Growth and Proliferation of Human Osteoblasts on Different Bone Graft Substitutes An In Vitro Study

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The purpose of this study was to investigate the effect of different bone graft substitutes onto the growth and proliferation pattern of bone cells derived from human iliac cancellous bone. Five different bone graft materials were used to investigate the effect on the proliferation of osteoblasts in vitro*: phytogene hydroxyapatite (Algipore®), α-Tricalcium phosphate (Bio-Base®), bovine hydroxyapatite (low temperature) (Bio-Oss®), bovine hydroxyapatite (high temperature) (Osteograf®), and bovine hydroxyapatite (high temperature) enhanced with p-15, synthetic peptide (PepGen p-15®). The osteoblasts were derived from human iliac cancellous bone and seeded with the different bone substitutes. The cell proliferation and viability (WST-1), alkaline phosphatase as an early marker of osteoblast proliferation, was evaluated after 6 and 9 days. The cultures were examined for cell growth pattern and morphology by normal light and scanning electron microscopy. The human osteoblasts showed a different proliferation pattern according to the type of applied bone graft substitute. PepGen P-15 showed the highest proliferation and differentiation rate followed by Osteograf, Algipore, and Bio-base. Bio-Oss showed the lowest. These results were confirmed by electron microscopy and light microscopy evaluation in which similar growth pattern were observed. Distinct bone graft materials have different impact onto the proliferation pattern of human osteoblasts* in vitro*. (Implant Dent 2004;13:171–179)*

Key Words: bone substitutes, human osteoblasts, proliferation

Bone replacement graft materials have played an important role in regenerative dentistry for many years.[1–4] In dentoalveolar surgery, bone is often needed to fill bony defects or to enable sinus lift augmentation[5–7] followed by implant placement. Although autogenous bone graft is an ideal material as a result of osteogenic, osteoinductive, and osteoconductive properties, it has some limitations such as donor site morbidity and additional surgical expense. For small amounts of bone, various donor sites within the oral cavity have been reported: the chin, the linea obliqua, or the zygoma.[8,9] At these locations, limited bone can be grafted under local anesthesia with minor donor site morbidity. If larger volumes of bone are required, then the iliac crest or the tibia[10] is often selected. Here, substantial amounts of spongy and cortical bone can be harvested. As a major disadvantage of the iliac crest, grafting can only be performed under general anesthesia and often with obvious donor site morbidity, which could cause pain for several days.[11]

As a result of these potential problems and the intention to avoid donor site morbidity, bone graft substitutes have been developed. In recent years, various bone graft substitutes derived from human, bovine, and plants or synthesis are being used for the reconstruction of bone defects and ridge augmentation in oral and maxillofacial surgery.[1,2,12,13] These substitutes can be implanted alone, or they can be mixed with autogenous bone, which could be harvested during the preparation of an implant site or from another donor site within the oral cavity. By mixing bone graft substitute material with autogenous bone, a significant increase of the augmentable material can be achieved, which often enables the clinician to refrain from iliac crest grafts. The single use of bone graft substitutes for augmentation reasons is also an important indication.

Several bone graft substitutes have been developed and have been used for the replacement of autografts or in combination with them or platelet-rich plasma (PRP).[14] Many reports of the clinical application have

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ISSN 1056-6163/04/01302-171
Implant Dentistry
Volume 13 • Number 2

DOI: 10.1097/01.ID.0000127522.14067.11

been published, which mainly reveal good clinical success.[1,13,15,16] The experimental studies on bone graft substitutes focus on technical properties like the particle size, porosity, and the surface structure, which are stated for the biologic behavior.[17,18] There are little data that directly compare the physiological or histologic behavior of different graft substitutes.[19–22]

The aim of this study was to evaluate the response of human osteoblasts to 5 different bone graft materials *in vitro*. Using different parameters, the impact of Algipore®, Bio-base®, Bio-Oss®, Osteograf®, and PepGen P-15® onto human osteoblasts *in vitro* was studied.

Materials and Methods

Isolation of Human Osteoblasts

Iliac cancellous bone was obtained from a 42-year-old woman undergoing cyst enucleation at the mandible followed by autogenous bone graft transplantation from her iliac crest. After grafting 1 cm^3 of spongy bone from the iliac crest, the piece of bone was washed with phosphate-buffered solution (PBS; pH 7.4) and placed in 0.2% collagenase solution for 30 minutes in a 37°C water bath. Collagenase was used for enzymatic digestion and release of osteoblasts from the bone matrix. After 30 minutes of incubation, the cell suspension was decanted and discarded. The bone specimen was filled with fresh collagenase solution and incubated for another 60 minutes. After incubation, supernatant was centrifuged at 1000 RPM for 5 minutes and the cells were released into culture flasks.

Cell Culture

The extracted cells were grown at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM), 10% Fetal Calf Serum (FCS), 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin, 2 mM L-glutamine, and 2.5 mM herpes buffer and changed 3 times a week. The second passage cells were plated at a density of 2×10^4 cells/well in 24-well plates. After 72 hours of cell inoculation, 30 mg of 5 different bone substitutes, phytogene hydroxyapatite (FRIOS-Algipore®, DENTSPLY Friadent, Mannheim, Germany, The Clinician's Preference, Golden, CO), α-tricalcium phosphate (Bio-Base®, Centerpulse, Freiburg, Germany), low-temperature bovine hydroxyapatite (Bio-Oss®, Geistlich Biomaterials, Switzerland), high-temperature bovine hydroxyapatite (Osteograf®/N, DENTSPLY Friadent Ceramed, Lakewood, NJ), and high-temperature bovine hydroxyapatite enhanced with p-15, synthetic peptide (PepGen p-15®, DENTSPLY Friadent Ceramed) were added. Cells were also added to polystyrene plates without any bone substitutes to serve as a control group. Culture medium was changed 24 hours after plating and at 72-hour intervals thereafter. Ascorbic acid and β-glycerophosphate were added to culture medium for bone cell mineralization.

WST-1 and determination of alkaline phosphatase and type I collagen were performed at 6 and 9 days after cell plating. Light microscopy and electron microscopy evaluation were performed 21 days after plating.

Determination of Cell Proliferation and Viability (WST-1)

WST-1 reagent (Roche, Mannheim, Germany) was used for the determination of cell proliferation and viability. WST-1 is a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenase in viable cells. The cultures were incubated for 1 hour at 37°C. The optical density was measured at 450-nm wavelength thereafter.

Determination of Alkaline Phosphatase

Alkaline phosphatase kit (Sigma, Munich, Germany) was used for the determination of the alkaline phosphatase, a parameter for osteoblast activity. It was assayed by hydrolysis of p-nitrophenol phosphate by the enzyme, yielding p-nitrophenol and inorganic phosphate. The levels of alkaline phosphatase were presented as Sigma unit/mL of medium.

Phase-Contrast Microscopy (LM)

The cell cultures with the bone graft substitutes were evaluated by using a Zeiss phase-contrast microscope at 5-fold magnification.

Scanning Electron Microscopy (SEM)

The cultures were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After washing 3 times with 0.1 M cacodylate buffer, the cultures were dehydrated in 30%, 50%, 70%, 90%, and 100% ethanol, 10 minutes each time. Subsequently, they were subjected to critical point drying and sputter-coated with gold-palladium and examined using a AMRAY 1610 T scanning electron microscopy at a voltage of 12 kV.

Statistical Analysis

All values were expressed as means $\pm$ standard deviation for 6 probes of each group. Student *t* tests were performed. The graph display is done by Tukey boxplots, a diagram that summarizes data using the median, the upper and lower quartiles, and the extreme values (outliers).

Results

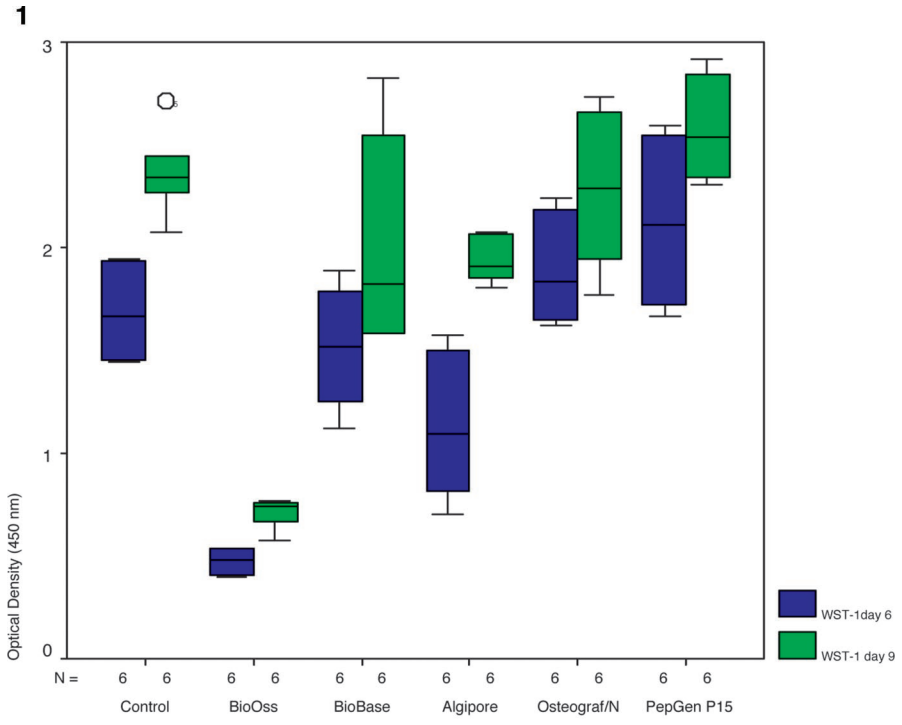
Cell Proliferation and Viability (WST-1)

Cell proliferation and viability were measured using WST-1 reagent and the results are presented in Figure 1 as Tukey boxplots.

Cells being incubated together with PepGen p-15® showed higher levels of WST-1, at day 6 (2.1 ± 0.4) and day 9 (2.6 ± 0.3), compared with the control group without any bone substitute (1.7 ± 0.2 and 2.5 ± 0.2, respectively). It was followed by the Osteograf®, BioBase®, and Algipore® groups. The group cultured with Bio-Oss® showed the lowest level with 0.5 ± 0.1 on day 6 and 0.7 ± 0.1 on day 9, compared with all other groups inclusive the control group. Measurement of WST-1 continuously increased in the Bio-Base, Osteograf, and PepGen p-15 group after adding the substitute material.

Determination of Alkaline Phosphatase

Figure 2 shows the Tukey boxplots of the amount of alkaline phosphatase of the cells cultured with different graft materials on days 6 and 9. The control group without any bone substitutes showed higher levels of alkaline phosphatase on day 6 (38.2 ± 17.0) and day 9 (63.8 ± 1.6) than those in all other experimental groups. Among the experimental groups, the



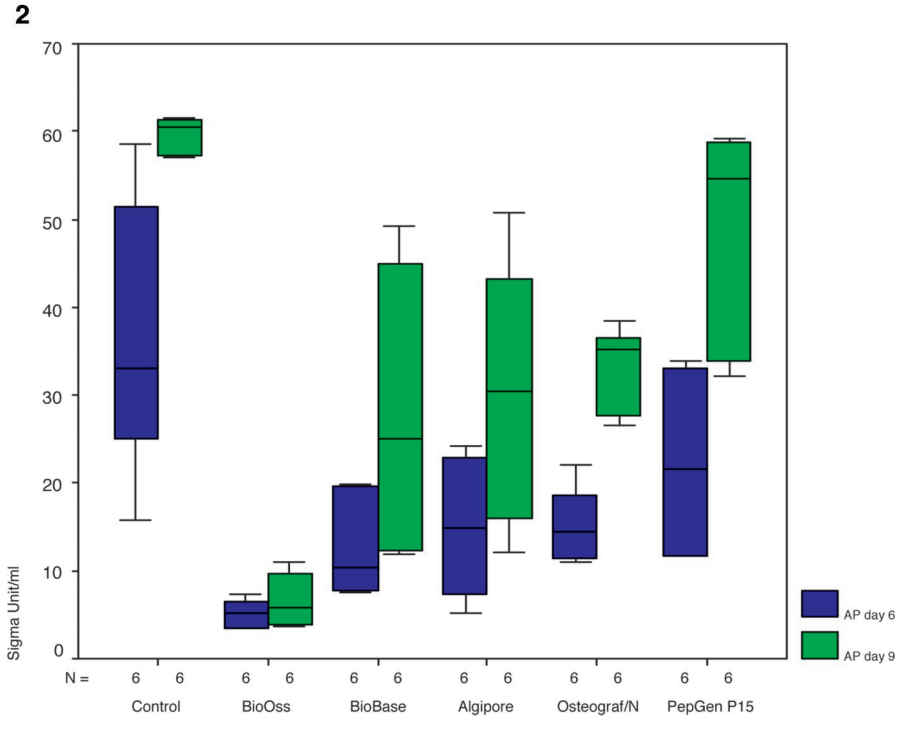


Fig. 1. Cell proliferation and viability measured by the WST-1 test. Increase of cell proliferation between days 6 and 9. Highest values were on day 9 for PepGen-P15.
Fig. 2. Determination of alkaline phosphatase. Increase between days 6 and 9. Highest values for the control group followed by PepGen-P15.

highest amount of alkaline phosphatase was observed in the group cultured with PepGen P-15®, 23.2 ± 11.1 and 52.1 ± 12.7, followed by those with Osteograf/N, Algipore®, Bio-Base®, and Bio-oss®.

Investigation of Cell Growth Pattern by Phase-Contrast Microscopy

The growth and proliferation pattern of osteoblasts cultured together with different bone graft materials was examined using a phase-contrast microscope 21 days after plating (Figs. 3–7).

There were different growth and proliferation patterns for each material. The cultures with PepGen p-15® showed the best cell growth among all experimental groups. In this group, the cells formed multicellular layers around the graft material and interparticular bridges between them. Multicellular layers were also observed in cultures with Osteograf, but few. The groups with Algipore and Bio-Base showed moderate cell proliferation. In contrast, the cells on Bio-Oss® showed poor proliferation.

The granules of Algipore®, Osteograf®, and PepGen p-15® were firmly attached to the cultured cell layer, but those of Bio-Oss® and Bio-Base® were not attached.

Investigation of Cell Growth Pattern by Scanning Electron Microscopy

The cell growth and proliferation pattern were also investigated by SEM 21 days after platting (Figs. 8–12).

The PepGen p-15® group showed the best cell growth among all experimental groups. In this group, the cells formed multicellular layers around the graft material and interparticular bridges between them. Most particle surfaces were covered with a sheath-like complex of matrix and cells. The cells were elongated and arranged parallel. Highly dense collagen fibers were observed in a high-magnification view. These structures could be seen only in the PepGen p-15® group. In some areas, newly deposited minerals were also observed.

Multicellular layers were also observed in cultures with Osteograf®/N, but little. The cells on Algipore and Bio-Base showed moderate cell proliferation. In contrast, the cells on Bio-Oss® showed poor proliferation. The granules of Algipore®, Osteograf®, and PepGen p-15® were firmly attached to the cultured cell layer, but those of Bio-Oss® and Bio-Base® were not attached.

Discussion

Bone graft materials can support the formation of new bone by the process of osteogenesis, osteoinduction, and osteoconduction.[23,24] Osteogenesis

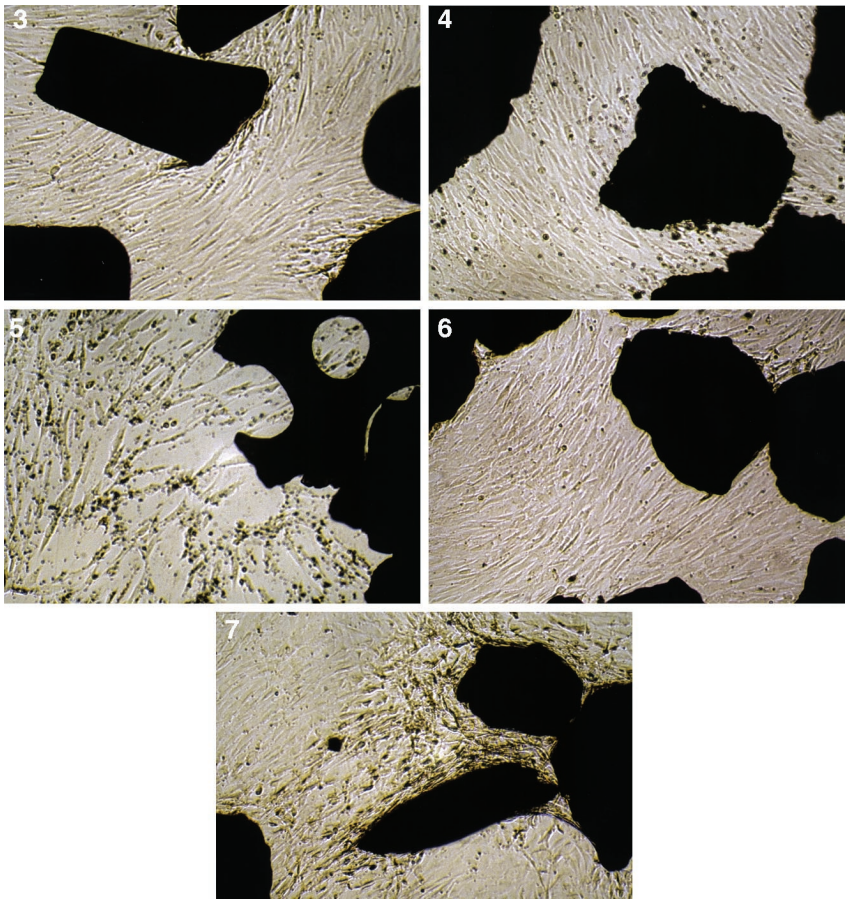


Fig. 3. LM of osteoblast cell-culture with Algipore. Monocellular layers between particles with focal accumulation of cells on rough surface areas.
Fig. 4. LM of osteoblast cell-culture with Biobase. Monocellular layers without accumulation.
Fig. 5. LM of osteoblast cell-culture with Bio-oss. Less abundant monocellular layer.
Fig. 6. LM of osteoblast cell-culture with Osteograf/N. Monocellular layers between particles with focal accumulation of cells on rough surface areas.
Fig. 7. LM of osteoblast cell-culture with Pepgen-P15. Multicellular layers between particles with accumulation of cells on the granular surface.

is the direct formation of bone by osteoblasts. Osteoinduction describes the mechanism of transforming mesenchymal cells into osteoblasts, which will produce new bone. Finally, osteoconduction is the process of bone apposition from existing bone.

Autogenous bone has long been considered to be the gold standard of bone graft materials because this forms bone by all 3 processes: osteogenesis, osteoinduction, and osteoconduction.[24] These grafts are harvested from the same patient, which means that a second surgical wound site would be necessary. As an alternative, vertical bone distraction[25] can be used, but this technique requires advanced surgical skills and cannot be used for every indication.

If bone graft substitutes are used, certain technical and biologic demands have to be fulfilled. These are biocompatibility, cell adherence, spacer function, tissue integration, and easy handling. There are many different substitute materials available. These can be classified into biologic or synthetic origin. The biologic materials are collagen or mainly hydroxyapatite derivatives. The collagens (Collos®; Ossacur, Oberstenfeld, Germany) are often accompanied by allergic reactions, which restrict clinical application. Hydroxyapatites are widely researched and documented but they are often mistaken for synthetic materials even if they are of natural origin. Biologic hydroxyapatites can be divided into grafts produced at low (<450°C) or high temperature (>450°C).

Hydroxyapatite produced at low temperature from bovine bone (Bio-oss®) allows a high porosity with large granules size for a better adherence of osteoblasts and the persistence of protein structures. These morphologic properties serve as a good spacer function and as an appropriate bone formation-guiding matrix. Controversial literature on the residual proteins and their biologic behavior are available. Some studies[26–29] state that residual proteins in hydroxyapatite have osteoinductive properties. On the other hand, residual protein structures are suspicious for transmitting certain diseases.[26–29] If hydroxyapatites are produced at low temperature from algaes (Algipore®), a similar morphology of the granules can be obtained but without the risk of transmitting uncertain proteins or prions, which in theory might be responsible for diseases like Jakob-Creutzfeldt. Hydroxyapatites produced at a higher temperature (Osteograf®/N, PepGen-p15®) show significant smaller porosity, but without any remaining protein structures. The disadvantage of the reduced porosity is partly compensated for by the small-sized granules.

Bone graft materials of synthetic origin can be divided into hydroxyapatites, tricalcium phosphates, calcium sulfates, and bioglasses.

Synthetic hydroxyapatites (Osteograf®/LD, DENTSPLY Friadent Ceramed, Ostim®, Haereaus-Kulzer, Hanau, Germany) show variable particle sizes from approximately 18 nm (Ostim®), which is stated as nanocrystalline up to 500 μm. For nanocrystalline materials, it has been found that immediate phagocytosis and new bone formation occur, but quite often inflammatory reactions can be seen.[30] Synthetic materials with larger particle size result in a very condensed defect fill. To overcome this effect, graft materials of large particle size are often mixed with demineralized freeze-dried bone allografts (DBDFA).[2]

Tricalcium phosphates can be divided into α-tricalcium phosphates (Biobase®; Centerpulse, Freiburg, Germany) or β-tricalcium phosphates (Bioresorb®, Oraltronics, Bremen, Germany, Cerasorb®, Curasan, Kleinostheim, Germany). It has been shown that α-tricalcium phosphates

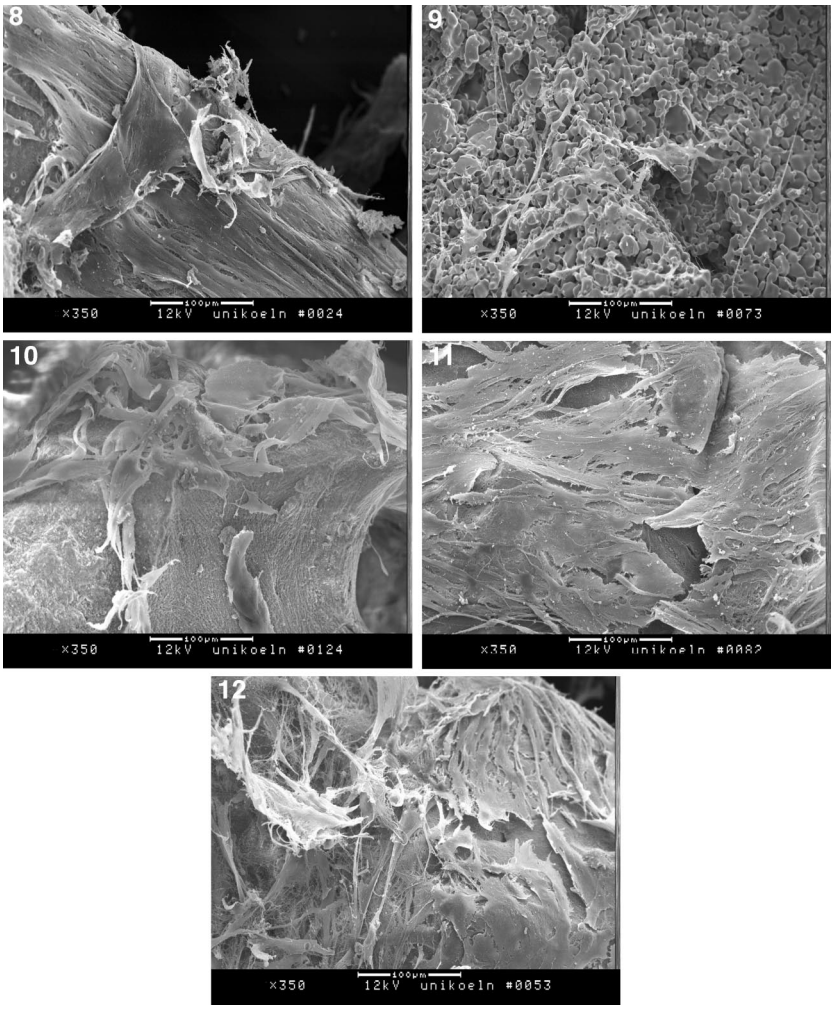


Fig. 8. Scanning electron microscopy of osteoblast cell-culture with Algipore. Granular surface fully covered with osteoblast cell sheets.
Fig. 9. Scanning electron microscopy of osteoblast cell-culture with Biobase. The granular surface is covered with singular cells.
Fig. 10. Scanning electron microscopy of osteoblast cell-culture with Bio-oss. The granular surface is covered with singular cell sheet.
Fig. 11. Scanning electron microscopy of osteoblast cell-culture with Osteograf/N. The granular surface is fully covered with osteoblast cell sheets.
Fig. 12. Scanning electron microscopy of osteoblast cell-culture with Pepgen-p15. The granular surface is fully covered with multilayer osteoblast cell sheets.

are resorbed slower than β-tricalcium phosphates, but that for α-tricalcium phosphates, macrophages are still present after many years. For tricalcium phosphate, contradictive reports about the rate of new bone formation and resorption are available. For both materials, there are contradictory reports on the rate of bone formation with respect to their resorption rate.[20,31]

Bioglasses show a good bioconductivity ("Ceravital") but as a result of the morphologic structure, with sharp edges, the complication rate for soft tissue management is increased.[32]

Bone formation is related to the adherence of osteoblasts to the graft material, which depends on the morphology of the graft surface structure.[33] The adherence can be enhanced by coating the graft material with certain cell-binding peptides. Interaction with collagen modulates cell proliferation and differentiation. For the cell-binding domain of type I collagen, a 15-chain peptide is responsible. In PepGen-p15®, a 15-chained peptide is located at the surface of the bone graft material to increase the adherence of the osteoblasts.[34–38]

Basic research on the osteointegration of implants has shown that the course of osteointegration is significantly distinctive by the morphologic structure of the implant surface.[39–41] Osseointegration starts with the formation of noncollagen proteins (Osteopontin, bone sialoprotein) at the rough implant surface, followed by the adherence of calcium phosphate to 1 or both proteins. This process is followed by the crystallization period, which induces the formation of collagen, and finally, the mineralization of collagen matrix. The investigated materials in this study showed similar characteristics of the surface.

Algipore, Biobase, Osteograf N, and PepGen P15 presented a high value for the cell proliferation test. The better results for PepGen P15 might be explained by the mechanism of enhanced cell proliferation resulting from the type I collagen binding of the P15 peptide.[34–38]

The initial mineralization represented by the alkaline phosphatase on days 6 to 9 again showed similar results for Algipore, Biobase, and Osteograf/N and the highest levels for PepGen P15. The adhesion of osteoblasts to the graft material coated[34–36] with PenGen-p15 was stronger than to noncoated materials.[12] The positive effect of P15 on cell proliferation and differentiation has also been shown in fibroblast cultures.[35,36]

These findings were confirmed by the light microscopy and SEM findings. On the rough granules surface, a good cell adhesion and formation of collagen fibers were observed. The poor results for the viability and differentiation markers for Bio-oss might be explained by the relative smooth surface of the granules, which does not support adherence of the osteoblasts.

Other studies have shown similar results for Bio-oss like for Algipore and PepGen P15. Compared with the present study, these cell cultures were put temporarily under vacuum condition to fill the porous structure of the graft material with cell medium.[42,43] By this approach, a better cell adhesion to the granula surface was reported.

Conclusions

Distinct bone graft materials have different impacts on the proliferation patterns of human osteoblasts *in vitro*. The morphology of the graft surface appears to have a major impact. The advantage of this study protocol was the ability to compare the osteoinductive properties of various bone graft materials onto human osteoblasts. The results suggest that bone graft materials with a porous structure or coated with a collagen analogue show the best differentiation and proliferation of osteoblasts *in vitro*. Further clinical and *in vitro* studies are necessary to confirm these findings.

Acknowledgments

The authors thank Prof. Dr. M. Noack and Mrs. Knof-Urbanek for providing facilities and excellent assistance on scanning electron microscopy performed at the Department of Periodontology and Conservative Dentistry, University of Cologne, Germany.

Disclosure

Dr. Jörg Neugebauer claims to have a financial interest in Friadent whose products Algipore®, PepGen p-15®, and Osteograf® are mentioned in this article. All other authors claim to have no financial interest in any company or any of the products mentioned in this article.

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Abstract Translations [German, Spanish, Portugese, Japanese]

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Wachstum und Vermehrung menschlicher Osteoblasten: ein Laborversuch an unterschiedlichen Ersatzsubstanzen zur Knochengewebstransplantation

ZUSAMMENFASSUNG: **Zielsetzung:** Im Laborversuch sollte festgestellt werden, wie sich unterschiedliche Knochenersatzmaterielien auf das Wachstums- und Vermehrungsmuster von aus dem menschlichen iliakalen Spongiosa gewonnenen Knochengewebszellen auswirken. **Methoden und Materialien:** Um im Laborversuch die jeweiligen Auswirkungen auf die Osteoblastenvermehrung zu untersuchen, wurden fünf unterschiedliche Knochenersatzmaterialien herangezogen: phytogenes Hydroxylapatit (Algipore®), α-Trikalziumphosphat (Bio-Base®), Rinderhydroxylapatit (niedrige Temperatur) (Bio-Oss®), Rinderhydroxylapatit (hohe Temperatur) (Osteograf®) sowie Rinderhydroxylapatit (hohe Temperatur), angereichert mit dem synthetischen Peptid p-15 (PepGen p-15®). Die Osteoblastengewinnung erfolgte aus dem Knochengewebe des menschlichen Iliakalspongiosa. Sie wurden gemeinsam mit den verschiedenen Knochengewebsersatzstoffen kultiviert. Nach 6 und 9 Tagen wurden Erhebungen bezüglich Zellvermehrung und Lebensfähigkeit der Zellen (WST-1) sowie zur alkalischen Phosphatase als frühzeitiger Indikator einer Osteoblastenvermehrung vorgenommen. Die Kulturen wurden mittels Lichtmikroskop und unter dem Rasterelektronenmikroskop hinsichtlich bestimmter Zellwachstumsmuster und Zellmorphologien untersucht. **Ergebnisse:** Die Anwendung der unterschiedlichen knochengeweblichen Transplantatersatzstoffe führte zu unterschiedlichen Osteoblastwachstumsmustern. Die höchsten Werte zu Vermehrung und Differenzierung wies PepGen P-15 auf. In der Rangliste folgten darauf Osteograf, Algipore und Bio-Base. Bei Bio-Oss waren die Werte am schlechtesten. Eine elektronenmikroskopische Untersuchung sowie die Bewertung mittels Lichtmikroskop wiesen ähnliche Wachstumsmuster auf und verifizierten damit die bisher erzielten Ergebnisse. **Schlussfolgerung:** Im Laborversuch wirken unterschiedliche Materialien zur Knochengewebstransplantation unterschiedlich auf die Vermehrungsmuster menschlicher Osteoblasten.

SCHLÜSSELWÖRTER: Knochengewebsersatzstoffe, menschliche Osteoblasten, Vermehrung

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Crecimiento y proliferación de osteoblastos humanos en diferentes substitutos de injertos de hueso: Un estudio in vitro

ABSTRACTO: **Propósito**: Investigar el efecto de diferentes substitutos de injertos de hueso en el crecimiento y proliferación de las células de hueso derivadas de hueso canceloso ilíaco humano. **Métodos Y Materiales**: Se usaron cinco materiales para injerto de hueso para investigar el efecto sobre la proliferación de osteoblastos in vitro: hidroxiapatita fitógena (Algipore®), fosfato α-tricálcico (BioBase®), hidroxiapatita bovina (baja temperatura)(Bio-Oss®), hidroxiapatita bovina (alta temperatura) (Osteograf®) y hidroxiapatita bovina (alta temperatura) mejorada con p-15, un péptido sintético (PepGen p-15®). Los osteoblastos fueron derivados de hueso canceloso ilíaco humano y sembrados con los diferentes sustitutos de hueso. La proliferación y viabilidad de las células (WST-1), fosfatasa alcalina como primer indicador de la proliferación de osteoblastos se evaluó después de 6, y 9 días. Los cultivos fueron examinados para determinar la configuración de crecimiento celular y morfología a través de microscopía electrónica con barrido y de luz normal. **Resultados**: Los osteoblastos humanos demostraron diferentes maneras de proliferación según el tipo de sustituto de injerto de hueso aplicado. PepGen P-15 demostró la tasa de proliferación y diferenciación más alta seguido por Osteograf, Algipore y Bio-base. Bio-Oss demostró la más baja. Estos resultados fueron confirmados por microscopía electrónica y evaluación con microscopio de luz donde se observaron configuraciones de crecimiento similares. **Conclusión**: Distintos materiales de injertos de hueso tienen un impacto diferente sobre la configuración de la proliferación de los osteoblastos humanos in vitro.

PALABRAS CLAVES: sustitutos de hueso, osteoblastos humanos, proliferación

Crescimento e Proliferação de Osteoblastos Humanos em Diferentes Substitutos de Enxerto Ósseo: um Estudo In Vitro

RESUMO: **Objetivo:** Investigar o efeito de diferentes substitutos de enxerto ósseo no modelo de crescimento e proliferação de células ósseas derivadas do osso esponjoso ilíaco.

Métodos & Materiais: Cinco diferentes materiais de enxerto ósseo foram usados para investigar o efeito na proliferação de osteoblastos in vitro: hidroxiapatita fitogênica (Algipore®), @-fosfato tricálcio (BioBase®), hidroxiapatita bovina (baixa temperatura) (Bio-Oss®), hidroxiapatita (alta temperatura) (Osteograf®), e hidroxiapatita bovina (alta temperatura) aumentados com p-15, peptídeo sintético (PepGen p-15®). Os osteoblastos eram derivados de osso esponjoso ilíaco humano e semeados com os diferentes substitutos ósseos. A proliferação celular e a viabilidade (WST-1), fosfatase alcalina como marcador precoce de proliferação de osteoblastos foram avaliadas após 6 e 9 dias. As culturas foram examinadas com vistas ao modelo de crescimento celular e morfologia por luz normal e microscopia de elétron por escaneamento. **Resultados:** Os osteoblastos humanos mostraram diferentes modelos de proliferação de acordo com o substituto de enxerto ósseo aplicado. O PepGen P-15 mostrou a taxa mais alta de proliferação e diferenciação, seguido pelo Osteograf, Algipore e Bio-base. O Bio-Oss mostrou a mais baixa. Estes resultados foram confirmados por microscopia de elétron e a avaliação de microscopia de luz e o modelo de crescimento semelhante foram observados. **Conclusão:** Materiais de enxerto ósseo distintos têm impacto diferente no modelo de proliferação de osteoblastos humanos in vitro.

PALAVRAS-CHAVE: substitutos ósseos, osteoblastos humanos, proliferação

異なる骨移植代用物質上のヒト骨芽の成長と増殖：生体外試験

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要約：

目的：本研究の目的は、異なる骨移植代用物質がヒト腸骨海綿骨から取られた骨細胞の成長・増殖に与える影響を調べることにあった。

素材と方法：骨芽増殖への影響の生体外試験のために、植物性ヒドロキシアパタイト（Algipore®）、リン酸αトリカルシウム（Bio-Base®）、ウシ・ヒドロキシアパタイト（低温）（Bio-Oss®）、ウシ・ヒドロキシアパタイト（高温）（Osteograf®）、p-15合成ペプチド（PepGen p-15®）で強化されたウシ・ヒドロキシアパタイト（高温）の、5つの骨移植物質が使用された。骨芽細胞はヒト腸骨海綿骨から採収され、異なる骨代川物質が移植された。アルカリフォスファターゼを骨芽細胞の早期増殖マーカーに使い、細胞増殖と生存力（WST-1）が6日後と9日後に評価された。培養物について通常光と走査電子顕微鏡によって細胞増殖パターン調査と形態学的調査が行われた。

結果：ヒト骨芽細胞は、骨移植代用物質の種類によって異なる増殖パターンを示した。PepGen p-15が一番高い増殖率と分化率を示し、Osteograf、Algipore、Bio-Baseの順でこれに続いた。増殖率と分化率が一番低いのはBio-Ossだった。電顕、光学顕微鏡の観察でこれらの結果が確認され、同様の成長パターンが認められた。

結論：各種の骨移植物質は、生体外でヒト骨芽細胞の増殖パターンにそれぞれ異なる影響を与える。

キーワード：骨代用物質、ヒト骨芽細胞、増殖

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