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The influence of scaffold architecture on chondrocyte distribution and behavior in matrix-associated chondrocyte transplantation grafts

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ABSTRACT

Scaffold architecture and composition are important parameters in cartilage tissue engineering. In this *in vitro* study, we compared the morphology of four different cell-graft systems applied in clinical cartilage regeneration and analyzed the cell distribution (DAPI nuclei staining) and cell–scaffold interaction (SEM, TEM). Our investigations revealed major differences in cell distribution related to scaffold density, pore size and architecture. Material composition influenced the quantity of autogenous matrix used for cellular adhesion. Cell bonding was further influenced by the geometry of the scaffold subunits. On scaffolds with widely spaced fibers and a thickness less than the cell diameter, chondrocytes surrounded the scaffold fibers with cell extensions. On those fibers, chondrocytes were spherical, suggesting a differentiated phenotype. Fiber sizes smaller than chondrocyte size, and widely spaced, are therefore beneficial in terms of improved adhesion by cell shape adaptation. They also support the differentiated stage of chondrocytes by preventing the fibroblast-like and polygonal cell shape, at least briefly.

1. Introduction

Cartilage tissue engineering is determined by the specific biomechanical properties of the native tissue, its high content of extracellular matrix (ECM), and the lack of innervation and vascularization. The cells responsible for ECM metabolism, the chondrocytes, are very rare in native cartilage and encapsulated in the ECM. Therefore, chondrocytes are not available for defect healing under natural conditions, which frequently leads to clinical problems. Tissue engineering-based cartilage defect healing overcomes this problem by implanting chondrocytes gained from small cartilage biopsies and propagated *in vitro*. During proliferation in monolayer conditions, however, chondrocytes dedifferentiate and cease producing the mechano-biologically indispensable matrix proteins collagen type II, aggrecan and others [1–3]. To obtain a hyaline cartilage tissue, this phenotype switch has to be reversed (Under 2D conditions and during proliferation, chondrocytes cannot redifferentiate). Conditions mimicking the natural environment, such as three-dimensionality [4,5], mechanical stress [6–8], low oxygen supply [9,10], growth factors [11–13], and specific matrix molecules [14,15], can induce chondrocyte redifferentiation.

In terms of three-dimensionality, chondrocytes are arrested to 3D-scaffolds for implantation. Apart from the regeneration effect, scaffolds are useful for the immobilization of the cells, for a broader distribution of the cells in the defect, and for facilitating the handling during surgery. The method using 3D-scaffolds to implant the patient's own chondrocytes into the cartilage defect is termed "matrix-associated autologous chondrocyte transplantation" (MACT) [16]. It is presently performed with either natural or synthetic polymer-based scaffolds [13,17–19]. Although MACT is presently the best method for regenerating cartilage defects >2 cm^2, there is still room to improve and accelerate the healing process.

In this respect, previous research has focused on alternative scaffold production such as temperature-regulated hydrogel formation [20] or on the examination of new sources for scaffold materials (chitosan [21], silk [22], celluloses [23], jellyfish collagen [24], carbon fibers [25]). Promising techniques include surface or bulk modification of the biomaterial composition (composite scaffolds [26,27], surface modification [28,29] and coating with

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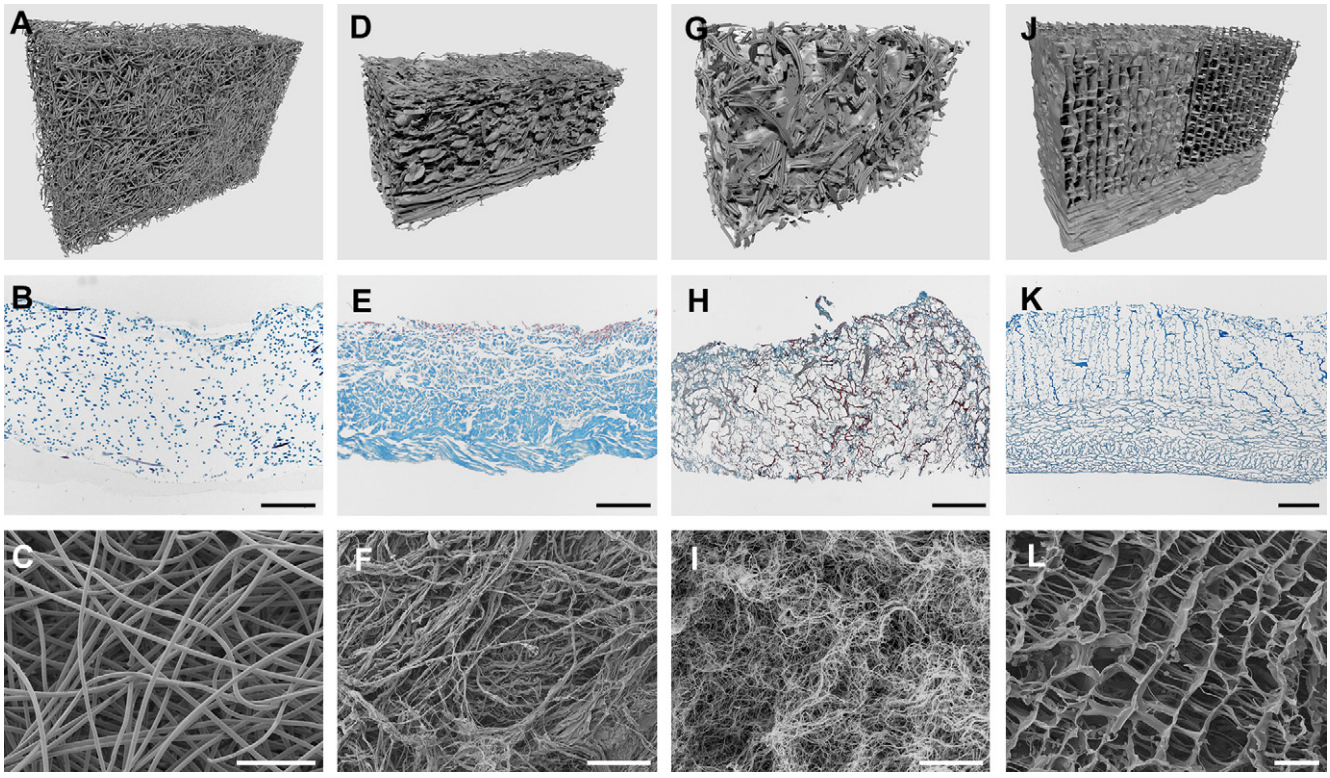

Fig. 1. Morphology of the four scaffold types: hyaluronan web (A–C), collagen fleece (D–F), collagen gel (G–I) and collagen sponge (J–L). A scheme demonstrates the general architecture of the scaffolds (A, D, G and J). Histological sections (B, E, H and K) and the SEM top view (C, F, I and L) show the difference in material density, fiber size and the orientation of the scaffold elements. Scale bars: (B) 500 μm, (C) 200 μm, (E) 200 μm, (F) 300 μm, (H) 1000 μm, (I) 30 μm, (K) 500 μm and (L) 100 μm.

bioactive molecules [30]). Other upcoming approaches are nanofiber technologies [31] and designed materials [32,33]. On the cellular level, the influence of growth factors, hormones and cytokines [11,34], the effect of mechanical stimulation [35,36] and the use of alternative cell sources, such as stem cells [37] and chondrocytes of debrided cartilage [38], have been investigated.

Despite the rapidly growing variety of scaffold systems, only a few of them are in clinical application. On the contrary, the materials used for patient cartilage defect healing are still first-generation scaffolds based on vertebrate ECM molecules or synthetic polymers. Despite the long-term clinical and cell culture-experience with those materials, their morphology and their influence on chondrocytes remain poorly investigated. In particular, comparative analyses including more than two of those clinical materials are missing.

The present study therefore uses morphological methods to compare graft systems that are frequently applied for cartilage defect treatment in clinics. Four ECM-based graft types with very different scaffold morphology were chosen, and the influence of the scaffold parameters on cell distribution and cell adhesion was analyzed.

2. Material and methods

2.1. Grafts

Residuals of four different types of MACT grafts were used (10 samples/group): Hyalograft®C autografts (Fidia Advanced Biomaterials, Italy), a hyaluronan web; Chondro-Gide® (Geistlich Biomaterials, Switzerland; MACI© Verigen, Denmark and Genzyme, Boston) a collagen type I/III fleece; the collagen type I gel CaReS® (Arthro Kinetics Biotechnology GmbH; Austria); and Novocart® 3D (TeTeC, Germany), which is a collagen type I sponge containing chondroitin-sulfate. Clinical studies were approved by the local ethical board and patient consent was given (147/2003, 148/2003, 420/2003, 307/2006). The surgeries were all done in the same hospital by one surgeon. Samples were obtained immediately after surgery and prepared for light, scanning and transmission electron microscopy (LM, SEM, TEM).

The grafts are produced by the companies as follows:

Hyalograft®C autograft: The patient's chondrocytes are isolated from biopsies and multiplied in monolayer culture. Afterwards the cells are seeded onto the surface of the Hyalograft®C web at a density of $1 \times 10^6/cm^2$ and cultivated for at least two weeks.

Chondro-Gide®: After isolation of chondrocytes from biopsies, chondrocytes are propagated in monolayer and seeded on the rough side of the fleece. Cell number and cultivation time are not exactly given, but according to personal communication, 3D cultivation time is about one week.

CaReS®: As opposed to the three other graft types investigated in this study, the patient's chondrocytes are mixed with the gel directly after isolation from the biopsies without monolayer cultivation. 3D cultivation is performed for two weeks (10–14 days).

Novocart® 3D: Patient's chondrocytes are isolated from full depth cartilage cylinders, multiplied in monolayer and seeded in a density of $1.45 \times 10^6/cm^2$ onto the scaffold. Those constructs are cultivated for about two days under 3D conditions before sending the graft to the hospital.

2.2. Histology/histochemistry

Histological sample processing was done according to the standard procedure of our lab [39]. Samples were fixed with 7.5% paraformaldehyde, dehydrated in a gradient series of alcohol, rinsed in xylol and infiltrated with paraffin. Deparaffinized sections were stained with alcian blue and fast red.

2.3. Vibratome thick sections and DAPI-staining

Hand-sectioned slices of glutaraldehyde-fixed graft samples were embedded in gelatine, fixed overnight with 4% paraformaldehyde and mounted on sample holders. Sections of 100 μm were then cut with an FTB-vibracut (FTB), immersed in DAPI nuclei stain and imaged with an Olympus BX41 epifluorescence microscope.

2.4. Electron microscopy

Standard fixation was performed with 2.5% glutaraldehyde (GA) in 0.1 M sodium cacodylate buffer solution (pH 7.3) at 4 °C overnight as primary fixation, then rinsed in 0.1 M sodium cacodylate buffer (pH 7.3) and postfixed in 1% osmium tetroxide (OsO_4) either with or without 1% potassium ferrocyanide for 2 h at room temperature. Before dehydration the samples were rinsed in the same buffer. Alternatively, ruthenium hexammine trichloride (RHT) fixation was used for better visualization of the extracellular matrix, especially protoeoglycans [40,41]. Therefore, samples were fixed in the presence of 0.7% RHT in both the primary and the postfixative. Fixation