

# Determination of the viability and activity of bone cells within a three dimensional scaffold matrix using the Bionas® 2500 analyzing technology

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## INTRODUCTION:

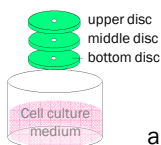
In orthopaedic surgery large bone defects (due to trauma, tumor or infection) need to be filled with autologous bone and allografts. These are only limited available and connected with peri-operative morbidity and risks. Therefore, large challenges are suspended on synthetic, porous, three-dimensional bone scaffolds which have to fulfil mechanical requirements on the one hand as well as permeability for three-dimensional cell settlement on the other hand. Problematic within these three-dimensional constructs is the uneven nutrient and oxygen supply resulting in insufficient proliferation rate in the core of cell seeded scaffolds.

In the present study we therefore addressed the question, whether 3D scaffolds are migrated evenly from the periphery to the core of the construct by means of a 3D sandwich culture model and the **Bionas® 2500 analyzing system**.

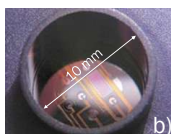
## METHODS:

In order to measure the metabolic vitality of human osteoblasts within a three-dimensional biocomposite, acidification and respiration rates in different construct layers were analyzed using the **Bionas® 2500 analyzing system**. Therefore, MG-63 cells were seeded on sterile cylindrical hydroxyapatite scaffolds. With the aim to distinguish between different depths within the construct, the matrix cylinder was divided into three discs. Each disc was loaded with  $1.13 \times 10^6$  cells (i.e.  $3.5 \times 10^6$  cells per construct), put on top of each other (Fig. 1 a) and incubated at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. After two weeks of cultivation the sandwich was disassembled, the upper, middle and bottom disc transferred in the **Bionas® metabolic chip SC1000** (Fig. 1 b) and both the acidification rate and oxygen consumption of cells were determined from each disc (respectively from each construct layer) in parallel. The cells on matrix constructs were supplied with medium by the automatic fluidic system. The medium is metabolized and nutrients converted to ATP. The cellular energy metabolism abstracts oxygen from and decreases the pH of the medium, respectively. The more acidification and oxygen consumption, the higher is the cellular activity within the according scaffold layer.

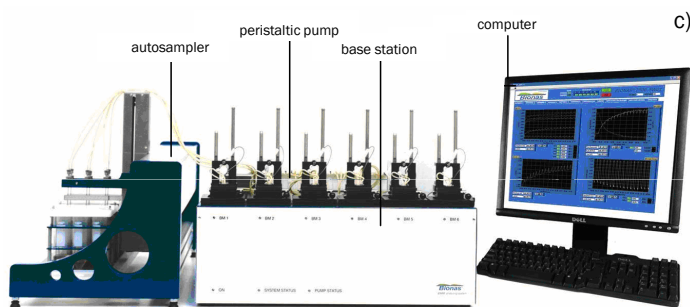
To verify the metabolic chip data, mitochondrial activity of chondrocytes on biomaterials was tested by WST-1 assay (Roche, Mannheim, Germany) according to the manufacture's instruction. With the LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes, Eugene, US), the proportion of live and dead cells were determined.



a)



b)

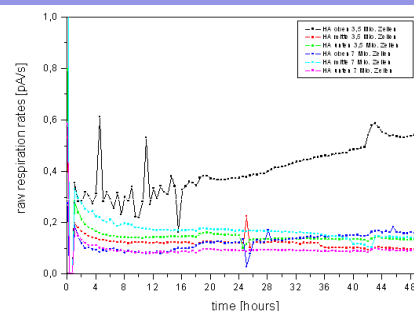


c)

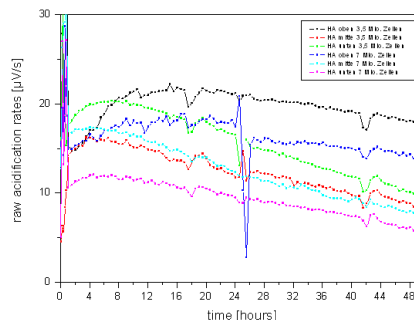
**Figure 1:** Three-dimensional hydroxyapatite sandwich system (a), the **Bionas® metabolic chip SC1000** (b) and the **Bionas® 2500 analyzing system** (c).

## RESULTS:

To measure the metabolic vitality of human osteoblasts within a three-dimensional biocomposite, acidification and respiration rates in different construct layers were analyzed using the **Bionas® analyzing system**. Cell activity on hydroxyapatite discs was recorded over a period of 50 hours. After the first eight hours leaving the test device stabilizing, the upper disc showed the highest respiratory rate followed by the bottom and the middle disc (Fig. 2 a). Equally, the acidification rate was the highest for the upper and the bottom disc whereas the middle disc showed the lowest acidification rate (Fig. 2 b). For the termination of the experiment 0.2% Triton X-100 was applied. The results were verified by the cell viability assay WST-1, where active cells cleave the tetrazolium salt WST-1 to formazan. For the upper membrane the cleavage was the highest, followed by the bottom and middle membrane (Fig. 3). With LIVE/DEAD® staining the most living cells were detected on the upper disc. The middle and bottom discs showed a similar colonization with MG-63 cells, even though more dead cells were detectable (Fig. 4).

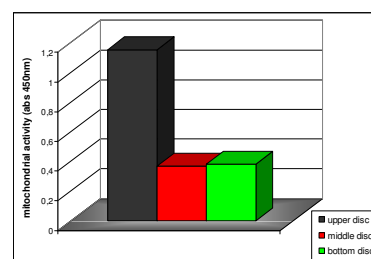


a)

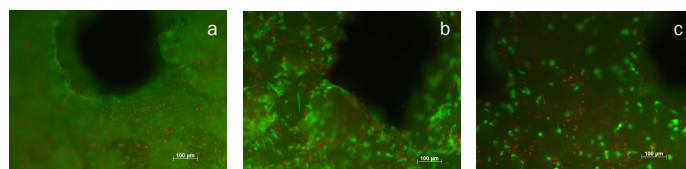


b)

**Figure 2:** Measurement of (a) respiration and (b) acidification rates for human osteoblasts (MG-63 cells) in different depths of three-dimensional scaffolds.



**Figure 3:** Mitochondrial activity of MG-63 cells on hydroxyapatite discs in different construct depths measured by WST-1-test after two weeks cultivation.



**Figure 4:** Live/Dead® staining of MG-63 cells on hydroxyapatite in different construct depths (upper membrane (a), middle membrane (b), bottom membrane (c)).

## DISCUSSION and CONCLUSION:

In summary, inside the three-dimensional biomaterial the metabolic activity of bone cells is considerably lower than in the periphery. That is attributed to the limited supply with nutrients and oxygen in large-scale cell-seeded scaffolds. These limitations affect various cellular mechanisms, including cell cycle, cell proliferation, apoptosis and glucose metabolism.

Our results demonstrate that the supply and therefore the viability of cells in the core of large 3D scaffolds is suboptimal in a static system. In conclusion, attention has to be paid on development and optimization of dynamic 3D cell culture systems. For that, the **Bionas® metabolic chip technology** is a beneficial tool to validate cultivating parameters within a 3D construct regarding cell viability and activity.

## Correspondence:

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