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Long-term maintenance of human articular cartilage in culture for biomaterial testing

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Abstract

Cartilage is a tissue that derives its unique mechanical and biological properties from the combination of relatively few cells and a large amount of a complex extracellular matrix. Furthermore, cartilage tissue is comparatively slow to respond to changes or harmful influences. To date, the optimal generation and long-term maintenance of cultured human articular cartilage for in vitro testing of biomaterials, poses an experimental difficulty. Experiments using cultured isolated chondrocytes in combination with scaffolds often fail to yield results comparable to the in-vivo situation. Consequently, our aim was to develop a culture method that allows in vitro maintenance of human hyaline cartilage explants in an optimal quality over an extended period of time. Such a culture could, for example, be used to determine the long-term effect of a new scaffold on intact cartilage, as an in vitro model for repair processes and to investigate biomaterial integration.

In this study we compared conventional static cultures with and without serum supplementation to a serum-free perfusion culture for the ability to maintain human articular cartilage explants in a morphologically intact and differentiated state over an extended period of time of up to 56 days. Results were evaluated and compared by morphological, histochemical and immunohistochemical methods.

The experiments showed that short-term maintenance of cartilage in a differentiated state for up to 14 days is possible under all culture conditions tested. However, best long-term culture results for up to 56 days were obtained with perfusion culture under serum-free conditions. Such a perfusion culture system can be used to perform biocompatabilty tests in vitro by long-term coculture of biomaterial and intact human articular cartilage.

Keywords: Biocompatability; Bioreactor; In vitro test; Cell culture; Human articular cartilage; Tissue Culture; Perfusion; Differentiation

1. Introduction

Hyaline cartilage has a very limited capacity for intrinsic functional repair [1]. Small chondral defects have been reported to regenerate by cell migration [2], but untreated large full-thickness defects in most cases progress to osteoarthritis with an eventual need for total knee replacement surgery [3,4]. Surgical and biological attempts have been made to induce significant and durable repair response in cartilage injuries. Such treatments include attempts to recruit progenitor cells from the bone marrow by penetration of the subchondral bone [5,6] or transplantation of osteochondral grafts, periosteum or perichondrium [7,8]. The therapies show acceptable short-term results but the repair tissue is fibrous and long-term data are usually unfavorable. The use of cultured cells in the autologous chondrocyte transplantation (ACT) technique is a promising

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alternative technique [9]. Autologous chondrocytes are expanded in vitro and injected under a periosteal flap sewed onto the defect. As shown by a large number of treated patients, ACT delivers good to excellent long-term clinical results well correlated with the formation of hyaline-like repair tissue within the injury site [10]. However, the method could be considerably improved using an advanced biomaterial instead of the periostal flap [11]. The procedure could generally be simplified and the open joint surgery could be replaced by a much safer arthroscopic operation [12,13]. Therefore, a number of flexible biomaterials needs to be evaluated for such purposes [14–17].

In order to test the biocompatibility of scaffolds and membranes that should be used within the joint in direct contact with articular cartilage, a good in vitro model will be necessary [18]. Such a model would have to allow long-term culture experiments because cartilage tissue can take weeks or months to show detectable pathology when exposed to harmful influences [19,20]. Long-term studies could also be valuable to investigate the so-far unknown underlying mechanisms of regeneration. Animal studies can yield very good insight [21] but the systems are highly complex and experimental parameters are difficult to control. As the transferability of findings from animal experiments to human tissue is problematic [22] in vitro models using human tissue should be used to complement and refine the results.

Consequently, our aim was to develop an in vitro model based on a tissue-specific culture that allows long-term maintenance of highly differentiated pieces of human cartilage for experimental purposes. In the present study, conventional static culture techniques were compared to a perfusion culture with respect to the ability to maintain human articular cartilage explants in a morphologically intact and highly differentiated state over short, intermediate and extended periods of time. All cultured explants were evaluated by a set of histochemical, morphological and immunohistochemical methods.

2. Materials and methods

2.1. Preparation of explants

Intact and healthy human articular cartilage was obtained from the femoral trochlear region of patients (four females/ two males; aged 21–39) undergoing patella regrooving operations. In this type of operation, a fairly large amount of healthy cartilage is removed. The patients had no history of cartilage damage or degeneration. Sample collection was performed with patient's consent under the ethical approval S040–01 of the Ethical committee of the medical faculty of Gothenburg University.

Pieces of cartilage were transported in phosphate buffered saline (PBS) from the surgical operating room and transferred to DMEM/F12 medium (Gibco BRL Life Technologies, Germany) immediately. A stainless steel punch with an inner diameter of 3 mm (Miltex Instruments, PA, USA) was used to punch out full thickness cylindrical explants of 3 mm diameter and 3 mm length (Fig. 1a). The average weight of these explants was 90 mg. Subchondral bone was not included in the preparation because it was not present in all material obtained from operations and because it would make sectioning of the samples much more difficult. Fresh explants from all six patients were processed and examined immediately to serve as controls.

2.2. Static culture

Static culture of the explants was performed in 12 well tissue culture plates (Becton Dickinson, Germany). Explants were placed into 1 ml of culture medium, each in separate wells of the culture plate. The three different culture media used were DMEM/F12 (Gibco BRL Life Technologies, Germany) + 50 μg/ml ascorbic acid + 50 μg/ml gentamycin + 2 μg/ml fungizone (Sigma, Germany) as serum-free base medium. Base medium supplemented with 10% fetal calf serum (FCS, Gibco BRL Life Technologies, Germany), and base medium supplemented with 10% autologous human serum (HS). Culture was performed in a humidified incubator (Haereus, Germany) at 37 °C. Culture medium was renewed every 3–4 days. Six individual samples were cultured in parallel in each medium for a period of 14, 28, 42 and 56 days.

2.3. Perfusion culture

Perfusion culture of the explants was performed in specially designed culture containers (Minucells and Minutissue, Germany) that allow continuous medium perfusion (Fig. 1b and c). The containers were placed on a heating plate (Medax, Germany) to maintain a constant temperature of 37 °C. Serum-free DMEM/F12 + 50 μg/ml ascorbic acid + 50 μg/ml gentamycin + 2 μg/ml fungizone + 35 mM HEPES (Sigma, Germany) was continuously perfused through the container using an IPC-N 8 peristaltic pump (Ismatec, Germany) at a rate of 1 ml/h. Used medium was not reperfused but collected as waste. The addition of 35 mM HEPES buffer was necessary to maintain the medium at a physiological pH of 7.4 under room atmosphere (0.3% CO_2). Six individual samples were cultured in parallel in each medium for a period of 14, 28, 42 and 56 days.