IMMODGEL

Local immunomodulation around implants by innovative auxiliary hydrogel-based systems encapsulating autologous and phenotype controlled macrophages

Summary

IMMODGEL aimed to identify adverse immune reactions to implantable biomaterials with a particular focus on titanium and silicone. By developing innovative immunomodulatory systems as novel therapeutic strategies, the project outcomes will significantly decrease the implant and medical device failure caused by such reactions.

Immune reactions to implants, biomedical devices, engineered tissues and transplants are a big obstacle in the biomedical field. For instance, electrodes can lose their functionality due to host immune responses, while the need to evade rejection of transplants narrows down the sources for transplantable tissues and contributes to the persistent donor tissue shortage (see Image 1).

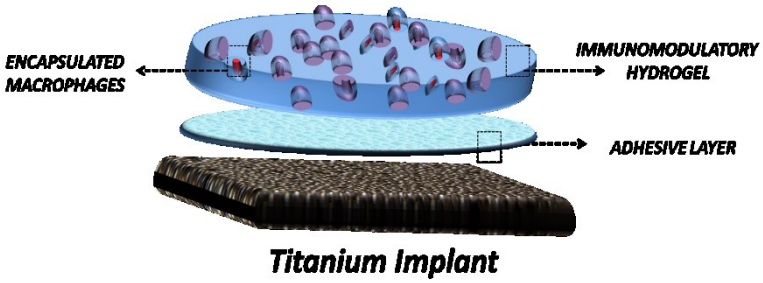


Image 1: Simplified schematic representation of the IMMODGEL system

The IMMODGEL project aimed at exploring the nature and the underlying mechanisms of adverse reactions with systems biology approaches. Such an understanding enabled the development of immunomodulatory therapeutic systems with biomaterials using tissue engineering and regenerative medicine methodologies. Our aim was to design immunomodulatory systems applicable to different situations (transplants, implants, biomedical devices, etc.). The design consists of an immunomodulatory hydrogel which contains encapsulated macrophages and is anchored to the implant surfaces and/or cytokine cocktail releasing adhesives/coatings. The system additionally presents antimicrobial properties via release of antimicrobial agents from the adhesive component.

The third axis of research in IMMODGEL (aside from systems biology level understanding of immune responses to titanium and therapeutic immunomodulation) consisted in the development of enabling technologies that can provide the tools for future research on immunomodulation, diagnostics related to immune reactions to implants and overall immunology research. For this end, founded on the data generated, IMMODGEL developed a patient-specific immunoprofiler in the form of an on-chip system that is currently at the stage of validation based on multiplex cytokine quantification and Mimotope Variance Analysis (MVA). Additionally, a "Foreign Body Response on-a-chip" has been designed and tested to predict patients´ specific responses to implant materials (tested with miniaturized titanium samples). The project also developed immunocompetent engineered tissues (respiratory epithelium and 3D hydrogel based model systems containing resident macrophages) to have in vitro models of immune reactions to biomaterials.

The key innovation was the development of IMMODGEL as an auxiliary system to improve the outcomes of implantation that has been validated in vitro and in vivo within the timeframe of the project. This innovation will help reducing the cost of implant complication and related medical costs in Europe. We have provided potentially marketable therapeutic, diagnostic systems and easily-transferable research tools together with a significant improvement in the understanding of the underlying causes of adverse reactions to biomaterials as well as methods to control them. The activities resulted in 41 scientific publications, 4 patent applications and more than 100 dissemination activities.

Project context and main objectives

Organ/tissue damage and loss are important clinical problems, where the current gold standard is transplantation. One of the main problems with transplants is the use immunosuppressants (IS) for preventing rejection. This results in several side effects such as increased tumor formation risk, higher susceptibility to infections and IS-related toxicity. According to Health Resources and Services Administration (HRSA) reports, depending on the target organ, the success rate of transplants can change between 50 to 90%, also many patients suffer while waiting for a suitable donor due to immunological concerns. Moreover, even though the survival rates are high, the deterioration of the transplant is not completely evitable. Therefore, there is an unmet need for locally applicable systems that can attenuate immune response towards transplants without having systemic effects. Beyond transplantation, implants, transplants and implantable biomedical devices have become mainstream solutions for a wide variety of health problems, and their use in medical practices either for therapeutic applications, prevention or diagnosis is increasing constantly. However, often adverse immune reactions against these foreign materials are observed in the host body. These can lead to dramatic immediate outcomes like immense pain, excessive inflammation or even rejection of the implanted material/tissue.

Overall these adverse immune reactions cause the following symptoms: i) delayed recovery, ii) deterioration of patients' life quality following implantation iii) unresolved persistent chronic health problems, and iv) additional health problems due to side effects of rejection and/or inflammation. Beyond the obvious problems posed on the patient's well-being, problems related to adverse immune reactions represent a massive burden on the healthcare system as they usually require additional surgeries, subsequent treatments and in turn long hospitalisation of the patients.

A series of cellular and molecular events following biomaterial implantation poses an important bottleneck for developing breakthrough implantable solutions. Moreover, chronic inflammation and also the local microenvironment created by it can be detrimental for the long-term functionality of the implants, especially complex systems with several parts such as *in vivo* biosensors and artificial biopancreas systems. With inflammation increasingly recognized as a crucial component influencing regeneration, immunomodulation or immuno-engineering has emerged as a potential solution to overcome this key challenge in biomedical engineering. IMMODGEL proposed to develop a new therapeutic strategy based on the design and development of an auxiliary system which will be able to modulate the immune system response around implanted materials or tissues once attached on them. This immunomodulation will trigger remodelling around them and improve their integration in the host body.

This therapeutic strategy was based on harnessing the component of the immune response, namely inflammation that is an inevitable consequence of implantation/transplantation and is closely linked to the clinical outcomes. Upon implantation, immune cells migrate to the implantation site and initiate a localized inflammatory response. Although inflammation is an indispensable element in tissue regeneration, a chronic inflammatory response will significantly limit natural healing, which is quite common in the case of implants.

Among the variety of immune cells, monocytes and macrophages play a particularly critical role that determines successful tissue–implant integration or implant failure. In particular, macrophage polarity steers the microenvironment toward inflammation or wound healing via the balanced induction of different macrophage phenotypes. Classically, activated M1 macrophages are associated with a pro-inflammatory response. By contrast, alternatively activated M2 macrophages are associated with an anti-inflammatory and regenerative response which induces angiogenesis and proliferation. Therefore, harnessing macrophage polarity presents a unique opportunity to control inflammation, prevent rejection, and accelerate integration of biomaterials and medical devices. Different macrophage phenotypes with distinct functional properties have been identified. For instance, M1 macrophages are induced by interferon gamma (IFN-γ) from T helper 1 (T_H1) cells, $CD8^+$ cytotoxic T cells (CTLs) or natural killer (NK) cells in the presence of microbial products such as lipopolysaccharide (LPS). M1 macrophages have pro-inflammatory and anti-tumour functions and secrete high levels of pro-inflammatory cytokines such as interleukin 12 (IL-12) and IL-23. On the other hand, M2 macrophages are induced by IL-4 and/or IL-13, which are mainly secreted by T_H2 cells or polymorphonuclear cells such as mast cells. M2 macrophages have anti-inflammatory and pro-wound healing activities and secrete large amounts of the anti-inflammatory cytokine IL-10. Currently, macrophage polarization is most commonly controlled via exposure to biochemical factors. Specifically, the M1 macrophage phenotype is typically induced through interferon gamma (IFNγ) or lipopolysaccharide (LPS) stimulation, while the M2 macrophage phenotype is typically induced through interleukin-4 (IL-4) or interleukin-13 (IL-13) stimulation. The M1/M2 polarization of macrophages has been described in a generalized manner and the optimum macrophage differentiation status for remodelling is not known, and thus one of the objectives was the determination of the optimum and a long-lasting phenotype based on the induction by cytokine cocktails.

Beyond cytokine based macrophage control, in IMMODGEL, one of the objectives was to determine whether physical stimuli such as encapsulation or presentation of micropatterns can control macrophage polarization. Recent advances in biomaterials science have identified that a biomaterial's design can be leveraged to instruct the host's immune system. For example, novel biomaterial surfaces, improved immune-instructive biomaterials and incorporating immune modulating cells could influence the wound healing process. In addition, several physical and biochemical factors have recently been reported to regulate macrophage polarization such as pore size, mechanical stimulation and extracellular matrix proteins (ECM) among others. However, the underlying mechanisms of how biomaterials steer macrophage polarity has remained poorly understood. The biomaterials *in vivo* will be in close contact with macrophages and characteristics such as surface chemistry and topography may have a critical role in initiating pro- or anti-inflammatory immune responses. Controlling biomaterial surface attributes provides a powerful tool for modulating the phenotype and function of immune cells with the aim of reducing detrimental pro-inflammatory responses and promoting beneficial healing responses. For this end, one of the objectives was to identify the specific surface topography features (surface micropatterns) that can induce M2 phenotype in incoming macrophages or induce M1 to M2 conversion.

The concept of IMMODGEL was that it is possible to design and engineer a stand-alone immunomodulatory system. This product can be attached to any implant to achieve local immunomodulation and thus protect the implant, transplant or tissue engineered product from the adverse effects of immune response while preventing systemic effects and subsequent infection risks. This is achieved via *in vitro* phenotype control of autologous macrophages which can be used to orchestrate the inflammation cascade. More precisely, the design of a cellular microenvironment that induces M2 phenotype was the main goal. Through this, it was possible to convert local reaction from an inflammatory route to a remodelling route. For achieving this, the objective in IMMODGEL was to develop a transferable, 3D hydrogel for macrophage (M2) encapsulation which can fix the encapsulated macrophage phenotype for 3 weeks with more than 90% viability.

One of the most common implant materials is titanium which is used in dentistry, as permanent tooth implants, and in orthopaedics as knee and hip replacement. Despite its biocompatibility, implants based on this metal commonly fail due to bad osseointegration, peri-implantitis, osteolysis etc. Depending on the target area, the rate of failure can be in the range of 2% to 10%. Currently there are only a few generic indicators (such as smoking, diabetes, factors that are known to affect healing overall) that are in use in order to indicate an elevated risk of implant failure. In an ageing society, the effects of implant failure have more reverberating effects as the problems related to implants mean additional health problems for ailing patients. One of the objectives of IMMODGEL was to determine a profile of specific phenotypic markers that describes the macrophage state around titanium implants.

In line with the silver economy initiative of the European Commission, it is important to provide tools to the implant practitioners to inform and guide elderly patients in order to minimize the adverse effect of such implantation procedures which otherwise are improving life quality. In IMMODGEL, another objective was to detect the patients prone to implant failure which affects a significant number of patients via development of personalized immunoprofiling tools such as a novel personalised multiplex diagnostic antibody-based array to identify personalized immune response to titanium implants.

One of the persistent problems around nondegradable metallic and polymeric implants is failure of macrophages to resolve the inflammation and their tendency to stay in a state, named “frustrated phagocytosis”. Implanted materials can induce a mixed pro/anti-inflammatory phenotype, supporting chronic inflammatory reactions accompanied by microbial contamination and resulting in implant failure. Several materials based on natural polymers for improved interaction with host tissue or surfaces that release anti-inflammatory drugs/bioactive agents have been developed for implant coating to reduce implant rejection. However, no definitive, long-term solution to avoid adverse immune responses to the implanted materials is available to date. The prevention of implant-associated infections or chronic inflammation by manipulating the macrophage phenotype is a promising strategy to improve implant acceptance. For this end, in IMMODGEL, the objective was to develop a polyelectrolyte multilayer based attachment system with cytokine release capacity for simultaneous anti-inflammatory and antimicrobial effects.

For the development of biomaterial based solutions, one potential route is the use of natural polymers with known anti-inflammatory functions. Hyaluronic acid (HA) plays a multi-faceted role in cell migration, proliferation and differentiation at micro level and system level events. In addition to its biological functions, it has advantageous physical properties which result in the industrial endeavors in the synthesis and extraction of HA for variety of applications ranging from medical to

cosmetic. A specific reason for the increase in use of HA based structures is their immunomodulatory and regeneration inducing capacities. Thus, in IMMODGEL one of the objectives was to harness the advantageous properties of HA in both coating and hyrogel formulation.

The expected added value of IMMODGEL project was: i) Decreased risk of chronic inflammation, ii) Improved remodelling response by shortening of the inflammatory cascade, iii) A temporal control over the remodelling of the implant, iv) Applicability to different structures which can provide a comprehensive solution to immunological responses without the need to change the design of the original implants.

Additional expected outcomes of the project were: i) Elucidation of Macrophage behaviour within 3D hydrogels, ii) Determination of the synergy between physical control and cytokines to obtain long term control over cell phenotype while keeping product cost feasible, iii) Development of complex immuno-competent tissue models to better understand activities of immune cells in artificial tissue settings, iv) Development of benchmarks for trial of immunomodulating implants, v) Utilization of bioprinter technology to augment the capacities of other implants.

The main results achieved in each of the 6 RTD Work Packages as well as dissemination/exploitation activities are summarized in the following pages.

Work Package 1

Systems immunology: identification of macrophage-mediated chronic inflammatory reactions to titanium implants

Objectives

- Identification of specific profile of inflammatory responses of macrophages to titanium by system immunology approach
- Identification of the optimal cytokine cocktail needed to obtain this specific phenotype for subsequent *in-vivo* immunomodulation
- Design and development of a multiplex antibody-based diagnostic chip to determine individual differences in the adverse reactions to titanium implants as a means of "Personalized immunomodulation

Main results

1. Using systems immunology approach we have identified adverse reactions of human macrophages on polished ad porous titanium. Affymetrix microarray analysis revealed that a total of 1690 genes were differentially regulated by polished titanium and 4648 genes were differentially regulated by porous titanium in M2 macrophages. Major detrimental reactions of macrophages induced upregulation of pro-inflammatory factors CSF1, TNFSF14, YKL40 and chitotriosidase, and extracellular matrix destroying matrix metalloproteinases with broad substrate specificity (MMP7, MMP8, MMP9). The microarray data were confirmed by RT-PCR, ELISA, activity assays. Both polished and porous titanium surfaces showed similar pro-inflammatory effects. Titanium had also a beneficial effect on macrophage programming by the induction of the expression of metallothioneins contributing to the non-inflammatory intracellular bacterial killing.
2. We have developed a cytokine cocktail consisting of IL4/IL10/TGFβ1 (M2Ct) that induced a long-term anti-inflammatory and pro-healing phenotype in human primary monocyte-derived macrophages. However, in the absence of M2Ct in the medium macrophages underwent rapid pro-inflammatory re-programming upon IFNγ stimulation. Therefore, loading and release of the cytokine cocktail from a self-standing, transferable gelatin/tyraminated hyaluronic acid based release system was developed to stabilize macrophage phenotype for in vivo applications in implantation and tissue engineering. The M2Ct cytokine cocktail retained its anti-inflammatory activity in controlled release conditions.
3. We developed the antibody-based multiplex assays for rapid identification of the direction of macrophage activation by measuring the concentration of TNFα and IL1β (for M1 phenotype) and IL1Ra andCCL18 (for M2 phenotype). The multiplex assays can be used for the identification of patient –specific M1/M2 profile of macrophage activation in response to implant materials.

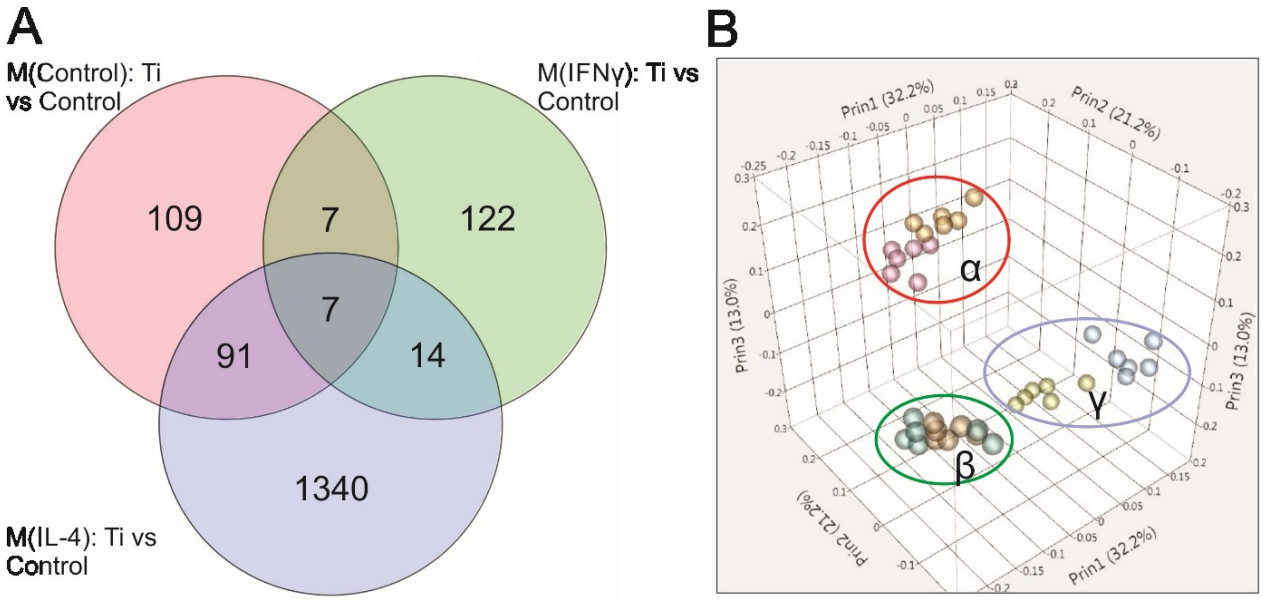


Figure 1. Microarray gene expression analysis of macrophages on polished titanium. α - clustering of data from M(Control) (pink – Control; orange - Titanium); β - clustering of data from M(IFNγ) (green – Control; brown - Titanium); γ - clustering of data from M(IL-4) (yellow – Control; cyan – Titanium)

Conclusions

1. We identified both detrimental and beneficial reactions of macrophages to titanium, identified macrophages as target cell for the immunomodulation in implant microenvironment, and demonstrated the proof-of-principle for generation of programmed anti-inflammatory macrophages as cell therapy tool in regenerative medicine.
2. The multiplex assays can be used for the identification of patient –specific M1/M2 profile of macrophage activation in response to implant materials.

Work Package 2

Effect of Topography on Macrophage Phenotype

Objectives

- Optimisation of the surface micropatterning for macrophage phenotype control
- Determination synergistic cytokine/micropattern interactions for macrophage phenotype control
- In-vitro modelling of Interaction between encapsulated macrophages and incoming macrophages

Main results

Several topographies such as microwells, micropillars and microgrooves with a wide range of dimensions were produced and screened with model monocytes and primary macrophages. Additionally, CTP developed the EHP technique for the fabrication of micropatterns with the dimensions and characteristics required for this project. This work led to the selection of 2 types of surface micropatterns as optimal (microgrooves/ridges with the dimensions: 20 µm depth, 20 µm groove, 10 µm ridge width; and micropillars with the dimensions: 20 µm depth, 20 µm pillar diameter, 5 µm distance between pillars).

With these micropatterns, further work was undertaken to determine the synergistic effects of cytokine induction and surface micropatterns. Using conventional methods for the assessment of macrophage activation, the effect of patterns was found to be subtle and outweighed by the cytokine microenvironment. However, using an unbiased gene microarray screening approach, it was found that microgrooves/ridges and micropillars induced distinct gene expression profiles, which in turn were different from those observed on unpatterned hydrogel. The greatest changes were observed in metabolic processes, DNA transcription and repair as well as protein translation and post-translational modification.

Finally, investigation of the effect of encapsulated phenotype-controlled monocytes/macrophages on incoming monocytes, endothelial cells and fibroblasts demonstrated increased endothelial cell growth in the presence of encapsulated M2 macrophages and increased fibroblast growth in the presence of encapsulated M1 macrophages.

Conclusions

Two micropatterns were selected and their effect on macrophage behaviour elucidated. The synergistic effect of patterns and the cytokine cocktail optimised in WP1 on macrophages was assessed. The patterns were found to alter fundamental cellular processes such as metabolism, transcription and translation, while the cytokine microenvironment was found to modulate conventional readouts of macrophage activation such as cell surface marker expression and cytokine profile. Hydrogel-encapsulated phenotype-controlled macrophages were able to modify the behaviour of incoming cell types such as endothelial cells and fibroblasts.

Outlook

We can reproducibly introduce these patterns to macrophage-laden hydrogels developed in WP3 and these surface patterns can be used in the final therapeutic system as described in DoW. In addition, we have shown the need for unbiased screening when determining the activation state of macrophages in contact with biomaterials.

Work Package 3

Development of physical constituents of IMMODGEL (Adhesive polyelectrolyte, cell encapsulating hydrogel)

Objectives

- Development of an effective encapsulation system for macrophages which ensures their viability and long-term health.
- Development of an adhesive system, which is non-toxic, versatile and high adhesive properties
- Attachment of the cell-laden gels to different implant configurations.
- Optimization of Release of Cytokine Cocktail in the 3D structure (Differential release from adhesive coating and the gel body)

Main results

1. 4 different hydrogel configurations based on enzymatically crosslinked gelatin, methacrylated gelatin, Tyraminated Hyaluronic acid, Tyraminated gelatin and HA-aldehyde were developed which can keep macrophages alive up to 21 days. The physical and mechanical properties of the hydrogels and the macrophage response to the hydrogels were quantified. The hydrogel composition, concentration and degradability were shown to have significant effects on monocyte/macrophage behaviour.
2. 3 different adhesive/release platform formulations based on polyelectrolyte multilayers (PLL/HA-aldehyde, Polyarginine/Hyaluronic acid) or self-standing spin coated films (based on gelatin and additives such as tyraminated-HA and Polyarginine) were developed and the release of cytokines such as IL-4 was successfully demonstrated up to 21 days. Macrophage phenotype can be changed by the release from these platforms. We also discovered that Polyarginine/HA multilayers additionally have antimicrobial properties.
3. To establish the contact between the implant and the IMMODGEL system different wet adhesives based on gelatin, PEG/tannic acid, DOPA-lysine artificial peptides and maleimide chemistry were developed. Although most of the systems were able to keep the IMMODGEL structure adhered to titanium and other biomaterial surfaces; the best option was DOPA-lysine due to its superior biocompatibility.

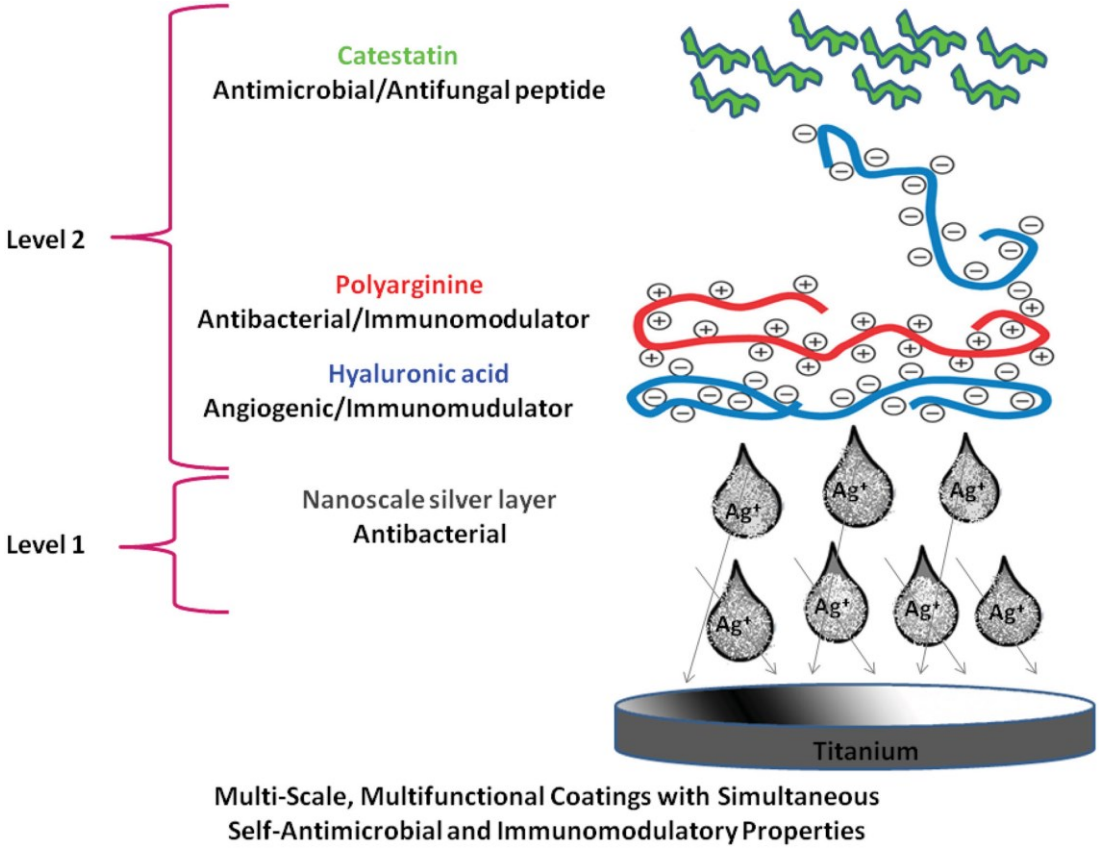


Figure 1. Multifunctional immunomodulatory coating formulations

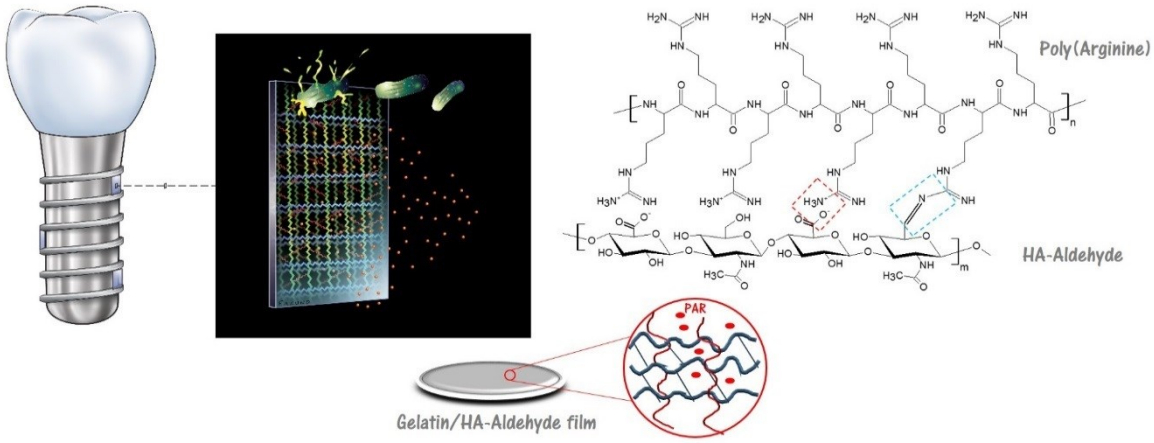


Figure 2. One of the coating formulations with concomitant antimicrobial and anti-inflammatory activity.

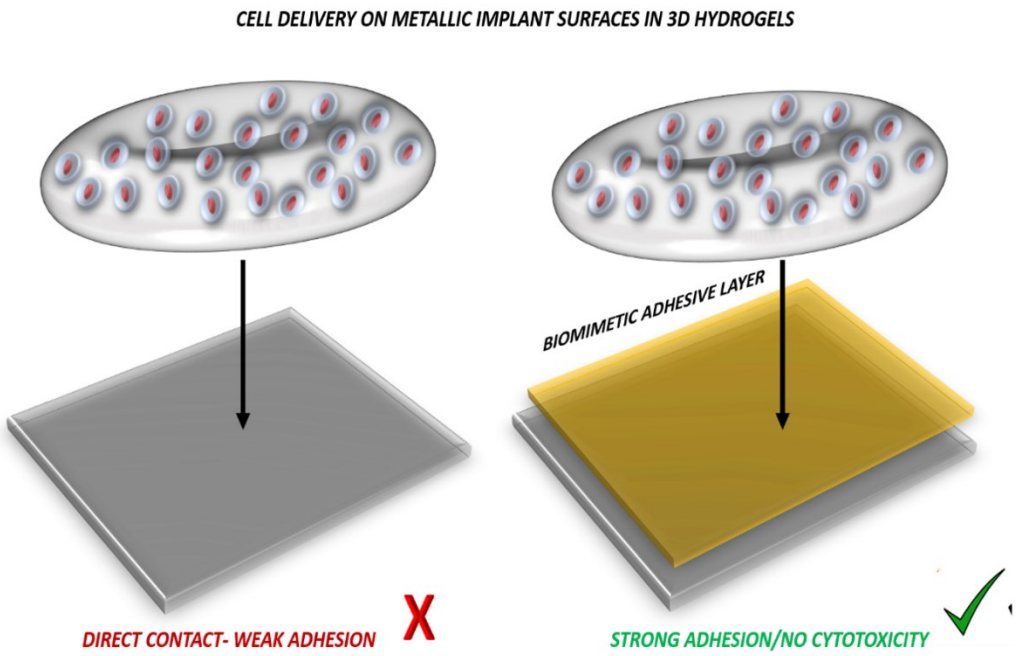


Figure 3. Mode of action of the DOPA-Lysine based adhesive

Conclusions

A set of formulations for immunomodulatory hydrogels, auxiliary cytokine release systems and wet adhesive for the application of the overall IMMODGEL system to implants is developed.

Work Package 4

Control of macrophage phenotype subsets in 3D hydrogel systems

Objectives

- Elucidation of the effect of the encapsulation conditions on macrophage with different gel constituents and physical properties.
- Determination of degradation kinetics of the gels by macrophages for determination of the duration of the immunomodulation
- Determination of the specific cytokine conditions to obtain the desired M2 sub-phenotype within hydrogels.
- Optimization and Re-adjustment of the cytokine release within the hydrogel in the presence of macrophages

Main results

1. The following hydrogel properties were analysed in relation to their effect on encapsulated monocyte/macrophage phenotype: Hydrogel concentration, degradability, stiffness, composition, crosslinking system (co-crosslinked composites, interpenetrating networks), pre-differentiation, presence of cytokines and cytokine cocktails. Overall, we have found that macrophages prefer gel concentrations between 3%-7%, non-degradable hydrogels induce M1 differentiation whereas degradable hydrogels induce M2 differentiation through integrin and MMP mediated interactions with the hydrogels. Stiffer hydrogels induce M1 differentiation; gel composition significantly affects the differentiation (for example presence of HA induces CD44 expression) and incorporation of cytokines boosts the effects relayed by the gels.
2. We have developed formulations for both hydrogels and the release platforms that are stable in the presence of macrophages up to 21 days. We successfully achieved the integration of the release platform of cell-laden hydrogels were and proved a positive effect of the release platform on the encapsulated macrophages.
3. The cytokine cocktail developed in WP1 was successfully loaded to the release platform and it was shown to be efficient in controlling macrophage phenotype when released. A 10x concentrated version of the cocktail is needed for in vivo experiments.

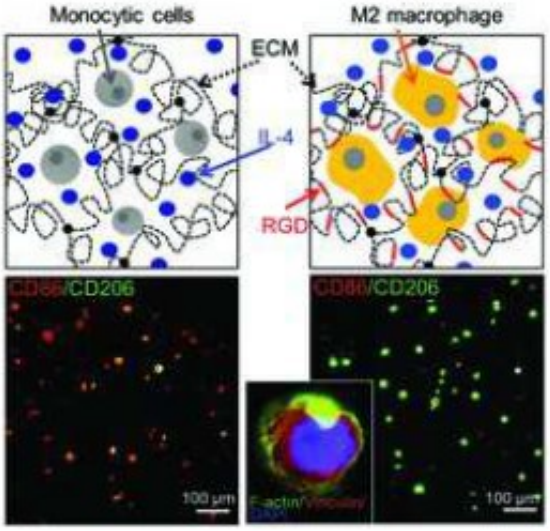


Figure 1. The effect of the nature of the encapsulating hydrogel on macrophage phenotype

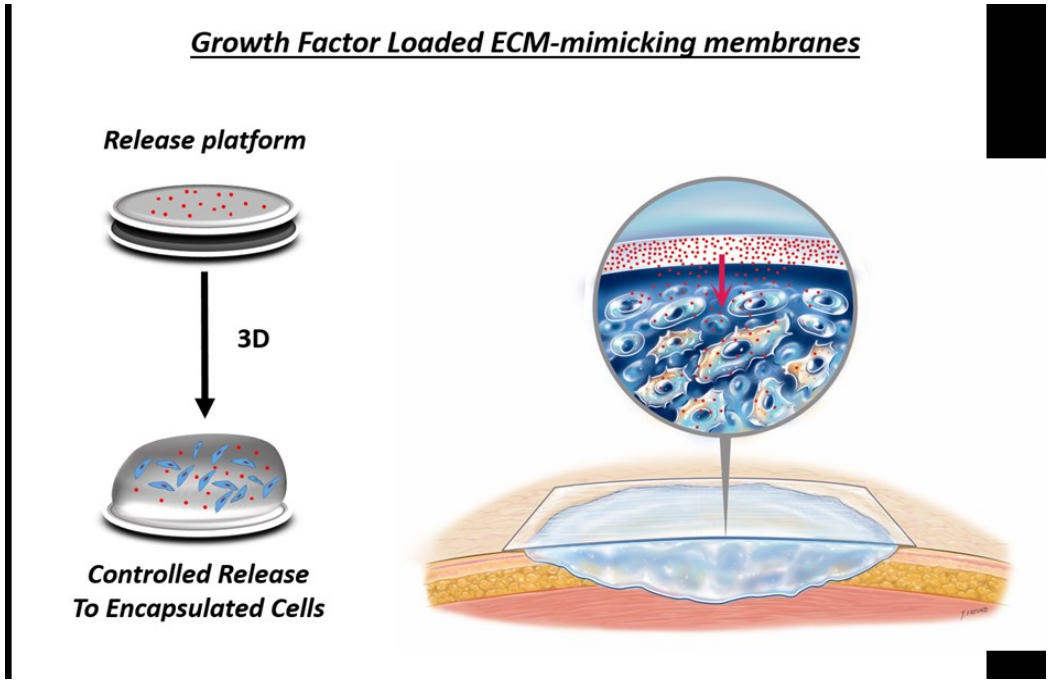


Figure 2. The demonstration of the mode of action of the auxiliary release system in conjunction with macrophage-laden hydrogels.

Conclusions

The physicochemical properties of hydrogels have a significant effect on macrophage phenotype and they can be used for phenotype control for both M1 and M2 macrophages in conjunction with cytokines.

Work Package 5

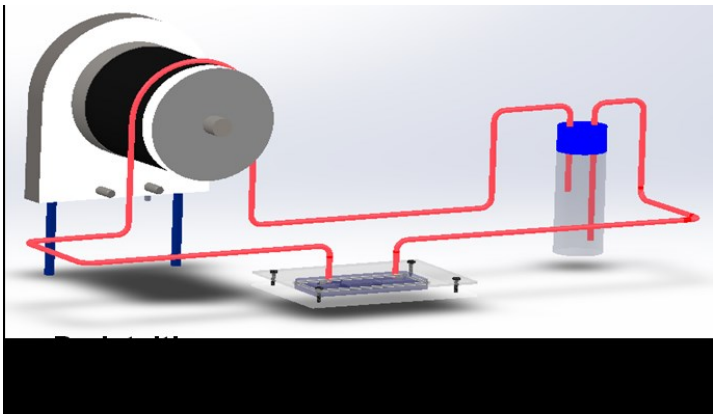
Enabling Technologies: Foreign Body Response on-a-chip, Immunocompetent Artificial Tissues, Bioprinted Immunomodulatory Hydrogels

Objectives

- Develop foreign body response on a chip platform to reproduce the in vivo immune microenvironment and accurately test foreign body responses in vitro
- Develop implant materials, coatings, and tissue constructs that will allow the modulation of host immune responses
- Develop bioprinting strategies to fabricate well-defined immunomodulatory hydrogels

Main results

1. A foreign body response on a chip platform has been developed featuring a double-layer microfluidic device; one simulating the tissue chamber where cytokines are released and implants are embedded, and another simulating the blood stream where monocytes circulate, sandwiching a layer of porous membrane populated with confluent endothelial cells functioning as the vascular wall.
2. The device was found to reproduce the monocyte-endothelium and monocyte-implant interactions, including activation, migration, and differentiation, using THP-1 cell line.
3. It was further found that the release of MCP-1 from the bottom chamber where the implant is located, could induce more transendothelial migration of the THP-1 cells from the top chamber, indicating that the device is able to model the inflammation environment for attracting monocytes.
4. The migrated THP-1 cells showed pronounced M1 differentiation on the surface of the implant Ti beads, indicating the faithful recapitulation of the chip device of the foreign body response to biomaterials.
5. Similar results have also been obtained with primary monocytes derived from peripheral blood mononuclear cells (PBMCs), indicating the translational potential of the device in personalized screening of foreign body responses.
6. Immunomodulative implant materials and coatings have been developed to release desired cytokines that will be able to differentiate macrophages into M2 lineage.
7. A series of bioprinting strategies have been developed to be able to achieve deposition of immunomodulatory hydrogels in the well-defined patterns in 2D and 3D.
8. The bioprinting strategies include multi-material to achieve structural complexity, microfluidic bioprinting to generate microfibrous structures, and sacrificial bioprinting and hollow tube bioprinting for perfusable vascular structures. A strategy for direct bioprinting of shear-thinning gelatin methacryloyl bioinks has been developed as well.



The foreign-body-on-a-chip concept.

Conclusions

Artificial systems including in vitro models of foreign body response on a chip and immunomodulatory materials have been successfully developed.

Outlook

1. Improvement of the foreign body response on a chip platform to incorporate other biological components such as stromal tissues to better mimic the microenvironment of implant materials in vivo.
2. Bioprinting will be combined with immunomodulatory hydrogels to enable patterning of these materials on implant surfaces.

Work Package 6

In vivo test of the immunomodulatory system with titanium/PDMS implants

Objectives

- Application of immunomodulatory hydrogel on different kind of implants (tracheal implant, subcutaneous implants) and stability of the coating for at least 2 weeks.
- Proof of concept of immunomodulatory system developed in IMMODGEL around titanium and/or PDMS implants after implantation (subcutaneous implants on mice).
- Proof of concept and demonstration of the positive effects of immunomodulation to develop tracheal implant (3D printed PDMS tracheal implant coated with immunomodulatory hydrogel implanted on rat)

Main results

1. The immunomodulatory hydrogel has been successfully applied on the surface of tracheal and subcutaneous implants and was stable for at least 2 weeks.
2. 48 mice have been implanted with a 3D printed PDMS implant with and without immunomodulatory hydrogel for 2 (1^{st} group) and 3 weeks (2^{nd} group) using 3 different conditions for each group:

Condition 1: 8 mice as control (only PDMS)
Condition 2: 8 mice with PDMS + Hydrogel without supplementation with cytokine cocktail 2 (PDMS + Hydrogel)
Condition 3: 8 mice with PDMS+ with supplementation with cytokines cocktail 2 (PDMS + Hydrogel + cytokine cocktail 2)
The comparison between condition 2 and 3 has shown that the presence of immunomodulatory hydrogel (hydrogel + cytokines) had an effect on the systemic level since we have observed a significant decrease of 7 pro-inflammatory cytokines in the blood. The presence of the immunomodulatory hydrogel has also decreased the level of local inflammation around the implants as demonstrated by histology results.

3. 24 rats have been implanted with a 3D printed tracheal patch with and without immunomodulatory hydrogel for 21 days using 3 different conditions (figure 1):

Condition 1: 8 rats as control (only PDMS: CONTROL)
Condition 2: 8 rats with PDMS + Hydrogel without supplementation with cytokines cocktail (PDMS + hydrogel)
Condition 3: 8 rats with PDMS + Hydrogel with supplementation with cytokines cocktail 2 (PDMS + Hydrogel + Cyt)

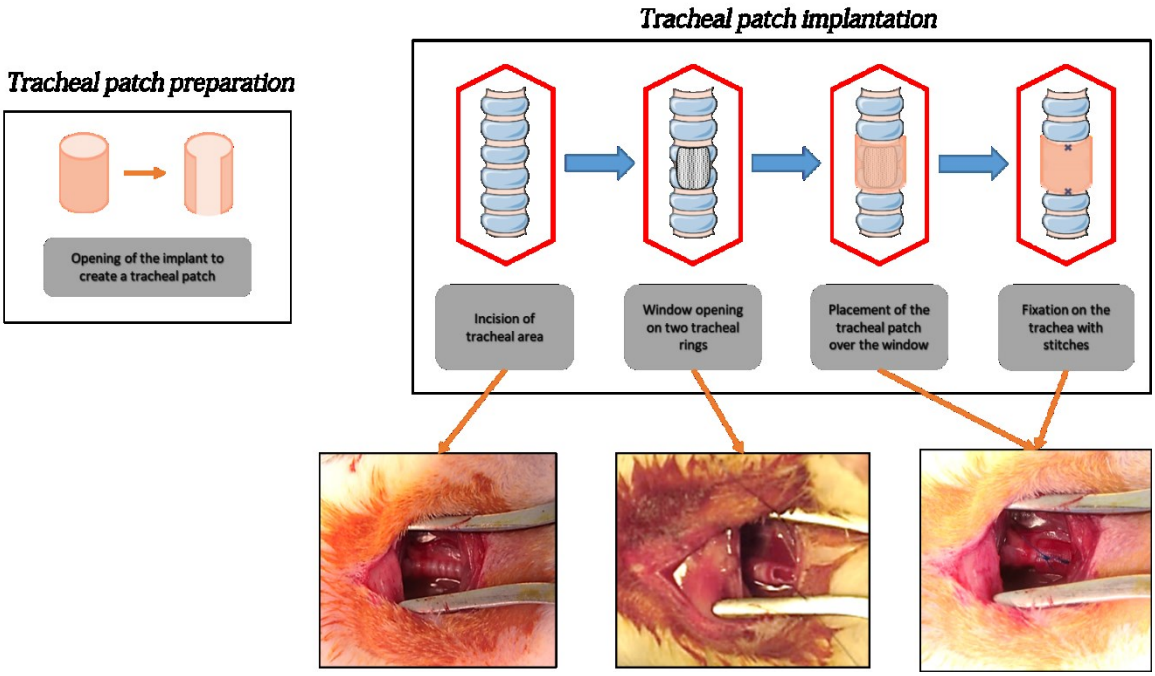


Figure 1. Schematic definition of the partial tracheal resection and subsequent implant implantation

In vivo tests on rat model have demonstrated the efficacy of this implant to repair tracheal defect. Moreover, the presence of the immunomodulatory coating on the outer part of the implant has shown positive effects by 1) increasing the survival rate and 2) decreasing the systemic inflammation after 21 days. Histology analyses have confirmed the trend observed in systemic level concerning the inflammation. With the presence of the immunomodulatory hydrogel at the surface of the implant we have decreased the local inflammation. Moreover, histology data have also shown a positive effect of the hydrogel by itself compared to PDMS control.

Conclusions

In this WP, the efficacy of the IMMODGEL system have been demonstrated in vivo with two sets of implantations. The first one concerned subcutaneous paravertebral implants on mouse model and the second concerned orthogonal implantation on rat with the elaboration of tracheal patch to repair partial tracheal defect under immunomodulatory conditions. The positive effect of IMMODGEL system was demonstrated to modulate the inflammation in both systemic and local levels.

Work Package 7

Dissemination, Exploitation, Management of Intellectual Property and Trainings

Objectives

- To raise interest in the IMMODGEL concept from potential users in Europe and beyond
- To achieve an early up-take of the project results
- To disseminate IMMODGEL aims, evolution and results through printed and electronic sources
- To manage and protect the consortium's foreground IP while minimising the risk of patent, trademark or any IPR infringement
- To assure a successful technology and knowledge transfer between the academic and industrial partners

Main results

1. A series of internal training sessions between partner organisations were organized during the first period of the project. Thus, the partners gained important hands-on experience for their joint tasks in the project.
2. Creation and continuous maintenance of the project website www.immodgel.org and an IMMODGEL Twitter account @immodgel and design of a project flyer providing a quick overview of the project and its core objectives and intentions.

Screenshot 1: Project website - Homepage

3. Over 40 scientific publications in relevant and high-impact scientific journals (e.g. Advanced Healthcare Materials, Journal of Tissue Engineering and Regenerative Medicine, ACS Biomaterials Science&Engineering), including 8 open access articles
4. 8 press releases and 14 articles in the popular press.
5. Project promotion with around 83 talks oral presentations, poster presentations at around 42 international events and conferences (e.g.: E-MRS Spring Meeting, May 2017, Strasbourg, France | World Biomaterials Congress, May 2016, Montreal, Canada | 27th European Conference on Biomaterials, September 2015, Kraków, Poland)
6. Organisation of IMMODGEL's final conference in the scope of TERMIS-EU Meeting, June 2017, Davos, Switzerland
    - 7 poster presentations
    - Dedicated session on "Immunomodulation and immune engineering in regenerative medicine"
    - 6 oral presentations

Overview of Dissemination Activities in the course of the project:

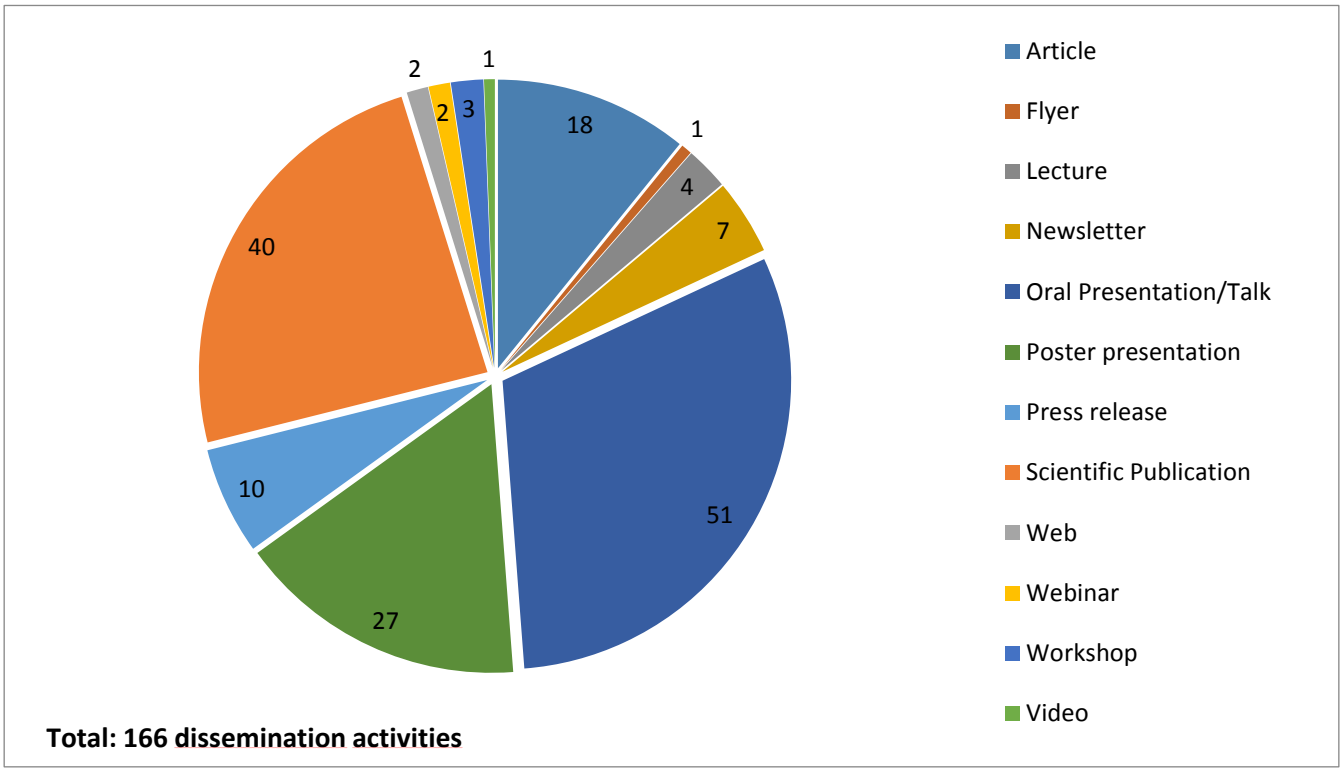


Figure 1. Overview of dissemination activities during the IMMODGEL project

7. All potential foreground expected in the IP workshop has been realized -including the filing of four patents. The industrial partners of the project are keen to pursue potential commercial exploitation following further maturation of the findings and future IPs following the exploitation plan developed within this WP.

A list of all scientific publications generated within IMMODGEL is available under http://www.immodgel.org/publications.html The list contains links to the respective journal issues.

All press releases are listed under http://www.immodgel.org/press-releases.html .

Highlighted articles and media:

"**Schulterschluss für medizinischen Fortschritt**" (German for *Alliance for medical progress*) Article in the regional newspaper Mittelbadische Presse (www.bo.de)

MITTELBADISCHE PRESSE | www.bo.de | Dienstag, 2. Juni 2015

Wirtschaft

Schulterschluss für medizinischen Fortschritt

Kooperationsserie (Teil 8): Mit dem Immodgel-Projekt sollen unerwünschte Immunreaktionen bei Implantaten verringert werden

Im achten Teil unserer Serie über die grenzüberschreitende Wirtschaftskooperation am Oberrhein stehen die medizinische Forschung und die Medizintechnik im Mittelpunkt. Es geht um die Entwicklung von Kehlkopfimplantaten, bei denen unerwünschte Immunreaktionen seitens der Patienten auf ein möglichst geringes Maß reduziert werden sollen. Neben der Straßburger Firma Protip Medical sind sechs weitere Partner aus Europa und einer aus den USA dabei, darunter – wie bei dem am 26. Mai vorgestellten Orgelbau-Forschungsprojekt – das Steinbeis-Europa-Zentrum mit Beratungsstellen in Karlsruhe und Stuttgart.

VON REINHARD RECK

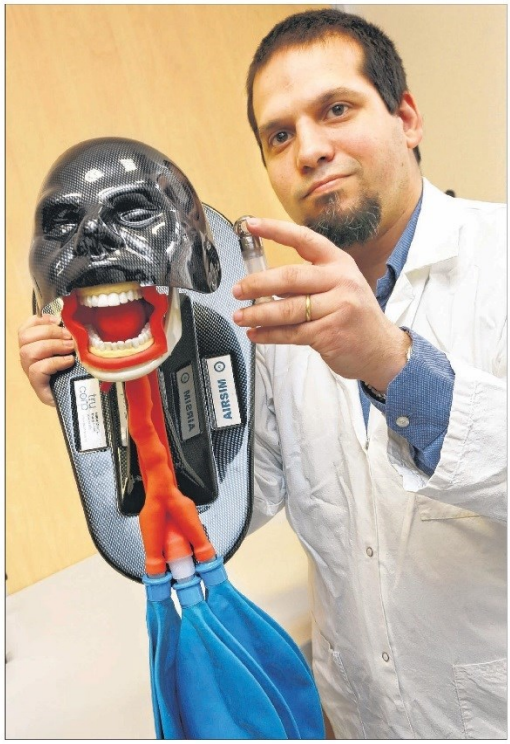
Der Forscher Nihal Engin Vrana hält in seinem Büro das Silikonmodell eines Kehlkopfes in der Hand und zeigt ein Implantat für diesen Körperteil. »Der Einsatz von Kehlkopfimplantaten ist von zunehmender Bedeutung«, betont der gelernte Ingenieur und Biologe, der für die Straßburger Firma Protip Medical arbeitet. Jeweils 4000 derartiger Implantate würden jährlich in Deutschland und Frankreich eingesetzt – etwa wegen Kehlkopfkrebs, Schluckstörungen oder anderer Probleme.

Aber es gibt bei Implantaten generell Schwierigkeiten – nicht nur wegen der aufwendigen Operationen. Häufig stellen die Mediziner unerwünschte Immunreaktionen von Patienten fest. Diese verursachen nicht nur Schmerzen, sondern können auch den Heilungsprozess nach dem Einsetzen des Implantats erheblich verzögern.

Das Ziel: Die Verringerung von unerwünschten Immunreaktionen bei Implantaten.

»Wir suchten nach einer Möglichkeit, um diese Immunreaktionen so weit wie möglich zu reduzieren«, so Vrana, dessen Firma sich unmittelbar neben dem Straßburger Bürgerhospital befindet. Der Wissenschaftler hatte eine konkrete Lösung im Blick: Er wollte Titan-Implantate entwickeln, die optimal die Voraussetzungen erfüllen – ein Gebiet, über das er schon vor seiner Arbeit in Straßburg an der Universität Dublin geforscht hatte.

Dafür brauchte Vrana aber noch weitere Partner. Denn das Unternehmen Protip mit seinen zehn Mitarbeitern betreibt in der Hauptsache Materialforschung, untersucht also, welche Stoffe für Implantate besonders geeignet sind. Um das Projekt zu verwirklichen, sind allerdings noch zahlreiche andere Arbeiten zu leisten – beispielsweise das Durchführen von Bluttests und nicht zuletzt die Produktion der Implantate.



Innovation in der Medizintechnik: Der Straßburger Forscher Nihal Engin Vrana demonstriert den Einsatz eines Kehlkopfimplantats aus Titan und Silikon an einem Modell. Foto: Ulrich Marx

So wandte sich der Straßburger Forscher an das Steinbeis-Europa-Zentrum (SEZ), das auch für viele andere Gemeinschaftsprojekte die Koordinierung übernimmt und Hilfestellung bei der Beantragung von Fördergeldern der Europäischen Union leistet. So entstand das Projekt »Immodgel«, wobei der komplette wissenschaftliche Name des Vorhabens »Lokale Immunmodulation in der Implantatumgebung durch innovative hydrogelbasierte Systemes« lautet. Das von 2013 bis 2017 laufende Projekt ist zunächst besonders auf Kehlkopf- und teilweise auch auf Zahnimplantate fokussiert. Es handelt sich um ein innovatives System, das aus chemischen (Hydrogel) und biologischen Komponenten (Zellen des Immunsystems) besteht.

»Herr Vrana von Protip Medical kannte bereits Einrichtungen, die an einer Beteiligung interessiert waren«, erläutert Mercedes Dragovits von der Karlsruher SEZ-Beratungsstelle, die als Verantwortliche für Immodgel fungiert. Das SEZ stellte unter anderem den Kontakt zu der in Mannheim angesiedelten medizinischen Fakultät der Universität Heidelberg her, und Dragovits übernahm die Projektkoordination. Nach einigen Vorarbeiten gelang es, ein aus acht Partnern bestehendes Konsortium zu bilden. Es sind nicht nur Einrichtungen aus den EU-Ländern Frankreich, Großbritannien, Deutschland, Estland und der Tschechei dabei. Auch das Brigham and Women's Hospital im US-amerikanischen Boston beteiligt sich. In dieser Stadt wurde das Projekt sogar als herausragendes Beispiel für die Forschungskooperation zwischen der EU und den USA im Gesundheitsbereich vorgestellt.

Mit Unterstützung des Steinbeis-Europa-Zentrums beantragten die Partner auch mit Erfolg Mittel von der EU-Kommission. Insgesamt wird für die vierjährige Laufzeit ein Betrag von rund 7,5 Millionen Euro investiert. 5,8 Millionen davon kommen von der Europäischen Union – aus dem Programm »FP7«. »Ohne diese Unterstützung hätten wir unser Vorhaben nicht verwirklichen können«, so Nihal Engin Vrana. Schließlich brauche man beispielsweise sehr teure medizinische Geräte. Allein die Durchführung eines Affymetrix-Bluttests koste rund 10000 Euro.

»Die geringe Entfernung zwischen Straßburg und Mannheim erleichtert alles enorm.«

»Jetzt haben wir ein gut eingespieltes Team von Spezialisten, wobei jeder seine Kompetenz mit einbringt«, meint Vrana. Die Zusammenarbeit verlaufe reibungslos, da alle ein starkes Interesse am Gelingen des Vorhabens hätten. Insbesondere lobt der Vertreter von Protip Medical die Kooperation mit der medizinischen Fakultät der Universität Heidelberg. Die Rollen sind klar verteilt: Protip erstellt in Straßburg Titan-Teile für ein Implantat, diese werden dann auf deutscher Seite mit Blutproben von Patienten getestet. Die Testergebnisse erhalten wiederum die Forscher in der Elsass-Hauptstadt und nehmen an den Titan-Teilen Veränderungen vor, falls unerwünschte Immunreaktionen oder Probleme anderer Art festgestellt wurden. »Die geringe Entfernung zwischen Straßburg und Mannheim erleichtert alles enorm«, so Vrana. Bei Problemen könne man sich schnell gegenseitig besuchen.

Später kommen noch weitere Partner ins Spiel – beispielsweise die Firma Contipro Biotech im tschechischen Dolni Dobrouc, die Beläge für Implantate herstellt.

So werden die Forschungen unter Einbeziehung aller Beteiligten in einem internationalen Kreislauf vorangetrieben. Dank der eigens entwickelten Tests zur Feststellung der Immunreaktionen seitens der Patienten können durch Immodgel Implantate erstmals individuell angepasst werden, um unerwünschte Reaktionen so gering wie möglich zu halten.

Auch Mercedes Dragovits vom Steinbeis-Europa-Zentrum meint, dass die Zusammenarbeit zwischen den Teilnehmern aus ganz unterschiedlichen Ländern reibungslos verläuft: »Bei der Kommunikation gibt es ohnehin keine Probleme, da alle fließend Englisch sprechen.« Hin und wieder kommt es zu Treffen von Vertretern der beteiligten Einrichtungen – in Karlsruhe, Nottingham, Brüssel oder anderswo. Ansonsten finden jeden Monat Telefonkonferenzen statt, um Bilanz zu ziehen und über das künftige Vorgehen zu beraten. »Bei der Terminierung der Telefonkonferenzen müssen wir natürlich – besonders wegen unserer Kollegen in den USA – die Zeitverschiebung berücksichtigen und aufpassen, dass keiner schon Feierabend hat«, erklärt Dragovits schmunzelnd.

»Das Projekt stellt eine wesentliche Innovation dar, die nicht nur die Stärke und Dauer von Entzündungen, die durch Implantate ausgelöst werden, verringern kann«, resümieren Mercedes Dragovits und Nihal Engin Vrana. Auch der Heilungsprozess nach der Operation werde verbessert. Das trage nicht zuletzt zu einer Kostensenkung bei. So hofft man, die bei Immodgel erzielten Resultate auch für andere Implantate verwerten zu können.

Lesen Sie diesen und die anderen Artikel dieser Serie im Internet unter www.bo.de/bruckenschlag
Zu Immodgel: www.immodgel.org
Weitere Projekte: http://download.steinbeis-europa.de/publikationen/25-kooperationen_bw.pdf

Wirtschaft: Brückenschlag am Oberrhein

Eine Serie der Mittelbadischen Presse

Das lesen Sie nächstes Mal:

Chancen mit einer grenzüberschreitenden Ausbildung (9. Juni).

HINTERGRUND

Das Steinbeis-Europa-Zentrum

Das Steinbeis-Europa-Zentrum (SEZ) mit seinen rund 50 Mitarbeitern wurde 1990 auf Initiative des Europabeauftragten des baden-württembergischen Wirtschaftsministeriums gegründet. Hauptaufgabe der Einrichtung, die Beratungsstellen in Karlsruhe und Stuttgart hat, ist es, Unternehmen den Weg nach Brüssel zu erleichtern und in den Genuss von EU-Fördermitteln zu gelangen. Dabei hat das SEZ gerade auch kleinere und mittelgroße Unternehmen im Blick, die von einem Technologietransfer profitieren können. Hochschulen und Forschungseinrichtungen hilft das Steinbeis-Europa-Zentrum bei der Antragstellung für grenzüberschreitende Projekte und deren Durchführung.

Das SEZ ist in viele Kooperationsnetzwerke eingebunden. Dazu gehören das Netzwerk der etwa 1000 Steinbeis-Transferzentren sowie das Enterprise Europe Network, dem rund 600 Organisationen in über 50 Ländern angehören. 2014 flossen mit Unterstützung der Einrichtung Projektmittel der EU-Kommission in Höhe von 8,3 Millionen Euro in den Südwesten.

www.steinbeis-europa.de

14. April Interview mit Jean-Louis Hoerlé über die Kooperation.

21. April Die Staustufe Iffezheim – ein Erfolgsprojekt.

28. April Software aus Kehl für den Europarat in Straßburg.

5. Mai Beta- und Gammastrahlung – Brückenschlag erwünscht.

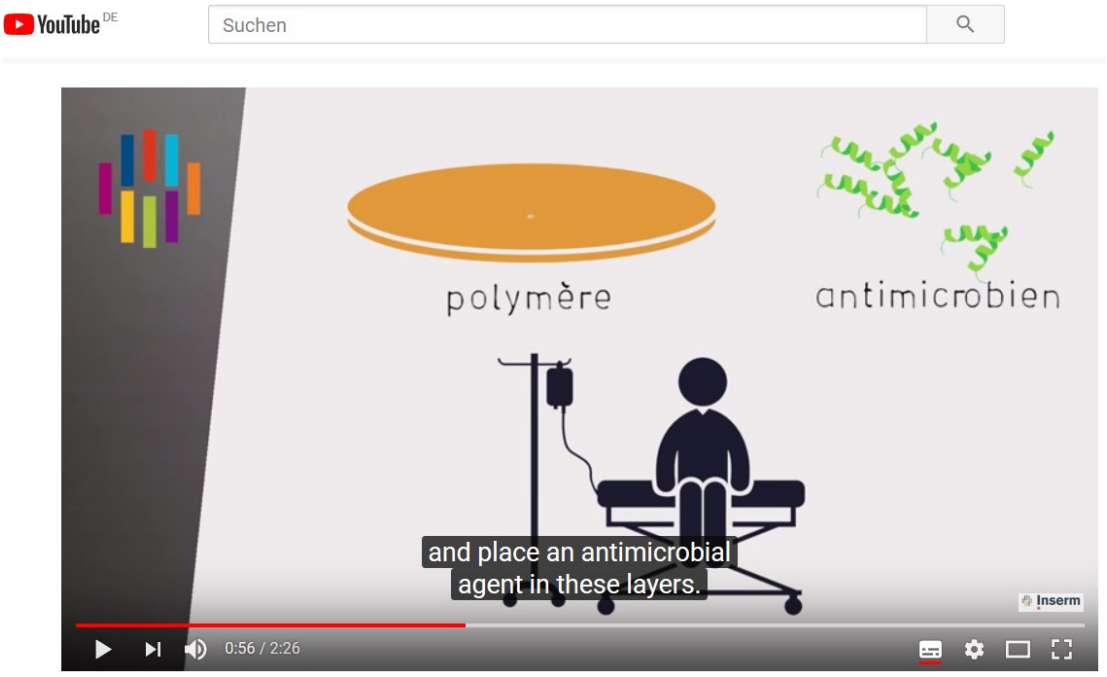
12. Mai Anwälte aus zwei Ländern suchen den Kontakt.

19. Mai Grenzüberschreitende Zusammenarbeit im Bankensektor.

26. Mai Bessere Technik für Orgeln mit EU-Forschungsvorhaben.

2. Juni Projekt gegen unerwünschte Immunreaktionen.

Interview to Philippe Lavalle – available on YouTube - Issued by INSERM´s Press department



Webinar "Building an Evidence Base" – including presentations from the scientific coordinator Dr. Nihal Engin Vrana (Protip) and from the administrative coordinator Dr. Mercedes Dragovits (Steinbeis 2i GmbH) – hosted by TERMIS-EU (Tissue Engineering and Regenerative Medicine – International Society) in the frame of the Pre-Accelerator Programme (PAP) – available on YouTube

Conclusions

In the course of the four-year IMMODGEL project the partners performed over 166 dissemination activities. The project partners have been especially active in disseminating the project via oral presentations at international conferences as well as via scientific publications (total of 40 papers as this report was being written). The partners´ efforts were balanced during the project but increased steadily. The IMMODGEL final conference during TERMIS-EU Meeting in Davos, Switzerland significantly raised the project's visibility and reinforced the long-term collaboration of the partners. Moreover, it provided the partners with an opportunity to improve their network for future projects and commercial undertakings.

An exploitation strategy based on workshops involving the whole consortium and regular patent search results was developed. The main exploitation outcome of the project will consist in the creation of a start-up based on the protected foreground generated by the IMMODGEL partners.

Potential impact of the IMMODGEL project

Implant technology has become widespread and several types of implants, such as hip implants, knee implants are being implanted in more than a million cases worldwide. The decreasing cost of dental implants has increased their demand in Europe, and an additional several billions of Euros growth in this field is expected in the next 5 years. In a field (dental) which is valued at more than 2 billion Euros and where over 40% of global demand is in Europe, improvement of the current protocols via immune response control as developed in IMMODGEL will have great positive economic consequences. The removal of adverse immunological reactions, a very common problem in the field of biomedical engineering, can also augment the direct investment from public and private sector in areas where they would be normally considered too risky.

Prognostic or Susceptibility/Risk, Monitoring Biomarkers: The occurrence of mild and severe inflammatory processes of implants are on the rise. There are several contributing factors to this trend such as the overall ageing society, the availability of the implants to a wider range of demographics and a longer expected implant life time. The exact reasons in detail are still unknown, however, it is clear that the lack of diagnostic methods to actually determine high-risk patients and treat them accordingly is playing a big role in this problem. IMMODGEL aimed to fill this gap by studying in which form the generated scientific knowledge of the personalized set of candidate genes will be of the best use.

The immunomodulatory system targeted in this project consisted of 1) autologous macrophages with a controlled phenotype 2) a composite hydrogel for macrophage and cytokine encapsulation and 3) a polyelectrolyte coating which will allow adhesion of the encapsulated macrophages to any implant.

We have optimized several encapsulation systems and polyelectrolyte coatings that fit the requirements of the final therapeutic system. We have also developed the protocols for theassembly of the structures and their in vivo testing. Encapsulation of macrophages has not been undertaken before the IMMODGEL project in the literature; thus IMMODGEL has provided the initial conditions for future research in this area. Cell adhesion plays an integral role in enabling communication between cells and their microenvironment. However, their role in monocyte to macrophage differentiation and particularly macrophage polarization are yet to be fully understood. Here, we hypothesized that integrin-mediated cell–biomaterial interactions could play a key role in macrophage polarization. Given that in vivo these events take place in the context of ECM and in 3D, obtaining a clear understanding of the role of integrins in macrophage polarization in a 3D microenvironment will be more physiologically relevant than in a 2D environment. We have used two distinct hydrogel systems to probe the effect of cell–biomaterial interactions on macrophage polarization in a 3D environment. We investigated whether macrophage polarity can be controlled through integrin-mediated biomaterial-based programming. Our findings will pave the way for future endeavors in elucidating immune system/biomaterial interactions in 3D configurations.

We have devised specific cytokine cocktails for precise control of macrophage phenotype that have been tested in 3D conditions. Artificial tissue model development for the testing of immunomodulation has been done. We have provided the biomaterial community with new tools such as incoming macrophage models and a foreign body response on a chip system for incorporating the immunology aspect in their activities.Uncontrollable activation of macrophages in the microenvironment of implants and engineered tissues is a significant problem leading to poor integration of implants and artificial tissues. We demonstrated that self-standing, transferable thin films are perspective tools for controlled release of

anti-inflammatory cytokine combinations and can be used to down-modulate macrophage activation on implant surfaces. We also show that optimized cytokine cocktails induces long-term anti-inflammatory and pro-healing phenotype in human primary monocyte-derived macrophages. This cocktail formulation could be loaded on films and promoted favorable M2-like macrophage phenotype with low responsiveness to pro-inflammatory stimuli. Such self-standing release systems can be used for prolonged local control of macrophage phenotype upon implantation.

Via systems immunology, we were able to determine specific markers that can define the tissue response to medical grade titanium implants. This can be of significant prognostic value of the fate of such implants and will facilitate the clinicians and immunologists to holistically understand the adverse immune responses to implants, how these are controlled by macrophages and how to make judicious choices regarding host-implant compatibility. We also developed immunoprofiling methods based on cytokine measurements and epitope characterisation which can also be used for pre-implantation diagnostics applications.
A foreign body response (FBR) on-a-chip system has been developed that will significantly reduce the research cost and the need for animal tests by replacing the compulsory biocompatibility tests with a sophisticated in vitro test. The initial design of the FBR on-a-chip system is ready and the future iterations are currently underway within the framework of future projects.

Personalized Implants: Macrophages are master cells of the innate immune system that protect all tissues of our body from exogenous danger of different origins by induction of inflammation and elimination of foreign materials and organisms. Moreover, there are other cases of macrophage and metal based complications such as myofascitis due to the Aluminium content in vaccine adjuvants. As the immune system of each individual differs, it is important to have ways to evaluate the possible responses of the individual patients. The discoveries in IMMODGEL will allow the stratification of the population with regards to the response to Ti allowing to protect the patients from complications. It is also possible to provide suggestions for possible modifications of commercially available implants to make them more amenable for a given patient thus paving the way of personalized implants. Finally, it was our aim to utilize its predictions for the development of therapeutic solutions for implant related problems which can then be further extended to transplants and biomedical devices, particularly those of titanium and other metals/alloys, polymers, ceramics and eventually to engineered tissues. It can also open the door for looking beyond the innate immunity and into adaptive immunity via effects of lymphocytes.

The results obtained on model implants can provide a necessary framework for a wide range of biomedical materials. By developing a system that can be applied to any kind of implant, transplant or biomedical device, the project will expand the capabilities of immunomodulation. In this sense, IMMODGEL will have a widespread impact and can improve the chances and success of many other European healthcare products. Our results up to now are in line with this claim and we hope to deliver the proposed impact following the animal tests.
IMMODGEL will yield high socio-economic impacts by significantly improving the health and quality of life of patients in EU and world-wide, and by decreasing therapy costs due to adverse immunological reactions. This research will not only improve the outcomes of implants and biomedical devices, but will also provide meaningful improvement of non-communicable diseases (NCDs). In an ageing society, by improving the lifetime of implants and biomedical devices, immunomodulatory systems can provide a

meaningful saving on healthcare costs. The project will also help the development of personalized medicine or stratified medicine approaches to implantology and development of necessary tools to achieve the personalization.
The proposed system can be used in conjunction with all types of implants, particularly with the implants such as dental, orthopaedic, spine or larynx implants. Applicability to different structures can provide a comprehensive solution to adverse immunological responses without the need to change the design of the original implants. This will also allow reducing the need of animal experimentation due to the elimination of immune system related failures which will not only have positive economic impacts but also ethical ones. Also, the development of a diagnostic tool to quantify immune response to implants will decrease health costs by predicting and preventing possible excessive immune reactions. Creation of new employment opportunities followed by the newly developed technologies and the know-how will also offer a positive economic impact on the society.

The development of this unique immunomodulatory system and relevant know-how is expected to provide significant opportunity for the industrial partners to both maintain and build further on their existing market positions. The overall implant industry is projected to increase between 5 and 10% per year (a growth of 20% is expected only in dental implant industry for the next 5 years), therefore a product that enhances the success of these surgeries and reduces several other associated health risks will fetch benefit of lower health sector costs and improved health conditions. For commercial exploitation and benefit to the industrial partners; Protip Medical has developed IP and new competencies thanks to the project. One of the technologies developed in IMMODGEL (patented) is under pre-clinical testing at the moment for eventual up-scaling and potential commercialisation. Protobios has entered into a potential new market (biomaterial diagnostics) and Contipro has found a new area for utilisation of their Hyaluronic acid based products as base material
System Immunology and Bioengineering have a continuously increasing importance due to the issues related to immune-competence, associated reliability and success issues, lack of broader industrial familiarity with wide-scale solutions and embedded commercial interests. The research undertaken in IMMODGEL project has generated academic and industrial interest. The development and application of an immunomodulatory system to control the adverse immune reactions in the host body will demonstrate their effectiveness for different types of surgical applications. The ability of implants to alleviate chronic disease related problems is significantly reduced by adverse immunological reactions to them, which makes immunomodulation a highly impactful area of technological development. This is expected to encourage further academic research and additional industry funding into academia to further lead in this technology.

Benefit to other academic research fields – Diagnostic and Immunological research is of great interest to researchers in many differing fields. Assessment of undiscovered fields of adverse immune reactions and host: implant interactions against specific cases will open up research options to academics which may not be immediately evident. For example, we have demonstrated a new means of classifying macrophage populations using image based machine learning. Given the heterogeneity of macrophage phenotype and current limitations of the machine learning approach it may be too early to suggest use of image analysis as an alternative to conventional cell phenotyping. However, our data provide strong evidence for the ability of high content and automated image analysis approaches for accurate, less resource intensive and

fast phenotyping of functional diverse cell populations. Moreover, the data organized and information collected during the project will support the developement of other systemic researches.
Biomaterial-mediated immunomodulation by programming macrophage polarity is a promising tool in tissue engineering, regenerative medicine and implantology to decrease adverse immune reactions, accelerate implant integration, facilitate tissue regeneration and increase implant lifetime. Overall, biomaterial-based control over macrophages represents a novel technique to obtain a fundamental understanding of macrophage behavior and is a strong therapeutic tool for immunomodulation for implants, drug, and cell delivery systems.
Engineering a tissue requires the design of the optimal conditions for the cells in the target tissue involved. Additionally, it is also crucial to develop methods to recruit and direct the host cells that will take an active role in the integration of the implanted tissue. A modular ECM-based system such as IMMODGEL can be used as a component of engineered tissues for directional release of cytokines and growth factors to attain precise microenvironment control. Development of a system composed of two modular components decouples the release and cell loading, which allows a higher degree of control over the encapsulated cell behavior.

Silver Economy, welfare: An aging population brings new challenges to health professionals in general and dentists in particular. It is well known that dental implants are likely to become used within a larger population pool and that a given individual may have several implants in the mouth. IMMODGEL will make it possible to access to safer titanium dental implant procedures thus allowing the access to implants to a wider population range and improving its welfare.
Controlling Healthcare Cost: Inflammation around the implants can cause severe peri-implantitis. A study in 2015, states that the treatment of peri-implantitis can cost several thousand Euros depending on the treatment method. A better understanding of the patient's innate response profile can result in a decrease of implant related complications. Moreover, this "complications" problem is not limited to dental implants as they are also known to exist in further implants and in various target organs. For example, implant related problems have been on the rise during the last few years (~5 000 complications out of 200 000 implantations for cardiovascular devices) which caused an increase in long term mortality (about 2%). In average, such complications cost about 15.000 dollars each. Similarly, in total hip replacement, a postsurgical treatment has a cost of 50.000 dollars (on average) with a rate of incidence of 2% (in the extreme cases the replacement of the implant or amputation of the limb could be necessary). By providing an innovative way to predict adverse immune reactions (reactions that in most cases are indirectly responsible for the infections as the implant is not integrated well), IMMODGEL will open the door to such a practice in other fields of implantology (hip, knee, cardiac, etc.) therefore contributing to a larger use of personalized medicine and a better control of healthcare costs.
HA is an important tool in biomaterial research as evidenced by its widespread use in surgical applications as a filler, as hydrogel material, cell carrier and drug delivery system. The ease of isolation/production of HA and its extensive biological activities makes it a very attractive target for therapeutic biomaterial based system development. The presence of several sites amenable to chemical modification on HA chains further improves its practical use as a wide array of derivatives can be developed. The current state of research has put emphasis on 3 directions in the use of HA in biomedical applications: i) injectable formulations that will improve the delivery and defect conformation for both drug and cell delivery applications ii) development of new derivatives of HA that enables further control over physicochemical and biological properties of the final structures iii) Harnessing the biological activities of HA for more

controlled biological effects such as controlled retention of multiple growth factors or specific, directed differentiation of cells to desired phenotypes. Thus, in the upcoming years, it is reasonable to expect the development of more derivatives of HA for applications in regenerative medicine, particularly in immunomodulation, angiogenesis, nerve regeneration and hybrid materials containing HA. As a HA provider, IMMODGEL partner Contipro will benefit from the use of HA in immunomodulatory formulations.

As an outlook, the next in line in the sophistication of engineered tissues can be the inclusion of immune system components. Nearly all tissues have resident macrophage populations which has been shown to be an important factor in tissue homeostasis and healing upon injury. Recently, there has been a growing focus on the control over innate immune response in the microenvironment of implanted materials particularly through well-established macrophage polarization pathways that have been shown to have a crucial role in vascularization of implanted scaffolds. Immunoassisted tissue engineering approaches can harness the ability of innate immune cells to resolve inflammation and promote regeneration and healing. This can be achieved by exploiting the phenotypic plasticity of immune cells either via controlled delivery of specific phenotype inducing cytokines or direct co-delivery of phenotype controlled immune cells together with the cells relevant to the target organ function. A new focus on establishing a cross-talk with the host immune system, rather than trying to evade it, could pave the way for more functional and fast-integrating artificial tissues. Concomitant use of new developments in temporal control of multiple growth factor/cytokine delivery; advanced bottom-up assembly methods of engineered tissues such as robotic assembly; use of bioactive miRNAs within scaffolds; and micro/nanoscale topographical and chemical control of scaffold features for inducing anti- or proinflammatory immune cell phenotypes would provide the tools for engineering multicellular organs and establishing in vitro organoids that faithfully model physiological conditions with immune system components. These efforts would bring forth the aspects of ‘regenerative immunology’ in regenerative medicine.

IMMODGEL project provided a better understanding of the significant role of innate immune cells in tissue remodeling and regeneration for regenerative medicine and implants. A wide variety of strategies are available to orchestrate the initial immune response to implanted structures as detailed above. However, because the immune response is tightly regulated both spatially and temporally, more elaborate techniques will be necessary to attain optimal functional integration. Apart from control of the innate immune responses, the next line of control can be achieved at the level of adaptive immunity via B cell and T cell responses. Some of the most commonly used biomaterials, such as synthetic polymers and ceramics, can be spared of adaptive immunological responses owing to the lack of potential immunogenic components. However, new generations of biomaterials, where hybrids of organic and inorganic components, synthetic peptide structures, and cell-responsive polymeric components are increasingly used, necessitate the consideration of the adaptive immune responses to biomaterial structures. For a designed self-assembling polypeptide chain, for example, it is important to know whether the sequence and structure of the design resembles an antigen and can therefore be a functional epitope that triggers adaptive immunity. To optimize the adaptive immune response to biomaterials, a better understanding of the mechanism of events related to adaptive immunity, such as leukocyte attachment, is needed which can be outlook studies after IMMODGEL.

It is becoming increasingly important to ask fundamental immunological questions in the context of biomaterial development. Topographical and/or chemical design of biomaterial surfaces with respect to the APC responses can pave the way for a new generation of ‘cell instructive’ materials with

immunomodulatory properties with a wide range of clinical applications. In future, high-throughput systems which will be key to better mimic the FBR in vitro will allow the elucidation of biomaterial-specific responses in real-time and will therefore substantially improve our ability to identify, predict, and control the immune response to implanted biomaterials. We believe, as the concepts surrounding the biocompatibility of biomaterials evolve, that the focus will shift from an evasion of the host immune system to an orchestrated interaction with it. This will also enable the discovery of new biomaterials.

Project website address: www.immodgel.org

Contact:

Scientific coordinator:
Dr. Nihal Engin Vrana
e.vrana@protipmedical.com
Protip Medical SAS
France

Administrative coordinator
Dr. Mercedes Dragovits
dragovits@steinbeis-europa.de
Steinbeis 2i GmbH
Germany