Project Periodic Report

Grant Agreement number: 309962

Project acronym: HydroZONES

Project title: Bioactivated hierarchical hydrogels as zonal implants for articular cartilage regeneration

Funding Scheme: Collaborative Project: Large-scale integrating project

Date of latest version of Annex I against which the assessment will be made: 23.02.2015

Periodic report: 1st ☐ 2nd ☒ 3rd ☐ 4th ☐

Period covered: from 01.07.2014 to 31.12.2015

Name, title and organisation of the scientific representative of the project's coordinator:

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Declaration by the scientific representative of the project coordinator

I, as scientific representative of the coordinator of this project and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

- The attached periodic report represents an accurate description of the work carried out in this project for this reporting period;
- The project (tick as appropriate)[1]:
  - ☒ has fully achieved its objectives and technical goals for the period;
  - ☐ has achieved most of its objectives and technical goals for the period with relatively minor deviations;
  - ☐ has failed to achieve critical objectives and/or is not at all on schedule.
- The public website, if applicable
  - ☒ is up to date
  - ☐ is not up to date
- To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 4) and if applicable with the certificate on financial statement.
- All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 2.4 (project management) in accordance with Article II.3.f of the Grant Agreement.

Name of scientific representative of the Coordinator: Prof. Jürgen Groll

Date: 20.02.2016

Signature of scientific representative of the Coordinator: .....

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For most of the projects, the signature of this declaration could be done directly via the IT reporting tool through an adapted IT mechanism.

[1] If either of these boxes below is ticked, the report should reflect these and any remedial actions taken.

Section 1 - Publishable summary

HydroZONES

Logo:

Project title: Bioactivated hierarchical hydrogels as zonal implants for articular cartilage regeneration

Website: www.hydrozones.eu

Contractors involved HydroZONES consortium:

Coordinator: Prof. Jürgen Groll

Address of the Coordinator: University Hospital Würzburg
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COMPLETE LIST OF PARTICIPANTS

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03	UKL-HD	Prof. Wiltrud Richter	University Hospital Heidelberg	D	Hospital
04	UOXF	Prof. Helen Byrne	University of Oxford	UK	University
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06	UU	Prof. Wim Hennink Dr. Tina Vermonden Prof. René van Weeren	University of Utrecht	NL	University
07	IPF	Prof. Carsten Werner	Leibniz-Institut für Polymerforschung Dresden e.V.	D	Research Institute
08	PIL	Artur Jorge dos Santos Mateus	Polytechnic Institute of Leiria	P	Research Institute
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10	GABO:mi	Birgit Fuchs	GABO:mi Gesellschaft für Ablauforganisation:milliarium mbH & Co. KG	D	SME
11	HCS	Michael Barfuß	Health Care Support	D	SME
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1.1 Summary description of project context and objectives

Degeneration of cartilage is a major cause of chronic pain, lost mobility and reduced quality of life for millions of Europeans and over 151 million osteoarthritis (OA) sufferers worldwide. Yet, despite intensive research, no clinical therapy is available that leads to healing of cartilage defects. Treatment to achieve cartilage regeneration (hyaline) and not only repair (fibrous) remains a great challenge.

Current cartilage implants cannot establish the hierarchical tissue organisation that appears critical for normal cartilage function. We hypothesise that a biomimetic zonal organisation of the implants themselves is critical to induce native tissue hierarchy and thus achieve cartilage regeneration.

HydroZONES represents an interdisciplinary consortium that adopts a strategy to regenerate articular cartilage based on biofunctional implants that mimic the tissues zonal structure and function.

As cartilage is a matrix-rich hydrogel-like tissue, our strategy relies on biofunctional degradable hydrogels that will be endowed with cartilaginous bioactive cues in a zonal manner. Degradable and clinically used thermoplastic polymers will be selected for mechanical reinforcement of the hydrogels. HydroZONES will follow and compare cell-free and cell-loaded hydrogels, comparing chondrocytes and bone marrow derived mesenchymal stem cells (BMSCs) for their efficacy.

Scaffolds that pass our stringent and well-documented in vitro and in vivo screening will undergo long-term pre-clinical in vivo testing in minipigs and horses, which will set a new international standard for pre-clinical testing of cartilage implants. These experiments will also verify whether zonal implants are able to outperform non-zonal implants, a question that is of great relevance to the field of regenerative medicine in general, as most tissues are organised hierarchically.

Defined endpoint of HydroZONES is the positive long-term pre-clinical evaluation of at least one construct, according to pre-clinical regulatory affairs and in GMP quality, to generate an optimal position for directly entering clinical trials after project end.

Cutting edge bioreactor technology and advanced dynamic in vitro cell culture systems will be employed for in vitro testing of the constructs and results will be used as input for realistic in silico modelling.

Second major aim of HydroZONES is the development of a predictive 3D in vitro assay for chondral implants, validated against our in vivo results, together with the hardware (prototype of a semi-automated test-system) to perform the assay.

HydroZONES ideally combines partners with scientific excellence, experience in automatisation conform to GMP, QM and regulation, pre-clinical testing and clinicians, as well as industry with experience in clinical trials. HydroZONES will generate economical impact for different markets along the product developmental line and advance European biomaterials industry on several levels. It will generate a dramatic added value to OA patients and increase the quality of life for millions of EU citizens.

HydroZONES will balance the combination of advancing European biomaterials industry with scientific excellence and verify or falsify a radical new strategy for implant design, the recapitulation of the hierarchical tissue organization for improved performance, at the example of cartilage.

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

This section focuses on work performed in the second funding period (month 19-36).

In WP1, partners 1, 6 and 7 have 3D-printed and/or cast their hydrogels having final dimensions of 6 mm diameter and 2 mm height (cylindrically shaped) as uniform shape to be used for experiments in WPs 4, 5 and 6. The different hydrogels can be loaded with MSC's and/or chondrocytes with high cell survival. Composite scaffolds based on thermoplast scaffolds provided by partner 12 were prepared both with and without cells, including zonal designs.

The tasks of WP2 have been completed, but technical support and adjustment will continue. Within WP3, deeper understanding of the chondrogenic potential of the different gels was obtained, including the additional "backup" gel system GelMA / gellan. These examination were extended to printed constructs and to layered systems, where clear effects of a zonal organization on cellular behavior of encapsulated gels could be observed.

In WP 4, *in vitro* test systems for static and dynamic culture of osteochondral explants were established and used for first testing of chondrocyte loaded scaffolds. A working prototype for mechanical loading of osteochondral explants in the culture platform, allowing for loading of three samples in parallel, has been developed. Cartilage tissue remained alive over a culture period of 84 days in the system with no significant change in GAG and collagen content.

For the biocompatibility and biofunctionality screening in WP5, zonal hydrogels from all WP1 partners were tested in the ectopic *in vivo* model for 6 weeks. For ectopic implantation of constructs embedded in porcine osteochondral tissue sample, a systematic study design was conceived that comprises investigation of TGFβ effect on the different cell types, feasibility of chondrocyte and BMSCs combination, the influence of thermoplast scaffolds (CCT scaffolds) and to evaluate printed gels. At the moment, all implants are being analyzed by histology and immunohistochemistry. Regarding the ectopic implantation of porcine osteochondral tissue samples, the tissue plugs remained vital 6 weeks after implantation, with normal bone remodelling and active osteogenesis in the bone part of the porcine tissue.

Work in WP6 concerns the large animal models and commenced at month 30. Subcutaneous testing showed that all developed hydrogels, casted and printed, are biocompatible. Host cell invasion is seen in combination with CCT scaffolds. Printed constructs of P(AGE/G)HA-SH and MHL are degraded differently by different animal species in subcutaneous testing (horse vs. mouse). In minipigs all tested hydrogel/CCT- glue combinations could not be fixed within the cartilage defects. Hence, focus was laid on the fixation of constructs. Additional fixation of printed porous constructs with a membrane is no option as constructs break during suturing. It was shown that PDGF attracts more cell to ectopic StarPEG/Heparin constructs in mice and is recommended for use in a large animal test study.

A systematic approach to validate outcomes of research & development processes has been described as a quality management tool for the consortium members in WP7. Integrated into the phase model of EN 13485 for the development process, a Validation Master Plan has been developed. The tools and the knowledge have been delivered to the consortium members.

Several training activities were performed, and students were exchanged between partners. Dissemination was intensified and numerous scientific publications were published. Regarding project management, general progress is good and according to plan. The major focus is now on WP6. While it is still on track at the end of the second reporting period, decisive experiments regarding fixation of the scaffolds are running and will show whether the time plan for the long term in vivo examinations can be kept.

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)

Expected final results and their scientific impact

HydroZONES pursues the development of materials physically suitable for printing and, at the same time, supporting cell survival and material-driven differentiation. The lack of such materials has been acknowledged as one of the bottlenecks holding back advances in the field of bioprinting. These materials will facilitate the generation of organised 3D tissue equivalents, which can be applied as implants, as *in vitro* screening assays, or to further our understanding of tissue regeneration in a more physiological environment. **HydroZONES** will provide further insight in the modulation of cell therapy by means of biomaterial directed differentiation, facilitating the elucidation of processes that control organisation and chondrogenesis, as well as mineralisation of the neo-tissue in the defect.

HydroZONES will also deliver a more physiological *in vitro* cartilage defect model that will open new opportunities for studying the governing processes of tissue regeneration and repair. The current status quo of cartilage tissue engineering can produce cartilaginous structures that lack the specific organisation of the native tissue. The consortium proposes a concept based on biomaterial-directed differentiation and bioprinting technology to restore the organisational features of the tissue.

Spreading awareness of the project and socio-economical impact

As the project is still in the preparation phase of the large animal studies, the socio-economic impact achieved so far is not high yet. However, multiple dissemination activities have been undertaken to spread awareness of the project in the scientific community and the general public.

In case of success especially within the large animal experiments, the potential socio economic impact of HydroZONES is great. Damage of articular cartilage or intervertebral disc cartilage occurs frequently and is followed by a process of OA, ultimately leading to pain and joint malfunction. OA causes severe loss in quality of life in approximately 40 million European Citizens. As such, it is the leading cause of disability, a more frequent cause of activity limitation than heart disease, cancer or diabetes. It accounts for more disability among the elderly in Europe than any other disease. It is in 6.6% of cases associated with severe psychological distress and a major cause of the high work disability benefits. While the total direct annual costs of OA in the US are estimated at $89.1 billion, the indirect costs are also high, largely a result of work-related losses and home-care costs. For example, the impact of arthritic diseases on earnings increased in recent years, with $108 billion of earnings being lost in 2003 in the United States alone. In France, direct costs of OA exceeded €1.6 billion in 2002 and accounted for 13 million physician visits. That year's figures represented a 156% increase in costs over 1993, which was for more than 90% due to an increase in the number of patients, rather than to an increase of costs per patient. HydroZONES will help to reduce costs for European healthcare systems by the generation of cheaper, potentially cell-free, alternatives based on instructive biomaterials to regenerate the complex tissue organisation at the defect site.