Section 1 - Publishable summary

HypOrth

Logo: 

Project title: New approaches in the development of Hypoallergenic implant material in Orthopaedics: Steps to personalised medicine

Website: www.hyporth.eu

Contractors involved (HypOrth consortium):

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1.1 Summary description of project context and objectives

- Identification of adverse immune reactions (AIR) to implant material and differentiation of AIR from low grade infection in the context of prosthesis loosening; inclusion of epidemiological and clinical expertise and the finding of diagnostic biomarkers for AIR
- Understanding of mechanisms of adverse immune reaction and develop predictive computational models
- Finding of predictive biomarkers for application in personalised medicine
- Testing of conventional and new material combinations for implant and coating on different cell systems: bone cells and immune cells for biocompatibility, antibacterial properties and evoking immunological parameters including the newly identified biomarkers
- Evaluation of data, production of prototypic hypoallergenic implant

Joint replacement is one of the most successful procedures in orthopaedics. Although many improvements were made, tissue reactions to biomaterials, infection and lacking fixation are still main reasons for failure and revision surgery. Various materials - considered as “ideal” to wear resistance (e.g. CoCr-alloys) or “bioinert” (Ti-alloys) – are found to induce adverse tissue reactions or to support biofilms. Lymphocyte-mediated response to biomaterials and wear products was shown to initiate inflammation and subsequent development of pseudotumours and osteolysis. Patients with a known metal allergy are at higher risk of developing sensitivity to the biomaterial. Bone loss and soft tissue masses with necroses have detrimental effects on the health and quality of life of European citizens.

Local reactions to biomaterials involve bone cells, cells from the monocytic lineage, and lymphocytes. The activation/inhibition of the different cells may be caused by the bulk material, particulate debris or ionic wear released from implants.

HypOrth will help to understand local adverse reaction to total joint replacements and to improve the integration of potential hypoallergenic implants with improved biocompatibility. It is necessary to understand the underlying mechanisms of cellular reactions induced by wear. HypOrth uses cell lines as culture models to test cell reactions to implant materials and wear products. 3 different patient cohorts from OVGU and UT will provide the possibility to transfer the cell line data to isolated material and cells from patients 1. with well-functioning implants, 2. receiving an implant, and 3. experiencing implant loosening.

HypOrth has completed patient recruitment after 36 months. A central patient database was successfully created by UT that securely guarantees data transfer between OVGU and UT. Access is also available for DTI and Progenika for microbiological and genomic/transcriptomic evaluation. Periprosthetic tissue samples from cohort 3 were thoroughly analysed for possible infection. Cell cultures from cell lines and PBMC's from patients are also established. Extraction methods for DNA and RNA from revision tissues have been validated. The Affymetrix analyses for blood have been completed, those for periprosthetic tissue are currently being processed. A survey of different biomaterials and surfaces from different polymers, ceramics and metals have been included into the study analyses. Those biomaterials and surface preparations (potential prototype material as hypoallergenic implants and surface modifications) have been tested with PBMC and various cell lines. Wear particles and ionic wear chosen for cell studies reflect the present knowledge of agents that induce periprosthetic AIR.

Implant materials and surface coatings have been fabricated in sample size and were monitored. The surface preparation is durable and securely attached to the biomaterials. Wear and fretting corrosion tests are established and were validated. A wear test simulator has been established. Using the machine, final prototypes are currently been tested.

1.2 Work performed since the beginning of the project and the main results achieved so far

WP1Period 1 focused on the development of the study protocol and standard operating protocols (SOPs), drafting and sharing with partner, finalising protocols. In addition, we discussed the details for clinical information that should be collected. At the same time, we developed online data collection system to be utilised in Estonia and Germany. This database was based on Filemaker software. We initiated collection of patient information and biological materials. During **period 2** we continued data collections. Filemaker based database was not reliable and users reported frequent crashes. Therefore, we decided to rebuild the database using RedCap platform. Redcap made data collection and storage very safe. During period 2 we finished data collection. In **period 3** we focused on independent quality control of clinical database. Epidemiological analysis of clinical data and description of the study cohort. Database is ready for wider international use for new osteoarthritis studies.

WP2 In the first period PBMCs from 159 patients (OVGU) and 108 patients (UT) were collected. Determination of immune status of 159 patients of OVGU was performed. Tissues from 83 patients were embedded in paraffin blocks. Related to **the second period** there was no differences in cell numbers (WBC; lymphocytes; monocytes; granulocytes T-suppressor cells; T-helper cells; NK cells; B cells and T lymphocytes HLA) between analysis groups. Activation parameters CD4/HLA-DR, CD4/CD25, CD4/CD69 and also CD8/HLA-DR, CD8/CD25, CD8/CD69 of 21 patient PBMCs did not change between patient pools or patient analysis groups after incubation on different material discs. For migration marker HEV no differences between analysis groups were found. Analysing chemokine receptors CCR4, CCR6 and CCR7, only CCR7 is differently expressed between analysis groups 1 and 3 by multinuclear giant cells. Further we found significant higher expressions of CD11c and CD68 in tissues of analysis group 3 compared to group 1. **In the third period** for interaction analysis of cells, monocyte- lymphocytic co-cultures (MM-6 and Jurkat) and isolated human PBMCs were incubated with different substances ($CoCl_2$, $CrCl_3$, CoCrMo particles, Titanium particles, UHMWPE particles) with different time durations. No difference in the number of synaptic contacts between analysis

groups was found by co-staining of CD2/CD58, CD40CD40L, CTLA-4/CD86. Furthermore, co-stained tissues from patients also revealed no differences in cell contacts between analysis groups. Testing migration and cell contacts in presence of CoCrMoNi particles using live-cell-imaging, monocytes and lymphocytes do not differ between analysis groups concerning migration parameters (way, distance, speed) and contact parameters.

WP3 Patients in pool III are preoperatively diagnosed with prosthetic loosening. Even though these patients were assessed to be aseptic, sometimes the analysis of perioperative obtained clinical specimens show a low-grade infection. In **reporting period 1***,* a SOP for molecular biological analysis of such samples was established, tested, and implemented at DTI (task 1) and it was used to supplement standard culture. During **reporting period 2** clinical specimens of all 104 patients in patient pool III were analysed for infecting microorganisms, resulting in seven infected patients – six found by culture and four by molecular testing (task 2). As part of task 3, an a priori list of candidate biomarkers for the identification of patients with low grade infections was created. In **reporting period 3** all available clinical, microbiological and transcriptomic data and cytokine profiles and patient pool III were collected, analysed and interpreted. As we were more successful in excluding patients with low grade infections than expected (expected 20, actually enrolled 7), the low number of enrolled, infected patients allows us to identify markers with large effect size, only. None of the biomarker candidates did allow a certain distinction of analysis group 3 and 4. However, data might warrant further investigation of the antimicrobial peptide hepcidin as a potential marker for low-grade infection (task 3).

WP4 In the **first period:** blood serum and tissue samples of patients were ongoing collected and prepared for Bio-Plex analysis. **In the second period** in blood serum no cytokine was found with a significant difference between analysis groups 1 and 3. In contrast to blood, there was found a decreased median expression of Fractalkine, IL-7, sCD40L, IL-4 and VEGF in patient tissues of analysis group 3. In contrast, the cytokine IL-8 showed an increased median expression in analysis group 3 compared to 1 and 2. **In the third period** we determined the amount of IL-8 in the blood from 286 HypOrth patients (118 patients in analysis group 1, 90 patients in AG 2, 78 patients in AG 3) using ELISA assay. We found a weak correlation of IL-8 blood concentration with implant age in knee group but not in hip group. Furthermore, we determined the correlation of IL-8 blood concentration (ELISA) with IL-8 tissue expression (PCR and Affimetrix assays, (WP 05) from 166 patients. IL-8 blood concentration of knee surgery group correlate significantly with IL-8 determined in tissue. In our patient cohort IL-8 indicates the implantation duration of knee joint. IL-8 in blood may serve as a surrogate parameter for IL-8 in tissue.

WP5 In **period 1** 657 RNA samples received from Uni Magdeburg and Uni Tartu have been tested so far using both Affymetrix and Dinamics Arrays. The analysis of the results allowed to select a genelist of 188 genes that are somehow involved in the development of adverse immune reactions. In **period 2** Validation experiment on these 188 genes was done. Real Time pPCR used a 96:96 Dynamics Array design for 9216 individual PCR reactions. The 188 genes, together with the enriched biological processes and KEGG pathways that show probable explanations for AIR result in a final list of 81 genes for further analysis which were also analysed for validation. In **period 3** a final candidate gene list contains 15 genes that will be used to model the AIR (P3). These are being confirmed. Several prototype materials are being used for treatment of different cell lines to evaluate their impact on the expression of these genes.

WP6 Period 1 and **period 2** have been focused on the preparations for a model development. In **period 3**: the focus has been on the development of a simple model involving only type of joint and age at primary TEP implantation, which is able to explain almost 40% of the variance of the observed implant age at revision. We have not been able to detect a further molecular process to predict implant survival.

WP7 During the **first period** we collected DNA samples from patients, extracted DNA from different patient pools and performed quality control. Not all samples were available at the end of the 1st period, because sample collection was still ongoing. However, we decided to start with the genome-wide DNA analysis in order to save time. In addition, we

later decided to use genome-wide genotyping instead of exome sequencing. Exome sequencing is more suitable for rare phenotypes and analysis of families and it does not capture intronic variations. Therefore, we decided to perform genome wide genotyping of AIR samples. During the first period we genotyped large part of pure cohort. During the **second period** we continued with extraction of DNA from newly recruited patients and performed genotyping. For the end of 2nd period we had almost all samples genotyped and analyzed. We performed initial association analyses. During genotyping we lost data from 12 subjects because of the poor quality of DNA. We decided to recover DNA for these patients and try to re-genotype them. During the **third period** we successfully extracted additional DNA for 12 missing samples and we completed genotyping. We performed association analysis for the AIR phenotype (pool 3 versus pool1 and 2). We modelled the implant survival time in relation to the patients' genotype adjusted for the age of patient. As a result, we identified several significant genetic loci responsible for the AIR. Potentially, genotyping for these loci could help to identify patients with higher risk for implant loosening and revision surgery. Functional studies are needed to identify biological function of the identified genes. Moreover, larger patient cohort is needed for further genomic studies and we started to find international collaborators. We presented results in the 19th EFORT Congress.

WP8 In **period 1** the testing methods were proposed for tribological and corrosion characterisation for reference and new materials, and the set of samples was fabricated by INOP and Mathys. Design and fabrication of new hip-join simulator were completed. In **period 2** the tests of reference materials defined by Mathys were performed: standard tribological tests using Block-on-ring tester; corrosion characterisation. Wear mechanisms of various reference material combinations were studied. Reference materials were tested on hip joint simulator. Structural characterisation (SEM, AES, TEM, XPS, XRD and EDS) of reference implant materials was carried out (MTA EK). In **period 3** all tests of reference and two tests of new hip joint endoprosthesis were done. Standard tribological tests using new Block-on-ring tester of new material combinations and fretting corrosion characterisation were performed. All materials of new materials needed for biological assays were provided by Mathys, and all microbiological tests were performed.

WP9 This work package oversees all Ethical aspects of all work packages following the guidelines of FP7 (Charter of Fundamental Rights of the European union; European Group on Ethics in Science and New Technologies (EGE)). In **period 1** ethics votes of local IRB's have been obtained. Data protection plan in the database is described. Log In requires personal passwords, log files save login footprints. Pseudonymisation is described. Data management fulfils criteria of ISO/IEC 27001:2005 standards. In **period 2** the study is registered in an ICMJE registry, the German Clinical Trials Registry (DRKS) (No. DRKS00010616). In **period 3** data protection and following ethical guidelines is continuously surveilled. All tasks have been fulfilled, no deviation of Description of Work is observed.

WP10 In **Period 1** after size agreement re. sample design, the feasibility of different surface treatments on the samples was investigated to guarantee that they correspond to implant surfaces. Seven polymers, 8 metals and 5 ceramic samples with different surface finish (polished, corundum blasted, machined or coated) were produced for DTI and OVGU (during period 1). For each material, more than 400 HypOrth samples – in total > 8500 samples will be produced, packaged and sterilized in due time. In **Period 2** Polymer, metal and ceramic samples for DTI and OVGU were produced. Reference and novel materials and coatings are provided for antibacterial and hypoallergenic testing. For novel samples, Mathys assessed nearly 40 different promising novel technologies. 12.000 samples were manufactured. The decision was taken to restrict the number of different samples for biological and microbiological testing. There will be 21 reference samples and at most 9 novel samples (during period 2). Materials for testing in WP8 (e. g. pin-on-disc (fretting corrosion) and block-on-ring (simple wear)) were provided from Mathys in medical grade quality) (during period 1 and 2). In **Period 3** in summary, 30 different materials and coatings were produced and tested. About 14000 samples made of implant type polymers, metals, ceramics and different coatings were manufactured for all biological and microbiological tests at DTI and OVGU (during period 1, 2 and 3). Based on these results, a number of endoprothesis prototypes for hip, knee and shoulder joints were produced (P3) and tested. In addition, materials

needed for specific testing e. g. pin-on-disc (fretting corrosion) and block-on-ring (sliding wear) were provided in medical grade quality (during the three periods). Implant samples made of reference materials (from period 1 and period 2) and new (promising) materials in **period 3** were provided for joint simulation wear studies, too.

WP11 During the **first period** the website was launched in August 2013 as information and communication platform with open and restricted areas. The public access area provides information for academic and industrial researchers and the press and the public. A project logo has been developed. During **period 2** the consortium produced a project flyer in printed form as well as two project posters that were used to advertise the project on conferences. During the **period 3** the Hungarian Scientific Society (partner MTA EK) chaired the ECERS congress and represented our project with a dedicated HypOrth booth providing information for the congress participants.

WP12 In **Period 3** one amendment request has been submitted; regular phone conferences were carried out with the coordinator and relevant partners; two f2f meetings have been organized.

1.3 The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far)

Total joint arthroplasty is one of the most beneficial procedures in orthopaedic surgery. However, a certain number of patients is experiencing adverse reactions to the implant materials. A consequence for these patients is revision surgery with the exchange of the endoprosthesis. HypOrth is contributing to increase the knowledge on adverse immune reactions (AIR) to implant materials. The database on the different patient pools has successfully been developed and patient samples have been collected from more 419 patients. The project has performed “omics” analyses on the patient samples in the various situations when bearing total joint arthroplasties as well as a preventive strategy for AIR.

Furthermore, HypOrth has identified candidate biomarkers for the diagnostic of AIR to implants. Cell cultures have confirmed immunohistochemistry and cytochemistry by cellular cytokine expression of patients’ cells. Those results will be used to develop tests as diagnostic tools in the differential diagnosis of AIR vs. septic loosening. There is potential for Interleukin-8 as a diagnostic tool to distinguish between aseptic loosening and well-functioning implants. Probably, there might be further biomarkers in the capsular tissues.

The surface characterizations, wear test methods and simulators will enable us to establish methods for testing new materials and surface coating or modifications of implants that are intended to prevent AIR. These test methods can address modified surfaces or ceramic implants, respectively, used for total hip, knee, or shoulder arthroplasties. The tests are performed under ISO-/ASTM standards, respectively.

HypOrth has already developed implant surfaces including ceramics, polymers, pure titanium, or titanium particulate coatings. Those surfaces and implant materials are being tested in material tests as well as cell culture experiments. From these results, prototypes are being designed. A very unique surface coating will be realized by using sea shells as a source for calcium/ hydroxyapatite coating including trace elements to enhance osseointegration and mimic biocompatibility. This technology has been proven to be efficient and effective.

The dissemination process of the science performed has already caused major attention of scientists, industry, and politicians. This is represented by scientific publications and presentations, as well as requests by industry and the project visits e.g. by the President of the State of Saxony-Anhalt or the establishment of the center of Competence ofr Orthopaedics 4.0 (KOU 4.0) in Magdeburg.

It can be assumed that the initiative HypOrth has direct impact on the health of European citizens but also on the technology transfer by stimulating metal forming industries. Already today, the prototype surfaces may have assuring superior properties to existing technologies.