CLINICAL ORAL IMPLANTS RESEARCH

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The use of acellular dermal matrix membrane for vertical soft tissue augmentation during submerged implant placement: a case series

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Abstract

Objective: To evaluate the efficiency of acellular dermal matrix membrane to augment vertical peri-implant soft tissue thickness during submerged implant placement.

Material and methods: Forty acellular dermal matrix-derived allogenic membranes (AlloDerm, BioHorizons, Birmingham, AL, USA) and 42 laser-modified surface internal hex implants (BioHorizons Tapered Laser Lok, Birmingham, AL, USA) were placed in submerged approach in 40 patients (15 males and 25 females, mean age 42.5 ± 1.7) with a thin vertical soft tissue thickness of 2 mm or less. After 3 months, healing abutments were connected to implants, and the augmented soft tissue thickness was measured with periodontal probe. The gain in vertical soft tissue volume was calculated. Mann–Whitney *U*-test was applied and significance was set to 0.05.

Results: All 40 allografts healed successfully. Thin soft tissue before augmentation had an average thickness of 1.54 ± 0.51 mm SD (range, 0.5–2.0 mm, median 1.75 mm), and after soft tissue augmentation with acellular dermal matrix, thickness increased to 3.75 ± 0.54 mm SD (range, 3.0–5.0 mm, median 4.0 mm) at 3 months after placement. This difference between medians was found to be statistically significant ($P < 0.001$). Mean increase in soft tissue thickness was 2.21 ± 0.85 mm SD (range, 1.0–4.5 mm, median 2.0 mm).

Conclusions: It can be concluded that acellular dermal matrix membrane can be successfully used for vertical soft tissue augmentation.

Soft tissue augmentation is very widely used procedure in many fields of implant dentistry. It was shown to be effective in developing the adequate width of attached tissues or increase of soft tissue volume due to esthetic reasons. Recently, it was shown that soft tissue graft might reduce bone remodeling at implants installed immediately after tooth extraction (Caneva et al. 2013). Autogenous connective tissue grafts from palatine for a long time have been a standard grafting material with very successful outcome (Dordick et al. 1976; Studer et al. 2000; Orsini et al. 2004; Sanz et al. 2009). However, some studies show obvious disadvantages of this approach. Harvesting procedure results in prolonged healing time at the donor site and therefore to an increased patient's morbidity (Griffin et al. 2006). Ongoing pain and numbness for several weeks after the surgery is frequently indicated by patient (Del et al. 2002). In addition, in some patients, anatomical limitations preclude harvesting of appropriate quality and quantity grafts (Soileau & Brannon 2006). These issues led to the use of alternative grafting techniques with allogenic materials, which also have shown to be successful (Dordick et al. 1976; Gapski et al. 2005; Wilson et al. 2005; Lorenzo et al. 2012). Therefore, one of the most widely used and researched resources from the family of allografts is acellular dermal matrix (ADM) derivative membrane. This material is donated from human skin without epidermis and cells, and serves as a matrix that supports revascularization, cell repopulation, and tissue remodeling. ADM may be used for soft tissue augmentation if a root coverage procedure, an enlargement of keratinized tissue, deepening of the vestibule, or augmentation of localized alveolar defects is indicated (Wei et al. 2000; Aichelmann-Reidy et al. 2001; Batista et al. 2001; Harris 2003). However, the use of ADM membrane for vertical peri-implant tissue augmentation has not been researched comprehensively. Usually, buccal

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aspect of soft tissue fell in the scope of interest of many authors, while vertical soft tissue thickness did not received much attention. Vertical soft tissue augmentation might be important for crestal bone preservation, as Linkevicius et al. have proved that thin soft tissue might be responsible for crestal bone remodeling, during formation of biological width around implants (Linkevicius et al. 2009a,b, 2010). In addition, it was shown that thick peri-implant soft tissue biotype may have a positive effect on the esthetic outcome of the implant treatment (Kan et al. 2003, 2010). Thus, the aims of these clinical case series were (1) to evaluate the performance of ADM derivative graft in vertical soft tissue augmentation during submerged implant placement and (2) to measure the increase of vertical soft tissue thickness after augmentation.

Material and methods

Patients

Partially edentulous patients, requiring implant treatment, were recruited for this study in Vilnius Implantology Center Clinic, Vilnius, Lithuania. The protocol of this study was approved by Vilnius regional ethical committee for biomedical trials, Vilnius, Lithuania (No.158200-07-512-149). Patients were enrolled if they fulfilled local inclusion criteria: thin mucosal tissues (2 mm or less), covering edentulous alveolar ridge, missing teeth in lower jaw posterior area, minimum of 6 mm bone width and 8 in height, healthy soft tissue (BOP < 20%, PI < 25% CPITN < 2), minimum of 4 mm keratinized soft tissues buccolingually, no bone augmentation procedures before and during implant placement, and finally, successful healing of the ADM membrane. Patients were excluded if they were less than 18 years of age, had medical contraindication for implant surgery, had history of periodontitis, were smokers, reported uncontrolled diabetes and/or alcoholism, and were taking medicine, influencing tissue healing. Each patient received verbal and written instructions, and signed the informed consent form with permission to use obtained data for research purposes before participating in the study.

Soft tissue thickness measurement and augmentation

One hour prior to the surgery, patients received prophylactic dose of 1 g amoxicillin (Ospamox; Biochemie, Kiel, Germany) and continued to take 0.5 g of antibiotics three times daily for 1 week after the intervention. All surgical procedures were performed by the same surgeon (A.P.). Surgery was performed under local anesthesia of 4% articaine 40 ml solution with adrenaline (Ubistesin, 3M ESPE, Seefeld, Germany). An incision with scalpel No. 15 in the center of edentulous ridge was performed, leaving at least width 2 mm of keratinized tissues bucally. Next, a full-thickness buccal flap was raised, and vertical thickness of soft tissues was measured with 1.0 mm marked periodontal probe (UNC; Hu-Friedy, Chicago, IL, USA). The probe was positioned in an upright position to the bone crest in the center of the future implant placement. If vertical soft tissue thickness was 2 mm or less, the soft tissues were considered to be thin and patient was included into the study (Fig. 1). After the measurement, the place of probe touching the bone crest was gently marked with a pilot drill. Then, a full-thickness lingual flap was raised to completely expose the implantation site. Internal hexagon implants with laser-modified surface (BioHorizons Tapered Laser Lok, Birmingham, AL, USA) were placed according to recommendations of manufacturers, and cover caps were screwed to implants. ADM-derived allogenic membrane (AlloDerm, BioHorizons) was used for vertical soft tissue augmentation.

ADM membrane is described as an acellular dermal matrix derived from donated human skin with removed both the epidermis and the cells. It has two distinct surfaces: basement membrane side is rough and will not readily absorb blood; and dermal side, which is smooth and will absorb blood. Standard dimensions' (20 × 40 mm) membrane with thickness varying from 0.89 to 1.65 mm was rehydrated with sterile saline solution for 10 min. Then membrane was folded one time that the basal side would be inside the double-layered matrix, while the dermal side would be exterior to contact with soft tissues and bone. Membrane was individually shaped to fit the implantation site and positioned over the alveolar ridge. It was extended mesiodistally to neighboring teeth, buccaly −10 mm and lingually for 5 mm beyond the implant margin to completely close implantation site (Fig. 2). Coronal periosteal-releasing incisions were made; flaps were approximated and sutured without tension with 5/0 sutures (Assucryl; Assut Medical Sarl, Lausanne, Switzerland). Primary wound closure was always achieved (Fig. 3). Patients were instructed to rinse the operated site for 1 minute with 0.12% chlorhexidine digluconate solution (Perio-Aid; Dentaid, Barcelona, Spain) twice a day for 4 weeks. Patients were asked to refrain from chewing or brushing the surgical area for 4 weeks after surgery. Sutures were removed 10 days after surgery (Fig. 4). Examinations were made every 2 weeks for

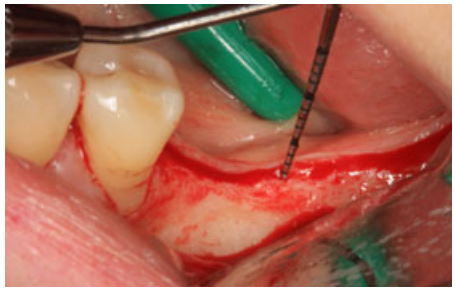

Fig. 1. Measurement of vertical thin soft tissues over the subsequent implant placement.

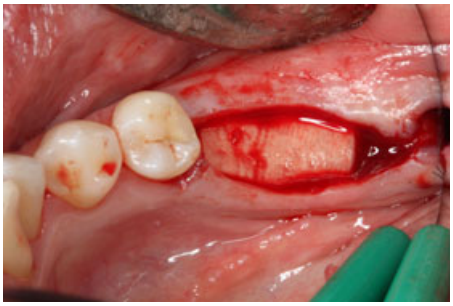

Fig. 2. Double-layer ADM membrane positioned on alveolar bone to completely close the implantation site.

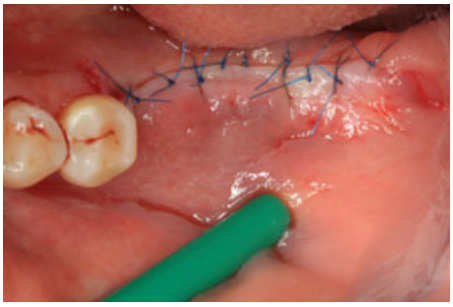

Fig. 3. Approximated and without tension sutured flaps, primary wound closure achieved.

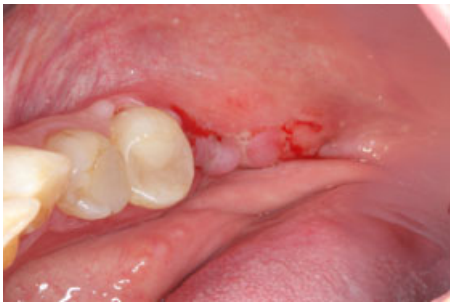

Fig. 4. Primary wound healing during suture removal.

3 months. After 3 months of healing final clinical examination, whether the membrane has reached the complete integration was performed (Fig. 5). The newly formed soft tissue was inspected for mobility. If there were no signs of inflammation and operated site appeared like healthy surrounding soft tissues, patient was scheduled for second-stage surgery. After infiltration of local anesthetic 4% articaine 40 ml solution with adrenaline (Ubistesin; 3M ESPE), an incision was made in the center of the bone crest. A full-thickness buccal flap was raised, and thickness of augmented soft tissues over the center of the implant was measured with a periodontal probe (Fig. 6). The probe was positioned in an upright position in the center of the implant healing screw. In case implant insertion angle was not straight, the probe was moved lingually to reach the lingual flap and measurement was made.

Then lingual flap was raised to completely expose the implant, and cover screw was removed. Healing abutment screw was covered with 0.12% chlorhexidine digluconate gel (Perio-Aid Gel; Dentaid) and connected to the implant. The excess of gel was carefully washed off with sterile solution. Flaps were approximated and sutured with single interrupted 5/0 sutures without tension (Assucryl; Assut Medical Sarl). No soft tissue excision during second-stage surgery was made. Patients were advised to minimize trauma to the site. The sutures were removed 7 days after surgery.

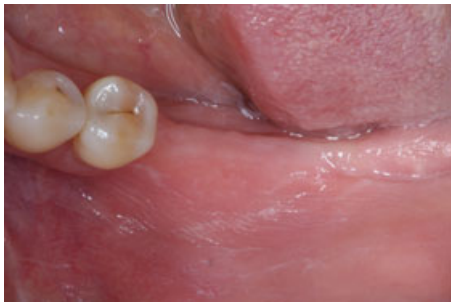

Fig. 5. Completely healed site without signs of inflammation. Operated site appeared like healthy surrounding soft.

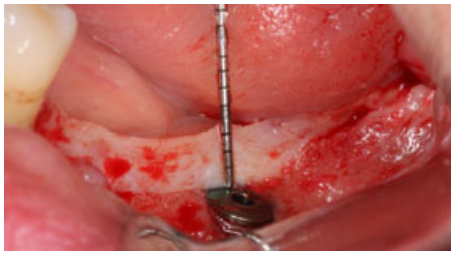

Fig. 6. Measurement of thickness of augmented soft tissues over the implant.

Statistical analysis

Data were analyzed using statistical software (SPSS 15.0 for Windows, Chicago, IL, USA). Descriptive statistics were calculated for the measurements as means, SEs, SDs, medians, and range of the measurements. Single patient was treated as a statistical unit. The normality of the distribution was tested and as variables appeared to be nonparametric, Mann–Whitney *U*-test was applied to find differences between thicknesses of thin soft tissues and augmented peri-implant mucosa. The median differences were considered statistically significant at $P \leq 0.05$ with a confidence interval of 95%.

Results

Forty patients, consisting of 15 males and 25 females with an average age equal to 42.5 ± 1.7, ranging from 21 to 53 years at the beginning of the experiment, were included into the study. Forty ADM membranes (AlloDerm, BioHorizons) were placed. All allografts survived: 39 healed uneventfully and 1 membrane showed an adverse reaction during initial stages of healing. The exposure of the membrane was diagnosed during suture removal. The uncovered part of the allograft was trimmed with surgical scissors; the site was irrigated with 0.12% chlorhexidine digluconate solution, and antibiotics intake was prolonged for an additional week. This patient was recalled every 3 days for inspection, till it was decided that there is no threat for the rejection of the graft. These measures led to normal wound healing in the secondary manner. At 3 months after placement, all grafts showed clinical signs of complete healing. The material had been completely covered by the surrounding epithelial and connective tissues, and the color and clinical characteristics of the tissues were compatible with a healthy condition. Augmented soft tissues were completely immobile. Thin soft tissue before augmentation had an average tissue thickness of 1.54 ± 0.51 mm SD (range, 0.5–2.0 mm, median 1.75 mm), and after soft tissue augmentation, thickness increased to 3.75 ± 0.54 mm SD (range, 3.0–5.0 mm, median 4.0 mm) (Table 1). This difference between medians was found to be statistically significant ($P < 0.001$) (Fig. 7).

Forty-two internal hex implants with laser-modified surface (BioHorizons Tapered Laser Lok) were placed simultaneously with soft tissue augmentation. Overall, the implant survival rate at the second-stage surgery was 100%.

Discussion

The results of these case series demonstrated that thin soft tissues could be successfully augmented in vertical direction with ADM membrane. The use of allograft simultaneously with implant placement in two-stage procedure resulted in statistically significant increase of peri-implant soft tissue height, measured during connection of the healing abutments. This procedure allowed the transformation of thin soft tissues with a mean thickness of 1.54 mm to thick soft tissues with 3.75 mm in thickness on average. The mean increase in vertical thickness was recorded to be 2.21 mm, which is very substantial and statistically significant. This is the first clinical observation to show the possibilities of ADM membranes in vertical augmentation of thin mucosa. However, it must be noted that it was not possible to have equal thickness of all used membranes, because each membrane is produced in thickness, varying from 0.89 to 1.65 mm. Therefore, every graft was folded once to be double-layered and to have thickness of 2–3 mm. The membrane was positioned in the way that dermal side would be in direct contact with the host tissues. It is believed that direct contact ensures quick infiltration of the graft with the blood and promotes revascularization and subsequent healing.

Similar study was conducted by Wiesner et al. (2010), which reported enlargement of soft tissues by 1.2 mm with connective tissue grafts for augmentation. Autologous grafts were taken from the palate and adapted to completely close the implantation site, as in the present study. It means that connective tissue grafts were placed on top of the

Table 1. **Soft tissue thickness before and after vertical augmentation with 40 ADM membranes**

Soft tissue thickness	Mean ± SE	±SD	Median	Min	Max
Thin tissues	1.54 ± 0.08	±0.51	1.75	0.5	2.0
Augmented soft tissues	3.75 ± 0.09	±0.54	4.0	3.0	5.0
Volume increase	2.21 ± 0.14*	±0.85	2.0	1.0	4.5

*Statistical significant difference (Mann–Whitney *U*-test, $P < 0.05$).

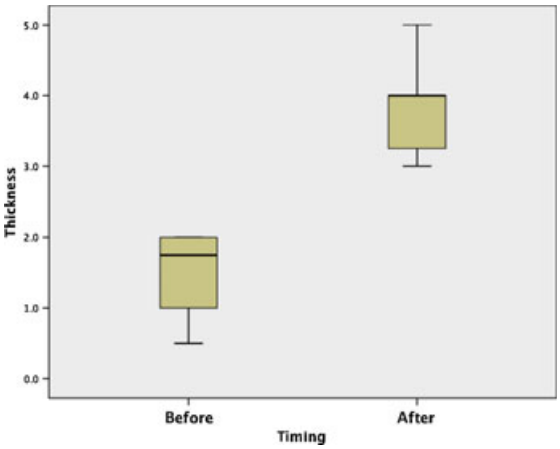

Fig. 7. Statistically significant difference between thin and augmented soft peri-implant tissues in box plot (Mann–Whitney *U*-test, $P < 0.001$).

implant and that could give vertical augmentation as well. However, the authors did not measure the vertical enlargement of peri-implant soft tissues, but examined buccal and lingual sites, which would represent soft tissue enlargement horizontally. Likewise, Caneva et al. (2013) have reported significant increase of peri-implant mucosa thickness after augmentation of vestibular implant site with connective tissue grafts in dog model. It seems that it is possible to augment peri-implant mucosa with autologous and allogenic grafts in several directions. Vertical soft tissue thickness is rather a new aspect of peri-implant mucosa to be addressed in the contemporary research. Usually, vestibular aspect of soft tissues around implants is the object of many studies. This is understandable, as the buccal site is the key to esthetics in implant dentistry. Similarly, keratinized soft tissues around implants are particularly well investigated due to their importance for long-term performance of dental implants (Wennstrom et al. 1994; Wennstrom & Derks 2012; Bengazi et al. 2013; Boynuegri et al. 2013; Lin et al. 2013). It must be stressed that vertical soft tissue thickness is a separate parameter, not to be confused with buccal tissue thickness or width of keratinized mucosa. The gain of volume in vertical direction of peri-implant soft tissues might be useful for several reasons. Thick peri-implant tissues allow formation of biological width without resorption of the crestal bone. It was demonstrated by Lindhe and Berglundh (Berglundh & Lindhe 1996) in an animal study and later confirmed by Linkevicius et al. clinical trials that thin soft tissues may predispose significant crestal bone remodeling, while naturally thick mucosa does not induce bone loss (Linkevicius et al. 2009a,b, 2010). From the other hand, the possibility that the observed increase in mucosal thickness is due to marginal peri-implant bone resorption cannot be excluded, although crestal bone level was not the scope of this study. In contrary, the data from recent study showed that thickening of thin mucosal tissues with allogenic membrane reduced crestal bone remodeling from 1.65 mm to 0.31 mm around regular connection implants (Linkevicius et al. 2013). In addition, the formation of the biological width in submerged implants starts after connection of the healing abutments; thus, crestal bone loss due to thin mucosal tissues usually cannot be present during the second-stage surgery. Clinical inspection of the augmented areas also showed that increase in soft tissue thickness was visible not only over the implant, but also in the edentulous alveolar ridge as well (Fig. 6). Therefore, it is not likely that the gain in vertical soft tissue height could result from crestal bone remodeling.

Another advantage of thin soft tissue thickening is that thick mucosa allows the formation of better restoration emergence profile, resulting in more esthetic and stable outcome (Belser et al. 1998). Historically, ADM has been successfully used to increase keratinized tissue, for a root coverage procedure, to deepen the vestibular fornix, and to augment localized alveolar defects (Wei et al. 2000; Aichelmann-Reidy et al. 2001; Batista et al. 2001; Harris 2003). However, results of this study should encourage clinicians to use this membrane for augmentation of vertical soft tissue volume as well. Several authors have studied the process of healing of ADM membranes. It was shown that after placement, there is blood infiltration of the graft through vascular channels, bringing host cells that adhere to proteins in the matrix. The host cells respond to the local environment, and the matrix is remodeled into the patient's own tissue. It has been documented that ADM is equivalent to autogenous connective tissue grafts in root coverage procedures (Gapski et al. 2005). (Luczyszyn et al. 2005) have shown that after 12 weeks, the ADM and the connective tissue seemed to be well integrated into a single highly vascularized structure, indicating almost complete incorporation of the ADM in an animal study. Furthermore, clinical trial indicates that after 3 months of healing, the acellular dermal alloplastic graft material displayed clinical and histologic features that were similar to the patient's own palatal donor material (Silverstein et al. 1999). Thus, the decision to make re-entry for healing abutment connection after 3 months of healing is justifiable. After 6 months of healing, histologic outcome shows similarity between connective tissue and ADM membranes (Cummings et al. 2005).

There are reports in the literature, which advocate one-stage approach, when soft tissue augmentation is performed simultaneously with implant placement (Caneva et al. 2013). However, this knowledge comes from animal studies and with an autologous connective tissue as a grafting material, which is considered a "gold standard" substance so far. Therefore, to reduce the possibility of complications, as during one-stage procedure grafting material might be more prone to infection and exposure, two-stage approach was selected in this study. In addition, the direct measurement of increased soft tissue thickness in exactly the same way, as it was done before soft tissue augmentation, was possible only during healing abutment connection procedure. Despite complete coverage of the membrane by mucoperiosteal flaps and flap approximation without tension after suturing, one case of prolonged and complicated healing of the membrane was registered. Although taken necessary measures ensured successful healing of the infected allograft, it might be still suggested to use more conservative two-stage approach in surgery with augmentation of the peri-implant mucosa. It is important to note that full-thickness mucoperiosteal flaps were raised during soft tissue augmentation, and double-layered membrane was positioned on denuded bone in the present study. There is evidence in the literature that a full-thickness flap might induce additional bone loss; hence, a split partial thickness flaps are advocated, when periosteum is not increased from

the bone (Fickl et al. 2008, 2011). However, results of the present clinical study show that placement of allograft on denuded bone does not cause any adverse reaction of the body during and after healing period. Furthermore, Wiesner et al. (2010) used partial thickness flap during soft tissue augmentation with connective autologous grafts; however, they reported 0.2 mm crestal bone loss more around test implants, compared to control sites with no augmentation. This shows that there might be more factors, which influence crestal bone loss more, than mucoperiosteal flap design.

One of the study limitations could be the precision of thickness measurements performed with the periodontal probe. The direct measurement of soft tissue thickness with periodontal probe was shown to be as reliable as ultrasonic or radiographic methods (Lawson & Jones 1998). In addition, using of probe is considered to be golden standard of measurement with its accepted accuracy in periodontology; therefore, there are no reasons why it should not be used for measurement of mucosal tissue thickness in a described method. It was shown that reliability of probing around teeth maybe influenced by the probing angle, the emergence profile of the tooth or restoration, the extent of inflammation on the tissues, probing force, etc. (Nyman & Lindhe 2003). These factors clearly were not involved in the measurement of soft tissue thickness before implant placement in the present study. In addition, a standardized calibrated probe was used for the direct measurements; therefore, the precision of the measurement with periodontal probe could be considered appropriate.

The measurement was performed one time; thus, reproducibility of the measurement was limited. However, this is due peculiarities of the study, as tissue measurements were performed during surgeries; therefore, it was not possible to make another measurement after some period of time, as it is usual in other studies. Another concern could be the standardization of the axis of the probe during the repeated measurement. The probe was always placed in upright position to the bone (first measurement during implant placement) and to the implant (second measurement during second-stage surgery); however, in some cases, this was not possible due to implant insertion axis. Nevertheless, the human factor remains in all clinical measurements. In addition, it is not clear whether the increase of soft tissue thickness would prevail after longer period of time. Further studies are needed to clarify the long-term performance of this material and technique on crestal bone stability around implants.

Conclusion

Within the limitations of this study, it can be concluded that ADM membrane can be successfully used for vertical soft tissue augmentation simultaneously with submerged implant placement. An average gain of 2.34 mm can be expected, depending on initial mucosa thickness. Good clinical integration of the material and complete resemblance to the surrounding healthy mucosal tissues can be expected as early as 3 months postoperatively.

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