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Cultivation of three-dimensional cartilage-carrier-constructs under reduced oxygen tension

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Abstract

Three-dimensional cartilage-carrier-constructs were produced according to a standard protocol from chondrocytes of an adult mini-pig. Experiments with different oxygen concentrations (21, 10 and 5%, v/v O_2) were performed and the constructs were compared qualitatively and quantitatively. The appearance of the cartilage obtained under reduced oxygen tension seemed to be closer to native cartilage with respect to shape of the cells, distribution of the cells within the matrix, smoothness of the surface, etc. The thickness of the cartilage formed by free swelling was always in the same range as for native cartilage (approximately 1 mm). Qualitatively the most stable attachment of the cartilage on top of the carrier was found for 10% O_2 (v/v). Especially at 5% O_2 (v/v) the attachment between cartilage and carrier was not sufficient. The constructs generated at lower oxygen tensions had a significantly higher amount of glycosaminoglycan per DNA, but still significantly less when compared to native cartilage. Furthermore, the cultivated cartilage contained a large amount of collagen type II. The experiments proved the applied concept for generation of cartilage-carrier-constructs and the usefulness of cultivation under reduced oxygen tension.

Keywords: Cartilage; Chondrocytes; Collagen; Glycosaminoglycan; Oxygen concentration; Tissue engineering

1. Introduction

Severe health problems can be caused by lack or damage of hyaline cartilage in joints especially in knee joints (Langer and Vacanti, 1993; Buckwalter and Mankin, 1997; Häuselmann and Hunziker, 1997; Petersen et al., 2003). The methods of tissue engineering allow the generation of cartilage tissue in vitro and open new strategies to restore damaged cartilage (Buschmann et al., 1992; Park and Ward, 1995; Sittinger et al., 1997). The aim of this work was to develop a protocol for cultivation of three-dimensional

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Nomenclature

b	thickness of cartilage matrix (m)
C_s	oxygen concentration at the surface of cartilage matrix (mol l^{-1})
D_{eff}	diffusion coefficient in cartilage matrix (m^2 s^{-1})
r	volume specific oxygen uptake rate (mol l^{-1})

Greek symbols

Φ_0	Thiele modulus for zero-order reaction and flat plate
η_0	effectiveness factor for zero-order reaction and flat plate

cartilage-carrier-constructs based on a concept suggested recently (Nagel-Heyer et al., 2005). The three-dimensional constructs were prepared by cultivation of chondrocytes on top of a solid carrier. This carrier shall support the tissue formation. Furthermore, in case of implantation the carrier is intended to fix the engineered cartilage in the bone. During a cultivation period the cells produce a three-dimensional cartilage matrix by free swelling. It has to be stressed that the cells are not entrapped in a macroporous scaffold as in alternative concepts (Darling and Athanasiou, 2003). The cultivation principle consists of the following steps (Nagel-Heyer et al., 2005): (a) chondrocytes are isolated from articular cartilage and expanded in monolayer culture and then seeded on a solid carrier to form a "primer layer"; (b) simultaneously chondrocytes are suspended in alginate gel and cultivated for 2 weeks to allow the formation of pericellular matrix; (c) the redifferentiated chondrocytes are then recovered from the alginate gel and sedimented on the "primer layer"; (d) the biphasic constructs are further cultivated for 3 weeks to allow free-swelling of the cartilage matrix.

The difficulty with any tissue engineered cartilage is maintaining it once it has been implanted. Cartilage must withstand compressive load but also support frictionless movement in the joint (Wondracek, 2003). The ability to overcome shear forces at the joint surface is crucial (Bobic, 1999). Therefore, a special conditioning during the cultivation to improve the matrix quality is very important. Furthermore, the chondrocytes undergo a process of dedifferentiation during proliferation, accompanied by a change in morphology, reduced synthesis of collagen type II and other matrix proteins and enhanced synthesis of collagen type I (for review see, Malda et al., 2003). The main goal of any tissue engineering concept for generation of implantable cartilage is the assurance of a chondrogenic phenotype, especially the secretion of cartilage matrix (proteoglycans and collagen type II). One possibility to improve the quality of tissue engineered cartilage is to induce a load for example by means of intermittent hydrostatic pressure or to reduce the oxygen tension in the gas phase to more physiological conditions (Hall et al., 1991; Carver and Heath, 1999a,b; Domm et al., 2002; Malda et al., 2004b). It is commonly accepted that the environment in the joint is characterised by a low oxygen tension. The effect of oxygen during the in vitro cultivation of chondrocytes is poorly understood and a controversial discussion is going on in the literature (Malda et al., 2003). In several studies chondrocytes were immobilised in alginate beads and cultivated under different oxygen concentrations in the gas phase (for review see, Malda et al., 2003). O'Driscoll et al. (1997) observed a limited collagen type-II production at very high (90% O_2) and very low (1–5% O_2) oxygen concentrations. Domm et al. (2002) showed a stimulatory effect in the matrix production for reduced oxygen tension (5% O_2). In a recent study from Malda et al. (2004b) pellets of chondrocytes were suspended in a stirred bioreactor under different oxygen concentration. They observed an increased production of glycosaminoglycan at 5 and 1% O_2 (v/v) in comparison to aeration with 21% O_2 (air). The increased glycosaminoglycan production was accompanied by a decrease of collagen type I. On the other hand, several studies on chondrocytes embedded in a three-dimensional matrix or scaffold have demonstrated an enhanced matrix formation, especially proteoglycan synthesis under more aerobic conditions (Obradovic et al., 1999; Ysart and Mason, 1994).

The main difference between the applied methodologies (alginate and pellet culture versus cartilage generated in three-dimensional scaffolds) can be seen in oxygen gradients in the vicinity and within the formed cartilage (Malda et al., 2003; Nehring et al., 1999). In case of alginate and pellet culture oxygen gradients at the surface of the constructs can be neglected and only oxygen limitations within the constructs are likely.