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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5. Teknologisk Institut (DTI) – Jan Lorenzen
6. Progenika Biopharma SA (Progenika) – Sergio Escorza
7. Mathys AG Bettlach (Mathys) – Daniel Delfosse
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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.

During the past 36 months HypOrth has completed patient recruitment. A central patient database was successfully created by UT. Data transfer between OVGU and UT is securely guaranteed. Information 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 has shown relevance for the inclusion of different polymers, ceramics and metals into the study. Those biomaterials and surface preparations (potential prototype material as hypoallergenic implants and surface modifications) are currently being 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 are 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 will be tested.

The dissemination process has been successfully commenced. HypOrth's homepage (www.hyyporth.com) is regularly visited. Several media (newspaper, university newsletters, orthopaedic journals etc.) reported the start of the project. Flyers for HypOrth have been created and are distributed. HypOrth has been declared as one of the "best practice" projects of the local EU platform in Sachsen-Anhalt. President of the state, Dr. Haseloff, visited the project in Magdeburg. Other scientific contributions, papers or Ph.D. theses acknowledge the support of the E.C. in HypOrth. Steering committee meetings and general assemblies took place at various partners' institution, most recently in Bilbao where a young investigator session gave excellent insights in the projects. 2 young investigators were awarded for the presentation.

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

WP1: For all work packages SOPs are finalised. The sensitive processes, e.g. sampling (blood, tissue, joint fluid), handling and transportation of materials to other centres were established. 419 patients have been recruited. Database is completed. Work sheets are prepared with database upgrade and upload information. Quality control of the data was performed.

WP2: Tissues from 159 patients (OVGU) and 108 patients (UT) were collected. Metabolic activity after material incubation is measured. Most materials except ATZ CaPt and TAV TPS showed similar activity as control. Analysis of migration markers HEV, CCR4, CCR6 and CCR7 was performed, no differences between analysis groups were found except chemokine receptor CCR7.

WP3: Clinical specimens of all 104 patients in cohort 3 were analysed for infecting microorganisms, resulting in 7 infected patients – 6 found by culture and 4 by molecular testing. An *a priori* list of candidate biomarkers was created. Serum cytokine profiles show no difference between infected and uninfected patients.

WP4: Plasma, serum, and tissue samples from patients were determined by Bio-Plex analysis. Serum cytokine concentrations were low in this patient group. No cytokine was found with a difference between analysis groups. Tissue cytokine concentrations were low as well but 6 cytokines were found regulated: Fractalkine, sCD40L, IL-4; IL-7; IL-8, VEGF.

WP5: Experiments to define wear particles and cell types of interest were designed and started. Progenika received 381 RNA purified samples and performed the evaluation (quality & quantity). Samples were hybridized to Affymetrix® GeneChip® microarrays. 184 candidate genes were selected for qPCR analysis. 9216 individual PCR reactions were analysed.

WP6: In contrast to the expectation the analysis of variables obtainable from blood samples did not show suitable candidates. This indicates that the loosening process may be more localized than systemic. Consequently, the model development must focus on local tissue reactions.

WP7: For exomic sequencing of the AIR cohort blood samples from 235 participants were collected in order to extract their DNA and RNA. 600.000 SNPs all over the genome were compared for frequencies of the DNA variants between patients.

WP8: Testing methods of materials were: Block-on-Ring Wear Test acc. to ASTM F77-98; Pin-on-Plate Fretting corrosion tests; Design of a new hip-joint simulator was completed. 2 tests of hip joints from Mathys were done. Simulator performance was validated. SEM, AES, TEM, XPS, XRD and EDS of reference materials were carried out. Coated Ti alloy was selected as substrate for novel hydroxyapatite (HA) biocoatings. Biofilm formation on 20 implant materials has reached 50% completion.

WP9: 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. The study is registered in a WHO or ICMJE registry, the German Clinical Trials Registry (DRKS) (No. DRKS00010616).

WP10: Polymer, metal and ceramic samples for DTI and OVGU were produced. Reference and novel materials and coatings are provided for antibacterial and hypoallergenic testing. 12.000 samples were manufactured. 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).

WP11: 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. The consortium produced a project flyer in printed form. A project logo has been developed.

WP12: one amendment request has been submitted, another one prepared; Regular phone conferences were carried out with the coordinator and relevant partners; 7 meetings were 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 will be able to perform "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 is prepared to develop candidate biomarkers for the diagnostic of AIR to implants. Cell cultures will confirm 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. In the patients' serum/blood there seem to be no biomarkers that are able to distinguish between aseptic loosening and well-functioning implants. Probably, there might be a biomarker 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.

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.