Novel roles of chondrocytes in the normal growth of maxilla and rapid palatal expansion

2022 Biomedical Research Awards (BRA)

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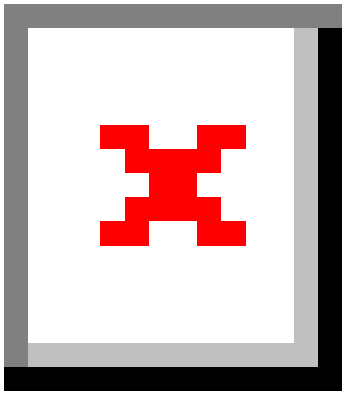
FollowUp Form

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:*

Novel roles of chondrocytes in the normal growth of maxilla and rapid palatal expansion

Award Type

Biomedical Research Award (BRA)

Period of AAOF Support
July 1, 2022 through June 30, 2024

Institution
Texas A&M College of Dentistry

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
PI: Yan Jing; Consultant: Peter Buschang

Amount of Funding
$30,000.00

Abstract
(add specific directions for each type here)

Rapid palatal expanders (RPE) are tools frequently utilized by orthodontists in cases with a constricted maxillary arch. They rely on expansive forces that separate the two palatine processes of the maxilla at the mid-palatal suture. RPEs are typically held in place for 6 months post expansion for stabilization of the suture. Since much of the expansion is due to dental and dentoalveolar tipping, there is a high probability of relapse. Treatments should accelerate the bone formation while increasing the expansion, so that the outcome will be more stable.

It has been shown that the mid-palatal suture of mice/rats mainly consists of secondary cartilage, and that tensional force applied to the suture induces new bone formation. However, the mechanism that controls this process is still unclear. Without a better understanding of the underlying mechanism of bone growth in response to sutural expansion, it is impossible to enhance the treatment effects.

Our long-term goal is to develop efficient strategies to accelerate the rapid palatal expansion during orthodontic/orthopedic treatment and to improve retention. The overall goal of this proposal is to determine the role of chondrocytes during normal maxillary bone growth and during mid-palatal expansion on mice. By using cell lineage tracing, our recent studies on TMJ condyle have demonstrated that chondrocytes directly contribute to TMJ condyle formation by transdifferentiating into bone cells. Moreover, the chondrocyte transdifferentiation is very sensitive to the alteration of TMJ mechanical loading. To demonstrate if the chondrocytes in mid-palatal suture function in a similar mechanism, we carried out a series of in vivo experiments using states-of-the-art techniques. We found multiple layers of chondrocytes at the mid-palatal suture, with numerous chondrocyte-derived bone cells in the adjacent bone in normal growing mice. In further, decreased loading in craniofacial area induced by soft diet dramatically reduced the chondrogenesis and chondrocyte-derived osteogenesis in mid-palatal suture.

Our central hypothesis, based on these findings, is that chondrocytes in mid-palatal suture directly participate in the growth of maxilla via a cell transdifferentiation mechanism, which is greatly accelerated in response to mid-palatal expansion.

Our study is significant because it will: 1) shed new light on the cellular and molecular mechanisms underlying maxillary bone growth and expansion during orthodontic and orthopedic treatment and 2) lay the foundation for developing novel approaches to accelerate rapid palatal expansion and enhance post treatment stability.

We plan to attain this goal by using a well-established mid-palatal expansion model on mice, combined with cell lineage tracing approach. In particular, we will evaluate the contribution of chondrocytes to maxillary

bone formation during normal state and mid-palatal expansion with lower and higher forces. We expect to find enhanced chondrogenic activity, followed by increased numbers of chondrocyte-derived bone cells with increasing amounts of force.
Our group is well-suited for this study since the PI (Jing) has the knowledge and expertise in craniofacial biology and orthodontics needed to successfully complete the proposed work. Dr. Jing will put 15% of her effort on research plan, including the management, design and execution of the project, and manuscript preparation. Dr. Buschang (consultant) will provide valuable suggestions on data interpretation and statistics. One technician in Dr. Jing's lab will assist with animal care, sample and data collection, ordering reagents, and daily management.

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".

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In the progress report in December 2022, we requested to modify our study goal, which was approved. The revised aim is "To determine the critical role of chondrocytes during normal postnatal maxillary bone growth and the long-term response to the decrease in masticatory force."
Animals
Twelve 3-week-old male Aggrecan-CreERT2 (AcanLineage); R26RTdTomato; 2.3Col1a1-GFP were randomly divided into two groups. Each group (n=6) maintained normal mice (hard diet, the control group) or powder food (soft diet, the experimental group) for 6 weeks. One time tamoxifen injections were given at 3 weeks. The sample size was based on published estimates assuming a power of 0.95 and alpha of 0.05, with a 10% difference and effect size of 2.4.
The research of interest (ROI) for μCT and cell transdifferentiation is the bone 100 μm away from the suture between the mesial side of the lingual roots of the 1st and 2nd molar on each side. The ROI for chondrogenesis is the suture between the mesial side of the lingual roots of the 1st and 2nd molar.

Results
There was no statistical significance between-group difference in weight prior to diet modification. During weeks four through six, mice from the soft food diet group weighed significantly less than the control (Fig.1A). However, there were no statistically significant differences between groups in total weight change from the start to end of the experiment (Fig.1B).

Soft Diet Demonstrated narrower maxillary Measurements and lower bone quality
The soft food mice had significantly less palatal width measured at MP (mesial-lingual cusp of 1st molar), AC (alveolar crest at the 1st molar), and AP (apex of mesial lingual root of 1st molar) than the control mice (Fig.2A). The 3D μCT image showed the palatal bone adjacent to mid-palatal suture of the soft food diet mice was more porous than the control bone (Fig.2B). Quantitatively, both bone volume (BV) and the bone volume to total volume ratio (BV/TV) were significantly reduced with significant less trabecular thickness but larger trabecular space in the soft food diet group, while there was no significant difference in trabecular number between groups (Fig.2C). In summary, the radiological analysis revealed narrower maxilla with reduced bone quality among mice fed softer diets.

The Chondrogenic Activity was Decreased in the Condyles from Soft Diet Mice

The confocal images showed fewer EdU+ cells in the mid-palatal sutures of soft diet mice than hard diet fed mice (Fig.3A), and quantitative results confirmed no significant difference in cell proliferation between groups (Fig.3D). The level of Aggrecan expression was similar in the mid-palatal suture of between groups (Fig.3B) with no significant difference of Aggrecan+ area to cartilage area (Fig.3E). However, Col10a1 expression in the mid-palatal suture was much lower with the soft diet compared to the hard diet (Fig.3C), with the ratio of Col10a1+ area to cartilage area significantly decreased (Fig.3F).
The number of Osx+ chondrocytes significantly decreased with a much lower density of Osx+ in cartilage in the soft diet group (Fig.4A white arrows & 4B). Additionally, the number of AcanLineage-differentiated Osx+ chondrocytes also significantly decreased, resulting in a much lower density of AcanLineage-differentiated Osx+ chondrocytes in mid-palatal suture (Fig.4B). In summary, these results show that a consistent soft diet had a mild effect on chondrocyte proliferation and differentiation, but significant impacts on chondrocyte maturation.

Soft Diet Reduces the Transdifferentiation of Chondrocytes into Bone Cells
We used both endogenous (2.3Col1a1-GFP expressed in mature osteoblasts and preosteocytes) and IHC (Osx expressed in osteoprogenitors, and DMP1 expressed in mature osteocytes) markers to evaluate the effect of dietary loading on chondrocyte transdifferentiation from early to late-stage bone cells. The confocal images of Osx IHC showed numerous red cells in the trabecular bone adjacent to mid-palatal suture of the control mice (Fig.4A, red arrows). Many displayed yellow nuclei, indicating cell transdifferentiation from chondrocytes to Osx+ bone cells. However, the number of red bone cells in the soft diet group was limited. The quantitative results confirmed significantly less AcanLineage-Osx+ bone cells in the soft diet mice, with no significant difference in non-AcanLineage-Osx+ bone cells to the control mice. The number of total Osx+ bone cells was also decreased in the soft diet group, although there were no statistically significant differences between groups. These results led to a lower ratio of AcanLineage-Osx+ bone cells to total Osx+ bone cells approaching significant difference (Fig.4C).
Confocal images of 2.3Col1a1-GFP illustrated similar qualitative results. In the subchondral bone of hard diet mid-palatal, there were many red and yellow chondrocyte-derived GFP+ bone cells (Fig.5A, white arrows), the number of which was much lower in the soft diet condyles. The quantitative results confirm the qualitative results. AcanLineage-derived 2.3Col1a1-GFP cells as well as total GFP+ bone cells were significantly higher in the hard diet group than soft diet group, whereas the number of non-AcanLineage-derived 2.3Col1a1-GFP cells was not significantly different between groups. This results in a non-significant difference in the ratio of AcanLineage-derived 2.3Col1a1-GFP cells to total 2.3Col1a1-GFP cells between groups (Fig.5B).
Lastly, we colocalized the cell lineage tracing with the DMP1 IHC signal. The confocal images showed numerous AcanLineage-derived-DMP1+ osteocytes in the control mice (Fig.5C, white arrows) but just a few in the soft diet mice. The quantitative data showed significantly fewer AcanLineage-derived-DMP1+ osteocytes in the soft food diet group compared to the control. There were no significant differences in non-AcanLineage-derived-DMP1+ osteocyte numbers between groups. There were also no significant differences in total DMP1+ cells with the different dietary loads, although the soft diet group was lower than control mice. The ratio of AcanLineage-derived-DMP1+ cells to total DMP1+ cells did not show a statistically significant difference between groups (Fig.5D).
Conclusion
The present study showed that chondrocytes in mid-palatal suture contribute to the transverse growth of maxilla by directly transdifferentiating into bone cells. A decrease in dietary loading inhibits chondrocyte maturation and cell transdifferentiation into early to late stage of osteogenic cells, resulting in reduced maxillary bone quality and width (Fig.6).
PS: The system does not allow me to upload files larger than 2MB. I have to save the images to a smaller size. If you need to view images with higher quality, please let me know.

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

Yes.

Were the results published?*
No

Comment: *The AAOF commends you on your success in grant funding but also encourages you to present and publish your scientific results to 1) contribute to advancing knowledge in orthodontics and 2) show evidence of your productivity in funded research.*

Have the results of this proposal been presented?*
No

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*
Some of the data obtained in this project have been used in my new BRA application due in October 2024. I am also preparing the manuscript with the data generated in this proposal for the application of 2025 Hellman, Sicher, Graber Research Awards.
Till now, I have received 3 awards from AAOF and 1 RAA supervised by me. The data generated from these studies not only helped me harvest more funding from NIH as a PI or Co-I, but also provides new research directions. As a junior faculty, I sincerely acknowledge the great supports from AAOF on my research career development.

Accounting: Were there any leftover funds?
$0.00

Not Published

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

Not Presented

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

Yes.

Internal Review

Reviewer comments

Reviewer Status*

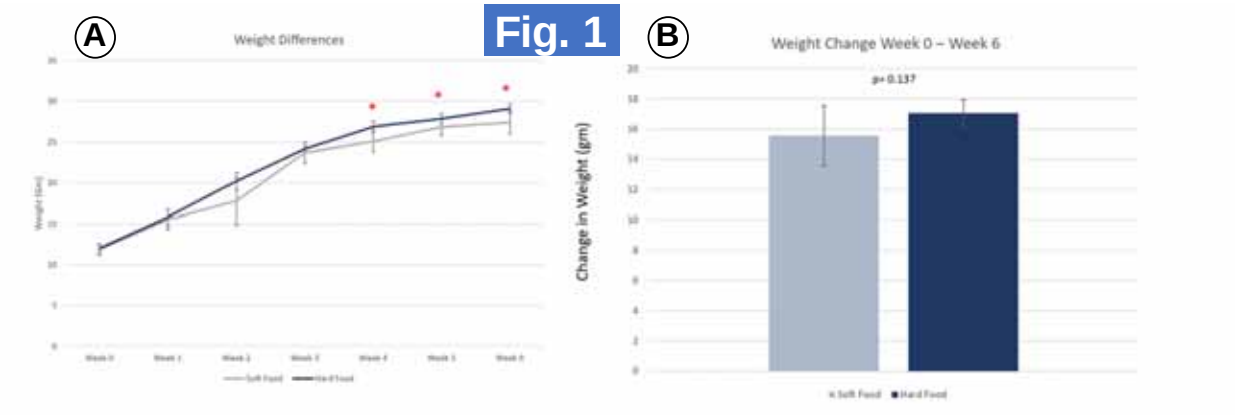
Approved

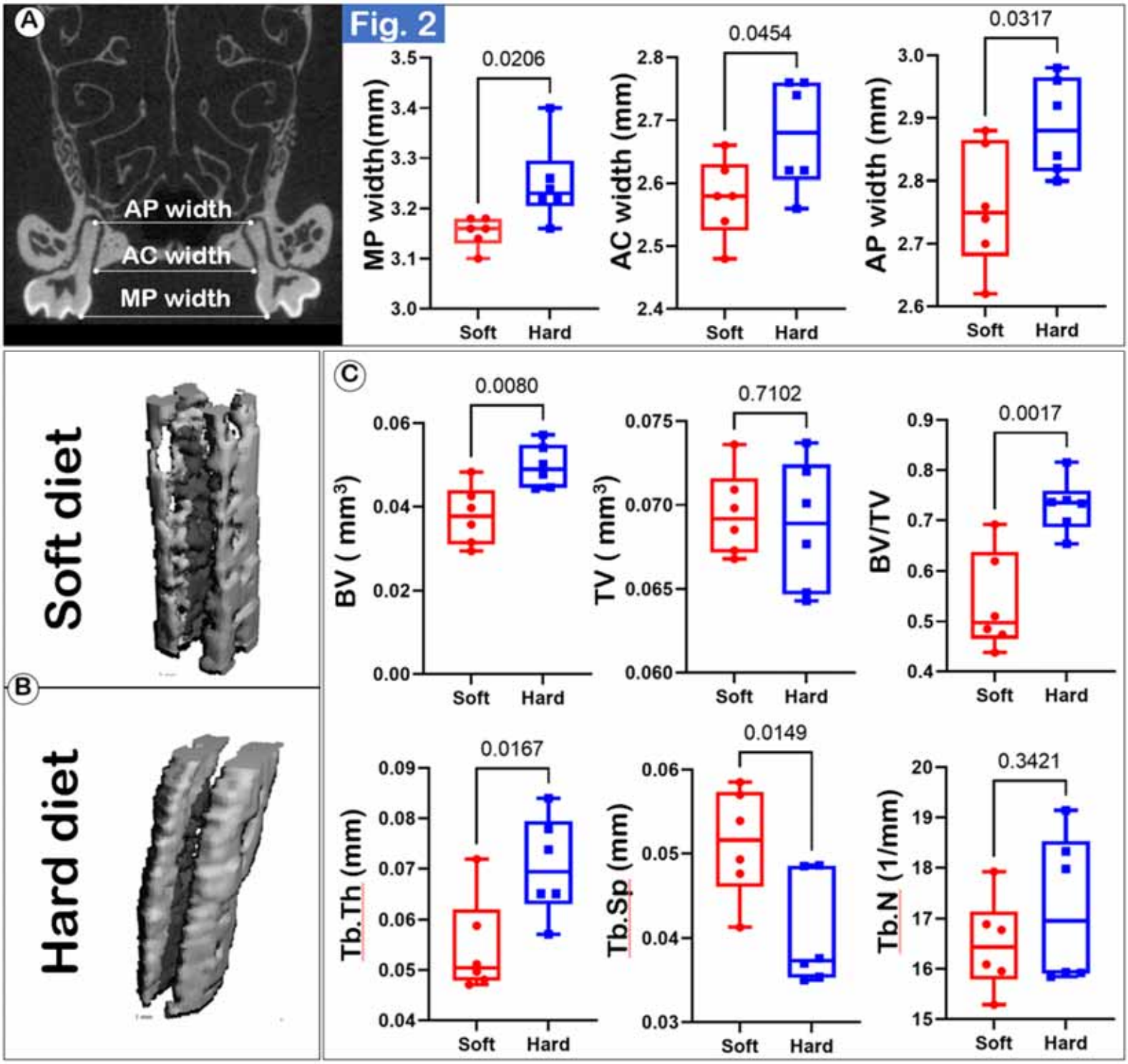
File Attachment Summary

Applicant File Uploads

- 20240620-final report-4.pdf

Fig. 1


A
Weight Differences
B
Weight Change Week 0 – Week 6
p= 0.137
Change in Weight (gm)


A
AP width
AC width
MP width
Fig. 2
MP width(mm)
0.0206
Soft
Hard
AC width (mm)
0.0454
AP width (mm)
0.0317
B
Soft diet
Hard diet
C
BV (mm3)
0.0080
TV (mm3)
0.7102
BV/TV
0.0017
Tb.Th (mm)
0.0167
Tb.Sp (mm)
0.0149
Tb.N (1/mm)
0.3421

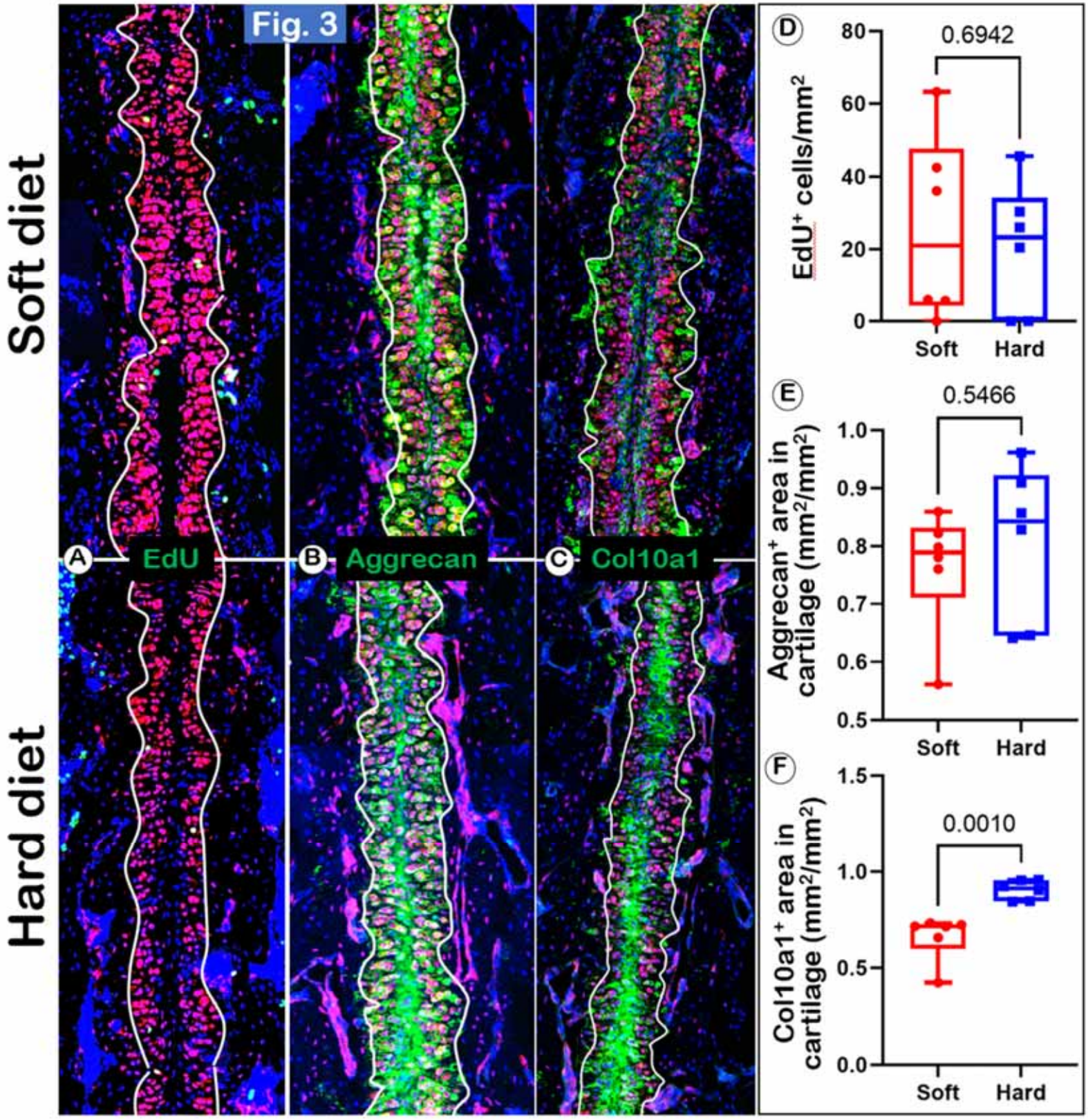
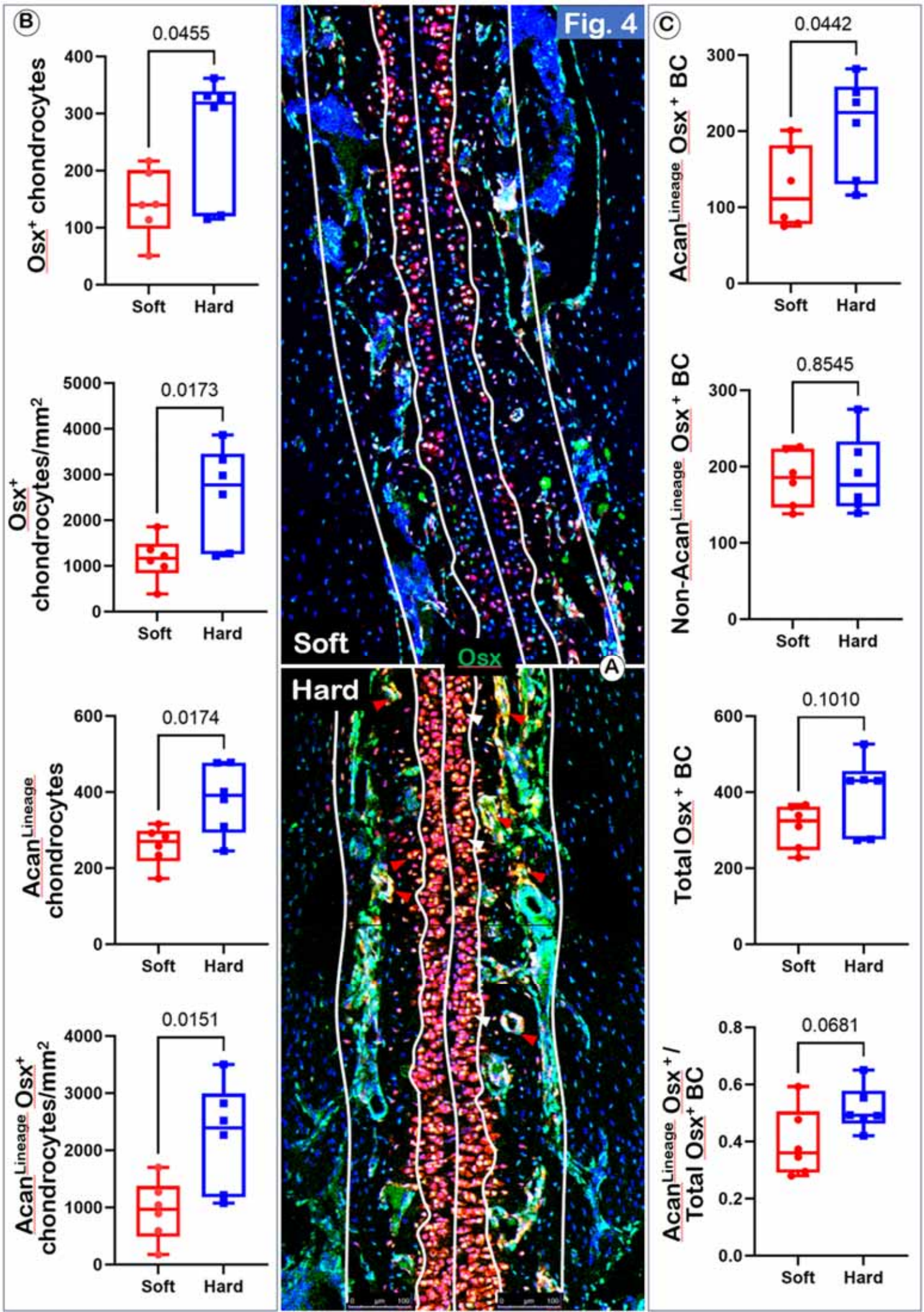


Fig. 3
Soft diet
Hard diet
A
EdU
B
Aggrecan
C
Col10a1
D
EdU⁺ cells/mm²
80
60
40
20
0
0.6942
Soft
Hard
E
Aggrecan⁺ area in cartilage (mm²/mm²)
1.0
0.9
0.8
0.7
0.6
0.5
0.5466
Soft
Hard
F
Col10a1⁺ area in cartilage (mm²/mm²)
1.5
1.0
0.5
0.0
0.0010
Soft
Hard


B
0.0455
Osx+ chondrocytes
400
300
200
100
0
Soft
Hard
0.0173
Osx+ chondrocytes/mm2
5000
4000
3000
2000
1000
0
Soft
Hard
0.0174
AcanLineage chondrocytes
600
400
200
0
Soft
Hard
0.0151
AcanLineage Osx+ chondrocytes/mm2
4000
3000
2000
1000
0
Soft
Hard
Fig. 4
Soft
Osx
A
Hard
C
0.0442
AcanLineage Osx+ BC
300
200
100
0
Soft
Hard
0.8545
Non-AcanLineage Osx+ BC
300
200
100
0
Soft
Hard
0.1010
Total Osx+ BC
600
400
200
0
Soft
Hard
0.0681
AcanLineage Osx+ / Total Osx+ BC
0.8
0.6
0.4
0.2
0.0
Soft
Hard

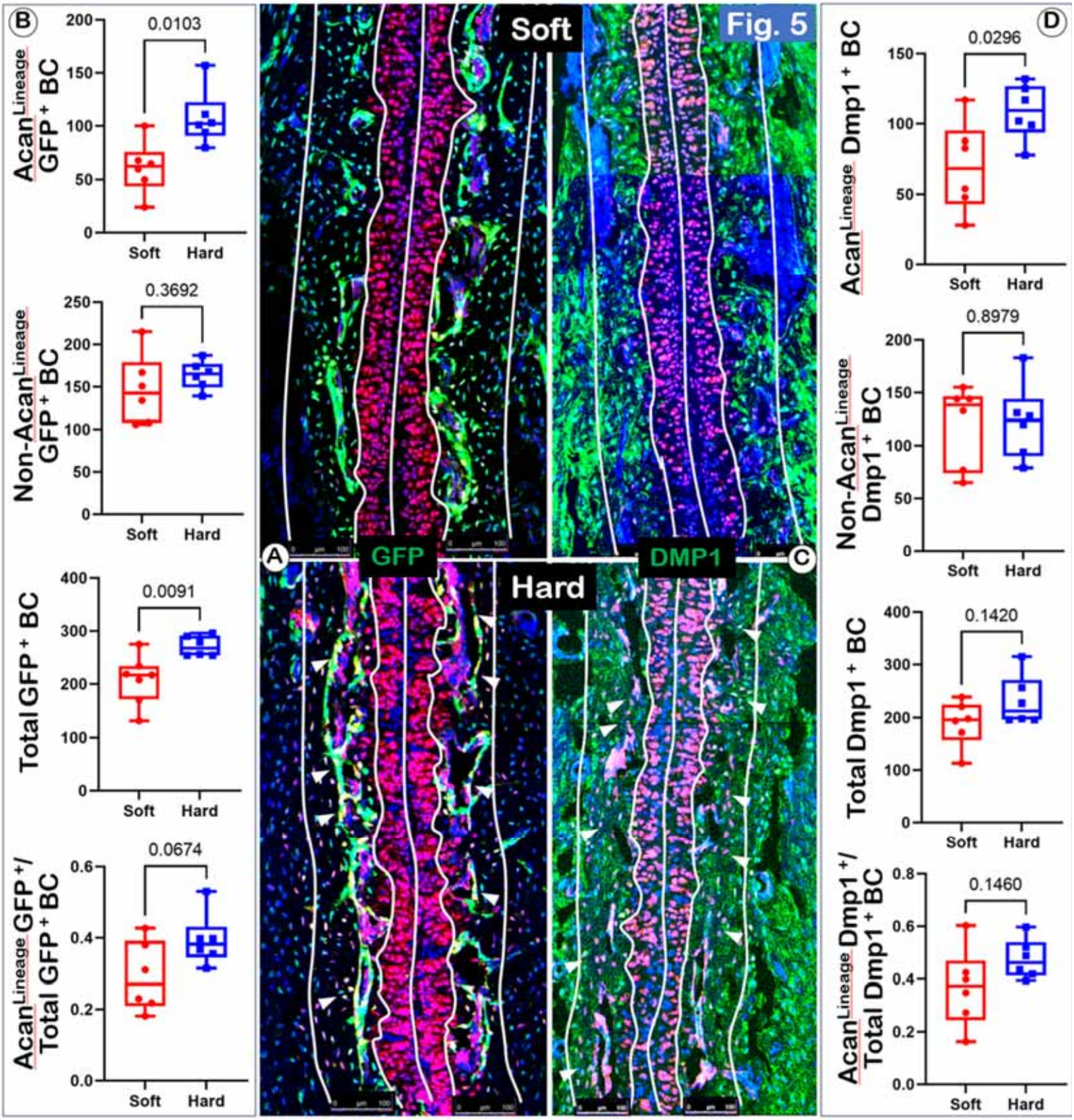
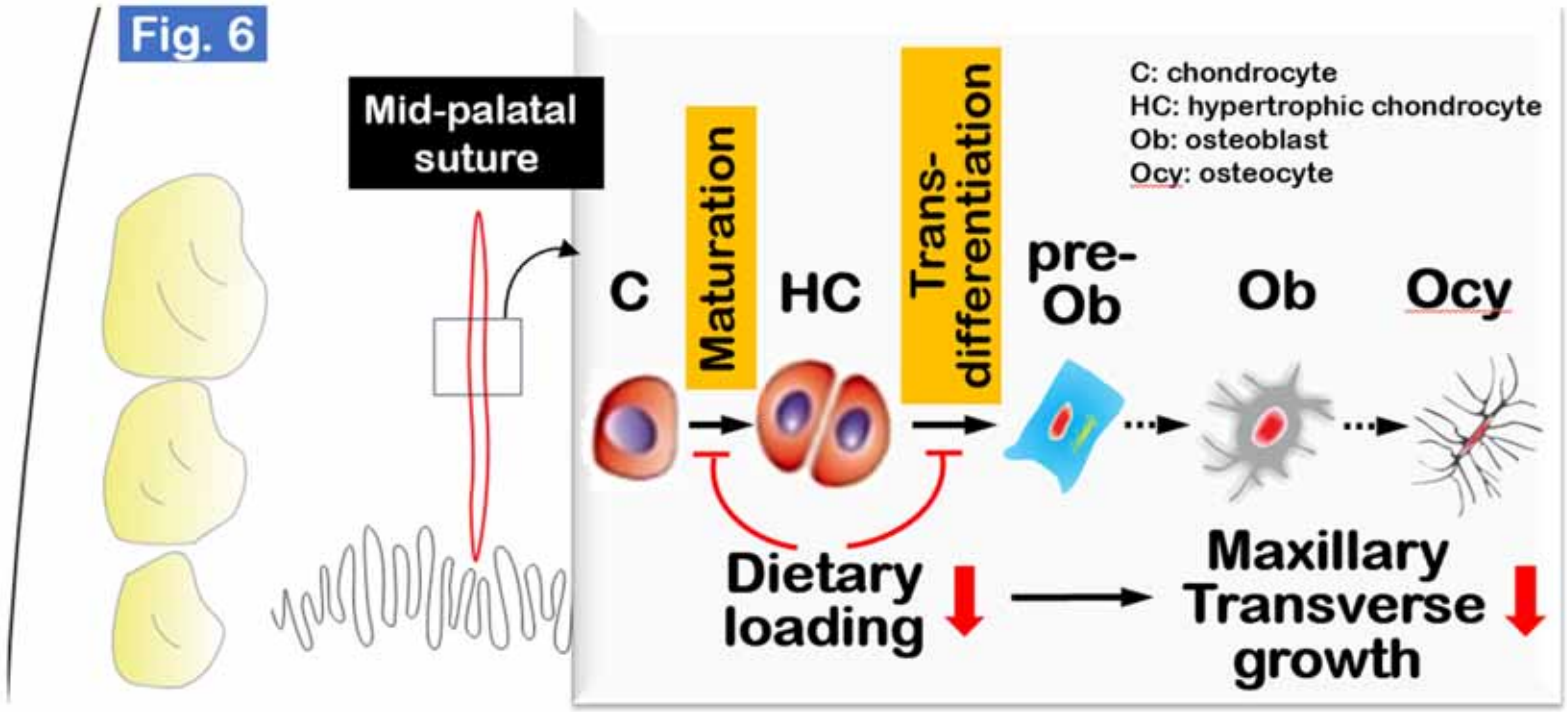


Fig. 5
Soft
Hard
GFP
DMP1
A
C
B
D
Acan Lineage GFP+ BC
0.0103
Soft
Hard
Non-Acan Lineage GFP+ BC
0.3692
Soft
Hard
Total GFP+ BC
0.0091
Soft
Hard
Acan Lineage GFP+/ Total GFP+ BC
0.0674
Soft
Hard
Acan Lineage Dmp1+ BC
0.0296
Soft
Hard
Non-Acan Lineage Dmp1+ BC
0.8979
Soft
Hard
Total Dmp1+ BC
0.1420
Soft
Hard
Acan Lineage Dmp1+/ Total Dmp1+ BC
0.1460
Soft
Hard

Fig. 6


Mid-palatal suture
C: chondrocyte
HC: hypertrophic chondrocyte
Ob: osteoblast
Ocy: osteocyte
C
Maturation
HC
Trans-differentiation
pre-Ob
Ob
Ocy
Dietary loading
Maxillary Transverse growth