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Oxygen-distribution within 3-D collagen I hydrogels for bone tissue engineering

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Abstract:

Tissue engineering (TE) approaches typically envisage the structural and functional reconstitution of previously damaged tissue *in situ*. An adequate three-dimensional environment is therefore of fundamental importance for the designated cells associated to the scaffold material. The sufficient supply with nutrients and oxygen *in vitro* and *in vivo* mark thereby critical challenges of TE. In this study, we intended to analyse the level of locally dissolved oxygen within 3-D cell-loaded collagen I gels *in vitro*. For the analysis of the oxygen levels *in situ*, we employed an optical fibre-based micro sensor setup, as well as a camera supported non-invasive optical sensor foils based technique. These complementary analytical tools enable the identification, localization, and temporal follow-up investigation of specified regions of interest within TE constructs. Human adipose-derived mesenchymal stem cells (hAdMSCs) cultured in collagen I gels under normoxic conditions were analysed periodically and kinetically up to 70 days – thereby revealing dynamic changes of the level of dissolved oxygen inside the gel constructs. Dependent on the applied cell concentration, the *in vitro* oxygen concentration (cO_2) within the gels reached physiological ranges (7 – 9 %) after 21 days, or 35 days of culture. The minimal cO_2 was measured after 35 days *in vitro*, featuring an oxygen level of 4.8 ± 1.3 %. Upon prolonged culture, a plateau-like status of the cO_2 around 8 – 9 % established, indicating a change in the physiological activity of the cells under investigation. The expression patterns of *BCL2*, *CASP3* and *MCM5* revealed significant differences among the proliferative and apoptotic stages of the cell-loaded samples at the investigated time points of 7 and 70 days in culture. In summary, these data show the temporary dynamic nature of the oxygen distribution in cell-loaded gel constructs. The applied technique is an ideal tool for the evaluation of multiple parameters affecting the oxygen distribution *in vitro*. We conclude that it takes 5 weeks for establishing an equilibrium of cO_2. Levels reached in a 3-D gel construct are comparable with physiological oxygenation ranges in bone-associated tissues.

Keywords: *in vitro* oxygenation level, mesenchymal stem cells, collagen I, bone tissue engineering, 3-D gel construct