NEW DESIGN OF PATIENT-SPECIFIC, ANTIMICROBIAL BIOACTIVE FINGER IMPLANTS FOR DURABLE FUNCTIONAL RECONSTRUCTION AFTER AMPUTATION

Marcin Dyner[1], Adam Byrski[2], Roman Major[2]*, Maciej Gawlikowski[3], Katarzyna Kasperkiewicz[4], Juergen M. Lackner[5], Aneta Dyner[6], Bogusław Major[2]

[1] Faculty of Science and Technology,
Jan Dlugosz University in Czestochowa,
Armii Krajowej Av, 13/15,
42-200 Czestochowa, Poland
[2] Institute of Metallurgy and Materials Science,
Polish Academy of Sciences,
Reymonta St. 25, 30-059 Krakow, Poland
[3] Silesian University of Technology,
Faculty of Biomedical Engineering,
Department of Biosensors and Processing
of Biomedical Signals,
Roosevelt Str. 40, Zabrze, Poland
[4] University of Silesia in Katowice,
Faculty of Natural Sciences, Institute of Biology,
Biotechnology and Environmental Protection,
Jagiellońska St. 28, 40-032 Katowice, Poland
[5] JOANNEUM RESEARCH Forschungsges.m.b.H.,
Institute of Surface Technologies and Photonics,
Functional Surfaces,
Leobner Strasse 94, 8712 Niklasdorf, Austria
[6] Medical Instruments Factory
CHIRMED Marcin Dyner,
Mstowska St. 8A, 42-240 Rudniki, Poland
*e-mail: r.major@imim.pl

Abstract

The absence of even a single finger results in a major impairment in the hand function (precise grasping, grip power), therefore significantly affecting the social and professional life of victims who are frequently young people. Finger amputation is a surgical treatment for ~69.000 patients in the EU after traumatic injury, in which replantation microsurgery fails due to the severity of tissue damage. The surgical reconstruction is currently possible only via autograft transplantation, e.g. a toe-to-hand transfer, thus leading to foot impairment. Some motion functional restoration is also possible using a bone-anchored silicone prosthesis but without the sense revalidation.

Our current research focuses on alternatives for surgical reconstruction by means of novel patient--specific, durable, biomimetic, bioactive and antibacterial implants for reconstructing lost bone and joints. The implant design – and the improved micro(neuro) surgery (beyond the project) – will consist in the fast successful rehabilitation, including the soft-tissue related mobility, the implantation of state-of-the-art nerve conduits as well as the aesthetic appearance.

Key issues for the long-term functionality of the biomaterial-based reconstruction of hard tissue are based on surgical demands, such as: (1) perfect integration of a bone-substituting metal with the surrounding bone tissue (a) with no signs of loosening due to stress shielding at the interface and (b) enhanced with protective activity against bacterial inflammation (antimicrobial properties and formation of vascularized bone tissue (ossification)) even months to years after the injury; (2) biomimetic finger joints based on non-wearing materials without ossification meant to prevent the loss of the motion function.

Keywords: finger implant, thin coatings, microstructure, cytotoxicity, microbiology

Introduction

Optimal treatment after accidental finger amputation

A traumatic finger (digit) amputation is the most common type of highly severe injury of the upper extremities [1-3]. The hand and digital amputations account for ~69.000 visits to emergency departments in the EU (~45.000 in the US) [4,5]. The absence of even a single finger (especially the thumb, ~15.000 patients/year in EU) results in major impairments in the hand function, i.e. the inability to perform more precise manoeuvres and engage in specific tasks and the decreased power of the grip, causing social inhibition and inadequate adaptation to society [6-8]. The multiple finger loss only increases the disadvantageous impact on life and work (i.e. productivity and income) [9].

Victims of severe finger injuries are usually young and in their prime income earning years [9]. Thus, choosing an appropriate treatment can bring substantial economic effects, not only to trauma patients but also to the lives of their families and society. Medically, an amputation is a relatively inexpensive and uneventful procedure, but in addition to the motion and sensing disability, the persistent pain is problematic. Although costly and microsurgically challenging, the gold standard for preserving the hand mechanics is replantation, offering substantial functional and aesthetic benefits [10-13]. It is performed in ~24% of all cases in Austria, 22% in EU28, 14% in the US and 29% in Japan with generally higher prevalence in the case of amputation of >2 digits. The procedure requires a long rehabilitation period and can result in functional deficits owing to persistent finger stiffness and limited sensation [14-17]. However, if the microsurgical reconstruction fails (14% of cases [14] or replantation is not an option due to the injury severity (e.g. after crushing without a clean-cut) (~38% of total cases), techniques such as finger pollicisation or a toe-to-hand transfer can also offer a good reconstructive alternative [18]. Some finger functionality is preserved with external silicone prostheses [19-21], if they connect to bone-anchored implants (in contrast to a snap prosthesis providing only aesthetic function) [21,22]. However, anchoring is rarely used due to the high risks of bacterial infection, prosthesis extrusion and osseoabsorption of the phalangeal stump [23,24].

Since immunogenicity risks prevent the use of any allografts and xenografts, the autologous reconstruction from patient's own donor tissue without the loss of other extremities (e.g. toes) is currently increasingly considered as a future alternative, especially for patients after the loss of the thumb or 2 and more fingers (estimated 15,000 cases/year in EU). While the autologous connective tissue, tendons and skin transfer from donor sites, including the vascularization, is considered a (micro-)surgical standard, the main challenge is the hybridization of this surgical approach with implants. The problematic issues consist in:

(1) the reconstruction of the finger bone segments as well as joints. When the available donor sites of bone are either strongly limited (e.g. from the iliac crest) or missing (for joints), it is necessary to apply bone- and joint-substitute materials. However, finger-bone-substituting implants (for reconstructing phalanx and metacarpal bones) have to be patient-tailored to match the length and anchoring geometry to the existing bone/joints. Finger-joint implants are currently available only for replacing arthritic joints. They are not suitable for the combination with any bone-substituting implants and have strong functionality limitations due to the material ossification (metal or polymer).

(2) the reduction of germ count and minimizing the rate of post-traumatic hand bone infections caused by bacteria introduced during the injury or the medical-surgical treatment (nosocomial, partly multi-resistant nosocomial *Staph. aureus & epidermidis, E. coli, Pseudomonas*) [25].

(3) the tactile function restoration: the loss of sensation of e.g. both digital nerves of the thumb (even if the motion is still possible without the amputation) equals 20% loss of the hand function [26]. Tactile sensibility can be restored by various neurovascular flap techniques [27] and a number of nerve guidance conduits and nerve protectant wraps approved by the US Food and Drug Administration (FDA) for clinical use in the peripheral nerve repair [28].

General demands for autologous tissue & implant scaffold based reconstruction

In order to reconstruct the finger using a durable, patient-tailored, biomimetic and bioactive implant (the R&D target of the fingerIMPLANT project, bullet points 1&2 from above), special focus must be laid on the highly complex reconstruction of motion, i.e. using structural implants (materials in demanded shape) for finger bone and joint reconstruction.

Soft-tissue related mobility, implantation of state-of-the-art nerve conduits and aesthetic appearance (bullet point 3) are mainly a task for the post-project clinical studies on adapting available microsurgical techniques.

Based on the consortium experience, the necessary patient-specific implants for reconstruction of bones, joints and tactile function must mandatorily be:

(1) non-cytotoxic and biocompatible for the long-term use, i.e. initially anti-microbial and bioactive to provide the proper bone neoformation,

(2) patient-tailored to the size and shape of the digital defect ("finger length"), the elasticity of surrounding bone at the interface to the existing bone in order to prevent stress shielding and degradation of the surrounding bone, and comparatively manageable for the surgeon,

(3) mechanically robust to provide sufficient strength against physical forces *in vivo* (similarly to the normal finger and hand motion).

The long-term surgical experience with tissue engineering, bone-substitute materials and artificial joints shows that any implant concept without metal- or ceramic-based, durable high-strength materials have failed in osteosynthesis and functional reconstruction due to heavy loads and a high number of load cycles (movements) affecting the extremities. Consequently, the following demands arise for the biomimetic design of bone and joint reconstruction:

Patient-tailored bone reconstruction implants

An excellent, patient-tailored adaptation of the implant to the patient-specific dimensional demands of the phalanx and metacarpal bones as well as providing fast axonal regrowth is essential to minimize the process of learning the new grasp, adding to an already huge burden of accepting the reconstructed extremity. Furthermore, arthroplasty in small bones generally shows that the implant stiffness has to be adapted to the natural bone stiffness in the areas of direct contact (anchoring sites) because the elasticity differences will result in the bone degradation caused by stress-shielding and therefore the implant loosening.

The most important characteristics that the implant must fulfil are:

(1) The response to loading similar to natural bone at the anchoring sites (Young's modulus (E) 25-30 GPa (ISO 6892-1) to prevent stress shielding by using design methodology for 4D printing (3D shape + directionally-optimized stiffness by cellular inner structure).

(2) Long-term mechanical and corrosion durability - tensile testing ISO 6892-1:2016: (tensile strength >800 MPa), fatigue limit (106 cycles, >400 MPa).

Tribologically and chemically durable artificial ceramic joint implants

The state-of-the-art silicone or PEEK polymer-based finger joint implants for arthritic joint replacement generally fail mechanically after 2-4 years [29-34], the CoCr or pyrolithic carbon joints fail due to loosening, ossification or wear after the similarly long use [35-38]. The demands are, thus, as follows:

(1) the non-osteoconductive material to prevent the osteoblast adhesion and ossification

(2) the highly smooth (Ra<10 nm) surface after the polishing post-treatment, both high hardness (>1200 HV) & toughness >6 MPa m0.5 for the minimized wear rate (<0.1 mm³/107 cycles, modified ISO 14242 test)) and the reduced anchoring sites of osteoblasts (start of ossification)

(3) the high-quality material for biomedical applications (bulk density >3.94 g/cm³, grain size <4.5 µm, flexural strength >400 MPa) (ASTM F603-12)

Thin bioresorbable, osteoconductive and antimicrobial coatings on metal implants

Generally, full metal-based bone-substitute implants impede the optimal interaction with the surrounding tissue due to the tissue capsula formation and the risk of persistent inflammation caused by the bacteria biofilm. So, to improve the implant/tissue interaction, metal implants may be coated with bioresorbable osteoconductive materials (like hydroxyapatite (HAp)) to enable leaching necessary osteoinductive ions dedicated for the faster bone neoformation during the slow dissolution in body fluids. This widely-used (e.g. for joint arthroplasty) safe approach of an *in vivo* bioreactor inside the patient's body, instead of the *ex vivo* cell cultivation with the excessive manipulation of cells, is mandatory for tissue engineering due to the rigid regulations.

Finally, such an approach leads to the key benefit i.e. the formation of a natural-like bone layer. However, state-of-the-art industrial coating technologies do not fulfil the demands of low-temperature processing to prevent distortion of small implant sizes. Further, materials like HAp are insufficient to protect deep wounds and bone destruction from the enormous risk of bacterial colonization in the cases of finger traumas. In spite of the initial antibiotic therapy, inflammation may occur even some weeks past the injury and/or the final reconstruction surgery in less vascularized regions with low blood supply, such as the implant surface. This constricts further bone formation and results in implant rejection if the emergency anti-biotic treatment is inefficient. Based on the joint substitute know-how, the medical demands for a coating technique at low temperatures (<120°C) are the following:

(1) the biodegradation of osteoinductive HAp within 15-20 weeks as a basis for neoformation of a thin layer (500 - max. 1000 μm) of natural-like vascularized cortical bone on the implant

(2) the local anti-microbial protection during these 15-20 weeks of the HAp biodegradation phase after implantation (decrease of *Staph. aureus & epidermidis, E. coli, Pseudomonas* from 1E5 to <1E0 /ml in 24 h, ISO 22196 test conditions) to prevent the biofilm formation and the finger implant loss.

The main objective of the study was to design and manufacture a prototype finger implant.

Materials and Methods

The research and development of the optimized, biomimetic implant based on finite element modelling is the origin of developing (i) high-elastic Ti-15Mo-5Zr-3Al scaffolds with partly bionic cellular structures as bone substitutes and (ii) ultra-tough, smooth, complex 3D-shaped ZrO_2-Al_2O_3 ceramic joint substitutes for additive manufacturing by adapted selected laser melting (SLM) and lithography-based ceramic manufacturing (LCM), respectively.

Microstructure analysis

The surface morphology was examined with scanning electron microscopy (SEM). Prior to the imaging step, the samples were coated with a gold thin film to prevent the tissue surface charging. The visualization was performed by FEI Versa 3D FEG SEM (FEI, Poland), with the 5.0 kV – 10.0 kV acceleration voltage and the electron beam current of 4.0 nA.

The study was performed from the cross-section using transmission electron microscopy (TEM) Tecnai G2 F20 (200 kV). The thin films for the TEM analysis were prepared by the focused ion beam technique (FIB) using gallium ions by the device QUANTA 200 3D Dual Beam.

Cytotoxicity

The task addresses issues of the possible cytotoxic effects of biomaterials, i.e. the fibroblasts necrosis in relation to the control group tested on the Ti6Al4V alloy with confirmed biocompatibility. The cytotoxicity test of samples was performed by the indirect method according to ISO 10993-5 on murine fibroblasts (L929 ATCC). The potential cytotoxic effect was determined according to the ISO 10993-5:2009 standards. Twenty-three samples measuring 1.5 cm^2 were placed in confluent mouse fibroblast (L929; ATCC) cultures (about 5x10^5 cells) and incubated for 48 h at 37°C.

Then the cells were stained with propidium iodide (PI). The images were taken with the Axio Imiger confocal microscope equipped with a camera and quantified using Axio-Vision 4.6 (Carl Zeiss MicroImaging). A statistical analysis (two-way ANOVA and Tukey post hoc test, P value smaller than 0.05 was considered as significant – Statistica 10.0 PL) was performed on three replicates from each treatment.

The number of live and necrotic cells was assessed by confocal microscopy using the propidium iodide (PI) marker MitoTrucker green which stains active mitochondria. This marker localizes the mitochondria independently of the mitochondrial membrane potential. The propidium iodide test is one of the more commonly used methods for cytotoxicity testing. To label mitochondria, cells are incubated with MitoTracker® probes which passively diffuse across the plasma membrane and accumulate in the active mitochondria. After labelling their mitochondria, the cells can be treated with an aldehyde-based fixative for the samples that require fixation to allow further sample processing. Some MitoTracker® probes are also preserved after permeabilisation with certain detergents during subsequent processing steps (e.g. Immunocytochemistry or *in situ* hybridisation). Propidium iodide penetrates into the cell only when the continuity of the cell membrane is breached. Upon entering the cytoplasm, it labels nucleic acids and upon excitation with green light, it turns the nucleus of the necrotic cell red.

Microbiology

The antimicrobial activity contact test was based on ISO 22196:2007(E). *Escherichia coli* strain ATCC 8739 (Gram-negative) and *Staphylococcus aureus* strain 6538P (gram-positive) were used, as recommended in the norm. The obtained results were visualized as antibacterial activity index (R) which represents the difference between the number of viable bacteria recovered from both untreated and treated specimens. To analyze the microbiological properties of the coatings according to ISO 22196, the samples were inoculated with a bacterial suspension of the units that formed a colony of approximately 2.5 x 10^5 – 1.0 x 10^6 / ml (cfu). *Staphylococcus aureus* (ATCC® 6538P™) and *E. coli* (ATCC® 8739™) strains were selected for the study. The samples were incubated for 24 h at 95% relative humidity and at 37°C. To determine the initial number of bacteria, the microorganisms were counted after having been applied to the biomaterial and quickly washed away from its surface.

Results and Discussions

The concept of the implant

The suggested implant design is presented in FIG. 1. The image shows the bone part and the joint part.

The material topography and microstructure

The surface topography images were obtained by scanning electron microscopy at an accelerating voltage of 2 kV, using a secondary electron detector. One of the images was taken with the table tilted to 52 degrees to obtain a three-dimensional image (FIG. 2). The microstructural tests were performed on the reference flat samples, not on the implant. The surface, shown in FIG. 2, corresponds to the surface dedicated to the bone fixation.
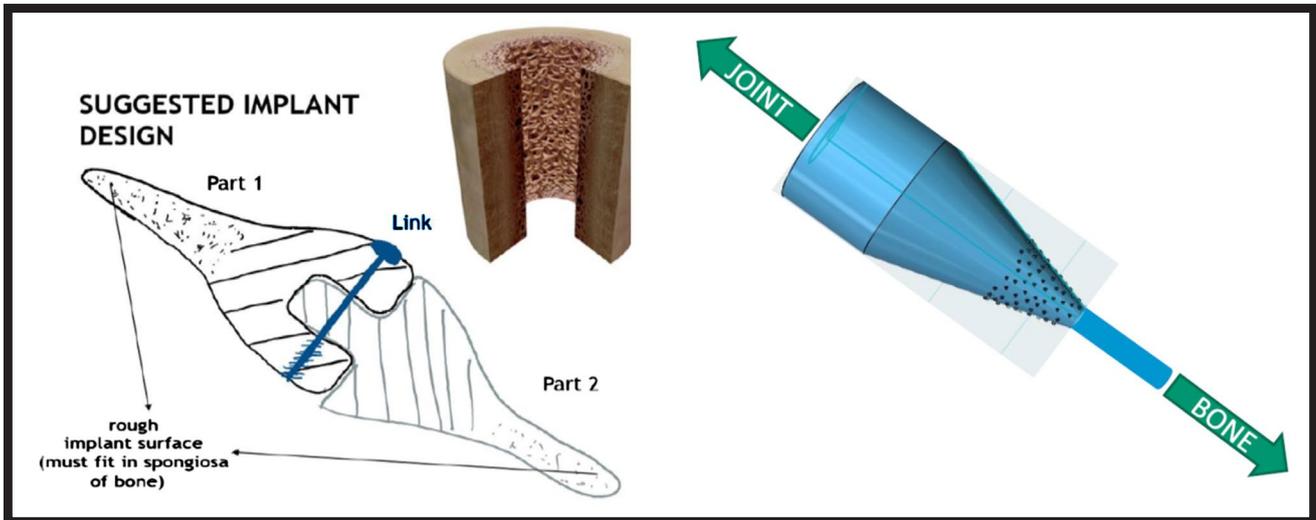
FIG. 1. The concept of the design: A - the suggested implant design, B - the concept of the implant-bone connection.
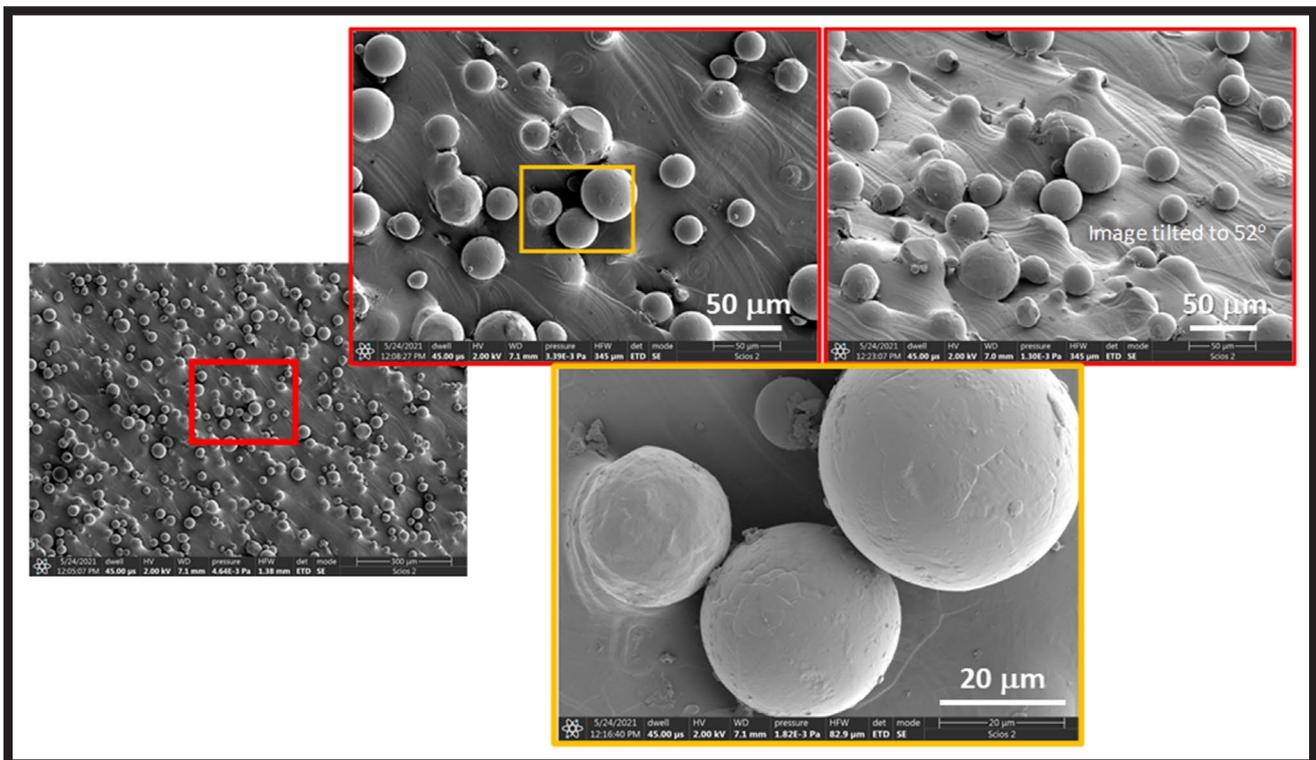

FIG. 2. SEM topography analysis of the materials intended for implants.

The detailed microstructural characterization was carried out using transmission electron microscopy on the cross-section (FIG. 3). A thin film was cut from the boundary between the substrate and the sphere. The sphere region showed the columnar growth of crystallites in the direction perpendicular to the sphere surface. Twinning was also shown, due to the tendency for twinning in hcp structures.

In order to better visualize the crystallites in the sphere area, the microstructural characterization was carried out in the dark field of observation from a selected diffraction reflector (FIG. 4).

Twinning is one of the main deformation modes in hexagonal close-packed (HCP) materials, and it has a great influence on mechanical properties.

The direct cytotoxic effect

The cytotoxic effect of the materials on the cells was determined according to the 10993 standard of the direct cytotoxicity analysis of materials. The tests were carried out using molecular probes of the mitotrucker type to test the level of mitochondrial activation and propidium iodide which labels necrotic cells (FIG. 5).

The graph was developed by the colocalisation function. The colocalization analysis is performed on a pixel by pixel basis. Every pixel in the image is plotted in the scatter diagram based on its intensity level from each channel. The colour in the scatterplot represents the number of pixels that are plotted in that region. In this example, the green intensity is shown on the x-axis and the red intensity is shown on the y-axis.
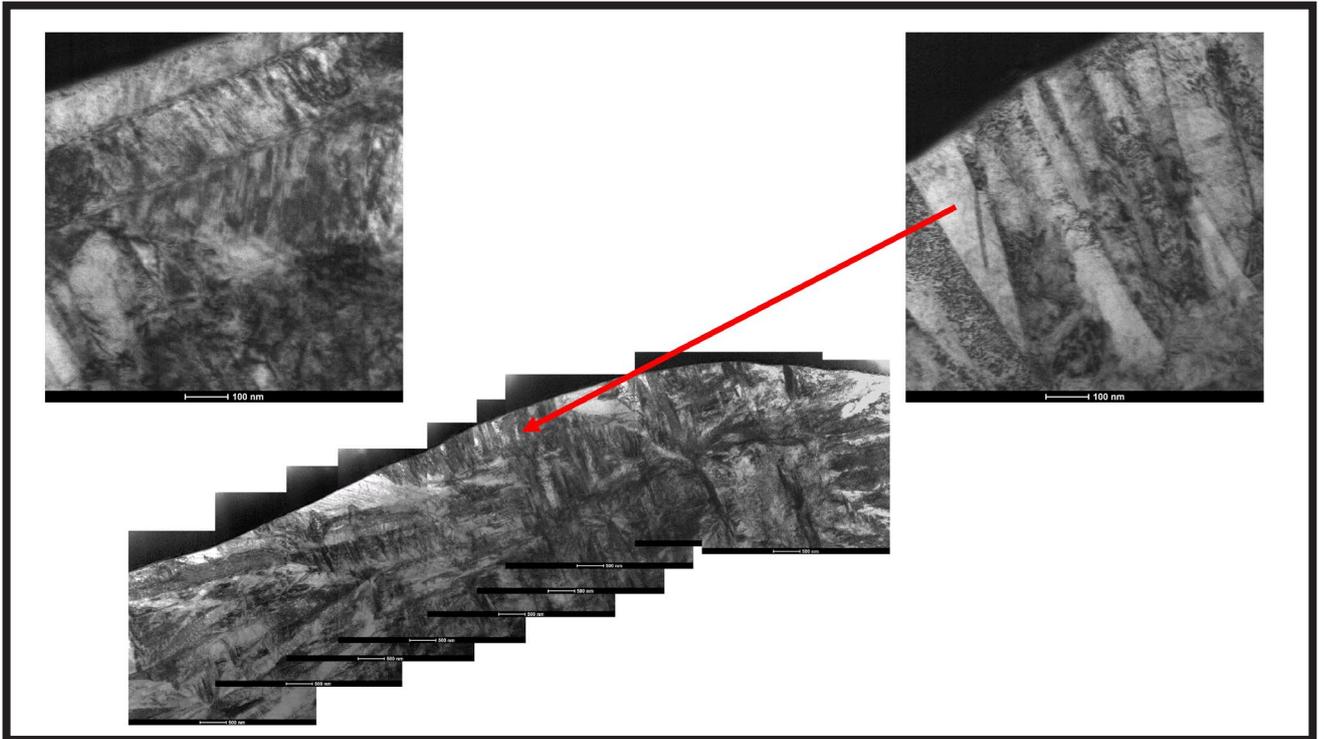
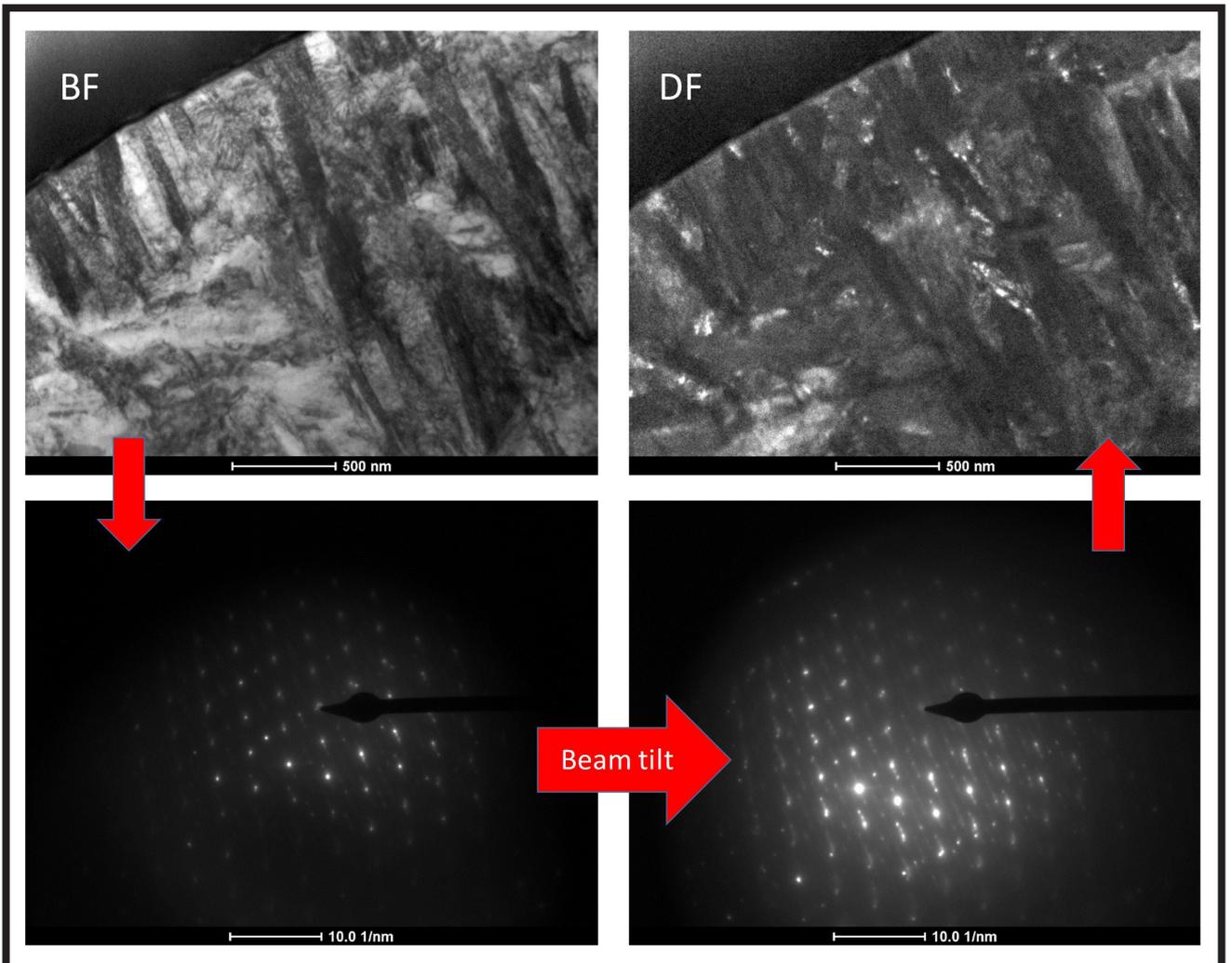**FIG. 3. TEM micrograph of the HCP structure of the coating.**

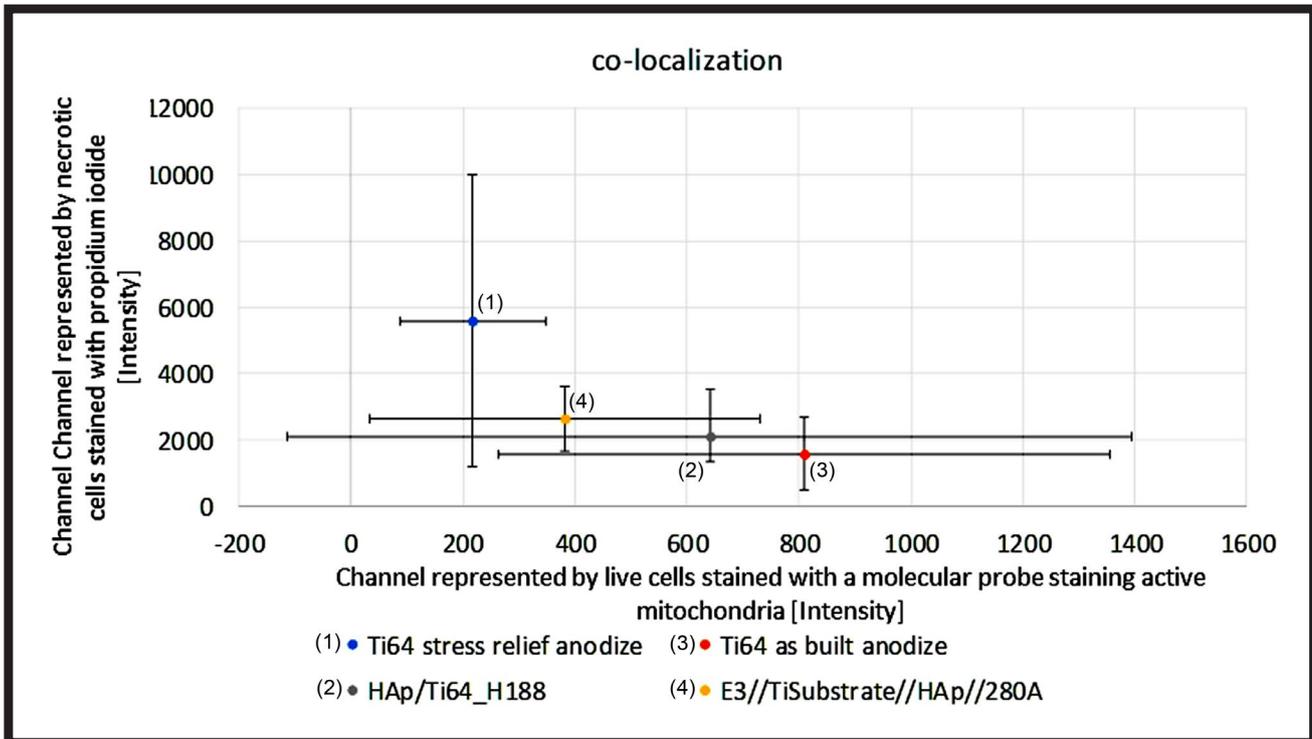**FIG. 4. Bright field image of the coating microstructure.**

FIG. 5. Cytotoxic effect of the tested materials on the cells.

TABLE 1. The antimicrobial activity assumption.

Biomaterial	Antibacterial activity R against:	
	E. coli	*S. aureus*
HAp/Ti64_H188	6.5	2.2
E3//TiSubstrate//HAp//280A	2.6	2.3

The microbiological effect

The antimicrobial activity value is presented in TABLE 1. The obtained results were visualized as antibacterial activity index (R) which represents the difference between the number of viable bacteria recovered from both the untreated and treated specimens. The material yields antibacterial properties if the calculated R value is greater than 2 (orders of magnitude). The higher the R index is, the better the antibacterial properties are.

Conclusions

The materials dedicated to the finger reconstruction were tested on the nanoscale and the microstructure was optimized for the proper overgrowth with the tissue. The microbiological tests showed good properties, i.e. antimicrobial properties for both the metallic substrate material and the hydroxyapatite-coated material. This is a very important characteristic of implant materials. The cytotoxicity tests did not show conclusive properties. A high probability of the necrotic comet formation and a large statistical scatter were observed, which may still indicate the low repeatability of the results. This feature will be refined in the near future, taking into account the positive microbiological aspects.

Acknowledgements

ORCID iDs

M. Dyner: https://orcid.org/0000-0002-9943-6335
A. Byrski: https://orcid.org/0000-0003-1152-5964
R. Major: https://orcid.org/0000-0003-3809-1908
M. Gawlikowski: https://orcid.org/0000-0002-6526-2656
K. Kasperkiewicz: https://orcid.org/0000-0002-9851-790X
J.M. Lackner: https://orcid.org/0000-0003-3931-232X
A. Dyner: https://orcid.org/0000-0001-7387-7178
B. Major: https://orcid.org/0000-0001-9471-1230

14

References

[1] J.P. Leigh, S.B. Markovitz, M. Fahs, C. Shin, P.J. Landrigan: Occupational Injury and Illness in the United States. Arch. Intern. Med. 157 (1997) 1557.
[2] K.C. Chung, M.J. Shauver: Table Saw Injuries. Plast. Reconstr. Surg. 132 (2013) 777e-783e.
[3] E. Davis Sears, K.C. Chung: Replantation of Finger Avulsion Injuries: A Systematic Review of Survival and Functional Outcomes, J. Hand Surg. Am. 36 (2011) 686-694.
[4] E. Mahmoudi, P.R. Swiatek, K.C. Chung, J.Z. Ayanian: Racial Variation in Treatment of Traumatic Finger/Thumb Amputation. Plast. Reconstr. Surg. 137 (2016) 576e-585e.
[5] J.M. Conn, J.L. Annest, G.W. Ryan, D.S. Budnitz: Non–Work-Related Finger Amputations in the United States, 2001-2002, Ann. Emerg. Med. 45 (2005) 630-635.
[6] C.J. Goodacre, G. Bernal, K. Rungcharassaeng, J.Y. Kan: Clinical complications with implants and implant prostheses. J. Prosthet. Dent. 90 (2003) 121-132.
[7] N. Shanmuganathan, M.U. Maheswari, V. Anandkumar, T.V. Padmanabhan, S. Swarup, A.H. Jibran: Aesthetic finger prosthesis. J. Indian Prosthodont. Soc. 11 (2011) 232-237.
[8] C. Aydin, S. Karakoca, H. Yilmaz: Implant-retained digital prostheses with custom-designed attachments: A clinical report. J. Prosthet. Dent. 97 (2007) 191-195.
[9] D. Boyle, D. Parker, C. Larson, L. Pessoa-Brandão: Nature, incidence, and cause of work-related amputations in Minnesota. Am. J. Ind. Med. 37 (2000) 542–550. http://www.ncbi.nlm.nih.gov/pubmed/10723048 (accessed May 28, 2019).
[10] G.M. Buncke, H.J. Buncke, C.K. Lee: Great Toe-to-Thumb Microvascular Transplantation After Traumatic Amputation, Hand Clin. 23 (2007) 105-115.
[11] H. Wang: Secondary surgery after digit replantation: Its incidence and sequence, Microsurgery. 22 (2002) 57-61.
[12] R.A. Bueno, B. Battiston, D. Ciclamini, P. Titolo, B. Panero, P. Tos: Replantation, Clin. Plast. Surg. 41 (2014) 385-395.
[13] L. Jazayeri, J.Q. Klausner, J. Chang: Distal Digital Replantation. Plast. Reconstr. Surg. 132 (2013) 1207-1217.
[14] S.J. Sebastin, K.C. Chung: A Systematic Review of the Outcomes of Replantation of Distal Digital Amputation. Plast. Reconstr. Surg. 128 (2011) 723-737.
[15] D. Fufa, R. Calfee, L. Wall, W. Zeng, C. Goldfarb: Digit Replantation: Experience of two U.S. academic level-I trauma centers. J. Bone Jt. Surg. 95 (2013) 2127–2134.
[16] M.J. Shauver, T. Nishizuka, H. Hirata, K.C. Chung: Traumatic Finger Amputation Treatment Preference among Hand Surgeons in the United States and Japan. Plast. Reconstr. Surg. 137 (2016) 1193-1202.
[17] R.D. Goldner, M. V Stevanovic, J.A. Nunley, J.R. Urbaniak: Digital replantation at the level of the distal interphalangeal joint and the distal phalanx. J. Hand Surg. Am. 14 (1989) 214-220.
[18] M.A. Pet, J.H. Ko, N.B. Vedder: Reconstruction of the Traumatized Thumb, Plast. Reconstr. Surg. 134 (2014) 1235-1245.
[19] V. Cervelli, D.-J. Bottini, A. Arpino, M. Grimaldi, M. Rogliani, P. Gentile: Bone-anchored implant in cosmetic finger reconstruction. Ann. Chir. Plast. Esthétique. 53 (2008) 365-367.
[20] B. Yeshwante, N. Parasrampuria, N. Baig: Prosthetic Rehabilitation of an Amputated finger. IOSR J. Dent. Med. Sci. 13 (2014) 10-17.
[21] N. Yadav, P. Chand, S.K. Jurel: Rehabilitation of single finger amputation with customized silicone prosthesis. Natl. J. Maxillofac. Surg. 7 (2016) 89-91.
[22] S. Sharma, G. Chand Baid, V. Lath, N. Gupta, S. Chandra Pankaj: Rehabilitation of an amputated finger - a case report. J. Evol. Med. Dent. Sci. 6 (2017) 744-748.
[23] A. Sierakowski, C. Watts, K. Thomas, D. Elliot: Long-term outcomes of osseointegrated digital prostheses for proximal amputations. J. Hand Surg. European Vol. 36 (2011) 116-125.
[24] P. Doppen, M. Solomons, S. Kritzinger: Osseointegrated Finger Prostheses. J. Hand Surg. European Vol. 34 (2009) 29-34.
[25] E. Drampalos, H.R. Mohammad, C. Kosmidis, M. Balal, J. Wong, A. Pillai: Single stage treatment of diabetic calcaneal osteomyelitis with an absorbable gentamicin-loaded calcium sulphate/hydroxyapatite biocomposite: The Silo technique, Foot. 34 (2018) 40-44.
[26] A.B. Swanson, C.G. Hagert, G.D. Swanson: Evaluation of impairment of hand function. J. Hand Surg. Am. 8 (1983) 709-722.
[27] L.-W. Lok, W.-L. Chan, Y.-K. Lau: Functional Outcomes of Antegrade Homodigital Neurovascular Island Flaps for Fingertip Amputation. J. Hand Surg. Asian-Pacific Vol. 22 (2017) 39-45.
[28] S. Kehoe, X.F. Zhang, D. Boyd: FDA approved guidance conduits and wraps for peripheral nerve injury: A review of materials and efficacy. Injury. 43 (2012) 553-572.