Received: 19 June 2021 | Revised: 4 October 2021 | Accepted: 2 February 2022

DOI: 10.1111/clr.13919

CLINICAL ORAL IMPLANTS RESEARCH WILEY

ORIGINAL ARTICLE

Clinical and histological evaluation of the use of acellular dermal matrix (ADM) membrane in peri-implant vertical soft tissue augmentation: A controlled clinical trial

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Funding information
The present study was supported by the National Natural Science Foundation of China (No. 30320192260, 81991500, 81991502)

Abstract

Objectives: The aim of this study was to clinically and histologically evaluate the efficacy of using acellular dermal matrix (ADM) for peri-implant vertical soft tissue augmentation at implant placement.

Materials and methods: Twenty patients were enrolled in this study. According to the initial thickness of vertical soft tissue, patients were assigned into the ADM group (≤2 mm) or the control group (>2 mm) prior to implant surgery +ADM grafting or implant surgery alone. Second-stage surgery was carried out 3 months later, and a small piece of ridge membrane was harvested for histological and immunohistochemical evaluation. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)-BB in peri-implant crevicular fluid (PICF) were also assessed 1 week, 1 month, and 5 months after second-stage surgery. Clinical parameters were recorded to evaluate peri-implant health at 1 week and 3 months after implant restoration.

Results: All 20 implants healed uneventfully and successfully. Soft tissue thicknesses were comparable in the two groups at second-stage surgery (3.20 ± 0.42 mm vs. 3.50 ± 0.58 mm). In the ADM group, the mean increase in soft tissue thickness was 1.85 ± 0.34 mm. Histological and immunohistochemical outcomes showed no differences between the two groups. VEGF and PDGF-BB levels in PICF were significantly lower in the ADM group 1 week after second-stage surgery ($p < .01$), yet they decreased in both groups later. The difference between the groups had disappeared by 5 months after second-stage surgery. The clinical peri-implant parameters were good and stable by the end of the study (3 months after restoration).

Conclusions: Our results suggested that using ADM at implant placement was effective in increasing the thickness of peri-implant vertical soft tissue and achieved comparable clinical and histological performance to the control group. However, the incremental soft tissue showed inferior angiogenic ability in the early stage of wound healing.

Jing Zang, and Li Su contributed equally to this work

Clinical Trial Registration; This study was registered at Chinese Clinical Trial Registry under code ChiCTR2000039769

1 | INTRODUCTION

Nowadays, dental implants offer a reliable therapeutic option for tooth replacement therapy. However, along with the widespread application of dental implants, the risk of peri-implant disease has emerged. Tantamount to the well-studied significance of peri-implant bone volume, the significance of the thickness of soft tissues around dental implants in protecting peri-implant health has also been under discussion in recent decades (Greenwell et al., 2005; Zigdon and Machtei, 2008; Giannobile et al., 2018).

Berglundh and Lindhe (1996) published a classic animal study and proposed that soft tissue around dental implants requires a certain thickness to form biological structures similar to the natural biological width around teeth. A prospective controlled study (Linkevicius et al., 2009) reported that thick alveolar mucosa (>2 mm) reduced the amount of peri-implant bone loss in the first year after loading.

The autologous subepithelial connective tissue graft (CTG) is generally accepted as the gold standard for soft tissue augmentation around both natural teeth and dental implants (Chambrone & Tatakis, 2015). However, this kind of procedure is always associated with complications such as hemorrhage, postoperative pain, and trauma at the donor site, while only a limited amount of tissue can be harvested (Caneva et al., 2013; Wiesner et al., 2010). Therefore, alternative strategies using allogenic and xenogenic materials have gradually been applied in clinical treatment and have been shown to be successful in improving gingival deformities around natural teeth (Haghighati et al., 2009; Paolantonio et al., 2002). Among them, acellular dermal matrix (ADM) is one of the most widely used and researched allograft materials harvested from human dermis and serves as a matrix that supports revascularization, cell repopulation, and tissue remodeling. The benefits of ADM include an unlimited tissue supply and negating the need for a second palatal surgical site. The theory behind the use of ADM for soft tissue augmentation is based on the nature of the extracellular matrix that supports cellular migration and revascularization from the surrounding host tissues. It has been suggested that ADM could mimic the native tissue microenvironment and create a stable structure (Boháč et al., 2018; Tavelli et al., 2019,2020). Moslemi et al. (2011) reported the results of a randomized clinical trial showing that the use of ADM produced similar outcomes in terms of root coverage and reduction in recession depth and recession width compared with CTG. Up to now, ADM has been widely and successfully used in improving natural soft tissue around teeth (Rahmani et al., 2006; Barros et al., 2015).

Unlike applications around natural teeth, vertical soft tissue augmentation in implant sites provides not only the necessary spatial distance to keep substantial pathogens from the coronal platform of the implant, but also the potential variation in biological sealing of the implant soft tissue cuff. It has already been reported in previous studies that ADM grafting can result in solid but inconsistent increases in soft tissue thickness (Puisys et al., 2015; Verardi et al., 2020). In addition, histological analysis of the incremental soft tissue has been very limited, but this is also essential in evaluating the use of ADM in peri-implant soft tissue augmentation. Therefore, the aims of the present study were (1) to evaluate the clinical and histological performance of ADM when used for peri-implant vertical soft tissue augmentation at the time of submerged implantation, as compared to the control group and (2) to further evaluate the healing process and the clinical parameters of the peri-implant soft tissue cuff generated from this augmented tissue.

2 | MATERIALS AND METHODS

2.1 | Study population

Partially edentulous patients seeking implant restoration were recruited in the Department of Periodontology, Peking University School and Hospital of Stomatology. This was a controlled clinical study, involving two groups of patients. Subjects were assigned to the ADM group if their edentulous mucosal tissue was thin (2 mm or less). Otherwise, they were assigned to the control group.

This clinical study was approved by the Institutional Review Board of Peking University School and Hospital of Stomatology (No. PKUSSIRB-202057114) and was in accordance with the CONSORT guidelines (Supplementary material). This study was registered at the Chinese Clinical Trial Registry under code ChiCTR2000039769. All the included patients had provided signed informed consent. The inclusion and exclusion criteria were as follows:

2.1.1 | Inclusion criteria

1. One single-tooth implant was scheduled in the posterior region.
2. At least 4 mm keratinized mucosa was present buccolingually.
3. No bone augmentation procedures had been performed before or during implant placement.
4. Non-smoker.

2.1.2 | Exclusion criteria

1. Uncontrolled periodontal disease.
2. Systemic diseases that could affect wound healing.
3. Pregnancy.

2.2 | Outcomes of interest

Primary outcomes: (a) changes in vertical soft tissue thickness between implant placement and second-stage surgery; (b) descriptive histological analyses and analysis of the percentage of VEGF-positive cells and micro-vessel density (MVD) in soft tissue samples.

Secondary outcomes: (a) total amounts/concentrations of VEGF and PDGF-BB in peri-implant crevicular fluid (PICF) at different time points after second-stage surgery; (b) clinical parameters after restoration including modified plaque index (mPII), modified bleeding index (mBI) (Mombelli et al., 1987), and probing pocket depth (PPD) (Glavind & Löe, 1967).

2.3 | Clinical procedures and data collection

2.3.1 | Three-dimensional (3D)-printed surgical guide

For patients recruited in the study, a cone-beam computed tomography (CBCT) scan was obtained and impressions of both jaws were taken using silicone impression material (Silagum, Hamburg, Germany) to fabricate stone cast models. The models were scanned using a laboratory scanner (Activity 880, Smartoptics, Oslo, Norway) creating STL files. The STL file of the model and the DICOM files from the CBCT were superimposed, and the surgical guide was designed using BlueSky Plan 4 (BlueSkyPlan.com, Libertyville, IL, USA). Surgical guides were printed with surgical guide resin (Med 610, Stratasys, Rehovot, Israel) using a 3D printer (Objet30 Prime, Stratasys) from the exported STL files (Figure 1a).

2.3.2 | Soft tissue thickness measurement and implant surgery procedure

All patients received a two-stage implant surgical approach by the same periodontist (YXQ). Implant surgery was performed under local anesthesia, a crestal incision with scalpel No. 12D was created under the guidance of a surgical guide (Figure 1b). Next, a full-thickness buccal flap was carefully elevated, and the vertical thickness of soft tissues was measured with a UNC-15 periodontal probe (Hu-Friedy) (Verardi et al., 2020). If the thickness was 2 mm or less, the patient was assigned to the ADM group and received an ADM graft for soft tissue augmentation during implant surgery. Otherwise, the patient would be assigned to the control group and receive implant surgery alone.

After measurement of the soft tissue thickness, a full-thickness lingual flap was raised to completely expose the implantation site. With the help of a surgical guide, Bicon implants (⌀ 4.5/5.0*6/8 mm, Bicon Dental Implants, Boston, MA, USA) were inserted with submerged healing following the manufacturer's instructions (Figure 1c). Before suturing the flap, soft tissue augmentation was performed in the ADM group. ADM membrane (RENOV, Beijing, China) was hydrated in warm saline solution (Figure 1d) and shaped to fit over the implanted area and below the flap (Figure 1e). Finally, flaps were sutured with 5/0 sutures, and primary closure was achieved in both groups (Figure 1f). Patients were instructed to keep to a soft-food diet postoperatively and to rinse the operated site with 0.12% chlorhexidine twice daily for 2 weeks. Patients were advised to take 250 mg of amoxicillin three times per day for 1 week, 300 mg of Ibuprofen twice daily for 3 days and then as needed. Sutures were removed 14 days after surgery.

2.3.3 | Second-stage surgery and soft tissue biopsies

Three months after implant surgery, patients were scheduled for second-stage surgery if there were no signs of inflammation and the operated site appeared healthy like the surrounding soft tissues. After local anesthesia, a surgical guide was used to

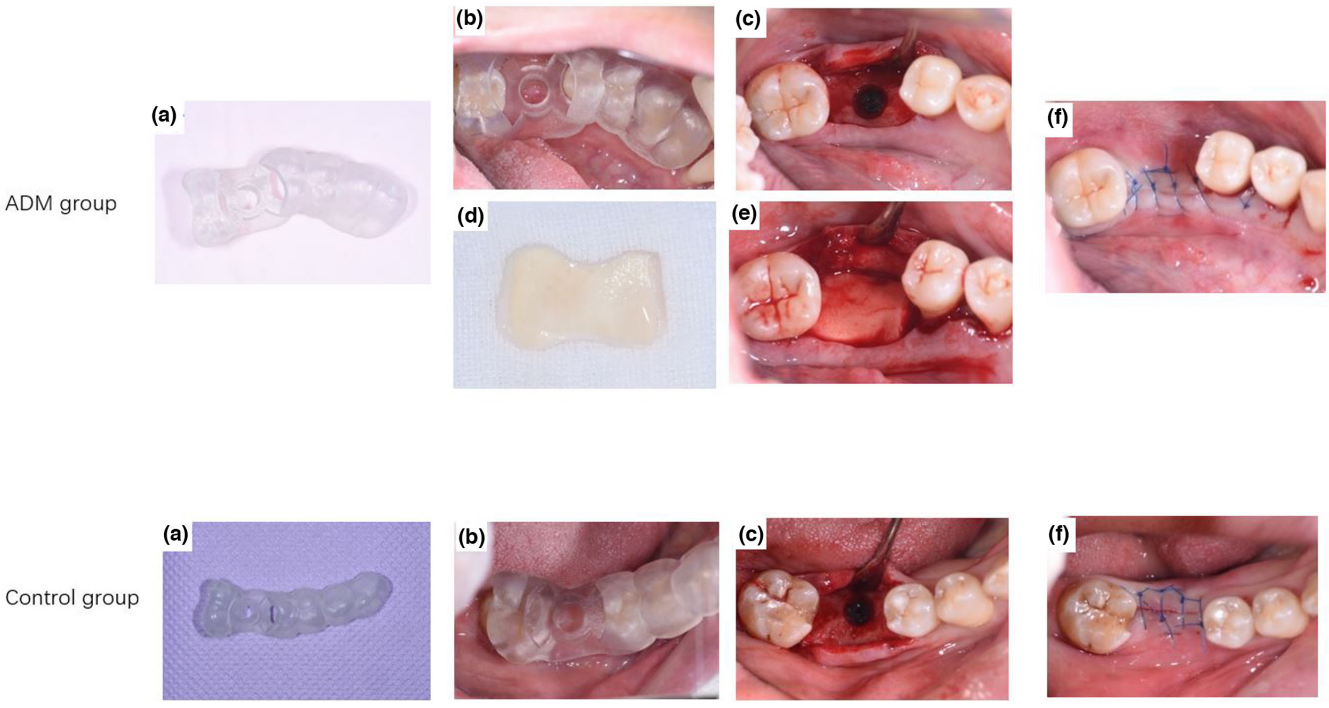

FIGURE 1 Photographic sequence of a representative clinical case for both the ADM and control group (a) Surgical guide. (b) Implant location was determined using surgical guide. (c) Healing plug was positioned over the implant. (d) ADM membrane was hydrated in warm saline solution. (e) Adapted ADM over the implant area. (f) Sutured tightly

determine the location of the implant. Soft tissue from the coronal portion of the implant (mesial–distal length: 5 mm; buccal–lingual length: 2 mm) was harvested without a flap with the healing plug exposed completely (Figure 2a,b). The vertical soft tissue thickness was then measured again using a UNC-15 periodontal probe (Hu-Friedy). After measurement, healing plugs were replaced by healing abutments (5*6.5 mm, Bicon) without the need for a suture (Figure 2c).

2.3.4 | Restoration process and clinical measurements

One month after second-stage surgery, patients were directed to prosthetic rehabilitation. All patients received a cement-retained Zirconia crown with the margin aligned to or above the gingival margin (Figure 2d). The crown and the abutment were cemented extraorally in order to guarantee the adhesive was completely cleared. Peri-implant clinical parameters were recorded 1 week and 3 months after implant restoration. mPII and mBI were assessed at buccal and lingual sites. PPD was assessed at four sites (mesial, buccal, distal, and lingual) using a PCPUNC15 probe (Hu-Friedy). Mean values of all sites were calculated for statistical analysis. An independent blinded, and calibrated examiner completed all the clinical measurements and was not informed about the aim of the study. The intraclass correlation coefficient (ICC) was 0.91, demonstrating high intra-examiner reliability.

2.4 | Histological & immunohistochemical evaluation and PICF analyses

All specimens were fixed in 10% neutral buffered formalin solution for further analyses. Following dehydration, specimens were embedded in paraffin and cut into 3 μm thick serial sections. One of the sections was stained with hematoxylin and eosin (H&E). A further two sections were used for immunohistochemical staining of VEGF and CD34, as follows. The sections were incubated overnight at 4°C with the monoclonal antibodies anti-VEGF and anti-CD34 (Servicebio technology Co., Wuhan, China). Scanned images of all slides were analyzed using the software Case Viewer2.4.0 (3DHISTECH Ltd., Budapest, Hungary). The number of VEGF-positive cells among at least 200 cells was counted in a central region at ×20 magnification; the values obtained were expressed as percentages of the total cells counted. For evaluation of MVD, all the morphologic structures with a lumen surrounded by CD34-positive endothelial cells were considered to be blood micro-vessels. MVD was determined by counting all the vessels at ×20 magnification within an examination area of 0.5 mm^2. Values are expressed as the number of vessels per mm^2. The immunoreactivity of the antibodies was evaluated by a blinded and calibrated observer who was not informed about the aim of the study. The ICC was 0.96, demonstrating high intra-examiner reliability. Counting was repeated three times, and for statistical analysis, the mean value obtained from the repeated counts was used.

PICF samples were gathered at three follow-up time points: 1 week after second-stage surgery, 1 month after second-stage surgery, and 5 months after second-stage surgery (3 months after restoration). The tip of a sterile paper point (No.30) was cut-off 0.5–1 cm before sampling. Samples were collected from buccal aspects of the healing abutment/crown after removing all supragingival plaque. The sample site was gently air-dried, and the area was carefully isolated with cotton rolls in order to protect samples from contamination. Paper points were inserted into the sulcus until slight resistance was felt then left in place for 30 s. Paper points contaminated with blood were discarded. PICF volumes were determined as described previously (Kuru et al., 2004). Paper points were placed into Eppendorf tubes and stored at −80°C until processing.

VEGF and PDGF-BB levels in PICF were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Meimian Industrial Co., Ltd.,) in line with the manufacturer's guidelines. The ELISA plates were assessed spectrophotometrically at 450 nm. Cytokine concentrations were calculated from the standard curve. Total amounts were calculated by multiplying concentrations and PICF volumes (Wei et al., 2004). PICF results are expressed as both total amounts and concentrations.

2.5 | Statistical analysis

To calculate the sample size for this study, data from a previous study were used (Puisys et al., 2015). It was reported that 3 months after using ADM, the mean increase in peri-implant vertical soft tissue thickness was 2.21 ± 0.85 mm. These values were employed, assuming a normal distribution, to perform a sample size calculation using the software G*power. If the expected difference between the ADM group and the control group at 3 months is 1 mm and the standard deviation is 0.5, the subsequent effect size is 1.43; thus, a total of 10 patients per group would be required (80% power, $\alpha = .05$).

The following time points were extracted for the PICF sampling and/or clinical parameter measurements: 1 week after second-stage surgery (T0), 1 month after second-stage surgery (T1), 1 week after restoration (T2), and 5 months after second-stage surgery (3 months after restoration) (T3) (Figure 3).

Descriptive statistics were calculated for the measurements as means, SDs, medians, and range of the measurements. A single implant was treated as a statistical unit. The normality of the distribution of other clinical variables (soft tissue thickness, mPII, PPD) was tested to be nonparametric. The differences of the medians between the two groups were evaluated using the Mann–Whitney *U* test and within a group with the Wilcoxon signed rank test.

Differences in VEGF expression and MVD between the ADM and control group were assessed by two-sample t-test. ELISA variables were analyzed by repeated-measures ANOVA. All data were

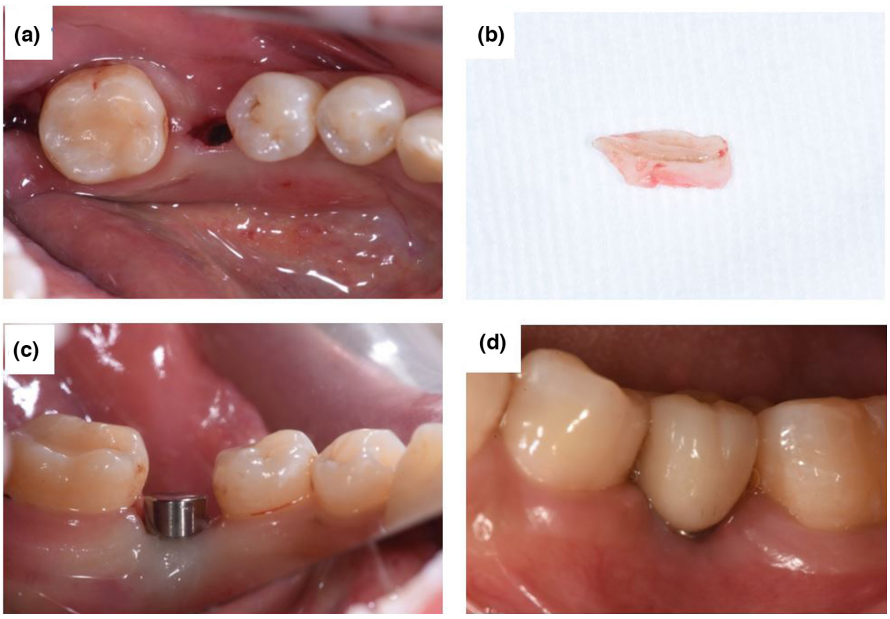

FIGURE 2 Second-stage surgery. (a) Healing plug was exposed. (b) Specimen harvested for histological examination. (c) Placed healing abutment(5*6.5 mm) without suture. (d) Implant restoration with crown margin above gingival margin

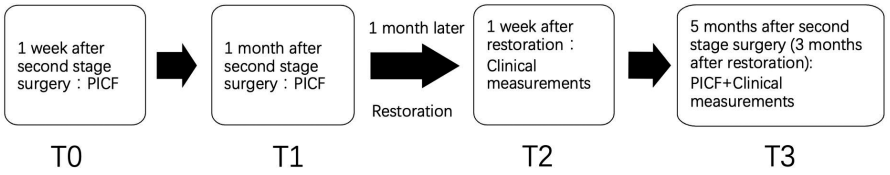

FIGURE 3 Different time points with corresponding procedures

TABLE 1 Measurements and changes of the soft tissue in the two groups before and after implant surgery

		ADM	Control	*p* Value
Before	Mean ± SD	1.35 ± 0.47	3.45 ± 0.64	<.01
	Median	1.50	3.25	
	Min, Max	0.5,2.0	2.5,4.5	
After	Mean ± SD	3.20 ± 0.42	3.50 ± 0.58	>.05
	Median	3.00	3.75	
	Min, Max	2.5,4.0	2.5,4.0	
Volume change	Mean ± SD	1.85 ± 0.34	0.05 ± 0.28	<.01
	Median	2.00	0.00	
	Min, Max	1.5,2.5	−0.5,0.5	

Abbreviations: ADM, ADM group; After, 3 months after implant surgery; Before, before implant surgery; Control, Control group; SD, standard deviation.

analyzed using statistical software (SPSS 13.0, SPSS Inc., Chicago, IL, USA). The significance level was defined as $\alpha = .05$.

3 | RESULTS

Twenty patients (ten patients per group), consisting of 12 males and 8 females with an average age of 41.9 ± 5.3, ranging from 26 to 48 years, were included in the study. All the participants completed the follow-up from implant placement surgery to T3. Slight pain and/or swelling were experienced by some patients in the ADM group. All twenty implants healed uneventfully without serious complications, and by the end of this study, the success rate of all the implants was 100%.

3.1 | Changes in vertical soft tissue thickness

Changes in soft tissue thickness are displayed in Table 1. The ADM group before augmentation had an average tissue thickness of 1.35 ± 0.47 mm (range, 0.5–2.0 mm, median 1.50 mm), and after soft tissue augmentation, thickness increased to 3.20 ± 0.42 mm (range, 2.5–4.0 mm, median 3 mm). The control group before implant surgery had an average tissue thickness of 3.45 ± 0.64 (range,

2.5–4.5 mm, median 3.25 mm), and during second-stage surgery, tissue thickness was 3.50 ± 0.58 (range, 2.5–4, median 3.75 mm). Thus, the thickness of soft tissue changed 1.85 ± 0.34 mm (range 1.5–2.5 mm, median 2.0 mm) and 0.05 ± 0.28 mm (range −0.5–0.5 mm, median 0.0 mm) in the ADM group and the control group, respectively ($p < .05$).

3.2 | Histological and immunohistochemical outcomes

In the ADM group, descriptive histological analysis showed organized epithelial structures and collagen fibers with rare inflammatory infiltrates 3 months after implant surgery. Samples were characterized by mature tissue and exhibited tissue organization. The results in the control group were comparable to those in the ADM group (Figure 4a,b).

Immunohistochemical staining revealed VEGF expression in all soft tissue samples at the cellular cytoplasmic level in epithelial and dermal cells (Figure 4c,d). In the ADM group, immunohistochemical analysis showed the proportion of VEGF-positive cells was 37.20 ± 3.09%, and in the control group, the percentage of VEGF-positive cells was 37.70 ± 3.79 (Table 2).

CD34 immunostaining was also observed in all samples (Figure 4e, f). MVD was 29.13 ± 4.34 and 32.73 ± 3.78 in the ADM group and the control group, respectively. The differences between the two groups were not statistically significant (Table 2).

3.3 | PICF substance level analysis and clinical measurements after restoration

The mean total amounts/concentrations of VEGF and PDGF-BB together with PICF volume are represented in Table 3. At T0, the total amounts of VEGF were 0.072 ± 0.004 pg and 0.137 ± 0.027 pg in the ADM group and the control group, respectively. PDGF-BB concentrations were 60.07 ± 1.47 pg/mL in the ADM group and 68.13 ± 1.72 pg/mL in the control group; PDGF-BB total amounts were 0.055 ± 0.004 pg and 0.114 ± 0.022 pg, respectively. ANOVA indicated that VEGF total amounts, and PDGF-BB total amounts/concentrations in the ADM group were significantly lower than those in the control group at T0. At T1, PDGF-BB total amounts were significantly lower in the ADM group. There were no significant differences between the two groups at T3 (Figure 5b,d). Compared with T1 and T3, VEGF total amounts/concentrations and PDGF-BB total amounts/concentrations at T0

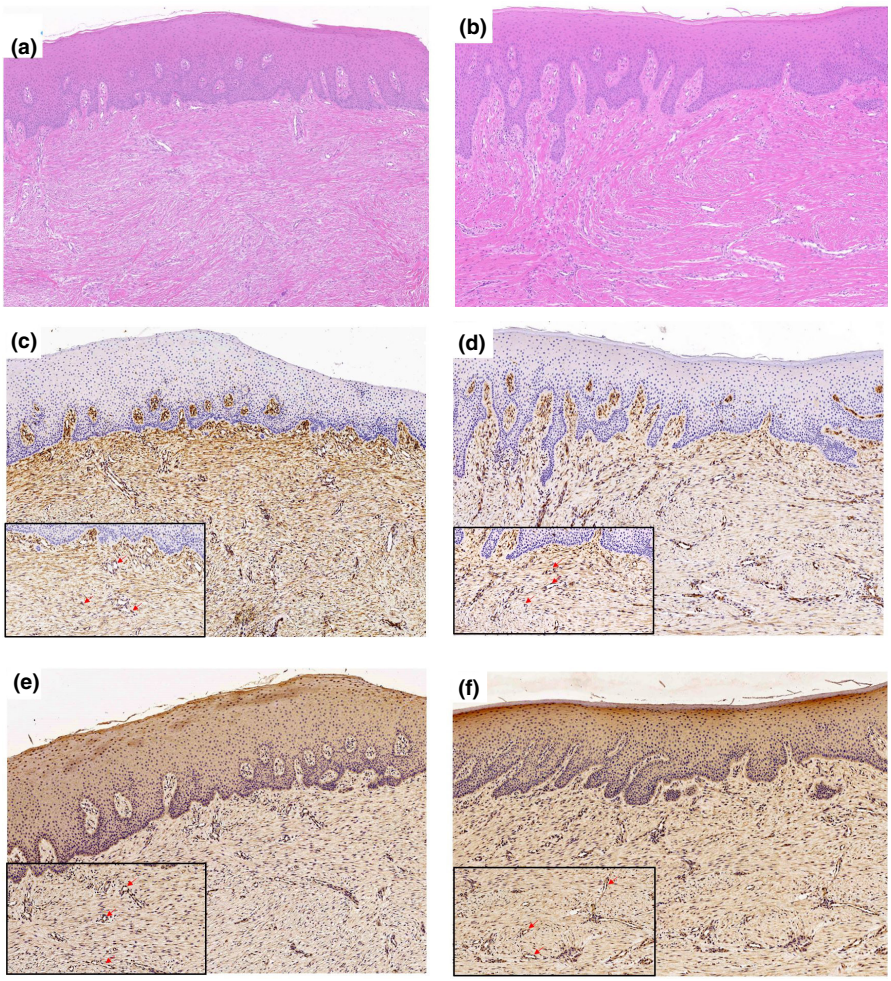

FIGURE 4 Hematoxylin and eosin staining (×5): (a) ADM group. (b) Control group. Both groups presented tissue organization. Immunohistochemical detection of VEGF (×5; insert,×20): (c) ADM group (d) Control group. Arrows indicate the VEGF-positive cells. Immunohistochemical detection of CD34 (×5; insert,×20): (e) ADM group (f) Control group. Arrows indicate the micro vessels

TABLE 2 Quantitative analysis of VEGF and MVD with immunohistochemical detection in the two groups (Mean ± SD)

Parameters	ADM group	Control group	*p* value
VEGF (%)	37.20 ± 3.09	37.70 ± 3.79	0.63
MVD (N/mm^2)	29.13 ± 4.34	32.73 ± 3.78	0.61

Abbreviations: N/mm^2, numbers of vessels per mm^2.

were significant higher and showed a time-dependent decreasing trend (Figure 5a,c). The PICF volumes in the two groups were also significantly higher at T0.

Peri-implant soft tissues were healthy at T2 and at T3. Inter-and intragroup differences were not statistically significant for any of the clinical measurements (mPII, mBI, and PPD) after restoration (Table 4).

TABLE 3 ANOVA results of VEGF and PDGF-BB within PICF in the two groups (Mean ± SD)

Parameters	Groups	T0	T1	T3
VEGF (pg/ml)	ADM (*n* = 10)	78.67 ± 3.77 **Aa**	63.49 ± 4.89 **Ba**	62.46 ± 4.55 **Ba**
	Control (*n* = 10)	82.34 ± 3.78 **Aa**	62.71 ± 5.27 **Ba**	63.00 ± 5.28 **Ba**
VEGF (pg)	ADM (*n* = 10)	0.072 ± 0.004 **Aa**	0.021 ± 0.003 **Ba**	0.014 ± 0.006 **Ca**
	Control (*n* = 10)	0.137 ± 0.027 **Ab**	0.027 ± 0.009 **Ba**	0.016 ± 0.006 **Ca**
PDGF-BB (pg/ml)	ADM (*n* = 10)	60.07 ± 1.47 **Aa**	53.52 ± 2.46 **Ba**	53.11 ± 3.03 **Ba**
	Control (*n* = 10)	68.13 ± 1.72**Ab**	53.60 ± 2.39**Ba**	53.48 ± 3.00 **Ba**
PDGF-BB (pg)	ADM (*n* = 10)	0.055 ± 0.004 **Aa**	0.018 ± 0.002 **Ba**	0.011 ± 0.004 **Ca**
	Control (*n* = 10)	0.114 ± 0.022**Ab**	0.022 ± 0.007 **Bb**	0.013 ± 0.005 **Ca**
PICF (μl)	ADM (*n* = 10)	0.92 ± 0.06 **Aa**	0.33 ± 0.04 **Ba**	0.22 ± 0.08 **Ca**
	Control (*n* = 10)	1.67 ± 0.31 **Ab**	0.42 ± 0.12 **Bb**	0.25 ± 0.09 **Ca**

Abbreviations: T0, 1 week after second-stage surgery; T1, 1 month after second-stage surgery; T3, 5 months after second-stage surgery.
Note: Horizontally, different capital letters indicate statistically significant differences among times of measurements according to the Bonferroni test at the 0.05 level.
Vertically, different small letters indicate statistically significant differences between the ADM group and the control group at the 0.05 level.

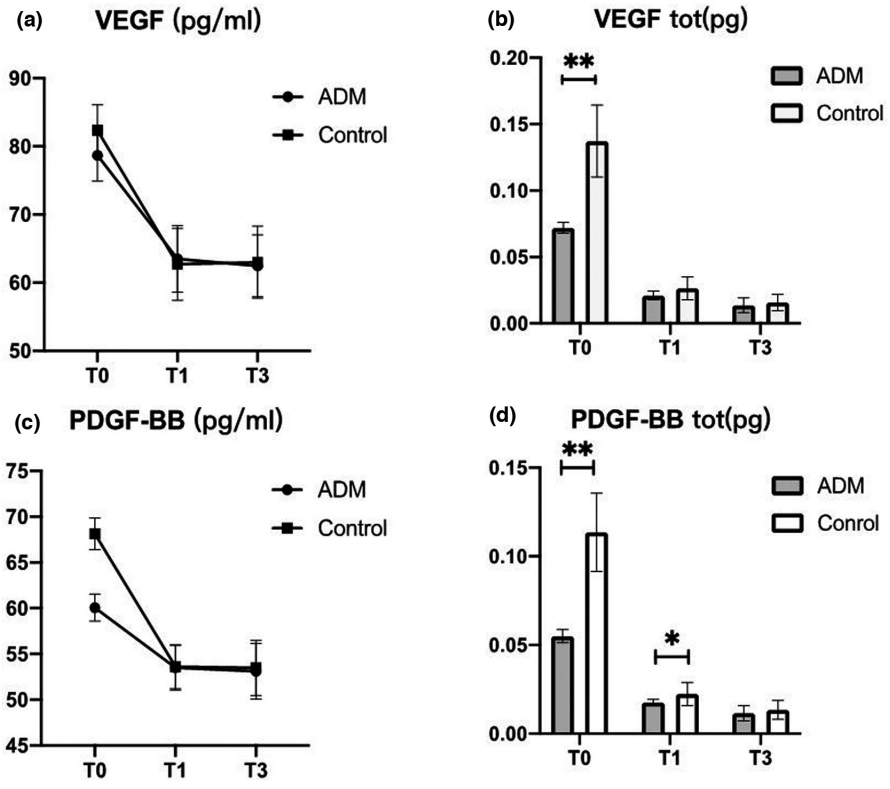

FIGURE 5 Levels of VEGF and PDGF-BB in PICF. tot: total amounts. T0:1 week after second stage surgery; T1: 1 month after second stage surgery; T3:5 months after second stage surgery. The horizontal black lines represent error bars. **= $p < .01$; *= $p < .05$

TABLE 4 Clinical measurements after implant restoration at different time points

	Parameters		ADM	Control	*p* Value
T2	mPII	Mean ± SD	1.10 ± 0.46	1.30 ± 0.59	.25[a]
		Median	1.00	1.50	
		Min, Max	0.50,2.00	0.00,2.00	
	mBI	Mean ± SD	0.85 ± 0.41	1.05 ± 0.37	.30[a]
		Median	1.00	1.00	
		Min, Max	0.00,1.50	0.50,1.50	
	PPD	Mean ± SD	3.23 ± 0.48	3.25 ± 0.49	.85[a]
		Median	3.25	3.25	
		Min, Max	2.50,4.00	2.25,3.75	
T3	mPII	Mean ± SD	1.05 ± 0.37	1.45 ± 0.44	.06[a]
		Median	1.00	1.50	
		Min, Max	0.50,1.50	1.00,2.00	
	mBI	Mean ± SD	0.70 ± 0.35	0.85 ± 0.53	.35[a]
		Median	0.75	1.00	
		Min, Max	0.00,1.00	0.00,1.50	
	PPD	Mean ± SD	3.08 ± 0.41	3.25 ± 0.24	.24[a]
		Median	3.00	3.25	
		Min, Max	2.25,3.75	3.00,3.75	
△T3-T2	△mPII	Mean ± SD	−0.05 ± 0.44	−0.15 ± 0.41	.33[a]
		Median	0.00	0.00	
		Min, Max	−0.50,0.50	−1.00,0.50	
		P [b]	0.71	0.26	
	△mBI	Mean ± SD	0.15 ± 0.47	0.20 ± 0.35	.97[a]
		Median	0.25	0.25	
		Min, Max	−1.00,0.50	−0.50,0.50	
		P [b]	0.32	0.10	
	△PPD	Mean ± SD	0.15 ± 0.39	0.00 ± 0.35	.48[a]
		Median	0.13	0.00	
		Min, Max	−0.50,0.75	−0.75,0.50	
		P [b]	0.26	0.86	

Abbreviations: T2, 1 week after restoration; T3, 3 months after restoration.

[a]Mann–Whitney *U* test

[b]Wilcoxon signed rank test.

4 | DISCUSSION

ADM membrane has long been used to improve mucogingival deformities (insufficient keratinized tissue and gingival recession) as an alternative to autogenous tissue. It has been indicated in many studies that ADM membrane possesses comparable clinical efficacy to autogenous tissue for root coverage procedures, with good long-term stability (Rahmani et al., 2006; Moslemi et al., 2011; Barros et al., 2015). Analogously, it is understandable that ADM membrane was gradually used to improve peri-implant soft tissues. Studies have already reported using ADM membrane to increase soft tissue thickness at buccal sites in order to improve the peri-implant esthetic outcomes. (Papi et al., 2020; Stefanini et al., 2020).

It should be stressed that the concepts of vertical soft tissue thickness and buccal soft tissue thickness are not exactly the same. Linkevicius and colleagues suggested that thin peri-implant vertical soft tissue, as measured from the bone crest in an apico-coronal direction, is associated with greater marginal bone loss than a thick tissue phenotype (Linkevicius et al., 2009, 2018; Puisys & Linkevicius, 2015). From then on, it was believed that adequate initial vertical soft tissue thickness (>2 mm) was beneficial to peri-implant health and thin soft tissue thickness (less than 2 mm) was responsible for crestal bone loss after implant restoration. In addition, over augmented soft tissue might also be detrimental to peri-implant health (Zhang et al., 2020). Consequently, we included cases with vertical soft tissue thickness more than 2 mm without grafting procedure as the positive control group in the present study. Previous studies

have already reported that using ADM could improve the thickness of thin peri-implant vertical soft tissue. However, their study outcomes, which were limited to clinical measurements, lacked histological evaluation of the incremental soft tissue. In the present study, we wanted to evaluate whether the ADM group could acquire comparable clinical and histological outcomes to the control group in the same period.

Our results indicated that the soft tissue thickness was comparable in the two groups at second-stage surgery (3.20 ± 0.42 mm vs 3.50 ± 0.58 mm) and a mean increase of 1.85 mm in the vertical soft tissue was observed at 3 months postoperatively in the ADM group. It has been suggested that ADM folding might enhance augmentation results. In one case series study (Puisys et al., 2015), ADM was folded and grafted above the submerged implant at the time of implant surgery. Three months after the operation, the average gain in vertical soft tissue thickness was 2.21 mm. In this study, the ADM group showed significant augmentation compared with the control group, so that ultimately the soft tissue thickness was comparable between the two groups by 3-month post-implant surgery (3.20 ± 0.42 mm vs 3.50 ± 0.58 mm). Thus, the incremental gain in thickness in this study was enough for the ADM group to achieve identical good clinical outcomes to the control group. Considering the potential postoperative morbidity accompanied with ADM folding, the necessity of it needs to be further investigated. It is also possible that augmentation results might be attenuated by time. According to a recent study involving second-stage surgery performed 6 months postoperatively, the reported vertical soft tissue gain was less than that observed in our study (1.33 ± 0.71 mm vs 1.85 ± 0.34 mm) (Verardi et al., 2020). Hence, further investigations are needed to determine the relationship between the length of postoperative time and the effect of augmentation.

Previous studies have evaluated the histological response of ADM when used around natural teeth. Resende et al. (2018) reported that an ADM group exhibited a lower percentage of cellularity and blood vessels but higher inflammatory infiltrates compared with a free gingival graft (FGG) group when used to treat a deficiency of keratinized tissue. The present study analyzed the histological results of peri-implant increased vertical mucosa and the results indicated that soft tissue appeared to be mature and organization in the ADM group, comparable to the control group. Few studies have reported histological analyses of tissue after using ADM for peri-implant soft tissue augmentation. Farina and Zaffe (2015) reported comparable histological outcomes in an ADM group compared with a non-grafted control group. Their results indicated that biopsies taken in both groups during second-stage surgery were characterized by mature tissue and the degree of inflammation was similar. Our results were in agreement with theirs. They also mentioned that a large number of vessels were found in the ADM group which were also detected in our study. VEGF expression and MVD were further evaluated through immunohistochemical analysis. The VEGF expression level in tissue represents the stimulation of angiogenesis during wound healing while the degree of angiogenesis can be evaluated by MVD (Aspriello et al., 2009; Bao et al., 2009). The results indicated that there was no significant difference in the percentage of VEGF-positive cells between the ADM group and the control group (37.2 ± 3.09 vs 37.7 ± 3.79); the MVDs were also comparable between the two groups (29.12 ± 4.34 vs 32.73 ± 3.78). There are no prior data available regarding soft tissue quality characterization based on quantification of VEGF expression and MVD in the augmented zone, making it impossible to compare our present study outcomes to prior research. Based on our results, we concluded that the degree of vascularization of the increased tissue in the ADM group was comparable with the control group 3 months postoperation.

In order to compare the function of the peri-implant vertical soft tissue in the ADM group to the control group after second-stage surgery, we analyzed VEGF and PDGF-BB expression in PICF. These two cytokines are considered to be key mediators of vascularization and angiogenesis (Coultas et al., 2005; Gamal et al., 2016). First, we found in this study that VEGF and PDGF-BB were obviously lower in the ADM group compared with the control group at T0 (1 week after second-stage surgery). At the next time points (1 month and 5 months after second-stage surgery), both cytokines showed similar low levels in the two groups. This could indicate that using ADM for peri-implant vertical soft tissue augmentation might slow down the process of vascularization at the very beginning, reminding clinicians that they should make every effort to control infection in the early phase postoperation. Second, these two cytokines both exhibited a markedly increased level 1 week after second-stage surgery and subsequently showed a clear, time-dependent decreasing trend. This phenomenon could be explained by previous studies which demonstrated that VEGF and PDGF-BB play important roles in the process of wound healing (Coultas et al., 2005; Matsuoka et al., 1989) and would be expected to return to low levels after implant restoration (Nogueira-Filho et al., 2014).

Vertical soft tissue is considered to participate in the formation of the peri-implant cuff after restoration. In order to evaluate whether its function of maintaining peri-implant tissue health in the ADM group was comparable to the control group, we examined the related clinical parameters after restoration. In the present study, all the patients maintained good oral hygiene after restoration with acceptable PII levels, and results showed that peri-implant tissues remained healthy in both groups. mBI and PPD values were comparably low in the two groups, indicating that no significant peri-implant inflammation was detected in any of the participants. Thus, we concluded that, in the ADM group, the function of protecting peri-implant tissue health was comparable to the control group in terms of clinical outcomes 3 months after restoration. However, further long-term observation will still be needed in the future.

To the best of the author's knowledge, this study is the first longitudinal clinical controlled study in humans that evaluated the effect of using ADM for vertical peri-implant soft tissue augmentation and investigated the outcomes using clinical and histological methods. In addition, it was also the first study to compare the PICF cytokines in the two groups to evaluate the function of peri-implant vertical soft tissue after second-stage surgery. In spite of this, there are still

some limitations in the present study including small sample size and limited follow-up period after restoration. Owing to the small sample size in the present study, the related outcomes should be interpreted with caution. Studies with larger sample size are needed in the future to further confirm the conclusions of this study.

5 | CONCLUSIONS

Within the limitations of this study, it can be concluded that the use of ADM at implant surgery was effective to thicken peri-implant vertical soft tissue. The soft tissue thickness 3 months post-ADM grafting was clinically comparable to the control group. This study showed that the ADM group achieved identical histological and immunohistochemical outcomes to the control group at 3-month post-implant surgery. However, the vertical soft tissue might have inferior angiogenic ability in the early stage of wound healing compared with the control group. Peri-implant clinical health after restoration was good and stable in the ADM group up to the last follow-up. Studies with larger sample size and longer follow-up time are needed to confirm these findings.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTION

Jing Zang involved in data curation, formal analysis, investigation, methodology, project administration, Validation, writing—original draft, and writing—review and editing. **Li Su involved in** resources, supervision, and writing—review and editing. **Qingxian Luan involved in** funding acquisition, investigation, project administration, resources, and supervision. **Guojing Liu involved in** data curation, formal analysis, investigation, project administration, and validation. **Shiyi Li involved in** data curation, investigation, and project administration. **Xiaoqian Yu:** involved in conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—original, and writing—review and editing.

AUTHOR CONTRIBUTION

Jing Zang: Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (supporting); Project administration (equal); Validation (lead); Writing – original draft (lead); Writing – review & editing (lead). **Li Su:** Resources (equal); Supervision (equal); Writing – review & editing (equal). **Qingxian Luan:** Funding acquisition (equal); Investigation (supporting); Project administration (supporting); Resources (equal); Supervision (equal). **Guojing Liu:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Project administration (supporting); Validation (equal). **Shiyi Li:** Data curation (supporting); Investigation (supporting); Project administration (supporting). **Xiaoqian Yu:** Conceptualization (lead); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (lead); Project administration (equal); Resources (equal); Supervision (equal); Validation (equal); Writing – original draft (supporting); Writing – review & editing (supporting).

ETHICAL APPROVAL

This present study was approved by the Institutional Review Board of Peking University School and Hospital of Stomatology (No. PKUSSIRB-202057114).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Zang, J., Su, L., Luan, Q., Liu, G., Li, S., & Yu, X. (2022). Clinical and histological evaluation of the use of acellular dermal matrix (ADM) membrane in peri-implant vertical soft tissue augmentation: A controlled clinical trial. *Clinical Oral Implants Research*, *33*, 586–597. https://doi.org/10.1111/clr.13919