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Effects of Bone Morphogenetic Protein-7 Stimulation on Osteoblasts Cultured on Different Biomaterials

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Abstract The objective of the present study was to investigate the effects of an in vitro stimulation of human osteoblasts by recombinant human bone morphogenetic protein-7 (rhBMP-7) on the collagen types and the quantity of the collagen cross-links synthesized in a three-dimensional culture on various biomaterials for bone replacement. Trabecular bone chips were harvested from human iliac crests, and cell cultures were established at standard conditions. One hundred and fifty nanograms per milliliter of rhBMP-7 was added. For the second passage a cell scraper was used to bring the cells into suspension, and 100 µl osteoblasts (at a density of 3.3×10^5) were transferred onto nine blocks of either Bio-Oss®, Tutoplast®, or PepGen p-15™. Blocks incubated with cells that were not treated with rhBMP-7 served as controls. Cell colonization of the biomaterials was observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) after a period of 2, 4, and 6 weeks. Throughout the experiment medium, supernatants were collected and collagen was characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Finally, the collagen cross-link residues hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) were quantified by HPLC. Within 4 weeks the cells became confluent on all of the studied biomaterials. All samples synthesized bone specific LP and collagen type I. However, in rhBMP-7-stimulated samples, the amount of HP and LP found was increased by 45% compared to non-stimulated samples. Cell proliferation and collagen synthesis was similar on the different biomaterials, but was consistently reduced in specimen not stimulated with rhBMP-7. In vitro stimulation of osteoblasts on Bio-Oss, Tutoplast, or PepGen p-15 with rhBMP-7 and subsequent transplantation of the constructs might lead to an enhanced osseointegration of the biomaterials in vivo. J. Cell. Biochem. 86: 90–98, 2002.

Key words: rhBMP-7; cell culture; biomaterials; collagen cross-links

Bone morphogenetic proteins (BMPs) are disulfide-bonded glycoproteins, are low in molecular weight, and are members of the transforming growth factor β (TGFβ) superfamily [Wozney and Rosen, 1998]. BMPs are powerful autocrine and/or paracrine local hormones that were recognized by their induction of heterotopic bone formation [Urist, 1995]. In case of a bone fracture, BMP synthesis is increased as a local response 24 h after the event followed by a limited period of 8–10 days of proliferation and cytodifferentiation of osteoprogenitor cells [Henricson et al., 1991]. BMPs are characterized by their ability to stimulate the differentiation of stem cells into chondrocytes or osteoblasts. Recombinant human BMP-7 (rhBMP-7 = recombinant human osteogenic protein-1 = rhOP1), stimulates osteoblast differentiation in vitro and induces bone formation in vivo [Yeh et al., 1998a,b].

Bone grafts are needed in surgery for various indications, such as the filling of bone cavities, the augmentation for dental implants, the regeneration of bone after tumor removal or trauma [Damien and Parsons, 1991; Qian and Bhatnagar, 1996; Bhatnagar et al., 1999; Mayr-Wohlfart et al., 2001], and many others. Alternatively, natural bone mineral (Bio-Oss), natural spongiosa bovine blocks (Tutoplast), and natural microporous xenogenic bone mineral coated with cell-binding peptide P-15™ (P-15: potent cell binding domain in the α1(I) chain of bone collagen type I, located in the sequence ^{766}GTPGPQGIAGQRGVV^{780} PepGen p-15™) are favored by some authors to facilitate

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the growth of new bone into osseous defects in certain clinical situations [Thaller et al., 1994; Qian and Bhatnagar, 1996; Berglundh and Lindhe, 1997; Açil et al., 2000]. These studies indicate that natural bone mineral can be used as an osseous graft material [Rey, 1990; Begley et al., 1993; Valdre et al., 1995; Açil et al., 2000]. However, there is need of further studies to evaluate how bone cells proliferate and elaborate matrix on these biomaterials (Bio-Oss, Tutoplast, and PepGen p-15). Furthermore, the effect of rhBMP-7 on the proliferation and extracellular matrix synthesis of osteoblasts on these biomaterials (Bio-Oss, Tutoplast, and PepGen p-15) mentioned has to be examined prior to clinical application. In the present study we planned to combine the osteoconductive effect of the biomaterials mentioned above with the osteoinductive properties of rhBMP-7 with the aim to test the potential of this model for the tissue-engineered growth of human bone.

Cell culture studies are an effective way to examine how osteoblastic cells interact with biomaterials. A classic way to prove the presence of osteoblast-like cells is the determination of the collagen types synthesized by the cell cultures studied [Açil et al., 1999]. Moreover, hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) are non-reducible cross-links of mature collagen, and the presence of LP in cell culture medium supernatants demonstrate the synthesis of bone specific extracellular matrix [Kuboki et al., 1992; Açil and Müller, 1994; Seitzer et al., 1995; Açil et al., 1997, 1999, 2000].

Our study was based on the hypothesis that osteoblast-like cells cultured under stimulation with rhBMP-7 on Bio-Oss, Tutoplast, or PepGen p-15 would produce identical collagen types but increased amounts of mature collagen and collagen cross-links as compared to non-stimulated samples [Açil et al., 1999, 2000; Bhatnagar et al., 1999]. To test this hypothesis, we cultured osteoblast-like cells from the human iliac crest on Bio-Oss, Tutoplast, and PepGen p-15 with and without the stimulation of rhBMP-7. The structural characteristics were to be examined by means of scanning as well as transmission electron microscopy. In addition, we analyzed the collagen types synthesized by SDS–PAGE and the amount of HP, LP, and hydroxyproline (Hyp) released into the medium supernatants by high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Human Bone Cell Culture

Human bone cells (hOB, osteoblasts) were gained from the trabecular bone of the iliac crest of children aged 8–10 years. These children received bone transplants in the course of reconstructive treatment of a cleft maxilla. In these operations, the donor site (iliac crest) needs to be closed up before surgery; it is started inside the oral cavity to avoid an infection of the iliac bone. Therefore, the amount of bone that needs to be raised is not exactly known. To avoid a reopening of the donor site after operation in the oral cavity, bone is raised in excess, routinely. The parents of the patients gave written consent to use bone for research purposes, in case the amount that was available exceeded the amount that was needed for osteoplasty. The specimens were dissected into fragments of 0.3–0.5 cm in diameter and extensively rinsed with PBS (phosphate-buffered saline; pH = 7.4). The fragments were seeded as explants into tissue culture flasks and cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Culture medium was Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS), 10^5 IU penicillin, 100 mg/L streptomycin, 2 mM L-glutamine, 100 nM dexamethasone (Biochrom, Berlin, Germany), and 1 mM L-ascorbic acid 2-phosphate (Sigma, Deisenhofen, Germany). Bone morphogenetic protein-7 dissolved in 20 mM sodiumacetate buffer (pH = 4.5) was added to a concentration of 150 ng/ml medium (Creative BioMolecules, Hopkinton, MA). Cells were defined as osteoblast-like cells by the determination of osteoblast markers (synthesis of osteocalcin and activity of alkaline phosphatase). Cells were subcultured in a second passage at a density of $3.3 \times 10^6/cm^2$. For the second passage, a cell-scraper was used to bring the cells into suspension. One hundred microliters containing 3.3×10^5 osteoblast-like cells [Açil et al., 2000; Springer et al., 2001] were transferred to a natural bone mineral block (Bio-Oss, Geistlich, Wolhusen, Switzerland) to a natural bovine spongiosa block (Tutoplast, Tutogen Medical GmbH, Erlangen, Germany) and to a natural microporous xenogenic bone mineral block coated with cell-binding peptide P-15 (PepGen p-15, OsteoGraf/N-300, CeraMed Dental, L.L.C., Lakewood, Co). Bio-Oss is a pure mineral derived from bovine spongy bone,

whereas Tutoplast is bovine mineral plus associated acellular bone matrix. PepGen p-15 is pure mineral comparable to Bio-Oss, but a cell-binding peptide is added [Thaller et al., 1994; Qian and Bhatnagar, 1996; Berglundh and Lindhe, 1997]. These materials are commercially available, and the size of the blocks was $10 \times 10 \times 20$ mm for all experiments. All experimental groups with and without rhBMP-7 were repeated three times.

Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase (ALP) is an enzyme thought to be important in the process of biomineralization. ALP promotes hydrolysis of phosphate containing substrates, produces orthophosphate, and increases deposition of calcium phosphate. Recently, ALP has been demonstrated to enhance mineralization of collagen sheets. Cells were seeded at 1×10^3 cells/cm^2 in Lab-Tek chamber slides (Nunc, Denmark), and cultured for 3 days. Alkaline phosphatase activity was determined by cytochemistry with the Sigma Diagnostic Kit (86-R, Deisenhofen, Germany), as described previously [Açil et al., 1999, 2000].

Osteocalcin Synthesis

Osteocalcin is the most abundant non-collagenous protein in mineralized bone matrix. The expression of osteocalcin was studied using monoclonal antibodies provided by Takara (Takara Shuzo, Co., Ltd., Japan), as described previously [Açil et al., 2000].

Extraction and Preparation of Pyridinolines From the Collected Supernatants Cell Culture Mediums

Five milliliters of aliquot was either lyophilized for subsequent preparation of pyridinolines or used for extraction of collagen (see below). For each time point three supernatants were available.

Each lyophilized sample was subsequently redissolved in 1 ml 6 N hydrochloric acid, as described previously [Açil et al., 1999, 2000].

Pyridinoline Standards and Analysis of Hydroxlysylpyridinoline (HP) and Lysylpyridinoline (LP)

The hydroxlysylpyridinoline (HP) and lysylpyridinoline (LP) content was quantified using external standards prepared from a commercially-available adult bovine bone gelatin (Deutsche Gelatine-Fabriken Baden, Germany) prior to sample application onto the chromatography system. HP and LP were purified by a preparative reverse-phase-column HPLC. Chromatography was performed on an HPLC system (Dionex, Idstein, Germany) at room temperature as previously described [Açil et al., 1999, 2000].

Analysis of Hydroxyproline (Hyp)

The assay was performed in a flat-bottomed 96-well plate. Ten microliters of each hydrolyzate were diluted with distilled water (1/5 to 1/40). Standard solutions of Hyp ranging from 1 to 5 μg/ml were formulated. Thirty microliters of sample or standard solution were pipetted into the wells of the plate. Sixty-nine microliters of 2:1 propan-2-ol distilled water were then added to all wells, as previously described [Açil et al., 2000].

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and the preparation of the specimen for these examinations were performed as described in our previous studies [Açil et al., 1999, 2000].

Extraction of Collagen Types I and V From the Collected Supernatants

In order to isolate collagen types I and V, 100 ml of the collected supernatants were precipitated in 4.5 M NaCl (pH = 7.5) and stirred at room temperature for 24 h. Then, the precipitations were centrifuged (12,000*g*, 1 h, 4°C). The pellet was dissolved in 0.5% acetic acid and then dialyzed against 0.5 M acetic acid and 0.2 M NaCl (pH = 2) for an additional 3 days. Limited pepsin digestion was performed to solubilize the collagen. Samples were stirred in a pepsin solution (0.5 M acetic acid, 0.2 M NaCl, 0.1 mg/ml, pH = 1.8; Boehringer Mannheim, Germany) at room temperature for 24 h. The samples were centrifuged at 12,000*g* for 1 h at 4°C. The supernatants were neutralized and stored at −20°C. This digestion procedure was repeated five times and all neutralized supernatants were pooled [Açil et al., 1997, 1999, 2000; Springer et al., 2001]. Again, the collagen was precipitated in 4.5 M NaCl. After centrifugation (at 12,000*g* for 1 h at 4°C), the pellets were resuspended in 0.5% acetic acid and

extensively dialyzed against 0.5% acetic acid and lyophilized.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE)

One to two micrograms of lyophilized collagen were dissolved to a final concentration of 2 mg/ml of sample buffer (63 mM Tris, 2% SDS, 10% glycerol, 0.01% bromophenol Blue, pH = 6.8) and SDS–PAGE was performed as described in our previous studies [Açil et al., 1997, 1999, 2000; Springer et al., 2001].

RESULTS

The cells of the different samples in the present study were defined as osteoblast-like cells by the determination of osteoblast specific markers: the presence of osteocalcin (data not shown), the alkaline phosphatase activity (data not shown), the collagen types synthesized (below), and the production of HP and LP, as below.

Biochemical analysis of the extracellular matrix (ECM) by HPLC demonstrated the presence of the cross-links HP and LP specific for bone type I collagen in all groups. Figure 1a,b shows representative chromatograms of the PepGen p-15 group. The HP, LP, and Hyp content of the medium supernatants of the three-dimensional cultures increased in a time-dependent manner and was notably higher in all of the rhBMP-7 treated groups (Table I). Within the groups treated with rhBMP-7 and within the groups not treated with rhBMP-7, there was no significant difference of the amount of HP, LP, and Hyp synthesized.

A sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of the α-chains of types I, II, III, and V collagen is shown in Figure 2. The α-chains of type I collagen demonstrated a typical pattern in that α2(I)-chains have a greater electrophoretic mobility than α1(I)-chains of the same molecular mass (95,000). For the identification of collagen III, delayed reduction was performed. Therefore, the α-chains of collagen III migrated less far than did the α1(I)-chains (apparent molecular masses 140,000–160,000). The α-chains of type V collagen have a molecular mass of approximately 125,000. On SDS–PAGE, mature collagen extracted from the pooled medium supernatants showed an identical electrophoretic pattern compared to those in human bone (Fig. 2). The pooled medium supernatants of all experimental groups did prove the presence of collagen type I (Fig. 2). Additionally, the samples obtained from the rhBMP-7 stimulated groups appeared to show a notably increased intensity of the bands on the acrylamide gel as compared to the non-stimulated groups (Fig. 2).

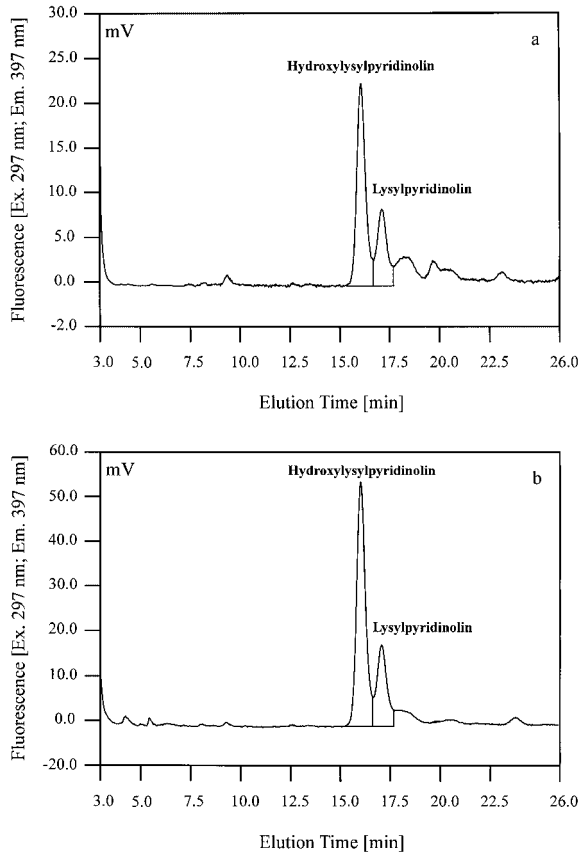

Fig. 1. Chromatograms of culture medium supernatants after 6 weeks of culture. **a**: PepGen p-15-group without rhBMP-7; **(b)** PepGen p-15-group with rhBMP-7. The samples were injected (200 µl, in 0.22% HFBA) and analyzed by HPLC. Hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) were eluted with a gradient from 18 to 20% acetonitrile for 15 min. The fluorescence intensity was measured using a Gynkotek RF 1002 spectrofluorimeter with excitation at 297 nm and emission at 397 nm. Please note the increased peaks of HP and LP in chromatogram b.

Scanning electron microscopy did reveal no difference of the structure of the cells cultured on Bio-Oss, Tutoplast, and PepGen p-15. However, in cultures stimulated with rhBMP-7, the cell density on the biomaterials appeared to be notably increased compared to cell cultures that were not stimulated with rhBMP-7 (Figs. 3a–f).

TABLE I. Analysis of Hydroxyproline (Hyp), Hydroxylylsylpyridinoline (HP), and Lysylpyridinoline (LP) in the Pooled Medium Supernatants After 6 Weeks of Incubation of Human Osteoblast-Like Cells on Bio-Oss, Tutoplast, and PepGen p-15

Incubation (6 weeks)	Hyp [μmol/ml]	HP [nmol/ml]	LP [nmol/ml]
Bio-Oss without rhBMP-7	7.6	88.7	17.8
Bio-Oss with rhBMP-7	10.8	128.6	26.2
Tutoplast without rhBMP-7	8.3	90.2	11.4
Tutoplast with rhBMP-7	12.3	133.1	27.6
PepGen p-15 without rhBMP-7	7.7	91.3	16.6
PepGen p-15 with rhBMP-7	11.2	130.3	25.9

The variation of the concentration measured in three repeats was $< 3\%$.

Transmission electron microscopy revealed an intense contact between the cellular membrane and the surface of the different biomaterials. Layers of osteoblast-like cells were indicating the three-dimensional organization of the cells on the natural bone mineral. No difference was apparent between the different materials. In the rhBMP treated samples, increased deposition of collagen fibrils in the extracellular space and an increased cell density was obvious as compared to samples that were not treated with BMP-7. As representative for all groups, TEM-Micrographs of PepGen p-15 incubated with osteoblast-like cells treated with (Figs. 4a–b) and without BMP-7 (Figs. 4c–d) are shown. This was in accordance with the data obtained by the collagen analysis (SDS–PAGE) and the quantitative analysis of the collagen cross-links HP and LP as well as the collagen marker Hyp (Table I).

DISCUSSION

The interaction of several matrices with cultured human osteoblast-like cells has been evaluated in various in vitro studies [Seitzer

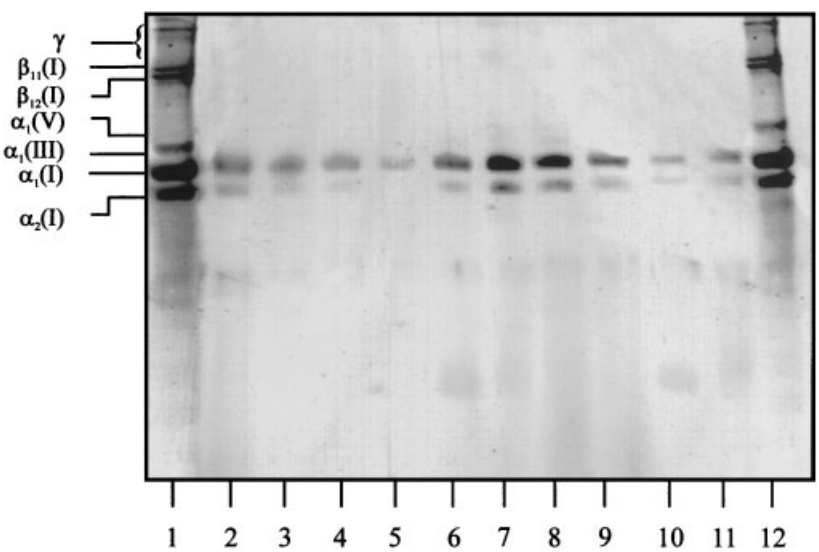

Fig. 2. SDS–PAGE of different collagen types showing their α-, β-, and γ-components. All samples were obtained from the pooled medium supernatants of the different groups after 6 weeks of incubation. The samples were dissolved in sample buffer with a concentration of 2 mg/ml (1 M Tris, 2% SDS, 10% glycerol, 0.25% bromophenol Blue, pH = 6.8). After heating at 95°C for 3 min, the samples were quenched on ice. **1** = standard collagen types I and III from human fibroblast-like cell culture; **2** = Tutoblast-group with rhBMP-7; **3** = Tutoblast-group without rhBMP-7; **4** = monolayer culture of osteoblast-like cells with rhBMP-7; **5** = monolayer culture of osteoblast-like cells without rhBMP-7; **6** = PepGen p-15-group without rhBMP-7; **7** = PepGen p-15-group with rhBMP-7; **8** = Bio-Oss-group with rhBMP-7; **9** = Bio-Oss-group without rhBMP-7; **10** = monolayer culture of osteoblast-like cells without rhBMP-7; **11** = monolayer culture of osteoblast-like cells with rhBMP-7; **12** = standard collagen types I and V from human fetal bone; 1 and 12 α-, β_{12}-, β_{11}-, and γ-components from human collagen extracts.

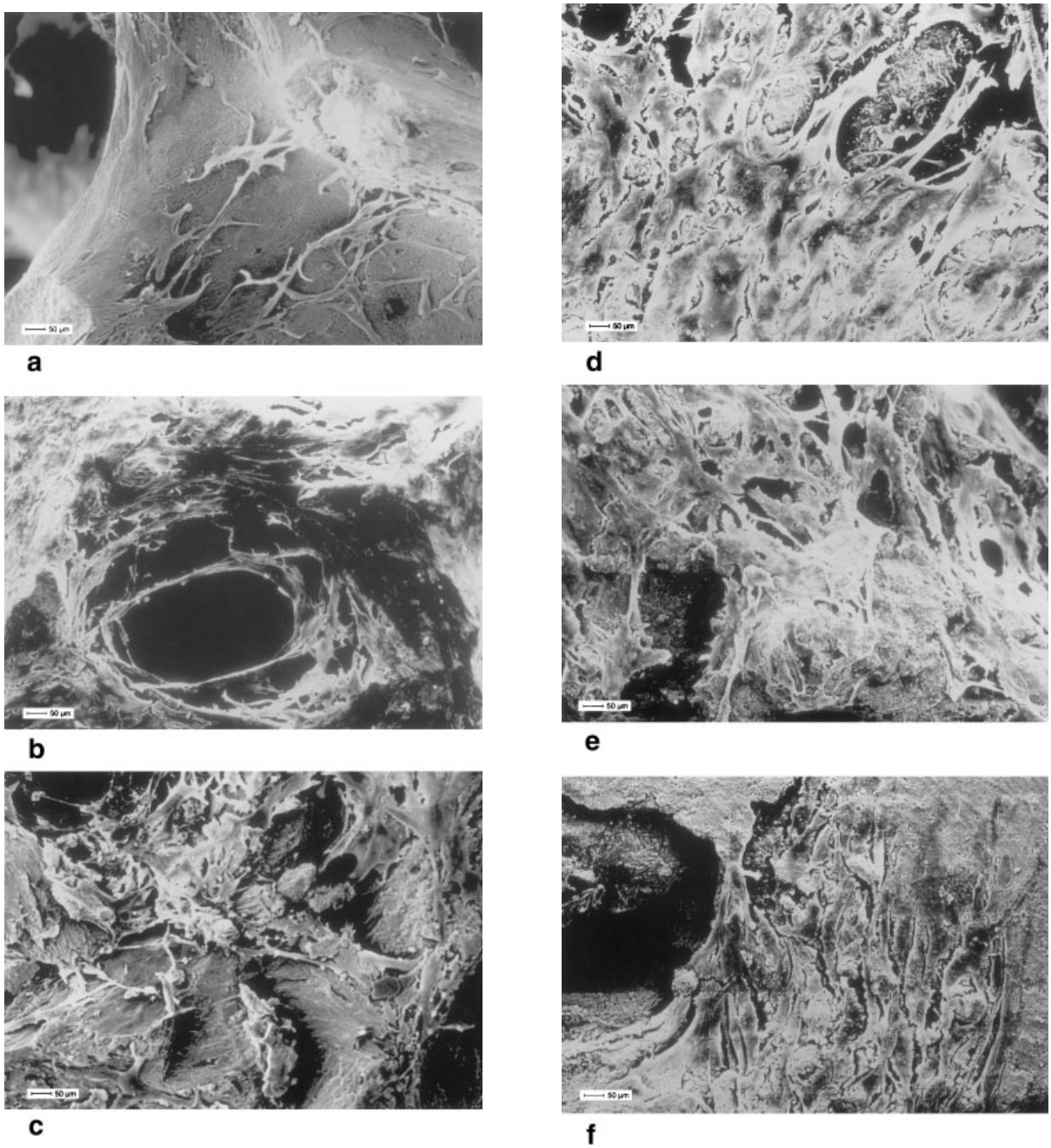

Fig. 3. Scanning electron micrographs showing the colonization of a Bio-Oss-block, a Tutoplast-block, and a PepGen p-15-block by osteoblast-like cells. **a**: Bio-Oss-block, cells after 4 weeks in culture without rhBMP-7, the porous surface is partially visible. **b**: Bio-Oss-block, cells after 4 weeks in culture with rhBMP-7, increased density of cells as compared to Figure 3a. **c**: PepGen p-15-block, cells after 4 weeks in culture without rhBMP-7, the porous surface is partially visible. **d**: PepGen p-15-block, cells after 4 weeks in culture with rhBMP-7, increased density of cells as compared to Figure 3c. **e**: Tutoplast-block, cells after 4 weeks in culture without rhBMP-7, the porous surface is partially visible. **f**: Tutoplast-block, cells after 4 weeks in culture with rhBMP-7, increased density of cells as compared to Figure 3e (original magnification × 1450, bar equals 50 μm).

et al., 1995; Zambonin et al., 1997; Yeh et al., 1998a,b; Açil et al., 2000]. In the present study, we investigated the effects of recombinant human bone morphogenetic protein-7 (rhBMP-7) on human osteoblast-like cells cultured on three different kinds of carrier biomaterials (Bio-Oss, Tutoplast, and PepGen p-15). Therefore, the attachment, proliferation, and matrix synthesis of the cells cultured on the different materials was examined by scanning and

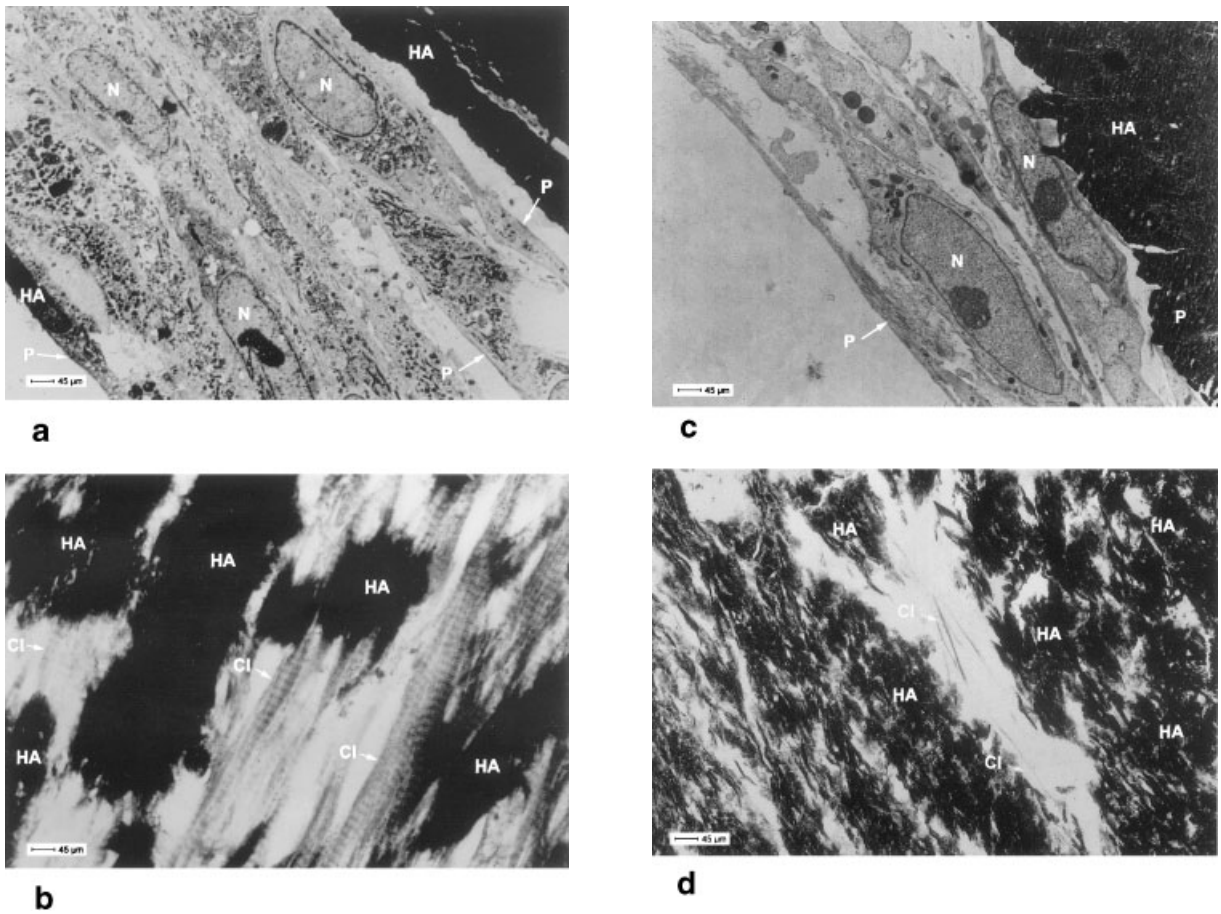

Fig. 4. **a**: Transmission electron micrographs after 6 weeks in culture with rhBMP-7. a: PepGen p-15-block, with BMP-7: Layers of osteoblast-like cells are indicating the three-dimensional organization of the cells in close contact with the surface of natural bone mineral (HA); N, nucleus; P, plasma membrane. **b**: PepGen p-15-block, with BMP-7: the extracellular space between the cells is occupied by collagen fibrils cut longitudinal in this illustration (Cl). **c**: PepGen p-15-block, without BMP-7: a reduced density of osteoblast-like cells was observed. **d**: PepGen p-15-block, without BMP-7: a reduced density of collagen fibrils was observed (original magnification × 26,000, bar equals 45 μm).

transmission electron microscopy as well as biochemical analysis.

After 6 weeks of incubation, SEM and TEM analysis revealed that osteoblast-like cells were able to colonize the biomaterial and to synthesize collagen. Our observations suggest that an organic bovine bone has a three-dimensional structure that provides the opportunity for bone formation within the material. Similar observations were reported for the colonization of a bovine-derived hydroxyapatite ceramic using human bone marrow cells and for a synthetic hydroxyapatite using rat calvaria cells [Zambonin et al., 1997]. Our findings supplement and extend a recent report on the early (up to 24 h) attachment and proliferation of neonatal rat calvaria cells on the same natural bone mineral (Bio-Oss) in particulate form [Stephan et al., 1999]. The present study demonstrates that attachment and proliferation of bone cells on the mentioned carriers can be stimulated by the application of rhBMP-7, additionally. In accordance with our results, Mayr-Wohlfart et al. [2001] reported that rhBMP-2 and rxBMP-4 (x = *Xenopus laevis*) have a favorable effect on the differentiation and proliferation of human primary osteoblast-like cells in cell culture.

We observed the formation of mature collagen fibrils in the control groups, which was increased in the rhBMP-7 stimulated groups. The formation of mature collagen reflects the complete processing of procollagen to mature triple helices, including the formation of two kinds of covalent non-reducible cross-links (HP and LP), which are formed by a sequence of post-translational modifications [Kuboki et al., 1992; Açil et al., 2000]. In the present study, biochemical analysis of the pooled medium supernatants

after pepsin digestion confirmed that the collagen produced was composed of collagen peptides that have intermolecular cross-links. In fact, we measured contents of HP, LP, and Hyp. As expected, HP, LP, and Hyp were increased in the rhBMP-7-stimulated group.

Bio-Oss is pure highly porous natural bone mineral [Valentini and Abensur, 1997; Açil et al., 2000]. PepGen p-15 is highly porous natural bone mineral coated with cell-binding peptide P-15 [Qian and Bhatnagar, 1996; Bhatnagar et al., 1999; Lallier et al., 2001]. Contrary to that, Tutoplast consists not only of bone mineral but also bears all components of natural bone except from vital bone cells [Hofmann et al., 2000]. It may not be possible to differentiate between extracellular matrix proteins synthesized by the human osteoblast-like cells during the experiments from proteins that were present in the specimen prior to the experiments. Therefore, data obtained in the Tutoplast-group were treated carefully and are not to be compared with the data of the other experimental groups. Still, we were able to show that in the medium supernatants of cultures on Tutoplast, the amounts of Hyp, HP, and LP were equivalent to those in the other groups (Bio-Oss and PepGen p-15).

In our study, SEM indicated that the density of the cell layer might be increased on PepGen p-15 as compared to Bio-Oss. This may be due to effects of cell-binding peptide P-15. However, biochemical analysis did show no differences between these two groups.

In the present study, we attempted to verify that the cells present in the explant cultures were osteoblast-like cells. Unlike osteoblasts in vivo, osteoblasts in a conventional monolayer culture are thought to be present under quite different conditions in which only a small amount of the ECM exists to support them [Zambonin et al., 1997; Açil et al., 2000]. It has been widely recognized that the composition and organization of the ECM profoundly affects the proliferation and the differentiation of cells and contributes to the unique physical and biomechanical properties of a tissue [Piez, 1984]. It is well known that intimate interactions occur between collagen and biological apatites in vivo [Zambonin and Grano, 1995]. In our study, osteoblast-like cells cultured on the three different biomaterials did show typical markers of osteoblast-like differentiation, proving that the biomaterials tested provide some of the crucial features of natural ECM. Moreover, the addition of dexamethasone to the culture medium may have suppressed fibroblasts [Milne et al., 1998; Açil et al., 2000].

Since the bone chips were not treated with collagenase prior to their placement in culture, multi-potent cells could have been present, as well [Zambonin and Grano, 1995; Açil et al., 2000]. RhBMP-7 is known to stimulate the differentiation of multi-potent cells into osteoblast-like cells [Asahina et al., 1996; Terheyden et al., 1997, 1999; Zhu et al., 1999; Caricasole et al., 2000; Springer et al., 2001]. Thus, multipotent cells stimulated by rhBMP-7 might have contributed to the higher amount of osteoblast-like cells as well as extracellular matrix proteins generated in the rhBMP-7 treated groups.

The rhBMP-7 stimulated three-dimensional human osteoblast-like culture system may be a useful in vitro assay system to study cell matrix interactions. It could be demonstrated that the three different carrier biominerals provide a favorable matrix for human osteoblast-like cells to attach, divide, and synthesize mature collagen. From a clinical perspective, the present study indicates the potential of the tested biomaterials for tissue-engineered production of human bone using bone cell autografts with or without differentiation factors. At present, in our department, studies are in progress evaluating the osteogenic potential of natural bone mineral in combination with differentiation factors in vivo.

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