Effects of Preparation Technique on Periosteal Microcirculation After Autologous Bone Augmentation in an Animal Model

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This controlled in vivo experimental study examines the impact of 2 periosteum preparation techniques on microcirculation during bone augmentation with isogenic bone grafts in rats. Twenty female Lewis rats were divided into 2 groups (n = 10 each). In one group, the periosteum was prepared with a conventional periosteal elevator; in the other, a piezoelectric device was used. After graft implantation at calvarial sites, intravital microscopy was performed postoperation (day 0) and on days 3, 8, and 28 immediately to assess microvascular parameters: functional capillary density, blood flow velocity, and vessel diameter. Statistical analysis was conducted using analysis of variance on ranks with $P < .05$. The piezoelectric device group showed higher mean values for functional capillary density, blood flow velocity, and vessel diameter than the conventional instrument group, though differences were not statistically significant. This study suggests that periosteum preparation with a piezoelectric device does not significantly differ from conventional methods regarding microcirculatory outcomes. Either method appears viable for preserving microcirculatory integrity during bone augmentation. Further research in larger models and clinical contexts is recommended to confirm these findings.

Key Words: periosteal microcirculation, bone remodeling, piezo-surgery, microcirculation

Introduction

In the past 15 years, dental implant placement has become a standard procedure in dentistry with excellent chances of success.[1–3]

As the indications for dental implants have broadened over recent years and available bone is sometimes insufficient to hold an implant, bone augmentation procedures are closely associated with dental implants. With the broader application of augmentation techniques, it was recognized that for successful bone augmentation postoperatively, it is essential that the periosteum functions as undisturbed as possible, as it provides all the humoral and cellular factors necessary for bone regeneration and formation. As the periosteum must be detached from the bone intraoperatively to insert an augmentation, an atraumatic surgical technique is essential for periosteum preparation. The increasing use of augmentation techniques has shown that adequate periosteal functioning after surgery is a key factor for successful bone augmentation, as the periosteum contains all humoral and cellular factors required for bone regeneration and the formation of new bone. The periosteum covers the outer surfaces of all bones except functional articular surfaces.[4–7]

Similarly, the endosteum lines the inner surfaces of bones.[4,6,7] Periosteum and endosteum comprise a thin layer of nonmineralized collagenous fibers and cell populations, including mesenchymal stem cells, osteoprogenitor cells, osteoblasts, and osteoclasts.[8] The osteoprogenitor cells and mesenchymal stem cells, periosteum, and endosteum play an essential role in bone remodeling and repair and help increase the width of bones throughout life.[4,5,9–12]

Histologically, the periosteum consists of a fibrous layer and an osteogenic (or cambium) layer, separated by a translucent layer with numerous capillaries.[4,5,11,13] The fibrous layer provides mechanical stability but contains many larger blood vessels entering the bone through nutrient foramina and supplying the bone marrow. Periosteal circulation offers up to 70%–80% of arterial supply and 90%–100% of venous return of the bone.[4,10] By contrast, the cambium layer has regenerative capacity.[10] The periosteum must be separated from the bone during surgery before the augmentation material can be inserted. Hence, an atraumatic approach to managing the periosteum is of great importance. Only if tissue is managed gently can the structural integrity and, thus, the regenerative potential of the periosteum be preserved to a large extent.[14,15] The focus of modern surgical procedures

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Table 1

Group design of the present study

Group	Periosteal Elevator	Piezo-surgery Device	Donor
N	10	10	8
Assessment Day	0; 3; 8; 28	0; 3; 8; 28	/

is on atraumatic methods that ensure gentle tissue handling and preparation.

Additionally, biologization, the assimilation into a biological framework, plays an increasing role. Until now, research has focused on bone, whereas less attention has been paid to the periosteum as an important factor in osteogenesis. Thus, meaningful studies still need to be made available.[16] So, investigating the effect of different preparation instruments on the periosteum is necessary.

The present study aimed to investigate the impact of two different periosteum preparation techniques on the microcirculation at the surgical site during bone augmentation in an isogenic rat model.

Materials and Methods

Animals and housing conditions

The study involved female Lewis rats obtained from Charles River Laboratories in Cologne, Germany. All experiments and animal care adhered to the German Animal Protection Act and the Guide for the Care and Use of Laboratory Animals.[17,18] The local animal welfare commissioner (Approval No: 14/1417) at the Animal Protection Division Office for Consumer Protection and Food Safety reviewed and approved the research applications. Twenty-eight female isogenic rats of the Lewis strain, weighing between 300 and 330 g, were housed in groups of 5 in cages, and after surgeries, they were individually housed (Table 1). All of them experienced identical stable climatic conditions throughout the experiment with a 12-hour light/dark cycle.

Surgical procedures and anesthesia

To investigate microvascular parameters (functional capillary density, blood flow velocity, and vessel diameter) after bone grafting, calvarial bone grafts from 8 isogenic animals were utilized as onlay grafts to augment the calvaria of rats in the study groups. All procedures were conducted under anesthesia, involving intraperitoneal injection of ketamine-xylazine (Ketavet, 75 mg/kg, xylazine, 25 mg/kg). The occipital hair was removed to enhance surgical visibility. Donor animals were euthanized for grafts, which were stored in sterile saline. Recipient animals underwent scalp incision, exposing the skull and periosteum.

The periosteum was horizontally cut and lifted using either a raspatory or a piezoelectric instrument (PR1, Mectron, Germany), and a graft was placed underneath. The periosteal preparation was performed using a piezoelectric surgical device (PR1) with a specialized tip for gentle tissue handling. The device was set to operate at a medium frequency (30–36 kHz) with output power carefully adjusted for soft tissue manipulation, allowing the periosteum to be lifted without impacting the underlying bone. Continuous irrigation was applied to control the temperature at the surgical site, reducing any risk of thermal damage. The piezoelectric tip was then positioned at the interface between the periosteum and bone. Using a controlled, sweeping motion, the device's gentle vibrations lifted the periosteum gradually, minimizing the need for force and reducing potential trauma to the tissue. Care was taken to avoid prolonged contact in any area, maintaining an even temperature and minimizing tissue stress. Sterile irrigation ensured the device remained cool throughout the procedure, preserving the periosteum's microcirculatory integrity and supporting optimal tissue health.

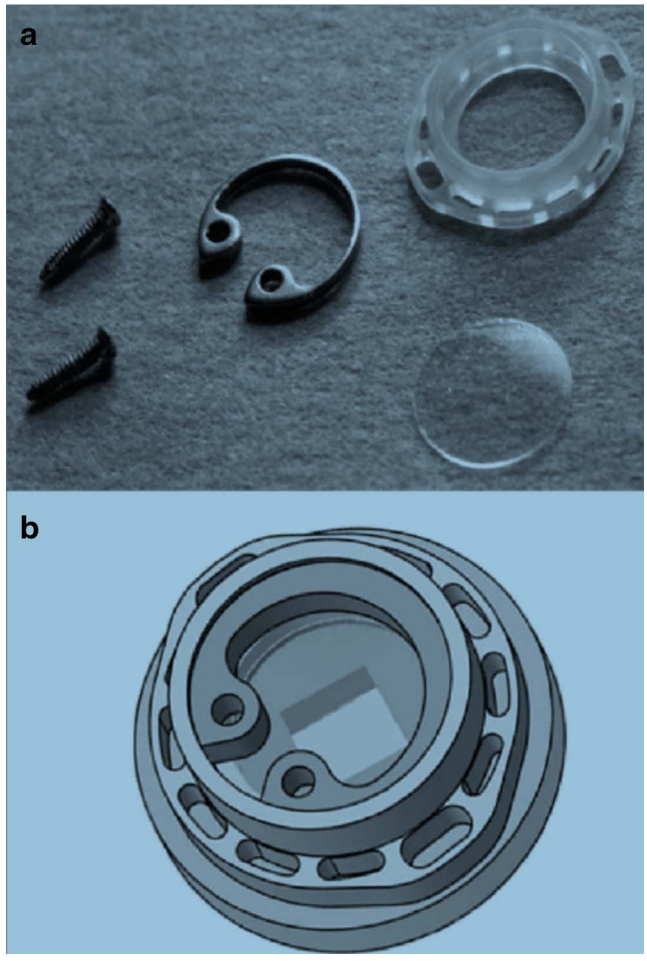

Figure 1. Observation chamber. (a) all parts; (b) in compound.

The graft, along with the observation chamber, was fixed using micro screws. The observation chamber comprises a basic frame with two bone screws, a glass coverslip, and a snap ring (Figure 1). The surrounding tissue was sutured (Ethicon-Vicryl sutures size 4.0, Johnson & Johnson). The procedure lasted approximately 25 minutes per animal. The temperature of the specimens was maintained at +36°C using a heating mat (ThermoLux; Witte + Sutor, Murrhardt, Germany).

Intravital fluorescence microscopy (IVM) and imaging

On specified days (3, 8, and 28) and under anesthesia, IVM of the periosteum was conducted using a modified microscope (AxioTech, Zeiss, Oberkochen, Germany) equipped with a mercury arc lamp and a blue filter set (450 – 490 nm). Microscopic images

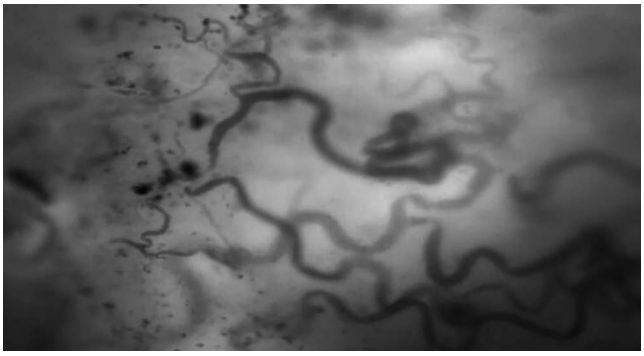

Figure 2. IVM using a modified Zeiss AxioTech microscope (Zeiss, Oberkochen, Germany) equipped with a mercury arc lamp and a blue filter set, making the vascular structures in the periosteum visible. IVM indicates intravital fluorescence microscopy.

were captured with a video camera (FK 6990 IQ-S, Pieper, Schwerte, Germany) and transferred to a DVD system (LQ-MS 800, Panasonic, Hamburg, Germany). To enhance visibility, animals were injected with a 1:1 mixture of rhodamine 6G (MG 476, Sigma Merck, Germany; 1 mg/mL in 0.9% NaCl solution) and fluorescein isothiocyanate-labeled dextran (FITC-dextran molecular weight: 150 000 Da; Sigma, Taufkirchen, Germany; 150 mg/mL in 0.9% NaCl solution) through the tail veins, enabling visualization of the vascular structures in the periosteum (Figure 2). Each animal was examined for 1 minute under various filters and magnifications, and the periosteum was recorded for subsequent evaluation.

Ethical considerations

All experimental procedures were conducted following ethical guidelines, and animals were euthanized with an overdose of anesthetic on day 28 to minimize suffering.

Statistical analysis

The statistical analysis of the microvascular parameters (functional capillary density, blood flow velocity, and vessel diameter) was conducted using IBM SPSS Statistics 23 (IBM, Armonk, New York, USA). An analysis of variance (ANOVA) test was employed to compare these parameters across different time points. Given the isogenic nature of the animals, the application of ANOVA on Ranks was justified by the homogeneity of variances observed in the preliminary tests. However, it is essential to note that whereas ANOVA assumes homogeneity of variances and normality of residuals, these assumptions were carefully checked and met. The statistical analysis results were reviewed and approved by the Institute for Armed Forces Medical Statistics at the Armed Forces Medical Academy. All procedural and mathematical assumptions for the use and interpretation of ANOVA were adhered to, ensuring the robustness and validity of the findings.

Results

The statistical analysis revealed no statistically significant differences between the periosteal elevator (PE) and piezoelectric device (PD) groups regarding functional capillary density, blood flow velocity, and vessel diameter. An independent statistician rigorously reviewed and validated all statistical analyses to confirm the methods' appropriateness and the results' reliability. Detailed results are illustrated in Figures 3 to 5 and Tables 2 and 3.

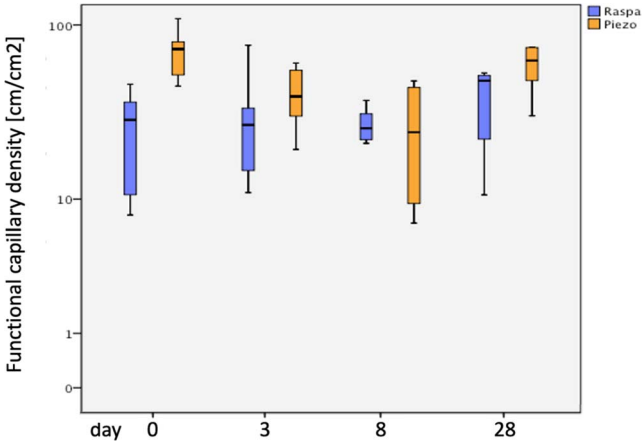

Figure 3. Box plot for the functional capillary density. On day 0, there is a significant difference between the groups, which leveled off on day 3.

Functional capillary density

Paired *t* tests were conducted to compare functional capillary density between the PE and PD groups. On day 0, a significant difference was observed, with the PE group showing a lower mean capillary density than the PD group (mean difference = −48.08, $P = .007$). The 95% confidence interval for this difference was −76.45 to −19.71.

On subsequent days, no statistically significant differences were found. Specifically, on day 3, the mean difference was −9.17 ($P = .519$); on day 8, it was −0.25 ($P = .972$); and on day 28, it was −26.24 ($P = .236$). The 95% confidence intervals for these differences included 0, indicating no significant difference between

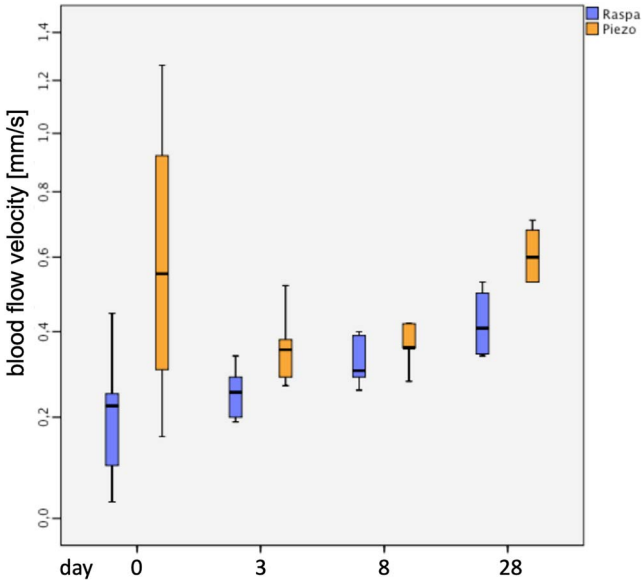

Figure 4. Box plot for the blood flow velocity. In the periosteal elevator group, mean blood flow velocity continuously increased from day 0 to day 28.

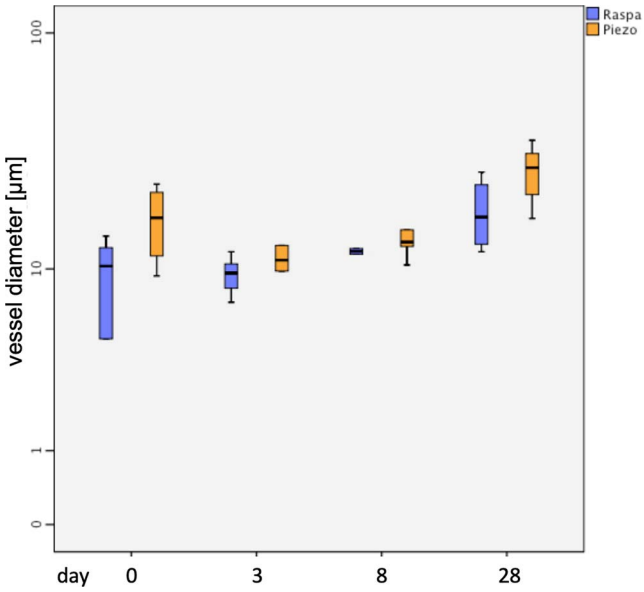

Figure 5. Box plot for the vessel diameter. The mean vessel diameter continuously increased from day 0 to day 28 in the periosteal elevator group.

the groups on these days. Tukey's HSD post-hoc test confirmed these findings, with no significant differences on days 3, 8, and 28 (*P* values of .995, 1, and .903, respectively). On day 0, the mean difference was -37.39, with a *P* value close to significance (.053).

Blood flow velocity

In the PE group, mean blood flow velocity steadily increased from day 0 to day 28, with a 19.55% increase between day 0 and day 3 and a 27.45% increase between day 3 and day 8.

In contrast, the PD group experienced a decrease in mean blood flow velocity by 44.35% on day 3 compared to day 0. This was followed by increases on days 8 and 28, with the most significant growth of 23.40% between days 8 and 28. Throughout the study period, the mean blood flow velocity was consistently higher in the PD group compared to the PE group.

Vessel diameter

The PE group gradually increased mean vessel diameter from day 0 to day 28, with an 8.48% increase between day 0 and day 3 and a most substantial 30.24% increase between days 8 and 28.

In the PD group, the mean vessel diameter was initially larger on day 0 but decreased by 22.84% on day 3. The diameter then increased on days 8 and 28, with the most significant increase of 76.79% occurring between days 8 and 28. At each time point, the mean vessel diameter was consistently larger in the PD group compared to the PE group.

Statistical assumptions and methodological considerations

All statistical tests were performed with attention to necessary assumptions, including sample size adequacy, normality of data distribution, and appropriate alpha levels for multiple comparisons. Power analysis was conducted to ensure sufficient sample size, and family-wise and manuscript-wise alpha levels were controlled to mitigate Type I and II errors. These considerations were integral to the validity of the findings reported herein.

Discussion

Repetitive quantitative in vivo analysis of periosteal microcirculation is a novel scientific method that can be used to objectively evaluate and directly compare the different surgical procedures investigated here.[9,19]

The present study aimed to investigate the impact of 2 different periosteal preparation techniques on the microcirculation at the surgical site during the augmentation of bone. To address the question of this study, an animal model was chosen to determine active vascularization. However, this investigation used a basic one-shot animal pilot research model. Although animal models are a common and sometimes necessary approach in biomedical research, this methodology has faced increasing scrutiny. Critics argue that animal research can often be avoidable, citing ethical concerns and questioning the relevance of animal models to human conditions due to differences in disease manifestation and physiological responses. In response to these concerns, we acknowledge the fallacy of false analogy, where results from animal studies may not always translate directly to human clinical outcomes. This study, however, was designed to mitigate such risks by implementing several methodological rigor measures, such as ensuring isogenicity to maintain genetic homogeneity, controlling environmental conditions, and utilizing intravital fluorescence microscopy (IVM) for precise and reproducible microvascular assessments. These controls help reduce variability and enhance the reliability of the findings.

Table 2

t test for paired samples—functional capillary density

	Paired Differences							
	Mean Value	Standard Deviation	Standard Error of The Mean	95% Confidence Interval for Difference		T	df	Sig. (2-Tailed)
				Lower	Upper			
Pair 1 PE0 − PD0	−48.08	27.03209	11.03581	−76.44844	−19.71156	−4.357	5	0.007
Pair 2 PE3 − PD3	−9.16833	32.4032	13.22855	−43.1734	24.83673	−0.693	5	0.519
Pair 3 PE8 − PD8	−0.25167	16.73099	6.8304	−17.80977	17.30643	−0.037	5	0.972
Pair 4 PE28 − PD28	−26.24	27.16733	15.68507	−93.72739	41.24739	−1.673	2	0.236

*df indicates degrees of freedom; PD, piezoelectric device; PE, periosteal elevator; Sig., significance.

TABLE 3

ANOVA and posthoc Tukey's HSD test for multiple comparisons—functional capillary density

				95% Confidence Interval	
Tukey's HSD Test	Mean Difference	Standard Error	Significance	Lower Bound	Upper Bound
Pair 1 PE0 - PD0	−37.39405	11.74783	0.053	−75.0513	0.2632
Pair 2 PE3 - PD3	−9.16833	12.1913	0.995	−48.2471	29.9104
Pair 3 PE8 - PD8	−0.25167	12.1913	1	−39.3304	38.8271
Pair 4 PE28 - PD28	18.052	14.16501	0.903	−63.4574	27.3534

*ANOVA indicates analysis of variance; HSD, honestly significant difference.

Furthermore, double-masked procedures were applied during data analysis to prevent observer bias. Alternative research methodologies have also been considered, but it was determined that animal models were essential for addressing the specific research question due to the unique physiological responses observed in live organisms, which are not replicable in vitro. The complex interactions within a living system, particularly involving bone grafting and microvascular parameters, necessitate an in vivo model to reflect the biological processes under study accurately. This research contributes to a foundational understanding that may inform further studies involving human subjects. The importance of rigorous ethical standards and the continuous evaluation of the necessity and relevance of animal models in research has been emphasized. Future studies should bridge the gap between animal models and human clinical research, ensuring that findings are robust and applicable across species. We hope to contribute valuable insights by addressing these critical considerations while aligning with ethical research practices.

Recent research by Remísio et al supports the relevance of osseointegration for titanium and zirconia implants. The findings reinforce the importance of periosteal health in ensuring implant stability. Moreover, the work by Fernandes et al on critical-size defect reconstructions demonstrates the significance of graft type in vascularization, aligning with the observed influence of periosteal preparation on microcirculation.[20,21]

A histological examination does not allow a statement on the dynamic situation of tissue perfusion; it only allows a static observation of the problem during sampling.[22] Histological data do not show whether perfusion has occurred. Hence, an animal model was chosen, and the rat proved to be an excellent model for answering these questions.

The data's statistical analysis showed no statistically significant differences between the two approaches to periosteal preparation regarding functional capillary density, blood flow velocity, and vessel diameter.

However, several calibration and validation steps were carefully implemented to ensure the reliability and validity of the microvascular parameters measured. Initially, interrater reliability was addressed by having multiple researchers independently assess a subset of the images. This approach helped guarantee consistency in measurements of functional capillary density, blood flow velocity, and vessel diameter. Cohen's kappa statistic was used to quantify the agreement between the raters, thus providing a robust measure of inter-rater reliability. Furthermore, intrarater reliability was examined by having the same researcher repeat the measurements on different occasions. This step was crucial for verifying that the measurements remained consistent over time when conducted by the same individual. The intraclass correlation coefficient was employed to assess this reliability aspect. In addition to these measures, construct validity was established by comparing the observed microvascular parameters with established benchmarks in the literature. By aligning the measurements with these benchmarks, it was ensured that they accurately reflected the underlying microvascular structures and functions intended to be measured.

Moreover, face and content validity were enhanced by engaging experts in microvascular research. These experts reviewed the methodology and measurement techniques, providing valuable feedback. Their insights were incorporated into the process, refining the measurement techniques and confirming that they adequately represented the constructs of interest.

To address the issue of Type I error (false positives) across multiple comparisons, we employed statistical techniques to control the familywise error rate. Specifically, we applied the Bonferroni correction method to adjust the significance level for multiple paired comparisons. Given the eight probability values reported across Tables 2 and 3, the adjusted significance level was set to $\alpha/8 = 0.00625$ to maintain an overall Type I error rate of 0.05. Furthermore, Tukey's HSD test was utilized for post-hoc analysis, which controls for Type I error while comparing all possible pairs. This ensures that the Type I error rate is within acceptable limits when conducting multiple pairwise comparisons.

Type II errors (false negatives) are inherently more challenging to control and are related to the power of the statistical tests performed. Although we applied stringent significance criteria to manage Type I error, the lack of significant findings on days 3, 8, and 28 might reflect insufficient statistical power rather than an absence of real differences. Future studies with larger sample sizes could provide more robust evidence to confirm or refute these findings.

In the periosteal elevator group, four animals died during the surgery. On day 28, the results for two others had to be excluded as they lost their observation chamber and graft.

In the piezoelectric device group, 3 animals died during the surgical procedure. The images obtained for 7 animals were included in the analysis. On day 3, one animal died during anesthesia. On days 3 and 8, the images of 6 animals from the piezo surgery device group and 6 animals from the periosteal elevator group were included in the analysis. On day 28, one animal from the piezo surgery group lost its observation chamber and graft.

The piezoelectric device group always showed higher values for all parameters than the periosteal elevator group. However, the differences were not significant. These findings correspond to

the results of earlier studies but need to reach the significance levels reported there.[23]

One possible explanation for the initial decrease in periosteal microcirculation is the formation of microthrombi and compromised perfusion at the surgical site. Early studies demonstrated the formation of microthrombi, which can partially or temporarily occlude blood vessels but do not damage them irreversibly.

The differences between the two groups in the present study suggest that the periosteum could be preserved more when the piezoelectric device is used.

Unlike similar investigations, the study presented here did not show significant differences between the two groups on days 3, 8, and 28.[23]

This result might be attributable to the mechanical stretching of the periosteum during subperiosteal graft placement. This deformation might have compromised perfusion in the region of the periosteal capillaries, which might have had a more significant effect on circulation at the surgical site than the instrument used for managing the tissue did. A possible solution might be to pre-stretch the tissue and the underlying periosteum using self-inflating hydrogel expanders before the surgical procedure and bone augmentation. This approach might prevent the periosteum from tearing. An additional periosteal incision for tension-free soft-tissue coverage may be unnecessary. The risk of periosteal perforation might be considerably reduced.[25]

In line with recent findings on bone biomaterials, studies like those by Motta et al underscore the promise of homologous bone grafts in reconstructive procedures, demonstrating that such grafts can be osteoconductive and viable alternatives to autogenous grafts. This aligns with the need to preserve periosteal integrity to promote healing and vascularization, as highlighted in the present study.[26] During the entire study period, no abnormal behavior was observed in any of the animals. Before surgery and all other procedures associated with intravital microscopy, all animals were anesthetized in a predetermined sequence, and the contrast agent was injected into a tail vein in the same sequence. This standardized approach was used throughout the experiments and allowed us to ensure an adequate perfusion period with contrast agent and uniformity of procedures. Microvascular perfusion can be investigated in vivo using polarographic oximetry, laser Doppler flowmetry, and gamma spectrometry.[17,23,27,28]

Additionally, Gasperini et al. demonstrated that biodegradable hydroxyapatite-based grafts with controlled degradation could enhance vascularization and promote bone formation. These findings support our emphasis on techniques that minimize periosteal damage, enabling better graft integration and microvascular health.[29] Previous studies have shown that intravital microscopy can effectively assess microcirculatory parameters.[9,19,23,25,27,30] Intravital microscopy allows examiners to assess microvascular structures and the perfusion of individual vessels directly, reliably, and reproducibly.[27]

Using an isogenic rat strain allowed us to directly compare different surgical procedures and their effects on the postoperative regeneration of periosteal microcirculation. Restoring and maintaining periosteal microcirculation is crucial to the success of bone grafts. Intravital microscopy is an established and valid method used in the present study to evaluate micro-perfusion reliably and reproducibly, as other studies have already shown.[31,32]

The periosteum is key in the blood supply to bone and bone grafts. Postoperative periosteal integrity and the rapid regeneration of periosteal microcirculation are crucial to successful grafting.[17] Every surgical procedure impairs periosteal perfusion and should, therefore, be performed as gently as possible to preserve the periosteum as much as possible.[28]

Conclusion

This study provides preliminary insights into how periosteal preparation techniques influence periosteal microcirculation during bone augmentation. Although differences in microcirculatory parameters were observed between piezoelectric and conventional instruments, these differences were not statistically significant, suggesting that mechanical tension from graft placement may substantially impact periosteal perfusion more than the preparation technique itself. Pre-stretching the periosteum with hydrogel expanders may help mitigate tissue stress, reducing periosteal tearing risks. While these findings offer a foundation for understanding periosteal preservation in bone grafting, further clinical research is needed to validate the results in human applications and optimize grafting techniques.

Acknowledgments

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Note

We declare that there are no conflicts of interest.

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