последующих публикациях. Есть основания полагать, что во всем этом не последнюю роль играет и спиральная симметрия, как один из основополагающих законов в строении живых организмов, а также и в структуре Вселенной.

При сравнительном анализе известных анатомо-гистологических особенностей строения эмалевой призмы и позвоночного столба, как оказалось, существует больше общих совпадающих признаков, чем принципиальных отличий. И это касается не только их формы и строения, но биомеханики. Кроме того, для эмалевых призм также характерны сужения и варикозные расширения, что необходимо учитывать при исследовании реакции эмали на механические воздействия, так как, вероятно, что области сужений являются именно теми участками, в которых может концентрироваться избыточное напряжение при поперечной нагрузке. Данное предположение, возможно, послужит дополнительным объяснением в случаях сколов эмали или перелома бугров коронок зубов и изыскания новых методов профилактики таких осложнений и особенностей моделирования при реставрациях.

Библиография

1. Лейзер Д. Создавая картину Вселенной. (Пер. с англ.) — М.: Изд-во «Мир», 1988. — с. 15-37.
2. Брейден Г. Божественная матрица: время, пространство и сила сознания. — С: София, 2008, 256 стр.
3. Чиркова Э. Н. Волновая природа регуляции генной активности. Живая клетка как фотонная вычислительная машина. http: // www. rusphysics. ru./dissertation/310/.
4. Хидирбегишвили О. Э. Парадоксы современной кариесологии. МЭСТРО №4 (9). http://www.e-stomatology.ru/pressa/periodika/maestro/10/#2.
5. Гаврилов Е. И., Щербаков А. С. Ортопедическая стоматология. — М.: Изд-во «Медицина», 1984. — 576 с.
6. Боровский Е. В., Грошиков М. И., Патрикеев В. К. Терапевтическая стоматология. — М.: Изд-во «Медицина», 1973. — с. 25-29.
7. Леман Клаус М., Хельвиг Э. Основы терапевтической и ортопедической стоматологии. 1-е издание на русс. языке под ред. проф. С. И. Абакарова, проф. В. Ф. Маккева. — 1999. — с. 42.
8. Боровский Е. В., Леонтьев В. К. Биология полости рта. — М., 1991.
9. Anqker L., Swain M.V., Kilpatrick N. Micro-mechanical characterization of the properties of primary tooth dentine. J. Dent 2003; 4: 261-267.

THE LATE EFFECTS OF THE DEPROTENIZED BOVINE BONE BLOCKS IN COMBINATION WITH RECOMBINANT HUMAN PLATELETDERIVED GROWTH FACTORBB AND GUIDED BONE REGENERATION FOR VERTICAL AUGMENTATION

Victor Pălărie,
Cercetător științific.

Laboratorul științific de inginerie tisulară și culturi celulare. Catedra stomatologie ortopedică, chirurgie oro-maxilo-facială și implantologie orală. Universitatea de Stat de Medicină și Farmacie "Nicolae Testemițanu"

Summary

Bioactive optimizations of deprotenized bovine bone (DBB) with growth factors as well as with guided bone regeneration (GBR) — techniques are promising options to enhance prognosis of vertical bone augmentation. The aim of the study was an evaluation of the late phases events of the vertical bone augmentation with DBB in combination with recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and GBR: new bone volume (NBV), new vertical bone height (VBH) and bone implant contact (BIC). In 6 rabbits, a DBB-block was fixed with a dental implant on the tibia bone. The following groups were included: DBB, DBB + collagen membrane, DBB + rhPDGF-BB and DBB + rhPDGF-BB + collagen membrane. A total of 12 samples were examined after 6 weeks. The results indicate that the addition of rhPDGF to DBB-blocks have a good potential to maintain bone formation for vertical augmentation. Furthermore, the findings illustrate that after six weeks, GBR with a collagen membrane is the key to maximize the new bone volume and height.

Key words: deprotenized bovine bone, guided bone regeneration, vertical augmentation, recombinant human platelet-derived growth factor-BB, collagen membrane, dental implant.

Rezumat

EFECTUL ÎNTÎRZIAT AL BLOCULUI OSOS BOVIN DEPROTEINIZAT ÎN COMBINARE CU FACTORUL DE CREȘTERE PLACHETAR RECOMBINANT UMAN-BB ȘI GHIDAREA REGENERĂRII OSULUI ÎN URMA AUGMENTĂRII VERTICALE

Combinarea activă între osul bovin deproteinizat (DBB), factori de creștere și regenerarea osoasă ghidată (GBR) reprezintă tehnici promițătoare în prognoza augmentării oasoase verticale. Scopul studiului este de a studia procesele de remodelare din fazele tardive în urma augmentării verticale cu DBB în combinare cu factorul de creștere plachetar recombinant uman — BB (rhPDGF-BB) și GBR: volumul osului nou format (NBV), înălțimea osului nou creat (VBH) și contactul implant — os (BIC). La 6 iepuri, blocul DBB a fost stabilzat cu un implant dentar în tibia. Următoarele grupuri au fost incluse în studiu: DBB, DBB +membrana de colagen, DBB + rhPDGF-BB + și DBB + rhPDGF-BB + membrană din colagen. În total 12 probe experimentale au fost examinate după 6 săptămâni. Rezultatele indică faptul că adăugarea de rhPDGF-BB la blocul DBB are un potențial benefic pentru a menține formarea osului în urma augmentării verticale. În plus, rezultatele ilustrează după șase săptămâni, GBR cu membrană de colagen reprezintă cheia succesului pentru a maximiza volumul și înălțimea osului nou format.

Cuvinte cheie: os bovin deproteinizat, regenerare osoasă ghidată, augmentarea verticală, factorul de creștere plachetar recombinant uman — BB, membrană din colagen, implant dentar.

INTRODUCTION

Resorption of the alveolar ridges following tooth extraction, periodontal aggression and trauma is a physiologically undesirable and probably avoidable phenomenon[1-3]. Reconstruction of vertical defects and atrophies in human and animal trials has been studied extensively by evaluating healing events via histological, radiological and clinical methods. But in fact, of these studies the regeneration of severe localized edentulous atrophic ridges remains a challenging procedure [4-10]. The available modalities for vertical reconstruction of the bone started to be compromised by different intraoperative and postoperative discomforts. "Gold standard" autogenous grafts require invasive techniques for the harvesting bone and often from the extraoral regions. Despite of the well known of advantages of autografts, like its capacity for osteoconduction as well as -induction and restricted immune reaction, there are also significant drawbacks, like induction of a secondary defect at the donor site, followed by possible infection and morbidity at the donor[11, 12]. The resorption of such grafts has reported to be up to 50 % of the total volume of reconstructed site [13]. The bone splinting and horizontal alveolar distraction are an alternative technique to harvesting operation[14, 15]. But these techniques have limitations due to non-toleration of the devices and the small amount of gained bone, especially when the vertical augmentation is indicated[16]. The alternative use of various bone substitute materials is possible [17, 18]. Deprotenized bovine bone (DBB; Bio Oss, Geistlich Pharma, Wolhusen, Switzerland)) shows a resistance to resorption following placement into bony defects or as an onlay graft. This may provide long-term preservation of the vertical and interproximal bone height as well as of the corresponding esthetics [19, 20]. It has also been shown to induce periodontal and periimplant bone regeneration, especially when used in conjunction with membranes. During bone regeneration by osteoconduction of the DBB-graft, pluripotent cells differentiate into osteoblasts, which can than produce osteocytes [21]. The implantation of DBB-blocks may provide additional volume stability [22, 23].

An additional bioactive optimization of DBB as a scaffold for the delivery of growth factors such as platelet-derived growth factor (PDGF) is an interesting option to induce further osteoinduction [10, 24-26]. PDGF was discovered as a major mitogenic factor present in serum, secreted from the α-granules of platelets activated during the coagulation of blood [17]. It works by means of angiogenesis and chemo taxis [27]. The results of animal studies as well as randomized controlled trials demonstrated the efficacy of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) for regeneration of cranial and ridges defects [1, 8, 28]. Therefore, protein therapeutics with rhPDGF-BB have a significant potential to treat conditions affecting bone. Because angiogenesis was observed to affect bone formation at ridge defects during the initial weeks, the evaluation of early stages of wound healing might be a particular interest for the assessment of the biologic activity of this factor [27]. The most common methods of ridge reconstructions include grafting procedures with coverage of a barrier membrane (guided bone regeneration (GBR)). When using resorbable membranes together with an underlying, osteoconductive material, a gain in marginal bone was reported in several studies. Collagen membranes maintain a temporary barrier function under provision of nutrient diffusion for cell proliferation and differentiation; it was proven that they are supporting an early transmembraneous angiogenesis. The degradation of those membranes starts shortly after implantation [28].

However, very little evidence exists regarding the early bone healing process and influence of the DBB-rhPDGF-BB complex and DBB alone during GBR procedures in vertical bone defects. The aim of the present study was to define the sequential healing

events and the effects of GBR with and without addition of rhPDGF-BB that occur during initial stages at vertical bone augmentation in rabbits with DBB as a vehicle carrier. The hypothesis is that there is a difference in histological formation of the new bone growth above DBB-rhPDGF-BB and DBB alone in proposed sites for the study. Furthermore, a difference between the outcomes of bone regeneration adjacent to materials covered and none covered with collagen membrane was assumed.

MATERIALS AND METHODS

Experimental setup

Deprotenized bovine bone blocks (DBB; Bio-Oss®, Geistlich Pharma AG, Wolhusen, Schweiz), recombinant human platelet-derived growth factor-BB (rhPDGF-BB; Sigma, St. Louis, USA) as well as resorbable, non-crosslinked collagen membranes (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Schweiz) were used for bone augmentation. One dental implant (3.5X11.5 mm NobelActive, Nobel Biocare, Zürich, Switzerland) was used in each study site to stabilize bone blocks and to study implant placement in bone defects.

Experimental animal-model

Six, 9 month old, 4-5 kg, New Zealand white rabbits were used. After approval of the ethic committee, the surgical part of the project was performed at the State University of Medicine and Pharmacy "N. Testemitanu", Chisinau, Moldova. The animals were operated under a general anaesthetic by intramuscular injections of a combination of a dose of 35mg/kg ketamine and a dose of 5mg/kg xylazine. The experiments were conducted using the tibia model via an anterior transdermal approach on both sides (n=12). In all animals, local bone of the proximal tibia was exposed and carefully skimmed with a straight fissure carbide bur under copious irrigation with sterile 0.9% physiologic saline. For each study site, in the middle of the DBB-block, a hole appropriate to the diameter of the implant (3.5 mm) was drilled and the implant was precautiously inserted (figure 1).

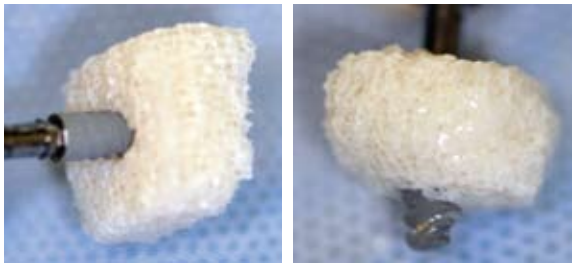

Figure 1: Dental implant inserted into the Bio-Oss®-block

The block was cut into a size of 10 mm x 10 mm with a height of 5 mm and screwed down on the bone (figure 2).

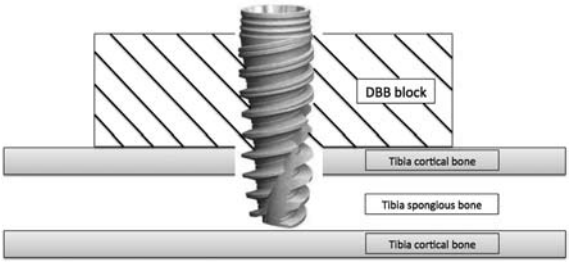

Figure 2: Schema of the DBB block together with the implant inserted in the shallow defect.

The animals were randomly allocated to 2 groups with one time point of healing according to study design and observation periods (table 1). DBB was randomly soak-loaded with 0.5 ml rhPDGF-BB [50] or animal blood. To evaluate differences when using the collagen membrane, a split-leg-design was used: in the left tibia, always only the periosteom was closed over the defect. In the right tibia, the additional collagen membrane was used (table 1).

Table 1: Schematically design of the animal experiments (total n=12)

Group	Procedures	Group size (total n=24)	Time of harvesting the specimens
3a (left)	DBB only	3	6 weeks
3b (right)	DBB+membrane	3	6 weeks
4a (left)	DBB+rhPDGFBB	3	6 weeks
4b (right)	DBB+rhPDGFBB+membrane	3	6 weeks

The mucoperiosteal flaps, muscles, subcutaneous tissue and skin were advanced, repositioned anatomically and fixed via interrupted and mattress sutures with Vicryl 4-0 (Ethicon GmbH, Norderstedt, Germany).

Biopsies and histological procedures

The animals were sacrificed at the 6 weeks after surgery with an excess dose of Pentobarbitone at 100 mg/kg. Samples were harvested and fixated with 4% paraformaldehyde. The specimens were cut in appropriate bony pieces after immersion fixation for four weeks and prepared for histological examination. Briefly, all samples were cut down by a commercial water cooled saw (Exakt Hamburg, Germany) to a thickness of 5 mm perpendicular to the axis of the placed dental implants. The bone slices were immediately embedded in PMMA (Technovit 7100, Heraeus Kulzer, Hanau, Germany) and then grinded to a thickness of 30 to 50 µm. The specimens were stained with Toluidine Blue and then examined using a Leica DM8000 M microscope (Leica Microsystems, Heidelberg, Germany). For histomorphometrical calculations, all slides were digitalized.

Histomorphometry

For histomorphometrical examinations, slides with the implant cut in the middle were used. The following parameter were assessed:

1) Volume of new formed bone at the augmented site. For this, the relation between the total volume of the primary augmentation (5 mm x 5 mm) and the new formed bone was evaluated on the left and the right side of the implant (%). Total values were calculated. DBB-particle were not counted as new bone.
2) Newly mineralized, maginal bone growth (in mm) on 5 equally distributed points at the left and 5 at the right side was measured. DBB-particle were not counted as new bone.
3) Bone implant contact (BIC) was measured by counting all pixels of the implant contour occupied by bone. BIC was expressed as the percentage of the perimeter of the implant cross section [53]. BIC was calculated for the left and the right side as well as a total value.

Statistics

A one-way analysis of variance (ANOVA) with Bonferoni simultaneous post-hoc test was conducted to compare groups; each group consisted of 3 implants under examination. For all parameter, the left and the right side of the implant as well as the total bone values were examined. The 6 weeks results were compared within the groups only. The nature of this experiment was exploratory; therefore, we report descriptive *p-values* of tests. P-values of $p \leq 0.0125$ were termed to be significant. The analyses were conducted using SPSS version 20.0 (SPSS, Chicago, IL, USA).

RESULTS

The postoperative healing was uneventful in all animals. No complications such as swellings, fractures, infections or allergic reactions were observed within the study period. No premature exposure of the augmented bone was seen. All animals could be included in the descriptive statistical analysis.

Volume of new-formed bone at the augmented site

After six weeks, in group3a, only 2.47% new formed bone was seen (SD: 2.47%; 0-4.89%). In group3b, the calculated mean total new bone was 28.48% (SD: 7.8%; 19.8-34.9%), in group 4a 5.3% (SD: 1.89%; 4.04-7.48) and in group 4b 35.54% (SD 4.79%; 30.87-40.43%) respectively. For the left side, group 4b was significantly better than all other groups (all: $p<0.0001$). For the right side, group 3b was significantly better in new-formed bone than the non-membrane groups (all: $p=0.002$). Group 4b showed a significantly better bone growth than group 3a ($p=0.016$). For the total augmented site, both membrane-groups (3b and 4b) showed a significant higher bone growth than the non-membrane groups (both: $p<0.01$). The difference between group 3b and 4b was not significant ($p<0.667$).

Newly mineralized, marginal bone growth

After six weeks, in group 3a, the mean marginal bone growth was 0.4 mm (SD: 0.14 mm; 0.31-0.56 mm). In group 3b, 2.02 mm (SD: 0.39 mm; 1.61-2.39 mm), in group 4a, 0.62 mm (SD: 0.21 mm; 0.4-0.81 mm) and in group 4b, 1.87 mm (SD:0.14; 1.78-2.04 mm) were calculated. On all sides and for the total values, group 3b as well as group 4b (membrane groups) had a significantly higher mean bone height then the non-membrane groups (all: $p \leq 0.01$). The difference between group 3b and 4b was not significant ($p>0.652$).

Bone implant contact (BIC)

After 6 week the mean BIC of 75.1% (SD: 20.3%; 27.9-96.7%) could be measured.

BIC in relation to augmentation

After 6 weeks, the mean BIC was 57% (SD: 26%; 27.8-77%) in group 3a, 87.5% (SD:11.9%; 74.1-96.7%) in group 3b, 83.2% (SD 6.5%; 75.9-88.3%) in group 4a and 72.5% (SD: 2.4%; 45.2-90.8%) in group 4b. At that point, no significant differences between the groups were seen anymore.

All values are presented in figures 3-7 and tables 2-4.

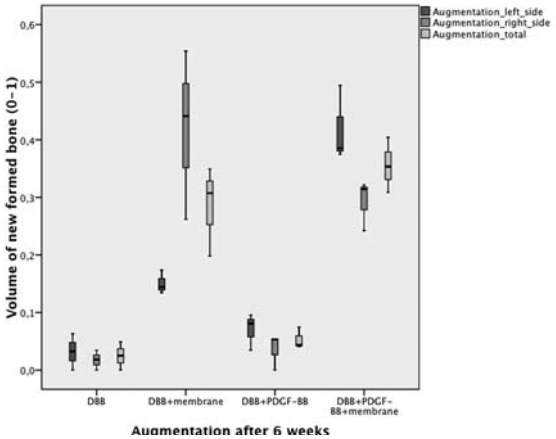

Figure 3: Boxplots showing the amount of new formed bone in the different groups after 6 weeks (0=0%, 1=100%)

Figure 4: Histological specimen (toluidine blue, x20) of a sample from the DBB+rhPDGF-BB+membrane-group. The new formed woven bone above the cortical frontier can be clearly distinguished. Residual particles of DBB areseen in the new bone tissue as well as above.

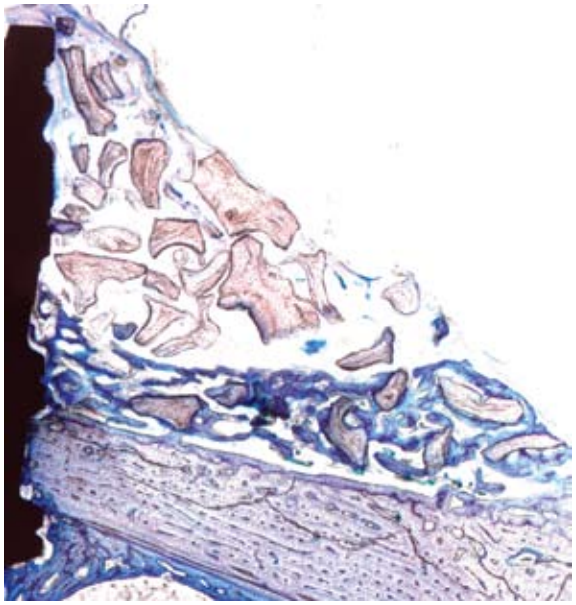

Figure 5: Histological specimen (toluidine blue, x20) showing the new bone growing from the underlying cortical bone around the DBB-particles.

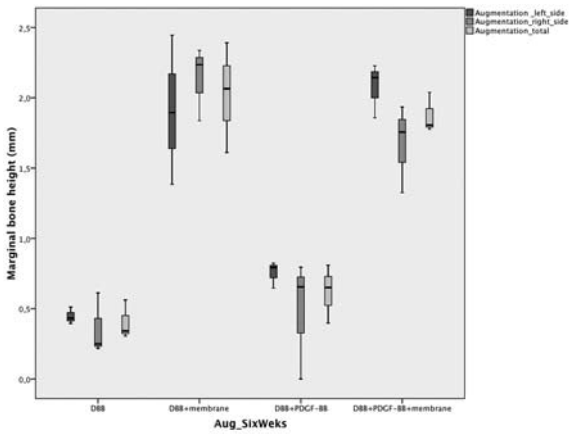

Figure 6: Boxplots of new marginal bone height (mm) after 6 weeks on the left and the right side of the implant as well as total values

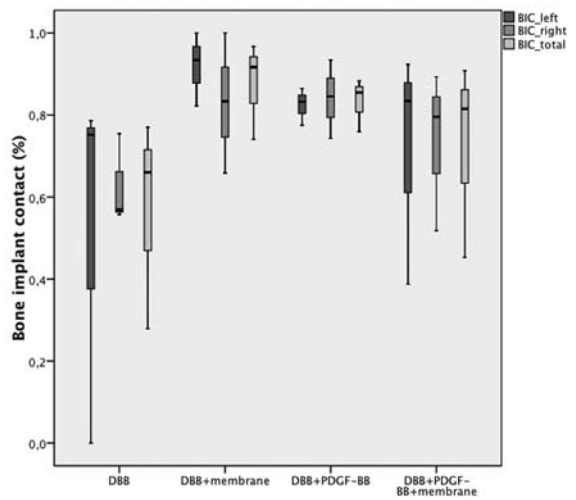

Figure 7: Boxplots of bone implant contact (%) after 6 weeks on the left and the right side of the implant as well as total values

Table 2: Mean volumes of new formed bone (%) after 6 weeks at the left and right implant side as well as total values. Standard deviations (SD) are given.

Side of the implant	Group	Volume of new formed bone (%)	SD
Left	3a	3.19	3.17
	3b	15.07	2.03
	4a	7.02	3.18
	4b	41.82	6.6
Right	3a	1.75	1.71
	3b	41.9	14.73
	4a	3.6	3.1
	4b	29.26	4.37
Total	3a	2.47	2.44
	3b	28.49	7.8
	4a	5.31	1.89
	4b	35.544	4.78

Table 3: Mean volumes of new marginal bone height (mm) after 6 weeks at the left and right implant side as well as total values. Standard deviations (SD) are given.

Side of the implant	Group	Marginal bone growth (mm)	SD
Left	3a	0.45	0.06
	3b	1.91	0.53
	4a	0.75	0.1
	4b	2.08	0.19
Right	3a	0.36	0.22
	3b	2.14	0.26
	4a	0.48	0.42
	4b	1.67	0.31
Total	3a	0.4	0.14
	3b	2.02	0.39
	4a	0.62	0.21
	4b	1.87	0.14

Table 4: Bone implant contact (%) after 6 weeks at the left and right implant side as well as total values. Standard deviations (SD) are given.

Side of the implant	Group	Bone implant contact (%)	SD
Left	3a	51.3	44.4
	3b	91.9	0.9
	4a	82.3	4.5
	4b	71.5	28.7
Right	3a	62.7	11.1
	3b	83.1	17.1
	4a	84.1	9.6
	4b	73.9	19.5
Total	3a	57	25.8
	3b	87.5	11.9
	4a	83.2	6.5
	4b	72.5	24.1

DISCUSSION

The bony vertical augmentation for functional as well as aesthetic reconstruction is a widespread, though critical method as long-term stability is desirable. Therefore, the study aimed to determine the effect of rhPDGF-loaded DBB on late bony healing after vertical augmentation in the rabbit tibia. Additionally, the effect of GBR surgery with and without rhPDGF — BB adjunct was assessed. The used animal model is well established for vertical augmentation and implant examination purposes [25]. Though, to the best of our knowledge, this is the first experimental rabbit study reporting on late bone growth for vertical augmentation using rhPDGF — BB soaked DBB-blocks fixed with dental implants in combination with GBR procedures. The tension on the soft tissue covering the defect after intraoral augmentation in similar to the tension in the tibia model. *Schwarz et al.* conducted a similar study at chronic-type lateral ridge defects in the dog mandible. Though, It has to be kept in mind, that their study size was low (n=2), not reaching any statistical power [20]. DBB is a xenogenic bone material with a high degree of biocompatibility; its structure is similar to cancellous bone. After slow remodelling over time, incorporation into native bone has been described; this can be supported by the findings of our study. It has been widely discussed, that the high regenerative potential of autologous bone transplants is due to the transfer of several vital cell type (mesenchymal stem cells, osteoblast as well as their precursor cells) and local autologous growth factors [10, 11]. Therefore, an additional bio-functionalization of the DBB, for example with rhPDGF-BB is a nearby option. Details about the absorption and the release kinetics of rhPDGF — BB and DBB-blocks are still unknown. *Bateman et al.* examined that the in vitro absorption of PDGF to β-TCP-carriers occur in a concentration as well as time-dependent manner. The *in vitro* release was — with a release of approximately 45% after 10 days — slower than the *in vivo* release [29]. Therfore, the use of rhPDGF-BB soaked DBB seems appropriate.

After six weeks, the effect of the membranes on new bone volume as well as on new vertical bone height seems to be major than the PDGF-effect. *Simion and co-workers* observed a beneficial effect of a natural bone mineral with addition of rhPDGF-BB four months after vertical ridge augmentation in dogs. In their study, the best effect was achieved without coverage of a collagen barrier membrane. The authors conclude that the membrane excludes osteogenic cells derived from the periosteum [8, 26]. These findings are in contrast to the results of our study as well as to the current findings regarding early trans membraneous angiogenesis [24] as well as GBR techniques [26]. *Schwarz et al.* could show that the collagen membrane did not interfere with bone induction by rhBMP-2 [21]. In summary, the separation of the periosteum (providing essential cell resources for rhPDGF mediated bone formation) does not influence early new bone formation in a negative way. The results rather lead to the hypothesis, that the collagen membrane might only primarily exclude the vascularization as well as the ingrowth of osteoprogenitor cells from the periosteum [15, 22]. The assumed additional membrane functions such as stabilization of the blood coagulum and to keep away unwanted soft tissue cells [24-26] does not seem to enhance early bone growth. After six weeks, a transmembraneous neo-vascularization with higher mineralization of new bone and a biodegradation of the respective collagen membrane might have taken place. Additionally, the collagen membrane may stabilize particulated bone graft materials at non-self-contained defects [19] such before vertical augmentation. Mechanical immobility is needed to achieve biological healing [17]. As solid DBB-blocks were used, this stabilization may have a smaller impact as for DBB granules. *Schwarz et al.* could show that the collagen membrane degrades after 4-6 weeks of healing in dogs [25]. Accordingly, after six weeks, the positive membrane-effect was evident. This is in contrast to the results of *Rothamel et al.*, though this group used a cross-linked collagen membrane with long-barrier function compared to the non-crosslinked membrane with resorption after shorter time in our study[18]. It can be considered as a drawback for membrane use that an early exposure of collagen membranes to the oral environment may jeopardize the outcome due to infection or rapid disintegration. This complication is still common [26]. *Jensen and Terheyden* stated in a review, that the incidence of soft tissue dehiscences was higher for non-resorbable than for resorbable membranes [21].

Previous studies could not see a difference between vertical bone growth in DBB-blocks or DBB-blocks pre-treated with either BMP or VEGF [10]. This supports the findings of the present study as, after three weeks for the total values and after six weeks for all values, no significant differences between the DBB and the DBB+rhPDGF-BB-group were seen.

New vertical bone growth showed to be from the contact area of the inserted material. This shows again [10] the need of direct bone-transplant-contact for successful augmentation. Furthermore, an additive effect of the implant surfaces on initial ossification was measured. This is in accordance to prior studies [13].

Conclusion

The present study indicates that the addition of rhPDGF to DBB-blocks has a good potential to maintain early bone formation for vertical augmentation. Furthermore, the findings illustrate that after six weeks, GBR with a collagen membrane is the key to maximize the new bone height.

Acknowledgements: The surgical part of study was performed with help offered by Prof. V. Topalo and Prof. V. Nacu from Chisinau, Moldova; the histological and statistical evaluations of the present manuscript were performed with support of Dr. P.

Kammerer and Prof. B. Al Nawas from Maiz, Germany. The Osteology Foundation; Geistlich Pharma AG, Wolhusen, Switzerland and Nobel Biocare, Zürich, Switzerland, supported the biomaterials and dental implants, as well as insertion kits.

Conflict of interest: The author declares that he has no conflict of interest.

REFERENCES

[1] Nevins M, Garber D, Hanratty JJ, et al. Human histologic evaluation of anorganic bovine bone mineral combined with recombinant human platelet-derived growth factor BB in maxillary sinus augmentation: case series study. Int J Periodontics Restorative Dent 2009; 29: 583-91.

[2] Cawood JI, Stoelinga PJ, Blackburn TK. The evolution of preimplant surgery from preprosthetic surgery. International journal of oral and maxillofacial surgery 2007; 36: 377-85.

[3] Araujo MG, Sonohara M, Hayacibara R, Cardaropoli G, Lindhe J. Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. Journal of clinical periodontology 2002; 29: 1122-31.

[4] Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol 2005; 32: 212-218.

[5] Draenert FG, Kämmerer PW, Palarie V, Wagner W. Vertical Bone Augmentation With Simultaneous Dental Implantation Using Crestal Biomaterial Rings: A Rabbit Animal Study Clin Impl Dent Rel Res 2011; In print.

[6] Felice P, Marchetti C, Iezzi G, et al. Vertical ridge augmentation of the atrophic posterior mandible with interpositional bloc grafts: bone from the iliac crest vs. bovine anorganic bone. Clinical and histological results up to one year after loading from a randomized-controlled clinical trial. Clinical oral implants research 2009; 20: 1386-93.

[7] Canullo L, Trisi P, Simion M. Vertical ridge augmentation around implants using e-PTFE titanium-reinforced membrane and deproteinized bovine bone mineral (bio-oss): A case report. The International journal of periodontics & restorative dentistry 2006; 26: 355-61.

[8] Simion M, Rocchietta I, Kim D, Nevins M, Fiorellini J. Vertical ridge augmentation by means of deproteinized bovine bone block and recombinant human platelet-derived growth factor-BB: a histologic study in a dog model. Int J Periodontics Restorative Dent 2006; 26: 415-23.

[9] Tonetti MS, Hammerle CH. Advances in bone augmentation to enable dental implant placement: Consensus Report of the 6th European Workshop on Periodontology. Journal of clinical periodontology 2008; 35: 168-72.

[10] Schmitt C, Lutz R, Doering H, et al. Bio-Oss(R) blocks combined with BMP-2 and VEGF for the regeneration of bony defects and vertical augmentation. Clinical oral implants research 2011.

[11] Blokhuis TJ, Arts JJ. Bioactive and osteoinductive bone graft substitutes: definitions, facts and myths. Injury 2011; 42 Suppl 2: S26-9.

[12] Schaaf H, Lendeckel S, Howaldt HP, Streckbein P. Donor site morbidity after bone harvesting from the anterior iliac crest. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 2010; 109: 5-8.

[13] Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. Int J Oral Maxillofac Implants 2009; 24 Suppl: 237-59.

[14] Swennen G, Schliephake H, Dempf R, Schierle H, Malevez C. Craniofacial distraction osteogenesis: a review of the literature: Part 1: clinical studies. International journal of oral and maxillofacial surgery 2001; 30: 89-103.

[15] Misch CM. Comparison of intraoral donor sites for onlay grafting prior to implant placement. The International journal of oral & maxillofacial implants 1997; 12: 767-76.

[16] Klesper B, Lazar F, Siessegger M, Hidding J, Zoller JE. Vertical distraction osteogenesis of fibula transplants for mandibular reconstruction--a preliminary study. Journal of cranio-maxillofacial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery 2002; 30: 280-5.

[17] Klein MO, Kammerer PW, Scholz T, et al. Modulation of platelet activation and initial cytokine release by alloplastic bone substitute materials. Clinical oral implants research 2010; 21: 336-45.

[18] Zhou X, Zhang Z, Li S, Bai Y, Xu H. Osteoconduction of different sizes of anorganic bone particles in a model of guided bone regeneration. The British journal of oral & maxillofacial surgery 2011; 49: 37-41.

[19] Tarnow DP, Magner AW, Fletcher P. The effect of the distance from the contact point to the crest of bone on the presence or absence of the interproximal dental papilla. Journal of periodontology 1992; 63: 995-6.

[20] Steigmann M. A bovine-bone mineral block for the treatment of severe ridge deficiencies in the anterior region: a clinical case report. The International journal of oral & maxillofacial implants 2008; 23: 123-8.

[21] Boyne PJ, Marx RE, Nevins M, et al. A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. Int J Periodontics Restorative Dent 1997; 17: 11-25.

[22] Chris Arts JJ, Verdonschot N, Schreurs BW, Buma P. The use of a bioresorbable nano-crystalline hydroxyapatite paste in acetabular bone impaction grafting. Biomaterials 2006; 27: 1110-8.

[23] Por YC, Barcelo CR, Salyer KE, et al. Bone generation in the reconstruction of a critical size calvarial defect in an experimental model. Annals of the Academy of Medicine, Singapore 2007; 36: 911-9.

[24] Stockmann P, Park J, von Wilmowsky C, et al. Guided bone regeneration in pig calvarial bone defects using autologous mesenchymal stem/progenitor cells — A comparison of different tissue sources. Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery 2011.

[25] Rocchietta I, Dellavia C, Nevins M, Simion M. Bone regenerated via rhPDGF-bB and a deproteinized bovine bone matrix: backscattered electron microscopic element analysis. The International journal of periodontics & restorative dentistry 2007; 27: 539-45.

[26] Simion M, Rocchietta I, Dellavia C. Three-dimensional ridge augmentation with xenograft and recombinant human platelet-derived growth factor-BB in humans: report of two cases. The International journal of periodontics & restorative dentistry 2007; 27: 109-15.

[27] Nevins ML, Reynolds MA. Tissue engineering with recombinant human platelet-derived growth factor BB for implant site development. Compendium of continuing education in dentistry 2011; 32: 18, 20-7; quiz 28, 40.

[28] Moore DC, Ehrlich MG, McAllister SC, et al. Recombinant human platelet-derived growth factor-BB augmentation of new-bone formation in a rat model of distraction osteogenesis. The Journal of bone and joint surgery. American volume 2009; 91: 1973-84.