The Role of NF-kB in Mesenchymal Stem Cells during Orthodontic Tooth Movement

2023 Research Aid Awards (RAA)

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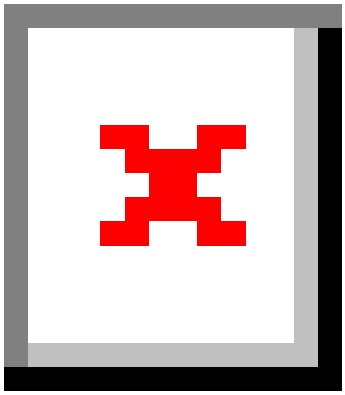
FollowUp Form

Award Information



In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*

The Role of NF-kB in Mesenchymal Stem Cells during Orthodontic Tooth Movement

Award Type

Research Aid Award (RAA)

Period of AAOF Support
July 1, 2023 through June 30, 2024

Institution
University of Pennsylvania

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
Dr. Hyeran Helen Jeon

Amount of Funding
$6,000.00

Abstract
(add specific directions for each type here)

Abstract

Title The Role of NF-kB in Mesenchymal Stem Cells during Orthodontic Tooth Movement

Orthodontic tooth movement (OTM) is a sterile inflammatory response induced by tissue damage caused by mechanical force. Orthodontic bone remodeling involves bone resorption by osteoclasts and new bone formation by osteoblasts in response to compression and tension respectively. It is mediated by a variety of inflammatory cytokines, RANKL, osteoprotegerin (OPG), and transcription factors such as RUNX2. The periodontal ligament (PDL) between teeth and alveolar bone contains stem cells supporting tissue turnover. Gli1+ cells in adult mouse PDL are multipotential stem cells, giving rise to PDL, alveolar bone, and cementum. They support periodontium tissue turnover and injury repair and maintain the homeostasis of PDL during OTM. Gli1+ cells express RANKL under compression and are proliferated and differentiated into osteoblastic cells under tensile force. Gli1 inhibition blocked OTM significantly, confirming an important role of Gli1+ cells on orthodontic bone remodeling. However, little is known on how stem cells in PDL regulate the mechanical force-induced bone remodeling in vivo.

The nuclear factor kappa B (NF-kB) is a central regulator of inflammation and bone homeostasis and controls cell proliferation, apoptosis, and differentiation. NF-κB induces the expression of various proinflammatory genes, including those encoding cytokines and chemokines. Our previous study showed that NF-kB expression increases on compression and tension side during OTM. Inhibition of NF-kB in PDL fibroblasts and osteoblasts significantly blocked OTM with reduced osteoclastogenesis and decreased RANKL expression. Interestingly, a recent study supports that the sterile inflammation-induced bone resorption must precede the anabolic response to tensile force through a coupling mechanism. As osteoclastogenesis is largely regulated by inflammatory markers, we will investigate if orthodontic force increases osteoclasts formation through activation of inflammatory markers via NF-kB. We hypothesize that blocking the inflammatory markers with NF-kB inhibition might inhibit osteoclastogenesis, reduce bone resorption and subsequent new bone formation.

Therefore, we propose a central hypothesis that the NF-kB activation in MSCs is essential in mechanical force-induced bone remodeling by i) increasing the proinflammatory cytokines, which initiates osteoclastogenesis

and subsequent coupled new bone formation. We will test this hypothesis by lineage-specific deletion of NF-kB in MSCs using the genetically modified mice. The hypothesis will be tested using the following aim:

Aim 1. To evaluate the effect of lineage specific NF-kB deletion in MSCs during the orthodontic tooth movement. We will examine both Gli1-CreERT2+.IKKβL/L and Gli1-CreERT2-.IKKβL/L mice. To induce Cre activity, we will administer tamoxifen orally to 8-week-old mice for 5 consecutive days. For control we will give corn oil only instead of tamoxifen in the same manner. One week after the last administration we will carry out orthodontic tooth movement with 10-week-old mice. An orthodontic appliance delivering 10 g of force will be placed between the right first molar and both incisors to displace the first molar mesially in both Gli1-CreERT2+.IKKβL/L and Gli1-CreERT2-.IKKβL/L mice. The contralateral unloaded sides will serve as the control. The animals will be equally divided into two different time points: 5 and 12 days of orthodontic loading (n = 8 for each group). Quantitative measurement will be performed with microCT, tartrate-resistant acid phosphatase (TRAP) stain, and immunofluorescence stain using the antibodies against NF-kB p65, RANKL, IL-1β, Runx2, and osteocalcin.

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".
OR
Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

RESULTS.pdf
This manuscript has been submitted and is currently being revised. We uploaded the results separately.

Were the original, specific aims of the proposal realized?*

Firstly, we examined Gli1-CreERT2+ ROSA26/Ai9 mice for lineage tracing. Mesenchymal stem cells (MSCs) are genetically altered in those mice to permanently express TdTomato with tamoxifen administration, allowing us to map these cells' fate in vivo. We confirmed that this Cre-Lox system functioned effectively in these mice.

Secondly, we successfully generated the experimental Gli1-CreERT2+.IKKβL/L and control Gli1-CreERT2-.IKKβL/L mice. Tamoxifen was administered as previously described, and NF-kB expression was examined using immunofluorescence staining. Our lab has established mouse orthodontic tooth movement models and published several papers (PMID: 36013326, 29483595, 33852725). However, we encountered issues managing the mouse breeder cages. Sample collection is ongoing, and we are awaiting more pups. The upcoming resident will take over and continue the project.

Thirdly, while waiting for more samples, Macy Cruz participated in the project "The Comparison of Bone Remodeling during Maxillary Expansion between Young and Middle-Aged Mice" to investigate the age-related MSC responses to mechanical force. Maxillary expansion procedure was conducted on 6-week-old (young) and 12-month-old (middle-aged) mice with 25g of force. Mice were euthanized at 0, 3, 7, and 14 days and examined using MicroCT, TRAP stain, immunofluorescence stain, and TUNEL assay. Results showed that both age groups had successful midpalatal suture openings. However, young mice exhibited earlier and more

intense osteoclast activity, higher RUNX2 and osteocalcin expression, and a higher percentage of Gli1+Ki67+ cells, while middle-aged mice showed increased MSC apoptosis. We have submitted our findings to the journal 'Bone' and are currently in the revision stage. We presented our findings at the 2024 AAO William R. Proffit Resident Scholar Award and won 3rd place. The AAOF support was acknowledged in the presentation.

Comment: *The AAOF commends you on completing this project successfully and on being recognized for the impact of your work at the AAO meeting. We encourage you to continue your pursuit of knowledge that advances our specialty and hope you will seek funding from the AAOF for future studies.*

Were the results published?*

No

Have the results of this proposal been presented?*

Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*

Firstly, the AAOF RAA has significantly supported our research project, aiding in generating experimental mouse strains: Gli1-CreERT2+.ROSA26/Ai9 and Gli1-CreERT2+.IKKβL/L mice. Additionally, it facilitated the purchase of reagents for the experiments. During initial delays in obtaining experimental samples, we completed the additional project, yielding valuable data. We believe our findings will enhance the understanding of MSC responses during orthodontic tooth movement and maxillary expansion and aid in developing personalized orthodontic treatments.

Accounting: Were there any leftover funds?

$0.00

Not Published

Are there plans to publish? If not, why not?*

We have submitted our findings to the journal 'Bone' and are currently in the revision stage.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*

Tittle:
Comparison of Bone Remodeling in Midpalatal Suture during Maxillary Expansion between Young and Middle-Aged Mice

Authors:
Hyeran Helen Jeon1*, Mary Cruz Contreras Salas1, Kyungjoon Park2, Lindsay Fisher2, Sara Ha2, Fionna Chan2, Dana T. Graves3

The 2024 AAO Annual meeting, New Orleans

Was AAOF support acknowledged?

If so, please describe:

Yes

Internal Review

Reviewer comments

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

- RESULTS.pdf

3. RESULTS

3.1. Suture gap, Intermolar widths, Bone Volume and Bone Volume Fraction

In response to tensile force, the width of the midpalatal suture notably expanded from day 0 to day 14 in both young and middle-aged mice ($p < 0.05$) (Table 1 and Figure 3A-3C). Initially, in young mice, the suture width measured 0.09 ± 0.01 mm, increasing to 0.19 ± 0.04 mm by day 14 ($P < 0.05$). Similarly, in middle-aged mice, the suture width was 0.06 ± 0.01 mm on day 0, reaching 0.25 ± 0.03 mm by day 14 ($P < 0.05$). Notably, on day 7 ($P < 0.05$, oral third) and day 14 ($P < 0.05$, middle and nasal thirds), the suture width in middle-aged mice significantly exceeded that in young mice. However, differences in suture widths between young and middle-aged mice were statistically insignificant on days 0 and 3 ($P > 0.05$). Intermolar widths at the crown level showed a significant increase from day 0 to day 14 in both young and middle-aged mice ($p < 0.05$), whereas no significant changes were observed at the root level.

Table 1. MicroCt Measurements. *Y: 6-week-old mice and A: 12-month-old mice

Mice		Suture width (mm)			Intermolar Width (crown) (mm)	Intermolar Width (root) (mm)	BV/TV
Duration	Group	Oral third	Middle third	Nasal third			
Day 0	Y	0.09 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	2.74 ± 0.02	3.05 ± 0.06	0.54 ± 0.02
	A	0.06 ± 0.01	0.04 ± 0.004	0.05 ± 0.01	3.01 ± 0.01	3.09 ± 0.06	0.68 ± 0.01
Day 3	Y	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.005	3.14 ± 0.10	3.18 ± 0.10	0.47 ± 0.01
	A	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	3.17 ± 0.04	3.18 ± 0.06	0.64 ± 0.02
Day 7	Y	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	3.04 ± 0.07	2.99 ± 0.09	0.52 ± 0.03
	A	0.14 ± 0.02	0.14 ± 0.03	0.14 ± 0.03	3.18 ± 0.08	3.12 ± 0.12	0.57 ± 0.03
Day 14	Y	0.19 ± 0.04	0.17 ± 0.04	0.19 ± 0.04	3.17 ± 0.06	3.22 ± 0.07	0.43 ± 0.02
	A	0.25 ± 0.03	0.30 ± 0.03	0.31 ± 0.04	3.53 ± 0.06	3.37 ± 0.07	0.40 ± 0.05

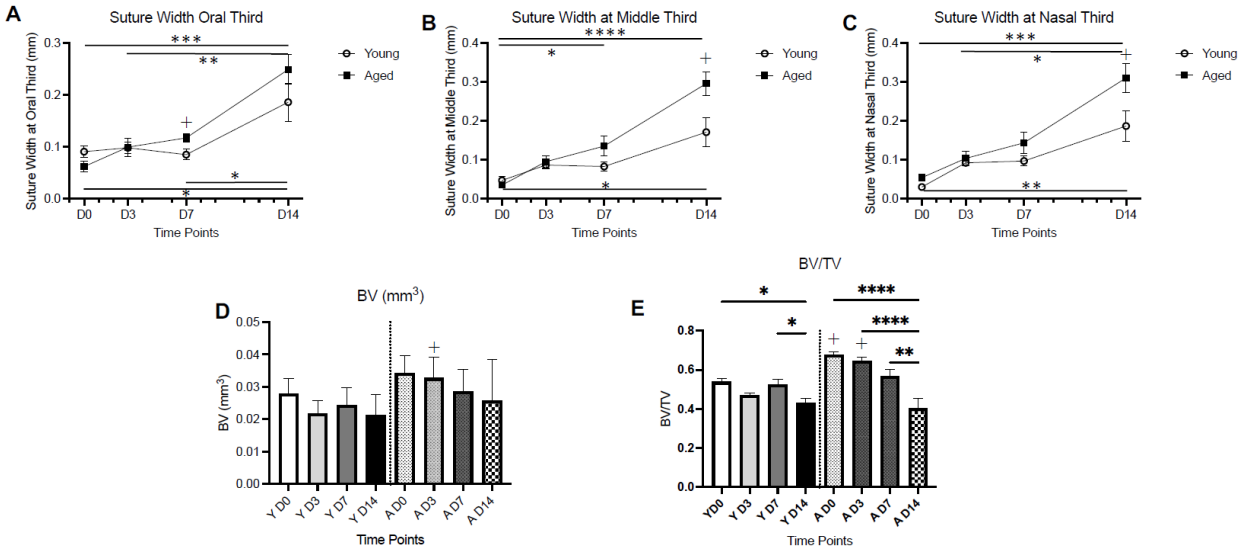


Figure 1. MicroCT measurements. *(A-C) Suture width (oral, middle, and nasal thirds). (D) Bone volume (mm^3). (E) Bone volume fraction. *P<0.05, **P<0.01, ****P<0.0001, $^+$P<0.05 vs. matched time point between young and middle-aged mice.*

In addition, we examined the bone volume and bone volume fraction (BV/TV) at the level of the maxillary first molars bilaterally (Figures 3D and 3E). The middle-aged mice had a higher BV/TV compared to the young mice on days 0 and 3 ($p<0.05$), which is consistent with findings in the TRAP-stained samples (Figure 4A). Both age groups showed a significant decrease in BV/TV over the entire experimental period ($P<0.05$ for young mice and $P<0.0001$ for middle-aged mice). The middle-aged mice presented a more dramatic decrease in BV/TV than the young mice from day 0 to 14.

3.2. Osteoclast Formation

Osteoclast formation was examined along the bone surface in the midpalatal suture, periosteal membrane and bone marrow area in the TRAP-stained sections (Figures 4A and 4B). Interestingly, there was rare osteoclast formation along the bony surfaces in the midpalatal suture. In young mice osteoclasts were predominantly located in the bone marrow area and along the periosteum, while osteoclasts in middle-aged mice were primarily located along the periosteum. In the bone marrow area, osteoclastogenesis peaked on day 3 in young mice, while the osteoclast formation in the middle-aged group reached a maximum on day 7 and decreased thereafter. On day 3 young mice demonstrated significantly more osteoclast formation than middle-aged mice in the bone marrow area ($p<0.05$). Along the periosteal membrane, Osteoclast formation in young mice peaked on day 3, maintained until day 7 and decreased thereafter, while middle-aged mice showed a peak on days 7 and 14 ($p>0.05$). The difference in the osteoclast number between young and middle-aged mice was not statistically significant along the periosteal membrane ($p>0.05$).

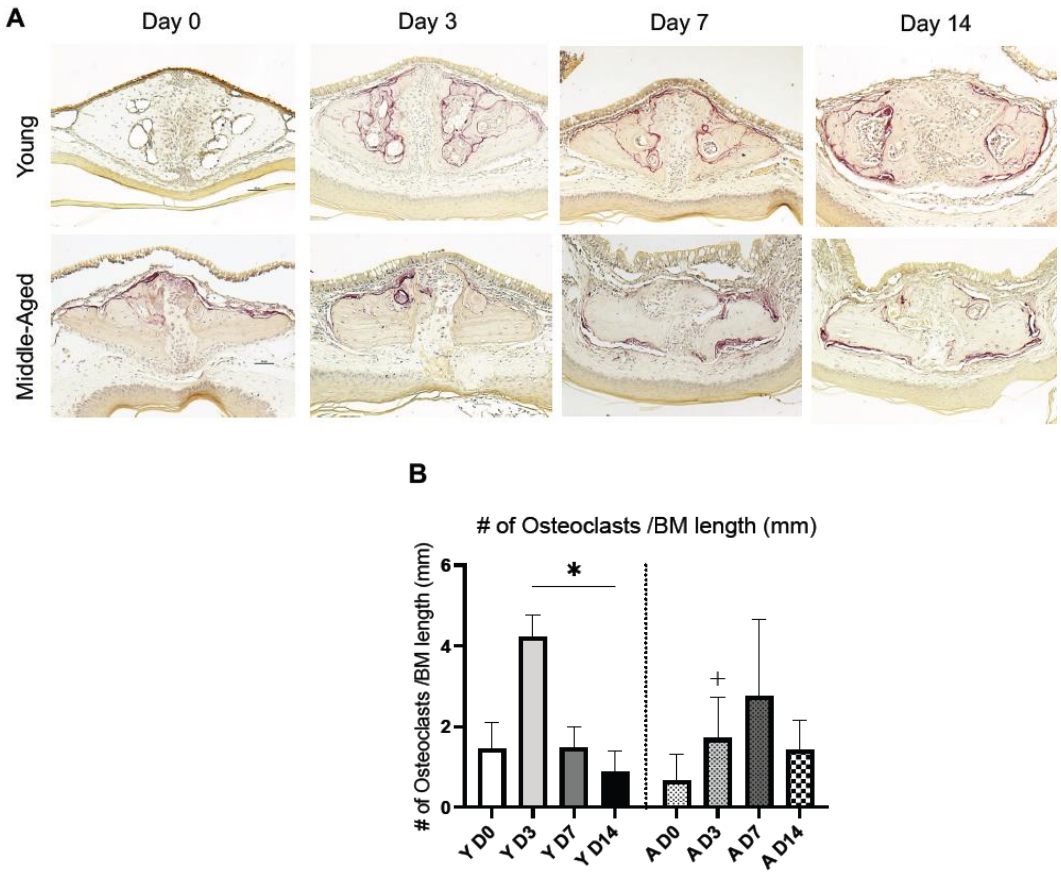


Figure 2. Osteoclast formation. *(A) TRAP-stained images (x20). Bar, 50 μm. (B) The number of osteoclasts in the bone marrow area. *P<0.05, ⁺P<0.05 vs. matched time point between young and middle-aged mice.*

3.3. Expression of RUNX2 and Osteocalcin

To investigate the osteogenesis process during maxillary expansion, we assessed the expression of RUNX2 and osteocalcin, early and late osteoblast markers, in the midpalatal suture area (Figure 5A-C). The young mice demonstrated a significant increase in the percentage of RUNX2-immunopositive cells in the midpalatal suture on day 14 compared to day 0 ($p<0.05$). The middle-aged mice presented a slight increase until day 7, but generally lower expression level compared to the young mice group ($p>0.05$). Similarly, the expression of osteocalcin-immunopositive cells in young mice is higher than in middle-aged mice. The difference on day 7 was statistically significant ($p<0.05$).

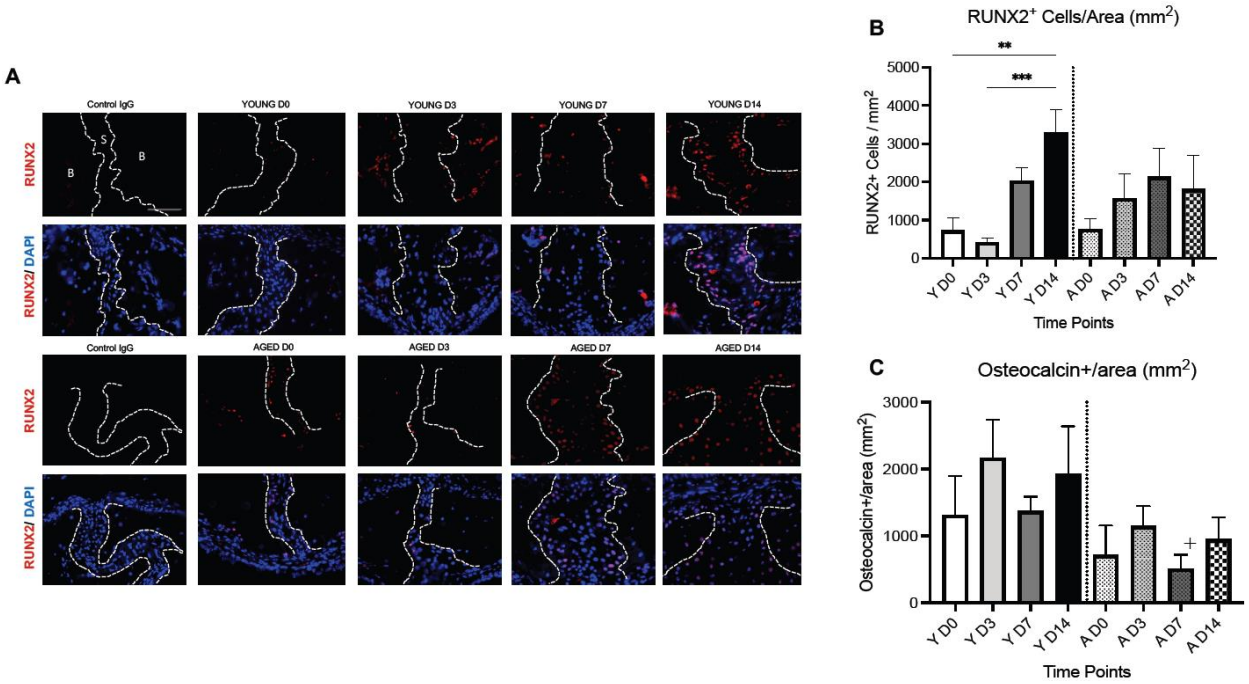


*Figure 3. RUNX2 Immunofluorescence stain. (A) Representative images in the midpalatal suture. B: bone and S: midpalatal suture. 40x. Bar, 50 μm. (B) The percentage of RUNX2⁺ cells per area. (C) The percentage of osteocalcin⁺ cells per area. **P<0.01, ***P<0.001. ⁺P<0.05 vs. matched time point between young and middle-aged mice.*

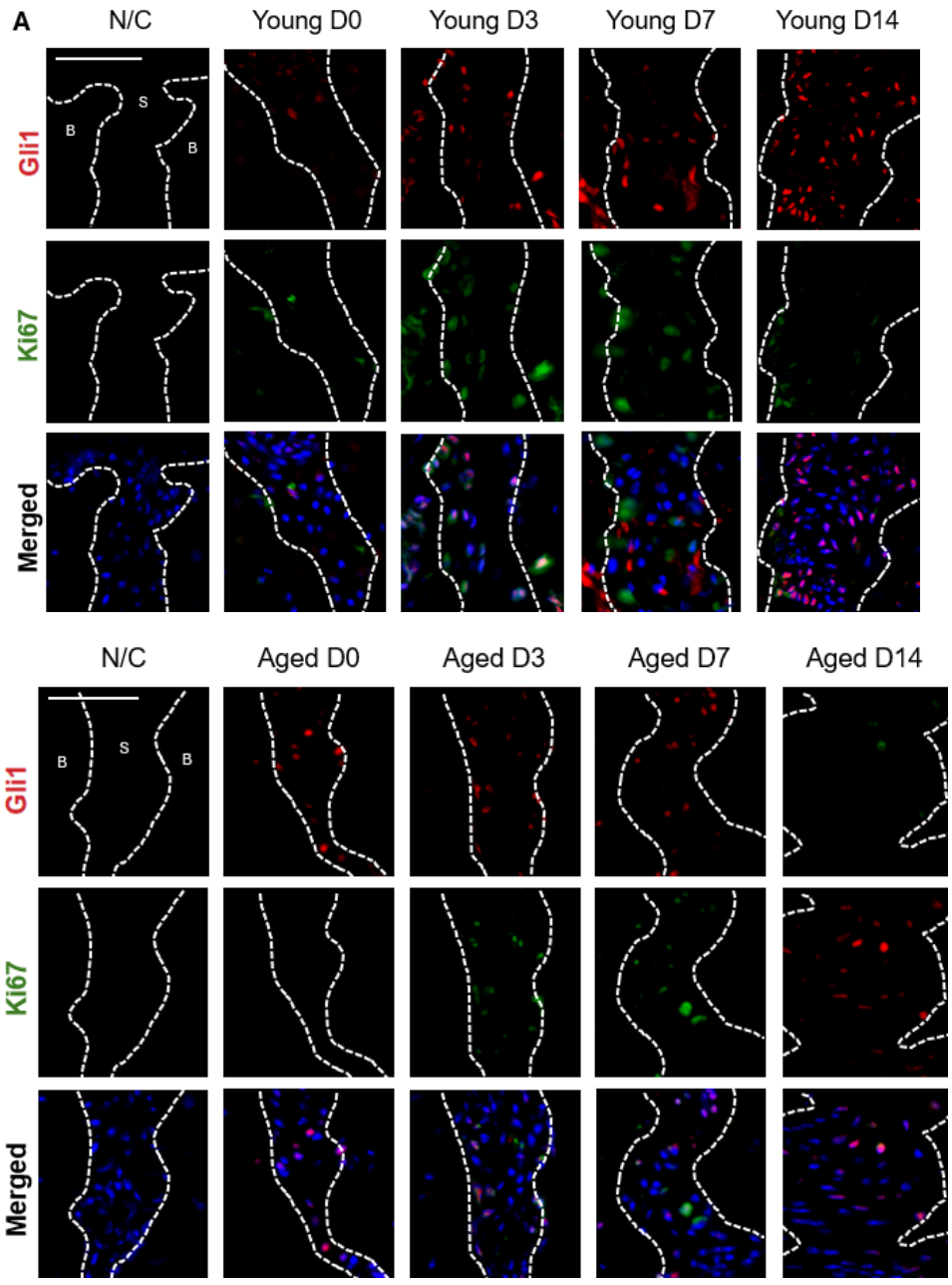
3.4. MSC Proliferation and Apoptosis

Cell proliferation, differentiation, and apoptosis are critical during tissue remodeling [92]. The young mice demonstrated that the percentage of Gli1-immunopositive cells significantly increased on day 3, slightly decreased on day 7, and then increased until day 14 after maxillary expansion (Figures 6A and 6B). In contrast, the middle-aged mice demonstrated an initial increase of Gli1-immunopositive cells on day 3 and decreased thereafter.

The young mice demonstrated that the percentage of Ki67-immunopositive cells significantly increased on day 3 and gradually decreased (Figure 6C). The middle-aged mice showed a slight increase in Ki67-immunopositive cells ($p>0.05$). On day 3 the percentage of Ki67-immunopositive cells in young mice is significantly higher compared to middle-aged mice ($p<0.05$).

The percentage of Gli1+Ki67+ double immunopositive cells showed a similar pattern with the expression of Ki67 (Figure 6D). The young mice had a significant increase on day 3 and decreased thereafter. The middle-aged mice presented a slight increase in the percentage of Gli1+Ki67+ immunopositive cells on day 3 and remained similar level. The percentage of Gli1+Ki67+

immunopositive cells in young mice was significantly higher than in middle-aged mice on day 3 ($p<0.05$).



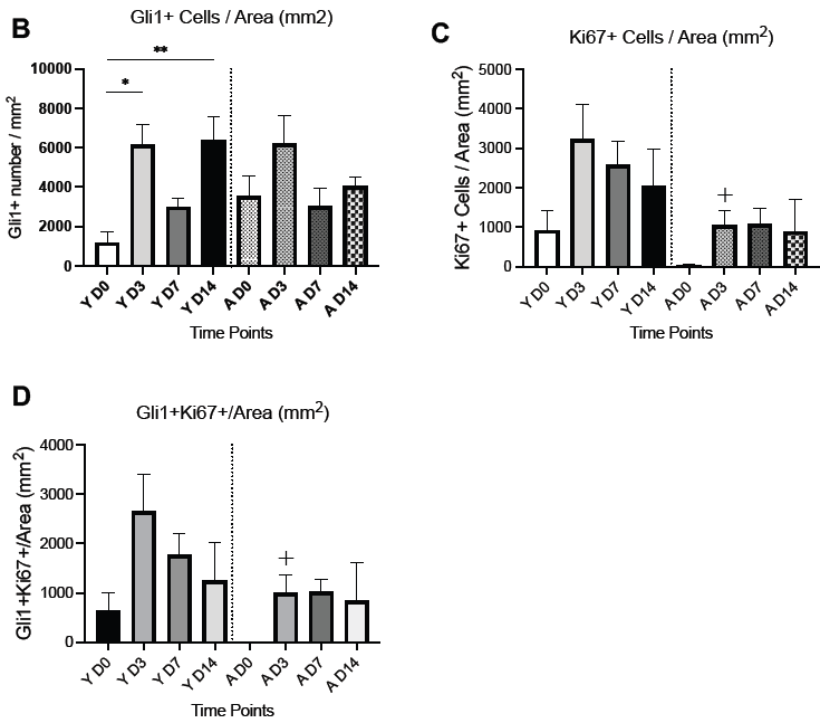
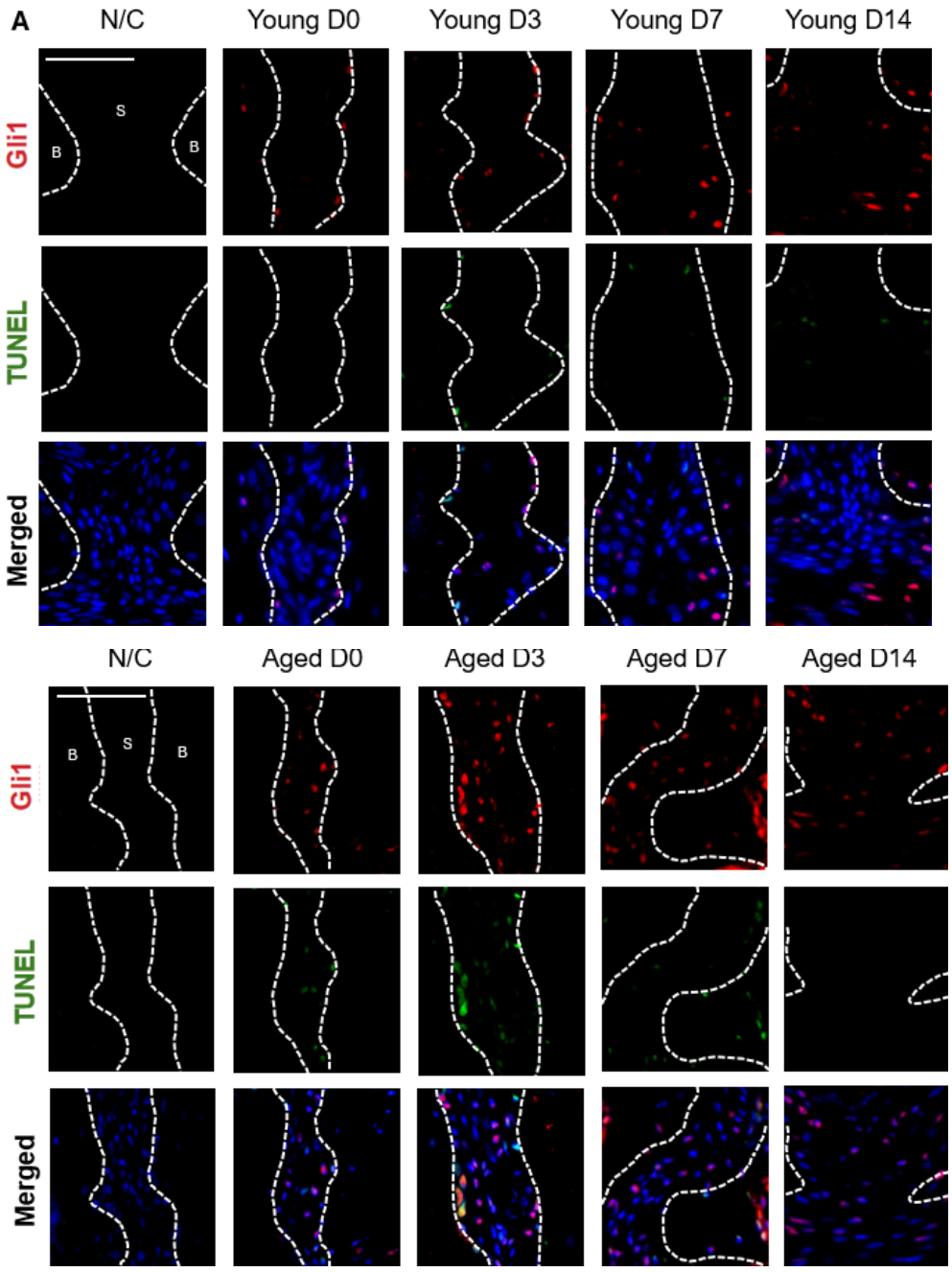


*Figure 4. Gli1/Ki67 Immunofluorescence stain. (A) Representative images in the midpalatal suture. B: bone and S: midpalatal suture. 40x. Bar, 50 μm. (B) The percentage of Gli1-immunopositive cells. (C) The percentage of Ki67-immunopositive cells. (D) The percentage of Gli1/Ki67-immunopositive cells. *P<0.05, **P<0.01. +P<0.05 vs. matched time point between young and middle-aged mice.*

In contrast, the percentage of TUNEL-positive cells increased on day 3 and decreased thereafter in both young and middle-aged mice (Figure 7A-C). The middle-aged mice group showed much higher TUNEL expression on day 3, compared to young mice ($p>0.05$). Similarly, the percentage of Gli1+TUNEL+ immunopositive cells peaked on day 3 and decreased thereafter in young and middle-aged mice. The middle-aged mice presented a higher expression of Gli1+Ki67+ immunopositive cells than young mice on day 3 ($p>0.05$).


A
N/C
Young D0
Young D3
Young D7
Young D14
Gli1
TUNEL
Merged
S
B
B
N/C
Aged D0
Aged D3
Aged D7
Aged D14
Gli1
TUNEL
Merged
B
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B

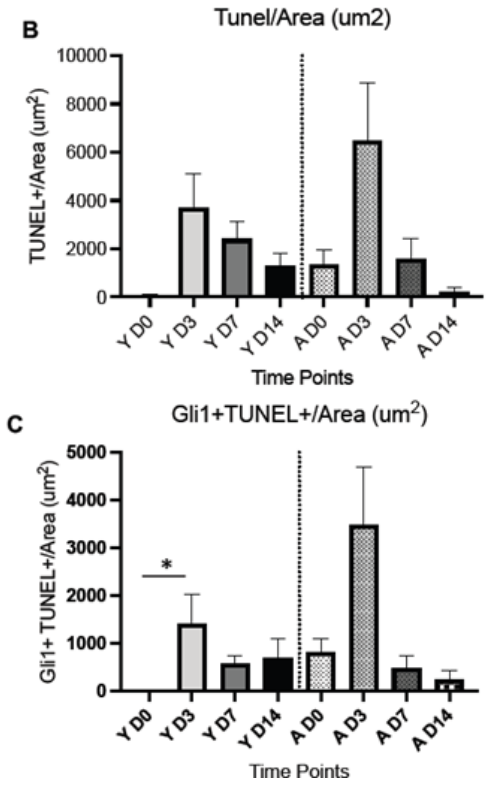

*Figure 5. Gli1/TUNEL Immunofluorescence stain. (A) Representative images in the midpalatal suture. B: bone and S: midpalatal suture. 40x. Bar, 50 μm. (B) The percentage of TUNEL-positive cells. (C) The percentage of Gli1/TUNEL-immunopositive cells. *P<0.05.*