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ORIGINAL RESEARCH

Regulation of Periodontal Ligament Autophagy by Super-Activated Platelet Lysate in a Rat Maxillary Expansion Model

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Purpose: To investigate the regulatory effect of Super-Activated Platelet Lysate (sPL) on autophagy of periodontal ligament stem cells (PDLSCs) during rat maxillary expansion, with implications for optimizing orthodontic treatment outcomes.

Methods: Fifty-four male Sprague-Dawley rats were randomized into control, maxillary expansion model, and sPL-treated groups. After expansion model establishment, arch width was monitored, and maxillary samples were collected on days 3, 7, and 14 post-expansion. Histological staining (H&E, TRAP) and immunological assays (IHC/IF) were performed to evaluate periodontal tissue morphology, osteoclast activity, and autophagy-related marker (LC3B, LAMP2, mTOR) expression.

Results: All rats adapted well after expander installation with no mortality. Arch width increased significantly over time. H&E staining showed narrowed periodontal space, disorganized fibers, and localized bone resorption in the model group on days 3 and 7, with stabilization by day 14. The sPL group exhibited improved tissue morphology by day 7 and stabilization by day 14, though with slightly increased osteoclast activity. TRAP staining revealed elevated osteoclast numbers in the model group on days 3 and 7, decreasing by day 14, whereas the sPL group had fewer osteoclasts initially but more by day 14 (all $p < 0.05$). IHC indicated increased LC3B expression in both groups on day 3, with significant intergroup differences. By day 7, LC3B decreased in the sPL group but remained elevated in the model group. On day 14, the sPL group showed significantly higher LC3B than the model group. LAMP2 expression mirrored LC3B, while mTOR showed an inverse trend. IF confirmed LC3B expression in CD90-positive cells within the periodontal ligament.

Conclusion: sPL can modulate PDLSCs autophagy during maxillary expansion, a novel regulatory mechanism that may mitigate alveolar bone complications and shorten orthodontic treatment duration. This finding highlights sPL's potential as a therapeutic agent in orthodontic practice.

Keywords: sPL, autophagy, maxillary expansion, PDLSCs, OTM

Introduction

Maxillary Transverse Deficiency (MTD) is a common dentofacial deformity. Its clinical manifestations include anterior crossbite, posterior crossbite, dental crowding, V-shaped arch, and masticatory dysfunction.[1] MTD significantly impairs facial aesthetics and orofacial function. Maxillary expansion is currently the primary treatment for this condition; however, it is often accompanied by complications such as alveolar bone dehiscence, fenestration, horizontal bone resorption, and root resorption. These complications are mainly caused by dental side effects during expansion (eg, tooth inclination and buccal alveolar crest resorption) rather than the desired skeletal remodeling.[2]

During maxillary expansion, mechanical loading causes localized hypoxia, which in turn triggers autophagic activity. As an evolutionarily conserved homeostatic mechanism, autophagy removes damaged organelles and misfolded proteins through the formation of autophagolysosomes, thereby enhances cell survival under stress conditions. Existing studies

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have confirmed that mechanical stress-induced autophagy within a specific force threshold serves as a key adaptive response in orthodontic tissue remodeling.[3] Orthodontic tooth movement (OTM) essentially involves mechanical force-mediated periodontal remodeling, which requires coordinated responses from periodontal ligament fibroblasts, periodontal ligament stem cells (PDLSCs), and osteoblasts.[4]

Among these cell types, PDLSCs are mesenchymal stem cells derived from the periodontal ligament, with high proliferative capacity, self-renewal ability, and multilineage differentiation potential. Their responsiveness to orthodontic forces during tooth movement has been well documented.[5] Platelet-rich plasma (PRP), an autologous blood concentrate, promotes tissue regeneration by releasing growth factors and has been widely used in orthopedics, dermatology, and dentistry.[6] However, the regenerative efficacy of PRP's limited in some cases.[7] Superactivated platelet lysate (sPL), an optimized product of PRP prepared via ultra-low-temperature freeze-thaw cycles, can achieve high-efficiency release of growth factors and has superior regenerative capacity compared with PRP. Although sPL can directly activate cells and accelerate tissue repair, its specific mechanism in regulating PDLSC autophagy during orthodontic remodeling remains unclear.

Based on the above background, we hypothesize that sPL may regulate the autophagic activity of PDLSCs during maxillary expansion, thereby modulating periodontal tissue remodeling and reducing expansion-related complications. To verify this hypothesis, this study establishes a rat maxillary expansion model to examine the expression levels and tissue localization of key autophagy-related proteins in periodontal tissues after sPL intervention. The specific objectives of this study are: (1) to clarify the effect of sPL on autophagy in periodontal tissues during maxillary expansion; (2) to explore the regulatory role of sPL in PDLSC autophagy under orthodontic stress; (3) to elucidate the underlying mechanism by which sPL modulates periodontal remodeling through regulating autophagy. This study aims to provide theoretical and experimental support for optimizing clinical maxillary expansion protocols and reducing related complications.

Materials and Methods

Animal Experiments

A total of 63 male SD rats, aged 6 weeks, were obtained from Liaoning Changsheng Biotechnology Co., Ltd. The rats were housed in the Experimental Animal Center of the First Affiliated Hospital of Harbin Medical University under controlled conditions: a temperature of 21–25°C, relative humidity of 45–55%, and a 12-hour light/dark cycle. The animals were provided with ad libitum access to food and water throughout the experimental period. This study strictly adhered to the requirements of the Chinese National Standard "Guidelines for the Welfare and Ethical Review of Laboratory Animals" (GB/T 35892–2018), and all animal experimental protocols were approved by the Animal Experimental Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Approval Number: 2021040).

Preparation of sPL

sPL was provided by Tianqing Stem Cell Co., Ltd. Briefly, healthy SD rats (n=12) were anesthetized with 3–5% isoflurane for induction and 1.5–2.5% isoflurane for maintenance (with oxygen flow rate of 1–2 L/min during induction and 0.5–1 L/min during maintenance).[7] Then, 80 mL of blood was collected via cardiac puncture, and the blood samples were anticoagulated with 3.8% sodium citrate. After blood collection, the rats were euthanized by increasing the isoflurane concentration to 5–7% with sustained oxygen supply until respiratory arrest was confirmed.[8] The anticoagulated whole blood was centrifuged at 1000 rpm for 10 min at room temperature to remove red blood cells. The remaining blood components were thoroughly mixed and subsequently centrifuged at 2300–3300 rpm for 15 min at 18–20°C to separate the plasma. The upper plasma layer was collected, and the platelet count in the lower PRP layer was determined. The platelet concentration in the PRP was then adjusted to 1×10^{12} cells/L using the reserved plasma. To activate the platelets, the PRP was incubated with $CaCl_2$ and low molecular weight heparin sodium in a 37°C, 5% CO_2 incubator for 1–2 h, followed by freezing at −80°C for 1–2 h. The mixture was then thawed at 37°C for 5 min and

incubated at 4°C for 1 h to facilitate fibrin coagulation. Finally, the sample was centrifuged at 4°C, and the supernatant was filtered to obtain the final sPL product, with a volume of approximately 20 mL.[7]

Preparation of Maxillary Expansion Appliance

A rat maxillary first molar expansion appliance (0.016-inch Australian wire) includes an anterior loop, bilateral 2-mm circular loops (slow force release), and force arms. The oval anterior loop (fitting maxillary incisors) was bonded for fixation. Force arms (spanning anterior teeth to distal end) had buccal terminals bent for molar interdental insertion (Figure 1A).

Establishment of Rat Maxillary Expansion Model and sPL

Fifty-four male SD rats (6–8 weeks old, 180±10 g) were randomly divided into blank, model, and sPL groups (n=18 each). Under chloral hydrate anesthesia (0.3 mL/100 g),[9] a maxillary expansion appliance pre-activated with 0.49 N buccal force was bonded to the incisors after surface preparation and inserted into the molar interdental space (Figure 1B). Starting on the day of modeling, the sPL group received periodic intraligamentary injections of sPL (50 μL/site) into the first molars every other day (7 total injections), while the model group received equivalent saline. All rats were maintained on soft food with daily monitoring. On days 3, 7, and 14, six rats per group were first euthanized with 5–7% isoflurane[8] (with sustained oxygen supply until respiratory arrest was confirmed) for maxilla collection, followed by fixation in 4% paraformaldehyde and decalcification.

Tissue Sampling and Measurement of Tooth Movement Distance

Before model establishment, the distance between the bilateral maxillary molars of the rats was measured (recorded as D1). Before sacrifice, the intraoral expansion appliance was removed, and the distance between the bilateral molars was measured again (recorded as D2). The transverse movement distance of the rat maxillary first molars (recorded as D3) was calculated by subtracting D1 (the maxillary arch width before tooth movement) from D2.

Histopathological Staining

Maxillary bone samples were trimmed, embedded in paraffin, and sectioned for subsequent staining. Hematoxylin and eosin (H&E) staining was performed to observe periodontal tissue morphology and measure periodontal ligament width. Tartrate-resistant acid phosphatase (TRAP) staining was used to identify and count osteoclasts. For the detection of autophagy-related proteins, immunohistochemical (IHC) staining was conducted using the following primary antibodies: LC3B (ab192890, Abcam), LAMP2 (ab199946, Abcam), and mTOR (ab192890, Abcam), followed by incubation with a secondary antibody (ab6721, Abcam). Additionally, immunofluorescence (IF) staining was employed to analyze the localization and expression intensity of autophagy-related proteins in periodontal ligament stem cells, using primary

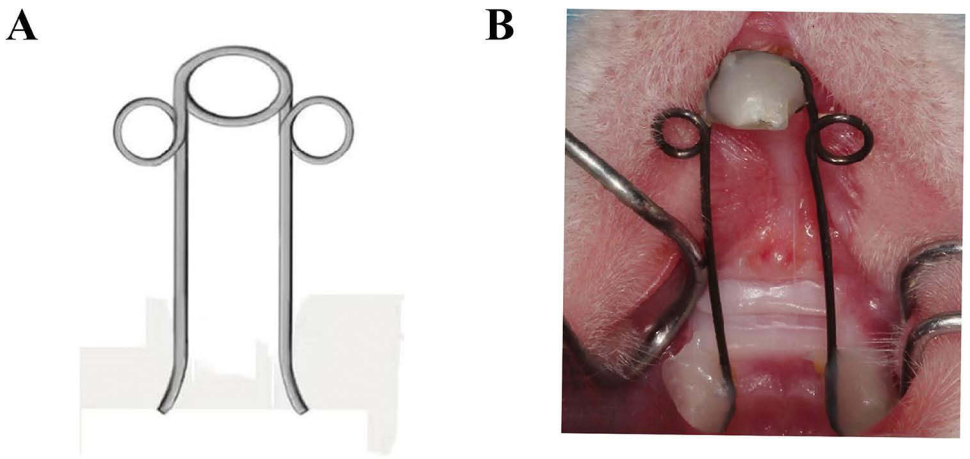

Figure 1 Design of the maxillary expansion appliance and schematic diagram of the rat model establishment. (**A**) Illustration of the maxillary expansion appliance. (**B**) Schematic representation of the appliance bonded to the rat maxillary incisors.

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antibodies against CD90 (A38930, Naturebio) and LC3B (ab192890, Abcam), along with corresponding secondary antibodies (ab150116/ab150073, Abcam) and DAPI (ab285390, Abcam) for nuclear counterstaining. All images were observed and captured using a Leica DM3000 LED optical microscope.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 software. Measurement data are presented as the mean ± standard deviation (mean ± SD). For comparisons among multiple groups, an ordinary one-way analysis of variance (ANOVA) was applied. If the ANOVA indicated a statistically significant difference, Tukey's post-hoc test was used for pairwise comparisons between groups. $P<0.05$ was considered statistically significant. The optical density of the target area and the fluorescence intensity in relevant images were measured, counted, and analyzed using ImageJ software.

Results

Living Status and Body Weight Growth Curve

Appliance-fitted rats showed reduced food intake and weight loss in the first 3 days, then recovered steadily. Their weight differed significantly from the blank group but not the sPL group (Figure 2A and Table 1). Throughout the experiment, all rats grew well with no deaths observed.

Maxillary Expansion Distance

After the installation of the expansion appliance, with the increase in expansion duration, the maxillary arch width of rats in the model group increased significantly, and tooth movement was obvious. There was a significant difference between the model group and the blank group, but no significant difference between the model group and the sPL group (Figures 2B, C and Table 2).

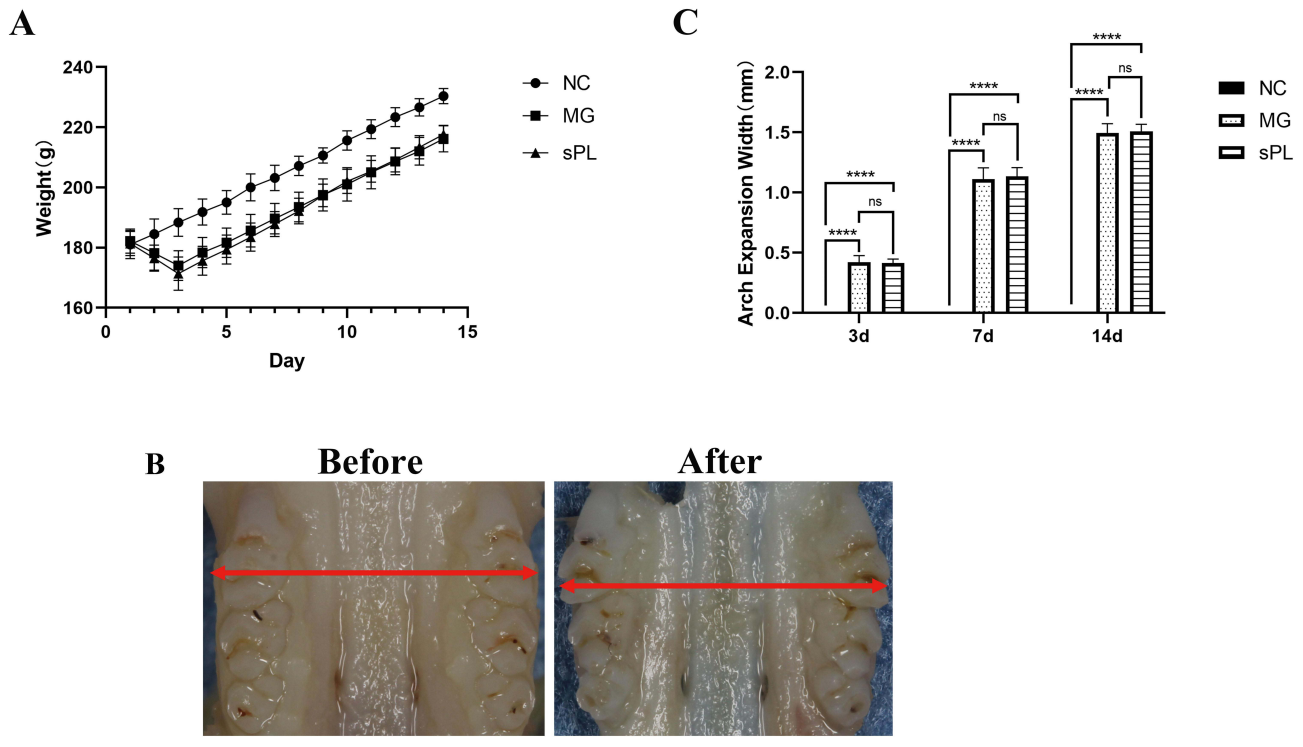

Figure 2 General conditions and maxillary expansion outcomes. (**A**) Body weight changes of rats in each group. (**B**) Representative photographs showing the maxillary arch width before and after expansion. (**C**) Quantitative measurement of the intermolar width.

Notes: Red arrows in B indicate the reference line for measuring the distance between the bilateral first maxillary molars in rats in the pre-intervention (Before) and post-intervention (After) samples, respectively. Values in A and C are expressed as the mean ± SD, and mean + SD, respectively. Significance levels are denoted as ****P < 0.0001.

Abbreviation: ns, not significant.

Table 1 Body Weight Changes of Rats in Different Experimental Groups After Maxillary Expansion (Days 1–14)

	NC	MG	sPL
1 days	181±4.73	182.17±4.02	181.33±4.03
2 days	184.5±4.97	178.17±5.67	176.5±4.32
3 days	188.33±4.63	174±4.98	171.33±5.57
4 days	191.83±4.36	178.33±5.01	175.67±4.84
5 days	195±3.95	181.67±4.89	179.33±4.8
6 days	200±4.47	185.67±5.43	183.5±4.68
7 days	203.17±4.22	189.67±5.09	187.83±4.12
8 days	207.17±3.19	193.5±4.93	192.17±4.26
9 days	210.67±2.58	197.5±5.32	197.33±3.78
10 days	215.67±3.2	201±5.55	202±4.05
11 days	219.33±3.14	205±5.51	205.33±3.61
12 days	223.33±3.14	208.67±4.41	209.17±3.97
13 days	226.67±2.88	212±4.65	213.33±3.88
14 days	230.33±2.58	216.17±4.36	217.67±3.01

Notes: Data are presented as the mean ± SD. Body weight was measured daily from day 1 to day 14 post-operation, and the unit is gram (g).
Abbreviations: NC, normal control group; MG, model group; sPL, sPL-treated group.

Table 2 Changes in Maxillary Arch Width of Rats After Expansion in Different Experimental Groups

Group	3 Days	7 Days	14 Days
NC	0±0	0.42±0.05	0.42±0.03
MG	0±0	1.11±0.09	1.14±0.07
sPL	0±0	1.5±0.08	1.51±0.06

Notes: Data are presented as the mean ± SD (unit: mm). Measurements were taken at 3, 7, and 14 days after expander installation.
Abbreviations: NC, normal control group; MG, model group; sPL, sPL-treated group.

HE Staining

Over 3, 7, 14 days of expansion, H&E staining showed (Figure 3A) distinct PDL remodeling across groups. The blank group maintained normal PDL structure without inflammation throughout. The model group exhibited progressive PDL damage and bone resorption, with partial recovery by day 14. The sPL group had milder PDL disruption on day 3 and accelerated structural recovery by day 14.

Significant differences in PDL width were detected between the model group and both the blank and sPL groups at all three time points of expansion (Figure 3B and Table 3).

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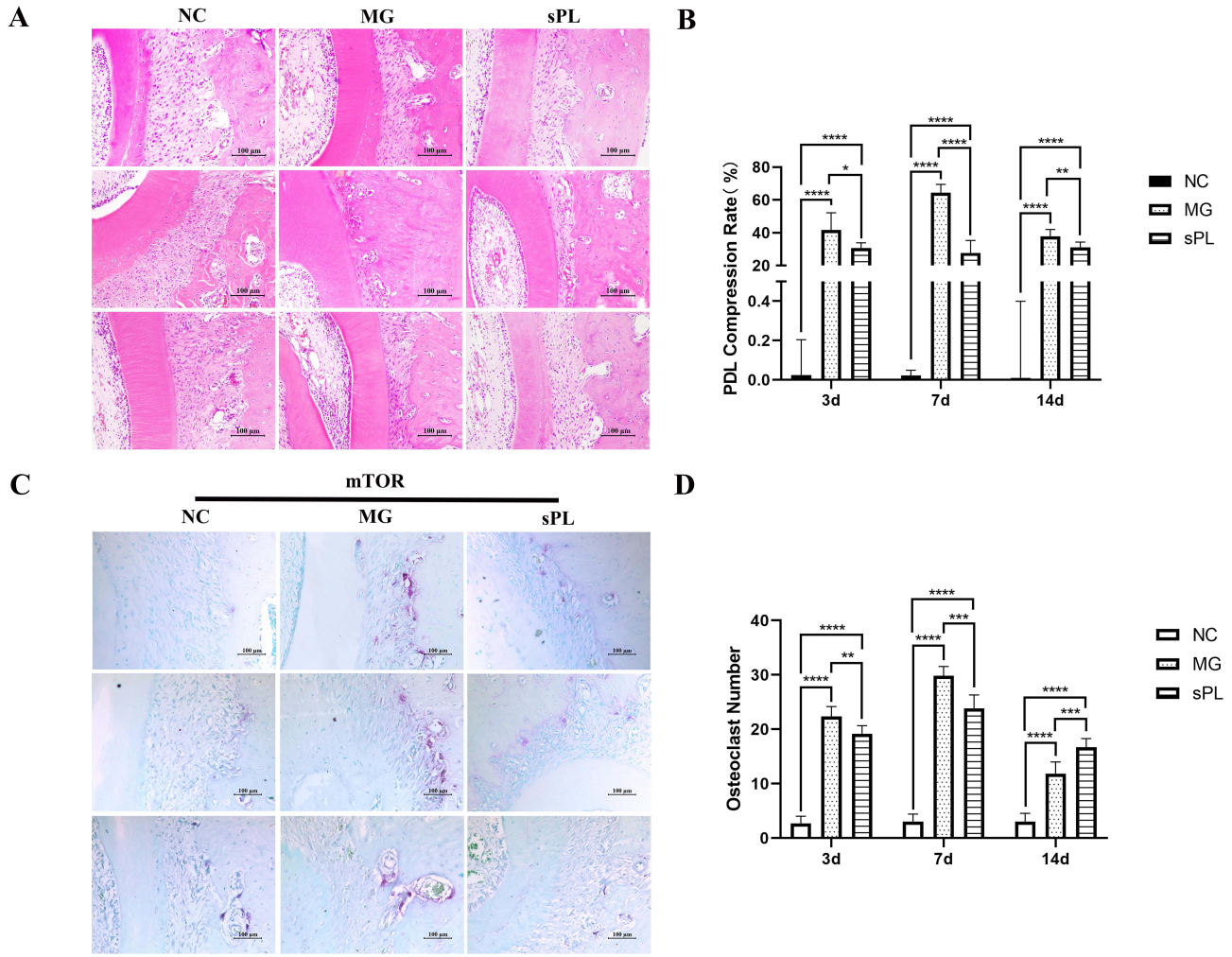

Figure 3 Histological evaluation of periodontal remodeling and osteoclast activity. (**A**) Representative H&E-stained sections of the periodontal ligament on the pressure side. (**B**) Statistical analysis of the PDL compression rate. (**C**) Representative TRAP-stained images identifying osteoclasts. (**D**) Quantitative analysis of osteoclast numbers. **Notes**: Values are expressed as the mean + SD.; Scale bars = 100 μm; Significance levels are denoted as *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Abbreviation: ns, not significant.

TRAP Staining

TRAP staining results (Figure 3C): No obvious TRAP-positive multinucleated osteoclasts were observed in the blank group at any time point (3, 7, or 14 days). In the model group, pressure-side osteoclast numbers increased on day 3, peaked on day 7, and declined on day 14, with cells localized to alveolar bone hyalinized zones. The sPL group showed

Table 3 Quantification of Periodontal Ligament Width and Osteoclast Count in Rats After Maxillary Expansion Across Experimental Groups

Group	Periodontal Ligament Width (μm)			$TRAP^+$ Osteoclast Count (cells/field)		
	3 Days	7 Days	14 Days	3 Days	7 Days	14 Days
NC	0.03±0.18	41.78±10.38	30.76±3.18	2.67±1.37	22.33±1.86	19.17±1.47
MG	0.02±0.03	64.4±5.03	27.73±7.54	3±1.41	29.83±1.72	23.83±2.48
sPL	0.01±0.39	37.8±4.24	31.14±3.15	3±1.55	11.83±2.14	16.67±1.63

Notes: Data are presented as the mean ± SD. Periodontal ligament width is expressed in micrometers (μm); $TRAP^+$ osteoclast count is expressed as the number of cells per high-power field. Measurements were taken at 3, 7, and 14 days after expander installation.
Abbreviations: NC, normal control group; MG, model group; sPL, sPL-treated group.

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the same temporal trend, but had significantly fewer osteoclasts than the model group on days 3 and 7, and significantly more on day 14 (Figure 3D and Table 3).

IHC Staining

Immunohistochemical analysis of LC3B (Figure 4A, B and Table 4). On day 3, model group LC3B expression was significantly higher than the blank group; sPL group LC3B was elevated but lower than the model group. On day 7, model group LC3B declined slightly but remained higher than the blank group, while sPL group LC3B was markedly reduced. By day 14, model group LC3B returned to blank group levels, whereas sPL group LC3B was significantly upregulated. The expression pattern of LAMP2 was consistent with that of LC3B (Figure 4C, D and Table 4). Conversely, mTOR expression exhibited an opposite trend to LC3B (Figure 4E, F and Table 4).The expression trend of LAMP2 was consistent with that of LC3B (Figures 4C and D), while the expression trend of mTOR was opposite to that of LC3B (Figures 4E and F).

IF Staining

Immunofluorescence results showed that LC3B was co-expressed in CD90-positive cells, and the co-localization signal of the two was mainly distributed in the periodontal ligament. The trend of positive LC3B expression in the model group and the sPL group on days 3, 7, and 14 was consistent with the trend observed in LC3B immunohistochemical staining (Figures 5A, B and Table 5).

Discussion

This study investigated the dynamic autophagic process in periodontal tissue and its regulatory mechanisms by sPL using a rat model of orthodontic maxillary expansion. Our results show that sPL alleviates the stress response of periodontal tissue induced by orthodontic force by modulating key autophagy-related proteins. These findings provide new experimental evidence for sPL as a potential adjunctive therapy to reduce orthodontic treatment-related side effects.

MTD is a common orofacial condition in children and adults, often treated with maxillary expansion. However, this procedure frequently causes complications such as alveolar bone loss and gingival recession, which impair clinical outcomes and long-term stability.[10] These adverse effects suggest that orthodontic forces may induce excessive stress and injury to periodontal supporting tissues during tooth movement.[11] Thus, understanding the biological responses of periodontal tissues to mechanical loading is crucial for developing targeted protective strategies and improving treatment safety.[12]

Autophagy, an evolutionarily conserved process that maintains cellular homeostasis under stress, has been increasingly studied in orthodontic force-induced periodontal remodeling.[13] When force is applied, the periodontal ligament (PDL) on the compression side undergoes structural disorganization and progressive compression, leading to mechanotransduction, followed by cellular ischemia and hypoxia.[14] These conditions rapidly activate autophagy, whereby damaged organelles and misfolded proteins are delivered in lysosomes to facilitate nutrient recycling and homeostasis restoration.[15] Studies in GFP-LC3 transgenic mice further show that autophagic activity is mainly elevated on the pressure side of tooth movement, implying a potential role of autophagy in compression-induced bone resorption rather than in tension-induced bone formation.[16] In areas with high orthodontic stress, excessive autophagy may trigger apoptosis and abnormal bone metabolism.[17] This could underlie the pathological mechanisms of common expansion-related sequelae, such as root resorption and horizontal alveolar bone loss.

OTM is widely practiced, but most rodent studies have focused on the mesiodistal direction.[18–20] To address this gap, we developed a novel transverse expansion device applying 50g light force, achieving stable buccal movement and arch width increase based on Martineli et al.[21] The PDL is a force-responsive tissue central to remodeling.[14] Wang et al reported reduced PDL cells and increased osteoclasts after OTM, with autophagic markers Beclin-1 and LC3-II peaking at 1 hour. Consistently, Wan et al[22] showed that cyclic tensile stress induces transient autophagy activation in human PDL cells, peaking at 3 hours.

Notably, oxidative stress during cardiac arrest[23] reveals a Beclin-1/LAMP2 dynamic that regulates autophagosome clearance - a mechanism potentially conserved in orthodontic force contexts. Blawat et al.[24] Also established mTOR's

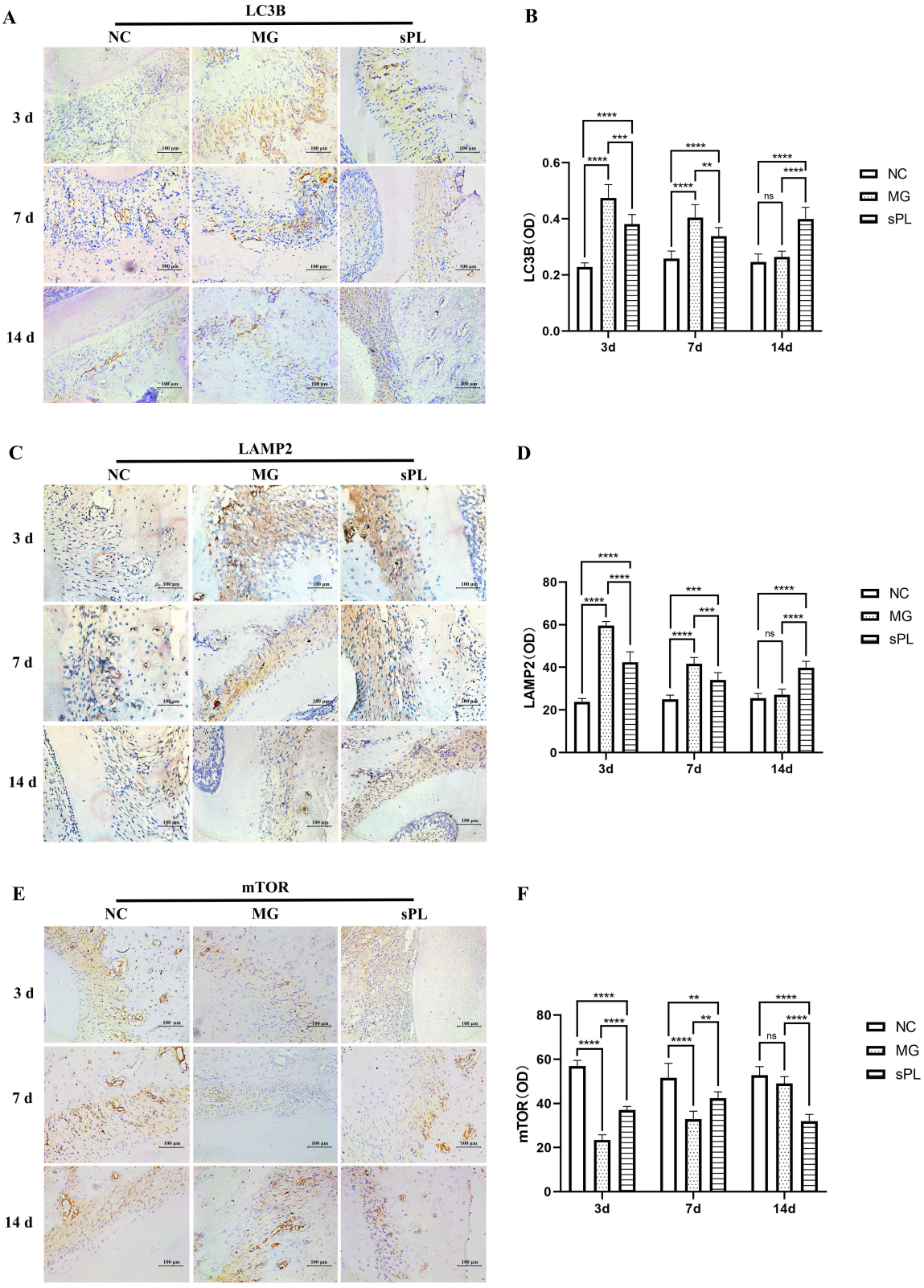

Figure 4 Immunohistochemical analysis of autophagy-related protein expression. (**A**) Representative IHC staining images showing the expression of LC3B. (**B**) Quantitative analysis of LC3B expression levels. (**C**) Representative IHC staining images showing the expression of LAMP2. (**D**) Quantitative analysis of LAMP2 expression levels. (**E**) Representative IHC staining images showing the expression of mTOR. (**F**) Quantitative analysis of mTOR expression levels.
Notes: Values are expressed as the mean + SD.; Scale bars = 100 μm; Significance levels are denoted as **P < 0.01, ***P < 0.001, ****P < 0.0001.
Abbreviation: ns, not significant.

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Table 4 Quantitative Immunohistochemical Analysis of Autophagy-Related Protein Expression in Periodontal Tissues of Rats from Different Experimental Groups Following Maxillary Expansion

Group	LC3B Expression (IOD)			LAMP2 Expression (IOD)			mTOR Expression (IOD)		
	3 days	7 days	14 days	3 days	7 days	14 days	3 days	7 days	14 days
NC	0.23±0.01	0.48±0.05	0.38±0.03	23.86±1.53	59.61±1.89	42.4±4.93	56.89±2.64	23.49±2.32	37.04±1.58
MG	0.26±0.03	0.41±0.05	0.34±0.03	25.06±1.89	41.68±2.96	34.11±3.36	51.59±6.58	32.91±3.61	42.37±2.93
sPL	0.25±0.03	0.27±0.02	0.4±0.04	25.6±2.15	27.07±2.68	39.8±3.05	52.84±3.88	49.13±3.03	32.06±2.98

Notes: Data are presented as the mean ± SD. Protein expression levels were determined by immunohistochemistry and are expressed as integrated optical density (IOD) values. Measurements were taken at 3, 7, and 14 days after expander installation.
Abbreviations: NC, normal control group; MG, model group; sPL, sPL-treated group.

role in regulating autophagy in mechanically stimulated PDL cells, hinglighting its key modulatory function across autophagosome nucleation, elongation, and maturation.[25] Collectively, these findings indicate that autophagic regulation in PDL stem cells is critical for tissue adaptation during orthodontic force application.

Mechanistically, sPL-derived growth factors (PDGF, TGF-β1, VEGF) temporally regulate autophagy via the mTOR pathway. PDGF activates mTOR through PDGFR, suppressing excessive autophagy at 3d.[26] TGF-β1 modulates mTOR time-dependently: at 14d, it downregulates PTEN to attenuate mTOR activity, promoting autophagic recovery.[27] VEGF contributes by activating mTOR via VEGFR2 and enhancing angiogenesis, indirectly supporting ordered autophagy.[28]

sPL is a promising periodontal regenerative material. Its growth factors can modulate autophagic pathways and upregulating osteogenic and angiogenic gene expression.[29–31] In our study, immunohistochemical analysis showed that sPL intervention restored autophagic homeostasis, as reflected by altered expression of key autophagy-related proteins (LC3B, LAMP2, mTOR), with positive signals mainly localized in the PDL. Immunofluorescence co-staining further demonstrated significant overlap between CD90 (a PDL stem cell marker) and LC3B in the pressure-side periodontium, indicating that PDLSCs are the primary cells with aberrant autophagy under orthodontic force. Importantly, local sPL administration effectively modulated this autophagic activity and improved periodontal tissue structure. These findings are consistent with reports on PRP, which enhances cell migration, proliferation, osteogenic differentiation, and autophagosome activation-specifically upregulating LC3B and Beclin-1 expression to promote autophagy-associated regeneration.[32] Thus, sPL emerges as a potential novel therapy to fine-tune autophagy, reduce orthodontic side effects, and improve treatment efficiency.

Notably, these preclinical findings hold significant implications for clinical orthodontic practice. Clinically, maxillary expansion-induced side effects (eg, alveolar bone loss and root resorption) remain major concerns affecting treatment safety and patient compliance.[33] sPL, as a bioactive material derived from platelets, has advantages of good biocompatibility and easy preparation, which is conducive to clinical translation. If validated in subsequent large animal and human-related studies, sPL could be developed into a localized adjunctive agent—for example, applied as a gel or membrane during maxillary expansion procedures to regulate PDLSC autophagy in situ. This would provide a targeted strategy to protect periodontal supporting tissues, reduce treatment-related complications, and improve long-term treatment stability, especially for patients with high risk of periodontal damage (eg, those with thin gingival biotype or initial alveolar bone loss). However, it is crucial to recognize that these promising implications are based on preclinical data, and the inherent limitations of the animal model employed in this study should be cautiously acknowledged, as they may constrain the direct translatability of current findings to clinical settings.

Although widely used in preclinical periodontal research, the rat model differs markedly from humans in periodontal tissue structure. Key disparities include gingival thickness, PDL fiber arrangement and alveolar bone density, which may alter the model's response to maxillary expansion and sPL intervention.[34] Moreover, autophagic regulatory pathways in rodent PDL stem cells (PDLSCs) do not fully recapitulate those in humans, as species-specific variations in the expression of autophagy-related genes.[35] Additionally, the standardized housing and dietary conditions of laboratory rats cannot replicate the complex clinical environment.[36] Key variables absent in laboratory settings, such as individual

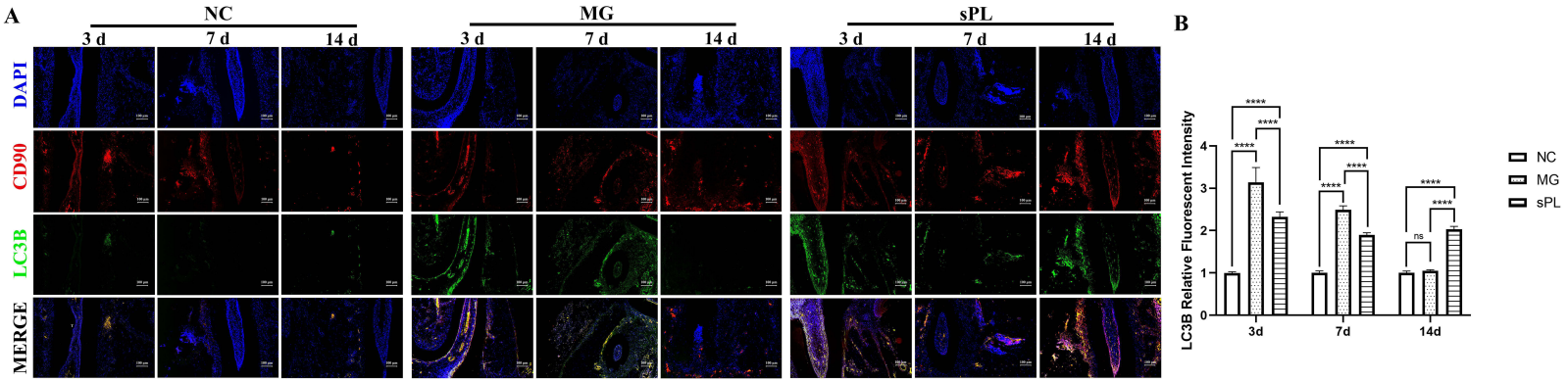

Figure 5 Immunofluorescence analysis of LC3B expression in CD90-positive periodontal ligament stem cells. (**A**) Representative IF images showing the co-localization of CD90 (red) and LC3B (green). Nuclei are counterstained with DAPI (blue). (**B**) Quantitative analysis of LC3B fluorescence intensity in CD90-positive cells.

Notes: Values are expressed as the mean + SD.; Scale bars = 100 μm; Significance levels are denoted as ****P < 0.0001.

Abbreviation: ns, not significant.

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Table 5 Quantitative Analysis of LC3B Fluorescence Intensity in Periodontal Tissues of Rats from Different Experimental Groups

Group	3 Days	7 Days	14 Days
NC	1±0.03	3.14±0.35	2.32±0.12
MG	1±0.05	2.49±0.09	1.89±0.06
sPL	1±0.05	1.05±0.03	2.03±0.07

Notes: Data are presented as the mean ± SD. LC3B fluorescence intensity was determined by immunofluorescence staining and quantified using ImageJ software in CD90-positive cell regions. Measurements were taken at 3, 7, and 14 days after expander installation.
Abbreviations: NC, normal control group; MG, model group; sPL, sPL-treated group.

differences in oral hygiene, systemic health status, and age-related alterations, can all modulate periodontal tissue responses and autophagic activity.[37]

Meanwhile, this study also has limitations regarding the clinical translatability of its rat model. Species-specific differences in periodontal anatomy and autophagy regulation limit direct extrapolation to humans,[38] and controlled experimental conditions cannot replicate individual patient variability.[39] The short expansion period may not fully reflect long-term clinical autophagy dynamics, and complex in vivo cellular interactions make it difficult to isolate PDLSC-specific autophagy from other cell types.[40]

Future work will adopt a stepwise approach: validate findings in large animal and disease models, and use in vitro CRISPR-Cas9-mediated gene editing on DPSCs to confirm autophagy's essential role. We will standardize DPSC preparation (isolation, culture, characterization) to reduce batch variations, explore gene-edited DPSCs integration, and evaluate their in vitro/ex vivo stability/therapeutic potential.[41] Translationally, individual blood composition variations[42] and lack of standardized protocolsremain challenges.[43] Subsequent research will focus on optimizing sPL concentration and developing personalized regimens to facilitate clinical translation.[44]

Conclusion

In conclusion, this study demonstrates that local sPL administration effectively modulates PDLSCs autophagic activity during orthodontic expansion by regulating key autophagy-related proteins (LC3B, LAMP2, mTOR). Specifically, sPL - upregulated LC3B/LAMP2 enhances autophagic flux to clear damaged components, while downregulated mTOR inhibits excessive cell stress—collectively reducing alveolar bone resorption, improving periodontal regeneration, and highlighting sPL's potential as an adjunctive therapy to alleviate orthodontic alveolar bone complications and boost treatment efficiency.

However, further in vitro mechanistic studies, large-scale preclinical (higher-order animal models) and human clinical trials are needed to confirm these findings, optimize sPL dosage/frequency, deepen understanding of its role in orthodontic tissue remodeling, and validate its translational potential.

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Each author fulfilled the criteria for authorship by making significant intellectual contributions to the study's conception, design, data acquisition, analysis, and/or interpretation. Additionally, every author contributed to the writing and critical revision of the article, endorsed the final version of the manuscript to be published, consented to its submission to this journal, and agrees to be accountable for ensuring the integrity and accuracy of the entire work.

Disclosure

The authors report no conflicts of interest in this work.

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