

Improved control of a prosthetic limb by surgically creating electro-neuromuscular constructs with implanted electrodes

Jan Zbinden^{1,2}, Paolo Sassu^{1,3,4}, Enzo Mastinu^{1,2,5}, Eric J. Earley^{1,2,6}, Maria Munoz-Novoa^{1,7}, Rickard Brånemark^{8,9,10}, Max Ortiz-Catalan^{1,2,11*}

¹ Center for Bionics and Pain Research, Mölndal, Sweden.

² Department of Electrical Engineering, Chalmers University of Technology, Gothenburg, Sweden.

³ Department of Hand Surgery, Sahlgrenska University Hospital, Mölndal, Sweden.

⁴ Department of Orthoplastic, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy.

⁵ The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy.

⁶ Osseointegration Research Consortium, University of Colorado, Aurora, CO, USA

⁷ Center for Advanced Reconstruction of Extremities, Sahlgrenska University Hospital, Mölndal, Sweden.

⁸ Center for Extreme Bionics, Biomechatronics Group, MIT Media Lab, Massachusetts Institute of Technology, Cambridge, USA.

⁹ Department of Orthopaedics, Gothenburg University, Gothenburg, Sweden.

¹⁰ Integrum AB, Mölndal, Sweden

¹¹ Bionics Institute, Melbourne, Australia.

This is the author's version of the work. It is posted here with permission of the AAAS for personal use, not for redistribution.

The definitive version was published in:

Science Translational Medicine

Volume 15, Issue 704

12 July 2023.

DOI: [10.1126/scitranslmed.abq3665](https://doi.org/10.1126/scitranslmed.abq3665)

Improved control of a prosthetic limb by surgically creating electro-neuromuscular constructs with implanted electrodes

Jan Zbinden^{1,2}, Paolo Sassu^{1,3,4}, Enzo Mastinu^{1,2,5}, Eric J. Earley^{1,2}, Maria Munoz-Novoa^{1,6}, Rickard Brånemark^{7,8,9}, Max Ortiz-Catalan^{1,2,10*}

¹ Center for Bionics and Pain Research, Mölndal, Sweden.

² Department of Electrical Engineering, Chalmers University of Technology, Gothenburg, Sweden.

³ Department of Hand Surgery, Sahlgrenska University Hospital, Mölndal, Sweden.

⁴ Department of Orthoplastic, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy.

⁵ The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy.

⁶ Center for Advanced Reconstruction of Extremities, Sahlgrenska University Hospital, Mölndal, Sweden.

⁷ Center for Extreme Bionics, Biomechanics Group, MIT Media Lab, Massachusetts Institute of Technology, Cambridge, USA.

⁸ Department of Orthopaedics, Gothenburg University, Gothenburg, Sweden.

⁹ Integrum AB, Mölndal, Sweden

¹⁰ Bionics Institute, Melbourne, Australia.

One Sentence Summary

Surgically created electro-neuromuscular constructs allow for the intuitive control of all five fingers of a prosthetic hand after a transhumeral amputation.

Abstract

Muscles remnant after amputation are the most common source of control signals for prosthetic hands. This is because myoelectric signals can be intuitively generated by the user at will. However, in higher amputation levels, such as transhumeral, not enough muscles are left to generate myoelectric signals to control the many lost joints, thus making intuitive control of

30 the prosthetic joints (*e.g.*, wrist and fingers) unattainable. Here, we show that severed nerves
31 can be divided along their fascicles and redistributed to concurrently innervate different types
32 of muscle targets, particularly native denervated muscles and non-vascularized free muscle
33 grafts. We instrumented these neuromuscular constructs with implanted electrodes accessible
34 via a permanent osseointegrated interface allowing for bidirectional communication with the
35 prosthesis while also providing direct skeletal attachment. We found that the transferred nerves
36 effectively innervated their new targets as proven by a gradual increase of myoelectric signal
37 strength. This allowed for individual flexion and extension of all fingers of a prosthetic hand
38 by a patient with a transhumeral amputation. Improved prosthetic function in tasks
39 representative of daily life was also observed. This proof-of-concept indicates that the amount
40 of motor neural commands can be considerably increased by creating electro-neuromuscular
41 constructs using distributed nerve transfers to different types of muscle targets and implanted
42 electrodes, and thus could enable the next generation of more highly integrated
43 neuromusculoskeletal prostheses.

44 Introduction

45 Upper limb amputation substantially changes the way in which one interacts with the
46 environment, with the most proximal amputations suffering from the highest impairment as
47 function decreases with every lost joint. Mechatronic joints can now be combined to replace
48 limbs lost to amputations. However, commanding artificial joints in a similar manner to
49 biological ones has remained challenging. Myoelectric signals extracted from residual muscles
50 in the stump have been the preferred source of control in mechatronic prostheses (*a.k.a*
51 myoelectric prostheses). The standard clinical solution is for prosthetic users to modulate the
52 activity of an antagonistic pair of remanent muscles to drive the activation of a single degree
53 of freedom (DoF) prosthetic joint. The problem is that there are not enough remanent muscles
54 to drive several prosthetic joints in an intuitive manner. For example, the opening and closing
55 of a prosthetic hand can be driven by the contraction of the biceps and triceps muscles in a
56 transhumeral amputation, but no other muscles are left to control the prosthetic elbow, wrist,
57 or individual fingers.

58 Surgically reconstructing the extremity can ameliorate the problem of insufficient control
59 signals by creating new myoelectric sites . Nerve transfers to native but denervated muscles,
60 also known as targeted muscle reinnervation (TMR) (2), have gained popularity in the past
61 decade. This procedure repurposes remnant muscles at the stump to amplify neural motor
62 commands to the missing joints. This is achieved by denervating a muscle with dispensable
63 biomechanical function, and then reinnervating it with a major nerve severed by the amputation
64 (*i.e.*, a nerve transfer). Once the muscle has been reinnervated, electrodes on the surface of the
65 skin can be used to record neural commands to the missing limb as the reinnervated muscles
66 are large enough to electrically radiate transcutaneously. A downside of this approach is that
67 all the motor information normally contained in the severed nerves is reduced to a single

68 myoelectric signal. Therefore, reconstruction with nerve transfer to native muscles has only
69 allowed for up to six independent control signals (3), which are enough to drive a mechatronic
70 elbow, wrist, and end effector (each using a pair of signals/electrodes). However, dexterous
71 manipulation of objects is out of reach with this approach as no additional information is left
72 for individual finger control.

73 Recently, another surgical approach has been suggested in which rather than transferring the
74 severed nerve to a native muscle, said nerve is longitudinally dissected into several fascicles
75 and then each fascicle is wrapped with a free muscle graft. This strategy, also known as
76 regenerative peripheral nerve interfaces (RPNIs) (4, 5), aims at extracting more motor neural
77 information at the cost of accessibility. Accessibility here is a problem because the free muscle
78 grafts are too small to be recorded with surface electrodes, and thus the implementation of such
79 an approach in prosthetic devices requires the use of implanted electrodes (6). Implanted
80 electrodes have shown to improve prosthetic control over their skin-surface non-invasive
81 counterparts (7–9), but bring an additional obstacle to clinical implementation, namely the need
82 for a safe, reliable, and long-term stable transcutaneous interface between the implanted
83 electrodes and the prosthesis (10). The lack of such an interface has hindered the clinical
84 adoption of implanted electrodes and the more refined surgical techniques for artificial limb
85 replacement.

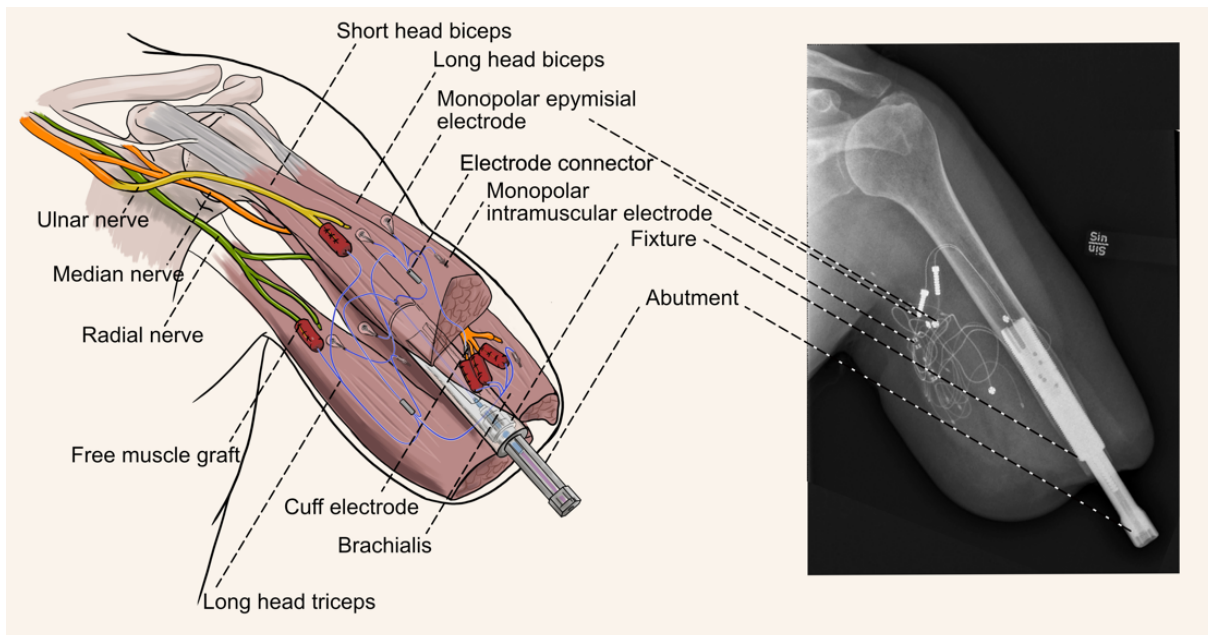
86 In this study, we present a novel combination of surgical reconstruction procedures combined
87 with a long-term stable neuromusculoskeletal interface that allows for bidirectional
88 communication between electro-neuromuscular constructs and the artificial limb (Figure 1). In
89 a patient with a transhumeral amputation, we transected the neuromas of the three major nerves
90 at the residual limb (median, ulnar, and radial), and then dissected each nerve longitudinally in
91 two groups of fascicles. One group was transferred to reinnervate native muscles that we first
92 denervated. Electrodes were then implanted in these (to be) reinnervated native muscles, as

93 well as in the other available unreconstructed muscles. The other group of fascicles was further
94 dissected to expose individual fascicles that were then used to reinnervate free muscle grafts
95 harvested from the lower limb. These grafts housed an intramuscular electrode each. These
96 newly created electro-neuromuscular constructs were used to extract motor commands directed
97 to the missing hand allowing for the intuitive flexion and extension control of all five fingers
98 of a prosthetic hand in a patient with a transhumeral amputation (demonstration in Movie S1).
99 In addition, improved prosthetic function while performing tasks normally found in daily life
100 was also observed (demonstration in Movie S2).

101 Results

102 Surgical procedure and neuromusculoskeletal interface

103 A 51 years-old male who lost his left arm above the elbow due to a traumatic injury participated
104 in this study. He was provided initially with a conventional myoelectric prosthesis using skin-
105 surface electrodes but used it rarely due to discomfort related to the socket attachment. In
106 addition, he was unsatisfied with the limited and unreliable myoelectric control provided by
107 the surface electrodes. We addressed these problems with the combination of surgical
108 reconstruction procedures together with an electromechanical interface for prosthesis
109 attachment and bidirectional communication with implanted electrodes. This
110 neuromusculoskeletal interface was implanted in three different stages. First, a titanium fixture
111 was implanted intramedullary and left undisturbed to osseointegrate (Figure 1). Six months
112 later, a percutaneous component (abutment, Figure 1) was coupled into the fixture allowing for
113 the skeletal attachment of the prosthetic arm, thus addressing the patient's socket-related
114 discomfort. The limited and unreliable prosthetic control was then addressed in a third surgery
115 by reconstructing the neuromuscular structures within the residual limb and instrumenting
116 them with implanted electrodes (Figure 1).



117

118

Figure 1 Schematic illustration and X-ray of a highly integrated human-machine interface.

119

120 The median nerve was dissected longitudinally in four groups of fascicles to reinnervate the
 121 brachialis muscle and three free muscle grafts (Figure 1). Similarly, the ulnar nerve was divided
 122 into two parts to innervate the short head of the biceps muscle and one free muscle graft (Figure
 123 1). The radial nerve was divided into two parts to then reinnervate the lateral head of the triceps
 124 muscle and one free muscle graft (Figure 1). The neuromas located at the end of these nerves
 125 were transected prior dissection and transfer. The non-vascularized muscle grafts were
 126 obtained from the vastus lateralis muscle in the thigh.

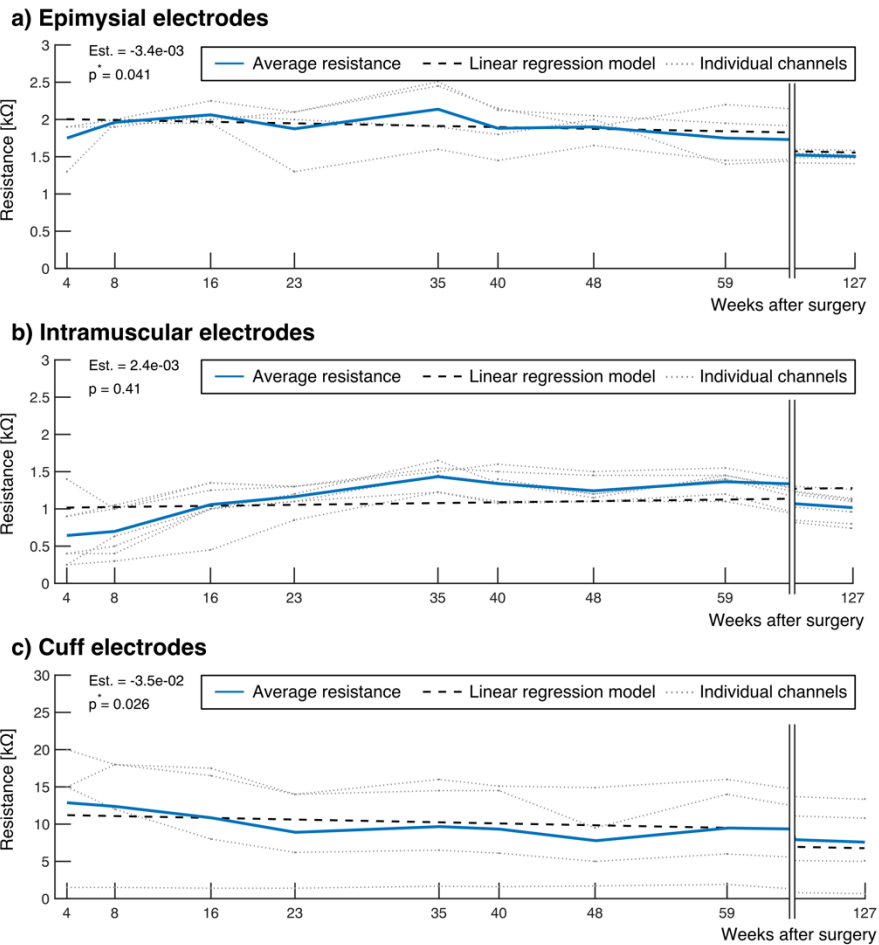
127 Intramuscular electrodes were inserted in native muscles as well as in the free muscle grafts.
 128 Epimysial electrodes were implanted on each head of the biceps muscle, and on the lateral and
 129 long heads of the triceps muscle (Figure 1). A cuff electrode with a mixed-tripolar contact
 130 configuration (II) was implanted around one of the fascicles of the median nerve (Figure 1).
 131 Communication between the implanted electrodes and the outside of the body was achieved

132 through the osseointegrated implanted within which feedthrough mechanisms allowed for
133 bidirectional signals transmission while sealing the interface (9).

134 A revision surgery for the skin interface was performed to remove excess granulation tissue at
135 week 65 post-implantation. The patient was treated with antibiotics after superficial skin
136 infections at week 94, 135, and 171 post-implantation, and after a deep soft-tissue infection at
137 week 116 post-implantation.

138 Neuromusculoskeletal interface stability

139 The stability of the neuromusculoskeletal interface was examined by monitoring the electrical
140 resistance of each implanted electrode over time. The resistance of the epimysial electrodes
141 ranged between 1.3-1.9 k Ω at four-weeks post-implantation and stabilized at 1.4-1.6 k Ω
142 (Figure 2a) two-years post-implantation. Similarly, the resistance of the intramuscular
143 electrodes ranged between 250-1400 Ω at four-week and then 740-1300 Ω (Figure 2b) two-
144 years after implantation. The cuff electrode has two types of contacts (11): ring and discrete.
145 The resistance of the ring contact was 1.5 k Ω at four-week and 1.5 k Ω two years post-
146 implantation, and the resistance of the three discrete contacts ranged between 15-20 k Ω at four
147 weeks and 5.1-14 k Ω after two years (Figure 2c). The resistance values for the intramuscular
148 electrodes did not show a statistically significant change over time ($p = 0.41$). The resistance
149 values of the epimysial and cuff electrodes decreased slightly during the second year ($p = 0.041$
150 and $p = 0.026$, respectively). One intramuscular electrode was considered an outlier and
151 excluded from the aforementioned range and further analyses, since it increased from 1.3 k Ω
152 to 141 k Ω between 40- and 127-weeks post-implantation. This abnormally large increment was
153 most likely due to damage to the external connector pin. For an overview of all resistance
154 values see Tables S1.



155

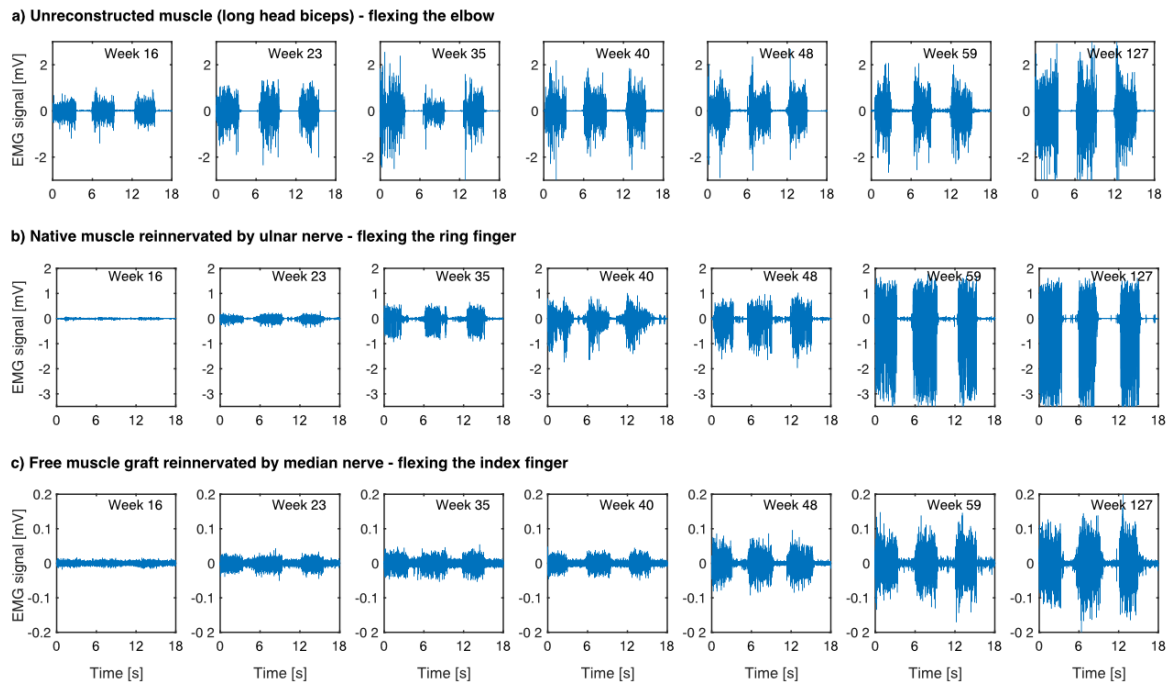
156 *Figure 2 Electrical resistance of epimysial, intramuscular, and cuff electrodes over time. (a) Resistance of the epimysial*
 157 *electrodes on unreconstructed and reinnervated native muscles (n=4). (b) Resistance of the intramuscular electrodes in*
 158 *unreconstructed muscles, reinnervated native muscles, and reinnervated free muscle grafts (n=8). (c) Resistance of the ring*
 159 *and three discrete contacts the cuff electrode around a fascicle of the median nerve (n=4). The parameter Est. denotes the*
 160 *slope of the linear regression.*

161

162 Reinnervation development and myoelectric signals quality

163 The development of the signal quality of the different implanted electrodes was quantified by
 164 calculating the signal-to-noise ratio (SNR). As expected, weeks were required for the
 165 functional reinnervation of all muscular constructs, as opposed to the unreconstructed muscles
 166 from which electromyographic (EMG) activity was readily available directly after
 167 implantation. Relevant signs of reinnervation were observed 23 weeks post-implantation

168 (examples in Figure 3). Exceptions were the muscles reinnervated by the radial and median
 169 nerves, which began to display information about single finger actuation at around 40 weeks
 170 post-implantation.

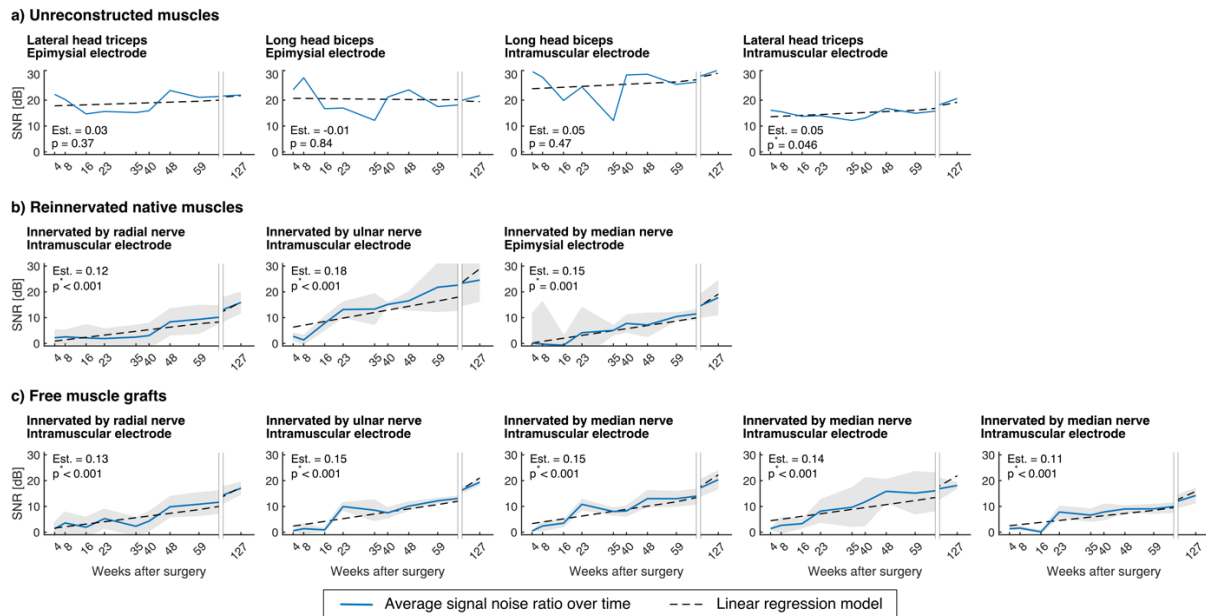


171

172 *Figure 3 Representative examples of the raw EMG signals over time. (a) Raw EMG signals for electrodes in an*
 173 *unreconstructed muscle, (b) a reinnervated native muscles, and (c) a free muscle graft. Each EMG recording consists of three*
 174 *seconds sequences of three active movement and rest periods.*

175 Soon after the surgery, the four electrodes placed in unreconstructed muscles displayed an
 176 average SNR of 23.3 ± 6.1 dB (Figure 4a) and remained nearly constant over time with an
 177 average SNR of 24.1 ± 5.4 dB at the end of the experiment period. The average SNR of the
 178 three electrodes in the reinnervated native muscles started at 1.6 ± 5.4 dB and increased to
 179 19.4 ± 6.4 dB (Figure 4b). All three electrodes in reinnervated muscles showed a significant
 180 increase in SNR over the 127 weeks of experimental period ($p = 5.8e-9$, $p = 3.8e-6$, and $p =$
 181 $1.3e-4$ for the radial, ulnar, and median electrodes, respectively). The electrodes in the five free
 182 muscle grafts started with an average SNR of 1 ± 0.8 dB and developed up to an average SNR

183 of 17.8 ± 2.4 dB (see Figure 4c). A significant SNR increase was observed over the two-year
 184 period since electrode implantation for the radial ($p = 2.7e-11$), ulnar ($p = 8.4e-10$), and the
 185 three median electrodes ($p = 2.4e-14$, $p = 9.3e-5$, $p = 1.5e-7$, respectively). For an overview of
 186 all SNR values see Tables S2.



187

188 **Figure 4 Signal to noise ratio development over time.** Change of signal to noise ratio of (a) four electrodes in unreconstructed
 189 muscles ($n=1$ movement), (b) three electrodes on/in reinnervated native muscles ($n=6$, $n=3$, and $n=4$ movements from left to
 190 right, respectively), and (c) five electrodes within reinnervated free muscle grafts ($n=6$, $n=3$, $n=5$, $n=3$, and $n=3$ movements,
 191 from left to right, respectively). The grey areas depict the standard deviation around the average values over the number of
 192 movements. For all signal to noise ratio calculations, only data of the main movements that anatomically corresponded to the
 193 electrode placement/innervation site were considered (see Methods for more details). The parameter Est. denotes the slope of
 194 the linear regression,

195 Decoding of motor volition

196 We decoded the intended movements of the missing arm and hand in two groups: nine gross
 197 movements and eleven finger movements. Gross movements consisted of hand open/close,
 198 wrist pro/supination, wrist flexion/extension, elbow flexion/extension, and no movement (rest).
 199 Finger movements consisted of all five fingers flexion/extension and no movement. We used
 200 information from all electrodes to compute offline and real-time classification performance by

201 Linear Discriminant Analysis. The *Motion Test (2)* as implemented in the open source platform
202 BioPatRec (12) was used to evaluate offline and real-time decoding.

203

204 Gross movements

205 The offline accuracy steadily increased within the first 23 weeks after implantation and
206 remained between 95.8% and 99.3% (see Figure 5a). The patient successfully completed the
207 *Motion Test* with gross movements for the first time 16 weeks post-implantation (see Figure
208 5b). Over the duration of the experiment, the completion time for a single gross movement
209 decreased from 4.0 ± 0.1 s to 1.8 ± 0.7 s with a minimum of 1.2 ± 0.2 s (see Figure 5c).
210 Correspondingly, the online accuracy of classifying all eight gross movements increased
211 significantly from $36 \pm 4\%$ to $66 \pm 16\%$ ($p = 0.005$), with a maximum of 75% 48 weeks after
212 implantation (See Figure 5d). For an overview of the Motion Test metrics for gross movements
213 see Tables S3.

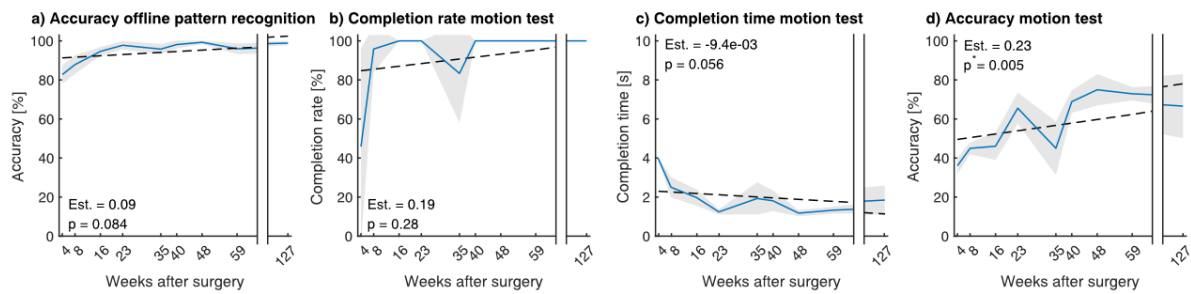
214

215

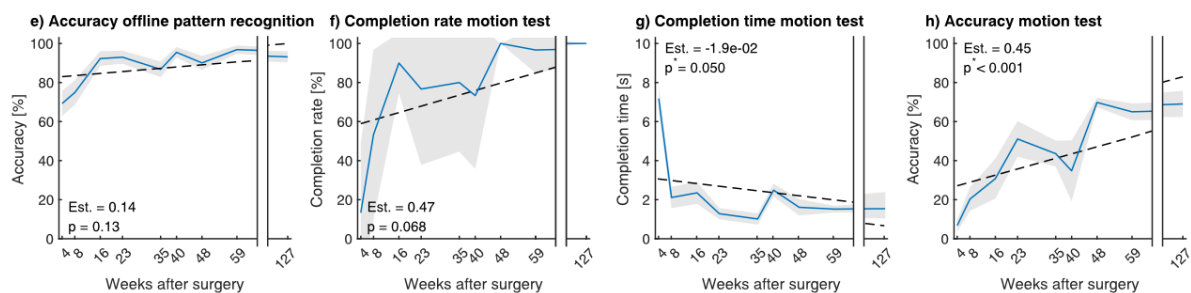
216 Single finger movements

217 The offline accuracy increased within the first 16 weeks and then remained between 86.7-
218 96.8% over the next two years (see Figure 5e). The average completion rate of the *Motion Test*
219 for single finger decoding increased from $13 \pm 35\%$ to 100% between the first and the last
220 training session (see Figure 5f). The average time for completion significantly decreased ($p =$
221 0.05) from 7.0 ± 0.6 s down to 1.6 ± 0.6 s (see Figure 5g). The average online classification
222 accuracy increased significantly ($p = 1.9e-5$) from $6 \pm 3\%$ to $69 \pm 6\%$ (see Figure 5h). For an
223 overview of the Motion Test metrics for finger movements see Tables S4.

Gross movements (Four degrees of freedom)



Single finger movements (Five degrees of freedom)



225

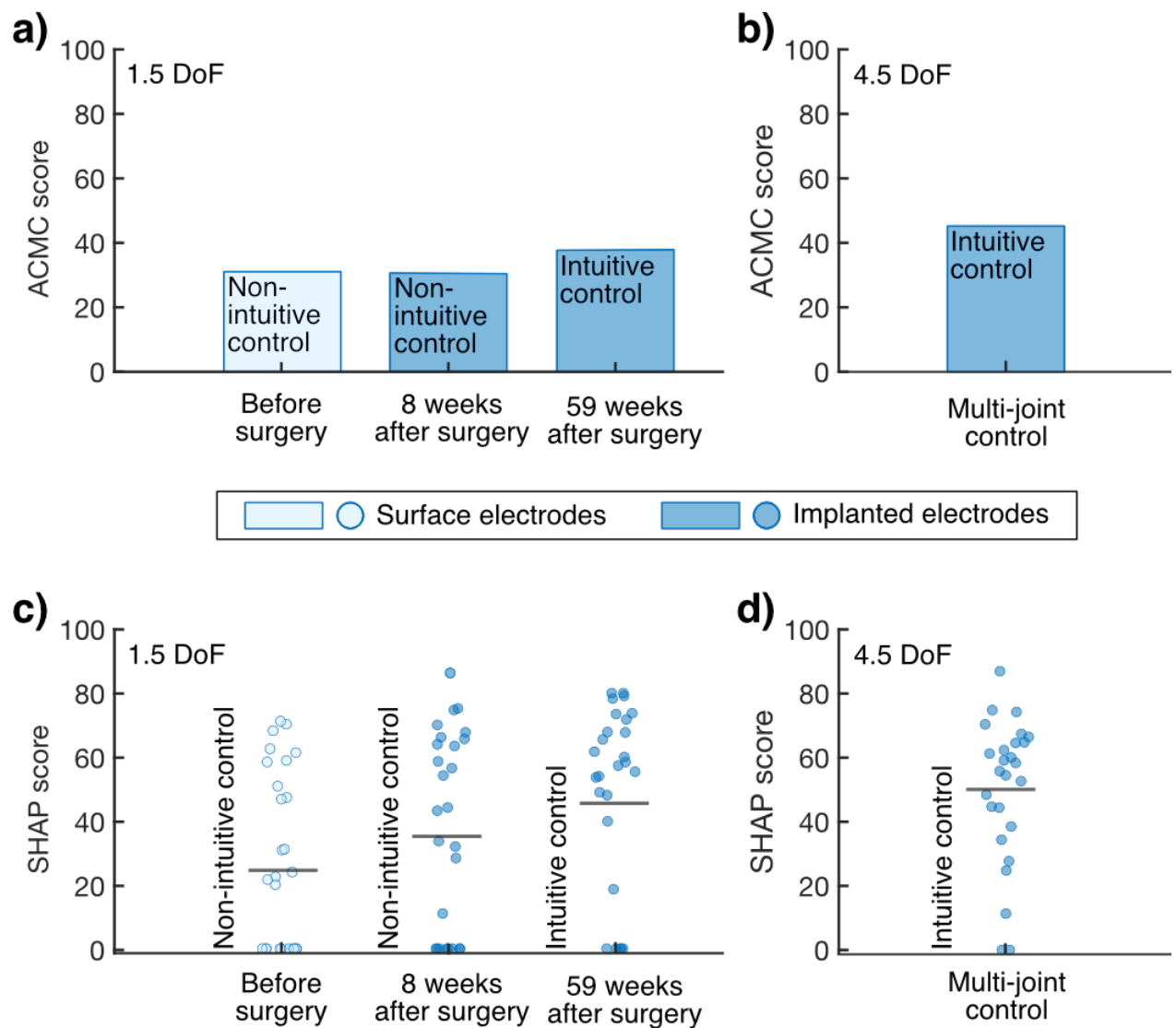
226 **Figure 5 Offline and online decoding performance.** Decoding performance using gross (hand open/close,
 227 supination/pronation, wrist flexion/extension, and elbow flexion/extension) and single finger movements over a two-year
 228 period. (a) Offline accuracy of nine gross movements ($n = 9$ movements, 100 repetitions). (b) Motion test completion rate for
 229 gross movements ($n = 9$ movements, 3 trials), where 100% indicates that all required movements were performed. (c)
 230 Completion time (time between the first decoded movement and twenty correct predictions of the promoted movement) and (d)
 231 accuracy (percentage of correct predictions) for the four degrees of freedom during the motion test. (e) Offline accuracy of
 232 individual fingers ($n = 11$ movements, 100 repetitions). (f) Completion rate, (g) completion time, and (h) accuracy for the
 233 motion test for individual finger decoding ($n = 11$ movements, 3 trials each). The parameter Est. denotes the slope of the linear
 234 regression.

235 Functional outcomes

236 We measured prosthetic function using the Assessment of Capacity for Myoelectric Control
 237 (ACMC) (13) and found it to improve by 16.7% at 59 weeks after the intervention (32.2 to
 238 37.6, see Figure 6a) using intuitive control with signals from the newly created myoelectric
 239 sites. Worthy of notice is that no improvement was observed earlier at 8 weeks after the
 240 intervention (32.2 to 30.3, see Figure 6a) using nonintuitive control with signals from

241 unreconstructed native muscles. The APMC was performed using a conventional prosthesis
242 with socket attachment and surface electrodes before the intervention, and skeletal attachment
243 and implanted electrodes after the intervention. The prosthesis consisted of a single degree-of-
244 freedom (DoF) myoelectric hand (MyoHand VariPlus Speed, Ottobock) and an elbow
245 (ErgoArm, Ottobock) with a myoelectric locking system (1.5 DoF combined). When taking
246 advantage of all the myoelectric sites by using a 4.5 DoF control scheme (allowing for
247 simultaneous and proportional control of the thumb, index finger, middle/ring/little fingers,
248 wrist rotation, and elbow lock/unlock), the patient achieved the highest improvement at 39.8%
249 (45.0 APMC score, Figure 6b). This was despite using such a system for the first time
250 (untrained). The 4.5 DoF prosthesis consisted of a multi articulated myoelectric hand
251 (BeBionic, Ottobock), a wrist rotator (Ottobock), and an elbow (ErgoArm, Ottobock) with a
252 myoelectric locking system. Conversely, the Southampton Hand Assessment Procedure
253 (SHAP) score improved both at 8 and 59 weeks after intervention by 42.6% and 82.7%,
254 respectively (24.9 to 35.5 and 45.8, Figure 6c). Using the 4.5 DoF control scheme with the
255 electro-neuromuscular constructs, the SHAP scored doubled to 50.1 (101.2%) compared to
256 conventional surface electrodes (see Figure 6d). For an overview of the functional outcome
257 values see Tables S5.

258



259

260 *Figure 6 Results of functional tests. (a) ACMC outcome, scored between 0 and 100, for a 1.5 DoF control scheme using*
 261 *surface electrodes before surgery and implanted electrodes at week 8 and 59 after surgery. A higher score indicates a higher*
 262 *capacity for myoelectric control. (b) ACMC outcome when using a 4.5 DoF simultaneous and proportional control scheme*
 263 *with the electro-neuromuscular constructs and without prior training. (c) Outcome of SHAP for surface electrodes before*
 264 *surgery and implanted electrodes at week 8 and 59 after surgery. The circles indicate the linear Index of Function, scored*
 265 *between 0 and 100, for each task of the SHAP ($n = 26$ tasks). The horizontal black line shows the weighted linear Index of*
 266 *Function (W-LIOF), where a higher score represents greater prosthetic functionality during activities of daily living. (d)*
 267 *Outcome of SHAP ($n = 26$ tasks) when using a 4.5 DoF simultaneous and proportional control scheme with the electro-*
 268 *neuromuscular constructs and without prior training.*

269 Discussion

270 In this study, we present a novel combination of surgical reconstruction procedures together
271 with a long-term stable neuromusculoskeletal interface. Thanks to the wired access to the
272 implanted electrodes provided by the neuromusculoskeletal interface, it was possible to track
273 the signals development over a period of two years. The signals development was analyzed in
274 terms of stability (*i.e.*, electrical resistance), quality (*i.e.*, signal-to-noise ratio), and feasibility
275 for advanced prosthetic control based on myoelectric pattern recognition (offline and in real-
276 time decoding). Aside from confirming the long-term stability of the interface and remarkable
277 strengthening of the myoelectric signals over time, we have shown that nerve transfer to
278 denervated native muscles, and non-vascularized free grafted muscles, increased the number
279 of myoelectric sources for prosthetic control to ultimately allow for independent control of all
280 five fingers (demonstration in S1 Movie).

281 The main limitation of our study is that only one patient was treated with this new surgical
282 approach. Here, we did not aim at comparing the surgical nerve transfer to native muscles
283 versus free muscle grafts, but rather to evaluate the feasibility of doing this concurrently.
284 Worthy of notice is that even if the nerve transfers would have not been successful, the patient
285 would have still enjoyed improved prosthetic control and sensory feedback using
286 unreconstructed sites (7, 14), which was an important ethical aspect to be considered in this
287 investigation.

288 The final number of reinnervation sites depended on the number of fascicles that can be
289 dissected without compromising naturally occurring fascicle crossings within the nerve trunk.
290 Larger diameter nerves, such as the median nerve, allow for more dissectible fascicles (four in
291 our study) than smaller ones, such as the radial nerve (two fascicles in our study). The number
292 of dissectible fascicles can vary from person to person and the level of amputation. Electrode

293 selection, on the other hand, depends on the type of muscular target. The free muscle grafts are
294 non-vascularized and are therefore dependent on blood diffusion from surrounding tissue for
295 survival, albeit vessels within the innervating fascicle can also contribute. Placing an epimysial
296 electrode would compromise blood diffusion in a relatively large surface area, and therefore
297 intramuscular electrodes are preferred for this muscular target. Native muscles are vascularized
298 and both epimysial and intramuscular electrodes have been employed successfully in this case.
299 We use the electric resistance to each electrode as a metric of long-term stability of the interface
300 as extreme high or low values would indicate an interruption of continuity (open circuit) or a
301 loss of independent connection (short circuit), respectively. Both scenarios would compromise
302 the use of the implanted electrodes and interface for prosthetic control. We found that the
303 electrical resistance of the implanted electrodes (including leads and feedthrough connectors)
304 remained stable within functional bounds. These results are in accordance with the long-term
305 electrical stability of previously-reported subjects that received neuromusculoskeletal
306 interfaces (7, 9).

307

308 We found that the transected nerves effectively innervated the native muscles and free muscle
309 grafts at about 5 months (ulnar and median nerves) and 11 months (radial nerve) post-surgery.
310 The innervation process led to distinct myoelectric signals appearing on the different electrodes
311 and a notable increase in SNR. As expected, the epimysial electrodes placed on
312 unreconstructed native muscles, not having to reinnervate, featured myoelectric signal activity
313 soon after the surgery and exhibited a stable and slightly increasing SNR over the whole 127
314 weeks.

315 In a previous study with two patients with transhumeral amputation (15), we tracked the
316 reinnervation of the radial and ulnar nerves into denervated native muscles reaching an average

317 SNR of 20 dB and 26 dB, respectively. Here, we report an average of 19.4 dB for similarly
318 reinnervated native muscles, despite using monopolar as opposed to bipolar electrodes as in
319 our previous study. When restricted on the number of possible electrode contacts in an implant
320 system, using monopolar electrodes allows for instrumenting more targets with the downside
321 of reducing SNR as compared with a bipolar configuration. Our results indicate that this is an
322 acceptable tradeoff when considering that more information can be obtained by adopting a
323 monopolar strategy.

324 In a study by Vu *et al.* (6), seven patients with upper limb amputation underwent RPNI surgery
325 for the treatment of neuroma pain. Three of their patients were implanted with intramuscular
326 electrodes with percutaneous leads one to three years after surgery. They reported an average
327 SNR of 4.2 dB with fine wire electrodes, 68.9 dB (after a three-year reinnervation period), and
328 21.0 dB (after a one-year reinnervation period) with intramuscular bipolar electrodes. These
329 SNR values are comparable with our findings for similarly reinnervated free muscle grafts at
330 two-years after surgery despite using monopolar electrodes (max. 21.7 dB, average 17.8 dB),
331 and therefore expecting lower SNR values compared to a bipolar configuration.

332 The two patients with intramuscular electrodes in native and reconstructed muscles in Vu *et*
333 *al.*'s study were able to control some finger movements (thumb, ring finger, and small finger
334 flexion, thumb opposition, and concurrent ab- and ad-duction of all fingers) albeit limited to
335 four movements at the time (6). These patients had transradial amputations and therefore some
336 of the native muscles responsible for finger control were available, as opposed to a
337 transhumeral amputation where all muscles related to finger control are lost. For a person with
338 a transhumeral amputation that underwent TMR, Osborn *et al.* (16) demonstrated in a video
339 recording the real-time individual flexion of up to three fingers at the time, with combined
340 extension, effectively decoding four movements. In comparison to these studies, we believe
341 that the considerable increase in prosthetic control reported here (independent flexion and

342 extension of all five fingers) was made possible by combining different surgical techniques
343 and thereby creating notably more sources for myoelectric control.

344 Over the duration of our investigation, the patient's ability to differentiate hand movements
345 improved steadily. Our patient successfully completed *Motion Tests* with gross movements (4-
346 DoF) already 16 weeks post-implantation, with faster completion times and increasing
347 accuracy over time. Concurrently with the innervation of the radial nerve, the subject
348 completed the *Motion Test* with single finger movement (5-DoF) at 48 weeks post-
349 implantation. Similar completion times and accuracies were achieved in both sets of
350 movements (gross and single fingers).

351 Kuiken *et al.* performed TMR surgery on five subjects (three with shoulder disarticulation and
352 two with transhumeral amputation) and investigated real-time decoding of ten elbow, wrist,
353 and hand movements using surface electrodes (2). They reported an average offline
354 classification accuracy of 95 % and *Motion Test* completion rates of 86.9 % and 96.3 % with
355 completion times of 1.54 s and 1.29 s, for four hand movements and for six elbow and wrist
356 movements, respectively. Another study by Cipriani *et al.* also demonstrated the feasibility of
357 real-time finger control (17), albeit in transradial amputees as opposed to transhumeral. They
358 reported the decoding of seven movements, four of which were flexing individual fingers, with
359 an average *Motion Test* completion rate of 79 ± 16 %. In comparison with these studies, we
360 assume that our patient was able to achieve higher completion rates (100%) owing to the
361 combination of surgical reconstruction and permanently implanted electrodes.

362 Reliability in daily use has been reported as the main factor for prosthesis acceptance (18). And
363 at a first glance, our reported *Motion Test* accuracy (69-75%) might suggest low reliability,
364 despite being higher than previously reported to yield acceptable controllability (19). It is worth
365 noticing that decoding accuracy is not synonymous with controllability. Spurious

366 misclassifications are common in decoders but do not translate necessarily to noticeable errors
367 during prosthetic control as classification output is continuously overwritten and low-pass
368 filtered by the motors in the prosthesis. For the *Motion Test* experiment, the patient was asked
369 to perform pre-selected movements intuitively during the recording session, without prior
370 training of the movements. Exploring the signal space together with the patient to find and train
371 more distinguishable, yet still intuitive, signal patterns for the different movements would
372 likely lead to an improvement in decoding consistency.

373 The pre-defined movements for the *Motion Test* experiment were selected to showcase the
374 potential of the surgical reconstruction approach. To increase reliability during home-use, a
375 subset of all the movements can be chosen instead. For example, only actuating the thumb and
376 index finger individually and actuating the middle, ring, and little finger as one movement
377 decreases the complexity of control drastically while still offering considerable functional
378 improvements.

379 A sequential decoding scheme, meaning that only one movement is output at the time, limits
380 functionality during home-use. Simultaneous control would increase the functional benefits
381 during home use, especially for finger movements (closing individual fingers sequentially to
382 perform a grasp would be slow, unintuitive, and frustrating). Adding proportionality could
383 further contribute to reliability and intuitiveness.

384 Based on the above-described considerations, we used a 4.5 DoF (thumb, index finger,
385 middle/ring/little fingers, wrist rotation, and elbow lock/unlock) simultaneous and proportional
386 control scheme to evaluate the functional benefits of a higher DoF controller made possible by
387 the additional myoelectric sources created by the surgical reconstruction. The use of the
388 neuromusculoskeletal prosthesis resulted in an increase of 12.8 points (however, the minimum
389 detectable change is 14.5 points (20)) from 32.2 to 45.0 during the ACMC, and a doubling of

390 the SHAP score from 24.9 to 50.1 compared to the standard two-site surface EMG electrode
391 prosthesis used prior to the surgical intervention. Even when using the same control scheme
392 (1.5 DoF direct control), the intuitive control and the added reliability from having the
393 electrodes implanted led to an increase in prosthetic functionality (ACMC score increased from
394 32.2 to 37.6 and the SHAP score increase from 24.9 to 45.8) compared to the two-site surface
395 electrode prosthesis used before the intervention. These results indicate that electro-
396 neuromuscular constructs using implanted electrodes can lead to greater capacity for
397 myoelectric control and thereby functionality for everyday tasks (demonstration in S2 Movie).

398 The improvements in functionality were achieved despite the patient only having used such a
399 prosthesis for a couple of hours. We therefore assume that the intuitive control granted by the
400 newly created myoelectric sources greatly facilitated learning a more complex control scheme.

401 Given the improvement in functionality we observed when the patient used the 1.5 DoF
402 prosthesis over an extended time at home, we foresee that prolonged use of the 4.5 DoF
403 prosthesis could lead to further functional improvements to facilitate activities of the daily life.

404 Due to constraints on the patient's availability, we performed only one set of functional tests
405 during each follow-up. Therefore, a limitation of our study is that we did not disentangle the
406 separate contributions to improvement of 1) electrode type (surface vs implanted); 2)
407 intuitiveness of control (native residual muscles vs surgically reconstructed myoelectric sites);
408 and 3) potential learning effects (21). The SHAP but not the ACMC scores showed
409 improvement early post-intervention when the control scheme was non-intuitive, and both
410 scores showed improvement later at week 59, when the control was intuitive using the new
411 surgically reconstructed myoelectric sites and the patient was more experienced. Further
412 systematic work is needed to disentangle the contribution of each variable.

413 We observed undesired crosstalk between myoelectric sites that can be attributed to several
414 causes, such as the placement of the electrodes; the location of the electro-neuromuscular
415 constructs; the use of monopolar electrodes; and the nature of the neuromuscular constructs
416 themselves as they often share neural information related to the same movements. Greater
417 attention to the placement of electrodes and neuromuscular constructs can reduce signals
418 crosstalk, but not entirely. Intraoperative identification of nerve fascicles during reconstruction
419 remains challenging but achieving such a deed would considerably improve the creation of
420 optimal neuromuscular control sources. On the engineering side, recent advances in source
421 separation algorithms have shown to be a promising approach for decoding movement intent,
422 offering insights into the discharge patterns of motor neurons (22, 23). Indeed, it has been
423 shown that neural and myoelectric information can be combined to achieve more independent
424 control of different prosthetic joints (24, 25).

425 In this proof-of-concept study, we have demonstrated the feasibility of transferring severed
426 nerves to native muscles and free muscles grafts, resulting in long-term stable electro-
427 neuromuscular constructs using implanted electrodes that can be safely and reliably accessed
428 using a neuromusculoskeletal interface. Safety and reliability are prerequisites for the clinical
429 implementation of new prosthetic technologies. By merging surgical and engineering
430 technologies, our approach allows for a more comfortable use of the prosthesis (26), for a more
431 functional load transfer between the prosthesis and the skeleton (27, 28), for a more reliable
432 and precise control of the prosthesis (8, 9), and opens possibilities for a more advanced and
433 dexterous control of a prosthetic hand supported by tactile sensory feedback (9, 29). This
434 research contributes to the wider clinical application of osseointegration and advanced surgical
435 reconstructions to improve artificial limb replacement.

436 Materials and Methods

437 Study design

438 This single-person case study investigated if creating electro-neuromuscular constructs using
439 distributed nerve transfers to different types of muscle targets instrumented with implanted
440 electrodes can increase the number of myoelectric sources for prosthetic control. The main pre-
441 specified study objective was to assess if additional myoelectric sources could be created by
442 transferring nerves to native and free grafted muscles and be used to decode motor volition (as
443 measured by the Motion Test). The secondary pre-specified objective was to assess the
444 innervation and development of the different surgical constructs over time (measured by the
445 signal to noise ratio (SNR) over time). In addition, we evaluated prosthetic function during
446 tasks representative of daily life (measured by the ACMC and SHAP).

447 One patient with a transhumeral amputation due to a traumatic injury (July 2015) participated
448 in this study. The first surgical stage, implanting the titanium fixture, was performed in
449 September 2017. The percutaneous component of the implant system was coupled into the
450 fixture in February 2018. And the third surgical stage (the basis for the presented study),
451 reconstructing the neuromuscular structures within the stump and instrumenting them with
452 electrodes, was performed in December 2018.

453 The study was designed to collect data over a period of one year at eight periodic follow-ups
454 (7.8 ± 2.9 weeks between lab visits) and at a follow-up two years (127 weeks) after
455 reconstruction and electrode implantation. Functional outcome data was collected before
456 surgery and at 8 and 59 weeks after the surgery. An additional round of functional tests with a
457 multi-DoF prosthesis was conducted 215 weeks after surgery as requested during the peer-
458 review process of this article.

459 During the time of this two-year study, the patient first used a prosthesis allowing for 1.5 DoF
460 (hand open/close and elbow lock/unlock) for daily home-use. At week 65, the patient was fitted
461 with an additional DoF (pronation/supination) (21). 181 weeks after the surgery, the patient
462 was also provided with an active elbow, allowing him to control a total of 3 DoF for daily
463 home-use. In all cases, a direct control approach was used, allowing for simultaneous and
464 proportional control of the individual DoF.

465 The study protocols were carried out in accordance with the declaration of Helsinki. The signed
466 informed consent was obtained before conducting the experiments. The study was approved
467 by the Regional Ethical Review Board in Gothenburg (Dnr. 18-T125).

468

469 [Surgical reconstruction and implantation](#)

470 The radial nerve was exposed through a curvilinear incision proximally in the arm at its exit
471 from the triangular interval. Branches to the lateral, long, and medial heads of the triceps were
472 identified with the help of a nerve stimulator and isolated. The motor branch to the lateral head
473 of the triceps was left intact, whereas the main branch to the long head was severed 5 mm
474 before its entrance into the muscle and marked for later neurotization.

475 The main trunk of the radial nerve was then followed distalward and its stump retracted
476 proximally. Once the large end neuroma was excised, the healthy-looking fascicles were split
477 longitudinally into two halves. One half was anastomosed to the motor branch supplying the
478 long head of the triceps. The other half lay in the center of a non-vascularized muscle graft
479 which was folded around the nerve following the principle of an RPNI (4, 5).

480 An anterior curvilinear incision in the arm gave access to the two major nerves, median and
481 ulnar, as well as the musculocutaneous nerve that was identified in the groove between the long

482 and short heads of the biceps muscle. The long head maintained its original innervation
483 whereas the motor branch of the short head was severed 5 mm before its entrance into the
484 muscle. The distal stump of the ulnar nerve was identified, retracted proximally, and its
485 neuroma excised. Similarly, to the radial nerve, the ulnar nerve was split longitudinally into
486 two halves: one was sutured to the motor branch of the short head of the biceps, and the
487 remaining half was placed in a non-vascularized muscle graft.

488 The distal stump of the median nerve was identified and retracted proximally, and the large
489 neuroma stump was excised. The nerve was divided longitudinally into four groups of
490 fascicles: one was reserved for sensory feedback and wrapped with an extraneural electrode;
491 the remaining three were utilized to reinnervate non-vascularized muscle grafts. All the free
492 muscle grafts were harvested from the vastus lateralis muscle in the homolateral thigh and had
493 a standard dimension of 5X3X1.5 cm.

494 Neuromusculoskeletal interface stability

495 The stability of the neuromusculoskeletal interface was examined over time by monitoring the
496 electrical resistance of each implanted electrode. The resistance was computed by applying
497 Ohm's Law to the voltage caused by a single current-controlled electrical pulse with known
498 parameters (100 or 200 μ A current amplitude and 100 μ s pulse width). The voltage was
499 measured via an oscilloscope and an isolated differential probe.

500 Myoelectric signals acquisition

501 During each follow-up, two EMG recording sessions were performed from all implanted
502 muscular electrodes. The recording sessions were performed using BioPatRec (12), an open-
503 source platform for research on myoelectric pattern recognition, and the Artificial Limb
504 Controller, an embedded system for controlling prosthetic devices (30). EMG monopolar data

505 was sampled at 500 Hz with 16-bit resolution and online high-pass and notch filtered at 20 Hz
506 and 50 Hz, respectively.

507 For each recording session, the subject was asked to comfortably sit in front of a computer and
508 follow on-screen instructions to perform a pre-selected set of movements. This study focused
509 on two different sets of movements, defined as “gross” and “single finger” movements. The
510 gross movements set included open and close hand, supination and pronation, flex and extend
511 wrist, and flex and extend elbow. The single finger movements set included flexion and
512 extension of all digits, *i.e.*, thumb, index, middle, ring and pinky fingers. For every set, each
513 movement was repeated three times, alternating 3s of muscular effort and 3s of resting time.
514 The subject was asked to perform each movement at approximately 70% of maximum
515 voluntary contraction.

516

517 Myoelectric signals quality

518 The development of the signal quality of the different implanted electrodes was quantified by
519 calculating the signal-to-noise ratio (SNR). For each recorded channel, the recording session
520 was divided into segments containing the voluntary contraction information of each movement,
521 and segments containing the resting state signal. Solely the steady-state of the EMG signal was
522 considered, thus the SNR was calculated on the middle 70% samples of each segment (see
523 Mastinu *et al.* for a similar approach (15)). Furthermore, only the movement data that
524 anatomically corresponded to the electrode placement was considered to calculate the SNR of
525 each of the individual electrodes. For calculating the SNR of the two electrodes on/in the
526 unreconstructed lateral head triceps only the recorded elbow extension movement, and for the
527 two electrodes on/in the unreconstructed long head biceps, only the recorded elbow flexion
528 movement were used. For the electrodes placed on/in sites innervated by fascicles of the radial

529 nerve, recordings of pronation, extend elbow and extension of all fingers were used if activation
530 was present. Hand, ring finger and pinky flexion movements were used to calculate the signal
531 to noise ratio values of the electrodes on/in sites innervated by fascicles of the ulnar nerve. For
532 the electrodes in sites innervated by fascicles of the median nerve, supination, hand flexion as
533 well as thumb, index finger, and middle finger flexion were considered if activation was
534 present. Finally, the root-mean-square values were calculated and then the SNR was obtained
535 according to the formula (1):

$$SNR_{dB} = 10 * \log_{10} \frac{EMG_{RMS, Movement(s)}^2}{EMG_{RMS, Rest}^2} \quad (1)$$

536

537 We considered native muscles and free muscle grafts to be effectively innervated at a sustained
538 SNR of four and higher.

539

540 Myoelectric pattern recognition

541 Offline pattern recognition accuracy

542 The EMG data from the recording sessions was segmented into overlapping time windows of
543 200 ms (50 ms time increment). The Hudgins' set of features (31) (mean absolute value, zero
544 crossing, waveform length, and slope changes) was extracted from each time segment. The
545 obtained features were randomly divided into training, validation, and test sets with a ratio of
546 40%, 20%, and 40%, respectively. Then, these sets were fed to a Linear Discriminant Analysis
547 (LDA) classifier for supervised training. Finally, the training was repeated 100 times and the
548 resulting averages, for both sets of movements, were reported.

549 Real-time pattern recognition accuracy

550 The real-time classification accuracy was assessed via the *Motion Test* introduced by Kuiken
551 *et al. (2)* as implemented in BioPatRec (12). In this test, the subject was asked to perform
552 movements randomly prompted on a screen. For every requested movement, 20 non-
553 contiguous classifications had to be correct within 10 seconds to deem the particular movement
554 completed. From this, several metrics were calculated:

- 555 • Completion Rate as the percentage of completed motions,
- 556 • Completion Time as the time between the first correct prediction and the completion of
557 the motion
- 558 • Real-time accuracy as the percentage of correct predictions over the total number of
559 predictions during the completion time.

560 The Motion Test was repeated three times for each set of movements, gross and single finger
561 movements. The average results and standard deviation over the three trials are reported.

562 Functional outcomes

563 Prosthetic functionality was evaluated before and after the surgical intervention using the
564 Assessment of Capacity for Myoelectric Control (ACMC)(13) and the Southampton Hand
565 Assessment Procedure (SHAP)(32). The ACMC measures a person's ability to perform daily
566 tasks, scoring 22 aspects of prosthetic use on a 4-point rating scale with a maximum of 66
567 points per task. A normed composite score between 0-100 can be obtained from the raw score
568 via Rasch analysis, where a composite score above 57.2 is classified as "extremely capable".
569 The SHAP consists of two parts: the first part involves 12 tasks where the subject grasps and
570 relocates abstract-shaped objects and the second part involves 14 tasks of activities of daily
571 living (ADLs), such as turning a door handle, unbuttoning a shirt, and opening a jar. The

572 execution times of all 26 tasks are used to calculate the weighted linear Index of Function (W-
573 LIF), a normed score where a score of 100 indicates normal hand function (33).

574 The patient's habitual two-site surface EMG electrode prosthesis (one electrode pair placed on
575 the bicep and triceps, respectively) with a direct control scheme was used for the pre-operative
576 tests, while the neuromusculoskeletal interface was used for post-operative tests.

577 A 1.5 DoF direct control scheme based on electrodes in the native biceps and triceps (i.e., non-
578 intuitive control) was used to control the prosthesis for the tests at 8 weeks after surgery. And
579 at 59 weeks after surgery, a 1.5 DoF direct control scheme based on signals from the surgically
580 created myoelectric sites was used to allow for intuitive opening (mapped to the signal from a
581 free muscle graft reinnervated by median nerve) and closing (mapped to the signal from a
582 native muscle reinnervated by ulnar nerve) of the prosthetic hand and lock/unlocking of the
583 elbow (mapped to the signal from the native biceps). In both cases, the patient used the same
584 control during daily life prior to the tests.

585 To demonstrate the functional benefits of the higher DoF controllers, the patient also performed
586 the tests using a 4.5 DoF simultaneous and proportional controller. The 4.5 DoF controller
587 allowed individual control of the thumb, index finger, middle/ring/little fingers, wrist rotation,
588 and elbow lock/unlock. For example, simultaneous activation of the thumb and index allowed
589 the patient to pinch, and simultaneous movement of all fingers allowed for hand opening and
590 closing. The control scheme was comprised of a multi-layer feed forward neural network
591 capable of multi-label classification (19) and a post-hoc proportionality algorithm taking the
592 predicted class and the current mean absolute value into account. Prosthetic function with the
593 4.5 DoF controller was tested without prior training.

594

595 Statistical analysis

596 To evaluate the development of the electrode impedance, the signals quality, and the offline
597 and real-time pattern recognition accuracies over time, we performed a linear regression
598 analysis as implemented in the “*fitlm*” function of MATLAB’s Statistics toolbox (Mathworks,
599 USA). For each linear regression analysis, we report the estimate (the slope of the linear
600 regression) and the *p*-value (outcome of the *t*-statistic with the hypothesis test that the estimate
601 is different to zero).

602

603 List of Supplementary Materials

604 Table S1. Overview of electrical resistance of epimysial, intramuscular, and cuff electrodes
605 over time

606 Table S2. Overview signal to noise ratio development over time

607 Table S3. Overview of offline and online decoding performance of gross movements

608 Table S4. Overview of offline and online decoding performance of finger movements

609 Table S5. Overview of results of functional tests

610 Movie S1. Single finger control of prosthetic hand

611 Movie S2. Functional outcomes: 4.5 DoF simultaneous and proportional control

612 References

- 613 1. M. Ortiz-Catalan, Engineering and surgical advancements enable more cognitively
614 integrated bionic arms. *Sci. Robot.* **6**, 4–6 (2021).
- 615 2. T. A. Kuiken, Targeted Muscle Reinnervation for Real-time Myoelectric Control of
616 Multifunction Artificial Arms. *JAMA* **301**, 619 (2009).
- 617 3. L. A. Miller, R. D. Lipschutz, K. A. Stubblefield, B. A. Lock, H. Huang, T. W. Williams, R. F.
618 Weir, T. A. Kuiken, Control of a Six Degree of Freedom Prosthetic Arm After Targeted Muscle
619 Reinnervation Surgery. *Arch. Phys. Med. Rehabil.* **89**, 2057–2065 (2008).
- 620 4. M. G. Urbanek, Z. Baghmanli, J. D. Moon, K. B. Sugg, N. B. Langhals, P. S. Cederna,
621 Quantification of Regenerative Peripheral Nerve Interface Signal Transmission. *Plast.*
622 *Reconstr. Surg.* **130**, 55–56 (2012).
- 623 5. M. G. Urbanek, T. A. Kung, C. M. Frost, D. C. Martin, L. M. Larkin, A. Wollstein, P. S.
624 Cederna, Development of a Regenerative Peripheral Nerve Interface for Control of a
625 Neuroprosthetic Limb. *Biomed Res. Int.* **2016**, 1–8 (2016).
- 626 6. P. P. Vu, A. K. Vaskov, Z. T. Irwin, P. T. Henning, D. R. Lueders, A. T. Laidlaw, A. J. Davis, C.
627 S. Nu, D. H. Gates, R. B. Gillespie, S. W. P. Kemp, T. A. Kung, C. A. Chestek, P. S. Cederna, A
628 regenerative peripheral nerve interface allows real-time control of an artificial hand in
629 upper limb amputees. *Sci. Transl. Med.* **12**, 1–12 (2020).
- 630 7. M. Ortiz-Catalan, B. Hakansson, R. Branemark, An osseointegrated human-machine
631 gateway for long-term sensory feedback and motor control of artificial limbs. *Sci. Transl.*
632 *Med.* **6**, 257re6-257re6 (2014).

- 633 8. E. Mastinu, F. Clemente, P. Sassu, O. Aszmann, R. Brånemark, B. Håkansson, M. Controzzi,
634 C. Cipriani, M. Ortiz-Catalan, Grip control and motor coordination with implanted and
635 surface electrodes while grasping with an osseointegrated prosthetic hand. *J. Neuroeng.*
636 *Rehabil.* **16**, 49 (2019).
- 637 9. M. Ortiz-Catalan, E. Mastinu, P. Sassu, O. Aszmann, R. Brånemark, Self-Contained
638 Neuromusculoskeletal Arm Prostheses. *N. Engl. J. Med.* **382**, 1732–1738 (2020).
- 639 10. M. Ortiz-Catalan, R. Brånemark, B. Håkansson, J. Delbeke, On the viability of implantable
640 electrodes for the natural control of artificial limbs: Review and discussion. *Biomed. Eng.*
641 *Online* **11**, 33 (2012).
- 642 11. M. Ortiz-Catalan, J. Marin-Millan, J. Delbeke, B. Håkansson, R. Brånemark, Effect on
643 signal-to-noise ratio of splitting the continuous contacts of cuff electrodes into smaller
644 recording areas. *J. Neuroeng. Rehabil.* **10**, 22 (2013).
- 645 12. M. Ortiz-Catalan, R. Brånemark, B. Håkansson, BioPatRec: A modular research platform
646 for the control of artificial limbs based on pattern recognition algorithms. *Source Code Biol.*
647 *Med.* **8**, 11 (2013).
- 648 13. L. M. Hermansson, A. G. Fisher, B. Bernspång, A. C. Eliasson, Assessment of Capacity for
649 Myoelectric Control: A new Rasch-built measure of prosthetic hand control. *J. Rehabil. Med.*
650 **37**, 166–171 (2005).
- 651 14. A. Middleton, M. Ortiz-Catalan, Neuromusculoskeletal Arm Prostheses: Personal and
652 Social Implications of Living With an Intimately Integrated Bionic Arm. *Front. Neurobot.*
653 **14**, 18 (2020).
- 654 15. E. Mastinu, R. Brånemark, O. Aszmann, M. Ortiz-Catalan, in *2018 40th Annual*

655 *International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*,
656 (IEEE, 2018), vol. 2018-July, pp. 5174–5177.

657 16. L. E. Osborn, C. W. Moran, M. S. Johannes, E. E. Sutton, J. M. Wormley, C. Dohopolski, M.
658 J. Nordstrom, J. A. Butkus, A. Chi, P. F. Pasquina, A. B. Cohen, B. A. Wester, M. S. Fifer, R. S.
659 Armiger, Extended home use of an advanced osseointegrated prosthetic arm improves
660 function, performance, and control efficiency. *J. Neural Eng.* **18**, 026020 (2021).

661 17. C. Cipriani, C. Antfolk, M. Controzzi, G. Lundborg, B. Rosen, M. C. Carrozza, F. Sebelius,
662 Online Myoelectric Control of a Dexterous Hand Prosthesis by Transradial Amputees. *IEEE*
663 *Trans. Neural Syst. Rehabil. Eng.* **19**, 260–270 (2011).

664 18. E. M. Janssen, H. L. Benz, J. H. Tsai, J. F. P. Bridges, Identifying and prioritizing concerns
665 associated with prosthetic devices for use in a benefit-risk assessment: a mixed-methods
666 approach. *Expert Rev. Med. Devices* **15**, 385–398 (2018).

667 19. M. Ortiz-Catalan, B. Hakansson, R. Branemark, Real-Time and Simultaneous Control of
668 Artificial Limbs Based on Pattern Recognition Algorithms. *IEEE Trans. Neural Syst. Rehabil.*
669 *Eng.* **22**, 756–764 (2014).

670 20. H. Y. N. Lindner, A. Langius-Eklöf, L. M. N. Hermansson, Test-retest reliability and rater
671 agreements of assessment of capacity for myoelectric control version 2.0. *J. Rehabil. Res.*
672 *Dev.* **51**, 635–644 (2014).

673 21. E. J. Earley, J. Zbinden, M. Munoz-Novoa, E. Mastinu, A. Smiles, M. Ortiz-Catalan,
674 Competitive motivation increased home use and improved prosthesis self-perception after
675 Cybathlon 2020 for neuromusculoskeletal prosthesis user. *J. Neuroeng. Rehabil.* **19**, 1–11
676 (2022).

- 677 22. D. Farina, I. Vujaklija, M. Sartori, T. Kapelner, F. Negro, N. Jiang, K. Bergmeister, A.
678 Andalib, J. Principe, O. C. Aszmann, Man/machine interface based on the discharge timings
679 of spinal motor neurons after targeted muscle reinnervation. *Nat. Biomed. Eng.* **1**, 0025
680 (2017).
- 681 23. S. Muceli, K. D. Bergmeister, K.-P. Hoffmann, M. Aman, I. Vukajlija, O. C. Aszmann, D.
682 Farina, Decoding motor neuron activity from epimysial thin-film electrode recordings
683 following targeted muscle reinnervation. *J. Neural Eng.* **16**, 016010 (2019).
- 684 24. J. A. George, T. S. Davis, M. R. Brinton, G. A. Clark, Intuitive neuromyoelectric control of
685 a dexterous bionic arm using a modified Kalman filter. *J. Neurosci. Methods* **330**, 108462
686 (2020).
- 687 25. B. Ahkami, E. Mastinu, E. Earley, M. Ortiz-Catalan, Extra-neural signals from severed
688 nerves enable intrinsic hand movements in transhumeral amputations. *Under Rev.* .
- 689 26. K. Hagberg, E. Häggström, M. Uden, R. Brånemark, Socket Versus Bone-Anchored Trans-
690 Femoral Prostheses. *Prosthetics Orthot. Int.* **29**, 153–163 (2005).
- 691 27. H. Van de Meent, M. T. Hopman, J. P. Frölke, Walking Ability and Quality of Life in
692 Subjects With Transfemoral Amputation: A Comparison of Osseointegration With Socket
693 Prostheses. *Arch. Phys. Med. Rehabil.* **94**, 2174–2178 (2013).
- 694 28. A. Thesleff, E. Haggstrom, R. Tranberg, R. Zugner, A. Palmquist, M. Ortiz-Catalan, Loads
695 at the Implant-Prosthesis Interface During Free and Aided Ambulation in Osseointegrated
696 Transfemoral Prostheses. *IEEE Trans. Med. Robot. Bionics* **2**, 497–505 (2020).
- 697 29. E. Mastinu, L. F. Engels, F. Clemente, M. Dione, P. Sassu, O. Aszmann, R. Brånemark, B.
698 Håkansson, M. Controzzi, J. Wessberg, C. Cipriani, M. Ortiz-Catalan, Neural feedback

- 699 strategies to improve grasping coordination in neuromusculoskeletal prostheses. *Sci. Rep.*
700 **10**, 11793 (2020).
- 701 30. E. Mastinu, P. Doguet, Y. Botquin, B. Hakansson, M. Ortiz-Catalan, Embedded System for
702 Prosthetic Control Using Implanted Neuromuscular Interfaces Accessed Via an
703 Osseointegrated Implant. *IEEE Trans. Biomed. Circuits Syst.* **11**, 867–877 (2017).
- 704 31. B. Hudgins, P. Parker, R. N. Scott, A new strategy for multifunction myoelectric control.
705 *IEEE Trans. Biomed. Eng.* **40**, 82–94 (1993).
- 706 32. C. M. Light, P. H. Chappell, P. J. Kyberd, Establishing a standardized clinical assessment
707 tool of pathologic and prosthetic hand function: Normative data, reliability, and validity.
708 *Arch. Phys. Med. Rehabil.* **83**, 776–783 (2002).
- 709 33. J. G. M. Burgerhof, E. Vasluian, P. U. Dijkstra, R. M. Bongers, C. K. van der Sluis, The
710 Southampton Hand Assessment Procedure revisited: A transparent linear scoring system,
711 applied to data of experienced prosthetic users. *J. Hand Ther.* **30**, 49–57 (2017).

712

713 Acknowledgements

714 The authors thank the subject who participated in this study and his family for their time and
715 efforts. The authors further thank David Martín Espinosa for preparing the schematic
716 illustration in Figure 1.

717

718

719 Funding

720 Promobilia Foundation grant 19500 (MOC)

721 IngaBritt and Arne Lundbergs Foundation grant 2018-0026 (MOC)

722 Swedish Research Council (Vetenskapsrådet) grant 2020-04817 (MOC)

723 Author Contributions

724 M.O.C., P.S., and R.B. conceived the surgical approach. M.O.C., E.M., and R.B. developed
725 the technologies used in this study. P.S. and R.B. performed the surgeries. J.Z., E.M., E.E.,
726 and M.M.N conducted the experiments. J.Z. analyzed the data. J.Z. and M.O.C. drafted the
727 manuscript. All authors revised and approved the final manuscript.

728 Competing interests

729 J.Z., P.S., E.E., and M.M.N. declare no competing interests. E.M. and M.O.C. have consulted
730 for Integrum AB. M.O.C. and R.B. hold shares of Integrum AB. M.O.C. and R.B. are co-
731 inventors on patent # US9579222B2 entitled “Percutaneous gateway, a fixing system for a
732 prosthesis, a fixture and connecting means for signal transmission “, which is held by
733 Integrum AB.

734

735 Data availability

736 All data needed to evaluate the conclusions in the paper are present in the paper and the
737 Supplementary Materials.

738 **Figure captions**

739 **Figure 1 Schematic illustration and X-ray of a highly integrated human-machine**
740 **interface.**

741

742 **Figure 2 Electrical resistance of epimysial, intramuscular, and cuff electrodes over time.**

743 (a) Resistance of the epimysial electrodes on unreconstructed and reinnervated native muscles
744 (n=4). (b) Resistance of the intramuscular electrodes in unreconstructed muscles, reinnervated
745 native muscles, and reinnervated free muscle grafts (n=8). (c) Resistance of the ring and three
746 discrete contacts the cuff electrode around a fascicle of the median nerve (n=4).

747

748 **Figure 3 Representative examples of the raw EMG signals over time.** (a) Raw EMG signals
749 for electrodes in an unreconstructed muscle, (b) a reinnervated native muscles, and (c) a free
750 muscle graft. Each EMG recording consists of three seconds sequences of three active
751 movement and rest periods.

752

753 **Figure 4 Signal to noise ratio development over time.** Change of signal to noise ratio of (a)
754 four electrodes in unreconstructed muscles (n=1 movement), (b) three electrodes on/in
755 reinnervated native muscles (n=6, n=3, and n=4 movements from left to right, respectively),
756 and (c) five electrodes within reinnervated free muscle grafts (n=6, n=3, n=5, n=3, and n=3
757 movements, from left to right, respectively). The grey areas depict the standard deviation
758 around the average values over the number of movements. For all signal to noise ratio
759 calculations, only data of the main movements that anatomically corresponded to the electrode
760 placement/innervation site were considered (see Methods for more details).

761 **Figure 5 Offline and online decoding performance.** Decoding performance using gross
762 (hand open/close, supination/pronation, wrist flexion/extension, and elbow flexion/extension)
763 and single finger movements over a two-year period. (a) Offline accuracy of nine gross
764 movements (n = 9 movements, 100 repetitions). (b) Motion test completion rate for gross
765 movements (n = 9 movements, 3 trials), where 100% indicates that all required movements
766 were performed. (c) Completion time (time between the first decoded movement and twenty
767 correct predictions of the promoted movement) and (d) accuracy (percentage of correct
768 predictions) for the four degrees of freedom during the motion test. (e) Offline accuracy of
769 individual fingers (n = 11 movements, 100 repetitions). (f) Completion rate, (g) completion
770 time, and (h) accuracy for the motion test for individual finger decoding (n = 11 movements, 3
771 trials each).

772

773 **Figure 6 Results of functional tests.** (a) ACMC outcome, scored between 0 and 100, for a 1.5
774 DoF control scheme using surface electrodes before surgery and implanted electrodes at week
775 8 and 59 after surgery. A higher score indicates a higher capacity for myoelectric control. (b)
776 ACMC outcome when using a 4.5 DoF simultaneous and proportional control scheme with the
777 electro-neuromuscular constructs and without prior training. (c) Outcome of SHAP for surface
778 electrodes before surgery and implanted electrodes at week 8 and 59 after surgery. The circles
779 indicate the linear Index of Function, scored between 0 and 100, for each task of the SHAP (n
780 = 26 tasks). The horizontal black line shows the weighted linear Index of Function (W-LIOF),
781 where a higher score represents greater prosthetic functionality during activities of daily living.
782 (d) Outcome of SHAP (n = 26 tasks) when using a 4.5 DoF simultaneous and proportional
783 control scheme with the electro-neuromuscular constructs and without prior training.

784