



Project Periodic Report

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Section 1 - Publishable summary

HydroZONES



Logo:

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

Website: www.hydrozones.eu

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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 3D in vitro assay for chondral implants, validated against our in vivo results, together with the hardware to perform the assay.

HydroZONES ideally combines partners with scientific excellence, experience in automation 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 third funding period (month 37-48).

In WP1, partners 1, 6 and 7 have 3D-printed and/or cast their hydrogels in the relevant dimensions for WPs 4-6. The different hydrogels can be loaded with MSC's and/or chondrocytes with high cell survival, and focus in this reporting period was printability of cell loaded hydrogels. Aside of the soaked composite scaffolds reported earlier, another focus was now to achieve direct double printing of gels and thermoplasts which could be reached by partners 1 and 6.

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, and all gels use within the consortium were confirmed as suitable biomaterials for the generation of cartilage-like matrix *in vitro*. Printed, cell loaded hydrogels, also with zonal composition, were examined, as well as double-printed cell loaded hydrogel / PCL scaffolds. Effects of printing on the differentiation behavior were examined and identified, as well as differences for ECM deposition of the cells in the different gel systems.

In WP 4, the previously established *in vitro* test systems for static and dynamic culture of osteochondral explants were used for systematic testing of chondrocyte-loaded hydrogels under static conditions in porcine osteochondral explants. Differences in matrix production depending on hydrogel types were detected, with best results in natural hydrogels. The equine testing was established, and the platform is validated for accurate assessment of mechanical properties of osteochondral plugs, including calibration. The assessment of mechanical properties was compared and validated against MTS Criterion tensile tester and possible differences were eliminated.

For the biocompatibility and biofunctionality screening in WP5, a reproducible *in vivo* model of ectopic OC porcine plug for hydrogel testing was developed. All hydrogels of the consortium were tested and showed similar results in terms of chondrogenic potential. Generally, little pronounced chondrogenic stimulation independently of cell type or presence of TGF- β 1 was observed. Despite the presence of the electrospun membrane, invasion by fibrotic tissue is detected. Only pHPMA-Lac+HAMA hydrogels demonstrated to be the most resistant to cell mouse invasion and material degradation. Also CCT reinforcement did not show any improvement on chondrogenic differentiation.

Work in WP6 was focused on pre-examination and preparation of the planned long term studies. Cell free hydrogels showed only minor cell ingrowth *in vivo* and will thus not be used for further studies. A major focus has been the fixation of constructs in the chondral defects. Generally, the soaked gel-filled thermoplast scaffolds from partner CCT could not be fixed and will thus not be used further. However, self-printed PCL scaffolds show promising results as alternative. Generally, only fragments of gels remained in the originally planned site of implantation in the pig model. Prof. von Rechenberg, the scientific advisory board member of HydroZONES with major animal testing experience mainly in sheep models, was consulted and an alternative location was explored. While fixation worked better, this site resulted in bone erosion. Loss of hydrogel although secured with a cover may indicate premature degradation in minipig defects. In the equine model, unexpected immunogenic problems with commercially available fibrin glue enforced development of a procedure to produce autologous fibrin glue. This was less immunogenic, but not efficient for fixation. Accordingly, an osteochondral anchor for hydrogels is being developed by customized 3D printing for the equine experiments.

In WP7, the HydroZONES Quality Management System has been optimised and actualised and it has been validated by external experts. The tools and the knowledge are and will be delivered to the consortium members in request. Management systems and tools for quality management, risk management and regulatory affairs management of ATMPs' – including combined ATMPs as Tissue Engineering Products are made available to the HydroZONES team. These systems and tools can be adapted, installed and applied by each consortium member and those can be offered to later industrial partners as a package of regulatory affairs support.

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 despite few uncritical minor delays according to plan.

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-economic impact

Within the last project year, multiple dissemination activities have been undertaken to spread awareness of the project in the scientific community and the general public. Aside of scientific dissemination, the HydroZONES twitter-account is, together with the HydroZONES homepage, regularly used to spread news achieved within the project.

In case of success especially within the large animal experiments in the last project year, 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.