The sinus lift with phycogenic bone substitute
A histomorphometric study

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Abstract
Objectives: The aim of this histomorphometric prospective study was to ascertain the efficacy of phycogenic bone substitute in an augmented sinus. The process of graft healing, bone remodeling, and biomaterial replacement was examined.

Material and methods: The phycogenic material (fluorohydroxyapatite) made from calcium-encrusted sea algae was used for the sinus lifts. Twenty-four procedures were carried out (one-stage and two-stage equally) and 45 titanium stepped-screw implants were placed. The patients were followed for 12–23 months. In intervals of 6, 9, 12, or 15 months after the sinus lift, 24 graft specimens were taken with a trephine bur. These specimens were examined histomorphometrically.

Results: The grafting material was gradually resorbed and replaced by newly formed bone. Between the sixth and 15th month after the sinus lift, the percentage of newly formed bone grew linearly (from 15.5% to 40.8%) and the percentage of bone substitute decreased linearly (from 34.5% to 13%). After 15 months, the density of trabeculae in grafted bone corresponded to cancellous bone of good quality; however, the bone substitute was not completely resorbed during this period. No significant difference between the quality of the newly formed bone in the cases of the one- and two-stage sinus lifts was found.

Conclusion: Sinus lift carried out with phycogenic bone substitute was shown to be an effective method with limited invasiveness and a high survival rate of implants (97.8%).

Dental implants have achieved a high level of reliability and a considerable rate of success (Adell et al. 1990). However, not all areas of the jaw afford implants equally favorable anatomical conditions. The best results are observed in the voluminous, highly mineralized bone of the interforaminal region of the mandible (Adell et al. 1990). In contrast, the least favorable region is the posterior maxilla, where the bone is largely cancellous with a low level of mineralization (McCarthy et al. 2003). Its height is usually limited by the extended maxillary sinus. This anatomical handicap can be overcome with a sinus lift (Jensen et al. 1998).

Sinus lift was introduced by Boyne in the 1960s and Tatum popularized the operation (Jensen 1999). Early studies advocated the use of autogenous bone to ensure graft survival and bone formation in the augmented space (Xu et al. 2003). However, its use is restricted by the limited amount of graft material that can be harvested intraorally and the need for general anesthesia for bone harvesting from extraoral...
informed about their participation in the Helsinki committee, and patients were those who smoked more than 15 cigarettes, had ostitis or other serious general diseases, coagulopathy, acute maxillary sinusitis, or unsatisfactory oral hygiene, diabetes mellitus, or a layer of small fluorohydroxyapatite crystals on the surface of the biomaterial. The thickness of this layer was approximately 0.93 cm³/g (Schopper et al. 2003).

Biomaterials that are used for the reconstruction of bone are non-viable foreign bodies, which only provide scaffolding for the formation of new bone (Schopper et al. 2003). Appositional bone growth around biomaterials can be considered as a result of a host response. Thus, it is desirable that biomaterials provide stability until bone formation has been largely completed and thereafter become gradually replaced by vital bone during bone remodeling (Schopper et al. 2003).

The aim of this study was to ascertain the efficacy of phycogenic bone substitute in an augmented sinus, as the properties of this material in connection with sinus grafting have not been documented in detail till now. The process of graft healing, bone remodeling, and biomaterial replacement was examined with the help of histomorphometric methods.

Material and methods

Patient selection

The study was carried out on a group of 24 patients (12 men and 12 women, mean age 47.4 years, range 18–61 years) with a defect of dentition in the posterior maxilla. All the patients had a residual sinus floor of less than 6 mm in height and at least 5 mm in width. There were no patients with unsatisfactory oral hygiene, diabetes mellitus or other serious general diseases, coagulopathy, acute maxillary sinusitis, or those who smoked more than 15 cigarettes a day. The study was officially approved by the Helsinki committee, and patients were informed about their participation in the study according to the Helsinki declaration of 1994.

Treatment procedure

From April 2001 to June 2002, 24 sinus grafting operations were performed, 12 with simultaneous implant placement (the preoperative height of alveolar bone was at least 3 mm) and 12 with subsequent implant placement (the preoperative bone height did not reach 3 mm). In the case of the one-stage procedure, the initial healing period was 9 months. In the case of the two-stage procedure, the healing time between sinus lift and implant placement was 6 months. Implants were loaded after a subsequent 9 months. The sinus lift was carried out using the window technique (Artzi et al. 2001). Amoxicillin potentiated by clavulanat (500 mg orally three times per day) was prescribed for 6 days; the first dose was administered 2 h before surgery. In the case of the two-stage procedure, dental implants were placed in the second stage, after a prophylactic oral dose of 2.5 g amoxicillin 2 h before surgery. Tiaprenol acid 300 mg was prescribed three times daily for pain relief only if needed. All the surgical procedures were carried out in the University Hospital (Hradec Králové, Czech Republic) under local anaesthesia in a fully sterile hospital setting.

The phycogenic material Algipore was used for the sinus lift. Algipore (DENTSPLY Friadent, Mannheim, Germany) is made from calcium-encrusted sea algae Corallina officinalis (Bieniek 1990; Schumann 1997; Schopper et al. 2003) (Fig. 1). Biomaterial processing involves pyrolytical segmentation of the native algae and hydrothermal transformation of the calcium carbonate [CaCO₃] into fluorohydroxyapatite [Ca₅(PO₄)₃(OH,F)₁₋ₓ] (Schopper et al. 2003). During this production, the organic components are completely removed. The particles of the biomaterial contain a pore system with the mean diameter of pores being 10 µm. Every pore is limited by one layer of small fluorohydroxyapatite crystals with a size of 25–35 nm (Schopper et al. 2003). The specific pore volume averages 0.93 cm³/g (Schopper et al. 2003). The specific surface area reaches 32–50 m²/g, which is caused by its 65% porosity (Kasperk et al. 1988). Approximately one 2-ml package of Algipore with grain size 1–2 mm mixed with 0.5 ml of venous blood was used in each procedure.

Forty-five Frialit-2 implants (DENTSPLY Friadent) in the form of stepped screws with deep profile surface (DPS) grid-blasting and etching were placed in 24 augmented sinuses. Each patient received from one to three implants (mean 1.9) with diameters of 3.8, 4.5, or 5.5 mm, and lengths of 13 or 15 mm. Their stability was established by Periotest (Siemens AG, Bensheim, Germany) during the second-stage surgery. Marginal bone loss was measured to the precision of 0.2 mm using the long-cone paralleling technique of intraoral radiography carried out during the second-stage surgery and after 1-year loading. The implants were followed to April 2003; i.e., 12–23 months (mean 16.4 months) after insertion.

Harvesting of the specimens, histomorphometry

The quality of the newly formed bone was evaluated on the basis of histomorphometric analysis of biopsy specimens taken under local anesthesia using a 2 mm internal diameter trephine bur. The trephine bur was introduced through a short mucosal incision under copious cool saline irrigation. It was directed horizontally from the oral vestibule to the centre of the graft at least 3 mm above the supposed bottom of the alveolar recess. If implants were present, the bur passed in at least 1 mm away from the implant. A biopsy specimen was obtained 6, 9, 12, or 15 months after
grafting, one from each of the augmented sinus. As there were 24 procedures, six biopsy specimens were harvested at each time interval, three from both one- and two-stage operations. Cylindrical specimens were fixed in Burkhardt’s solution, dehydrated in increasing concentrations of ethanol, and embedded in methylmethacrylate, without decalcification. After morphological examination, area values for bone and biomaterial were obtained. Five subsequent 4-μm-thick sections were cut with microtome (Jung, Heidelberg, Germany) from each specimen. Three sections were stained with Giemsa stain and used for histomorphometric evaluation. The other two sections were stained with Gömöri and Ladewig stains, respectively, in order to evaluate qualitative features of the bone tissue. The quantity of bone and bone substitute was histomorphometrically evaluated after digitalization of the light microscopic picture using the software LUCIA M, version 3.0 [Laboratory Imaging, Prague, Czech Republic]. The percentage of the components of the harvested tissue [i.e., hard bone tissue, residual bone substitute, and fibrous tissue] was investigated with respect to the healing period. The linearity of the dependence was tested [Draper & Smith 1996] to find out if a linear regression model is applicable.

Results

All implants were stable at the second-stage surgery; the mean Periotest value was \( -2.9 \pm 1.5 \). The mean marginal bone loss at the second-stage surgery and after 1-year loading was \( 0.41 \pm 0.33 \) and \( 0.92 \pm 0.42 \) mm, respectively. At the end of the observation period, 44 implants were loaded without progressive resorption of marginal bone and without undesirable clinical symptoms. One implant from a 49-year-old healthy woman failed 4.5 months after loading. The success rate of the implant placements was 97.8%.

One specimen was taken incorrectly, missing the grafted area. This is why only 23 specimens were processed. Microscopically, the specimens showed trabeculae of lamellar cancellous bone, fibrous tissue, and particles of resorbing bone substitute with foci of new bone and osteoid formation on its surface [Figs 2–5]. The original host bone was represented by broad regular trabeculae of lamellar bone. Small irregular deposits of primitive woven bone and osteoid closely apposed or growing into the

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![Fig. 2](image2.png)

**Fig. 2.** Ingrowth of osteoid (blue, arrow) into the porous structure of the phycogenic bone substitute [asterisk]. 12 months, Giemsa staining, original magnification \( \times 400 \).

![Fig. 3](image3.png)

**Fig. 3.** Mature lamellar bone on the surface of phycogenic bone substitute [asterisk]. 15 months, Gömöri staining, original magnification \( \times 400 \).

![Fig. 4](image4.png)

**Fig. 4.** A trabecula of mineralized bone (blue) and osteoid (red) on the surface of phycogenic bone substitute [asterisk]. Note ingrowth of the osteoid into porous structure of substitute material [arrow]. 9 months, Ladewig staining, original magnification \( \times 400 \).

![Fig. 5](image5.png)

**Fig. 5.** Broad trabeculae of bone with a remnant of unresorbed phycogenic bone substitute [arrow]. 6 months, Giemsa staining, original magnification \( \times 200 \).

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porous substitute material were regarded as newly formed bone. These areas unequivocally prove the osseoconductive property of the substitute material. Deposits of the new bone on the surface of the substitute material sometimes showed a lamellar pattern similar to ‘creeping apposition’ seen on the surface of necrotic trabeculae in bone infarction. Neither inflammatory infiltrate nor foreign body reaction was present. The percentage of bone, the residual bone substitute, and fibrous tissue in biopsy specimens is shown in Fig. 6.

The percentage of bone and bone substitute in relation to the healing period is presented in Tables 1–3 and in Fig. 7. The regression analysis in which the independent variable is time was used. The use of the simple linear regression model has been justified by the test of linearity. This test indicates that such a model is the right one. The percentage of bone grows linearly with respect to time. The percentage of bone substitute decreases with the healing time. But, according to a one-sample t-test, even after 15 months it is different from zero. Even though the decrease may be described as linear, the low coefficient of determination (0.27) does not allow for any predictions as to when the resorption of bone substitute will be completed.

Statistical comparison showed no significant differences between the one- and two-stage sinus lift.

Discussion

The usefulness of sinus lift depends on the grafting material [Wheeler et al. 1996]. Autogenous bone has been documented as the gold standard [Boyne & James 1980]. If the non-autogenous material is used, the operation required for harvesting of the autogenous bone is eliminated. The bone substitute has to be resorbed and completely replaced with newly formed bone. The process of restructuring can be evaluated histomorphometrically. A number of studies have appeared concerning bovine [Valentini et al. 1998; Landi et al. 2000; Yildirim et al. 2000; Artzi et al. 2001; Hallman et al. 2001; Szabó et al. 2001] or synthetic hydroxyapatite [Artzi et al. 2001, β-tricalciumphosphate (Szabó et al. 2001; Zerbo et al. 2001), freeze-dried
demineralized bone [Landi et al. 2000], or bioactive glass [Schepers et al. 1998; Tadjoedin et al. 2000; Cordioli et al. 2001]. Even though results in these publications were highly variable, it has been proven that the percentage of bone in the grafting material during the healing period grows and the percentage of unre sorbed foreign residue is reduced [Landi et al. 2000]. After 6 months, 5.4–44.1% of bone and 7.7–34.5% of bone substitute [Landi et al. 2000; Tadjoedin et al. 2000; Yildirim et al. 2000; Szabó et al. 2001], and after 12 months, 28–43.7% of bone and 24.6–28% of bone substitute were registered [Valentini et al. 1998; Landi et al. 2000; Artzi et al. 2001]. After 16 months, the bone formed 45% of the mass, while bone substitute was completely resorbed in several cases [Tadjoedin et al. 2000].

The general properties of the phycogenic bone substitute Algipore have been researched by a number of authors. They state that the granules are vascularized in the tissue after only a few weeks and are then gradually resorbed and replaced with new bone through remodeling. This process is aided by the osseocconductivity of the material [Kasperk et al. 1988; Feifel et al. 1995; Schopper et al. 1999]. The doubts about the ability of bone growth into biomaterial with pore sizes smaller than 40 μm [Klawitter et al. 1976] were not verified. The suitability of the Algipore for sinus grafting was investigated by Schopper et al. [2003]. Their investigation demonstrated that the use of this biomaterial can trigger appositional bone formation in the maxillary sinuses of severely atrophic human maxillae even without additional bone harvesting. The results also showed that a pore size of 10 μm is capable of accepting bone growth into the bone substitute particles. The average amount of bone that had formed after a mean healing time of 7 months was 23% [Schopper et al. 2003]. This is in agreement with our investigation [15.5 ± 9.6% and 28.2 ± 16.8% after 6 and 9 months, respectively].

A horizontal approach for obtaining bone samples was preferred over the conventional vertical approach, because the implants were not always inserted simultaneously. However, this method is associated with a higher risk of missing the grafted area. In all cases, the sections were stained with Giemsa, Gomori, and Ladewig stains. The Giemsa stain was used for undecalcified metacrylate sections as a routine staining method because it gives better results and shows more cytological details than the hematoxylin and eosin stain. In addition, sections stained with Giemsa stain excellently show the structure of the porous substitute material and are suitable for histomorphometric evaluation. The Gomori stain shows the pattern of collagen fibers in bone trabeculae and it enables distinguishing the lamellar and the woven bone, respectively. The Ladewig stain is necessary to distinguish the mineralized bone matrix [blue] and the unmineralized osteoid [red]. This staining method shows ingrowth of osteoid into the structure of porous substitute material and the osteoid seams in the mineralized bony trabeculae.

In this study, the percentage of bone substitute after 6 months was 34.5%, gradually decreasing over the entire 15-month period of observation. The last measurement, taken 15 months after the sinus lift, reached a mean value of 13%. As Fig. 6 shows, an almost complete resorption of bone substitute in three specimens in a 9-month period or later was recorded. Statistical analysis of the results, however, leads to the conclusion that even after 15 months the bone substitute is not fully resorbed and that it is impossible to ascertain when this is going to happen. The impossibility of predicting this is most likely caused by major interindividual differences and a non-standard method of harvesting with the trephine bur. Haessler et al. [1999] estimate the complete resorption time of Algipore during sinus lift to be 5 years.

With increasing healing time, the percentage of newly formed bone grew linearly. After 15 months of healing, the percentage of trabeculae was 40.8%, which corresponds to cancellous bone of good quality [Trisi & Rao 1999]. This points to a good long-term prognosis for the implant.

In the case of the one-stage procedure, the newly formed bone was loaded from the ninth month on, and in the case of the two-stage lift, only from the fifteenth month on after the augmentation. It can be assumed that due to functional loading, the newly formed bone from the one-stage surgery will be of better quality than the bone from the two-stage surgery. This hypothesis, however, has been rejected statistically.

Long-term survival of implants in sinus grafting was studied by Tong et al. [1998]. Using meta-analysis, they evaluated 1149 implants placed 6 months to 5 years previously. The maximum number of failures was documented during the first 6 months. Implantation into grafted bone had a success rate 87–98%, which was higher than that of the implants placed in the native, low-quality bone of the posterior maxilla. Others have published similar findings [Wheeler 1997; Lorenzoni et al. 2000; Raghoebar et al. 2001]. The results of the present study, a 97.7% overall survival rate after 12–23 months, support these findings.

Conclusions

Sinus lift carried out with phycogenic bone substitute and stepped screw dental implants is considered a highly satisfactory method, which has a limited invasiveness and high success rate of implants (97.8%, 12–23 months following implant placement). Phycogenic bone substitute is gradually resorbed and replaced by newly formed bone. During the time period of 6–15 months, the percentage of newly formed bone grows linearly and the percentage of phycogenic material decreases linearly. After 15 months, the density of trabeculae in grafted bone corresponds to cancellous bone of good quality. Phycogenic bone substitute is not completely resorbed after 15 months. No significant difference between the quality of newly formed bone in the cases of the one-stage and the two-stage sinus lifts has been found.

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Résumé

Le but de cette étude prospective histomorphométrique a été d'analyser l'efficacité d'un substitut osseux phycogénique lors de l'épaisseur sinusale. Les processus de guérison osseuse, de remodelage osseux et du remplacement du biomatériel ont été examinés. Le matériau phycogénique (fluorohydroxyapatite) a été fabriqué à partir d'algues maritimes.
incrustées de calcium a été utilisé pour ces épaississements sinusiens. Trente-quatre processus ont été effectués (en une ou deux étapes) et 45 implants vis en titane ont été placés. Les patients ont été suivis durant 12 à 23 mois. Dans les intervalles des 6, 9, 12 ou 15 mois après l’opération, 24 spécimens de greffions ont été prélevés à l’aide d’un trépan. Ces spécimens ont été examinés histomorphométriquement. Le matériel greffé était graduellement résorbé et remplacé par de l’os néoformé. Entre le sixième et le quinzième mois, le pourcentage d’os néoformé augmentait linéairement de 16 ± 10% à 41 ± 15%, et le pourcentage de substitut osseux diminuait également linéairement de 35 ± 9% à 13 ± 10%. Après quinze mois, la densité des trabécules dans l’os greffé correspondaient à de l’os spongieux de bonne qualité; cependant le substitut osseux n’était pas complètement résorbé durant cette période. Aucune différence significative de qualité de l’os néoformé n’a été visible dans les cas opérés en une étape ou deux. L’épississement sinusal effectué avec un substitut osseux phyco-génique s’est avéré être une méthode efficace avec peu d’invasion et un taux de survie important des implants [97.8%].

Zusammenfassung

Ziel: Das Ziel dieser histomorphometrischen Langzeitstudie war, die Nützlichkeit eines phyco-genen Knochenersatzstoffes im damit aufgebauten Sinus zu bestätigen. Man untersuchte die Einheilung des Transplantates, die Knochenremodellierung und den Abbau des Biomaterials.


Resultate: Das Transplantationsmaterial wurde schrittweise resorbiert und mit neugebildetem Knochen ersetzt. Zwischen dem sechsten und fünfzehnten Monat nach der Sinusbodenelevation wuchs einerseits der Prozentsatz von neu gebildetem Knochen linear an (von 15,5 ± 9,6% auf 40,8 ± 15,3%) und der Prozentsatz des Knochenersatzmaterials nahm andererseits linear ab (von 34,5 ± 8,6% auf 13 ± 9,6%). Nach 15 Monaten entsprach die Dichte der Knochenbalkchen im augmentierten Knochen einem spongiösen Knochen von guter Qualität; das Knochenersatzmaterial war aber in dieser Zeitspanne noch nicht vollständig resorbiert worden. Zudem fand man keine statistisch signifikanten Unterschiede zwischen der Knochensubstanz des neugebildeten Knochens bei einer einphasigen und bei einer zweiphasischen Sinusbodenelevation.

Zusammenfassung: Die mit Hilfe eines phyco-genen Knochenersatzstoffes durchgeführte Sinusbodenelevation erwies sich als effiziente Methode mit geringer Invasivität und hoher Überlebensrate für die Implantate [97.8%].

References


Simunek et al. Sinus lift with phyrogenic bone substitute