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Biomaterials 20 (1999) 1743–1749

Biomaterials

Technical note

Tissue engineering of biphasic joint cartilage transplants

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Received 13 July 1998; accepted 18 March 1999

Abstract

In isolated posttraumatic or idiopathic joint defects the chondral layers and adjacent subchondral spongy bone are usually destructed. For regeneration we suggest the in vitro formation of a cartilage-coated biomaterial carriers (biphases) in order to fill the corresponding joint defects. In this study Biocoral®, a natural coralline material made of calcium carbonate, and calcite, a synthetic calcium carbonate, were used as supports for the cultivation of bovine chondrocytes in a three-dimensional polymer fleece. The cell-polymer-structure was affixed to the biomaterial with a fibrin-cell-solution. The artificial cartilage formed a new matrix and fused with the underlying biomaterial. The results indicate a promising technical approach to anchor tissue engineered cartilage in joint defects.

Keywords: Calcium carbonate; Resorbable polymers; Cartilage; Tissue engineering

1. Introduction

Loss of cartilage can occur in osteoarthritis, osteochondrosis dissecans, after trauma or in connection with rheumatoid diseases. In the most favourable case only, clinical therapy permits reattachment of a loosened cartilage-bone fragment e.g., in osteochondrosis dissecans or traumatic bone–cartilage lesions. The problem involved in any loss of cartilage of the large joints and its primary physiological replacement has been the focus in latest research. At present, however, the considerable pain and functional limitations usually found in joints altered by cartilage loss, necessitate either arthrodesis or even the implantation of a joint prosthesis.

A natural cartilage–cartilage pairing as a suitable joint contact is consequently desirable [1]. The new therapeutic approach is the induction of hyaline cartilage growth on an absorbable subchondral support material for regeneration of destroyed endogenous tissue. An induction of cartilage growth is requisite for this purpose [2–7], since according to the current state of knowledge, cartilage defects are only closed by scars of inferior cicatricial cartilage. The new materials and procedures should allow both cartilage bone regeneration and absorption of the biomaterials in course of time. The additional application of substances like cytokines bound to the biomaterials to accelerate the mentioned process could be beneficial.

Irregularly shaped cartilage defects often cause problems, since round transplants are commonly used and press-fitted into prepared round defects of corresponding diameters. Autologous transplantation cannot be performed with extensive cartilage loss [1]. If prosthetic care is not desired, cartilage must be cultivated and then inserted into the defect zone with a suitable support material. Chondrocyte transfers have already been clinically performed [1]. After isolation and cultivation in vitro without a biomaterial, the cells were injected into the defect zone and covered with a sutured periosteal flap. Since cartilage-like tissue has to form in vivo in this procedure, the corresponding joint cannot be stressed before a full cartilage consolidation has taken place.

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0142-9612/99/$ - see front matter
PII: S0142-9612(99)00061-7

On a biomaterial consisting of calcium carbonate obtained from the Porites coral, our group could demonstrate cartilage growth and regeneration in a rabbit model. However, the minced cartilage taken from the created defect was injected into the superficial pores of the implanted calcium carbonate cylinder without previous tissue engineering.

Neither a procedure nor a suitable biodegradable support to anchor engineered cartilage as a main prerequisite in joint repair, have been addressed in literature. The use of a natural coral or a calcium carbonate support synthetically produced by our group is tested in this study to overcome this substantial problem.

2. Materials and methods

2.1. Polymer fleece

For arranging the chondrocytes in a three-dimensional pattern a nonwoven material (Ethisorb 210) provided by Ethicon® (Norderstedt, Germany) (Fig. 1) consisting of polyglycolic acid (PGA) and poly-L-lactic acid (PLLA) in a 90 : 10 ratio and also containing small amounts of polydioxanon was used. Its biocompatibility was tested by our study group before [8,9].

2.2. Biomaterials

In the present study, two different biomaterials were used as carriers for the cultured cartilaginous layer: Biocoral®, a calcium carbonate from coralline material (Inoteb Co., St. Gonnery, France) and calcite a synthesised calcium carbonate (Federal Institute for Material Research and Testing in Berlin).

2.3. Cell preparation

Hyaline cartilage was obtained from macroscopically normal bovine femoral heads within 12 h of slaughter.

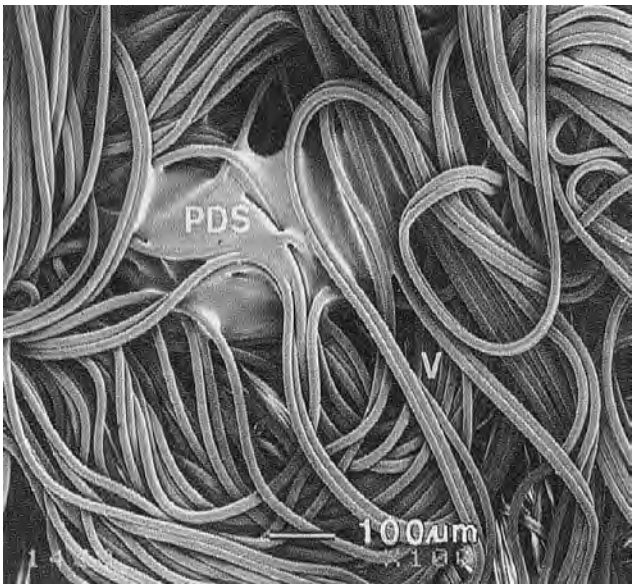

Fig. 1. Fiber structure of the resorbable polymer fleece (Ethisorb®) with PGA-fibres and polydioxanon-fixation-points (PDS).

The cartilage was diced into cubes of 1 mm^3. Chondrocytes were isolated using an enzymatic solution as described previously [10]. Briefly, the extracellular matrix of the diced cartilage was digested for 12–18 h at 37°C in the presence of 2 mg/ml Type II collagenase (Seromed, Berlin, Germany) and 0.1 mg/ml hyaluronidase (Serva, Berlin, Germany) in Ham's F12 medium. The resulting cell suspension was filtered through a nylon sieve with a pore size of 70 μm. The suspension was centrifuged at 1700 rpm for 7 min, washed twice in RPMI medium and resuspended in RPMI medium.

2.4. Tissue engineering and culture

Chondrocytes approximately tripled in one week as a monolayer culture and were subsequently treated with

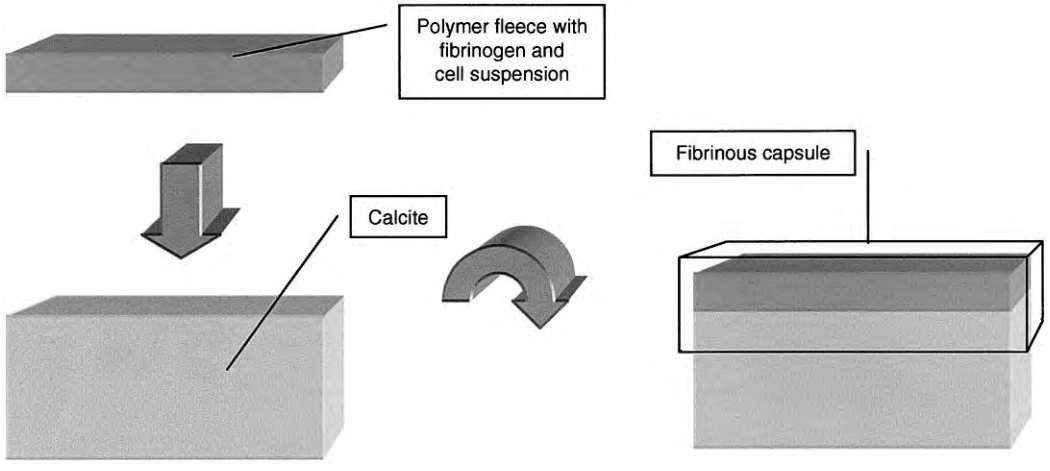

Fig. 2. The polymer fleece containing fibrinogen and cell suspension was mounted on a block of calcite and encapsulated with fibrin.

trypsin and washed twice in medium. These cells were brought to a density of approximately 20–25 million cells/ml in a 1 : 3.5 diluted fibrinogenic solution. The polymer fleece (Fig. 1) was repeatedly inoculated with this suspension over several minutes in order to achieve a high cell concentration. After seeding onto the two different biomaterials, the newly created biphases were fixed with 1 : 10 diluted thrombin and encapsulated (Figs. 2 and 3). Cultures were incubated in perfusion culture chambers at 37° [11,12] (Fig. 4(a)). Ham's F12 (Gibco) supplemented with 2% FCS and 50 mg/ml ascorbic acid was used as culture medium. A peristaltic pump was operated in on-off intervals of 30 min to guarantee constant supply of 1 ml fresh culture medium per 1 h. Fresh medium was taken from a cooled reservoir and used medium was pumped into a waste bottle (Fig. 4(b)). The temperature inside the perfusion culture system was generated by a heating plate to guarantee constant 37°C. The system was operated at room atmosphere. After keeping the specimens in perfusion culture for 7, 10, 14, 28, 42, 56, respectively, 70 days, the biphases were removed, fixed in Lillie's 7% formaldehyde solution pH 7.2, dehydrated in 70, 80, 96 and twice 100% ethanol,

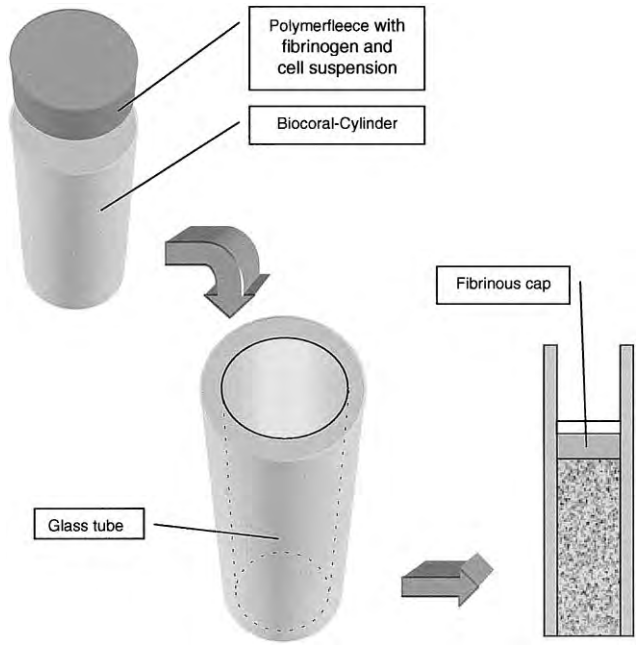

Fig. 3. A polymer fleece loaded with fibrinogen and cells was mounted on a block of natural coral and fixed within a glass tube.

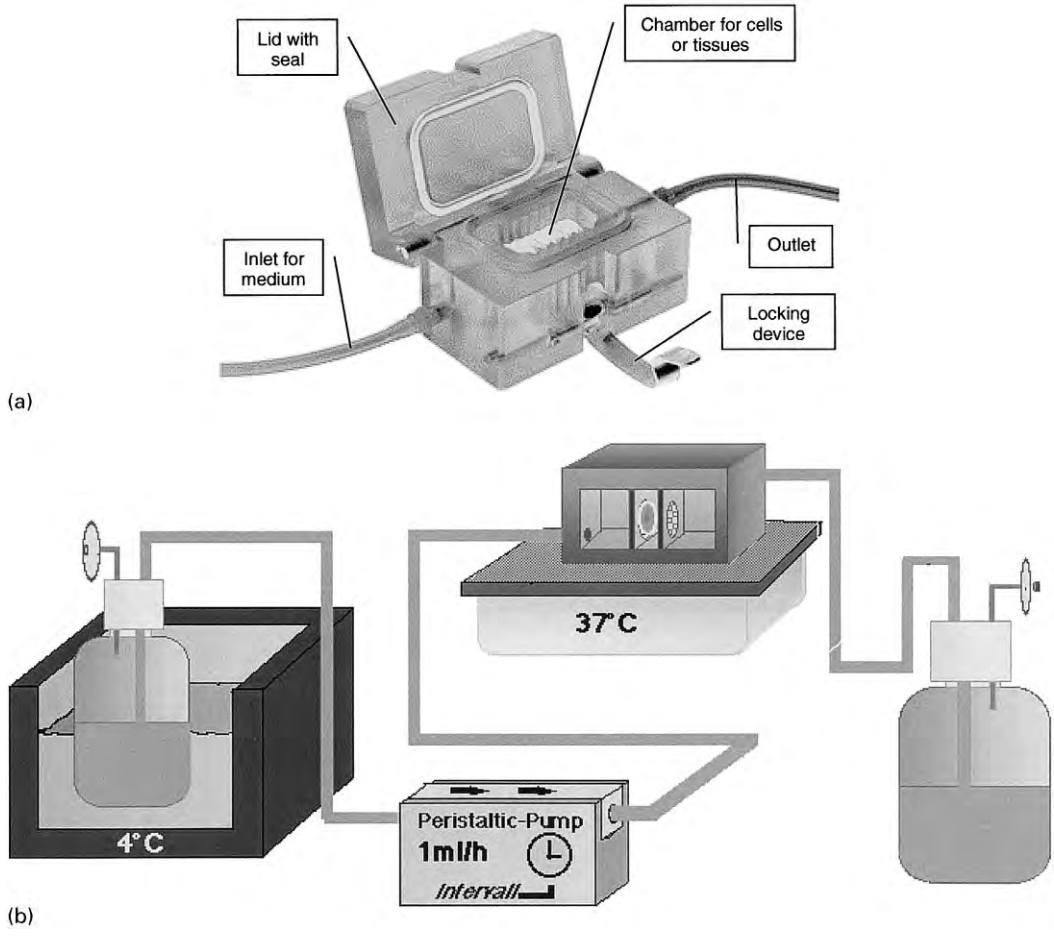

Fig. 4. (a, b) Perfusion culture chamber. A peristaltic pump delivers a constant flow of fresh culture medium from a medium reservoir as described before [16]. The culture chamber is placed upon a heating plate to guarantee a temperature of 37°C. Used medium is collected in a waste bottle.

soaked in polymethylmethacrylate monomer and polymerised into blocks for preparation of sawed 50 μm thick sections using a sawing microtome (Leitz® 1600, Wetzlar, Germany). The sections were stained in Giemsa, doublestained in Alcian/PAS respectively Kossa/Paragon solution and analysed in transmission light microscopy.

2.5. Calcite

Calcite was covered by the polymer fleece containing the cell suspension in fibrin as shown in Fig. 2.

2.6. Natural coral

A natural coral cylinder was inserted into a corresponding glass tube and fixed with the same thrombin solution after applying the matching polymer fleece (Fig. 3). This way the cartilage was solely supplied by medium from the surface and base.

3. Results

3.1. Tissue stability

The fleece adheres to the biomaterials after fixation by fibrin encapsulation (Figs. 5, 6(a) and (b)). The interrelation between cells, biomaterial, PGLA-fibres and polydioxanon-fixation-points are shown in Fig. 7(a) and (b). Apart from being evenly distributed in the polymer fleece, the chondrocytes also adhere to the biomaterial (Figs. 8 and 9). Fibres of the fleece are present in the early stages of cultivation and provide a three dimensional scaffold for the chondrocytes (Figs. 5, 6(a) and (b)). While matrix is produced by the cells, the fibres of the fleece are partly dissolved (Fig. 10) in later stages.

3.2. Tissue morphology

The proteoglycans and collagens synthesised by the chondrocytes are held in place by fibrin fixation and encapsulation as previously described [13]. Consequently a new matrix with metachromatic staining is formed (Figs. 6(b) and 11).

During the incubation periods of up to ten weeks, the cells remain vital, and display a round morphological appearance which is essential for production of cartilage like matrix by the chondrocytes [14].

3.3. Histological analysis

Both preparations (natural coral and calcite) show a rather even density and distribution of vital chondrocytes (Figs. 6(a), (b) and 8). In the interface between biomaterial and polymer fleece some chondrocytes are found to adhere to the biomaterial (Figs. 8 and 9). After

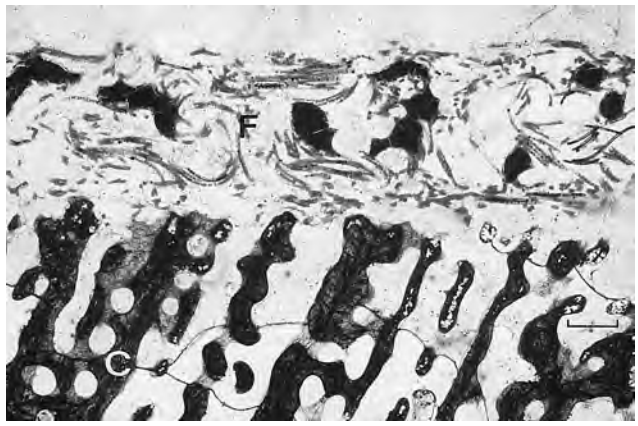

Fig. 5. Culture of bovine chondrocytes within a fleece (F) above porous natural coral (C) affixed by fibrin encapsulation, 10 days after seeding. Note the penetration of the cell suspension into to the biomaterial. Sawed section. Alcian/PAS staining. Bar 250 μm.

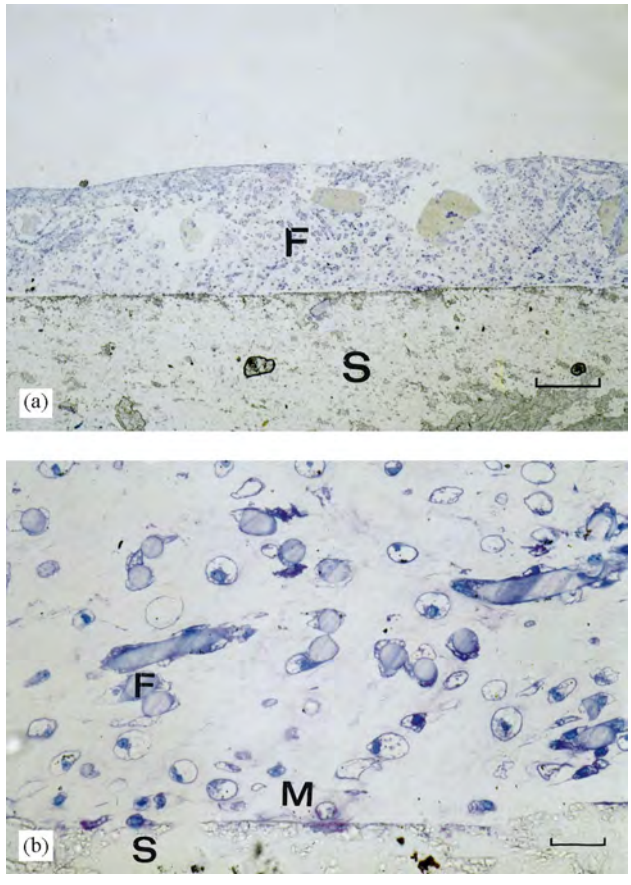

Fig. 6. Fleece (F) with chondrocytes at the surface of bulk calcite support (S) affixed by fibrin encapsulation, 42 days after seeding. Sawed section. Giemsa staining: (a) bar 250 μm, (b) metachromatically stained extracellular matrix (M). Bar 25 μm.

different periods of cultivation there is only little difference in the distribution of chondrocytes but synthesised intercellular matrix seems to increase gradually. As a sign of vitality and differentiation the chondrocytes synthesise

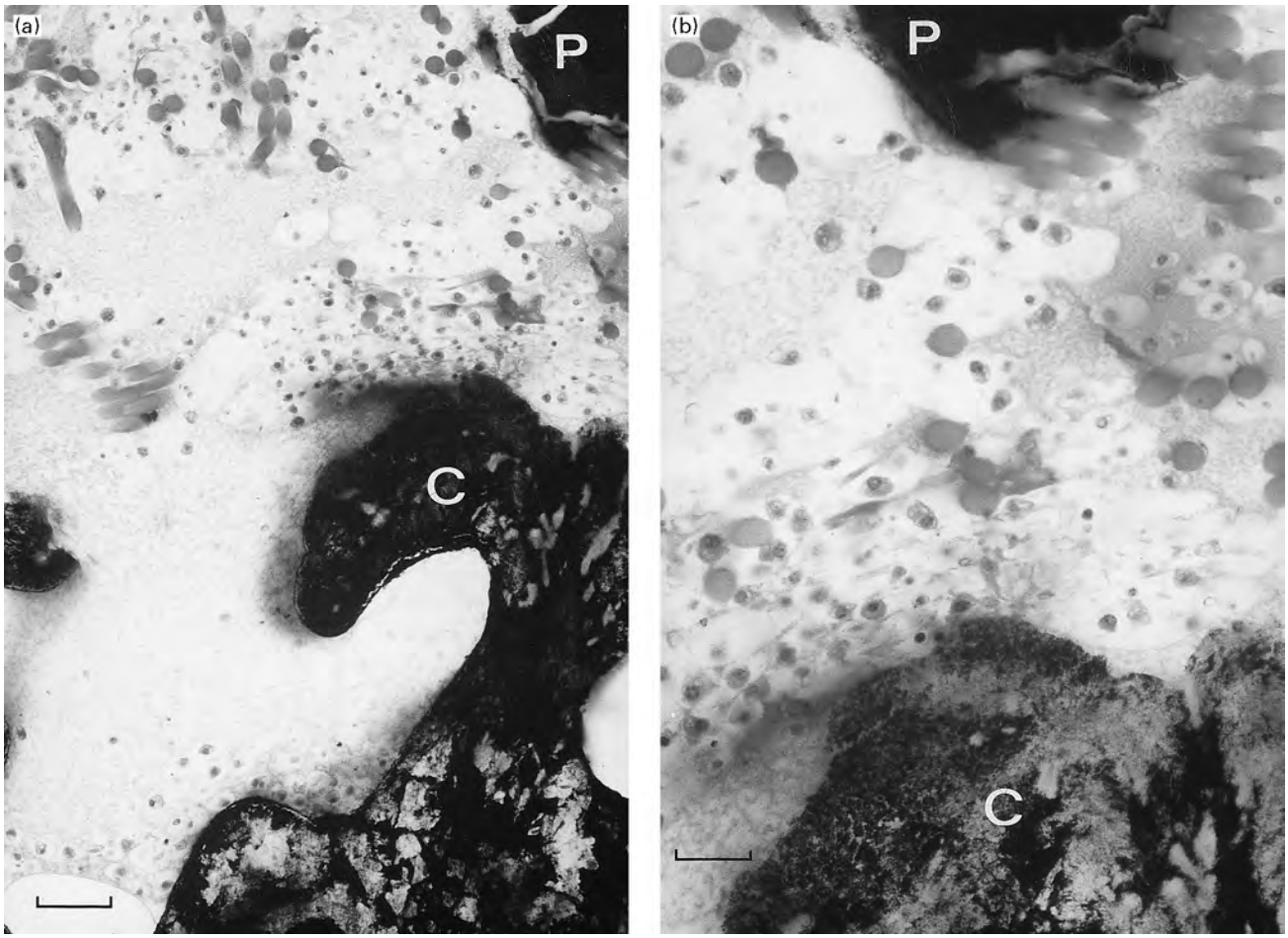

Fig. 7. Interrelation between cells, porous natural coral (C), PGA-fibres and polydioxanon-fixation-points (P), 28 days after seeding. Sawed section. Kossa/Paragon staining. (a) Bar 50 μm. (b) Bar 25 μm.

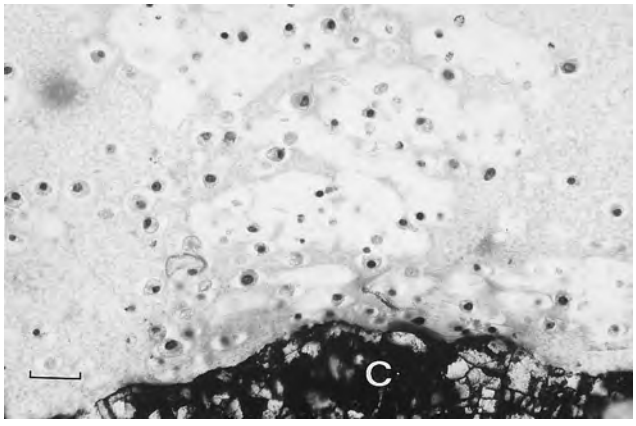

Fig. 8. Chondrocytes rather evenly distributed and adhering to the surface of natural coral (C), 7 days after seeding. Sawed section. Kossa/Paragon staining. Bar 25 μm.

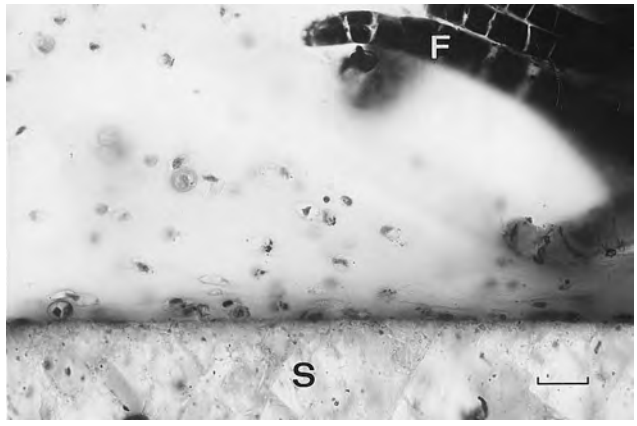

Fig. 9. Chondrocytes adhering to the surface of the calcite support (S), 14 days after seeding. Polymer fibres (F). Sawed section. Kossa/Paragon staining. Bar 25 μm.

ground substance which is staining metachromatically (Figs. 6(b) and 11) in an inhomogeneous distribution. Morphologically the viability and matrix production neither seem to depend on the two different medium supply techniques nor on the biomaterial. However, the porosity of the natural coral seems to have the advantage of a better penetration by the cell suspension into the biomaterial (Fig. 5).

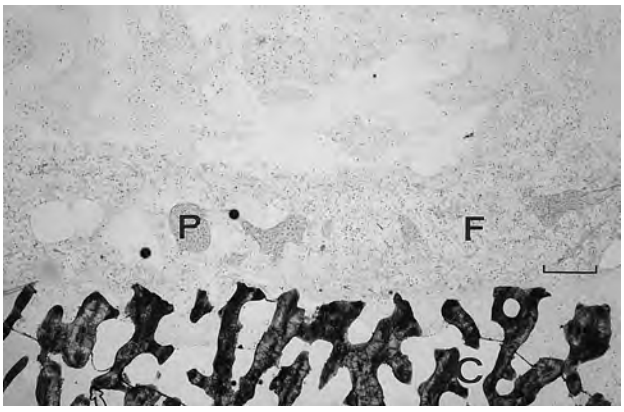

Fig. 10. Culture of bovine chondrocytes within a fleece (F) above porous natural coral (C), 28 days after seeding. PGA-fibers are partially dissolved, while polydioxanon (P) takes longer to resorb. Sawed section. Giemsa staining. Bar 250 μm.

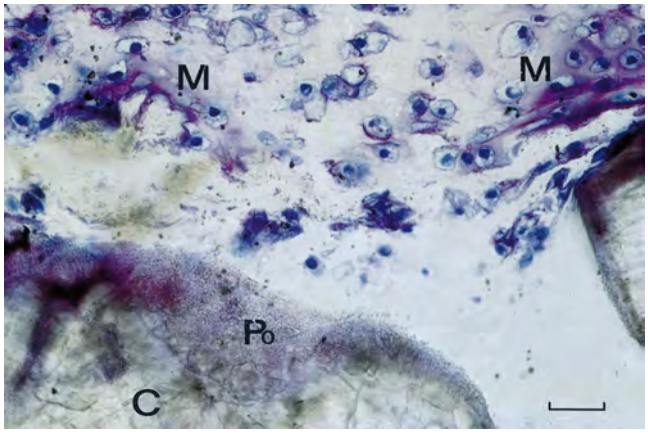

Fig. 11. Chondrocytes within metachromatically stained extracellular matrix (M), stained surface of natural coral (C) porosities (Po) after 70 days in culture. Fibres of the fleece already degraded and dissolved (see also Fig. 10). Sawed section. Giemsa staining. Bar 25 μm.

4. Discussion

Isolation, multiplication in a monolayer culture and subsequent dissemination in a three-dimensional polymer fleece should enable the regeneration of autologous cartilage in vitro without causing a rejection in the following implantation, presenting a promising prospective. The binding of cartilage cultured from isolated chondrocytes with bone-absorbable material creates an implant with considerably improved anchorage for the reconstruction of destroyed articular surfaces.

The in vitro creation of three-dimensional human chondrocyte structures was shown by Sittinger and Burmester [15,16] from our own interdisciplinary study group. Besides using the additional biomaterials (calcium carbonate), the specimen were encapsulated in agarose gel in the former study, while fibrin is used for encapsulation now in order to keep the problem of biocompatibility to a minimum. In contrast to agarose fibrin is also bioresorbable. The chondrocytes are distributed three-dimensionally with the aid of a resorbable fleece [13,16]. Of particular interest in the present study is the behaviour of artificial cartilage at the surface of a supporting porous biomaterial. The potential of both phases to interact in vitro in order to create a mechanically stressable junction is examined.

Since no significant morphological differences of the specimen are found after supplying the cultures with medium in two different ways, it can be assumed, that a medium supply solely from surface and base does not improve tissue morphology in this perfusion culture system. However, the porosity of the natural coral seems to have the advantage of a more stable interaction between the two phases since the penetration of the cell suspension into the biomaterial is superior. Additional toothing of the layers may also be promising in increasing shear stress stability of joint cartilage transplants.

So far we have shown that biphasic autologous joint cartilage transplants can be cultured. Further investigations have to focus onto the mechanical properties. Developing a suitable in vivo procedure including the integration of the bilayer constructs will be the next step. The attachment to neighbouring tissues and the replacement of the biomaterials by bone has to be analysed [17,18].

Moreover, sintering a biomaterial with a polarised porosity pattern comparable to a natural bone–cartilage-morphology may present an interesting alternative to currently available biomaterials.

Acknowledgements

This work is supported by the Deutsche Forschungsgemeinschaft, (DFG Bu 445/5-1), and the SONNENFELD-STIFTUNG, Berlin. The authors express their thanks to Mrs. M. Dilger, Mrs. I. Borchert and Mr. L. Oehring for excellent technical assistance.

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