AWARD NUMBER: W81XWH-16-1-0791

TITLE: Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface

PRINCIPAL INVESTIGATOR: Mark Pitkin

RECIPIENT: Poly-Orth International
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REPORT DATE: JANUARY 2022

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
JANUARY 2022	FINAL	**09/30/2016 - 09/29/2021**

4. TITLE AND SUBTITLE
Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0791

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
.
Poly-Orth International
Sharon MA 02067-1518

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The investigators wish the American Veterans and civilians with amputations can use powered prostheses with direct skeletal attachment and direct bidirectional neural control. Since 2004, their work has been devoted improving a skin-device and bone-device interface. Current research is designed as a translational study to develop Skin and Bone Integrated Pylon with Peripheral Neural Interface (SBIP-PNI) directly attached to the residuum and the powered prosthetic hand with bidirectional control. .

15. SUBJECT TERMS- NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	**24**	**19b. TELEPHONE NUMBER** *(include area code)*

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The investigators wish the American Veterans and civilians with amputations can use powered prostheses with direct skeletal attachment and direct bidirectional neural control. Since 2004, their work has been devoted improving a skin-device and bone-device interface. Current research is designed as a translational study to develop Skin and Bone Integrated Pylon with Peripheral Neural Interface (SBIP-PNI) directly attached to the residuum and the powered prosthetic hand with bidirectional control.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Direct skeletal attachment; powered prosthesis; neural interface; bidirectional control system.

3. **ACCOMPLISHMENTS:**
What were the major goals of the project?

Goals/Milestones
Year 1
 Manufacture the integrated pylons with peripheral neural interface (SBIP-PNI) for animal studies and fabricate the powered prostheses with sensory feedback

Milestones #1: Meeting the Poly-Orth specification and passing the QC tests – planned in Q2; current completion 100%

Milestone #2: Ship the implants to the Pine Acre Rabbitry/Farm (PARF) and to Georgia Institute of Technology (GIT – – planned in Q2; current completion 100%.

Comment: the site #2 for animal studies with pigs has been changed from PARF to DaVinci Biomedical Research, Lancaster, MA, with corresponding approval.

 Implant SBIP-PNI into cats - planned in Q4; current completion 75%
 Supply cats with powered prostheses with sensory feedback and initiate gait study- planned in Q4: will be completed in Q1 of Year 3
Year 2
 Conclude cat gait study with and without sensory feedback. Will be completed in Q1 of Year 3.
 Implant SBIP-PNI into Yorkshire Swine and conduct gait study with and without sensory feedback: Gait study without sensory feedback completed.
Year 3
 Perform mechanical testing of device skin and device-bone attachment Perform histological analysis of the samples
 Conclude pig gait study with and without sensory feedback
 Demonstrate infection free sustainable device-body interface with the SBIP-PNI
 Demonstrate that adverse events rate (AER) in animal study is lower than the established threshold
 Submit application for IDE to the FDA Comments/Challenges/Issues/Concerns
Year 4
 A no-cost one-year extension has been approved to pursue the new approach being developed for amputation/implantation in the porcine sub-study at the DaVinci Biomedical, which has more translational value and for additional trials at GeorgiaTech with more functional powered prostheses.
 Achieved infection-free dorsum implantation in pig study
 Developed a strategy for addressing the issue of skin mobility in the stump of pig during first weeks after implantation with Botulinum injections.
 Concluded cat gait study with and without sensory feedback with protection against infection.

Year 5

 A no-cost one-year extension has been approved to pursue the enhancement of the porcine model with Botulinum injections for temporary immobilizing skin movement around the transcutaneous implant.

 We found that injections in the four muscles of the distal thigh of the left hind leg with **MYOBLOC® (rimabotulinumtoxinB;** 5,000 Units/muscle) were sufficient to provide noticeable immobilization by the fourth week after the procedure. This conclusion was made based on the analysis of the dynamics of asymmetry in vertical ground reactions on the injected (left hind) and uninvolved (right hind) legs during gait over an instrumented walkway.

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

How were the results disseminated to communities of interest?

Abstracts and Publications

Park H, Islam MS, Grover MA, Klishko AN, Prilutsky BI, DeWeerth SP. A prototype of a neural, powered transtibial prosthesis for the cat: Benchtop characterization. Frontiers in Human Neuroscience. *Frontiers in Neuroscience* **12**: 471, 2018.

Jarrell J, Farrell BJ, Kistenberg RS, Dalton JF, Pitkin M, Prilutsky BI. Kinetics of individual limbs during level and slope walking with a *unilateral transtibial bone-anchored prosthesis in the cat. Journal of Biomechanics, **76**: 74-83, 2018*.

Park H, Klishko AN, Oh K, Dalton JF, DeWeerth SP, Pitkin M, Prilutsky BI. Cat locomotion with a powered prosthesis integrated with residua bone, skin, sensory nerves and muscles. In: Minisymposium of Society for Neuroscience Annual Meeting, San Diego, CA, 2018.

Pitkin, M., C. Cassidy, M. Shevtsov, J. Jarrell, H. Park, B. Farrell, J. Dalton, W. L. Childers, J. Temenoff, K. Oh, A. Klishko and B. Prilutsky (2019). Animal studies of the Skin and Bone Integrated Pylon with deep porosity for bone-anchored limb prosthetics with and without neural interface. Military Health System Research Symposium MHSRS-19-00758, Kissime, FL (presented).

M. Shevtsov, N. Yudintceva, M. Blinova, I. Voronkina, D. Suslov, O. Galibin, D. Gavrilov, M. Akkaoui, G. Raykhtsaum, A. Albul, E. Pitkin, M. Pitkin, Evaluation of the temporary effect of physical vapor deposition silver coating on resistance to infection in transdermal skin and bone integrated pylon with deep porosity, J Biomed Mater Res B Appl Biomater 107(1) (2019) 169-177 (attached to this report).

Prilutsky, B., H. Park, K. Oh, J. P. Dalton IV, S. P. DeWeerth, M. Pitkin and A. Klishko (2019). Bidirectional Control of a Sensing Powered Transtibial Prosthesis during Walking in the Cat. Society for Neuroscience (presented at the October 17-23 Meeting).

Childers LW, Jarrell JR, Klishko AN, Oh K, Grant CN, Jeffers MK, Herrin KR, Dalton JF IV, Pitkin M, Prilutsky BI. Ankle Power of Transtibial Bone-Anchored Prosthesis with Carbon Fiber and Fiberglass Passive Foot in Walking Cats. Congress of International Society of Biomechanics, Calgary, Canada, 2019.

H. Park, E. Latash, Y. Morkov, A.N. Klishko, S.P. DeWeerth, A. Frigon, B. Prilutsky, Cutaneous Sensory Feedback from Paw Pads Affects Balance Control during Split-belt Treadmill Locomotion in the Cat, Journal of Experimental Biology 222(14, jeb198648) (2019).

Klishko AN, Park H, Grenga G, Zhang C, Oh K, Herrin K, Dalton JF IV, Pitkin M, Prilutsky BI. Stimulation of residual sensory nerve modulates walking mechanics in the cat with bone-anchored transtibial prosthesis. *American Society of Biomechanics Annual Symposium*. Atlanta, August 07, 2020.

Pitkin M, Jarrell JR, Park H, Farrell BJ, Dalton JF IV, Childers LW, Temenoff JS, Oh K, Klishko AN, Prilutsky BI. Animal Studies of The Skin and Bone Integrated Pylon With Deep Porosity For Bone-anchored Limb Prosthetics With And Without Neural Interface. Military Medicine 186, S1, 688-695. https://doi.org/10.1093/milmed/usaa445. PMID: 33499499, (2021).

M. Shevtsov, D. Gavrilov, N. Yudintceva, E. Zemtsova, A. Arbenin, V. Smirnov, I. Voronkina, P. Adamova, M. Blinova, N. Mikhailova, O. Galibin, M. Pitkin, Protecting the skin-implant interface with transcutaneous silver-coated skin-and-bone-intergrated-pylon (SBIP) in pig and rabbit dorsum models, J Biomed Mater Res B Appl Biomater. DOI: 10.1002/jbm.b.34725 (2020).

Bohart, Z., C. Cassidy, D. Merrill, M. Villani, R. Villani, L. Cappabianca and M. Pitkin. "Temporary Botulinum immobilization of residuum muscles for facilitation of the initial ingrowth of skin to the porous Skin and Bone Integrated Pylon in the technology of direct skeletal attachment. Large animal model." Frontiers in Rehabilitation Sciences-Medical and Surgical Rehabilitation (2021) (Submitted) - **see Appendix 1.**

Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report.

1. The study protocol # DB-633, "Effect of botulinum neurotoxin serotypes A or B injections into thigh musculature of a swine," was approved by the IACUC at DaVinci Biomedical Research Products, Inc., Lancaster, MA, and by the USAMRMC Animal Care and Use Review Office (ACURO) on July 16, 2020, with further approval of Amendment 1 on March 25, 2021.

What do you plan to do during the next reporting period to accomplish the goals? If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

1. Unresolved issues with direct skeletal attachment of limb prostheses (osseointegration)

Traditional attachment of prostheses with a socket may be associated with pain, discomfort, and medical complications at the residuum-prosthesis socket interface especially in transfemoral amputees with short stumps. Bone-anchored limb prostheses (osseointegration) offer a number of advantages over socket-based prostheses for these amputees,[1] including Veterans and Service Members. The technology of osseointegration, with clear indications when traditional sockets cannot be properly fitted, relies on the integration of the residuum's bone with the titanium implant, and traces its origins to the 1950's in Sweden.[2,3]

A serious problem with this technology is the high infection rate at the interface of the skin with the implanted fixture (over 50%) and potentially low implant survival rate.[4,5] This infection rate is much higher than the average 2% to 4% rate of surgical site infection, which prevented the approval of this treatment in the US for many years.[6] On July 16, 2015, the Food and Drug Administration "…*authorized use of the first prosthesis marketed in the U.S. for adults who have amputations above the knee and who have rehabilitation problems with, or cannot use, a conventional socket prosthesis.* [7] At the same time, the FDA recognized that "…*the most common adverse event was infection"*.[7]

Another unresolved problem in prosthetics is that people with lower limb loss lose not only motor function of the missing limb but also tactile sensation of contact with the ground.

Although a powered prosthesis can provide ankle power, the person does not receive natural tactile information from foot/hand cutaneous afferents. It may be possible to provide tactile sensation from the limb prosthesis by electrical stimulation of residual sensory nerves triggered by contact with external environment and thus improve motor performance.[8-13] Thus, electrical stimulation of the residual sensory distal tibial nerve during the stance phase can potentially improve symmetry of locomotion with a unilateral prosthesis. The use of recorded muscle activity from residual or intact muscle to control a powered limb prosthesis can further improve symmetry of locomotion.[15,16]

2. Addressing these issues in the current project

The goals of our project have been three-fold: (1) Test the ability of the patented Skin and Bone Integrated Pylon (SBIP) to safely integrate with the skin and bone in natural loading conditions in cats and pigs; (2) Characterize quality of prosthetic locomotion; and (3) Test if SBIP with peripheral neural interface (PNI) between powered prosthesis and residual muscles and sensory nerves (SBIP-PNI) improves quality of locomotion in cats.

2.1. Results of Cat study

The mean bone and skin tissue ingrowth showed correlation with the duration of implant use. The animals that walked for over 153 days had bone and dermal tissues occupying 75.7±7.8% and 74.4±8.7% of implant porous space, respectively. One of 8 animals studied histologically had infection and demonstrated poor integration with residual tissue (36.3% and 26.0% of bone and dermal tissue ingrowth, respectively). Ground reaction forces, power and work of the prosthetic limb were reduced during walking with the stiff passive foot compared with intact locomotion (by 30-46%), whereas those of the contralateral hind- and forelimbs increased ($p<0.05$). This asymmetry was likely caused by the insufficient energy generation for propulsion by the prosthetic leg, as no signs of pain or discomfort were observed in the animals.[26]

The animal tested with the powered ankle prosthesis demonstrated the best performance – peaks of vertical ground reaction forces, ankle moment and power were closer to the intact values compared to passive prosthesis ($p<0.05$).

The duty factor of the prosthetic hindlimb was longer for Mode 2 than for Mode 1 or Mode 3 ($p=0.004$-0.050). The relative duration of the double hindlimb support phase when the contralateral hindlimb was the trailing limb was shorter in Modes 1 and 3 than in Mode 2 ($p=0.016$-0.041). We found no difference in peak of vertical ground reaction force of the prosthetic limb between the modes.

Histology analysis in this animal who had implant for 885 days is illustrated in (Figure 4). Healing characteristics appeared overall optimal as indicated by extensive bone ingrowth and apposition into the porous titanium implant, within the shaft and up to the amputation line (Rousselle S: Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface. Histology Report AGG20-018, Alizée Pathology, LLC, Thurmont, MD; available upon request). At the amputation line, the implant-skin-bone-interface was characterized by an anisotropic ingrowth of the native dermis. Deep ingrowth into the porous titanium overlying the bone was confirmed in fibrous connective tissue with small caliber blood vessels, or granulation tissue, with complete apposition between the bone, dermis, and implant shaft. There were no evidence of pocket formation, inflammation, or infection. The epithelium completely bridged the lateral aspects of the fibrous stump to meet the porous titanium. Granulation tissue extended perpendicularly and without downgrowth from the dermis over the

fibrous stump and overlying the bone.

2.2. Results of Pig study

In the DaVinci Biomedical sub-study, symmetry of loading the intact and prosthetic limbs during gait was 80±5.5% (Figure 2, C). Skin-implant interface was infection-free, but developed as a stoma type, not with deep ingrowth as anticipated, probably due to the high mobility of the skin and soft tissues in the pig's thigh.

A mechanical testing was performed post-mortem with the Instron testframe. In pull-out tests, the implant broke at 5,450 N pull-out force, keeping its in-bone portion non-detached. In two animals, non-treatable infection occurred within first 7 days after implantation.

In the Institute of Cytology pig dorsum sub-study with silver coated SBIP, compared to non-coated control, there was no evidence of adverse tissue responses (e.g., infection, hemorrhage, necrosis, exuberant fibrosis/scarification).[31]

Histology evaluation illustrated in Figure 3, Rows D-E, showed creation of a viable skin seal within the porous structure of the SBIP (Stanley JRL: Non-GLP Evaluation of Subcutaneous Titanium-Based Devices in Pig and Rabbit model. Report FEE18-604, Alizée Pathology, LLC, Thurmont, MD; available upon request). This antimicrobial feature of the silver-coated implant will be used in the next animals in anticipation of its positive effect during initial post-implantation period.

2.3. Discussion

The current studies in pigs showed that even the casting of the leg residuum did not limit the skin movement around the implant. That movement did not disrupt the skin seal once it has been developed inside of the deeply porous cladding of the SBIP implant. However, at the initial period after implantation the skin movement was compromising the skin ingrowth.

In the cat sub-study with SBIP implants, casting of the residuum immediately after implantation was sufficient for skin immobilization, and only one animal of six was infected (infection rate of 16.7%). In the sub-study with peripheral neural interface using the SBIP-PNI implants, 7 of 8 cats infected (infection rate of 87.5%). There were two general differences between the two groups of cats: type of implant and sex. The first group of female cats was implanted with SBIP, the second group of male cats was implanted with SBIP-PNI. The latter pylon has the channel through which the electrodes from inside the residual limb were connected to the external prosthesis. Although the external opening of the channel was sealed with dental cement at the end of surgery, movement of electrode leads insight the channel might have allowed outside pathogens to enter the residual limb and cause a low-grade infection, which prevented osseointegration of the bone with implant. However, we observed no signs of systemic infection in blood samples. The channel in SBIP-PNI implant connecting the outside prosthetic connector with inside electrodes was probably the main reason for such a low success rate (1 of 8 animals). Therefore, in future studies we will consider an additional silver protective coating on the channel's inner walls and develop a more protective interface within the control system.

2.3. Development of a new porcine model with temporary immobilization of the skin movement in the residuum

Detailed description of the study design and results are presented in a paper being submitted to Frontiers in Rehabilitation Sciences-Medical and Surgical Rehabilitation (08/25/2021) - **see Appendix 1.**

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16. Jarrell, J.R., et al., *Kinetics of individual limbs during level and slope walking with a unilateral transtibial bone-anchored prosthesis in the cat.* J Biomech, 2018. **76**: p. 74-83. DOI: 10.1016/j.jbiomech.2018.05.021. [PMCID: PMC6062466].
17. Shevtsov, M., et al., *Protecting the skin-implant interface with transcutaneous silver-coated skin-and-bone-intergrated-pylon (SBIP) in pig and rabbit dorsum models.* J Biomed Mater Res B Appl Biomater. , 2021. **DOI: 10.1002/jbm.b.34725**(109): p. 584-595.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- transfer of results to entities in government or industry;
- instances where the research has led to the initiation of a start-up company; or
- adoption of new practices.

Recommendations for implantation of the pylons with peripheral neural interface and on bidirectional control of powered prostheses are anticipated at the completion of the following project. A modified protocol for implantation with Botulinum immobilization will be proposed for clinical trials.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- improving public knowledge, attitudes, skills, and abilities;
- changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or
- improving social, economic, civic, or environmental conditions.

The investigators wish the American Veterans and civilians with amputations can use powered prostheses with direct skeletal attachment and direct bidirectional neural control, which could improve the quality of life and social integration of the patients.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals.

All modifications in studies have been approved by ACURO.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other).

Journal publications.

Park H, Islam MS, Grover MA, Klishko AN, Prilutsky BI, DeWeerth SP. A prototype of a neural, powered transtibial prosthesis for the cat: Benchtop characterization. Frontiers in Human Neuroscience. *Frontiers in Neuroscience* **12**: 471, 2018.

Jarrell J, Farrell BJ, Kistenberg RS, Dalton JF, Pitkin M, Prilutsky BI. Kinetics of individual limbs during level and slope walking with a *unilateral transtibial bone-anchored prosthesis in the cat. Journal of Biomechanics, **76**: 74-83, 2018*.

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H. Park, E. Latash, Y. Morkov, A.N. Klishko, S.P. DeWeerth, A. Frigon, B. Prilutsky, Cutaneous Sensory Feedback from Paw Pads Affects Balance Control during Split-belt Treadmill Locomotion in the Cat, Journal of Experimental Biology 222(14, jeb198648) (2019).

Pitkin M, Jarrell JR, Park H, Farrell BJ, Dalton JF IV, Childers LW, Temenoff JS, Oh K, Klishko AN, Prilutsky BI. Animal Studies of The Skin and Bone Integrated Pylon With Deep Porosity For Bone-anchored Limb Prosthetics With And Without Neural Interface. Military Medicine 186, S1, 688-695. https://doi.org/10.1093/milmed/usaa445. PMID: 33499499, (2021).

M. Shevtsov, D. Gavrilov, N. Yudintceva, E. Zemtsova, A. Arbenin, V. Smirnov, I. Voronkina, P. Adamova, M. Blinova, N. Mikhailova, O. Galibin, M. Pitkin, Protecting the skin-implant interface with transcutaneous silver-coated skin-and-bone-intergrated-pylon (SBIP) in pig and rabbit dorsum models, J Biomed Mater Res B Appl Biomater. DOI: 10.1002/jbm.b.34725 (2020).

Bohart, Z., C. Cassidy, D. Merrill, M. Villani, R. Villani, L. Cappabianca and M. Pitkin. "Temporary Botulinum immobilization of residuum muscles for facilitation of the initial ingrowth of skin to the porous Skin and Bone Integrated Pylon in the technology of direct skeletal attachment. Large animal model." Frontiers in Rehabilitation Sciences-Medical and Surgical Rehabilitation (2021) (Submitted) - **see Appendix 1.**

Books or other non-periodical, one-time publications. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

Other publications, conference papers, and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year

Nothing to report

(international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - data or databases;
  - biospecimen collections;
  - audio or video products;
  - software;
  - models;
  - educational aids or curricula;
  - instruments or equipment;
  - research material (e.g., Germplasm; cell lines, DNA probes, animal models);
  - clinical interventions;
  - new business creation; and

- other.

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Mark Pitkin
Project Role: PI
Researcher Identifier (e.g. ORCID ID): L-7934-2017
Nearest person month worked: 5
Contribution to Project: Dr. Pitkin has directed all aspects of the project

Name: Grigory Raykhtsaum
Project Role: Director of Engineering
Nearest person month worked: 3
Contribution to Project: Mr. Raykhtsaum was responsible for development and manufacturing of the SBIP-PNI pylons for animal studies

Name: Charles Cassidy
Project Role: Investigator
Nearest person month worked: 1
Contribution to Project: Dr. Cassidy is a surgeon on the project performing two procedures in Year I.

Name: Boris Prilutsky
Project Role: Director of the Georgia Tech study
Nearest person month worked: 1
Contribution to Project: Dr. Prilutsky has directed development of the powered prosthesis for animal studies and the animal trials with SBIP-PNI in Year I.

Name: Hangue Park
Project Role: Investigator/Postgraduate student of Georgia Tech
Nearest person month worked: 10
Contribution to Project: Dr. Park developed the powered prosthesis for animal studies.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

1. DaVinci Biomedical Research, 20 Maple St, Lancaster, MA 01523
    - Financial support: MR150015 Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface
    - Facilities and personnel collaborating on animal studies with pigs.
2. Advanced Manufacturing Products (ADMA), Hudson, OH
    - Financial support: MR150015 Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface
    - Facilities and personnel for sintering titanium SBIP-PNI pylons with selected specifications for animal studies
3. Georgia Institute of Technology, Atlanta, GA
    - Financial support: MR150015 Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface
    - Conducting animals study with cats wearing powered prostheses following DSA
4. T3 Labs, Atlanta, GA 30313
    - Financial support: MR150015 Integration of the Residual Limb with Prostheses via Direct Skin-Bone-Peripheral Nerve Interface
    - Facilities and personnel collaborating on animal studies with cats.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Nothing to report

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

See next page for Appendix 1.

Appendix 1

Temporary Botulinum immobilization of residuum muscles for facilitation of the initial ingrowth of skin to the porous *Skin and Bone Integrated Pylon* in the technology of direct skeletal attachment. Large animal model.

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ABSTRACT

Enhancing the technology of bone-anchored limb prosthetics, we present a modified porcine model for developing an infection-free integration between skin and a percutaneous bone implant. The deeply porous Skin and Bone Integrated Pylon (SBIP) presented an infection-free skin-implant interface both after implantation into dorsum and after implantation into the residuum after below-knee amputation. However, deep ingrowth of skin into the porous cladding of the SBIP was achieved better in the dorsal procedure, while implantation to the residuum sometimes developed a stoma, probably due to the high mobility of the skin and soft tissues in the pig's thigh. Uncontrolled high skin mobility during the first weeks after implantation constituted a limitation for the porcine animal model, which we tried to address in the current study.

As our previous studies showed that casting of the leg residuum did not sufficiently limit the skin's movement around the implant, we tested a modified protocol of the implantation, which included injection of Botox into the thigh muscles. During the course of the study we identified proper Botox componentry, dosage and period after injections to achieve a maximal effect of immobilization of the muscles affecting skin movements. To verify the immobilization, we used kinetic data on asymmetry of loading during gait with the Strideway system, Tekscan, Inc., Boston, MA.

We found that injections in the four muscles of the distal thigh of the left hind leg with **MYOBLOC® (rimabotulinumtoxinB;** 5,000 Units/muscle) were sufficient to provide noticeable immobilization by the fourth week after the procedure. This conclusion was made based on the analysis of the dynamics of asymmetry in vertical ground reactions on the injected (left hind) and uninvolved (right hind) legs during gait over an instrumented walkway.

Keywords: direct skeletal attachment; porcine model; skin immobilization; Botulinum injections.

INTRODUCTION

Bone-anchored limb prostheses offer a number of advantages over socket-based prostheses [1]. The technology of osseointegration relies on the integration of the residuum's bone with the titanium implant, and traces its origins to the 1950's in Sweden by Dr. Per-Ingvar Brånemark [2, 3].

A problem with this technology is the still high infection rate at the interface of the skin with the implanted fixture.[4-6]

Percutaneous porous devices used in bone-anchored prostheses have the potential for initial integration with the skin, as demonstrated in animal studies by various research groups [7-10]. Our studies have also investigated porous implants for direct skeletal attachment, focusing on the ability of implants to invite and sustain deep skin and bone ingrowth to promote an infection-free body-device interface while maintains required mechanical strength. The implant we developed with such features is called the Skin and Bone Integrated Pylon (SBIP) [8, 11-13]. The innovation of the SBIP lies in its patented combination of four key technological characteristics: *porosity*, *pore size*, *porosity volume fraction*, and *particle size*, and a provision for *passage for wired neural interface, and protective silver coating* [11, 14].

The parameter most distinct from the prior art, and which most meaningfully distinguishes the SBIP from other systems is the *porosity volume fraction* (*VF*), which quantifies how porous the implant is (formally, *VF* is the ratio of the volume of the porous portion to the entire volume of the device).

As the SBIP implants have been designed to encourage and enable deep skin permeation, there is a critical and vulnerable period – between implantation and full permeation – that requires methodological advances. Until the surrounding skin cells remodel within all of the implant's pores, special care to minimize the skin movements around the implant is required to protect the still non-occupied pores from bacterial infiltration [15-17]. Minimizing skin movements during initial period after transdermal implantation is especially important in the studies with large animals (pigs), since the activity of the massive musculature in the residuum and above may mechanically pull out the skin around the implant.

Our previous studies with pigs [18] showed that deep and sustainable ingrowth of skin into the porous cladding of the SBIP can be achieved after implantation into the pig's dorsum. As to implantation into the residuum of the leg, the skin developed a stoma around the implant [16, 17]. There is an excess of the movable skin and soft tissues in the pig thigh; simple casting did not successfully immobilize the skin while the skin seal was developing.

Since our overall intention is to establish a sustainable and safe skin seal to provide natural barriers against infection, we tested here a modified implantation protocol. The modification is the inclusion of pre-implantation injections of Botox to temporarily immobilize the muscles that affect the movement of skin in the implantation zone.

Botulinum toxins are approved by the FDA for application in human patients [19] and are frequently used in patients with spasticity of the upper and lower limbs due to upper motor neuron disorders, spinal cord injuries, multiple sclerosis, strokes, brain injuries, and cerebral palsy [20, 21]. Fewer reports are extant on botulinum toxin applications in pigs [22]. That makes it necessary to judiciously select the type of toxin and its dosage, which may differ from those recommended for humans [23-25].

In the current pilot study with three animals, we calibrated both the dose and optimal timing for the implantation, which is when the botulinum toxin reaches its maximum effect. This paper presents the leading hypothesis, study design and outcomes of the study.

STUDY DESIGN

The study protocol # DB-633, "Effect of botulinum neurotoxin serotypes A or B injections into thigh musculature of a swine," was approved by the IACUC at DaVinci Biomedical Research Products, Inc., Lancaster, MA, and by the USAMRMC Animal Care and Use Review Office (ACURO) on July 16, 2020, with further approval of Amendment 1 on March 25, 2021.

The purpose of this botulinum toxin study was to determine the period when the injection's immobilization effect was the greatest on the pig leg muscles. The contraction of these muscles can compromise the initial remodeling of the skin while a sustainable seal is developing after implantation of the transdermal implant into the leg's residuum.

The best timing for the implantation is when the immobilization effect is strongest. The asymmetry of loading between the uninvolved hind leg and the hind leg with injected botulinum toxin can be used to detect the maximum immobilization. The intensity of immobilization was quantified by an Asymmetry Index (AI) calculated from quadruped gait analysis data obtained with the Strideway System, Tekscan, Inc. Boston, MA. For each of the gait trials, the Strideway software, among other parameters of gait, generates a Symmetry Table as the ratios of the magnitudes of the various parameters for left and right legs. An ideal magnitude of IA in sound gait, when the load on the right and left legs is equal, is 1.00.

Reports in human applications of botulinum toxin injections indicate that immobilization is at its maximum 3-4 weeks after injections. We hypothesized that within this interval, neurocontrol over the muscle-coordinated activity during gait cycle will change the magnitudes of the loading of the injected leg, as detected in the increase in the IA.

Thus, the purpose of this botulinum toxin study was to confirm this hypothesis or to make the necessary modifications in the type of botulinum toxin or its dosage.

METHODS

1. Procedures.

We injected botulinum toxin A (Xeomin®), Merz Pharma GmbH & Co., Dessau, Germany, an incobotulinum product, equivalent to Botox® and Dysport® [26], and compared its effect with Botulinum toxin type B (MYOBLOCÒ Elan Pharmaceuticals, Inc, San Francisco, CA, USA), which showed better desired effect in pigs compared to toxin A in pig masseter muscles [22]. The injections were dosed at 8 units/kg, similar to human pediatrics and equivalent to the maximum allowed dose by the FDA in children to the lower limb [19].

Injections were performed using ultrasound guidance into the rectus femoris, vastus lateralis, vastus intermedius, and vastus medialis of the pigs in order to increase adherence of the below-knee prosthesis (see **Table 1**).

Application of the botulinum toxin treatment included injections into the distal musculature of the hind limb of the pig; daily monitoring during first 2 weeks and weekly monitoring of behavior and locomotor activity of the animal; gait analysis of the pre-procedure and following 2, 4, 6, 8 and 10 weeks after the injection.

Photos **A-F** (**Figure 1)** illustrate the procedure of the Botulinum study in Animal 1 **No. 1090**. **A** – Xeomin®, **B** – GE Ultrasound laptop machine for guidance of injections. **C, E** – finding a

Table 1. Injected muscles, toxin type and dosage

Injected muscles	**Toxin type and dosage**		
	IncobotulinumA (XeominÒ)	**RimabotulinumtoxinB (Myoblock®)**	
	Animal 1 No. 1090	***Animal 2 No. 1143F***	***Animal 3 No. 81-141F***
Rectus femoris	2.0 mL (100 units)	4.5 mL (7,500 units)	2.5mL (5,000 units)
Vastus lateralis	2.0 mL (100 units)	4.5 mL (7,500 units)	2.5mL (5,000 units)
Vastus intermedius	4.0 mL (200 units)	4.5 mL (7,500 units)	2.5mL (5,000 units)
Vastus medialis	2.0 mL (100 units)	4.5 mL (7,500 units)	2.5mL (5,000 units)
Gluteus maximus	2.0 mL (100 units)	NA	NA
Total units injected	12.0 mL (600 units)	18 mL (30,000 units)	4.0 mL (20,000 units)

spot for injection by moving the transducer with visual confirmation on the screen of the GE Ultrasound machine (**D**). **F** – Schematics of the injection spots in the study, 9-23-20.

2. Outcomes

Animal 1 No. 1090.

Application of incobotulinumtoxinA was performed between September and December of 2020, by injection of Botox A to the distal thigh musculature of the right leg.

Outcomes: The Xeomin® was ineffective. There was no muscle weakening and therefore, no effect on animal gait. For the duration of 3 months post injection and 3 months wash-out period the animal was normal. The animal's gait was normal throughout, with symmetrical kinematic and kinetic data compared between the involved and uninvolved legs

The conclusion was made based on results of the consecutive gait analysis that Botulinum toxin A doesn't provide sufficient immobilization of the leg muscles, and that a new injection with Botulinum toxin B was suggested with a modified dosage.

Animal 2 No. 1143F.

Since the previous injections of incobotulinumtoxinA proved ineffective for blocking muscular contraction, the second cycle of application of botulinum toxin B, a different serotype of botulinum toxin treatment **(MYOBLOC® (rimabotulinumtoxinB),** was performed on January 23, 2021:

Ultrasound-guided muscular injections were entered into the distal thigh of the right hind leg, with **MYOBLOC® (rimabotulinumtoxinB;** 5,000 Units/1 mL) diluted from 1 mL to 3 mL using injectable saline. Four (4) muscles were each injected with 7,500 units/muscle.

The animal recovered well from the injection procedure. The animal was observed 2x daily. Observations of animal and injection sites were normal. On Day 4, during AM checks the limb appeared normal. The animal was found lying down and not eating. The animal was unable to stand and was non-responsive. After a consult with AV, it was determined that there was

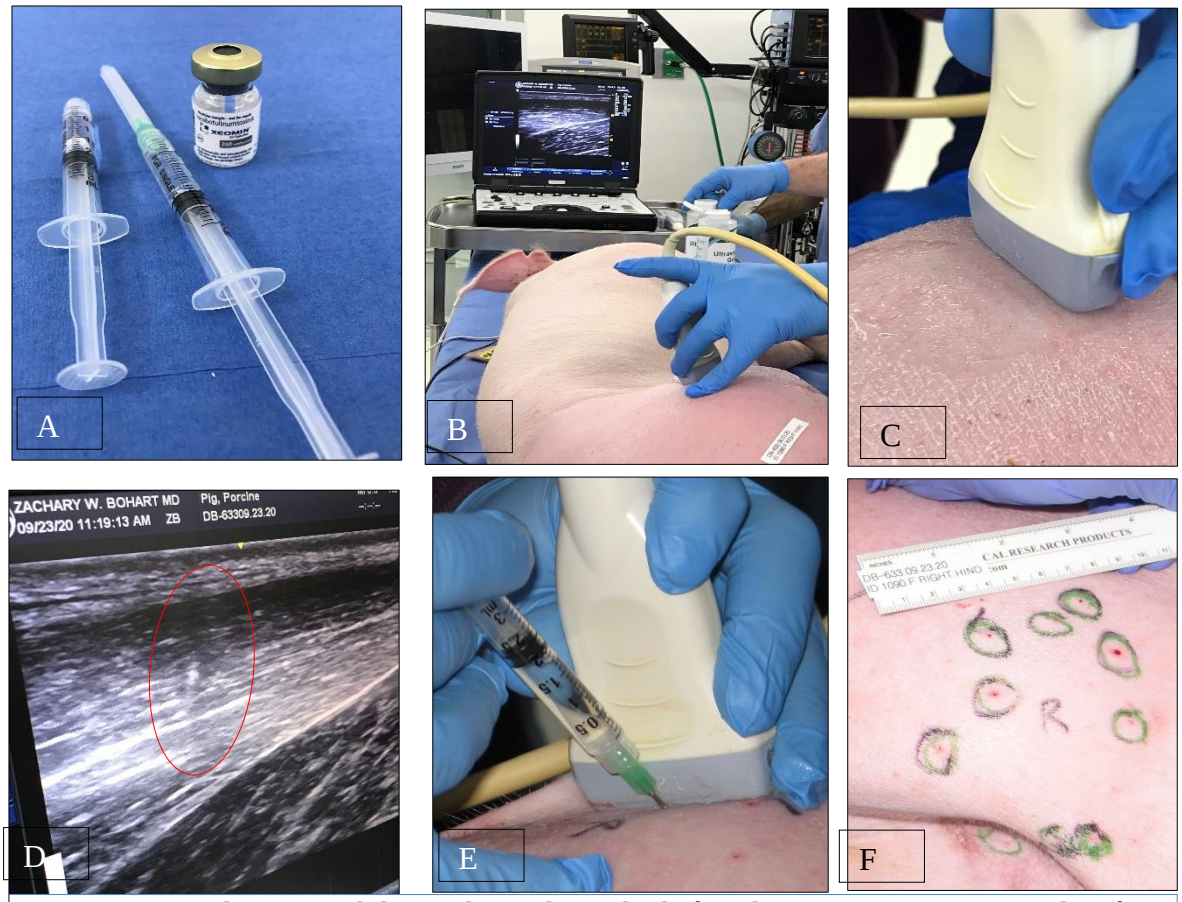

Figure 1. A study on immobilizing skin and muscles before the osseointegration procedure for a better integration of tissues at the skin-implant (SBIP) interface. **A** – Xeomin®, an incobotulinum toxin A product equivalent to Botox® and Dysport®. **B** – GE Ultrasound laptop machine for guidance of injections. **C, E** – finding a spot for injection with visual confirmation (**D**). **F** – Schematics of the injection spots in the study with the Animal 1 - No. 1090, 9-23-20.

toxicity. Animal was referred for unscheduled euthanasia. The animal was euthanized the same day.

Necropsy notes: T: 101.9F; HR: 120; RR: 20; CRT: >4 sec. Animal unable to stand, lethargic. Injection sites were normal. Laterally recumbent, paretic hind end and front end. Slightly cyanotic.

***Animal 3 No. 81-141F*.**

The third animal received a smaller dosage of **rimabotulinumtoxinB** than Animal 2; recovered and was tested with the Strideway gait analysis system.

Application of Botulinum treatment was performed on May 19, 2021.

UV-guided muscular injections were performed to the distal thigh of the left hind leg with **MYOBLOC® (rimabotulinumtoxinB;** 5,000 Units/1 mL) diluted from 1 mL to 0.5 mL using injectable saline. Four (4) muscles were each injected with 5000 units/muscle.

Table 2. Asymetry Index dymamics

Number of weeks after Botox B	Asymmetry Index (SI)	STDEV
0	1	0.08
4	1.47	0.17
6	1.23	0.17
8	1.02	0.19
10	0.94	0.14

Animal No. 81-141F had an uneventful recovery. Animal was observed 2x daily. The injection sites were normal throughout the survival period. On Day 6 , post-injections the animal started to become paretic. This paresis lasted 6 days during which the animal was tube fed and intermittently placed in a Panepinto sling. The animal made a full recovery and was able to complete all the gait analyses.

Weekly monitoring of behavior and locomotor activity of the animal demonstrated recovery from the injection and return to regular ambulation with the greatest asymmetry in kinematic and kinetic data at week 4 after injection procedure.

Gait analysis was performed five times: 2 days pre-procedure as a baseline, and 4, 6, 8 and 10 weeks after the injection procedure. The dynamics of the Asymmetry Index (AI) is shown in a Table 2 and is illustrated in the chart (**Figure 2.)** A distinct increase of AI occurred at week 4 after the injection. By weeks 8, and 10 , the AI recovered to the initial symmetry in loading of both hind legs.

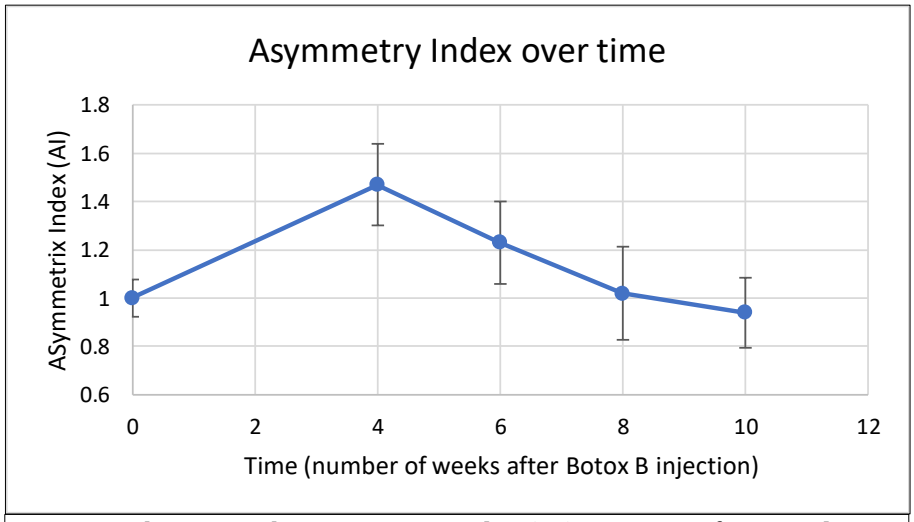

Figure 2. Changes in the Asymmetry Index (AI) as a ratio of maximal vertical ground reaction on the injected leg to the uninvolved leg. Animal 3 No. 81-141F.

The maximal AI at week 4 indicated that the loading on the injected leg by that time exceeded the loading on the uninvolved contralateral leg by 47±17%. Within this interval, the neurocontrol over the muscle-coordinated activity during gait cycle was affected by the Botulinum toxin, which did not allow the leg to be lifted as quickly as the contralateral leg. The animal spent more time loading this leg, which resulted in the higher magnitude of normal ground reaction.

DISCUSSION

We anticipated that by immobilizing the distal thigh muscles approximately 4 weeks before the transdermal implantation, the initial ingrowth of skin into the porous cladding will progress without being torn off by muscular movement. We will investigate the benefits of the pre-implantation Botulinum injections in our further studies in bone-anchored prosthetics with this

modified porcine model. The model with pre-implantation botulinum toxin injections may have higher translational value that the regular one, considering existing FDA approved Botulinum applications in humans.

CONCLUSIONS

1. Injections with incobotulinumtoxin[A] (Xeomin®) were ineffective at inducing any form of muscle weakness with effect to gait.
2. **MYOBLOC® (rimabotulinumtoxinB) injections proved toxic with the first dosage applied. A range finding study was recommended to identify the optimal dose to induce muscle weakness.**
3. Smaller dosage of **MYOBLOC® (rimabotulinumtoxinB)** showed safe outcomes of the injection and demonstrated the effect expected – asymmetry (47%±17%) in loading between affected and non-affected limbs 4 weeks after the injection (Figure 2) compared to baseline recording (Figure 1). Further observations showed recovery of the symmetry in gait parameters: as 23%±21% in 6 weeks, 2%±21% in 8 weeks and 3%±21% 10 weeks after the injection procedure (see **Table 2, Figure 2**).
4. Limitations of the study include small number of animals and the pilot selection of the dosage being found effective. For addressing these limitations further studies are suggested.

AUTHOR CONTRIBUTIONS

Z. Bohart – study design and injection methodology; C. Cassidy – translational value of the modified protocol; the DaVinci Biomedical team provided, animal husbandry, enrichment and veterinary care; M. Pitkin – study design.

FUNDING

This study was supported by the U.S. Department of Defense grant W81XWH-16-1-0791 and by Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH grants HD057492 and HD090768.

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