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Implementation of an Automated Manufacturing Platform for Engineering of Functional Osteochondral Implants

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Abstract

The EU Horizon 2020 project »JointPromise« proposes the development and implementation of an end-to-end automated production platform for three-dimensional joint implants, paving the way for tissue-engineered implants able to regenerate deep osteochondral defects. Currently, the manufacturing pipeline consists in manual production processes for microtissue cultivation, harvest and bioassembly into larger implants. In the conceptualizing stage of this project, the manual processes were translated into standard operating protocols (SOPs) and process design criteria like material flow and throughput as well as technical specifications of laboratory devices for an automated performance were elaborated.
Spheroid-based implants provide a novel approach in tissue engineering by aggregating progenitor cells into potent microtissues. After the differentiation of cartilaginous microtissues, functional joint implants are assembled via 3D bioprinting to match the complex structural organization of native cartilage tissue. The »JointPromise« platform includes suitable devices for cell and microtissue cultivation, harvest and implant production as well as quality control in an overall layout consisting of according pipetting units, incubator, centrifuge, bioprinter and high-speed microscope. After initiating the platform build-up, a control software for process controlling and monitoring during cell seeding, cultivation and harvest is implemented. Clinical feasibility and efficacy of osteochondral defect regeneration by the produced joint implants will subsequently be proven in large animal models.
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Keywords: Automation; Osteoarthritis; Tissue Engineering; Bioprinting; Stem Cells; Regenerative Medicine

1. Introduction

Regenerative medicine (RM) provides novel therapies to meet the rising demand for medical interventions due to the economic and societal burden of an ageing world population [1]. The 2017 Global Burden of Disease survey reported over 300 million cases of osteoarthritis (OA), one of the most prevalent chronic joint diseases worldwide resulting in predominantly progressive articular cartilage and subchondral degeneration [2,3,4]. While conventional therapy approaches consist of proper disease management including long-term pharmacotherapy for pain relief, end stage disease requires

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whole joint replacement surgery to retrieve mobility and reduce pain; Tissue engineered therapies of RM could enable the treatment of such life-constraining disabilities in the near future [2,5].

RM approaches such as restoring the bone-cartilage unit, are promising strategies to prevent the development and progression of the OA disease [4]. Manufacturing such living implants remains a challenge. Developmental engineering strategies starting from the aggregation of progenitor cells (or stem cells) in suspension and non-adherent platforms result in microtissues with the ability to differentiate autonomously and thereby trigger native regeneration cascades after implantation [6,7,8,9]. Additionally, the complex structural organization of native osteochondral tissue can be mimicked by 3D bioprinting of cartilage-like microtissues in living joint implants [10]. The production of Tissue Engineered advanced therapy medicinal products (ATMP) is currently based on research manual laboratory scale protocols, giving rise to risks of contamination, inconsistent product quality, high personnel expenses and lack of scalability. To minimize failure sources due to human errors and thereby enhance the productivity as well as generate a scalable, reproductive reliable process for the production of living osteochondral implants, »JointPromise« set out to develop an automated, GMP-compliant manufacturing platform.

Nomenclature

ATMP	Advanced Therapy Medicinal Product
CAD	Computer-Aided Design
GMP	Good Manufacturing Practice
HEPA	High-Efficient Particulate Air
HSM	High-Speed Microscope
PLC	Programmable Logic Controller
RM	Regenerative Medicine
SOP	Standard Operating Procedure
URS	User Requirement Specification

2. Conceptual platform design

2.1. Establishment and translation of SOPs

The conceptualizing stage of the production platform initiated with the translation of standard operating procedures (SOPs) for cell cultivation and harvest into automated process steps. Manual SOPs for chondrocyte differentiation out of progenitor cells from biopsy were elaborated based on the approach to mimic the structural complexity of native joint tissue. By producing progenitor cell aggregates which subsequently mature into microtissues, complex joint implants can be then assembled bottom-up with multi-step 3D bioprinting of microtissue-loaded bioinks.

The biomimetic multizonal joint implant is composed of transient cartilage (subchondral bone part) as well as stable, articular cartilage (joint surface cartilage) zones made of potent microtissue populations (see Fig. 1). The volume of each zone is crucial for the definition of the number of microtissue required per zone and will affect the structure of the bioprocess. Starting from the bottom, the presumptive bone zone consists of hypertrophic chondrocyte microtissues and provides ~80% of the implant volume (Fig. 1, i). This zone will give rise to bone through endochondral ossification for subchondral bone development which will be vascularized due to the end stage maturation of the tissue releasing proper biological cues and to improve integration with the host [9]. Next, a ~200 µm biofilm of prehypertrophic chondrocyte microtissues is deposited for the formation of a stable tidemark (ii) in conjunction with an articular cartilage layer composed of stable chondrocyte microtissues (iii). Finally, a single cell layer of surface chondrocytes is deposited on the implant surface to provide a lubricant layer (iv).

The establishment of SOPs for cell seeding, cultivation and harvest defined process design criteria regarding vessel volumes, material flow and the required throughput of the production platform. Following the SOPs, about 1200 microtissue spheroids can be produced within 21 days of culture out of 1 mL cell suspension per tissue culture plate. To reach the required productivity of around 100 tissue culture plates per implant, the production platform will need to process around 70 L of liquids during seeding and harvest processes and 5 L per cell media change to produce around 2.8M microtissue spheroids in 21 days. Combined with the technical requirements of the according laboratory devices for the automated performance of the elaborated SOPs, specifications for each device of the platform were elaborated in the User Requirements Specification (URS).

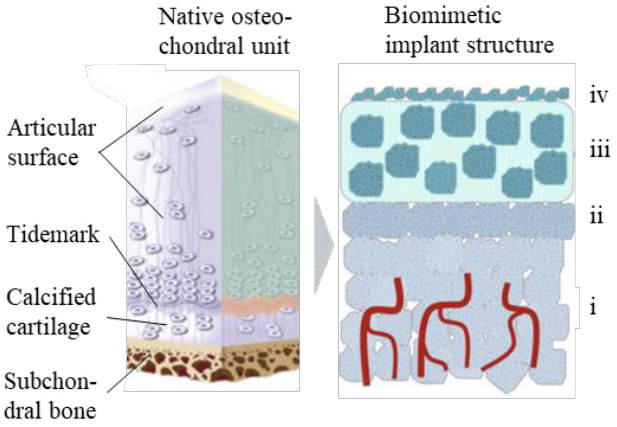

Fig. 1: Layers of the native osteochondral unit (left) and biomimetic implant structure (right) with presumptive bone zone of hypertrophic chondrocyte microtissues (i), biofilm of prehypertrophic chondrocyte microtissues (ii), articular cartilage layer of chondrocyte microtissues (iii) and single cell lubricant layer of surface chondrocytes (iv)

2.2. 2D platform layout

The previously described translation of SOPs into automated process steps results in the definition of the required technical specifications in the URS. A 6-axis robotic arm is required for the transport of the cell culture vessels and disposables to each device implemented in the production platform. To enable seeding of the cell aggregates according to the SOPs for cell cultivation and harvest and maintain a constant cell culture environment, a centrifuge and incubator

are incorporated. The liquid handling unit functions as an automated pipetting system for cell cultivation process steps such as rinsing, cell seeding, media change and microtissue harvest in falcon tubes and tissue culture plates. As the maximum pipetting volumes in liquid handling units are limited to 5 mL, larger liquid volumes have to be processed by an automated serological pipette with capacity of up to 50 mL for pipetting into larger vessels in order to maximize throughput. To translate manual interaction required for removing caps of disposables as falcon tubes or centrifugation bottles into an automated process chain, a modular decapping system needs to be developed. An automated quality control of the produced microtissues is implemented by a high-speed microscope (HSM). The HSM detects images in motion, enabling full well scans as well as a higher throughput in comparison to manual imaging. With image stitching and analysis, cell quality parameters such as aggregate size and distribution can be automatically evaluated via an own-written software. [11] A 3D bioprinter will finally be utilized for the assembly of functional living 3D joint implants out of previously cultivated cell aggregates mixed with biocompatible bioinks.

All above-mentioned platform components are then arranged in a 2D platform layout (see Fig. 2). The first approach of the platform layout was based on previous experience in automated cell production on the AUTOSTEM platform [12] and provided two interlinked areas with different clean room grades for enclosed or open cell processing according to SOPs and GMP compliance. The left side of the central gate containing the bioprinter and the control cabinet was planned to be located outside of the housing. All remaining platform devices were planned to be located on the right side of the central gate inside a defined clean room environment.

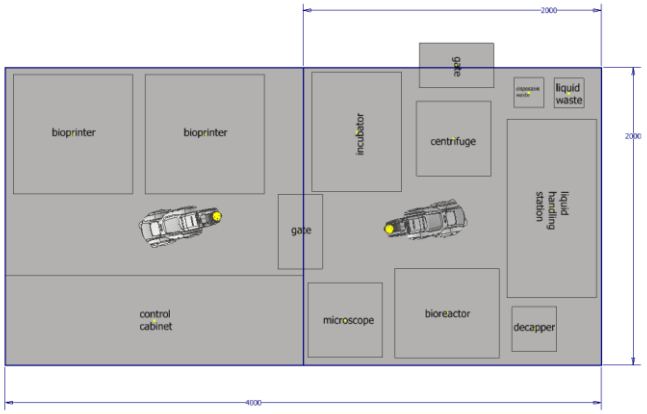

Fig. 2: First »JointPromise« 2D platform layout approach (2020)

2.3. Material flow and 3D platform model

Based on the first approach of device arrangement for the »JointPromise« production platform in the 2D layout, modifications in the process and device requirements were elaborated to optimize the platform design and productivity. As the 3D bioprinter was subsequently defined to contain an internal transportation, the left robotic arm is obsolete. In preliminary workshops, the processes of the bioreactor were modified to be carried out in the incubator. The resulting space is replaced by automated serological pipettes for large liquid volume handling as well as required disposable storage, liquid and solid waste containers. Regarding the arrangement of devices, two main changes were implemented: The incubator will be located next to the liquid handling unit as the new Hamilton Microlab Vantage system offers gripping systems for transferring externally provided tissue culture plates into the pipetting area. That way, plate transfer from the transfer position of the incubator to the liquid handling unit can be carried out without the robotic arm to parallelize process steps. Second, the accessibility of the centrifuge for maintenance purposes as well as disposable and waste storage for manual loading and emptying requires access on two platform sides. As the centrifuge can be loaded via a hatch at the top of the device, positioning below the platform tabletop provides a space-saving arrangement.

The above-mentioned considerations and amendments were implemented in the optimized arrangement of devices in the 3D layout. The CAD model of the »JointPromise« production platform combines all devices for cell cultivation, microtissue harvest and ATMP production in an overall layout (see Fig. 3).

A control cabinet containing programmable logic controllers (PLC) for electrical actuation of all devices as well as a housing providing hygienical environment standards and safety compliance complete the »JointPromise« platform. High-efficient particulate air filtration (HEPA) as well as hydrogen peroxide gassing for decontamination in the platform housing provide a safe, GMP-compliant production environment closed off from the human operator. Integrated gates for human interaction below the platform tabletop not only enable essential maintenance interactions, but also liquid and solid waste extraction and loading of process disposables to minimize contamination risks by human interaction in the production area above the tabletop.

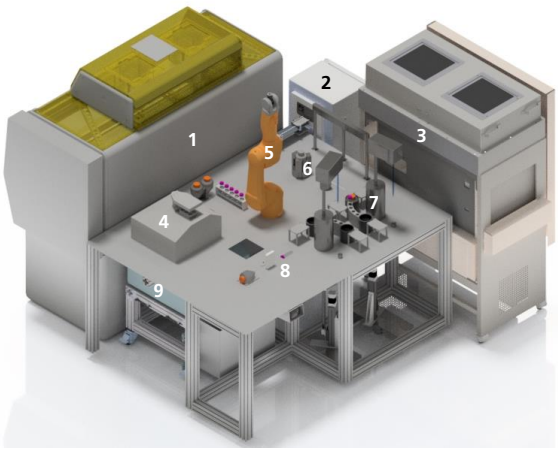

Fig. 3: Current »JointPromise« CAD model including liquid handling unit (1), incubator (2), 3D bioprinter (3), high-speed microscope (4), 6-axis robotic arm (5), decapper (6), automated serological pipettes (7), disposable depots and waste (8), centrifuge (9)

The selection of commercially available device options was based on the SOPs, throughput and GMP compliance, while the layout was defined through the material flow during cell seeding and aggregate cultivation (see Fig. 4a) and microtissue harvesting (b).

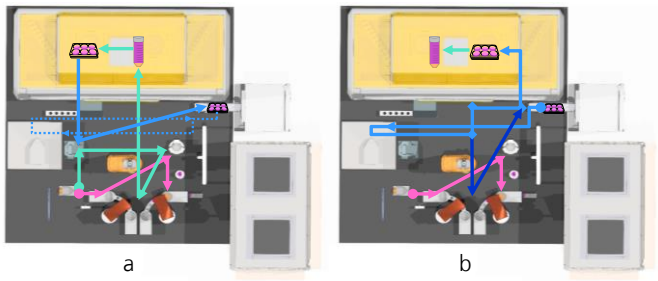

Fig. 4: Material flow visualization on top view of the »JointPromise« platform CAD model for cell seeding and aggregate cultivation (a) and microtissue harvesting (b). Pink lines for cell medium pathways, green lines for cell suspension, blue lines for seeded cells on tissue culture plates, dashed lines for quality control and dark blue lines for large liquid volume pathways

Overall, the requirements result in the following list of devices:

Table 1. Device list for the »JointPromise« automated production platform

Platform component	Manufacturer	Product line
Liquid handling unit	Hamilton Robotics Bonaduz, Switzerland	Microlab Vantage
Incubator	LiCONiC Instruments Mauren, Liechtenstein	StoreX
3D bioprinter	Poietis Pessac, France	Custom solution
High-speed microscope	Fraunhofer IPT Aachen, Germany	Custom solution
6-axis robotic arm	Stäubli International AG Pfäffikon, Switzerland	TX2 Stericlean
Decapper	Fraunhofer IPT Aachen, Germany	Custom solution
Automated serological pipette	Fraunhofer IPT Aachen, Germany	Custom solution
Disposable depots and liquid/solid waste	Fraunhofer IPT Aachen, Germany	Custom solution
Centrifuge	Sigma Laborzentrifugen GmbH Osterode, Germany	4-16KRL

The previously developed control software COPE (Control Operate Plan Execute) ensures a flexible connectivity of the devices, allowing to control and monitor processes of cell seeding, cultivation, harvest and bioprinting. Hardware modules are integrated into the executive control software architecture via agents following a plug-and-produce collaborative approach overcoming the diversity of vendor-dependent communication protocols and interfaces in commercially available devices. [13]

3. Conclusion and outlook

»JointPromise« aims to develop and implement an automated manufacturing platform for microtissue-based living implants for the regeneration of deep osteochondral joint defects. The automated cell production provides the required complexity and productivity to meet the rising demand of novel RM therapy approaches.

The implementation of the automated, GMP compliant production platform is bases on established SOPs and their translation into automated process steps. By combining resulting process design criteria with technical specifications, the device requirements were elaborated in the URS. The material flow according to the SOPs resulted in an initial 2D platform design based on previous experience in automated cell production. Final arrangement of devices was optimized in the overall 3D CAD model of the production platform completed by a control cabinet and housing for a defined hygienic environment and a recommended device list for GMP-compliant production was elaborated.

The initiated build-up of the »JointPromise« platform is followed by the implementation of the control software COPE for process controlling and monitoring during cell seeding, cultivation and harvest. Characterization of the resulting joint implants will be carried out with multiple technologies utilizing metabolomics.

Acknowledgements

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References

[1] Khademhosseini A, Langer R. Nat Protoc. 2016;11(10):1775-81.
[2] Bijlsma JW, Berenbaum F, Lafeber FP. Lancet. 2011;377(9783):2115-26
[3] Global Burden of Disease Study 2017. Institute for Health Metrics and Evaluation (IHME), Seattle, USA
[4] Lories RJ, Luyten FP. Nat Rev Rheumatol. 2011;7(1):43-9
[5] Cram P, Lu X, Kates SL, Singh JA, Li Y, Wolf BR. 1991-2010. JAMA. 2012;308(12):1227-36
[6] Sasai Y. Cell Stem Cell. 2013;12(5):520-30
[7] Fordham RP, Yui S, Hannan NR, Soendergaard C, Madgwick A, Schweiger PJ, et al. Cell Stem Cell. 2013;13(6):734-44
[8] Lenas P, Moos M, Luyten FP. Tissue Eng Part B Rev. 2009;15(4):381-94
[9] Nilsson Hall G, Mendes LF, Gklava C, Geris L, Luyten FP, Papantoniou I. Adv Sci (Weinh). 2019;7(2):1902295
[10] Hall GN, Tam WL, Andrikopoulos KS, Casas-Fraile L, Voyiatzis GA, Geris L, Luyten FP, Papantoniou I. Biomaterials. 2021;273:120820
[11] Schenk FW, Brill N, Marx U, Hardt D, König N, Schmitt R. Scientific reports. 2016;6(1):34038
[12] Ochs J, Barry F, Schmitt R, Murphy JM. Bioinsights. 2017;3(8):739-748
[13] Jung S, Ochs J, Kulik M, König N, Schmitt RH. Procedia CIRP. 2018;72:1245-1250