Comparative evaluation of different calcium phosphate-based bone graft granules – an in vitro study with osteoblast-like cells

Bio ceramics have long been applied for bone replacement. To adapt to surgical sites for bone defects involving complex shapes, granule types of ceramics are more desirable than block forms. Various in vivo studies have been performed to evaluate the biocompatibility as well as the potential to induce new bone formation by synthetic granule-shaped bone graft materials of different chemical composition. However, there are only few studies, dealing with in vitro examination of cellular attachment, proliferation and osteogenic differentiation on granule-shaped bio ceramics. As cellular attachment is necessary for proliferation of osteoblasts and their precursors, in vitro experiments might be a useful first step for the evaluation of novel granule-shaped bone graft materials, before subjecting them to comprehensive in vivo studies.

Numerous calcium phosphate-based bone graft materials have been developed during the last decades (LeGeros 2002). Main classes comprise hydroxyapatite [HA], β-tricalcium phosphate (β-TCP), bioglasses and mixtures thereof. Ceramics from phase-pure β-TCP are widely used in the field of bone regeneration because of their good biocompatibility, biodegradability and osteoconductivity. To further improve these promising properties, different modifications of β-TCP ceramics have been established. Silicon substitution in the crystal structures of tricalcium phosphate ceramics, for example, leads to improved osteoinductive properties.

Ceracell® is an open-celled highly porous bioceramic from β-tricalcium phosphate (β-TCP) under addition of bioglass and Osseolive®, an open porous glass ceramic with the general formula $Ca_3KNa(PO_4)_2$. The goal of this study was to characterize different modifications of the two bone graft materials in vitro in comparison to already established ceramic bone grafts Cerasorb M®, NanoBone® and BONIT Matrix®.

Materials and methods: Adhesion and proliferation of SaOS-2 osteoblast-like cells were evaluated quantitatively by determining DNA content and lactate dehydrogenase (LDH) activity and qualitatively by scanning electron microscopy (SEM). In addition, MTT cell-vitality staining was applied to confirm the attachment of viable cells to the different materials. Osteogenic differentiation was evaluated by measurement of alkaline phosphatase (ALP) activity as well as gene expression analysis of osteogenic markers using reverse transcriptase PCR.

Results: DNA content and LDH activity revealed good cell attachment and proliferation for Ceracell and Cerasorb M. When pre-incubated with cell-culture medium, also Osseolive showed good cell attachment and proliferation. Attachment and proliferation of osteoblast-like cells on NanoBone and BONIT Matrix was very low, even after pre-incubation with cell-culture medium. Specific ALP activity on Ceracell® and Osseolive® increased with time and expression of bone-related genes ALP, osteonectin, osteopontin and bone sialoprotein II was demonstrated.

Conclusions: Ceracell as well as Osseolive granules support proliferation and osteogenic differentiation in vitro and may be promising candidates for in vivo applications.

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Preparation of the novel materials Ceracell® and Osseolive®

Ceracell®
A well-dispersed slurry (solid content 60–70%) was prepared by a mixture of β-TCP powder manufactured as described elsewhere (Peters & Reif 2004) and 4% sodium-magnesium-silicate-bioglass powder (Federal Institute of Testing and Materials [BAM], Berlin, Germany). Polyurethane (PU) sponges (45 and 60 ppm, Zervos, 86911 Diessen, Germany) were immersed into the slurry.

Polyurethane foams were soaked with this suspension, on which surplus suspension becomes expressed (squeezed, rolled and centrifuged). Ideally, the slurry completely coats the material bars and ligaments. After drying (evaporation of the solvent from the slurry) the foam was burned out and the ceramic particles were sintered together at 1100°C for 4 h (Nabertherm N 100/H; Nabertherm, Lilienthal, Germany). As final product, a ceramic with the foam superstructure is achieved. The ceramic bars are hollow. The resulting porous ceramic bodies were crushed with a grinder to obtain granules of 1–2 mm particle size. In the following study, Ceracell® granules with different porosities were applied (see Table 1).

Osseolive®
The GB14 glass ceramic powder was prepared as described elsewhere (Berger et al. 1995). Briefly, CaO (30.67 wt%), P2O5 (43.14 wt%), Na2O (9.42 wt%), K2O (14.32 wt%) and MgO (2.45 wt%) were melted at 1600°C in a platinum pan, then crushed and milled. A well-dispersed slurry of GB14 powder (solid content 60–70%) was prepared by a mixture of ceramic powder and 4% sodium-magnesium-silicate-bioglass powder (Federal Institute of Testing and Materials [BAM]) (Osseolive II) or without the sodium-magnesium-silicate-bioglass powder (Osseolive II). PU sponges (80 ppm) were immersed into the slurry. After sintering at 1000°C for 1 h and cooling to room temperature, the resulting porous ceramic bodies were crushed with a grinder to obtain granules of 1–2 mm particle size.

Cerasorb M® granules were prepared as described in Peters & Reif (2004). BONIT Matrix® was a generous gift of DOT [Rostock, Germany], and NanoBone® was purchased from Artoss [Rostock, Germany].

Cell seeding and cultivation of cell-seeded samples
SaOS-2 cells (ATCC 243, DSMZ, Braunschweig, Germany) were expanded in McCoy’s 5A medium (Biochrom, Berlin, Germany), supplemented with 15% foetal calf serum [FCS], 10 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine [all from Biochrom] at 37°C and 5% CO2.

Samples of 0.12 cm³ of the different materials (n = 5 per time point and material for biochemical measurements, n = 5 per time point and material for gene expression analysis and some extra samples for microscopic evaluation) were seeded with 1.6 × 10⁵ cells in 200 μl of cell-culture medium per sample. In some experiments ceramic samples were preincubated for 24 h with cell-culture medium before seeding the cells. Next day, samples were transferred to 48-well plates and provided with 500 μl of fresh medium, containing 5 mM β-glycerophosphate and 0.05 mM ascorbic acid-2-phosphate (both from Sigma, Taufkirchen, Germany). Medium was changed every 1–3 days, depending on the number of cells. Samples for biochemical measurements (n = 5) after 1, 7, 14, 21 and 28 days of cultivation were washed twice with PBS, transferred to test tubes and frozen at −80°C until further analysis. Samples for gene expression analysis (n = 5) were taken after 1, 7, 14, 21 and 28 days of cultivation. Samples were washed with PBS and subjected to RNA isolation as described below. Additional samples for SEM investigation were taken after 1 and 28 days of cultivation. Cell cultivation experiments on the different granule-shaped materials were performed two times.

Biochemical analysis of enzyme activity (ALP and LDH) and DNA content
Frozen cell-seeded ceramic granules were thawed for 20 min on ice followed by lysis

Materials applied in this study
An overview of all examined materials is given in Table 1.

Table 1. Overview on the granule-shaped materials applied in this study

<table>
<thead>
<tr>
<th>Product name</th>
<th>Company</th>
<th>Composition</th>
<th>Particle size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerasorb M</td>
<td>Curasan Kleinostheim, Germany</td>
<td>Phase-pure β-TCP</td>
<td>1-2</td>
</tr>
<tr>
<td>Ceracell I</td>
<td>Curasan Kleinostheim, Germany</td>
<td>Phase-pure β-TCP with 4% sodium-magnesium-silicate-bioglass</td>
<td>1-2, pores 250-300 μm</td>
</tr>
<tr>
<td>Ceracell II</td>
<td>Curasan Kleinostheim, Germany</td>
<td>Phase-pure β-TCP with 4% sodium-magnesium-silicate-bioglass</td>
<td>1-2, pores 200 μm</td>
</tr>
<tr>
<td>Ceracell III</td>
<td>Curasan Kleinostheim, Germany</td>
<td>Phase-pure β-TCP with 4% sodium-magnesium-silicate-bioglass</td>
<td>1-2, pores 200-300 μm</td>
</tr>
<tr>
<td>Osseolive I</td>
<td>Curasan Kleinostheim, Germany</td>
<td>Glass ceramic Ca2KNa(PO4)2 with 4% sodium-magnesium-silicate-bioglass</td>
<td>1-2</td>
</tr>
<tr>
<td>Osseolive II</td>
<td>Curasan Kleinostheim, Germany</td>
<td>Glass ceramic Ca2KNa(PO4)2</td>
<td>1-2</td>
</tr>
<tr>
<td>BONIT Matrix</td>
<td>DOT, Rostock, Germany</td>
<td>HA/TCP (60%/40%), embedded in SiO2 matrix</td>
<td>0.6 × 4</td>
</tr>
<tr>
<td>NanoBone</td>
<td>Artoss, Rostock, Germany</td>
<td>HA (76%), embedded in SiO2 matrix (24%)</td>
<td>1-2</td>
</tr>
</tbody>
</table>

β-TCP, β-tricalcium phosphate; HA, hydroxyapatite.
with 1% Triton X-100 in PBS for 50 min on ice. During cell lysis each sample was sonicated for 10 s at 80 W with an ultrasonic processor UP 100H [Dr. Hielscher GmbH, Teltow, Germany].

One aliquot of the cell lysate was added to ALP reaction buffer, containing 1 mg/ml p-nitrophenyl phosphate (Sigma), 0.1 M diethanolamine, 1% Triton X-100 (pH 9.8), 1 mM MgCl₂ and the mixture was incubated at 37°C for 10 min. Finally, 1 M NaOH was added to stop the enzymatic reaction. After centrifugation at 16,000 g for 10 min the supernatant was transferred to a microtiter plate and the absorbance was read at 405 nm with a multi-function microplate reader (Spectra fluor plus, Tecan, Crailsheim, Germany). A calibration line was constructed from different dilutions of a 1 mM p-nitrophenol stock solution.

Another aliquot of the same cell lysate was mixed with LDH reaction buffer (Cytotox96 kit, Promega, Madison, WI, USA). After incubation for 30 min at room temperature 1 N acetic acid was added to stop the enzymatic reaction. Absorbance was measured at 492 nm.

Another aliquot of the cell lysate was mixed with Picogreen ds DNA quantitation reagent (Molecular Probes, Eugene, OR, USA) diluted 1 : 800 in TE buffer (reagent (Molecular Probes, Eugene, OR, USA) mixed with Picogreen ds DNA quantitation reaction. Absorbance was measured at 492 nm. Critical point drying was performed with a CPD 030 apparatus [BAL-TEC, Balzers, Liechtenstein]. Samples were mounted on stubs, coated with gold and imaged using a Philips XL 30/SEM with FEG (field emission gun), operating in SEM mode. The microscope was driven with an acceleration voltage of 5 kV and a working distance of 14 mm detecting secondary electrons.

### Statistics

Data on biochemical measurements are represented by the means of five individual samples. Error bars represent standard deviation. For individual comparisons, ANOVA was used. After performing Levene test for equal variance, means comparison was achieved using Tukey model. Differences with \( P < 0.5 \) were considered significant.

### Scanning electron microscopy

Cell-seeded granules were washed twice with PBS, fixed for 60 min with 2% glutaraldehyde in PBS, washed with distilled water, and dehydrated using a gradation series of ethanol/distilled water mixtures. Critical point drying was performed with a CPD 030 apparatus [BAL-TEC, Balzers, Liechtenstein]. Samples were mounted on stubs, coated with gold and imaged using a Philips XL 30/SEM with FEG (field emission gun), operating in SEM mode. The microscope was driven with an acceleration voltage of 5 kV and a working distance of 14 mm detecting secondary electrons.

### Results

#### Adhesion of osteoblast-like cells to the surface of different bone graft granules

Scanning electron microscopy investigations of the granules after 1 day of cultivation provided qualitative morphological data on cell adhesion. Samples of Ceracell with three different porosities as well as control samples of Cerasorb M showed long stretched cells, which were tightly attached to the surface of the material (Fig. 1). However, we were not able to detect attached cells on Osseolive samples, as well as on BONIT™, and NanoBone granules.

The adhesion of viable cells to the granules was furthermore evaluated by measurement of LDH activity after lysis of the cells. According to the SEM investigations, we found different amounts of cells on the materials. Samples, on which we were not able to detect cells with SEM, showed significantly lower LDH activity compared to control Cerasorb M \( (P < 0.001 \) for Osseolive I, Osseolive II, BONIT and NanoBone compared to Cerasorb M). (Fig. 2a).

#### Adhesion of osteoblast-like cells to different granule-shaped bone graft materials when pre-incubated with cell-culture medium

Pre-incubation of Osseolive granules with cell-culture medium prior to cell seeding led to a significant higher amount of attached viable cells after 24 h \( (P < 0.001 \) for both materials compared to Cerasorb M) \( (P < 0.001 \) compared to 2b). In contrast, the amount of viable cells attached to Cerasorb M, Ceracell, BONIT and NanoBone remained in the same range compared to non-incubated samples.

Cell adhesion to Osseolive samples after pre-incubation with cell-culture medium was also evaluated by SEM. After 24 h of adhesion we were able to detect cells, which were attached to the surface of the two different materials (Fig. 1). This is in contrast to the
adhesion experiment without medium pre-incubation. Despite the low values for LDH activity we were also able to detect few cells on BONIT after pre-incubation with cell-culture medium. However, cells attached to BONIT after 24 h of adhesion show a different morphology compared to cells on Osseolive samples. On Osseolive granules we observed more stretched cells compared to BONIT [Fig. 1].

Adhesion of viable cells to different granule-shaped bone graft materials was furthermore investigated by MTT staining after 24 h of cultivation. MTT is incorporated by viable cells and intracellularly converted into a dark blue formazan dye which cannot escape the cell because of its insolubility in water. Therefore, the MTT staining is applicable to visualize the distribution of viable cells macroscopically (Lode et al. 2008). We were able to detect living cells on all examined materials [Fig. 3].

**Proliferation of osteoblast-like cells on different bone graft materials**

Proliferation of SaOS-2 cells on the different granules was evaluated with two different assays. Determination of DNA content covers the whole amount of attached cells, whereas measurement of LDH activity reveals the presence of viable cells. In both cases, only such cells were included in the measurement, which are attached to the material. Cells growing on the polystyrene culture dishes were not involved, since the granules were removed from the culture dishes and washed before analysis.

**Proliferation on bone grafts, which were seeded without medium pre-incubation**

During 28 days of cultivation, the number of viable cells increased on samples of Ceracell...
with three different porosities, as well as control samples of Cerasorb M. While the number of viable cells initially dropped for these samples between day 1 and day 7 of cultivation, the increase of viable cells between day 7 and 28 was between 11- and 17-fold. Samples of Osseolive, BONIT and NanoBone did not reveal any notable increase of viable cell number, going along with the low number of viable cells, being detected after 1 day of cultivation [Fig. 4].

DNA determination brought out a similar pattern as LDH activity. Increase of DNA content was determined for samples, which also showed an increased number of viable cells. For Osseolive and NanoBone, the cell number calculated from DNA content was higher compared to the number of viable cells from LDH activity measurement; however, there was no increase in DNA content with cultivation time [Fig. 4].

Proliferation of osteoblast-like cells on samples of Ceracell with three different porosities as well as control samples of Cerasorb M was furthermore confirmed by SEM investigations of cell-seeded granules after 28 days of cultivation. Compared to day 1, much more cells were visible on the surface of the granules (Fig. 5). We did not detect attached cells on samples of Osseolive, BONIT and NanoBone after 28 days of cultivation.

**Osteogenic differentiation**

Activity of alkaline phosphatase (ALP) of osteoblast-like cells on bone grafts, which were seeded without medium pre-incubation

In the following study, only those materials were examined, which showed sufficient proliferation rates when seeded without medium pre-incubation (Cerasorb M as con-

![Fig. 3. MTT staining of different bone graft granules, pre-incubated with cell-culture medium and seeded with osteoblast-like cells after 1 day of cell adhesion. Stereomicroscopic image, magnification 20×.](image)

![Fig. 4. Number of cells attached to different bone graft materials during cultivation for 4 weeks, n = 5 (± SD of the mean). Cell number was calculated from lactate dehydrogenase (LDH) activity as well as from DNA content using a calibration line of known cell numbers.](image)

Proliferation of osteoblast-like cells on Osseolive samples was furthermore confirmed by SEM of cell-seeded granules after 28 days of cultivation. Compared to day 1, a much higher number of well spread cells were visible on the surface of the two examined materials (Fig. 5). Additional proliferation studies involving BONIT and NanoBone after pre-incubation with cell-culture medium brought out a decrease in number of adherent cells during the first week of cultivation for both materials, which was confirmed by DNA- and LDH determinations [data not shown].
trol and Ceracell samples with different porosities). Specific ALP activity (ALP activity related to the number of viable cells) increased for all examined materials. After 7 and 14 days of cultivation, specific ALP activity on Ceracell I, II and III was significantly lower compared to Cerasorb M control. However, after 21 and 28 days of cultivation no significant differences in specific ALP activity were detected between Ceracell and Cerasorb M control. Only after 14 days of cultivation specific ALP activity was higher compared to Cerasorb M (Fig. 6a). Highest ALP values were detected for all materials at day 28 of cultivation. Increase of specific ALP activity was around 10-fold for all examined materials.

ALP activity for samples pre-incubated with cell-culture medium

After pre-incubation of Osseolive samples with cell-culture medium overnight, we were able to determine ALP activity after cultivation of osteoblast-like cells over 4 weeks (Fig. 6b). Specific ALP activity for Cerasorb M control at days 7, 14 and 21 of cultivation was significantly higher than for all three osseolives (Osseolive I, II, III) (Fig. 6b). Specific ALP activity of Osseolive II was in the same range of Cerasorb M control only after 14 days of cultivation specific ALP activity was significant higher compared to Cerasorb M.

Gene expression of osteogenic markers

Analysis of gene expression for osteoblast-like cells, cultivated on materials without pre-incubation with cell-culture medium, was performed for cell-seeded samples of Ceracell with three different porosities as well as control samples of Cerasorb M. Due to the low number of attached cells, it was not possible to isolate a sufficient amount of RNA from the other samples (Osseolive, BO-NIT, NanoBone).

Gene expression of the osteogenic markers ALP, ON, OP and BSP II was detected for SaOS-2 cells on all investigated bone graft materials. There were no remarkable differences in ALP, ON and BSP II expression between the cells, attached to different bone graft materials, indicating that all examined materials support osteogenic differentiation (Fig. 7). After 21 and 28 days of cultivation gene expression of OP was higher on Ceracell samples compared to Cerasorb M control (Fig. 7).

Gene expression of osteogenic markers for samples pre-incubated with cell-culture medium

After pre-incubation with cell-culture medium, we were able to isolate sufficient amounts of RNA after 1, 7, 14, 21 and 28 days of cultivation of SaOS-2 cells on Osseolive samples. Gene expression analysis of osteogenic markers was performed in comparison to Cerasorb M as control, which was also pre-incubated with cell-culture medium before seeding. We detected gene expression of the osteogenic markers ALP, ON, OP and BSP II for Osseolive samples in a comparable amount to Cerasorb M (Fig. 8).

Discussion

This study reveals some interesting insights into the interaction of osteoblast-like cells with granule-shaped bone graft materials. Most previous in vitro studies involving granule-shaped bone graft materials are limited to cultivation of cells in the presence of the
Fig. 6. Specific alkaline phosphatase (ALP) activity of different bone graft materials seeded with SAOS-2 osteoblast-like cells, \( n = 5 \) (± SD of the mean). ALP activity was related to viable cell number determined by lactate dehydrogenase (LDH) activity measurement. [a] dry samples were seeded with cells, [b] samples were pre-incubated with cell-culture medium containing 15% foetal calf serum (FCS). Significant differences: *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).

Fig. 7. Gene expression of alkaline phosphatase (ALP), osteonectin, osteopontin, bone sialoprotein II (BSPII) and GAPDH for SAOS-2 osteoblast-like cells seeded on five different granule-shaped bone graft materials after 1, 7, 14, 21 and 28 days of cultivation. RNA of five cell-seeded samples was combined for each analysis. GAPDH was used as an internal control.

Fig. 8. Gene expression of alkaline phosphatase (ALP), osteonectin, osteopontin, bone sialoprotein II (BSPII) and GAPDH for SAOS-2 osteoblast-like cells seeded on five different granule-shaped bone graft materials after 1, 7, 14, 21 and 28 days of cultivation. Samples were pre-incubated with cell-culture medium before seeding of the cells. RNA of five cell-seeded samples was combined for each analysis. GAPDH was used as an internal control.

Material, thus cells which adhered to the cell-culture plastic are also included in the quantitative measurements (Rice et al. 2003; Kübler et al. 2004, Hattar et al. 2005, Turhani et al. 2005, Le Nihouanne et al. 2007, Herten et al. 2009). Our study exclusively covers cells which directly adhered to the respective granule-shaped material. A recent report of Rameis et al. (2009) also quantifies the number of cells attached to a granule-shaped biomaterial (Bio-Oss®) using radiolabelled cells; however, only adhesion over 3 h was monitored and there are no data on later time points of cultivation. We recently conducted an in vitro study, involving three different granule-shaped bone graft materials, again including Cerasorb M as a control material and studying solely cells attached to the material. (Bernhardt et al. 2011).

In the present study, we demonstrated cell adhesion and proliferation on Cerasorb M as well as on novel Ceracell granules with different porosities, even without pre-treatment of the ceramic materials. β-TCP ceramics support cell adhesion and proliferation (Liu et al. 2007, Ni et al. 2007) which was also shown for Cerasorb M before (Aybar et al. 2004, Neamat et al. 2009, Bernhardt et al. 2011). Recently, Cai et al. (2009) developed a porous β-TCP ceramic reinforced with bioglass, similar to Ceracell®. This bioglass reinforced β-TCP ceramic supported osteoblast adhesion and proliferation even to a higher extent compared to pure β-TCP ceramic.

In contrast to Cerasorb M and Ceracell, we only detected small amounts of attached cells on the glass ceramics (Osseolive I and II), as well as on the silica gel containing nanocrystalline composites BONIT matrix® and NanoBone®. Pre-treatment of biomaterials with serum-containing cell-culture medium is a common practice to improve cellular adhesion. Therefore, we performed a second set of experiments involving pre-incubation of all investigated bone graft materials with cell-culture medium containing 15% FCS. Using this setup, which is also recommended by some manufacturers of bone graft materials (but of course regarding autologous human serum), we found a significantly higher number of attached cells on the Osseolive® glass ceramics. Cell number increased steadily during further cultivation enabling us to study osteogenic differentia-
sion as well as osteogenic differentiation on this material. However, for the cited study compact discs of BONIT Matrix were applied instead of granules. Possibly, the material properties, especially the surface of the material are different for compact discs compared to granules. The eight different granule-shaped materials examined in this study as well show differences in total surface area and surface topography which might also have an impact on the different in vitro performance of the different materials.

The novel β-TCP samples containing 4% sodium-magnesium-silicate-bioglass (Ceracell I-III) turned out to be good substrates for osteoblast-like cells. Admittedly, cell numbers on the Ceracell substrates were considerably lower compared to Cerasorb M control; however, cell number increased steadily by a factor of 2–3 during 4 weeks of cultivation. Encouraged from these good results, we decided against pre-treatment of the samples with cell-culture medium to facilitate cell adhesion and proliferation. Furthermore, due to the incorporation of bioglass an increase in mechanical stability was reached, which is also reported by Cai et al. (2009). We examined Ceracell samples with different pore size (ranging from 200 to 350 μm), but did not detect any significant influence of this property on adhesion and proliferation of osteoblast-like cells.

To evaluate the osteogenic potential of the different granule-shaped bone grafting materials in vitro, we examined their impact on osteogenic differentiation. Generally, osteogenic differentiation is defined by three principal biological periods: cellular proliferation, cellular maturation and matrix mineralization. Different stages of osteogenic differentiation are characterized by specific osteogenic markers ALP is expressed during the post-proliferative period of extracellular matrix maturation. Osteoblasts turn out to be very low after pre-incubation. However, specific ALP activity of osteoblast-like cells was significantly higher on Osseolive samples with different porosity supported attachment and proliferation of osteoblast-like cells. We detected an increase of specific ALP activity with cultivation time and demonstrated gene expression of osteogenic markers on Ceracell even without pre-treatment of the samples with serum. Osteoblast-like cells were able to attach and proliferate on Osseolive granules only after pre-incubation of the samples with 15% FCS for 24 h.

Conclusion

The goal of this study was to characterize two classes of newly developed granule-shaped bone graft materials, Ceracell, a β-TCP based ceramic with addition of 4% sodium-magnesium-silicate-bioglass and Osseolive, a calcium alkali orthophosphate ceramic in comparison to the already established materials Cerasorb M, NanoBone and BONIT Matrix in vitro. The open porous Ceracell granules with different porosity supported attachment and proliferation of osteoblast-like cells. We detected an increase of specific ALP activity with cultivation time and demonstrated gene expression of osteogenic markers on Ceracell even without pre-treatment of the samples with serum. Osteoblast-like cells were able to attach and proliferate on Osseolive granules only after pre-incubation of the samples with 15% FCS for 24 h.

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References


