Final report Project 3775

A modular fluid flow bioreactor system to investigate the role of oxygen tension in tumor invasion and metastasis

PD Dr. Marietta Herrmann

Introduction

The state of reduced O_2 availability or the drop of O_2 tension below critical thresholds is described as hypoxia and associated with many pathological conditions. O_2 tension has a significant impact on most mammalian cells, causing transcriptional changes eventually affecting cellular functions (such as differentiation state, migration, secretory functions) and viability. Still, most *in vitro* cell culture experiments are performed under atmospheric O_2 tension (160 mm Hg), which markedly exceeds the O_2 partial pressure in most tissues. The expansion of cells in O_2 and nutrient rich environments leads to metabolic adaptation of cells, in particular to a shift from glycolysis to oxidative phosphorylation (OXPHOS) with implications for cellular functions as it is well established for mesenchymal stromal cells (MSCs), an important component of the bone metastatic tumor niche [Yuan, 2019]. This highlights the importance to consider oxygen tension for the development of physiological relevant model systems. Moreover, many primary tumors and bone metastasis contain hypoxic lesions, i.e. regions of reduced O_2 tension compared to the tissue surrounding the tumor [Gilkes, 2016]. Since hypoxia has been associated with poor survival in tumor patients [Vaupel 2009], it is evident that O_2 tension has a critical influence on tumor cell malignancy including their proliferation, invasion and metastatic properties [Gilkes, 2016; Zeng, 2015]. Metastasis and the presence of disseminated cancer cells are indicative for the cancer reoccurrence rate and mortality [Gilkes, 2016], but often not considered in studies of the primary tumor site.

Most conventional 2D and static cell culture models are not able to simulate the complex tumor environment at primary and metastatic sites and do not consider the role of O_2 tension, thus their translational value is limited.

Objectives of the project

In this collaborative project with the Chair for Tissue Engineering and Regenerative Medicine (TERM) and Fraunhofer ISC, we aimed to develop a fluid flow bioreactor which allows to expose different 3D tumor models to various O_2 levels. The project was based on existing bioreactor technology [Schuerlein, 2017] and established 3D tumor models based on co-cultures of tumor cell lines and stroma cells in biological decellularized intestinal scaffolds (*small intestinal submucosa + mucosa*, SISmuc) [Nietzer, 2016]. Another aim of the project was to provide a bioreactor platform to study tumor cells in a bone metastatic environment based on decellularized human bone scaffolds, dBone, recently established by the applicant [Pereira, 2020].

Results

I. Dynamic culture system for decellularized human bone scaffolds (dBone) and increasing cellular complexity in dBone scaffolds.

Our group recently established a protocol for decellularization of human femoral head trabecular bone specimens obtained from patients undergoing hip replacement surgery [Pereira, 2020]. The resulting scaffolds, referred to as dBone scaffolds, retain the physiological organic extracellular matrix composition and morphology of trabecular bone and thus present a culture substrate for bone residing cells that closely mimics the native cell environment. Mechanical loading is another characteristic feature of bone tissue and bone cells are highly qualified to respond and adapt to mechanical alterations. Thus, we here aimed to develop a bioreactor system suitable for dynamic culture of dBone scaffolds. We successfully applied a bioreactor system where dBone scaffolds could be cultured under continuous perfusion as well as cyclic compressive loading (Fig. 1A). In the recently published manuscript [Pereira, 2021], in silico modeling (Fig. 1B) was applied to model mean and local shear

stress parameters. Furthermore, the work highlighted an early adaptation of reseeded MSCs to the dynamic parameters. Overall, these results presented an important step towards the development of dynamic fluid flow culture model of bone metastasis.

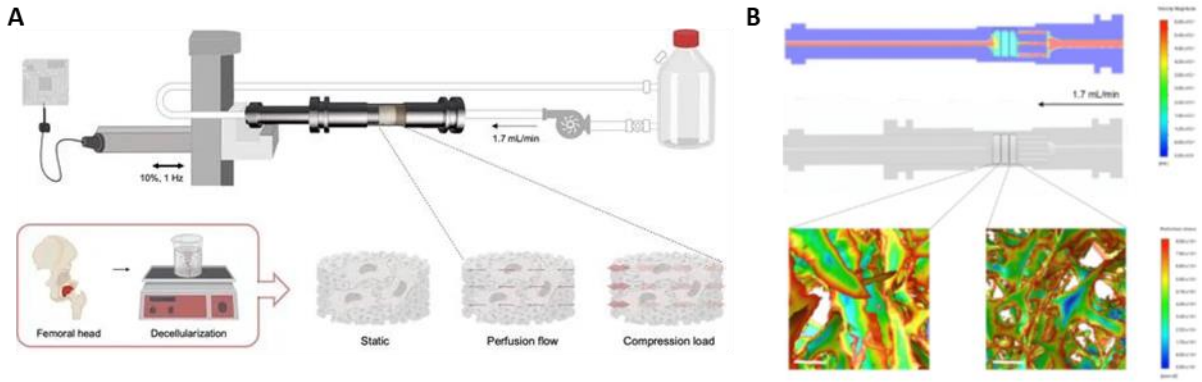

Figure 1. Dynamic culture system for dBone scaffolds [Pereira, 2021]. A. Schematic representation of the bioreactor platform. Three dBone scaffolds, reseeded with MSCs, are exposed to continuous fluid flow and mechanical stimulation by cyclic compression. Static cultures were performed as control. **B.** *In silico* modeling revealed a mean shear stress of 8.5 mPa that was evenly distributed in all three dBone scaffolds (top). In depth analysis of shear stress parameters revealed however high variations in shear stress dependent on the local trabecular morphology (bottom).

In order to further develop our culture system towards a physiological relevant bone metastasis model, we next aimed to include additional bone residing cells, namely osteoclasts, in our model system (Fig. 2). In parallel, co-cultures between hMSC and breast cancer cell lines, as well as triple-cultures of hMSC, osteoclasts and breast cancer cells were successfully established in SISmuc models.

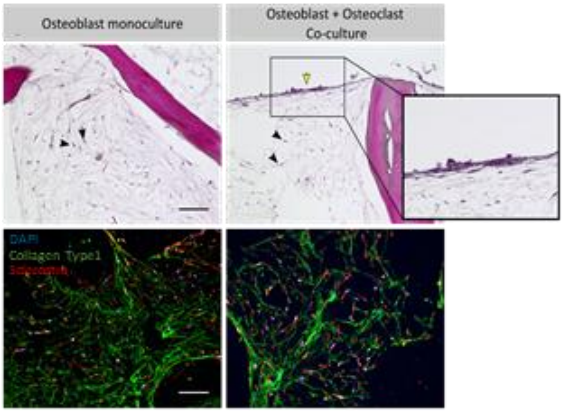

Figure 2. Co-cultures of MSC and osteoclasts in dBone scaffolds. hMSCs grow and differentiate into osteoblasts when cultured on decellularized bone; paraffin sections were stained with hematoxylin and eosin for histological analysis, and dapi, anti-sclerostin and anti-collagen I for immunohistochemical analysis. **Left panel:** dBone seeded with hMSCs differentiated into osteoblasts Right panel: Co-culture with osteoclasts, large cells can be found on top of the outer layer of hMSCs on the surface of the scaffolds; scale bars: 10 mm and 100 µm (Master thesis Lisa Bauer 2021).

II. Implementation of hypoxic culture conditions in a 3D dynamic fluid flow bioreactor system

In parallel to the step-wise establishment of dynamic culture conditions and cell co-culture in dBone scaffolds, we established a bioreactor chamber allowing to perform dynamic culture conditions under low oxygen tensions. The aim was to develop a system that would allow to integrate different previously established dynamic bioreactor systems, in particular the above-mentioned system for dBone scaffolds as well as the well-established SISmuc system [Nietzer, 2016].

The developed system is illustrated in Figure 3. It consists of a hypoxia chamber fitting different bioreactor systems (Fig. 3A, B, label 2). Furthermore, a flow-through-cell oxygen sensor is inbuilt in the fluid flow system just after the bioreactor chamber and thus allows to live monitor the oxygen tension within the bioreactor chamber.

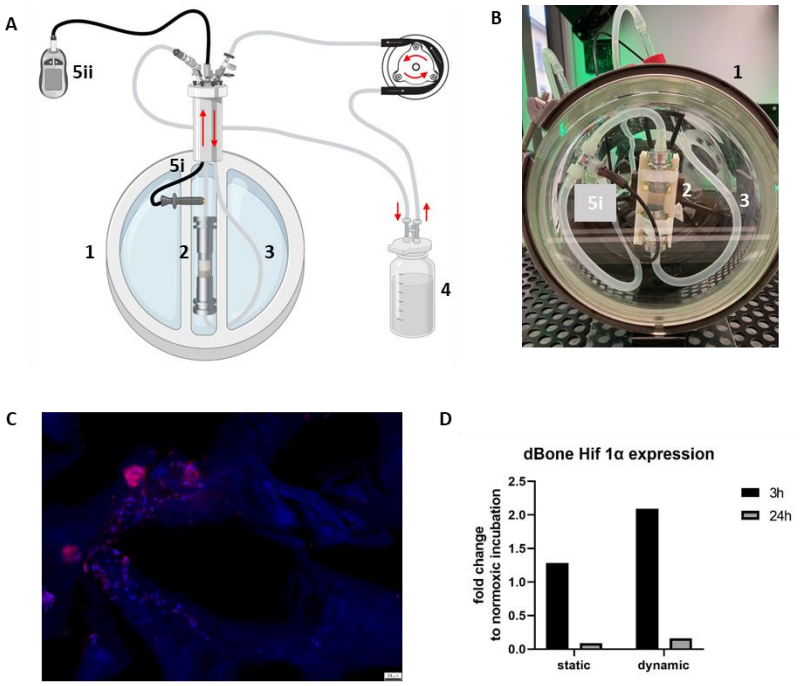

Figure 3. Newly developed bioreactor platform for integration of hypoxia and dynamic incubation. Illustration of the bioreactor platform as schematic representation (**A**) and photo of the dBone set-up within the hypoxia chamber (**B**). A gas tight glass chamber (1) is used to host the fluid flow bioreactor containing the scaffolds (2), a gas permeable silicon tube (3) allows saturation of the cell culture media with low oxygen levels before entering into the bioreactor chamber. Medium is continuously perfused from a media reservoir (4) outside the hypoxia chamber. Oxygen tension is measured using a flow-through-cell that is placed close after the bioreactor system (5i) and connected to an external measurement device (5ii) of the company PreSens Precision Sensing GmbH. **C.** The hypoxia probe primocidal was added to the culture medium. The image shows successful detection of the probe by a red fluorescent secondary antibody on cryosections of dBone scaffolds after 3h of dynamic culture in the hypoxia chamber. Cell nuclei are stained with DAPI (blue) **D**. Gene expression analysis was performed with Beta-2-Microglobulin as housekeeping gene and normalized to the controls at 21% oxygen tension by the $2^{-\Delta\Delta CT}$ method.

Preliminary experiments have been performed with the system, both with integrated dBone and SISmuc bioreactors. For this, scaffolds that have been pre-incubated with MSC for 2-3 weeks and were subsequently seeded with a layer of tumor cells and incubated for 3h or 24h under static and dynamic conditions. Standard cultures at 21% oxygen tension were compared to cultures in the hypoxia chamber that was flooded with gas containing 1% oxygen. Hypoxia in the model systems was successfully confirmed by immunofluorescently detection of the hypoxia probe primocidal (Fig.3C) and differentially regulation of the transcription factor Hif 1α (Fig.3D), which showed a slight upregulation after 3h followed by a strong contra-regularly downregulation after 24h both in dynamic and static conditions. While these experiments clearly provided a proof-of-concept, follow-up experiments will focus on the monitoring of oxygen parameters over time in the bioreactor system as well as the corresponding cellular responses.

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(The publication resulting from this project is highlighted)

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