening. Participants wore a solid microneedle  $\beta$ -lactam biosensor for up to 6 h while being dosed at steady state with oral phenoxymethylpenicillin (five 500 mg doses every 6 h). On arrival at the study centre, two microneedle sensors were applied to the participant's forearm. Blood samples (via cannula, at -30, 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240 min) and extracellular fluid (ECF; via microdialysis, every 15 min) pharmacokinetic (PK) samples were taken during one dosing interval. Phenoxymethylpenicillin concentration data obtained from the microneedles were calibrated using locally estimated scatter plot smoothing and compared with free-blood and microdialysis (gold standard) data. Phenoxymethylpenicillin PK for each method was evaluated using non-compartmental analysis. Area under the concentration-time curve (AUC), maximum concentration, and time to maximum concentration were compared. Bias and limits of agreement were investigated with Bland-Altman plots. Microneedle biosensor limits of detection were estimated. The study was registered with ClinicalTrials.gov, number NCT03847610.

**Findings** Ten healthy volunteers participated in the study. Mean age was 42 years (SD 14). Seven (70%) were men. Microdialysis and microneedle results were similar for phenoxymethylpenicillin ECF maximum concentration (0.74 mg/L vs 0.64 mg/L; 95% CI -0.24 to 0.44;  $p=0.53$ ), time to maximum concentration (1.18 h vs 1.10 h; -0.52 to 0.67;  $p=0.79$ ), and AUC (1.54 mg $\times$ h/L vs 1.67 mg $\times$ h/L; -1.10 to 0.85;  $p=0.79$ ). In total, 440 time points were compared with mean difference between measurements -0.16 mg/L (95% CI -1.30 to 0.82). Mean phenoxymethylpenicillin AUCs for free serum and microneedle PK were similar (1.77 mg $\times$ h/L [SD 0.59] vs 1.67 mg $\times$ h/L [1.00]; -0.77 to 0.97;  $p=0.81$ ). Median coefficient of variation between sensors within individuals was 7% (IQR 4-17). Limit of detection for the microneedles was estimated at 0.17 mg/L.

**Interpretation** This study is proof-of-concept of real-time, microneedle sensing of penicillin in vivo. Future work will explore microneedle use in patient populations, their role in data generation to inform dosing recommendations, and their incorporation into closed-loop control systems for automated drug delivery.

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## Introduction

Individualised approaches to drug monitoring can enhance antimicrobial dosing and the treatment of infection. Individualised dosing improves clinical outcomes, while minimising the adverse consequences of antimicrobial therapy, especially development of toxicity and antimicrobial resistance (AMR).<sup>1</sup> AMR poses a global threat to human health.<sup>2,3</sup> A major modifiable driver of AMR is suboptimal dosing of antibiotics.<sup>2</sup> The well reported observation of wide variations in individual

pharmacokinetic (PK)-pharmacodynamic target attainment has highlighted the need for enhanced methods of drug monitoring to facilitate individualised dose optimisation.<sup>4,5</sup>

Therapeutic drug monitoring is a cornerstone of individual dose optimisation. However, commercial assays are only available for a handful of antibiotics, targeting drugs with a narrow therapeutic index, such as vancomycin—a glycopeptide antibiotic—and the aminoglycosides. Therapeutic drug monitoring for these drugs

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### Research in context

#### Evidence before the study

We searched PubMed, Web of Science, and Google Scholar, from inception to May 24, 2019, for studies reporting methods for real-time antibiotic drug monitoring, using main search terms “drug monitoring”, “antibiotic”, “real-time”, and “continuous”. We only considered original research and review articles published in English. Although several biosensor and microneedle technologies have been reported in the literature, no in-human studies have been done to our knowledge. Invasive techniques, using biosensors mounted on central venous catheters inserted into ambulatory rodents have previously been described. Hollow microneedle technologies have been reported and tested *in vitro*; however, these technologies do not provide true *in-vivo* monitoring potential because of the requirement for extracellular fluid extraction via microsampling. Solid microneedle arrays have been used to show real-time glucose monitoring in healthy volunteers and patients with diabetes in proof-of-concept studies, suggesting that solid microneedle array antibiotic biosensors could potentially provide real-time, *in-vivo* antibiotic drug monitoring.

#### Added value of the study

This first-in-human study provides proof-of-concept that a minimally invasive, microneedle  $\beta$ -lactamase biosensors can be used for real-time, *in-vivo* antibiotic drug monitoring. This study provides evidence to support the wider exploration of microneedle antibiotic monitoring in patients and for a broader range of antimicrobial drugs. It is also the first step towards automated, individualised antibiotic dosing in humans through the application of closed-loop control systems.

#### Implications of all the available evidence

As well as supporting future exploration of real-time antibiotic monitoring, this study adds to the growing body of literature showing proof-of-concept for direct *in-vivo* monitoring of drugs using solid microneedle array biosensors. The implications of this research extend beyond antibiotics and even drugs, also providing evidence for biomarker monitoring *in vivo* using solid microneedle arrays.

is crucial for preventing toxicity. The use of therapeutic drug monitoring for individualised dosing of antibiotics, such as the  $\beta$ -lactams, can improve clinical outcomes.<sup>16</sup> Current methods of therapeutic drug monitoring for individualised antibiotic dosing present operational and technical barriers.<sup>5,7</sup> To address these issues, the development of wearable, real-time biosensor technology that can be deployed in a minimally invasive fashion and deliver continuous drug monitoring has been proposed.<sup>4</sup>

Microneedle technology has been under development for the past 20 years.<sup>8</sup> To date, the majority of applications for microneedles involve drug and vaccine delivery.<sup>8</sup> However, hollow microneedle arrays have been designed to perform microsampling of extracellular fluid (ECF) to facilitate analysis *ex vivo*.<sup>9,10</sup> This approach has similar analytical problems (eg, the need to extract ECF and *ex-vivo* analysis) as alternative approaches, such as microdialysis and therefore cannot be used to facilitate real-time drug monitoring. In contrast, solid microneedle arrays have been used for continuous monitoring of glucose and lactate directly in dermal ECF.<sup>11–14</sup> Therefore, it might also be possible to use this type of technology to monitor ECF antibiotic concentrations in humans.<sup>4,15</sup>

This study is a first in-human exploration of a  $\beta$ -lactam microneedle array biosensor for the monitoring of phenoxymethylpenicillin, a commonly used antibiotic, in healthy volunteers.<sup>15</sup> It aimed to show the reliability and potential of a minimally invasive microneedle array biosensor for continuous drug monitoring. The secondary objectives of the study were to explore the accuracy of the microneedle sensors against current gold standards of drug monitoring (ie, blood sampling via cannula and

ECF concentrations using microdialysis). Tolerability of wearing the microneedle array was compared with cannula and microdialysis catheter insertion using visual analogue scales.

## Methods

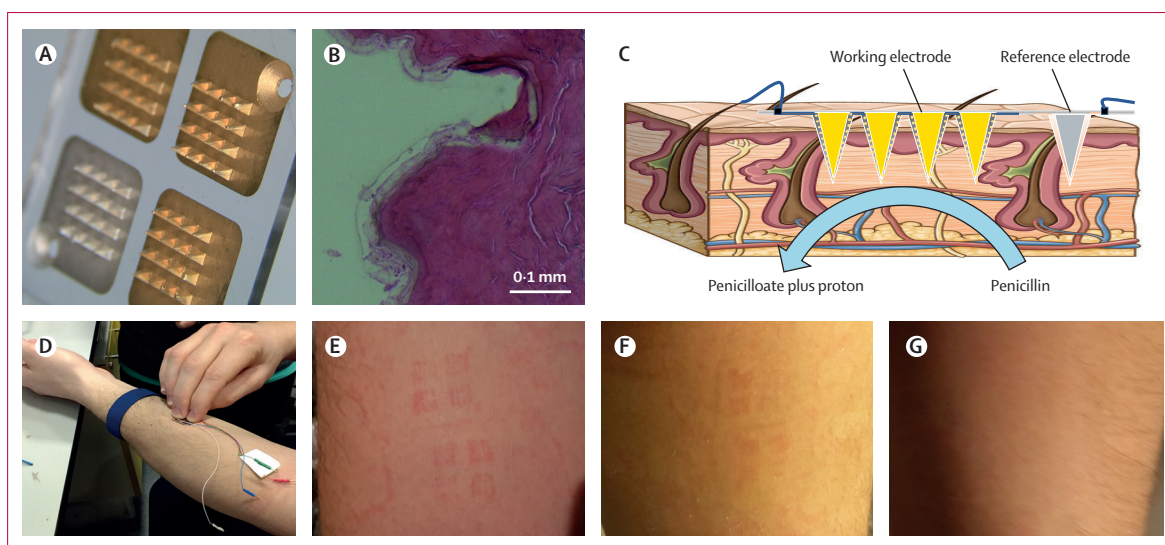
### Study design

This study was a first-in-human, proof-of-concept study to evaluate the ability of microneedle biosensors to do real-time drug monitoring in healthy volunteers. The study was done at the National Institute of Health Research/Wellcome Trust Imperial Clinical Research Facility (Imperial College London, London, UK).

The study protocol was reviewed and approved by London-Harrow Regional Ethics Committee.

### Participants

Between April and August, 2018, participants were identified through emails sent to a healthy volunteer database containing over 2000 potential volunteers, held at Imperial College Clinical Research Facility. Male and female healthy volunteers older than 18 years were eligible for inclusion. Volunteers were excluded if they had evidence of active infection, allergies to phenoxymethylpenicillin, were at high risk of skin infection (eg, atopic dermatitis) near the sensor site, or presented with anaemia (defined as haemoglobin <13 g/dL for men and <12 g/dL for women) during screening. If volunteers responded to the advertisement, they were initially screened via email followed by a screening visit at the Clinical Research Facility. Those meeting study criteria at screening were invited to participate in the study. All participants provided written, informed consent.



**Figure 1: Experimental setup**

(A) Microneedle base used for the study. (B) 20  $\mu\text{m}$  cross-section of human skin after microneedle application stained with haematoxylin and eosin. Examination under microscope shows penetration of the microneedles through the epidermis. (C) Schematic of the penetration of the microneedle array into the dermal-interstitial space. (D) Application of a microneedle array biosensor to the forearm with firm pressure for 60 s. (E) Time lapse of marks left after wearing a microneedle for 6 h immediately after removal, 1 h after removal (F), and 12 h after removal (G).

## Procedures

Healthy volunteers were recruited to wear a microneedle  $\beta$ -lactam biosensor for up to 6 h while being dosed at steady state with oral phenoxymethylpenicillin. Oral phenoxymethylpenicillin was the  $\beta$ -lactam chosen because of its convenience (ie, it can be delivered orally rather than by injection), well documented safety profile, and narrow spectrum of activity. Participants took five doses of phenoxymethylpenicillin (500 mg every 6 h, a standard treatment dose for bacterial infections in adults) on an empty stomach before attending the study, documenting doses on a self-reported diary card.

On arrival at the study centre, two microneedle sensors were applied to the participant's forearm. A cannula for blood sampling was inserted in a large vein in the antecubital fossa. A microdialysis catheter was inserted subcutaneously in the forearm close to the microneedle array. Participant baseline characteristics (height, weight, gender, age, and body-mass index [BMI]) were collected and glomerular filtration rate estimated using the Cockcroft-Gault equation. Participants were dosed on an empty stomach with 500 mg oral phenoxymethylpenicillin (ie, at time=0 min). The output from the microneedle array sensors was recorded for comparison to concomitant blood and tissue ECF PK samples. The blood and ECF samples were taken via a cannula and by microdialysis, respectively.

## Microneedle biosensors

The development and in-vitro validation of the microneedle array biosensors used in this study have been described in detail.<sup>15,16</sup> Figure 1 summarises the microneedle array structure and application process. The polycarbonate microneedle array structure is metallised

to produce four independent electrodes. Each electrode is created by first metallising the electrode areas with an adhesion layer of chromium (110 nm). Three of the electrodes are then coated with gold (150 nm) to create independent working electrodes. The fourth electrode is metallised with 150 nm of silver and then chloridised to act as a silver–silver chloride reference electrode. The working electrodes are subsequently modified by electrodeposition of iridium oxide to measure changes in pH.<sup>15,16</sup> Finally, a hydrogel layer containing an extended spectrum  $\beta$ -lactamase from *Enterobacter cloacae* (Sekisui Diagnostics, Tokyo, Japan) is added.<sup>15,16</sup> Sterilisation is achieved with Cobalt-60 gamma radiation.

The sensors are applied to the forearm with firm pressure for 60 s and secured in place with a transparent medical dressing. The sensors are connected to a potentiostat and the open circuit potential of the working electrode versus the reference electrode recorded. As phenoxymethylpenicillin in the ECF diffuses into the hydrogel layer, it is hydrolysed to penicilloate and a proton by  $\beta$ -lactamase. Therefore, as the concentration of phenoxymethylpenicillin in the tissue increases, the concentration of protons generated at the sensor surface increases. This process leads to a fall in the local pH at the sensor surface, which is detected as an increase in open circuit potential as the equilibrium of iridium oxide oxidation states (ie, the ratio of iridium + 3 to iridium + 4) within the sensor is shifted. For calibration of the working electrodes, a control electrode is also worn. The control electrode is identical to the working electrode but without  $\beta$ -lactamase enzyme in the hydrogel layer. Its use provides a background potential that is subtracted from the working electrode potential to compensate for any

time-dependent changes in the sensor output, which are independent of changes in analyte concentration (drift).

During the study period, each participant wore two microneedle devices. One containing working electrodes and one containing the control electrode. The open circuit potential (in mV) was recorded 200 times every second for each electrode. This measurement provided data for three phenoxymethylpenicillin sensors and one control array per participant. The electrochemical signal was recorded with an in-house instrument for potentiometric recording of biosignals (Imperial College London, London, UK).<sup>17</sup>

### Blood samples

A maximum of 15 blood samples were acquired before and after the final (sixth dose, time=0 min) dose of phenoxymethylpenicillin. Blood samples were taken via a cannula at times -30, 0, 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min. Samples were allowed to clot for 10 min and then placed on ice. They were centrifuged (at 3000 G for 10 min) within 30 min. The serum was stored at -80°C until analysed. Analysis was done using high-performance liquid chromatography tandem mass spectrometry at Bristol Antimicrobial Reference Laboratory (Bristol, UK). Both total and free (unbound) drug concentrations were determined. Free antibiotic concentration was determined for individuals by performing ultrafiltration (Centrifree ultrafiltration, 30 kDa molecular weight cutoff; Sigma-Aldrich, Schnellendorf, Germany). Each participant had free drug concentration determined at three collection timepoints and an average was calculated. The limit of quantification for the high-performance liquid chromatography tandem mass spectrometry assay was 0.10 mg/L.

### Microdialysis

ECF phenoxymethylpenicillin concentrations were determined through continuous microdialysis. A microdialysis catheter (CMA 63 microdialysis catheter, 20 kDa molecular weight cutoff; Linton Instruments, Norfolk, UK) was inserted into the subcutaneous tissue of the forearm after application of topical local anaesthetic. The catheter was perfused using sterile T1 perfusion fluid (sodium 147 mmol/L, potassium 4 mmol/L, calcium 2.3 mmol/L, chloride 156 mmol/L; Linton Instruments, Norfolk, UK) with a flucloxacillin internal standard (10 mg/L).<sup>18–20</sup> The rate of perfusion was 2 µL/min with microvials changed every 15 min during the study period. The first 30 min was designated the equilibrium period with samples obtained during this period excluded from the analysis. Microdialysis sampling commenced 1 h before phenoxymethylpenicillin administration (ie, time=0 min) and continued until the end of each study period (240 min). Microdialysis vials were stored at -80°C following collection until analysed. Analysis was done using high-performance liquid chromatography tandem mass spectrometry at Bristol Antimicrobial Research Centre

(Bristol, UK). The limit of quantification for the phenoxymethylpenicillin and flucloxacillin assays were both 0.10 mg/L.

In-vivo recovery of phenoxymethylpenicillin in the microdialysate solution was determined from the measured loss of the flucloxacillin internal standard across the microdialysis membrane for individual participants:

$$\text{Percentage of penicillin recovery} = 100 \times (C_{\text{in}} - \text{mean } C_{\text{out}}) / C_{\text{in}}$$

where  $C_{\text{in}}$  is the concentration of flucloxacillin measured in the perfusate and  $C_{\text{out}}$  is the concentration of flucloxacillin measured in the microdialysate for each individual volunteer. This in-vivo recovery was used to estimate actual tissue ECF concentration from the dialysate concentration.

### Outcomes

The primary outcome of this study was to determine whether a microneedle biosensor could be used to monitor phenoxymethylpenicillin concentrations in human dermal ECF.

### Data processing and analysis

Microneedle data, in mV, were recorded in real-time. All processing and calibration into phenoxymethylpenicillin concentration was done after data collection. Signal processing was done by firstly subtracting the control sensor from individual working electrodes to correct background drift. Noise was corrected for using first order locally estimated scatterplot smoothing (LOESS) filters in IgorPro8 (WaveMetrics, Lake Oswego, OR, USA).

Given the study aims, the small number of sensors, and potential person-to-person and sensor-to-sensor variation, separate calibration and validation cohorts would be challenging. We chose to split individual sensor data into independent calibration and validation sets. To calibrate individual sensors, a random selection of timepoints were plotted against corresponding microdialysis data. For each individual sensor, a first order polynomial was determined by linear regression between sensor output and microdialysis tissue concentration. This polynomial was then used to convert sensor output (mV) to phenoxymethylpenicillin concentration (mg/L) in the validation dataset. Concentration data from all microneedle sensor validation sets were then compared with microdialysis results at corresponding timepoints using Bland-Altman plots to examine for bias and limits of agreement.

As calibration of the microneedles was based on regression of microdialysis results, the method of concentration determination for the microneedle arrays is not entirely independent of the microdialysis data. However, the purpose of Bland-Altman plot in this study was to show the methods are in agreement over the entire dataset using independent measurements of calibration and validation. Therefore, while considering this limitation, we agreed that this method of evaluation remained valid

See Online for appendix

for initial comparison of the microneedles against the current gold standard, microdialysis.

Limits of detection of the microneedle biosensors were estimated as:

$$\text{Limit of detection} = \frac{(3 \times \text{Csd})}{M}$$

where Csd is the standard deviation of the y-intercept (C) of a first order polynomial generated by fitting a line of regression through the pooled microneedle array data and M represents the slope function for the same polynomial.<sup>21</sup>

Limit of quantification was estimated as:<sup>21</sup>

$$\text{Limit of quantification} = \frac{(10 \times \text{Csd})}{M}$$

The PK profiles of free serum, microdialysis, and microneedle data were determined and compared using non-compartmental analysis. This analysis was done with Pmetrics software, in R (R Core Development Team).<sup>22</sup> Figures were plotted using IgorPro8 to estimate the area under the concentration–time curve (AUC), maximum concentration (C<sub>max</sub>), and time to maximum concentration for each measurement method. Statistical analysis was done using *t* tests in R.

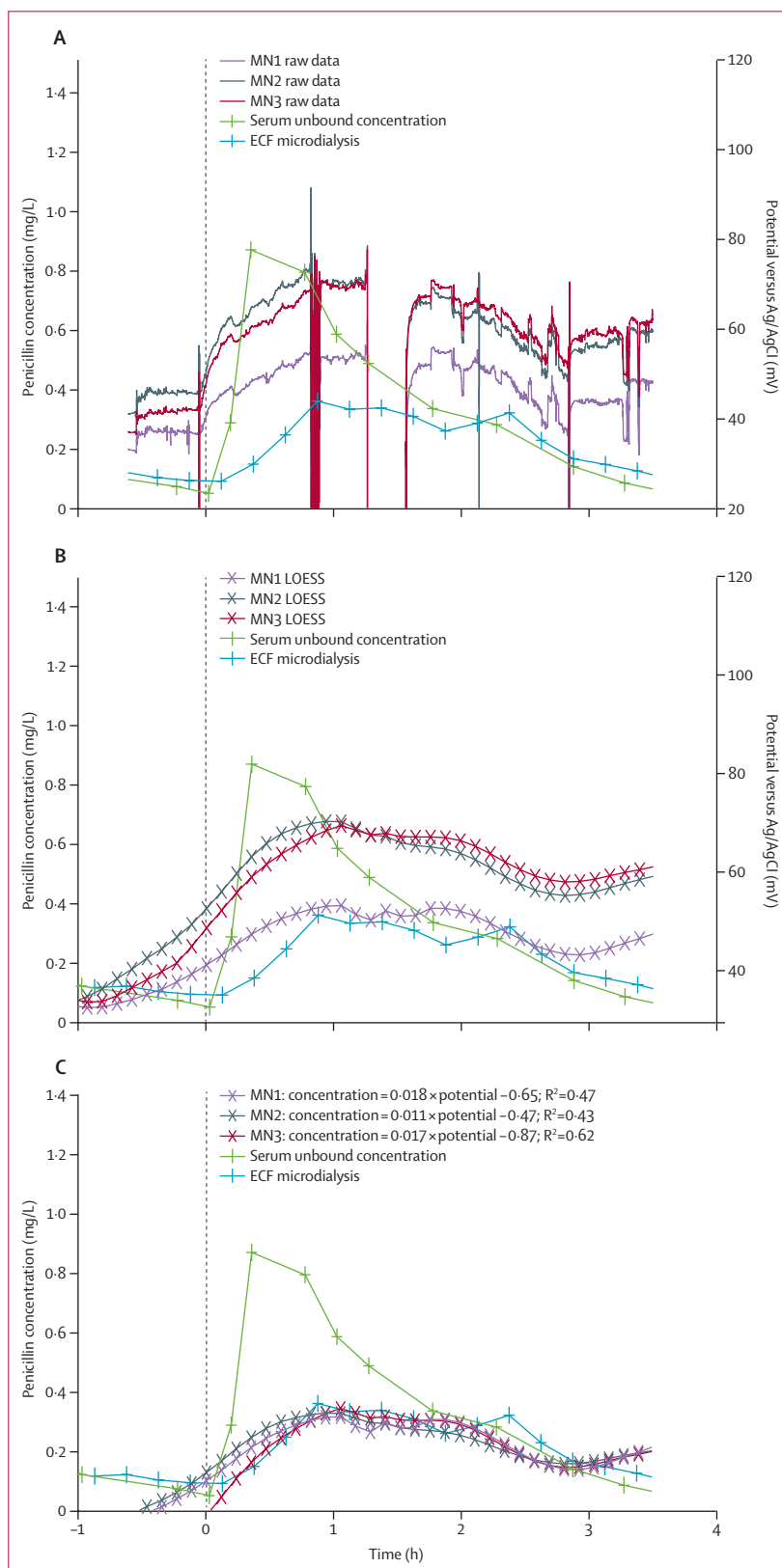
### Visual analogue scoring

During the study visit, participants completed visual analogue scoring from 0 to 10 cm, rating pain or discomfort caused by cannula, microdialysis catheter, and the microneedles. Visual analogue scoring was collected immediately and 3 h after insertion. Mean scores were visually compared. Statistical analysis was not done as it was not powered to account for type 2 errors.

The study was registered with ClinicalTrials.gov in February, 2019, number NCT03847610. Registration was delayed because of an administrative error during local registration that should have facilitated ISRCTN registration. The registered study protocol was not amended in anyway following ethics committee approval. The protocol is available in the appendix.

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the



	Age (years)	Gender	Unbound phenoxymethylpenicillin in serum (%)	Blood			Microdialysis			Microneedle		
				Cmax (mg/L)	Tmax (h)	AUC (mgxh/L)	Cmax (mg/L)	Tmax	AUC (mgxh/L)	Cmax (mg/L)	Tmax (h)	Average AUC (mgxh/L)
1	56	Male	33	2.04	0.47	2.20	0.85	0.15	2.92	0.79	0.15	2.94
2	43	Male	21	0.99	1.28	1.63	0.37	1.46	0.87	0.37	1.29	1.09
3	30	Male	25	0.48	0.75	1.10	0.25	1.51	0.55	0.22	1.01	0.58
4	65	Male	23	1.60	0.75	2.07	0.81	0.93	1.27	0.54	1.19	1.29
5	51	Male	25	2.23	0.50	2.16	1.16	0.93	2.08	0.99	0.49	2.10
6	52	Male	23	0.65	0.88	1.27	1.33	1.56	2.84	1.07	2.34	3.71
7	28	Male	21	0.90	0.37	1.38	0.36	0.88	0.84	0.33	1.02	0.83
8	51	Female	16	0.90	0.55	1.16	0.61	1.96	0.95	0.58	1.71	0.88
9	23	Female	22	2.05	0.95	3.09	NA	NA	NA	NA	NA	NA
10	23	Female	22	1.41	0.95	1.66	0.91	1.21	1.59	0.84	0.69	1.615
Mean (SD)	42 (14)	..	23 (4)	1.32 (0.60)	0.75 (0.26)	1.77 (0.59)	0.74 (0.37)	1.18 (0.53)	1.54 (0.88)	0.64 (0.30)	1.10 (0.65)	1.67 (1.00)

Cmax=maximum concentration. Tmax=time to maximum concentration. AUC=area under the concentration–time curve. NA=data not available.

**Table 1: Comparison of individual participant demographics and microneedle data**

	p value	95% CI
<b>Free blood vs microneedle</b>		
Cmax (mg/L)	0.0095	0.21 to 1.17
Tmax (h)	0.16	-0.87 to 0.17
AUC (mgxh/L)	0.81	-0.77 to 0.97
<b>Free blood vs microdialysis</b>		
Cmax (mg/L)	0.025	0.08 to 1.09
Tmax (h)	0.049	-0.86 to -0.01
AUC (mgxh/L)	0.53	-0.53 to 0.98
<b>Microneedle vs microdialysis</b>		
Cmax (mg/L)	0.53	-0.24 to 0.44
Tmax (h)	0.79	-0.52 to 0.67
AUC (mgxh/L)	0.79	-1.10 to 0.85

Cmax=maximum concentration. Tmax=time to maximum concentration. AUC=area under the concentration–time curve.

**Table 2: Statistical comparison of free blood, microneedle, and microdialysis pharmacokinetic parameters derived using non-compartmental analysis**

decision to submit for publication. The views expressed in this publication are those of the authors and not necessarily those of the National Health Service, the National Institute for Health Research, or the UK Department of Health.

**Results**

150 responses to the initial advertisement were received. Of these, 81 (54%) completed the email screening. Screening visits at the Clinical Research Facility were organised with 25 (31%) of 81 individuals. 17 (68%) were eligible for inclusion. Three (18%) of 17 withdrew after screening, and three were held on standby for the study. We had standby participants because we only needed to recruit ten participants in total and recruitment screening visits

were staggered. In total, 11 (65%) volunteers participated in the study. One volunteer withdrew consent to undergo microdialysis on the study day and was withdrawn from the analysis. The participant did not cite a reason for declining microdialysis catheter insertion. Therefore, ten volunteers were included in the study.

The mean age of participants was 42 years (SD 14) and seven (70%) of ten participants were men. Participants mean height was 174 cm (11), weight 74 kg (15), and BMI 24 kg/m<sup>2</sup> (3). Mean creatinine clearance was 114 mL/min (29).

Each participant wore a microneedle array containing three enzyme-coated sensors (30 sensors in total) and one array containing the control sensor (ten sensors in total). Data for 25 (83%) of 30 enzyme-coated sensors were available for analysis. In two cases (participant 1 and 5), one of the three working electrodes failed due to poor electrical connectivity. For participant 9, data from all three working electrodes became corrupted, meaning that data for this individual were not available for analysis.

Microneedle data were recorded for a mean of 3.74 h (SD 0.48). A mean of 13 blood samples (SD 1) and 14 microdialysis PK samples (3) were taken per participant. Figure 2A shows raw microneedle data for the three microneedle arrays (mV) plotted against free serum and ECF phenoxymethylpenicillin concentration (mg/L). Figure 2B shows LOESS smoothing of the microneedle data and figure 2C shows final calibrated microneedle data (mg/L) against free serum and ECF (microdialysis) determined phenoxymethylpenicillin concentrations. Table 1 describes the individual participant PK comparison of serum, microdialysis, and microneedle monitoring using non-compartmental analysis. Mean microdialysis recovery of phenoxymethylpenicillin was estimated at 23% (SD 4; table 2). Concentration data obtained from the microneedle sensors were similar to that from

microdialysis (mean AUCs  $1.67 \text{ mg}\times\text{h/L}$  [SD  $1.00$ ] for microdialysis vs  $1.54 \text{ mg}\times\text{h/L}$  [0.88] for microdialysis; 95% CI  $-1.10$  to  $0.85$ ;  $p=0.79$ ; tables 1, 2). Mean  $C_{\text{max}}$  was also similar between measurements for both techniques ( $0.64 \text{ mg/L}$  [0.30] vs  $0.74 \text{ mg/L}$  [0.37];  $-0.24$  to  $0.44$ ;  $p=0.53$ ; tables 1, 2).

Overall, there was a good agreement with a mean difference between microneedle and microdialysis measurements of  $-0.16 \text{ mg/L}$  and the majority of points were within the  $1.96 \text{ SD}$  range ( $0.82$  to  $-1.30$ ; figure 3). Deviation beyond the  $1.96 \text{ SD}$  range can be attributed to a single individual's microneedle array. Limit of detection was estimated at  $0.17 \text{ mg/L}$  and limit of quantification at  $0.55 \text{ mg/L}$ . Median coefficient of variation (CV) between sensors for the same individual was 7% (IQR 4–17).

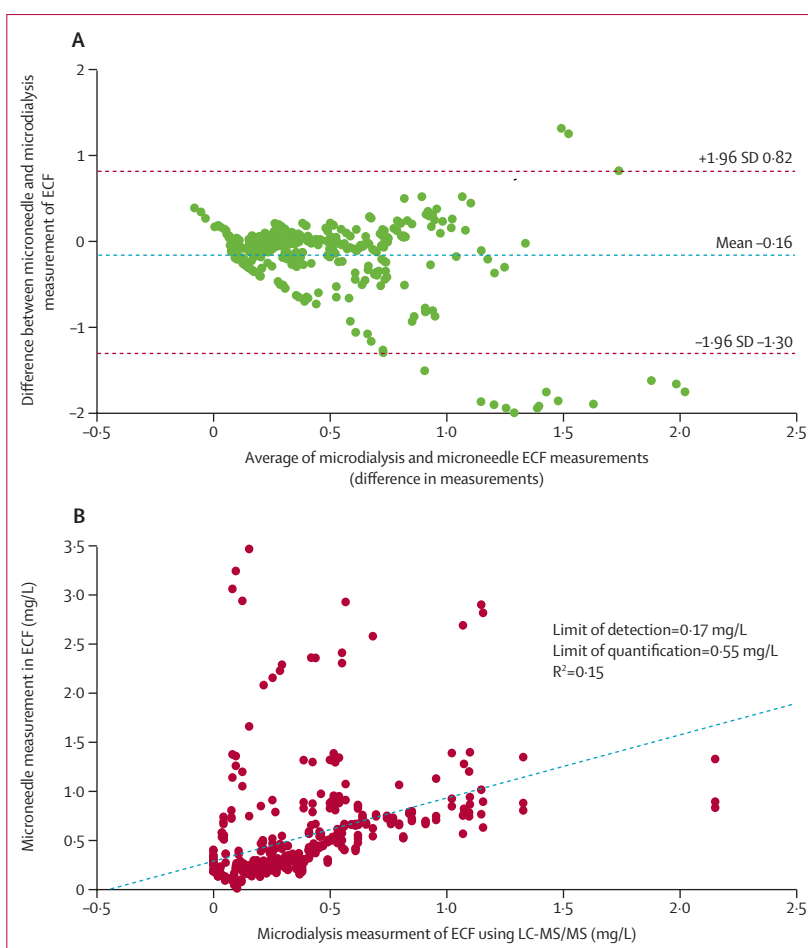
PK parameters for free serum phenoxymethylpenicillin concentration were compared with parameters determined using the microneedle biosensors. Phenoxymethylpenicillin protein binding was variable between individuals with a mean free phenoxymethylpenicillin fraction of 23% (SD 4; table 1; CV 18%). Free serum and microneedle PK had similar mean AUCs ( $1.77 \text{ mg}\times\text{h/L}$  [SD  $0.59$ ] for free serum vs  $1.67 \text{ mg}\times\text{h/L}$  [1.00] for microneedle; 95% CI  $-0.77$  to  $0.97$ ;  $p=0.81$ ; tables 1, 2). Free serum  $C_{\text{max}}$  was significantly higher than microneedle measurements of the ECF ( $1.32 \text{ mg/L}$  [0.60] vs  $0.64 \text{ mg/L}$  [0.30];  $0.21$  to  $1.17$ ;  $p=0.0095$ ; tables 1, 2).

All modalities of phenoxymethylpenicillin measurement were well tolerated. Mean visual analogue scoring for microneedles, microdialysis catheter, and cannula was low. At insertion, microneedle pain or discomfort scored a mean of  $0.9 \text{ cm}$  (SD  $1.5$ ) falling to  $0.1 \text{ cm}$  ( $0.2$ ) at 3 h. Cannula insertion scored  $0.2 \text{ cm}$  ( $0.2$ ) on insertion rising to  $0.5 \text{ cm}$  ( $0.8$ ) at 3 h. Microdialysis scored  $1.5 \text{ cm}$  ( $3$ ) at insertion and  $0$  ( $0$ ) at 3 h. No participants experienced any adverse events (data not shown).

## Discussion

In this first-in-human evaluation of microneedle antibiotic drug monitoring, we showed that a microneedle  $\beta$ -lactam biosensor could monitor changes in ECF drug, could be calibrated to drug concentration, and was well tolerated by healthy volunteers. PK profiles of ECF phenoxymethylpenicillin were similar between microdialysis and microneedle methods but showed highly variable PK between participants. AUCs of free antibiotic in serum and ECF were similar, but highly variable between participants. These results are in line with documented variability in the PK of phenoxymethylpenicillin.<sup>23</sup>

Real-time, minimally invasive, microneedle drug monitoring provides the ability to monitor and potentially react to changing drug PK within the individual and might support the development of individualised approaches to antibiotic dosing. Within our study, microneedle biosensors were calibrated against, and subsequently compared with, individual discrete tissue microdialysis measurements, which is the current gold standard for



**Figure 3:** Bland-Altman plot comparing agreement of ECF phenoxymethylpenicillin measurements between microdialysis and microneedle (A) and scatter plot for the estimation of lower limit of detection and lower limit of quantification for the microneedle biosensors (B)

ECF=extracellular fluid. LC-MS/MS=high-performance liquid chromatography tandem mass spectrometry.

determining ECF drug concentration. Using this method, the microneedle biosensors had high concordance with microdialysis in terms of phenoxymethylpenicillin PK profile and determining ECF drug concentration, providing a proof-of-concept for their application in wider clinical practice. Table 3 compares potential benefits of microneedle versus microdialysis methods for ECF drug monitoring.<sup>15,18,24–26</sup> Similarly, the potential benefit of microneedle technology over blood sampling is that no laboratory analytics are required, monitoring can be continuous, and the process is minimally invasive with no exposure to blood of other potential hazardous bodily fluids.<sup>9,10</sup>

Microneedle ECF antibiotic measurement might facilitate optimisation of other compartment drug concentrations in the future, including serum. Free (unbound) concentration of drug is the current gold standard, given that free drug is in equilibrium between body compartments, such as blood and ECF.<sup>27</sup> Therefore, the development of minimally invasive microneedle sensors might provide a mechanism to guide blood compartment

	Microneedles	Microdialysis
Disruption to local tissue	Minimal evidence of local tissue disruption	Potential for disruption of local tissues, inflammation, and bleeding during insertion <sup>18</sup>
Practicality for use in clinical practice	Easy application, no needles or skin puncture required	Requires training and puncture of the skin
In-vivo measurement	Direct detection in vivo	Not applicable (dialysate collected and analysed ex vivo)
Quantification	Direct quantification of drug concentration <sup>15,24-26</sup>	Estimation of drug concentration calculated often using an internal standard method <sup>18-20</sup>
Resolution of sampling	Up to 200 readings per second acquired in vivo	Samples are collected at timepoints (eg, every 15 min)
Laboratory analysis required	No	Yes, for both target and internal standard; often by use of HPLC techniques

HPLC=high-performance liquid chromatography.

**Table 3: Comparison of the microneedle and microdialysis methods for extracellular drug monitoring**

dose optimisation.<sup>4</sup> This study supports this argument by showing similar AUCs in serum and ECF. Through the greater exploration of this technique and linkage to other drug compartments, dose optimisation might become a reality.

Several outstanding variables must now be explored, including the effect of conditions such as sepsis on ECF volume expansion and pH change, and the ability of microneedles to detect both subtherapeutic and supratherapeutic drug concentrations accurately. Based on preliminary in-vitro analysis and data from this study, we anticipate that the microneedle biosensors should perform within appropriate limits of detection.<sup>15,16</sup> Furthermore, the use of a control sensor allows for changes in individual ECF pH to be controlled for in the calibration step.<sup>15,16</sup> Finally, microdialysis studies already provide data showing PK variability within ECF in critical illness. Given the variable PK of phenoxymethylpenicillin in ECF, this study provides initial evidence to support the appropriateness of microneedles to cope with wide ECF variations in sepsis.

The current study aimed to show the potential of microneedle sensors through calibration against individual microdialysis data. However, for wider roll out and evaluation when microdialysis is not available, alternative standardised calibration techniques are required. This process might be achieved through concurrent free drug analysis of serum allowing estimation and comparison of AUCs between compartments, or through standardised calibration of devices based on pooled data. Furthermore, the enzyme-based biosensor used in this study might not be appropriate for all antibiotics, such as aminoglycosides, or in the presence of enzyme inhibitors, such as amoxicillin–clavulanic acid or piperacillin–tazobactam drug combinations.<sup>4</sup> The selection and functionalisation of aptamer technology has already been shown to be possible for real-time drug monitoring and offers an alternative sensing modality for application to microneedles.<sup>28,29</sup> Finally, the combination of microneedle sensing with methods

of individualised antibiotic dosing, such as closed-loop control systems, is yet to be shown in vivo.<sup>4,30</sup>

The current study also has several limitations. This study was done in healthy volunteers using an orally administered phenoxymethylpenicillin designed to show proof of concept for real-time, minimally invasive monitoring of antibiotics in humans. Drug PK, especially in ECF, was highly variable between individuals making grouped sensor analysis challenging. Data from five (16%) of 30 microneedle electrodes were not available for analysis. Furthermore, the study of healthy volunteers makes generalisability to patients challenging, particularly in reference to changes that occur in sepsis and with polypharmacy. This study does, however, provide proof-of-concept for microneedle-based antibiotic therapeutic drug monitoring and will be further explored in patient populations now that the safety and potential reliability of such technology has been shown in vivo.

In conclusion, this first-in-human study of microneedle array biosensors for antibiotic therapeutic drug monitoring has shown that minimally invasive, microneedle sensors are able to continuously monitor ECF antibiotic concentrations in line with current microdialysis gold standards. The microneedle results showed similar free drug AUC when compared with serum AUC. The PK profiles were also similar between microneedle and microdialysis methods of ECF monitoring, with highly variable PK. Further work will explore the role of these sensors in obtaining necessary data to optimise  $\beta$ -lactam dosing in patient populations and as part of real-time dose optimisation using closed-loop control systems.

#### Contributors

TMR and AH conceived and designed the study. TMR led the study. SG, DF, SS, AC, and DOH produced the sensors used for the study. TMR, SG, DF, RW, and DOH supported participant recruitment, screening, and running of the study. MG provided pharmacy support. AM, AL, MB, and MK supported drug concentration determination. TMR drafted the first version of the manuscript. All authors contributed substantially to revisions and submission of the final manuscript.

#### Declaration of interests

We declare no competing interests.

#### Data sharing

Individual, anonymised data from the study are available on reasonable request from the authors, provided that it is in line with current ethical and intellectual property requirements surrounding the use of data.

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