Evaluation of the performance of a novel hydrostatic bioreactor

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***Abstract*— pH of cell culture medium and dissolved oxygen are important criteria for cell proliferation and differentiation and are also crucial for the performance of cell culture systems. Therefore, this study aims to investigate the performance of a novel hydrostatic bioreactor while measuring the changes of pH and dissolved oxygen in bicarbonate-buffered and HEPES-buffered cell culture media. pH changes of media samples, which were subjected to hydrostatic pressure with varying pressure amplitudes, frequencies and exposure times, were determined via a colorimetric approach. The concentration of dissolved oxygen was measured using a micro optical oxygen sensor. An increase in pressure, frequency and time resulted in an increase on pH. However, after an exposure time of 30 minutes no further increase in pH was observed and the pH of the media samples were adjusted to the cell culture environment of the incubator. Similar trends were observed for bicarbonate-buffered and HEPES-buffered media. Initial results suggest further that pressure had a stronger influence on pH than frequency.**

Keywords— **hydrostatic pressure, bioreactor, dissolved oxygen measurement, pH measurement**

I. INTRODUCTION

pH and dissolved oxygen concentration are vital parameters due to their importance during bioprocesses [1]. The pH value of cell culture medium influences cell proliferation and cell viability [2] as well as the synthesis of extracellular matrix [3]. Dissolved oxygen is known to affect cell cycle, cell growth and differentiation [4,5]. It has been hypothesized that hypoxia is the key parameter for three-dimensional cell culture, because of the limited solubility and diffusion properties of oxygen in liquids [6,7]. Thus, a lack of dissolved oxygen in cell culture medium could result in cell death [5]. Despite their importance, limited information seems to be available about pH and oxygen changes in three-dimensional cell culture systems. A novel bioreactor was used to apply hydrostatic pressure to cell culture environment. Hydrostatic pressure provides mechanical stimulation and native-like environment for cells and has been shown to stimulate cell differentiation and tissue formation [8,9]. Angele et al. (2003) found that subjecting human mesenchymal progenitor cells to hydrostatic pressure lead to an increased deposition of cartilaginous matrix proteins [10]. Cyclic hydrostatic pressure also induced chondrogenic differentiation in murine embryonic fibroblasts [11]. Hence, hydrostatic pressure might play an important role during cartilage formation. Moreover, intermitted hydrostatic pressure has been demonstrated to promote osteogenic differentiation [12]. This study aims to investigate the changes in pH and dissolved oxygen concentration in cell culture media in a novel hydrostatic bioreactor. The duration, pressure and frequency of the applied hydrostatic pressure were altered in order to determine their effect on pH and dissolved O_2.

II. METHODS AND MATERIALS

pH changes were determined as described elsewhere with modifications (Jang 2010). Briefly, a colorimetric attempt was used to quantify pH changes in DMEM cell culture medium, which follows the color change of Bromothymol Blue from yellow (pH 6.0) to blue (pH 7.6). Known volumes of Bromothymol Blue (BTB) (Sigma-Aldrich, UK) were added to medium samples (Table 1) in multiwell tissue culture plates. The absorbance was read at 610 nm wavelength in the conventional plate reader before applying hydrostatic pressure. To apply hydrostatic pressure, multiwell plates were inserted into the bioreactor without lid. The bioreactor was sealed and hydrostatic pressure was applied. Subsequently, at various time points, pressures and frequencies in order to investigate their influence on pH changes (n=8).

To relate absorbance changes to pH, absorbances were read for media samples with known pH and standard curves were plotted. pH values were adjusted using 5M HCl and 2M NaOH to prepare medium standard samples.

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Table 1: Medium samples buffered with Bicarbonate (BC) and 25 mM HEPES (HP) with Phenol red (+ PR) and without Phenol red (- PR) were used for the investigation of pH and O_2 changes applying pressure in a new hydrostatic bioreactor. In addition medium samples were supplemented with fetal calf serum (FCS).

Bicarbonate Buffered Medium	HEPES buffered Medium
BC + PR + FCS	HP + PR + FCS
BC - PR + FCS	HP - PR + FCS
BC + PR - FCS	HP + PR - FCS
BC - PR - FCS	HP - PR - FCS

Oxygen changes in various cell culture media (Table 1) were determined using a micro optical oxygen sensor (PreSens, Germany). Measurements were performed before applying hydrostatic pressure and then at various time points, pressures and frequencies in order to investigate their influence on O_2 changes.

III. RESULTS

The absorbance spectrum for BTB in media samples with known pH was measured between 300-700 nm (Figure 1). Depending on pH, Bromothymol blue is charged differently, which influences the absorption of visible light. Maximum absorption was observed at 610 nm. An increase in pH resulted in an increase of absorbance at 610 nm, which indicates a linear relationship between absorption and pH. Therefore, the absorption was measured at 610 nm for further experiments.

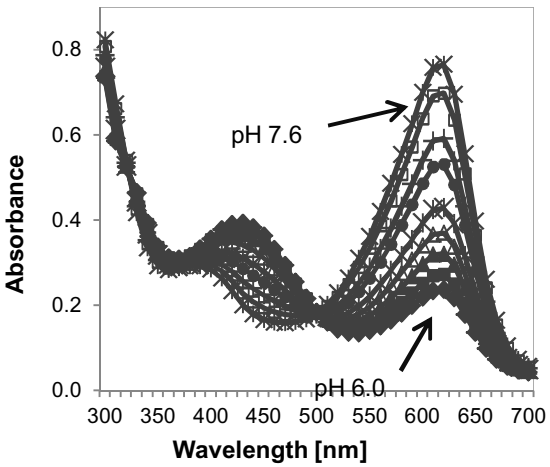

Figure 1: Absorbance spectrum BTB (A). The absorption spectrum of BTB of media samples with known pH was screened from 300 to 700 nm. Maximum absorption was observed at 610 nm

Absorbance was measured at 610 nm for medium samples with known pH (Figure 2). Linear correlation between absorbance and pH was found over a range of pH 6-8 which is well in the range of pH changes observed during cell culture [13]. Changes of the pH values of media samples were calculated by interpolating the measured absorbance from the standard curve.

The influence of frequency of hydrostatic pressure on pH of media samples within the bioreactor is shown in Figure 3 and Figure 4. pH values increased with frequencies up to 0.8 Hz. At higher frequencies pH values decreased. Similar trends were observed for bicarbonate-buffered (BC) and HEPES-buffered (HP) media with and without FCS. However, it seems that the addition of FCS lead to a stronger pH increase at 0.8 Hz frequency for BC-buffered media.

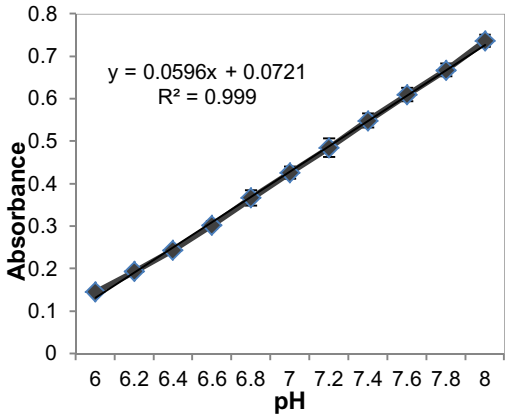

Figure 2: Standard curve for the determination of pH changes in medium (BC+PR+FCS) shown exemplarily (B). Standard deviations are shown as error bars (n=8).

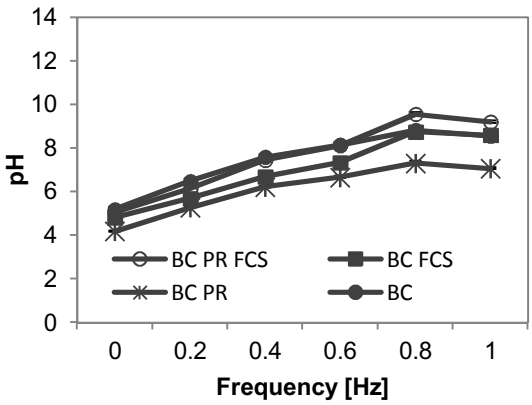

Figure 3: pH changes of bicarbonate-buffered cell culture media in dependency of frequency. Standard deviations are shown as error bars (n=8).

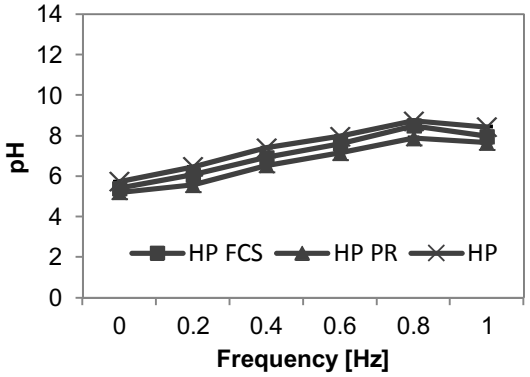

Figure 4: pH changes of HEPES-buffered cell culture media in dependency of frequency. Standard deviations are shown as error bars (n=8).

Altering the pressure amplitude and duration of applied hydrostatic pressure resulted in similar pH changes. An initial pH increase was observed for higher pressures and longer durations. However, no further pH increase was observed for media samples subjected to hydrostatic pressure for more than 30 minutes. This indicates that the media pH was adjusted to cell culture environment after 30 minutes in the bioreactor chamber.

IV. CONCLUSIONS

Changes in pH and dissolved oxygen in cell culture media were investigated when applying hydrostatic pressure at various pressure amplitudes, frequencies and exposure times. Differences in pH were colorimetrically indicated by Bromothymol Blue, a pH indicator, which changes its color from yellow (pH 6.0) to blue (pH 7.5). At different pH values Bromothymol Blue possess different charges, which are responsible for the colorimetric properties such as the absorption of light. Hence, the absorption spectrum of the cell culture medium is determined by its pH value. The higher the pH of the standard samples the higher the absorption at 610 nm. Cell culture media samples wer subjected to hydrostatic pressure for up to 1hour. At various times points (0 m, 10, 20, 30, 40, 50, 60 minutes) pH of the media was determined. The longer the cell culture media were subjected to hydrostatic pressure, the higher the pH. However, after 30 minutes no further increase in pH was observed, suggesting that the media pH had adjusted to the atmospheric conditions in the incubator. Media samples were exposed to hydrostatic pressure with amplitudes of 0, 50, 100, 150, 200, 280 kPa. Results showed that the higher the pressure the higher the pH. Similar trend was observed when samples were subjected to hydrostatic pressure with increasing frequencies (0, 0.2, 0.4, 0.6, 0.8 and 1.0 Hz). Bicarbonate-buffered and HEPES-buffered cell culture media showed similar trends for the increase in pH.

The increase in pH could be explained with the increased gas flow within the bioreactor chamber when hydrostatic pressure is applied. Higher pressures and frequencies might pump the atmosphere from the cell culture incubator (5% CO_2) faster in the bioreactor chamber, leading to an increase of gas exchange between the gaseous phase and liquid phase within the chamber and therefore and increase in pH. Longer durations gave more time for gas exchange between media and gaseous phase within the bioreactor chamber.

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REFERENCES

1. Jang J, Moon SJ et al. (2010). Colorimetric pH measurement of animal cell culture media. Biotechnol Lett 32:1599–1607
2. Xu X, Smith S, Urban J et al. (2006). An in line non-invasive optical system to monitor pH in cell and tissue culture. Medical Engineering & Physics 28:468–474
3. Wu MH, Wang J, Taha T et al. (2007) Study of on-line monitoring of lactate based on optical fibre sensor and in-channel mixing mechanism. Biomed Microdevices 9:167–174
4. Volkmar E, Drosse I, Otto S et al. (2008). Hypoxia in Static and Dynamic 3D Culture Systems for Tissue Engineering of Bone. Tissue Engineering: A 14(8):1331-1340
5. Nock V, Blaikie RJ, David T (2007). Micro-patterning of Polymer-based Optical Oxygen Sensors for Lab-on-Chip Applications. PACS: 47.61.Fg
6. Malda J, Klein TJ, Upton Z (2007). The roles of hypoxia in the in vitro engineering of tissues. Tissue Engineering 13: 2153
7. Arkudas A, Beier JP, Heidner K, et al. (2007). Axial prevascularization of porous matrices using an arteriovenous loop promotes survival and differentiation of transplanted autologous osteoblasts. Tissue Engineering. 13:1549
8. Elder BD, Athanasiou KA (2009). Hydrostatic Pressure in Articular Cartilage Tissue Engineering: From Chondrocytes to Tissue Regeneration. Tissue Engineering: B 15(1):43-53
9. Wang JHC, Li B (2010). Mechanics rules cell biology. Sports Medicine, Arthroscopy, Rehabilitation, Therapy & Technology, 2(16)
10. Angele P, Yoo JU, Smith C et al. (2003). Cyclic hydrostatic pressure enhances the chondrogenic phenotype of

human mesenchymal progenitor cells differentiated in vitro. Journal of Orthopaedic Research 21: 451- 457
11. Elder SH, Fulzele KS, McCulley WR (2005). Cyclic hydrostatic compression stimulates chondroinduction of C3H/10T1/2 cells. Biomechan Model Mechanobiol 3:141–146

12. Roelofsen J, Klein-Nulend J, Burger EH (1995). Mechanical Stimulation by intermittent hydrostatic compression promotes bone-specific gene expression in vitro. J. Biomechanics, 28(12):1493-1503
13. Zhou H, Purdie J, Wang T, Ouyang A (2010). pH Measurement and a Rational and Practical pH Control Strategy for High Throughput Cell Culture System. American Institute of Chemical Engineers Biotechnol. Prog., 26: 872–880