PROJECT FINAL REPORT

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EXECUTIVE SUMMARY

Despite significant achievements in the field of regenerative medicine and the enormous potential for engineered tissue products, significant hurdles have prevented cellular therapies from gaining widespread clinical adoption. Manufacturing related issues have been proposed as key challenges to be addressed for the translation of regenerative therapies to the clinic and the successful commercialization of engineered products. Similar to other biotechnology sectors (e.g., vaccines or recombinant protein production), bioreactor systems could play a central role in establishing engineered tissues in the clinic. In fact, by automating and streamlining manufacturing processes, bioreactors would allow product reproducibility, safety and standardization to be improved and possibly affect cost-effectiveness. This project aimed at the development, pre-clinical and clinical testing of a sensor-based bioreactor system for the production of functional, autologous engineered grafts with reproducible properties. The bioreactor-based manufacturing paradigm was addressed in the specific context of cartilage repair. Innovative features of the BIO-COMET tissue engineering strategy included: (i) an automated and controlled production system, (ii) bioreactor developed for regulatory compliance, (iii) simplified, streamlined, and scalable tissue engineering process, (iv) on-line monitoring of culture/quality parameters, and (v) data management for traceability. To achieve these goals, BIO-COMET brought together internationally renowned leaders in the field of regenerative medicine, from academic, clinical and industrial research institutions. This project will be instrumental in extending use of bioreactor-based platforms beyond cartilage tissue engineering, with the ultimate goal to facilitate broad utilization and commercialization of cell-based grafts as therapeutic solutions.

BIO-COMET CONCEPT AND OBJECTIVES

TODAY: Current tissue engineering paradigm

Despite the compelling clinical need to regenerate damaged tissues/organs, the impressive advances in the field of tissue engineering have yet to result in viable engineered tissue products with widespread therapeutic adoption. Manufacturing-related issues, including regulatory compliance, standardization, up-scaling, and cost-effectiveness, have been proposed as central challenges to be addressed for the commercial success of a cell-based engineered product. In fact, the central bioprocesses for engineering cell-based grafts have traditionally been, and continue to be, based on *conventional manual benchtop techniques*. These, due to the large number of manual and labor-intensive manipulations required, possess inherent risks of contamination, potential high intra- and inter-operator variability, limited scale-up opportunity, and high manufacturing costs in the long-term.

BIO-COMET CONCEPT: Bioreactor-based manufacture of tissue grafts

Bioreactors have the potential to overcome these limitations by: (i) providing a controlled physico-chemical culture environment, which tightly regulates the bioprocesses, *minimizing process and product variability*, (ii) including monitoring and data management systems which offer a high level of traceability and increased *compliance to regulatory guidelines*, and (iii) introducing automation which facilitates *safe and standardized production* methods and maximizes prospective *scale-up* and *cost-effectiveness* in the long-term. Thus, the bioreactor-based implementation of automated, controlled, and streamlined manufacturing processes, similar to other biotech sectors (e.g., the pharmaceutical industry), will be instrumental to *facilitate the broad utilization and commercialization of tissue grafts as therapeutic solutions.*

TODAY: *Cell therapy* for cartilage repair

While there are a variety of causes for degenerative joint diseases, such as osteoarthritis in an aging population, the onset of degeneration in nearly half of the cases has been associated with prior injuries to articular cartilage that have gone untreated. Autologous chondrocyte implantation (ACI), which is based on the injection of a suspension of chondrocytes into a cartilage defect, has been well-established in the clinic for the repair of cartilage injuries for nearly two decades. Although good clinical results are generally reported in short-term follow-up, the technique generates a repair tissue with inferior mechanical properties as compared to healthy cartilage, jeopardizing a durable repair in the long-term. Moreover, due to the *high costs*, *long rehabilitation time*, *large inter-patient variability of the chondrocyte quality* and a *lack of sound cost versus benefit data*, ACI has failed to gain acceptance by the health care sector and therefore suffers from very limited reimbursement coverage.

BIO-COMET CONCEPT: *Tissue therapy* for cartilage repair

As an alternative, a three-dimensional cartilage tissue graft, engineered *in vitro* to possess functional properties (i.e., to biochemically and mechanically mimic native cartilage), could reduce the initial rehabilitation time and result in a more durable repair in the long-term. To reduce the variability in the quality of the engineered tissue grafts, a cell source with more reproducible function such as *nasal chondrocytes* could be used. As compared to articular chondrocytes, nasal chondrocytes have a higher and more reproducible capacity to generate functional cartilaginous tissue, which is less dependent on the donor age, and show a similar response to physical forces resembling joint loading. The tools, technologies, and processes developed within BIO-COMET enable the production of standardized functional cartilage grafts and their assessment in pre-clinical and clinical models.

Main objectives of BIO-COMET

The main objectives of BIO-COMET were:

1. To integrate established research based technologies and processes into an automated and controlled bioreactor-based tissue manufacturing system, which is GMP compliant and conforms to regulatory guidelines for the production of engineered tissues for clinical use.

2. To produce functional human cartilage grafts in the developed bioreactor-based system for assessment in a multicenter clinical trial.

In order to replace manual cell culture techniques in the manufacturing process, the project strategy was NOT to develop complex and rather costly automated/robotic systems, which essentially mimic established manual procedures. Instead, we aimed *to streamline the individual bioprocessing steps into a novel manufacturing paradigm*. Specifically, to eliminate the typical phase of monolayer culture in plastic dishes and to expand the cells isolated from the tissue biopsy directly within the porous 3D scaffold and culture them in the same environment until the graft is sufficiently mature for implantation (Figure 1).

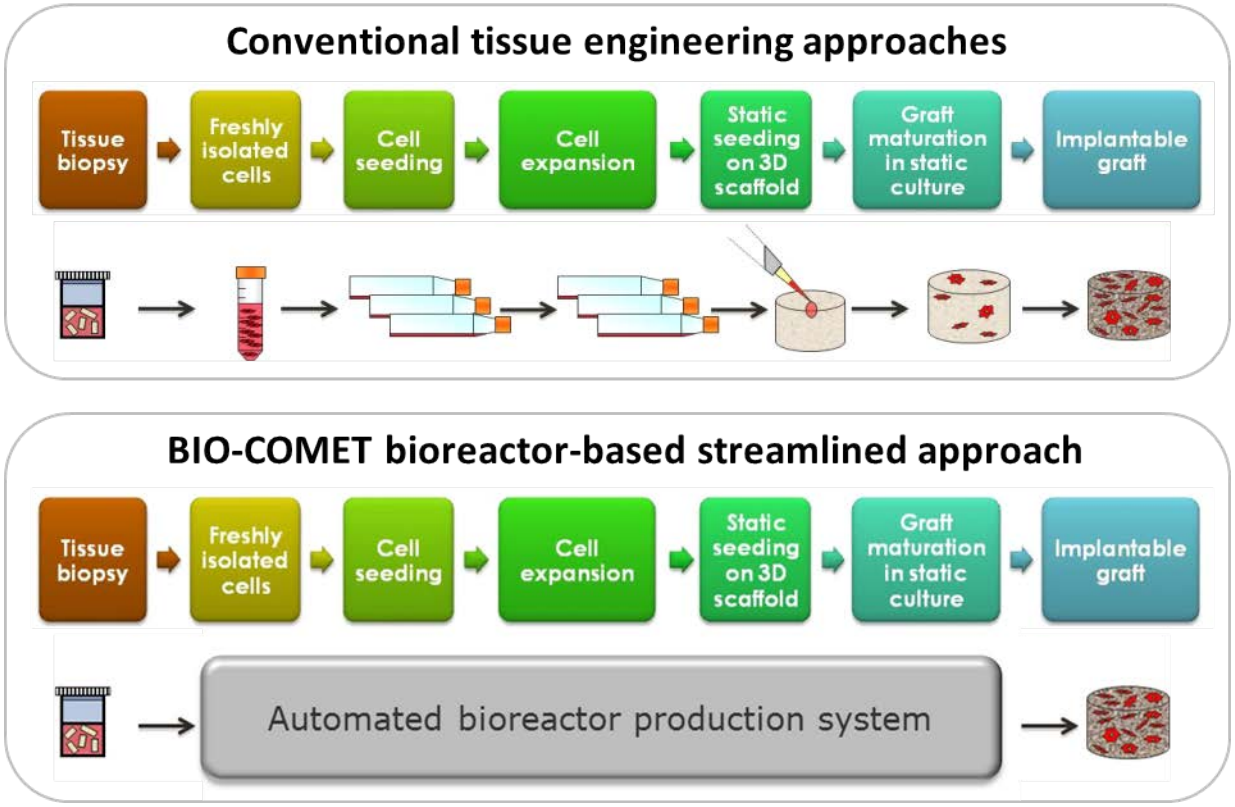

Figure 1: *Conventional tissue engineering approaches* are based on labor-intensive manual cell culture techniques, which possess inherent risks of contamination, high operator variability, limited scale-up potential and high manufacturing costs in the long-term. *BIO-COMET's bioreactor-based streamlined approach* bypasses the typical 2D cell expansion in plastic dishes and relies on a 3D perfusion culture principle. The bioreactor-based platform overcomes the above listed limitations and will facilitate the translation of tissue engineered products to the clinic and to successful commercialization.

BIO-COMET Innovation

Innovative features of the BIO-COMET tissue engineering strategy included: (i) an automated and controlled production system, (ii) GMP compliant bioreactor conforming to regulatory guidelines, (iii) simplified and streamlined tissue engineering process, (iv) on-line monitoring of culture/quality parameters, and (v) data management systems for traceability. The grafts were based on a leading orthopedic material (MioRegen) and were used in combination with new implantation techniques in critically sized cartilage defects. The consortium met with European regulators (European Medicines Agency, EMA) to present the BIO-COMET technologies and to define a roadmap for regulatory compliance for the clinical trial and for ultimate commercialization of the bioreactor and engineered tissue grafts.

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DESCRIPTION OF MAIN SCIENTIFIC AND TECHNICAL RESULTS

Work Package 1

We have designed and developed a prototype bioreactor-based manufacturing system to control the production of engineered cartilage grafts. A "Digestion Bioreactor" was designed to control and automate the digestion of a cartilage biopsy to yield a suspension of isolated chondrocytes (Figure 2). A "T-CUP Bioreactor" was designed to streamline the conventional tissue engineering culture processes by seeding the isolated cells onto a 3D scaffold, expanding the cells directly within the scaffold, and generating a

cartilaginous graft (Figure 2). Sensors were integrated into the system in order to monitor pH and oxygen during graft production, which could ultimately be used to generate data for in-process controls and/or release criteria for the engineered graft. Software and user-interfaces were also developed to allow simple and easy use of the bioreactors as well as including safety features to minimize risk of user error. The properties of a biomimetic composite scaffold, which is commercially available for the treatment of osteochondral defects, were modified to meet the requirements of our outlined clinical study and to be compatible for use within the T-CUP bioreactor. Using the modified scaffold and the developed bioreactor system, viable and cartilaginous grafts could be successfully generated.

Figure 2: (left, a-e) Digest bioreactor and (right) T-CUP bioreactor

Subsequently, the bioreactor-based manufacturing system was transferred to the Good Manufacturing Practice (GMP) test facility and used in four production runs to manufacture engineered cartilage grafts for our pre-clinical large animal study. These successful production runs provided crucial feedback, verifying the main specifications of the bioreactor systems while leading to the further optimization of specific design elements (e.g., optimized cartilage biopsy digestion, re-design for sensor integration in T-CUP).

After refining specific aspects of the bioreactor designs and processes, the manufacturing system was used for the production of engineered cartilage grafts for a second large animal study. The implementation of the bioreactor-based production system was again highly successful and provided further positive feedback to the technical partners. In parallel, qualification activities for the bioreactor system design were successfully completed, whereby design inputs were determined based on the critical-to-function characteristics of the system and design outputs assessed to verify that the design complied with the

product requirements. Although further refinements to the system and automated protocol design may be proposed based on the final study data from the second large animal trial, the qualification activities undertaken on the current prototype have demonstrated that the design of the system and automated protocol meets the critical to function requirements and specifications defined for the large animal study.

In addition, research activities continued to refine the process in order to simplify automation requirements and to facilitate regulatory compliance. Ascorbic acid (AA) is generally considered to be a critical culture medium supplement for cartilage tissue engineering. However, due to its instability, AA is added to the culture medium from frozen aliquots at the time of each medium change. For an automated manufacturing process, this would require a freezer and defrosting compartment, which poses challenges to the automation of liquid handling, significantly increasing complexity and costs. Therefore, we investigated the effect of AA on the chondrogenic differentiation and extracellular matrix production of human chondrocytes. The results of our studies demonstrated that the addition of AA to human chondrocyte cultures had only little influence on chondrogenic differentiation and the extracellular matrix accumulation and structural organization. Therefore, we conclude that AA may not be required for a cartilage tissue engineering process. The elimination of AA from this process would allow for a more simple, compact, and cost-effective bioreactor system.

Research activities were also aimed at assessing steps within the manufacturing process to facilitate regulatory compliance. Perichondrium is a closely adherent tissue overlaying nasal cartilage. In the BIO-COMET manufacturing process, the thin layer of perichondrium is removed, leaving only nasal cartilage. However, the assessment of the cellular composition of cell-based therapy products is required by regulators as a way to ensure appropriate quality and function of the final product. We have shown that cells from the perichondrium, which could potentially contaminate the starting material (nasal cartilage biopsy), had a negative impact on the quality of the final engineered cartilage graft, although to varying degrees between donors. Additional “titration” experiments were performed to investigate specific ratios of perichondrium cells/nasal chondrocytes, demonstrating increased effects as the number of perichondrium cells increased. Data from this study are central to optimize the BIO-COMET manufacturing process, as appropriate processing of the starting material could efficiently maximize the quality of the final product.

Work Package 2

In addition to the scientific and technological developments of BIO-COMET, considerable attention was focused on defining regulatory issues and compliance strategies required to undertake a clinical trial using a bioreactor-based manufacturing system. Key structures for this objective were established, including the Quality Assurance Team (QAT) and the Design Review Board (DRB). The main goals for establishing a quality assurance system have been achieved including a document management system (DMS) and approval structures as well as documents to ensure traceability of design changes and control of the graft production and quality related processes. Two key deliverables focusing on regulatory issues for the bioreactor-based manufacturing system have been achieved within the project.

Deliverable 2.1, Documentation for Bioreactor Development, Qualification and Validation centered on documentation of the design and qualification activities required to develop and manufacture the bioreactor systems developed during the project and used to produce tissue-engineered cartilage grafts. A project design record or *Design History File* (Figure 3) was established, comprising four phases: *Feasibility, Preliminary Design, Detailed Design and Design Qualification/Validation*. The first two phases, *Feasibility* and *Preliminary Design* involved the production of functional prototypes designed to translate manual cell

culture techniques to automated systems, and to challenge these systems in cartilage graft production for a large animal study. Successful production and implementation of the first system prototypes allowed grafts to be produced for implantation in this animal study. This work allowed the BIO-COMET team to identify aspects of the bioreactor-based manufacturing system requiring further optimization and development in order to move towards clinical application of the system. Commercialization activities in work package 7 and definition of regulatory issues within this work package have also helped to guide the development of system requirements.

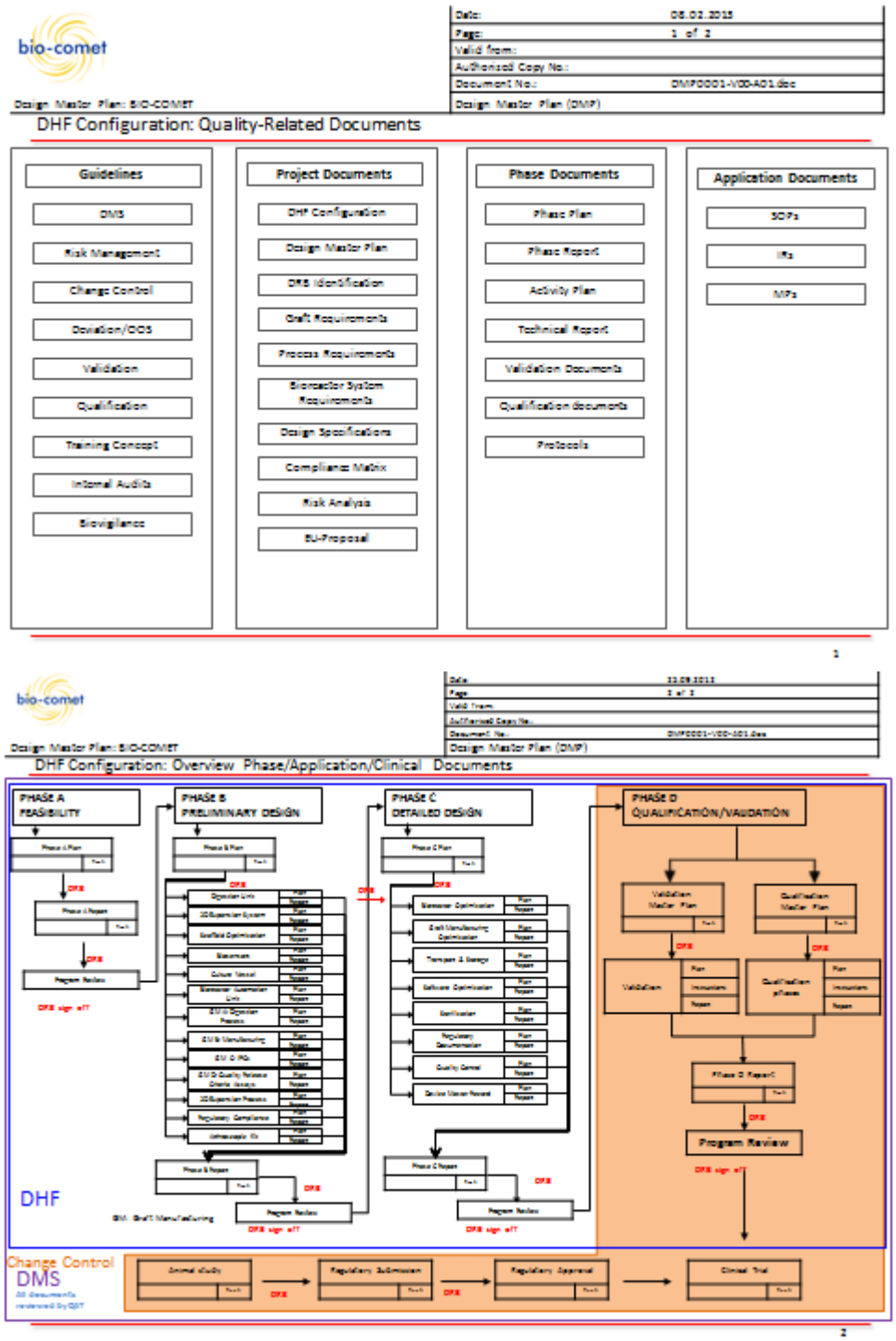

Figure 3: Design History File (DHF)

In developing the prototype systems, user requirements were drafted and design documents prepared, including those describing the assembly, bill of materials, sterilization and quality testing for construction of bioreactor disposables and control system. Other development documents prepared for the animal study

included Instructions for Use and Detailed Operating Procedures for installation, operation and troubleshooting of the systems. This work has allowed the team in the Detailed Design phase of the project, to assess the performance of the system and identify improvements to the specifications of the prototype system. In order to demonstrate design qualification, it has been documented that the design specifications or outputs of the bioreactor-based manufacturing system meet certain defined design requirements or design inputs. These design requirements have in turn been shown to comply with the top level user requirements from which they were produced.

Significant additional development work has been necessary in the Design phases of the project: modifications to system hardware and electronics have been introduced in order to overcome challenges related to performance reliability. Production of additional sensor and digest bioreactor disposables, as well as additional installation and remote system monitoring and support has also been required as a result of additional production runs performed as part of the large animal study. Although this work extended development activities beyond the timeline anticipated in the original work plan, it has allowed refinement of various building blocks of the bioreactor system that are of benefit to the quality of the cartilage graft product, as well as to the regulatory compliance and long-term commercial feasibility of the bioreactor based manufacturing system. Due to time constraints, it has not been possible to finalize all of the bioreactor-based manufacturing system requirements, and, as it is critical that all necessary development work be completed prior to qualification and validation of the production process, we have not been able to undertake a full process validation during the timeframe of the project.

In *Deliverable 2.2, Definition of Regulatory Issues for the Clinical Market,* the regulatory criteria and the compliance strategy required for clinical development using the bioreactor and related control instrumentation were defined. Regulations and guidance published by both the United States Food & Drugs Administration (FDA) and European Medicines Agency (EMA) have been reviewed in order to gain an international perspective on the likely regulatory pathway and strategy that would be taken for the BIO-COMET bioreactor system and cartilage replacement therapy. Potential issues that may be foreseen as a result of this review and requiring further clarification have been identified. Regulatory compliance considerations extended from the point of initial cell collection through to the implantation of the cell-based implant, as well as to the manufacturing equipment and environment. The likely designation, and therefore regulatory approval pathway, has been determined under both the European and US frameworks.

A meeting to present the new technologies being developed to representatives of the EMA's Innovation Task Force, and to take the opportunity to discuss with them potential regulatory issues that had been identified was held. These issues focused on questions relating to the *classification and qualification of bioreactors, In Process Controls (IPCs) and release criteria testing, production environment, plus the applicability of existing clinical trial data for grafts produced by a manual process.* Information has also been sought from the FDA on the regulatory framework surrounding the approval of a bioreactor-based manufacturing system.

The likely designations of both the bioreactors and the cartilage graft under two different regulatory frameworks (EMA and FDA) have been defined. The information received as agency opinions on the manufacturing and quality strategies has been in line with the position anticipated by the Consortium and on the whole has confirmed the validity of the approaches discussed. Significantly, no information was obtained that will fundamentally alter the agreed development pathway.

Work Package 3

Within Work Package 3, we aimed to develop methods that could ultimately be used to define product release criteria for the engineered cartilage grafts produced within the bioreactor system and for establishing in-process controls during the manufacturing process.

Release Criteria

With the goal of developing a non-invasive method to define product release criteria, we aimed to establish a relationship between specific extracellular matrix components released into the culture media and graft quality. A high ratio of collagen type II to type I is indicative of good chondrogenesis and, ultimately cartilage formation. This task was broadly divided into two phases; firstly to optimize and validate two existing collagen assays (for collagens type I and type II), and secondly to develop a new assay that may be an alternative to one or other of the existing assays.

In engineering small-scale cartilage constructs, we compared a non-collagenous polyglycolic acid scaffold with the collagen based FinCeramica MABI scaffolds, and found that collagen release into the culture media was due to cell synthesis rather than degradation of the MABI collagen scaffold. We also found that levels of collagens I and II in the media predicted respective collagen accumulation in polyglycolic acid-derived constructs.

We encountered possible interference and/or sensitivity issues with the collagen ELISA assays when bioreactor spent media and grafts were analyzed. Despite exhaustive attempts to resolve these issues by various media processing tests and assay re-design, we were unable to validate both ELISA assays, and concluded that interference of the MABI scaffold rather than sensitivity was preventing collagen detection. As an alternative, we optimized Western blot methodology and successfully detected collagens I and II in bioreactor media; and used immunohistological analysis to determine graft collagen content with semi-quantitative analysis of images. Analysis of media collected during the manufacturing of cartilage grafts for the large animal studies showed that collagen type II levels significantly increased in the 2nd week of differentiation, and that the collagen type II/I ratio correlates strongly with the cartilaginous content of grafts, thereby validating this outcome measure.

We have also identified link protein (Figure 4) as an alternative marker for assay development, and established a reliable protocol for measuring link protein in bioreactor spent media. This marker was detected in all samples collected during the production runs for the large animal study as well as in experimental tests based on human chondrocytes. Link protein levels strongly correlated with graft quality at a very early stage of engineering from both human and sheep cells, therefore enabling assessment well in advance of graft release for implantation. In addition, we showed that link protein correlated with the cartilaginous content of the chondral defect, following graft implantation in the knee, further validating this marker. Considering the robust nature of the technique, the strong correlation with graft quality early in engineering, and the correlation with outcome after surgery, we have identified link protein as an excellent candidate as a quality outcome measure.

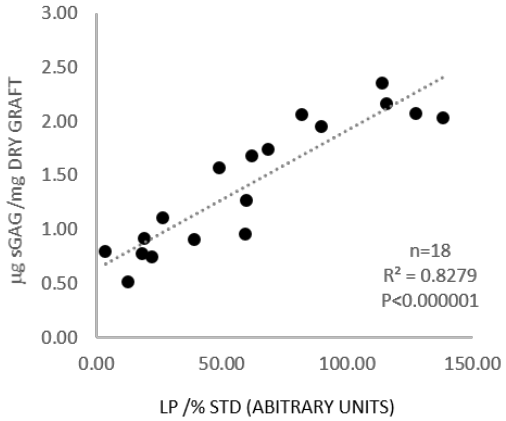

Figure 4: Link protein (LP) detected in culture media compared to the content of glycosaminoglycans (GAG) in the engineered cartilage grafts. Data collected from the manufacture of cartilage grafts for the first large animal study

In-process control (pH)

In conventional cell/tissue culture processes, culture medium is typically exchanged batch-wise at regular time intervals. This approach can lead to significant and uncontrolled fluctuations in pH. While pH has been shown to affect numerous cell types, there are currently no reports on the effect of pH on human nasal chondrocytes (NC). Thus, we aimed to assess the effects of defined pH levels during the proliferation of nasal chondrocytes. Implementing PreSens sensors to obtain non-invasive pH measurements, our experiments have shown that rather small differences in pH can significantly influence the expansion of nasal chondrocytes. After five days of expansion, cell numbers at pH 6.8 and 7.2 were only 45% and 76% of those obtained at pH 7.4, respectively. Interestingly, we next determined that the pH level applied during the proliferation phase also affected the subsequent re-differentiation capacity of nasal chondrocytes. Engineered cartilage generated from nasal chondrocytes expanded at pH 7.2 and 7.4 had the most uniform and intense staining for cartilage extracellular matrix components and the highest ratio of collagen types II:I mRNA compared to all other pH levels assessed (from pH 6.5 to pH 8.0). The results of these studies will allow us to implement non-invasive sensors and pH measurements as an in-process control to optimize and standardize graft quality. By maintaining the pH within the defined range, the processes can be more tightly controlled, leading to better standardization of graft quality and facilitating compliance with regulatory guidelines.

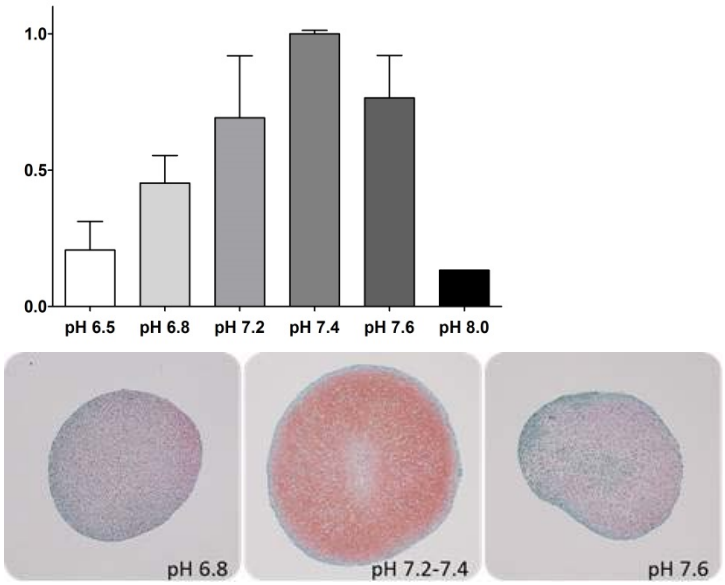

Figure 5: (top) Cell numbers after 5 days of expansion at different pH levels (values normalized to pH 7.4) and (bottom) representative Safranin-O stained pellets generated from nasal chondrocytes expanded at specific pH levels

In-process control (purity of nasal chondrocytes)

As previously described, nasal cartilage is surrounded by a layer of perichondrium tissue. Since the presence of residual perichondrium on the nasal cartilage tissue may negatively impact the quality of the final engineered product, appropriate processing of the starting material may efficiently maximize the quality of the final engineered cartilage graft. In addition, the assessment of the cellular composition of cell-based therapy products is required by regulators. Therefore, an assay to assess the purity (i.e., proportion of nasal chondrocytes to perichondrium cells) of the biopsy could be important for the quality of the final product.

We have assessed whether a gene expression assay could be used to discriminate populations of nasal chondrocytes from perichondrium derived cells. When analyzing the gene expression of different cell populations comprised of known ratios of nasal chondrocytes:perichondrium cells, we determined that both Aggrecan:Versican and HAPLN1:MFAP5 mRNA ratios progressively decreased with increasing percentages of perichondrium cells (HAPLN1: also known as CRTL1, cartilage link protein; MFAP5: also known as MGAP2, microfibril-associated glycoprotein-2). Therefore, assessing these mRNA ratios could provide a means of discriminating cell populations from different tissue biopsies. The collected data give positive indications that this approach could lead to the development of a reliable assay to quantify the purity of nasal chondrocytes derived from the biopsy.

Work Package 4

As described under Work Package 1, the BIO-COMET manufacturing process for producing engineered cartilage grafts for the large animal study was fully implemented and tested during a trial run at Holostem. Technical and process know-how was transferred from the different project partners to Holostem (Figure 6). Bioreactor and sensor systems were installed within Holostem facilities and Holostem personnel extensively trained to operate the bioreactors and perform required processes and quality assessments autonomously. Procedures for the packaging and the transport of cartilage biopsies and engineered grafts to/from Holostem and University of Zagreb were established within the project. Although the challenges associated with the transport were initially underestimated, additional procedures were put in place to

track all relevant data and ensure timely delivery across international borders. These procedures were assessed in test runs and found to meet the outlined quality parameters as provisionally defined, including the overall duration of transport and temperature within the transport container. The established quality assessments, tested throughout the production process, met defined specifications. Following the successful trial production run, production of grafts for the large animal study commenced.

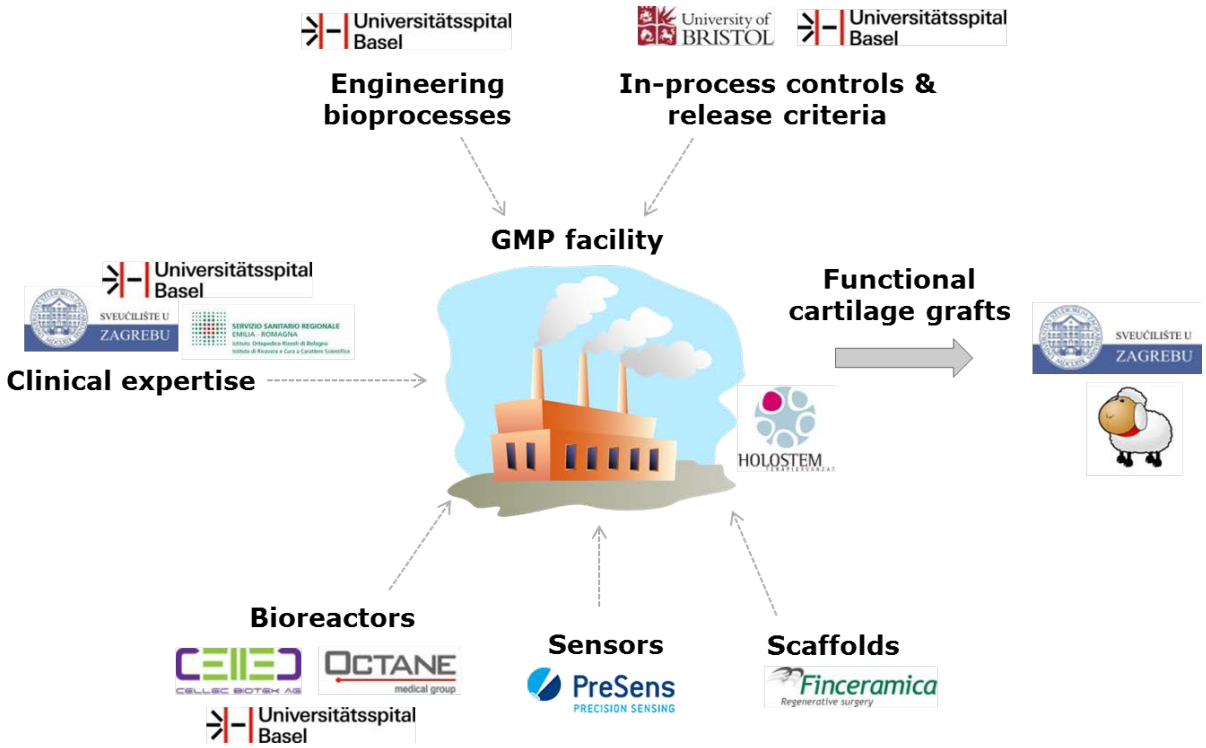

Figure 6: BIO-COMET expertise and transfer of knowledge to centralized manufacturing facility

For the first large animal study, a total of sixteen grafts were successfully produced at Holostem in four sequential production runs. The manufacturing process for each production run lasted five weeks. Four bioreactor systems were run in parallel for each production run, generating four independent grafts per run. The large animal study represented a key milestone for the bioreactor development as it served as a basis for testing the performance and functionality of the prototype system. Moreover, it allowed us to test the feasibility of the process and thereafter to refine specifications for both the bioreactor system and the process. Key requirements for the process were identified and enabled the further development of the bioreactor in compliance with regulatory criteria and GMP manufacturing rules.

The bioreactor-based manufacturing system was subsequently used to manufacture engineered cartilage grafts in three production runs for the 2nd large animal study. The implementation of the bioreactor-based production system was again highly successful, with all engineered grafts meeting the defined quality criteria.

In parallel, studies were performed to verify whether BIO-COMET protocols, which were previously established for the transport of sheep-based cartilage grafts, would be suitable for human grafts. Therefore, we engineered cartilage grafts based on human nasal chondrocytes, simulated the storage/transport conditions, and assessed graft quality after 1, 2, and 3 days. These studies indicate that the transport conditions implemented during the large animal studies are also suitable for the shipment of engineered grafts based on human nasal chondrocyte for up to 72 hours.

Cartilage grafts for both large animal studies were manufactured using the growth factors transforming growth factor beta (TGFβ1) and Fibroblast growth factor-2 (FGF2). Since GMP grade growth factors are significantly more expensive but expected to have similar effects to uncertified grades, grafts for both large animal studies were manufactured using uncertified grades. However, since GMP grade growth factors will ultimately be required for advanced clinical studies, we have assessed the effect of GMP grade TGFβ1 and FGF2 for human cartilage tissue engineering. We found that similar proliferation rates and matrix deposition were obtained with the GMP grade factors as compared to the uncertified grade used for the large animal studies. Therefore, GMP grade TGFβ1 and FGF2 will be suitable for use in the production of engineered cartilage grafts for clinical use.

Work Package 5

The aims of the large animal study were to determine the best surgical procedure for the fixation of engineered cartilage grafts and the most suitable cell source (nasal vs. articular chondrocytes). Chronic femoral condyle lesions in adult sheep were selected for the large animal model. It was a primary aim that the large animal study protocol was designed to ensure the use of the minimal number of animals and that animal care and all procedures were conducted according to the highest standards in veterinary medicine. The endpoint of the large animal study must generate valid results that could enable the translation of bioreactor engineered cartilage graft to the clinical practice. Selection of the proper implantation technique, tailored to the specific scaffold or graft requirements is crucial for improving the chances of a successful pre-clinical study and also guiding future clinical implantation procedure.

Determination of surgical procedures

Pilot studies on sheep helped fine tune the surgical procedures (choice of instruments, nasal and articular cartilage harvesting, graft implantation), as well as perioperative protocols (anesthesiological procedures, pain managements, antibiotic prophylaxis) and postoperative protocols.

A minimally invasive surgical approach was preferred since there is less risk of altering joint homeostasis anatomically and biomechanically, and also to determine an unfavorable joint environment. Two pilot studies performed by UZagreb and IOR showed the feasibility of using combined medial and lateral parapatellar surgical approaches to the knee of sheep. The chronic femoral condyle lesion method was defined (osteochondral lesion 6 mm wide and 5 mm deep), as was the technique for harvesting nasal cartilage. Techniques for graft implantation and fixation were determined (press fit technique without additional suturing). The study design is shown in Figure 7.

In brief, during a first procedure, two partial-thickness defects 4 mm in diameter were created on the lateral and medial femoral condyles of a sheep hind leg with a standard mosaicplasty kit. Cylinder shaped cartilage biopsies were harvested from the created defect. In the nasal chondrocyte-based group, nasal cartilage was harvested from the nasal septum. Cartilage samples were shipped to Holostem for further processing and production of the engineered cartilage grafts. After the first procedure the animals were transferred to a farm for a period of 6 weeks, during which time the construct was produced and shipped to Zagreb. After the 6 weeks, the initial 4 mm diameter partial-thickness defect site was converted to a 6 mm diameter osteochondral defect, 5 mm in depth to match the thickness of the engineered cartilage graft, and engineered constructs were implanted. Untreated empty defects (negative control) and cell-free scaffold implants served as controls. Sheep were sacrificed after 6 weeks, 3 months, and 12 months. Explants were assessed, evaluated with semi-quantitative macroscopic scoring and analyzed with histological staining, immunohistochemistry, biochemistry, and ELISA.

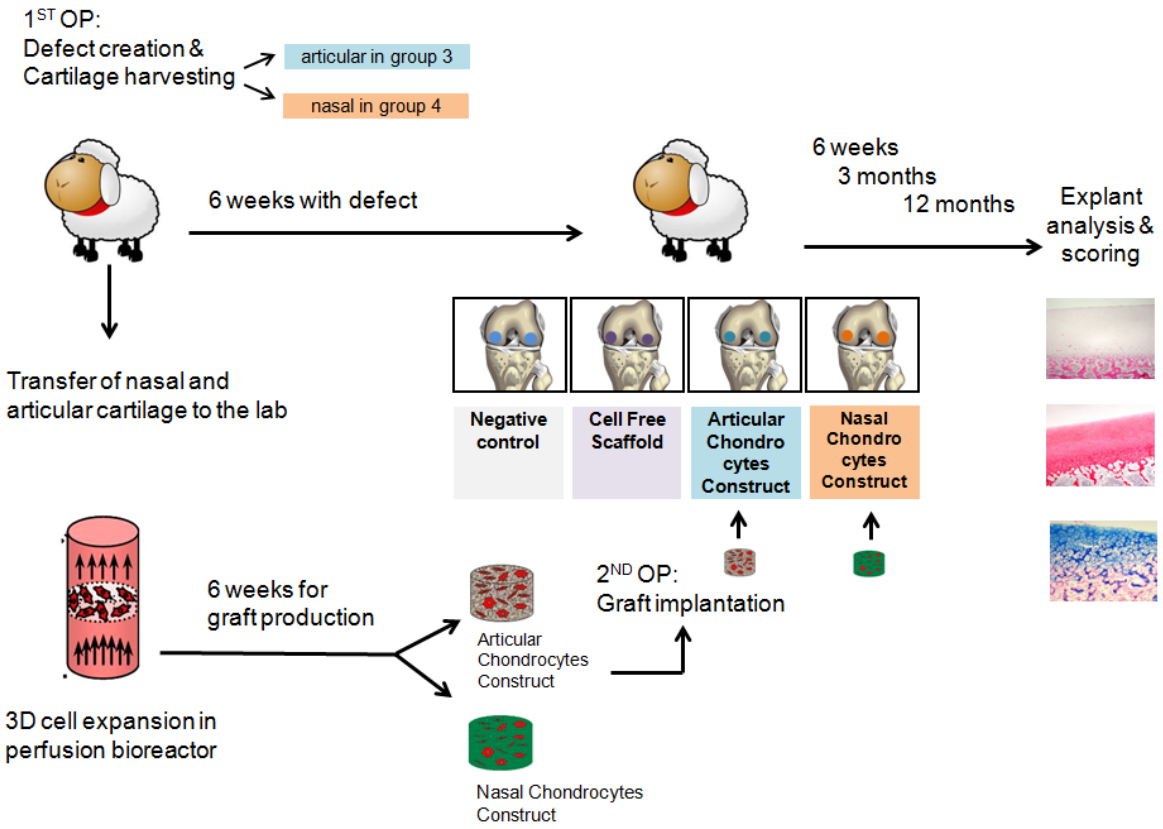

Figure 7: Schematic outline of sheep study

Generation of preclinical data and decision on cell source

Previous studies have demonstrated improved reproducibility and enhanced tissue quality of nasal vs. articular chondrocyte-based grafts. Together with these previous studies, we have generated additional data from our *in vitro* and pre-clinical studies which further support the use of autologous NC-based engineered tissues for the treatment of articular cartilage defects: (i) although derived from the neuroectoderm, nasal chondrocytes have the plasticity to adopt a stable HOX expression profile of an articular cartilage (mesoderm) site; (ii) sheep studies indicated that nasal chondrocytes could survive within an articular cartilage defect and contributed to the repair, which was significantly superior to the repair achieved by articular chondrocyte-based grafts.

HOX gene expression

In embryonic models and stem-cell systems, mesenchymal cells derived from the neuroectoderm can be distinguished from mesoderm-derived cells by their HOX-negative profile - a phenotype associated with enhanced capacity of tissue regeneration. Therefore, we investigated the expression of HOX genes in differentiated nasal and articular chondrocytes from adults (Pelttari et al., 2014). Quantitative RT-PCR confirmed expression of HOXC4, HOXC5, HOXC8, HOXD3 and HOXD8 in articular cartilage (AC), but only at baseline or undetectable levels in nasal cartilage (NC). The differential gene expression was observed from chondrocytes derived not only from native human cartilage, but also following de-differentiation and subsequent chondrogenic re-differentiation *in vitro*. Therefore, these set of markers could constitutively distinguish NC from AC.

We then investigated whether NC possess features of environmental plasticity and have the ability to adopt the HOX expression profile of a recipient site. We thus implanted human NC cartilaginous constructs into subcutaneous pockets of nude mice - a site of mesodermal origin (verified to include HOX-positive cells). After 5 weeks, the explanted cells (identified to be of human origin by Alu *in situ* hybridization) upregulated the expression of HOXC4, HOXC5 and HOXD8 to levels similar to that of native articular cartilage. Additionally, the induction of HOXC4 and HOXD8 expression at protein levels confirmed the HOX reprogramming capacity of NC. The *in vivo*-activated HOX genes remained expressed even after subsequent *in vitro* culture of the explanted construct for 42 days, highlighting the stability of the acquired phenotypic changes. Therefore, although derived from the neuroectoderm, nasal chondrocytes have the plasticity to adopt a stable HOX expression profile of an articular cartilage (mesoderm) site.

First Sheep study

Six week explants: Following a six week implantation period, explants of the cell-free scaffold implants showed apparent signs of inflammation upon gross examination, which was confirmed by histological evaluation. Similar to the cell-free group, explants based on either nasal or articular chondrocytes also showed signs of inflammation upon gross examination and histological evaluation. Explants of the negative control group (scaffold-free, empty defect) did not display signs of inflammation as observed with the scaffold-based implants.

3 month explants: Despite varying degrees of inflammation still observed in all explants after 3 months implantation in the sheep, defects treated with nasal chondrocyte grafts were filled with hyaline cartilage repair tissue (Figure 8). Within the hyaline cartilage repair tissue were rounded chondrocytes embedded within lacunae. Moreover, integration of newly formed cartilage with the surrounding cartilage could be seen on both sides of the defect. In contrast, defects treated with articular chondrocyte grafts contained little repair tissue, and the little tissue that was formed did not stain positively for glycosaminoglycans (a key component of articular cartilage).

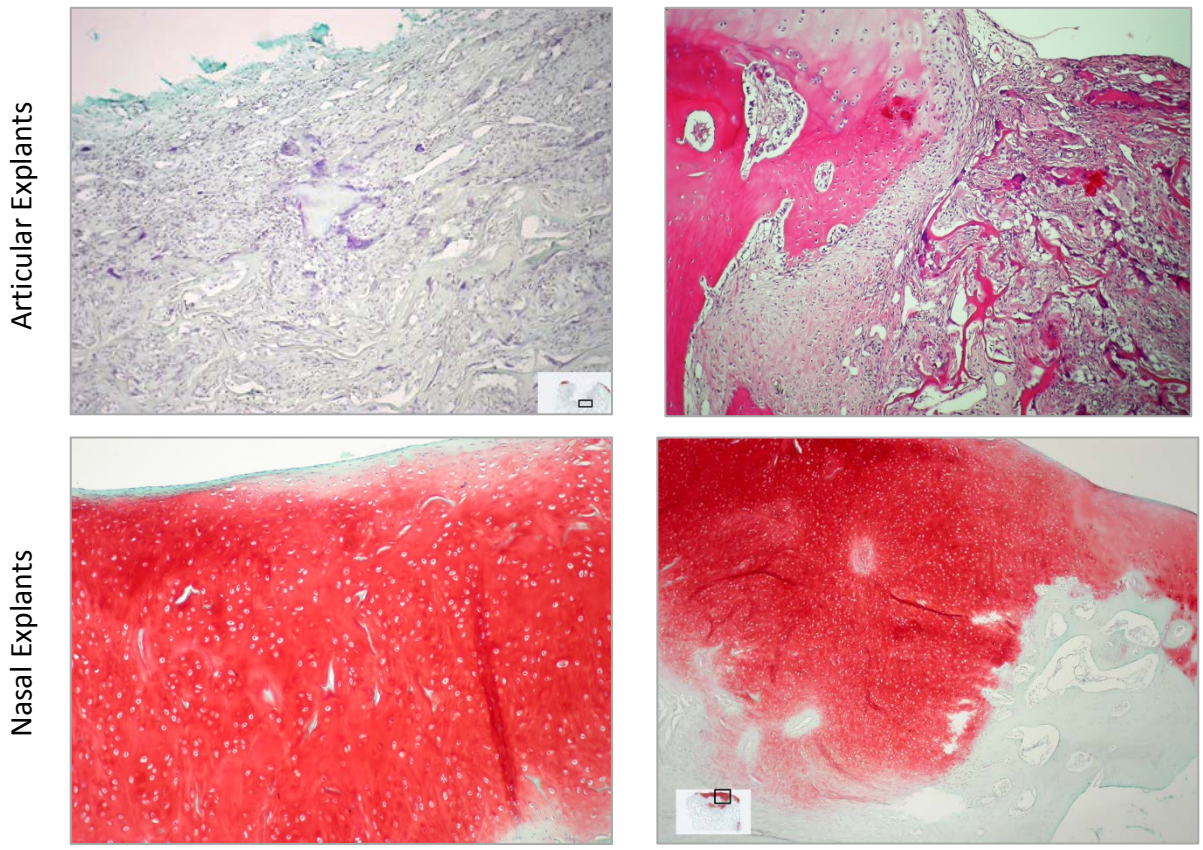

Figure 8: 3 month explants. *Articular chondrocyte-based explants*: (top left) repair tissue consisted of fibrocartilage; (top right) no integration between the repair tissue and surrounding tissue could be observed. *Nasal chondrocyte-based explants*: (bottom left) repair tissue consisted of hyaline cartilage; (bottom right) regions of integration between newly formed repair tissue and surrounding tissue were observed

12 month explants. Following 12 months implantation, no signs of inflammation were detected. Histological analyses showed good restoration of cartilage and subchondral bone in the group treated with nasal chondrocytes based grafts (Figure 9). Biochemical analysis supported the histological findings.

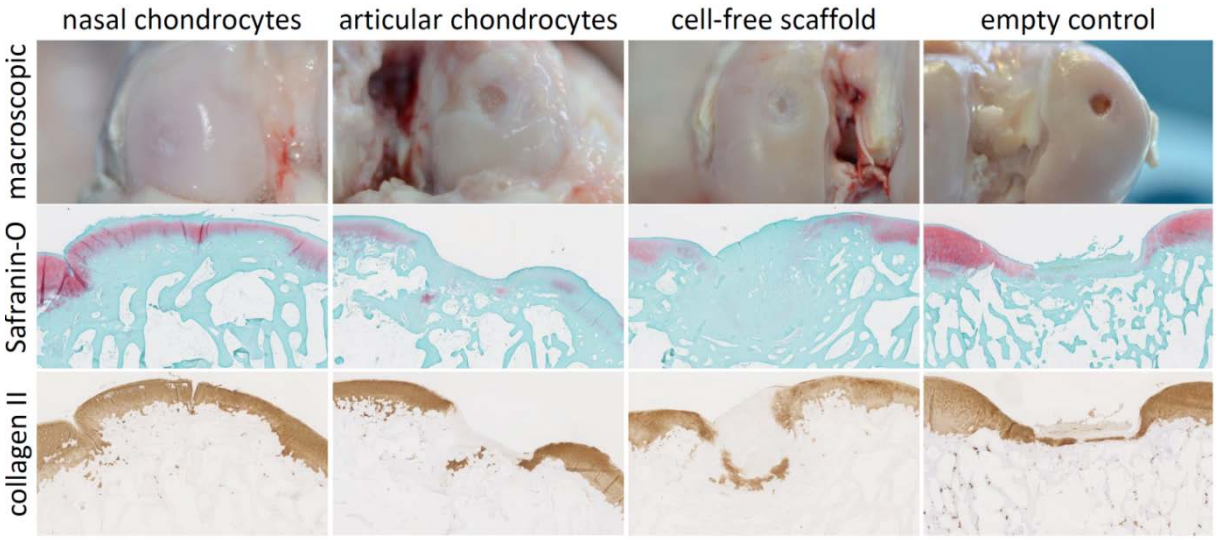

Figure 9: First sheep study, 12 month time point

A key feature observed in the repair of the nasal chondrocyte group was the impressive degree of integration between the repair tissue and the surrounding native cartilage tissue. In fact, no boundary

could be detected between the repair and native tissues. Moreover, chondrocytes in the repair tissue of the nasal group were embedded in lacunae and organized in columnae, therefore closely resembling the morphology of hyaline cartilage (Figure 10). In contrast, the defect site treated with articular chondrocyte implants was filled predominately with fibrous-like cartilage. No signs of integration could be observed in the articular chondrocyte-based explants.

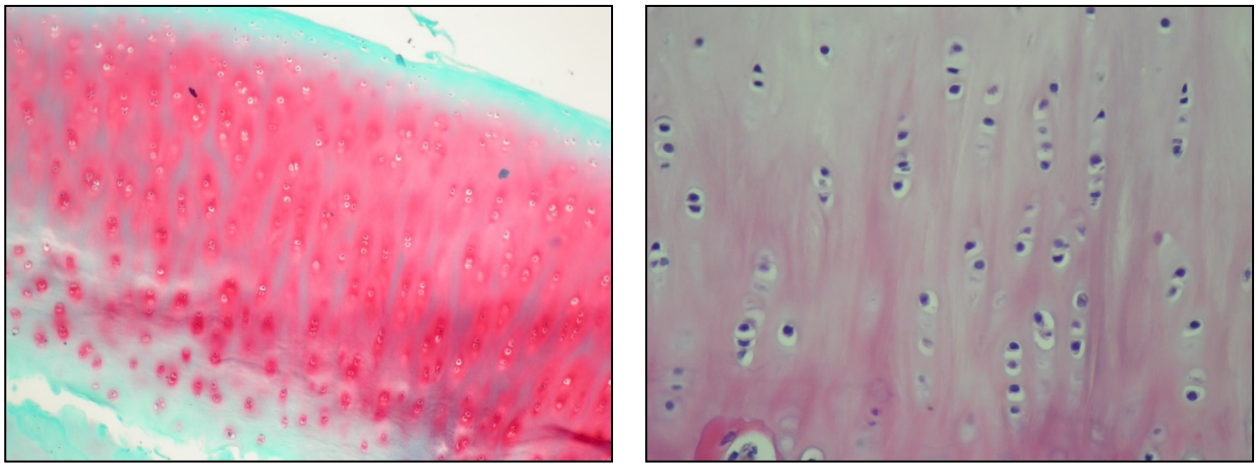

Figure 10: 12 month explants of NC group. (left) cartilage-cartilage integration between repair tissue and surrounding native cartilage; (right) chondrocytes embedded in lacunae and organized in columnae

Second sheep study

Considering varying degrees of inflammation were observed (at early time points) in both cell-based groups as well as the cell-free scaffold groups (i.e., all scaffold-based groups), the source of inflammation appeared to be scaffold related. Numerous chemical, mechanical, and biological assays were performed pointing to the release of magnesium-hydroxyapatite (Mg-HA) from the scaffold into the defect site as the likely source of the observed inflammatory response. Therefore, in order to minimize the amount of released Mg-HA, the scaffold was modified, reducing the amount of HA.

A second sheep study was subsequently performed with the modified scaffold. Since the nasal chondrocyte implants resulted in a significantly better repair than the articular chondrocyte implants, only nasal chondrocyte based grafts were assessed in the second sheep study. Engineered grafts were manufactured in the bioreactor-based system at Holostem facilities and implanted into the sheep at the University of Zagreb. Sheep were sacrificed after 3 months and 12 months.

After three months implantation, while there were signs of very mild inflammation (in terms of small infiltration of lymphocytes), the inflammatory response was significantly lower as compared to the first sheep study. Histology and immunohistochemistry results were comparable to the results of the first animal study. The nasal chondrocyte implants generated in the bioreactor showed better morphology of repair tissue compared to the cell-free scaffold group (Figure 11). Consistent with the histological assessments, collagen II and glycosaminoglycan contents were higher in repair tissues of bioreactor generated nasal chondrocyte implants.

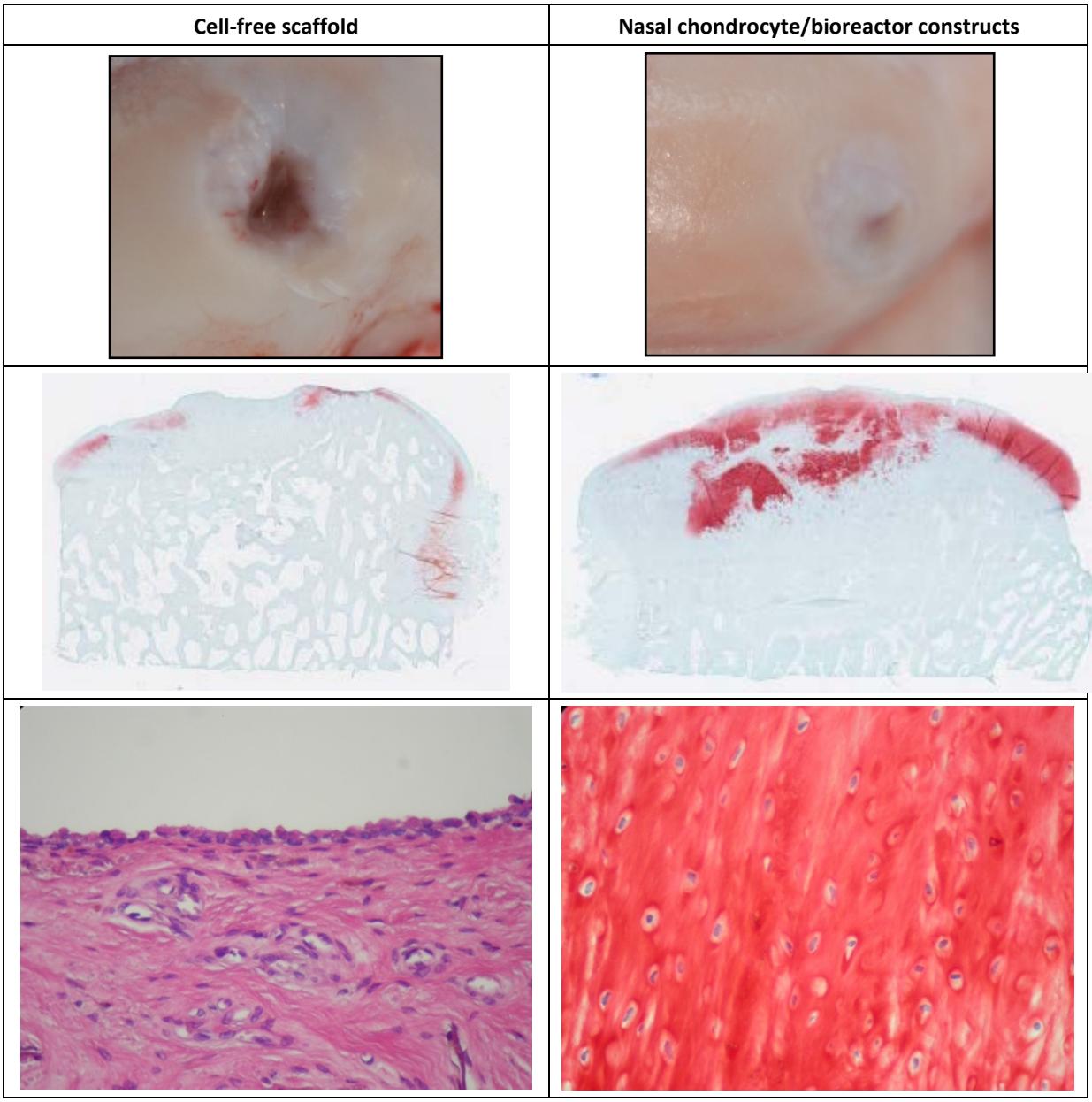

Figure 11: Second sheep study, 3 month time point. (top) macro images of repair sites; (middle) safranin-O staining of explants; (bottom) high magnification images showing native cartilage-like repair tissue in explants from bioreactor-generated constructs

Sheep from the 12 month time point were sacrificed in December 2015, and therefore explants will be analyzed outside the time frame of the BIO-COMET project.

Conclusion of sheep studies

The results obtained from both pre-clinical large animal studies indicate not only the feasibility of use of bioreactor generated nasal chondrocyte grafts, but also show superiority in terms of the quality of cartilage repair in a large animal model. The observed inflammatory response in the original animal study at early time points subsided with time and did not influence longer term cartilage healing. When the modified scaffold was used in the second large animal study, the inflammatory response was dramatically reduced. All the generated data are sufficient to support an application to regulatory bodies for future clinical trials.

Fibrin glue studies

In addition to the two main sheep studies, another sheep study was performed to evaluate *in vivo* biological effects of using fibrin glue for osteochondral scaffold fixation, both in terms of additional stability and physical protection against intra-articular environment. Thirty sheep were used for the study. All sheep were treated with cell-free scaffolds (defect size: 7 mm diameter, 5 mm depth) in both femoral condyles at the right knee, with standard technique. Group A: received scaffold implanted by press-fit only. Group B: the scaffold was implanted with autologous fibrin glue, obtained overnight by venous blood sample previously collected. Animal euthanasia was performed at 2, 4, 8 12 and 24 weeks of follow-up (N = 3 of each group at each follow-up) and all knees were explanted, carefully preserving joint integrity. Due to changes in regulations for conducting animal studies, the approval by the Italian National regulatory authority (Ministry of Health) was significantly delayed, and therefore the last group (time point 24 weeks) will be explanted at the very end of the project. Nevertheless, all others explants have been analyzed. While the osteochondral regeneration seems to be unaffected by fibrin, it appears that fibrin glue could have a potential protective effect in terms of synovial reaction to the implantation.

Application to the national authorities

As part of the application to the national authorities for approval of the clinical trial, an Investigator's Brochure (IB) must be prepared, compiling clinical and non-clinical data on the investigational product relevant to the study of the product in humans. A typical brochure includes information on dynamics (e.g., healing), kinetics (e.g., cell migration) and toxicology (e.g., local tolerance) from *in vitro* and *in vivo* studies (animal model) as well as clinical studies, if available. Therefore, we have compiled the results of BIO-COMET's large animal studies described above (to date), migration study, and tumorigenicity studies, which will be included in the Investigator's Brochure for application to national authorities for approval of a clinical trial.

Migration study

A study was performed to determine the potential for chondrocytes to migrate from an implanted engineered tissue into the surrounding tissues of a joint. Green fluorescent protein (GFP)-labelled autologous nasal and articular chondrocytes were seeded and cultured *in vitro* and subsequently implanted into full thickness cartilage defects generated in the trochlea of goats. Subsequent fluorescence-activated cell sorting (FACS) analysis of surrounding joint tissue to assess migration of GFP positive chondrocytes showed that the migration of cells into most surrounding joint tissues was not detected. Only a small percentage of cells could be detected in the fat pad. The data compiled is sufficient to address migration for inclusion within the Investigator's Brochure.

Tumorigenicity study

Due to the limited size of biopsies that can be clinically obtained, chondrocytes must be expanded *in vitro* in order to obtain a mature and functional cartilaginous tissue construct. Extensive proliferation is typically associated with cell de-differentiation. Even though de-differentiation is not synonymous of a carcinogenic process, a hypothetical concern can be addressed that these cells might become transformed in a rapid expansion culture environment. Therefore, to address these concerns, we have:

i.) Evaluated the tumorigenic potential of manufactured grafts based on a literature search of publications related to auricular or articular chondrocytes;
ii.) Performed an *in vivo* study with 3D pellet cultures (scaffold-free) to assess the tumorigenic potential of

in vitro cultured nasal chondrocytes;
iii.) Assessed the results to date of a UHBS clinical trial in which autologous engineered cartilage constructs have been implanted in a tumor site after tumor excision;
iv.) Outlined a tumorigenicity study plan to be conducted by UHBS after the results of the additional animal studies have been obtained.

Conclusion

The aim of this work has been to obtain sufficient pre-clinical data for inclusion in the Investigator's Brochure for the clinical trial. Collectively all the data generated in our *in vitro* studies and large animal studies showed excellent feasibility, efficacy and safety of the use nasal chondrocytes to repair cartilage defects. The generated data will be sufficient to be used for application for a clinical trial.

Work Package 6

Documentation for approval of a clinical trial

Due to the unexpected scaffold induced inflammation observed in our first large animal study, a second unforeseen and unplanned sheep study needed to be conducted. The additional activities associated with this second sheep study resulted in significant delays to and deviations in the original project time line and objectives. Since the final data from the second sheep study will not be available within the time frame of the BIO-COMET project, approval from the ethical committees and national authorities cannot be obtained and the clinical study initiated. Nevertheless, significant progress has been made to generate the key documents required to obtain approval of a clinical study. Documents have been drafted and agreed to a level from which they can be relatively easily updated and/or adapted for submission. The guidelines and regulations have been reviewed to ensure that the protocols and processes as defined are in accordance with the applicable laws. A draft of the clinical study protocol, a document required for approval by the Ethical Committee, has been prepared by BIO-COMET clinical partners.

Minimally invasive surgical kit

As described in work package 5, a minimally invasive surgical approach is preferred since there is less risk of altering joint homeostasis anatomically and biomechanically, and also to determine an unfavorable joint environment. Therefore, we aimed to initially to develop and later to modify an existing mini-invasive surgery kit that would be compatible with the BIO-COMET scaffold.

A mini-invasive surgical kit has been developed and a prototype manufactured. The prototype has been successfully tested in a cadaver using both mini-arthrotomy and arthroscopic surgical procedures. The results demonstrate the positive feasibility of a mini-invasive surgery using this kit for the engineered graft developed within BIO-COMET.

Work Package 7

Finally, in addition to our scientific, technical, and clinical activities, we are developing a strategic plan for the commercialization of the BIO-COMET cartilage graft and production system. Competitor products in the area of cartilage repair have been identified and other bioreactor cell culture systems on the market have been analyzed. Competitor price points have been summarized for products and treatment options within the same target market. The reimbursement landscapes in Europe and the USA have been investigated, and the reimbursement status of competitor products defined. Technological factors including graft properties and manufacturing issues have been assessed, including cost analyses of bioreactor-based

versus manual manufacturing, in addition to the BIO-COMET manufacturing process. Methods of potential cost reductions have been identified and a commercialization plan, including a clinical roll-out strategy, proposed.

Preparatory Actions for Commercialization

The main considerations for commercialization of the BIO-COMET bioreactor-manufactured cartilage graft have been addressed. Deliverables for this work package have highlighted key aspects of the commercialization plan and captured the implementation, marketing, sales and reimbursement strategies defined for this product.

Tissue-engineered technologies represent very attractive opportunities for improved therapies for the regeneration of damaged tissues and organs. However, many factors can impact the translation of tissue-engineered products from research, through clinical trials, to full commercialization. As products within the field of biotechnology take an average time to market of 10-15 years, have average development and production costs in the region of €0.7 billion, 90% failure rate by the clinical-trial stage, and a less than 30% chance of recouping development costs, the significance of the challenges is clear. Novel tissue-engineered products may have multiple elements contributing to risk during their commercialization. Careful consideration and analysis of the risk factors involved, and the mitigation of these challenges during development is critical.

Analysis of the target clinical indication and definition of the product target market has been a key activity of this work package. At the start of the project, a joint meeting of BIO-COMET's Clinical Advisory Board which includes external clinical advisors with expertise in orthopedics, cartilage repair and regenerative medicine, and the BIO-COMET Commercial Exploitation Committee took place to discuss clinical opportunities and potential limitations of a tissue-engineered cartilage graft. Several options for the target indication were proposed, and each was evaluated in terms of their relative technical and clinical risks as well as their potential impact level.

In order to moderate risk without detriment to the long term market potential of the graft construct, it was agreed that the target indication for the Phase I clinical trial would be *acute, symptomatic, isolated cartilage lesions on the femoral condyle, between 2–5 cm^2, ICRS-grade III-IV in adults (18-65* years). While manufacture of a larger graft (>3 cm diameter) would be likely to increase its market potential (as research conducted in this work package has shown), demonstration of feasibility and safety are the primary concerns for a Phase I clinical trial. It was noted that higher impact indications could be addressed later in clinical development, thereby enhancing the competitive position of the product.

Analysis of market potential and competitive landscape

Market analysis was conducted at regular intervals during the project to ensure that the Consortium was aware of new competitive products coming onto the market. This analysis highlighted the market potential for the BIO-COMET bioreactor-manufactured cartilage graft as well as describing the alternate options in the same market. As achieving reimbursement is a key aspect of clinical adoption on a large scale the reimbursement strategy was considered.

Competitor products in the area of cartilage repair were identified: those consisting of a cell suspension, a combination of cells and 3D scaffold, or acellular 3D matrices and membranes were all included. In addition, alternative cartilage repair methods such as osteochondral allograft, osteochondral autograft or debridement were also included when seeking to establish how competitive the BIO-COMET graft could be

compared to standard practice. Analysis of other bioreactor cell culture systems on the market showed that the majority of systems are designed only for suspension culture systems. Of the systems identified for 3D cell culture, none possessed all the features of the BIO-COMET bioreactor systems, e.g. tissue digest and bio-sensing.

The potential for tissue-engineered therapies remains highly attractive notwithstanding the risks involved in their development. Products in early stage development, such as the BIO-COMET cartilage graft, provide the opportunity for the barriers to commercialization to be considered and therefore to engineer innovative production systems and product characteristics that make it uniquely attractive to surgeons, patients and investors.

Technological factors influencing commercialization

We have also assessed the main technological advantages of the BIO-COMET graft in the context of its competing technologies, compared its projected price to competitors and considered how its unique production technology might be best utilized to manage production costs.

There are several key advantages associated with the BIO-COMET graft which have the potential to increase reproducibility, increase consistency and increase performance. The benefits of the BIO-COMET graft over its competitors were assessed in terms of both technical and regulatory advantages. A list of the relevant intellectual property was provided and a review of external IP performed to ensure that there is the freedom to practice. The IP portfolio from the combined BIO-COMET consortium is strong: this will be a barrier to competitors and will also support claims that this will be a superior next generation product for a potential partner.

Strategic business plan

Factors such as graft properties and manufacturing issues have been addressed, including cost analyses of bioreactor-based versus manual manufacturing, in addition to the BIO-COMET manufacturing process. For our strategic business plan, the market, implementation and reimbursement strategies were considered, and methods of potential cost reductions highlighted. Key conclusions of this plan were that while competition exists in the 1st phase target market, the potentially superior clinical function of a mature tissue-engineered graft will position the BIO-COMET graft as a next generation autologous chondrocyte implantation (ACI) product. Following this, the target indication could be expanded to either address markets with fewer competitive products or to treat defects which cannot currently be treated by the available ACI products.

While a very strong product offering can be achieved with the BIO-COMET graft, it will depend on positive pre-clinical and clinical results: successful completion of a Phase I trial will be important for achieving investment for later trials.

In order to be competitive, the implementation of approaches which reduce production costs, e.g., the use of a closed system in a production room with decreased environmental controls should be investigated as part of the process and bioreactor optimization. This will be necessary if the BIO-COMET graft is to achieve reimbursement and be a viable product.

While the primary focus of effort in these analyses has been in investigating the commercial prospects for a BIO-COMET graft, two partners have identified a market for spin-off products developed as part of their research.

PROJECT IMPACT

The expected impact of BIO-COMET will span from the clinical, scientific and technical sectors to the socio-economic and industrial areas, as detailed in the following sections.

Clinical impact

- The introduction of bioreactors in the manufacturing of engineered cartilage will open the possibility for scaling up and scaling out the processes while increasing safety, standardization and traceability;
- Cartilage repair based on reproducible and mechanically functional grafts, combined with a minimally invasive surgery, will reduce rehabilitation time and allow a more rapid return to normal life activities;
- The regeneration of a physiological, hyaline-like cartilage, as opposed to a less mechanically functional fibrocartilaginous tissue, will delay or eliminate the need for a prosthetic implant;
- These issues will then have a significant benefit both in terms of quality of life and reduction of healthcare costs;
- Large-scale functional grafts will be considered for the treatment of more advanced pathologies (e.g., osteoarthritis), in conjunction with surgical or pharmacological interventions aimed at resolving the etiology of the disease;
- Further scaling up of the generated grafts and extension of the subchondral layer will pave the way to the manufacture of customized biological prostheses.

Socio-economic impact

- The project will set the stage towards proving cost-effectiveness of cell-based cartilage repair techniques, which will in turn be essential to claim reimbursement by health insurances and/or social systems;
- The pioneering introduction of automated bioreactor systems in biomanufacturing will lead to tight interactions with the competent authorities and to the establishment of detailed guidelines in a field which is not yet clearly regulated;
- The project will exemplify the roadmap for a bioreactor-based translation of tissue engineering strategies into clinical products;
- The proof-of-principle in the context of cartilage repair will have an impact on the broad utilization and commercialization of cell-based grafts as therapeutic solutions for a variety of other indications (e.g., bone repair, epithelia reconstruction, etc.);
- The success of a few representative models of bioreactor-based delivery of tissue engineering into the clinic will accelerate commercial and healthcare provider interest.

Scientific and technical impact

- Retrospective correlation between the measured quality criteria of the graft and the pre-clinical outcome will be instrumental to investigate the mode of action of engineered cartilage tissues and to define the required properties for engineered cartilage grafts;
- Validation of in-line sensors for metabolic parameters will be relevant to standardize other cell culture systems;
- The 3D growth of functional cartilage tissues will be of interest as a system to model tissue formation/repair (e.g., to dissect biological pathways of development or to test specific drugs);
- The technologies and processes developed will be applied for a broad spectrum of other engineered products.

Steps required to achieve these impacts

There is no lack of medical discovery in the area of cell-based treatments, but there is a virtual absence of the technology platforms necessary to fully optimize the processes and enable translation toward clinical adoption. The partners of BIO-COMET are in this regard convinced that there will be no shortage of ways to collaborate on both technology and business to build and exploit the market opportunity. Thus, in order to effectively achieve the potential relevance and impact, the project critically requires the following competencies and activities to be brought together:

- Expertise and know-how in a variety of different interdisciplinary areas (e.g., engineering, biology, material science, medicine, management);
- State-of-the-art infrastructures and related trained personnel (e.g., prototype production, advanced industrial plant for material processing, large size animal facilities, GMP-accredited biomanufacturing facilities);
- Structured interactions between scientists/engineers/clinicians and the regulatory authorities;
- Structured project management, with maintenance of a cohesive focus;
- Structured management of intellectual property;
- Precise definition of instruments for visibility and dissemination.

From the considerations above, it is obvious that the scale and the breadth of an international action, bringing together the leading players in the different fields, is essential to reach the targeted goals. The participation of a non-European SME in the BIO-COMET consortium further reflected the need to recruit the globally most advanced groups in each sector, and give the potential of extending the exploitation plan beyond the European borders.

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DISSEMINATION ACTIVITIES

Dissemination activities within the BIO-COMET project have included:

- Organization of a workshop;
- Organization of a symposium;
- Organization of a conference
- Publication of a book chapter;
- Peer reviewed journal publications;
- Oral and poster presentations to scientific conferences and workshops, as well as lectures as part of instructional courses.

Details of all these publications and activities are recorded on the project website and below.

Workshop on 3D perfusion cell and tissue culture in basic research and clinical translation

Cellec hosted a half-day conference in April 2015 on 3D perfusion cell and tissue culture in basic research and clinical translation. This free event held in Basel, Switzerland attracted around 120 attendees from the scientific community and the media. Eleven invited speakers from nine affiliations presented perfusion bioreactor technologies.

In one session, various academic and industrial partners presented how they have successfully applied perfusion bioreactor technology within a wide spectrum of basic research projects. The other

session was dedicated to illustrating how automated bioreactor systems can be used to translate basic research into clinical applications.

ESSKA Lunchtime Symposium: BIO-COMET BIOreactor based Clinical Oriented Manufacturing of Engineered Tissues

The ESSKA (European Society for Sports Traumatology, Knee Surgery and Arthroscopy) Cartilage Committee organized a Consensus meeting in Verona, Italy in May 2015 to discuss early osteoarthritis and how it can be treated with biological solutions. World renowned orthopedic surgeons and researchers were invited to explore the topic through presentations and debates, including aspects of basic science, well-established treatments, through to future perspectives. Different treatments were examined from highly conservative perspectives through the use of stem cells, with the various therapeutic solutions currently available critically evaluated.

There were approximately 300 scientists (engaged in higher education and research) and clinicians in attendance. The audience was international, predominantly from Europe and the USA.

BIO-COMET organized a symposium during the conference to highlight the current challenges of bringing engineered cartilage grafts from the bench to the bedside and to present our bioreactor-based approach for the translation of engineered tissue grafts to the clinic. During the symposium, University Hospital Basel and University of Zagreb presented:

i) The project goals, objectives, and perspectives;
ii) Details of our bioreactor-based approach for manufacturing engineered tissue grafts; and
iii) The results of our pre-clinical model.

3rd International Conference on Regenerative Orthopaedics (ICRO): new insights in cartilage repair

The key findings of the BIO-COMET project were presented in detail at the 3rd International Conference on Regenerative Orthopaedics: new insights in cartilage repair. The conference was organized by the Croatian Academy of Sciences and Arts and the University of Zagreb's BIO-COMET partner in cooperation with the Section for Translational Medicine of the Croatian Orthopaedic Society. This one day meeting took place in November 2015 in Zagreb, and all of the BIO-COMET partners provided at least one speaker. The event attracted around 75 participants mainly from Europe, e.g., Croatia, Switzerland, Italy, Germany and the United Kingdom. Topics included animal models in cartilage research, imaging technologies in tissue engineering, nanocomposite scaffolds, bioreactor-based cartilage engineering and GMP facilities for bioreactor-based tissue engineering.

Bioreactors: enabling technologies for research and manufacturing: book chapter

Bioreactors: enabling technologies for research and manufacturing, M. Adelaide Asnaghi, Timothy Smith, Ivan Martin and David Wendt, in Van Blitterswijk & De Boer, Tissue Engineering 2nd Ed, December 2014, 2nd Ed., Elsevier, Netherlands, December 2014, ISBN: print book 9780124201453 ebook 9780124202108 (Chapter 12, pages 393-426).

Publications

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Scientific conferences and workshops

UHBS

1. **Bioreactor-based cartilage engineering**, David Wendt, at 3rd International Conference on Regenerative Orthopaedics and Tissue Engineering (ICRO): new insights in cartilage repair, 4 November 2015, Zagreb, Croatia (*Oral presentation*)
2. **Bioreactor-based manufacturing of engineered cartilage grafts: paradigm validation in a large animal study,** David Wendt, at World Conference on Regenerative Medicine, 21 October 2015, Leipzig, Germany (*Oral presentation*)
3. **Bioreactor-based manufacturing of engineered cartilage grafts: paradigm validation in a large animal study**, David Wendt, at TERMIS 2015, 4th World Congress: Past, present future: the evolution of regenerative medicine, 8-11 September 2015, Boston, USA (*Poster*)
4. **Controlled pH through automated medium exchange supports chondrogenic capacity of human nasal chondrocytes**, M. Adelaide Asnaghi, at TERMIS 2015, 4th World Congress: Past, present future: the evolution of regenerative medicine, 8-11 September 2015, Boston, USA (*Poster*)
5. **BIO-COMET Pre-clinical model**, Ivan Martin, at ESSKA Consensus Meeting's Lunch Symposium - Early osteoarthritis: all around biological solutions, 21-22 May 2015, Verona, Italy (*Oral presentation*)
6. **BIO-COMET Bioreactor system**, David Wendt, at ESSKA Consensus Meeting's Lunch Symposium - Early osteoarthritis: all around biological solutions, 21-22 May 2015, Verona, Italy (*Oral presentation*)
7. **Bioreactor-based approach for cartilage repair**, David Wendt, at Cellec Workshop: 3D perfusion cell and tissue culture in basic research and clinical translation, 15 April 2015, Basel, Switzerland (*Oral presentation*)
8. **Bioreactor-based, clinically oriented manufacturing of engineered tissues**, Ivan Martin, EU Commission Forum in association with MiME (Materials in Medicine), 8-11 October 2013, Faenza, Italy *(Oral presentation)*
9. **A tissue therapy model using nasal chondrocytes and bioreactor**, Ivan Martin, 11th World Congress of the International Cartilage Repair Society (ICRS), 15-18 September 2013, Izmir, Turkey *(Oral presentation)*
10. **Functions of bioreactors in the context of scaffold-based skeletal tissue engineering**, David Wendt, Summer School on Tissue Engineering and Regenerative Medicine, 8-12 July 2013, Riva del Garda, Italy *(Workshop)*

UZagreb

11. **Novel technology for articular cartilage repair-preclinical animal model**, Andreja Vukasović, at 3rd International Conference on Regenerative Orthopaedics and Tissue Engineering (ICRO): new insights in cartilage repair, 4 November 2015, Zagreb, Croatia (O*ral presentation*)
12. **Bioreactor-based tissue engineering in sports medicine**, Alan Ivković, at 5th International Congress of Sports Medicine, 23-25 October 2015, Maribor, Slovenia (*Oral presentation*)
13. **Bioreactor-based cartilage engineering**, Alan Ivković, at 24th Congress of the European Association of Tissue Banks, 1-3 October 2015, Split, Croatia (*Oral presentation*)

14. **Differences in morphology of articular and nasal septum cartilage harvested for use in bioreactor-based tissue engineering**, Alan Ivković, at 10th Biennial ISAKOS Congress (International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine), 7-11 June 2015, Lyon, France (*Poster*)
15. **Structural analysis of autologous osteochondral graft engineered in bioreactor in an ovine animal model**, Andreja Vukasović, at PhD Day University of Zagreb, School of Medicine, 22 May 2015, Zagreb, Croatia (*Poster*)
16. **Bioreactor-engineered cartilage graft for osteochondral knee lesion – study in sheep**, Andreja Vukasović, at SEEFORT – the SouthEast European Forum on Orthopaedics and Traumatology, 23-25 April 2015, Dubrovnik, Croatia (*Poster*)
17. **Cytokine expression in synovial membrane of sheep knee treated with tissue engineered osteochondral graft**, Maja Pušić, at SEEFORT – the SouthEast European Forum on Orthopaedics and Traumatology, 23-25 April 2015, Dubrovnik, Croatia (*Poster*)
18. **Bioreactor-based cartilage engineering**, Alan Ivković, at SEEFORT – the SouthEast European Forum on Orthopaedics and Traumatology, 23-25 April 2015, Dubrovnik, Croatia (*Invited speaker*)
19. **Regenerative orthopaedics and tissue engineering: current possibilities for clinical translation**, Alan Ivkovic, at 3rd Belgian Symposium on Tissue Engineering (BSTE), 19-20 March 2015, Leuven, Belgium (*Oral presentation*)
20. **Optimization of perfusion system for bone tissue growth on peptide hydrogel**, Panek M, Pribolsan L, Gotic M, Caput Mihalic K, Vukasovic A, Alan Ivkovic, Jurkin T, Marijanovic I, at 2014 TERMIS-AM Meeting, 13-16 December 2014, Washington, USA *(Abstract and poster)*
21. **Nose or knee? Morphological analysis of articular and nasal septum cartilage harvested for bioreactor-based tissue engineering**, Vukasovic A, Kostesic P, Hudetz D, Maticic D, Pecina M, Ivkovic A, at XXVI SICOT Triennial World Congress, 19-22 November 2014. Rio de Janeiro, Brazil *(Poster and abstract)*
22. **Ultrastructural analysis of sheep articular and nasal septum cartilage harvested for osteochondral tissue engineering in bioreactor**, Andreja Vukasovic, Kostesic P, Hudetz D, Maticic D, Pecina M, Ivkovic A, at 16th ESSKA Congress, 14-17 May 2014, Amsterdam, Netherlands *(Poster)*
23. **Evidence based evaluation of surgical cartilage repair technique**, Alan Ivkovic, at Ankara Cartilage Symposium, 25–26 April 2014, Ankara, Turkey *(Oral presentation)*
24. **Biphasic collagen - hydroxyapatite scaffold for treatment of osteochondral lesions of the knee – preliminary results of the sheep study**, Andreja Vukasovic, Kostesic P, Gusak V, Maticic D, Hudetz D, Jezek D, Pecina M, Ivkovic A, at ICRS Focus Meeting 2013 on Stem cells and scaffolds: a new frontier for joint regeneration, 5-6 December 2013, Bologna, Italy *(Poster)*
25. **Biosurgery in articular cartilage repair: knee a la Carte**, Alan Ivkovic, at Croatian Orthopaedic Society Symposium, 26 October 2013, Porec, Croatia (*Oral presentation*)
26. **Morphology of sheep articular and nasal septum cartilage for tissue engineering in bioreactor**, Vukasovic A, Kostesic P, Gusak V, Maticic D, Jezek D, Hudetz D Pecina M, Alan Ivkovic, at 11th World Congress of the International Cartilage Repair Society, 15-18 September 2013, Izmir, Turkey *(Poster)*

27. **Comparison between two ICRS histology scoring systems for cartilage repair**, Andreja Vukasovic, at 2nd International Conference on Regenerative Orthopaedics and Tissue Engineering (ICRO): new insights in cartilage repair, 21-22 September 2012, Opatja, Croatia *(Poster)*

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28. **Innovative treatment of cartilage lesions**, Elizaveta Kon, at 10th Biennial ISAKOS Congress (International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine), 7-11 June 2015, Lyon, France (*Lecture as part of an Instructional Course)*
29. **Osteochondral regeneration: a new MRI evaluation score MOCART-OC**, Giuseppe Filardo, at 12th World Congress of International Cartilage Research Society (ICRS), 8-11 May 2015, Chicago, USA (*Free paper*)
30. **Failures of osteochondral scaffold implantation in the knee: influencing factors and how to deal with surgical revision**, Francesco Perdisa, at 12th World Congress of International Cartilage Research Society (ICRS), 8-11 May 2015, Chicago, USA (*Poster*)
31. **Arthroscopic MACT for cartilage defects of the knee: prospective study at minimum 10 years of follow-up**, Elizaveta Kon, at American Academy of Orthopedic Surgeons (AAOS) Annual Meeting 2015, 24-28 March 2015, Nevada, Las Vegas, USA *(Poster)*
32. **Cell-free biomimetic scaffold for osteochondral defects: a prospective clinical study at 72 months of follow-up**, A. Di Martino, E. Kon, G. Filardo, L. Andriolo, F. Perdisa, A. Sessa, and M. Marcacci, at ESSKA World Meeting 2014, 14–17 May 2014, Amsterdam, Netherlands *(Poster)*
33. **Edema in matrix-assisted autologous chondrocyte transplantation: MRI evaluation at different follow-up times**, Francesco Perdisa, G. Filardo, E. Kon, A. Di Martino, F. Tentoni, M. Busacca and M. Marcacci, at ESSKA World Meeting 2014, 14–17 May 2014, Amsterdam, Netherlands *(Free paper)*
34. **Improvement of osteochondral scaffold fixation: mechanical stability evaluation in a human cadaver knee model**, Giuseppe Filardo, F. Perdisa, M. Drobnic, D. Ravnik, E. Kon and M. Marcacci, at ESSKA World Meeting 2014, 14–17 May 2014, Amsterdam, Netherlands
35. **Use of a three-layered biomimetic one-step scaffold to treat osteochondral lesions of the patella. Prospective study at 2 years of follow-up**, Elizaveta Kon, G. Filardo, V. Condello, C. Zorzi, A. Di Martino, F. Tentoni, L. Andriolo and M. Marcacci, at ESSKA World Meeting 2014, 14-17 May 2014, Amsterdam, Netherlands *(E poster)*
36. **Edema in matrix-assisted autologous chondrocyte transplantation: MRI evaluation at different follow-up times**, F. Perdisa**,** Giuseppe Filardo, E. Kon, A. Di Martino, F. Tentoni, M. Busacca and M. Marcacci, at 11th World Congress of the International Cartilage Repair Society, 15-18 September 2013, Izmir, Turkey *(Free paper session)*
37. **Patient profiling in cartilage regeneration: mid-term results and prognostic factors of MACT. A multicenter study**, Giuseppe Filardo, at 11th World Congress of the International Cartilage Repair Society, 15-18 September 2013, Izmir, Turkey *(Poster)*

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38. **Advanced bioreactors for application in clinical medicine**, Timothy Smith, at 3rd International Conference on Regenerative Orthopaedics and Tissue Engineering (ICRO): new insights in cartilage repair, 4 November 2015, Zagreb, Croatia (*Oral presentation*)

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39. Can we measure cartilage repair?, Anthony Hollander, at International Cartilage Repair Society Focus Meeting, 3-4 July 2014, Zurich, Switzerland *(Invited speaker)*

Theses (in progress)

1. **Structural analysis of bioreactor–engineered autologous cartilage graft for treatment of osteochondral defects in sheep model**, Andreja Vukasović, School of Medicine
2. **µCT and histological analysis of subchondral bone changes in a chronic osteoarthritic knee joint in a sheep model**, Petar Kostešić, Faculty of Veterinary Medicine
3. **Evaluation of intraarticular inflammatory markers following cartilage reconstruction with autologous osteochondral graft engineered in a perfusion bioreactor**, T. Petrović, School of Medicine

Other activities

1. **Wiederherstellungschirurgie**, TV Interview (in German), Ivan Martin, Telebasel 15 November 2013, Basel, Switzerland http://www.telebasel.ch/de/tv-archiv/&id=366845061 *(TV interview)*
2. **Hrvatska postaje centar izvrsnosti za podrucje tkivnog inzenjeringa - Croatia is a becoming centre of excellence in the field of tissue engineering**, Alan Ivkovic, *in* Zdravstveni vodic (Health Guide), December 2013 (article for the lay public).
3. **Startup Competition for Young Entrepreneurs,** 250 participants. Cellec. Awarded Young Entrepreneurs Prize NW Switzerland 2014
4. **Presentations to potential investors,** Cellec, in Switzerland
5. **Taking tissue engineering to the next level**, PreSens, October 2013 (*Flyer).*

PROJECT WEBSITE

The project website is at http://www.bio-comet.eu

PROJECT LOGO

CONTACT DETAILS

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