INTRODUCTION

Current upper-limb amputation prostheses have 2 key limitations: attachment to the amputation stump and intuitive, reliable prosthesis control. Present attachment solutions, including sleeves and harnesses, are sources of discomfort or pain for users, causing poor transpiration and pressure sores.1–3 Users also prioritize prosthesis function, including dexterous control and sensory feedback.1,2,4

One approach to improving prosthesis attachment is transcutaneous bone-anchors.6–8 Bone-anchors allow direct skeletal attachment of prostheses: the bone-anchor stem is implanted within the medulla of the residual bone and exits through the skin distally on the residual limb. Implant designs necessitate a 1-stage9,10 or 2-stage11–13 surgical procedure, and approaches to sealing the skin interface and avoiding ascending infections vary, including skin-to-bone healing12 or the formation of a skin–implant interface.9,14

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Commercially available active upper-limb prostheses provide body-powered or myoelectric control. Myoelectric control strategies, including pattern recognition and proportional regression approaches, can improve prosthesis function over on/off control with finite state machines for changing grip.25 Myoelectric control using skin surface electrodes has associated challenges. Signals vary due to changes in electrode location, skin conductivity due to perspiration, muscle movement relative to the skin surface, and limb shape changes. Avoiding skin surface electrodes will reduce complexity and discomfort for prosthesis users. Implantable systems, with electrodes on the muscle surface or within the muscle, can overcome these issues providing improved EMG (electromyography) quality and reduced variability.16–22 Transcutaneous bone-anchors can be used to create a hard-wired connection to implanted electrodes,17,24 avoiding wireless signal transmission and implanted electronics.

Surgical approaches can improve myoelectric control by directed reinnervation of residual muscles to amplify neural signals from nerves previously supplying the amputated limb.25 One approach: targeted muscle reinnervation (TMR) transfers upper-limb nerves to residual muscles of the torso or limb stump.26–32 An alternative, regenerative peripheral nerve interface, reinnervates free muscle transfers with transected residual nerves of the limb.21,32–36

This study assesses a combined approach for prosthetic attachment and control using a bone-anchored device and implanted epimysial electrodes. We have previously demonstrated this approach in a single animal model, to observe recovery of function over 12 weeks,23 and the present study validates this over an extended timescale with n = 6 animals. This study also investigates TMR alongside hard-wired epimysial electrodes in a single animal model, to observe recovery of function and signal quality over 12 weeks.

METHODS

The bone-anchored device is shown in Figure 1A. Devices were designed and manufactured as described previously.23 Transcutaneous bone-anchors with a porous flange were laser sintered from Ti-6Al-4V (Eurocoatings, Trentino, Italy)37 with 700 μm pore size and 300 μm strut size. Flange and tapered pin were hydroxyapatite plasma sprayed (Plasma Biotal, Tideswell, UK). A 2 mm diameter hole was drilled from the top-surface of the stem, exiting immediately below the porous flange.

Bipolar epimysial electrode arrays (Ardiem Medical Inc., Indiana, Pa.) with platinum–iridium electrodes were used (3.68 mm diameter, 10 mm center-to-center inter-electrode distance). Arrays have reinforced backing and an electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance. Arrays have reinforced backing and electrode distance.

Electrode arrays were implanted following a previously described procedure.23 In one further animal, the bone-anchor and epimysial array were implanted and TMR was carried out.

SURGICAL PROCEDURE

All in vivo procedures were carried out in compliance with The Animals (Scientific Procedures) Act UK, 1986 (revised 2013) and local guidance. All animals were skeletally mature female mule sheep. Sterile procedures were used throughout. A total of 8 animals were used for in vivo testing. In 7 animals, the bone-anchor and epimysial array were implanted following a previously described procedure.23 In one further animal, the bone-anchor and epimysial array were implanted and TMR was carried out.

BONE-ANCHOR IMPLANTATION

Anesthesia was induced with intravenous ketamine hydrochloride (Ketaset, Fort Dodge Animal Health Ltd., Sandwich, UK) and midazolam hypnovel (Roche Products Ltd., Welwyn Garden City, UK) and maintained with 2% isoflurane (IsoFlo) in oxygen. A 5 cm incision was made 10 cm inferior to the knee joint on the medial aspect overlying the left tibia. The bone-anchor was implanted trans-tibially: both cortices of the tibia were drilled, reamed, and the bone-anchor was press-fitted into the hole. A 2 mm gap between the flange and the tibia allowed the electrode cable to exit into the soft tissue (Fig. 1B).

ELECTRODE ARRAY IMPLANTATION

A 10 cm incision was made over the lateral compartment muscles of the left leg. The peroneus tertius muscle was identified.38 A subcutaneous tunnel was made between the lateral compartment incision and the bone-anchor incision, and the epimysial array was passed through the tunnel. The electrode was aligned with the long-axis of the muscle belly and sutured to the epimysium using 4-0 Prolene (nonabsorbable) suture (Fig. 1C).

TARGETED MUSCLE REINNERVATION

The peroneus tertius muscle was exposed and retracted laterally to expose the peroneal nerve. Nerve branches to the tibialis anterior muscle and to the lateral compartment muscles were identified. One of 3 nerve branches to the tibialis anterior muscle was transected close to the muscle. The transected nerve branch was freed from surrounding tissue until the branch joined the peroneal nerve. The motor nerve branch to the peroneus tertius muscle was identified and transected 1 cm proximal to the insertion point. Epineurial repair was performed using three 8-0 nylon monofilament sutures (S&T, Neuhausen am Rheinfall, Switzerland) between the distal portion of the transected nerve to the peroneus muscle and the transected branch to the tibialis anterior muscle (Fig. 2A, B). The epimysial array was sutured onto the epimysium of the peroneus tertius muscle as above. The native motor branch to peroneus tertius muscle was cauterized to prevent spontaneous reinnervation.

CLOSURE AND POSTOPERATIVE MANAGEMENT

All incisions were closed in 2 subcutaneous and 1 cutaneous layers using Vicryl (absorbable) suture (Ethicon Inc., Somerville, N.J.). Wounds were dressed with Mepitel (Mönlycke Health Care Limited, Bedfordshire, UK), sterile gauze,
 Silicone masking caps (Greentree Shercon, Tewkesbury, UK) were used to protect the external sockets.

**ELECTROMYOGRAPHY**

EMG was recorded during treadmill walking at 2 km·h⁻¹. For the 7 animals which had not undergone TMR, recordings were made 1, 2, 3, 4, 6, 8, 14, and 19 weeks following implantation. For the single animal which had undergone TMR, recordings were made weekly for 12 weeks. The bone-anchor was connected using a shielded cable. A reference electrode was placed over a suitable bony prominence: the left-leg hock joint (ankle).

At 19 weeks, skin surface EMG recordings were made for comparison. The peroneus tertius muscle was identified by palpation. The skin was shaved and cleaned with alcohol. Ag-AgCl gel surface electrodes (11 mm electrode diameter; .Vermed Inc., Buffalo, N.Y.) were applied with an interelectrode distance of 20 mm.

Recordings were made using a BIOPAC EMG100C differential electromyogram amplifier and an MP150 data acquisition system with AcqKnowledge version 4.1.1 software (all from BIOPAC Systems, Inc., Goleta, Calif.). Recording parameters were: 1000 samples per second, 100–500 Hz band pass, 50 Hz notch filter, and 500× amplification.

Signal-to-noise ratio (SNR) was calculated for 6 gait cycles per recording according to Equation 1 using MATLAB 2017b (The MathWorks, Inc., Natick, MA, USA).³⁹ Signal was identified visually; this was possible because at 2 km·h⁻¹ 1 gait cycle occurs approximately each second. SNR was used as an estimate of signal quality.

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Fig. 1. The bone-anchor and surgical procedure. A, The bone-anchor before epimysial array attachment. B, Bone-anchor inserted trans-tibially. C, Epimysial electrode array sutured to peroneus tertius muscle, with cable passing through a subcutaneous tunnel between the bone-anchor and the muscle.
signal noise = \frac{\sum v^2_{\text{signal}}}{\sqrt{N_{\text{signal}}}} - \frac{\sum v^2_{\text{noise}}}{\sqrt{N_{\text{noise}}}}

\text{PRETERMINAL MUSCLE STIMULATION}

Crosstalk was assessed by preterminal stimulation of adjacent muscles in animals which had not undergone TMR. Under general anesthesia, the peroneus tertius, tibialis anterior, peroneus longus, and gastrocnemius muscles and their associated motor nerve branch(es) were identified and exposed by dissection. Motor nerves were stimulated using 1 mA pulses (Medtronic Vari-Stim III, Medtronic Inc., Fridley, Minn.). Six readings per muscle were recorded from the epimysial array on the peroneus tertius as described above. Following muscle stimulation, the animals were euthanized by intravenous injection of 0.7 mg/kg sodium pentobarbitone (Pharmasol Ltd., Hampshire, UK).

\text{FORCE PLATE ANALYSIS}

For the TMR study, recovery of weight bearing was assessed using ground reaction force. Four days before surgery, and at regular intervals before or after EMG recording, the animal was walked across a force plate and at least 6 measurements for each hind limb (left and right) were recorded. Measurements were normalized for animal weight and reported as $F_{\text{max}}$/weight.

\text{IMPEDANCE SPECTROSCOPY}

Impedance spectroscopy was carried out as described previously\textsuperscript{23} from $10^4$ to $10^5$ Hz at 0.4 Vp-Hz at 0.4 Vp-p using an EVAL-AD5934EBZ impedance monitor (Analog Devices, Norwood, Mass.). Measurements were made before implantation, after 19 weeks in vivo under terminal anesthesia, and following explantation with 3 mm of surrounding tissue, in 0.9% saline solution.

\text{STATISTICAL ANALYSIS}

Values are reported or plotted as mean ± 95% CI unless otherwise stated. Statistical comparisons were carried out using nonparametric tests using SPSS for Linux version 20 and SPSS for Windows version 22 (IBM Corp., Armonk, N.Y.).

\text{RESULTS}

The single animal which had undergone TMR was euthanized after 12 weeks. A total of 5 animals were euthanized after 19 weeks. Two animals were euthanized before the intended endpoints. A single animal was euthanized at week 5 after diagnosis of a chronic infection of a surgical site for a different, concurrent study, and a single further animal was added to the study. A single animal was diagnosed with Johne’s disease, a paranatally acquired infection of ruminants not associated with this study\textsuperscript{40}; this animal was euthanized at 12 weeks.

\text{GROSS MORPHOLOGY}

Where bone-anchors were placed proximally on the tibia, skin movement caused sieving: an exposed flange with the epidermal layer within the flange, rather than above the flange. This complication was first observed at week 4 with thinning of the skin overlying the flange. In a single animal, the flange became visible at 8 weeks with dry necrosis of the skin, and no abnormal reddening, exudate, or other evidence of infection was observed. Where bone-anchors were placed in a more distal position, the skin–implant interface appeared stable. Fibrous capsules formed around the electrodes and cable in every animal overlying healthy muscle.

Following TMR, muscle atrophy was observed on the tibialis anterior and peroneus tertius muscles. The reinservated nerve was in continuity, and no reinnervation was observed from the cauterized end of the native motor branch to peroneus tertius muscle (Fig. 2C).
Histological analysis was previously reported in Dowling (2015)\(^1\) and is summarized here. The explanted bone-anchors showed well-vascularized dermal tissue integration throughout the porous flange (Fig. 3) with 71 blood vessels per mm\(^2\) (median, 95% CI, 49-98 mm\(^{-2}\)) infiltrating 90% of pores (median, 95% CI, 68%–100%). Where dermal tissue infiltration was poor, gaps at the tissue implant interface were observed. Downgrowth, where the epidermal cell layer descends along the implant shaft, was observed (5.36 mm median downgrowth from skin surface, 95% CI, 4.65–10.4 mm). Downgrowth through a thick skin section to the flange forms a sinus descending alongside the implant, and debris between the shaft and epidermis causes a pocket to form at the base of the sinus (see figure, Supplemental Digital Content 1, which displays epidermal downgrowth and sinus track formation along the implant shaft, http://links.lww.com/PRSGO/B180; and figure, Supplemental Digital Content 2, which displays debris impeding epidermal attachment at the base of a sinus, http://links.lww.com/PRSGO/B181).

Where downgrowth remained limited, contact between epidermal tissue and the bone-anchor shaft suggested a stable transcutaneous interface above the flange (see figure, Supplemental Digital Content 3, which displays epidermal attachment to the implant with limited downgrowth, http://links.lww.com/PRSGO/B182).

**EMG RECORDINGS**

EMG recordings typically show low noise, with the gait cycle readily identifiable (see Fig. 4A and figure, Supplemental Digital Content 4, which displays Raw EMG and power spectrogram of EMG recordings from epimysial electrodes and skin surface electrodes after 19 weeks, http://links.lww.com/PRSGO/B183). Coincident surface electrode recordings show additional activity, increased noise, and less readily identifiable gait cycle (Fig. 4B and SDC4). SNR was typically in the range 10 to 25 dB and was 18.7 dB (± 6.4 dB, 95% CI) after 19 weeks (see Fig. 5A and figure, Supplemental Digital Content 5, which displays summarized SNR data for each individual animal with the standard surgical procedure, http://links.lww.com/PRSGO/B184). SNR agreed with previous work\(^2\) reassessed using the present analysis methods (Fig. 5C). At 19 weeks, SNR was greater for epimysial EMG (19.6±7.4 dB) compared with surface EMG (6.65±7.63 dB), but the difference was not statistically significant by Wilcoxon signed rank test (Z = 15, 7; \(P = 0.0625\)) (Fig. 6).

**EVOLED CROSSTALK**

Crosstalk was assessed by stimulation of adjacent muscles under terminal anesthesia in animals which had not undergone TMR. Largest compound muscle action potentials (CMAPs) were observed when stimulating the peroneal nerve muscles: peroneus tertius (0.0338±0.0032 V), peroneus longus (0.0200±0.0019 V), and tibialis anterior (0.0077±0.0010 V). Smallest CMAPs were observed when stimulating the 13 gastrocnemius (0.0048±0.0012 V).

**EMG AND GAIT FOLLOWING TMR**

After 6 weeks following TMR, gait and EMG recovery had occurred. SNR was not different from recordings from animals without TMR after 6 weeks, but was lower before this time (Fig. 5B). Raw EMG shows some activity identifiable at 3 weeks (Fig. 7A), indicative of gait cycle; however, at low amplitude, it is not possible to differentiate crosstalk and reinnervation in this case. After 10 weeks, the gait cycle is readily identifiable (see Fig. 7B and figure, Supplemental Digital Content 6, which displays Raw EMG and power spectrogram of EMG recordings following TMR, http://links.lww.com/PRSGO/B185). Mean EMG frequency was lower following muscle reinnervation (Fig. 5D).

After 6 weeks, weight bearing recovered such that normalized ground reaction force was not significantly different between legs (\(P \geq 0.108\), by Wilcoxon signed rank test) (Fig. 8). From 1 week after surgery to 4 weeks after surgery, inclusive ground reaction force was significantly reduced on the left (operated) leg (\(P \leq 0.002\), \(\alpha_{Bonferroni} = 0.007\)) except at week 3 where normalized ground reaction force was not significantly different (\(P = 0.064\), by Wilcoxon signed rank test). This indicates that recovery of gait correlated with recovery of muscle function.

**ELECTRODE IMPEDANCE**

Mean electrode impedance before implantation was 1.3kΩ (1338±17 Ω); after 19 weeks, in vivo mean impedance was 2.2kΩ (2153±388 Ω); and following explant and fixation in formal saline, mean impedance was 3.1kΩ (3093±2063 Ω).

**DISCUSSION**

We have demonstrated epimysial EMG recording from 5 animals over 19 weeks, and a further 2 animals over 12 weeks and 1 animal over 5 weeks. The bone-anchored devices performed well, providing a transcutaneous cable route, while preserving the skin barrier. In a single animal, we observed EMG and functional recovery 6 weeks following TMR. The results presented in this paper fur-
ther support the use of bone-anchors as hard-wired portals for biosignal recording.

The present study uses a trans-tibial insertion to minimize recovery time, allowing functional recordings from 1 week following implantation. In practice, bone-anchored devices are intended for longitudinal, intramedullary insertion following amputation, and similar devices, without the electrode cable, are in use clinically and in veterinary practice.\(^9,42\) Contrasting forces, for example, lack of weight bearing, therefore make this trans-tibial in vivo model less useful for assessment of the bone-implant interface. However, it remains valid to assess the skin–implant interface overlying and within the porous flange. The present flange is cylindrical, and alternative flange designs, including those in use clinically, are dome shaped\(^9,37\); we expect that a dome shape will reduce skin damage and sieving due to movement and pressure at the flange edge.

The implant design, with electrode cable(s) exiting directly into soft tissue below the porous flange presents advantages over other designs. Alternative bone-anchored portals have a cable exit within the bone medulla, requiring an additional surgical hole in the bone cortex for cable routing to the muscles.\(^17,24\) All designs introduce a path for infection along the cable within the bone-anchor. The present design is sealed with epoxy resin and medical grade silicone; however, for clinical use, a hermetic seal is preferable to restore the complete barrier within the bone-anchor.

This study assessed TMR in a single animal, showing functional, weight-bearing, recovery coincident with EMG recovery, 6 weeks after surgery. This study coapted a tibialis anterior nerve branch to the peroneus tertius motor nerve, which both act to extend the hock joint. Tibialis anterior function was not monitored; however, only 1 of 3 tibialis anterior motor nerve branches was transected to minimize functional impairment. Change in weight bearing may be due to the surgical procedure, rather than deinnervation and reinnervation, and the observed response may be due to healing and learning; a training effect may explain why EMG and weight-bearing recovery are coincident; and once the animal learns to use altered musculature, it can achieve a more normal gait. A control condition where the peroneus tertius motor nerve and single branch of the tibialis anterior nerve are transected and ligated to prevent reinnervation would allow differentiation between learning and reinnervation effects.

EMG recovery was assessed by SNR; however, subtler changes in reinnervated muscle occur including reduced EMG frequency, in agreement with previous investigation.\(^43\) This is possibly attributable to an increase in slow-twitch motor units, thereby reducing force output per motor unit. In self-reinnervated muscles, la afferent connections are lost, preventing proprioception, coordination, and stretch reflexes\(^44–46\); conflicting evidence from surgically reconstructed agonist–antagonist pairs has shown muscle spindle activity correlated with muscle stretch.\(^55\) Although TMR and regenerative peripheral nerve interface muscles

![Fig. 4. Raw EMG recordings from epimysial electrode array (A) and skin surface electrodes (B) after 19 weeks. Example traces recorded coincidentally from the same animal.](image)
no longer have roles in skeletal movement, maintaining proprioception would be beneficial. Reinnervation can also achieve sensory feedback, through targeted reinnervation of cutaneous nerves. 

Crosstalk is the portion of the EMG signal due to activation of nearby muscles, rather than the target muscle. Peak crosstalk CMAP decreased with increasing electrode–muscle distance. Reducing recording volume with smaller interelectrode distances (10 mm in this study) and tripolar electrode configurations can reduce crosstalk. The increased impedance in vivo supports previous studies showing a similar change.

This study used bipolar epimysial electrode arrays limiting this study to one recording location. By combining reinnervated muscles with implanted multielectrode arrays, greater information about movement intention could be gained. We are developing an implantable EMG recording system, which can be combined with multielectrode arrays. The hard-wired bone-anchor approach is not limited to muscle recordings: it is suitable for neural recording where sampling rates ≥10 kHz per channel are required.

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Fig. 7. Raw EMG recordings following targeted muscle reinnervation. Recordings made at (A) 3 weeks and (B) 10 weeks. Note the change in voltage (y-axis) scale.

Fig. 8. Change in normalized ground reaction force per leg following TMR on left leg; Right leg (black) and left leg (red). Dashed vertical line showing date of surgery.
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