The effect of metaphyseal holes and interposition material on the longitudinal growth stimulation of long bones in a rabbit model

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Cite this article:
Bone Joint Res 2025;14(3): 236–244.

DOI: 10.1302/2046-3758.143.BJR-2024-0321.R2

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Aims
It remains unclear which factors influence overgrowth of the tibia, resulting from the metaphyseal hole created during anterior cruciate ligament (ACL) reconstruction in skeletally immature patients. This study aimed to investigate the effects of growth stimulation by creating a metaphyseal hole in a rabbit model, based on its distance from the physis and type of interposition material.

Methods
In Experiment 1, 38 skeletally immature male New Zealand white rabbits were randomized into one of four groups: a metaphyseal hole created at 5, 10, or 15 mm distal to the physis of the left proximal tibia with the hole filled with bone wax, or a sham control group. In Experiment 2, after establishing the distance associated with the most overgrowth, a defect was created at 10 mm distal to the physis in 20 additional rabbits, which were randomly assigned to have the defect filled with Tisseel, or be left unfilled. The rabbits were euthanized six weeks postoperatively.

Results
The length and rate of overgrowth were higher in the groups with holes drilled 5 and 10 mm distal to the physis compared to the sham group. A significant increase in new bone width was observed in the 10 mm distal hole group. Growth stimulation in both radiological and histological analyses was not significant in the unfilled and Tisseel groups. Valgus deformities were observed in all groups with metaphyseal holes compared to the sham group.

Conclusion
Creating a metaphyseal hole 10 mm distal to the physis of the proximal tibia can significantly stimulate longitudinal growth and induce tibia valga in rabbits; however, the effect varies depending on the type of interposition material. Notably, overgrowth was significant only in the group with holes filled with bone wax. Based on this study, the location of the metaphyseal hole during ACL reconstruction or physeal bar excision could be optimized.

Bone & Joint Research

Article focus

- To examine the effect of metaphyseal hole creation based on its location and type of interposition material on subsequent growth stimulation using a rabbit model.
- To understand the factors associated with overgrowth related to length discrepancy or valgus deformity of the tibia, resulting from the metaphyseal hole created during anterior cruciate ligament reconstruction in skeletally immature patients.

Key messages

- Creating a metaphyseal hole at 10 mm distal to the physis of the proximal tibia with the use of bone wax as an interposition material significantly stimulated longitudinal growth compared to the sham group.
- Valgus deformities were observed in all groups with metaphyseal holes created, regardless of their location and the type of interposition.

Strengths and limitations

- This study performed radiological and histological analyses to compare the overgrowth length, new bone width, and growth rate of the tibia based on the location of the metaphyseal hole and the type of interposition material.
- Our results are limited to a rabbit model and cannot be simply applied in clinical settings; large animal models should be designed to investigate the effect of more subdivided holes.

Introduction

Skeletally immature patients may experience overgrowth leading to length discrepancy or valgus deformity of the tibia following anterior cruciate ligament (ACL) reconstruction, whether through transphyseal or physeal-sparing techniques.[1–5] The pathophysiology of overgrowth has been mainly explained by elevated vascularity near the growth plate after surgical approach and drilling during the procedure. Similar to ACL reconstruction, physeal bar excision involves making an opening in the metaphysis and inserting interposition materials. A previous study analyzed the effects of physeal bar excision caused by fracture or infection, and observed a higher growth rate of the operated femur compared to the unoperated one.[6]

The factors influencing overgrowth following surgical procedures such as ACL reconstruction or physeal bar excision, specifically through the creation of a metaphyseal hole, remain unclear. The overgrowth following a long bone fracture is associated with extensive stimulation of the periosteum, and previous animal studies have shown that circumferential disruption of the periosteum can lead to significant overgrowth in long bones.[7–10] This phenomenon can be attributed to an increase in local blood supply and the release of mechanical constraints.[10,11] Additionally, instability caused by a fracture has been reported as a crucial factor contributing to overgrowth in a rat model.[12]

Lee et al[13] found that creating a metaphyseal hole and filling it with bone wax could stimulate longitudinal growth in the long bones of rabbits. By understanding how the location of the metaphyseal hole or the type of interposition material affects overgrowth, we may potentially select the metaphyseal drill hole to minimize or maximize overgrowth following ACL reconstruction or physeal bar excision. This study aimed to investigate the effect of metaphyseal hole creation on subsequent growth stimulation depending on: 1) its distance from the physis; and 2) the type of interposition material using a rabbit model.

Methods

Study design

We chose a rabbit model because the size of the rabbit in a previous study was the smallest among the readily available laboratory mammals that allowed various surgical procedures to be reproducible.[10] To eliminate potential biases in bone growth related to differences in sex, age, and hormonal factors, we obtained seven- to eight-week-old male New Zealand white rabbits, weighing between 2 and 2.5 kg, from commercial suppliers (Doo Yeol Biotech, South Korea).[14,15] All rabbit experiments were approved by the Committee on the Ethics of Animal Experiments of Yonsei University College of Medicine, and conducted in line with relevant guidelines and regulations. All rabbits were acclimated to the animal care facility for an average of seven days, approved for use in the study by a facility veterinarian (see Acknowledgements), and underwent the designated surgical procedures. An ARRIVE checklist is included in the Supplementary Material to show that the ARRIVE guidelines were adhered to in this study.

In Experiment 1, 38 skeletally immature rabbits were randomized into one of four groups: a metaphyseal hole created at 5, 10, or 15 mm distal to the physis of the left proximal tibia, or a sham control group. Bone wax was then used to fill the hole. In Experiment 2, after establishing the distance associated with the most overgrowth to reduce the number of rabbits killed, a defect was created at 10 mm distal to the physis in 20 additional rabbits, which were randomly assigned to either have the defect filled with Tisseel fibrin sealant (Baxter, USA) or left unfilled as an empty defect control. Four rabbits that either expired or exhibited minimal weight gain were excluded from the analysis. A total of 54 rabbits were included in five experimental groups and one sham group. The groups with metaphyseal holes 5, 10, and 15 mm distal to the physis consisted of nine, ten, and nine rabbits, respectively. The unfilled group had eight rabbits, while the Tisseel group had ten rabbits. Randomization was performed by generating random numbers in Microsoft Excel (Microsoft, USA). The sample size was set to the minimum required to adequately conduct the experiments, consistent with other studies using rabbits.[8,10]

Surgical procedures

Once the rabbits were under anaesthesia by isoflurane inhalation, a longitudinal skin incision approximately 1.5 cm in length was made over the medial aspect of the left proximal tibia. The muscles were elevated off the tibia, ensuring minimal disturbance to the periosteum. In the sham control group, the procedure was performed in this same manner. For each experimental group, a metaphyseal hole was created 5, 10, and 15 mm distal to the physis of the proximal tibia using 4 and 6 mm Steinmann pins sequentially under the image intensifier (GE OEC 9900 Elite; General Electric Healthcare, USA). The cancellous bone beneath the physis was then removed by curettage. The space of the metaphyseal

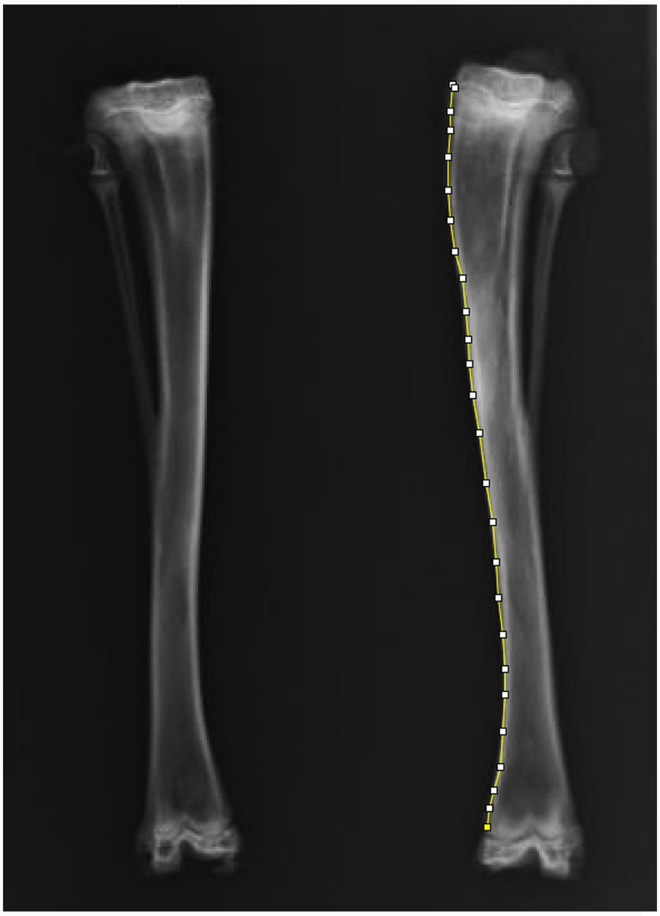

Fig. 1
Measurement of tibial length. The distance along the medial cortex between the proximal and distal physes was used to measure tibial length (yellow line).

hole was either left unfilled or filled with different interposition materials depending on the assigned group. After the surgery, antibiotics (Enrofloxacin 5 mg/kg subcutaneously) and analgesics (Meloxicam 0.2 mg/kg subcutaneously) were administered for one week. An Elizabethan collar was placed around the rabbits' necks to prevent self-injury at the surgical site. Six weeks after surgery, euthanasia was performed using intravenous injection of potassium chloride following deep anaesthesia.

Outcome measures

Radiological and histological analyses of both tibiae were performed. The overgrowth length, new bone width, and growth rate were measured as described below, and the difference between both sides was calculated.

Radiological analysis was performed to measure tibial lengths using a high-resolution REX-650R x-ray system (Listem, Korea) and CS-7 software (V1.10R00_026; Konica Minolta, Japan). The soft-tissues surrounding the bone were removed after harvest, and radiographs were taken in an optimal position. The tibial length was defined as the distance along the medial cortex between the proximal and distal physes, and was measured on both the operated and contralateral sides (Figure 1). Additionally, to evaluate possible angular deformities resulting from asymmetric overgrowth between the tibia and fibula, the angle formed by the intersection of the perpendicular lines of the proximal and distal physes of the tibia was measured, and valgus alignment was expressed as a positive value. All measurements were conducted in a blinded manner, with neither the purpose nor the methods of the study disclosed. The overgrowth length and angular differences were calculated by subtracting the length or angle of the contralateral limb from the values of the operated limb.

As for histological analysis, the growth rate was evaluated by the mineral apposition rate (MAR) using double labelling, where 25 mg/kg calcein and Alizarin Red S (Sigma-Aldrich, USA) were subcutaneously injected three and seven days prior to euthanization, respectively. To establish anatomical standardization, a ruler was placed next to the tissue to measure its length and width. For fluorescence analysis, the collected tibiae were fixed in 3.7% paraformaldehyde solution at room temperature for a week, and embedded in a plastic resin block using a mixture of ethanol and Technovit 7200 resin (Heraeus KULZER, Germany). Tissue blocks were made using a 3 cm segment of the proximal tibia, including the physis, taken at one-third of the total thickness. Coronal sections with a thickness of 50 μm ± 5 μm were obtained perpendicular to the longitudinal axis of the bone using an EXAKT diamond cutter (KULZER EXAKT 300; Heraeus KULZER). Fluorescence images of the labelled bone were captured using a Pannoramic 250 Flash III system (3DHistech, Hungary) and analyzed using Caseviewer software (3DHistech). The width between the labels was measured at three points per section.[16–18] The differences between the measurements made at the same point on the operated and contralateral sides were averaged and divided by four to obtain a MAR. After the histomorphometry analysis, the samples were extracted from the resin block and decalcified with 0.5 M EDTA for four weeks. After embedding in paraffin wax, 5 μm thick sections were cut in the coronal plane using a rotary microtome. The sections were sequentially stained with haematoxylin and eosin (H&E). The new bone width was measured as the distance between the trabecular bone and the end of the hypertrophic zone in the physis, using digital micrographs stained with H&E at a magnification of 100× (Aperio ImageScope, v12.3.0.5056; Leica Biosystems, USA).

Statistical analysis

Statistical analyses were performed using SPSS version 23 (IBM, USA). Descriptive statistics were collected as the mean and SD. The Shapiro-Wilk test was applied to check data distribution. An independent-samples *t*-test or Mann-Whitney U test was used to compare continuous variables between two groups. For multiple comparisons among more than two groups, one-way analysis of variance (ANOVA) with a Bonferroni post-hoc test or the Kruskal-Wallis test was used. Statistical significance was set at $p < 0.05$.

Results

Effect of location of the metaphyseal hole

The mean overgrowth of tibial length on the operated side was 0.70 mm (SD 0.56), 0.56 mm (SD 0.85), and 0.37 mm (SD 1.04) in the groups with metaphyseal holes 5, 10, and 15 mm distal to the physis, respectively (Figure 2a). There was no significant difference among the three groups ($p = 0.714$, one-way ANOVA). Statistical differences were observed in the

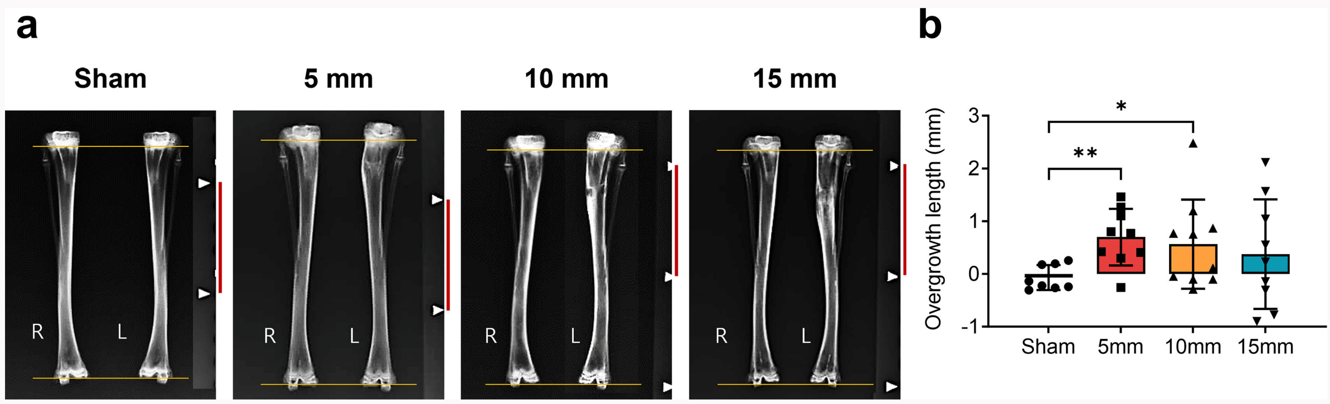

Fig. 2
Radiological analysis for evaluating tibial overgrowth length. a) Overgrowth length was measured as the difference between the operated and contralateral tibiae in each group according to the location of the metaphyseal hole. The distance between the arrowheads represents 5 cm. b) Bar chart shows the tibial overgrowth lengths. Statistical differences were observed in the 5 mm and 10 mm distal hole groups ($p = 0.002$ and 0.046, respectively, both independent-samples *t*-test) compared to the sham group. Error bars indicate the SD. *$p < 0.05$, **$p < 0.01$.

5 mm and 10 mm distal hole groups ($p = 0.002$ and 0.046, respectively, both independent-samples *t*-test) compared to the sham group (-0.07 mm (SD 0.24)) (Figure 2b). However, there was no significant difference between the sham and 15 mm distal hole groups ($p = 0.245$, independent-samples *t*-test).

In H&E staining, the new bone width was 54.93 µm (SD 74.55), 98.10 µm (SD 62.15), and 24.96 µm (SD 49.04) in the 5, 10, and 15 mm distal hole groups, respectively (Figure 3a). There was no significant difference among the three groups ($p = 0.142$, one-way ANOVA). The difference was significant between the 10 mm distal hole and sham groups (7.53 µm (SD 79.62); $p = 0.049$, independent-samples *t*-test) (Figure 3b). However, there was no statistical difference between the 5 mm distal hole and sham groups ($p = 0.295$, independent-samples *t*-test), and the 15 mm distal hole and sham groups ($p = 0.659$, independent-samples *t*-test). The MAR from three to seven days before kill was 34.96 µm/day (SD 3.23), 53.26 µm/day (SD 1.35), and 32.16 µm/day (SD 5.14) in the 5, 10, and 15 mm distal hole groups, respectively. There was no significant difference among the three groups ($p = 0.156$, Kruskal-Wallis test). The MAR was significantly higher in the 5 mm and 10 mm distal hole groups ($p = 0.047$ and 0.009, respectively, both independent-samples *t*-test) compared to the sham group (0.88 µm/day (SD 0.31)) (Figure 4). There was no statistical difference between the 15 mm distal hole (32.16 µm/day (SD 5.14)) and sham groups ($p = 0.073$, independent-samples *t*-test).

Effect of interposition materials in the metaphyseal hole

The metaphyseal hole was made 10 mm distal to the physis of the proximal tibia in both the unfilled and Tisseel groups, based on the results of overgrowth observed according to the location of the holes. Comparing three groups with different materials in holes created 10 mm distal to the physis, a significant difference in overgrowth length was observed only between the groups filled with Tisseel and those filled with bone wax ($p = 0.036$, one-way ANOVA with a Bonferroni post-hoc test). There were no differences in the new bone width and MAR ($p = 0.060$ and 0.102, one-way ANOVA and Kruskal-Wallis test, respectively). The mean overgrowth of tibial length was -0.18 mm (SD 1.23) in the unfilled group and -0.67 mm (SD 1.08) in the Tisseel group. There was no statistical difference from the sham in each group (Figure 5). In the unfilled group, the new bone width (27.54 µm (SD 41.71)) and MAR (14.26 µm/day (SD 2.29)) were not significantly different from those of the sham group ($p = 0.338$ and 0.087, respectively, both independent-samples *t*-test). In the Tisseel group, there was no significant difference in new bone width (21.66 µm (SD 75.27)) and MAR (8.33 µm/day (SD 0.66)) from the sham group ($p = 0.770$ and 0.059, respectively, both independent-samples *t*-test).

Angular deformity by the creation of a metaphyseal hole

Significant valgus deformities were observed in all groups compared to the sham group (-0.33° (SD 0.95°)) (Figure 6), and the angular differences were 1.62° (SD 1.77°), 1.47° (SD 1.47°), and 1.13° (SD 0.84°) in the groups with metaphyseal holes 5, 10, and 15 mm distal to the physis, respectively ($p = 0.013$, 0.006, and 0.005, respectively, all independent-samples *t*-test). There was no significant difference among the three groups ($p = 0.755$, one-way ANOVA). Statistical differences were observed in the unfilled (1.35° (SD 1.08°); $p = 0.005$, independent-samples *t*-test) and Tisseel groups (1.16° (SD 1.19°); $p = 0.009$, independent-samples *t*-test) compared to the sham, and there was no significant difference among the three groups with different materials ($p = 0.854$, one-way ANOVA).

Discussion

There is a paucity of studies regarding overgrowth following ACL reconstruction or growth stimulation after physeal bar excision. Previous studies have reported growth acceleration in long bone length following circumferential periosteal transection,[10,11,19] but the influence of cortical bone damage on subsequent overgrowth has rarely been studied.[12,20] Lee et al[13] reported the effect of metaphyseal hole creation and interposition with bone wax on overgrowth in rabbits. Nevertheless, the difference in the extent of overgrowth based on the location of the hole was not determined. To compare the effects of growth stimulation depending on the location of the metaphyseal hole, we created a hole at 5, 10, or 15 mm

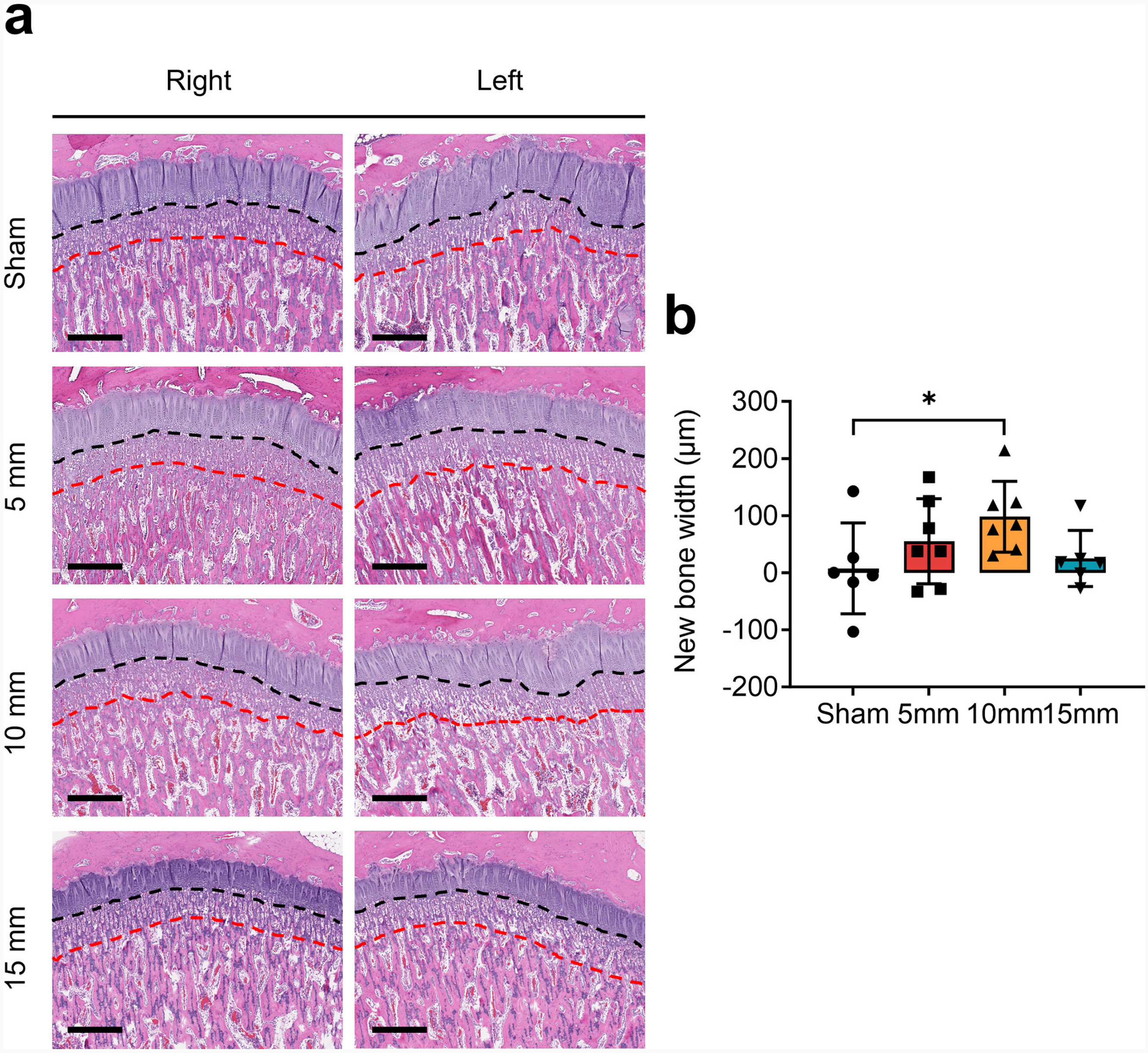

Fig. 3

Histological analysis for evaluating new bone width. a) Coronal sections stained with haematoxylin and eosin show the structure of growth plate. Area between the black and red dotted lines represents the new bone area. Scale bars, 500 µm. b) Bar chart showing the new bone widths. A statistical difference was observed between the 10 mm distal hole and sham groups ($p = 0.049$, independent-samples *t*-test). Error bars indicate the SD. *$p < 0.05$.

distal to the proximal physis of the tibia in each group. These specific distances were based on the potential effects on the adjacent physis, with a consideration that locations closer than 5 mm could result in direct damage to the physis during the procedure.

We observed significant overgrowth in the tibia with metaphyseal holes 5 and 10 mm distal to the proximal physis, compared to the sham. Additionally, the MARs were also higher in both groups, but there was a notable increase in new bone width observed exclusively in the 10 mm distal hole group. These results suggest that the metaphyseal hole located 10 mm distal to the physis may stimulate growth. However, care should be taken to avoid damage to the physis and medial collateral ligament when drilling close to the growth plate.

The operated tibia in the 5 and 10 mm distal hole groups was on average 0.67% and 0.54% longer, respectively, than the contralateral side. Halanski et al[10] conducted a comprehensive study using a rabbit model to compare the effects of relevant periosteal procedures and reported an average overgrowth of 1.5%, 1.7%, and 2.3% at eight weeks after periosteal transection, full release, and resection, respectively. However, their study cannot be easily compared with our study because of differences in study design and radiological measurements. Interestingly, the growth rate at six weeks after surgery in our 10 mm distal hole group was comparable to the growth rates at two weeks postoperatively in their study, and was higher than those at eight weeks postoperatively. These results suggest that our procedures may stimulate growth over a longer period than periosteal

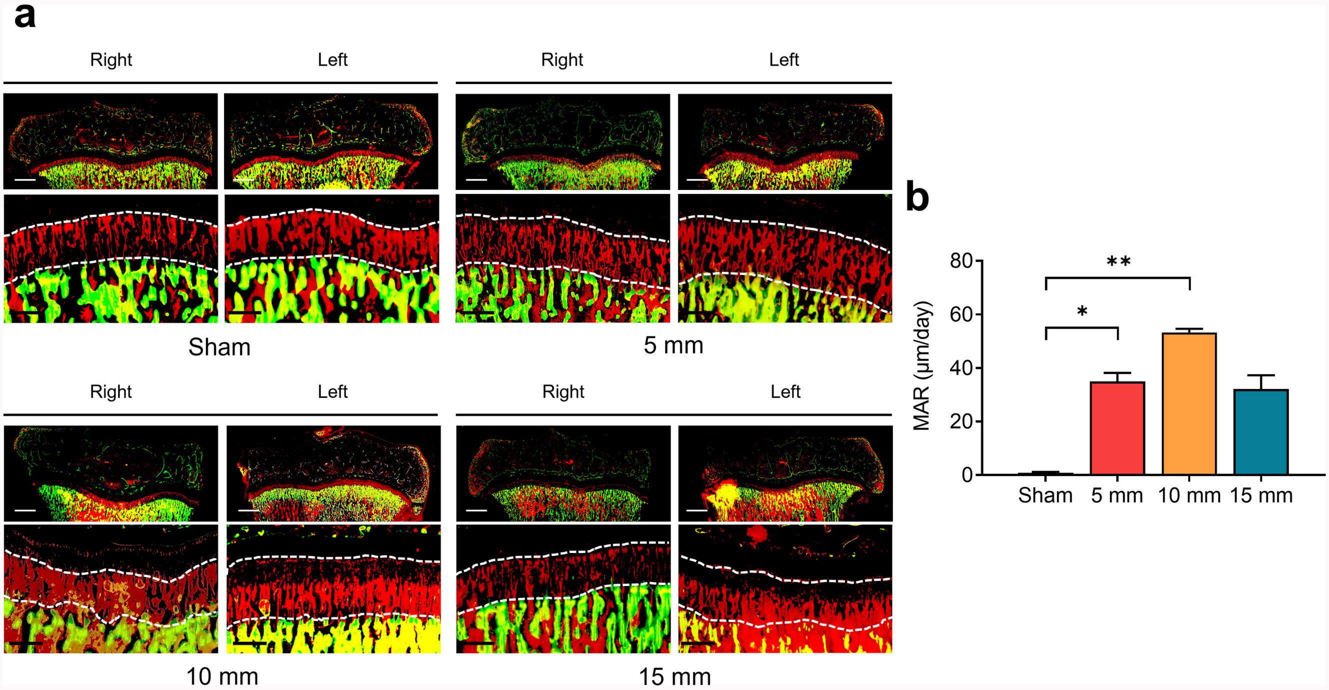

Fig. 4

Histological analysis for evaluating growth rate. a) Double labelling of sections shows green and red areas stained with calcein and Alizarin Red S, respectively. The mineral apposition rate (MAR) can be calculated by measuring the distance between the labels. White scale bars, 2,000 µm, and black scale bars, 500 µm. b) Bar chart shows the MARs. Statistical differences were observed in the 5 mm and 10 mm distal hole groups ($p = 0.047$ and 0.009, respectively, both independent-samples *t*-test) compared to the sham group. The error bars indicate the SD. *$p < 0.05$, **$p < 0.01$.

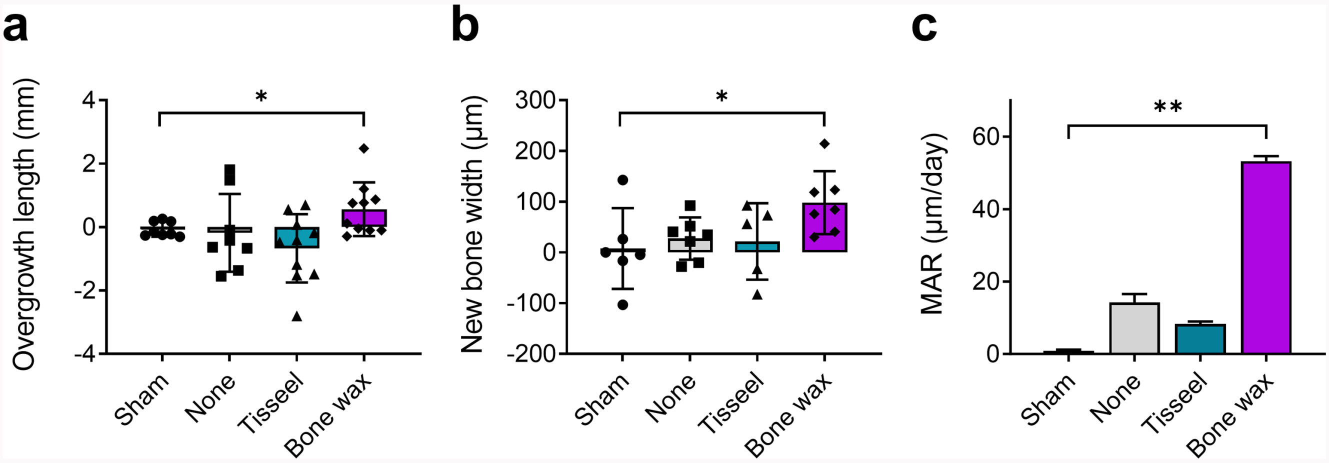

Fig. 5

Effect of interposition materials in the metaphyseal hole. a) Bar chart showing the tibial overgrowth lengths. b) Bar chart showing the new bone widths. c) Bar chart showing the mineral apposition rates. Statistical differences were observed between the bone wax and sham groups ($p = 0.046$, 0.049, and 0.009, respectively, all independent-samples *t*-test).The error bars indicate the SD. *$p < 0.05$, **$p < 0.01$. MAR, mineral apposition rate.

procedures. Limpaphayom and Prasongchin[11] reported that growth stimulation by periosteal stripping and division lasted three to five years in children with limb length discrepancy. Overgrowth after fractures was reported to occur mainly within the first 18 months and could last up to 3.5 years.[21] Further long-term follow-up studies are required to evaluate the growth rate over time and duration of growth stimulation.

The mechanism of growth stimulation following circumferential periosteal procedures has been described as a result of local hyperaemia and mechanical detethering of the physis.[8,10,11] Kaneko et al[16] indicated that an increase in number and size of hypertrophic chondrocytes could be related to stimulated bone growth after periosteal procedures in a mouse model of achondroplasia. However, the mechanism associated with periosteal stimulation does not fully explain overgrowth after ACL reconstruction or physeal bar excision. Moreover, several clinical studies have reported unstable fractures as a risk factor for overgrowth,[22–25] and a previous experimental study using young rats demonstrated that bony instability at the osteotomy site plays a critical role in subsequent longitudinal overgrowth.[12] Therefore, it is thought that the microinstability resulting from bilateral cortical

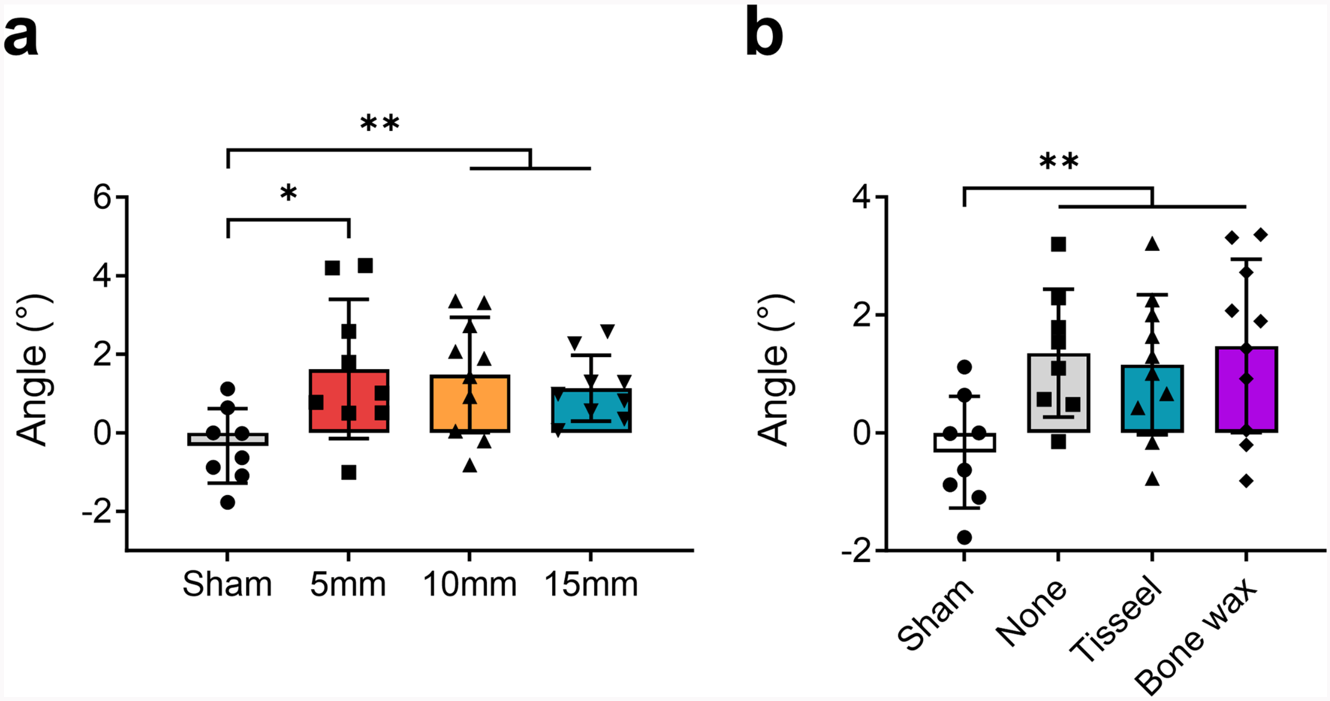

Fig. 6

Angular deformity by the creation of a metaphyseal hole. a) Bar chart showing the angular differences according to the location of the metaphyseal hole. Statistical differences were observed in the 5, 10, and 15 mm distal hole groups ($p = 0.013$, 0.006, and 0.005, respectively, all independent-samples *t*-test) compared to the sham group. b) Bar chart showing the angular differences according to the type of interposition material. Statistical differences were observed in the unfilled, Tisseel, and bone wax groups ($p = 0.005$, 0.009, and 0.006, respectively, all independent-samples *t*-test) compared to the sham group. The error bars indicate the SD. *$p < 0.05$, **$p < 0.01$.

drilling, along with partial periosteal damage, contributed to the growth stimulation in this study. However, overgrowth was not significant in the 15 mm distal hole group. This location is thought to be too far from the growth plate to cause microinstability or hyperaemia for stimulating longitudinal growth.

We found differences in overgrowth based on the type of material used to fill the metaphyseal hole. Among the three groups with a hole created 10 mm distal to the physis and filled with different interposition materials, significant longitudinal overgrowth was observed only in the group that used bone wax. If growth stimulation is affected by instability and the detethering effect caused by damaged cortical bone and partial periosteal release, adequate haemostasis on the bleeding metaphyseal surfaces will be required to delay healing and maintain the overgrowth effect. Bone wax is an inert agent widely used for local haemostasis, and works by mechanically occluding cut vessels or Haversian canals.[26] In the unfilled group, it is believed that rapid haematoma formation and subsequent bone healing prevent growth stimuli in metaphyseal holes without interposition. A previous study investigating the effects of repetitive periosteal transection on overgrowth noted that the duration of accelerated growth may be attributed to differences in healing time for the periosteal injury.[10] Tisseel is a commercially available fibrin sealant containing fibrinogen and thrombin, and is also used as a surgical haemostatic agent.[27] However, unlike bone wax, the fibrin sealant acts as a topical adhesive. The glue-like effect of fibrin sealant may interfere with growth stimulated by creating a metaphyseal hole. The slight viscosity and faster resorption within ten to 14 days,[27] compared to bone wax, which is minimally resorbed from the site of application,[28] may also contribute to early detachment and loss of sealing effectiveness. Despite its haemostatic effect, the decreased instability and rapid healing of periosteal damage caused by Tisseel appear to be responsible for the results contrary to those of the bone wax group.

Although our procedure does not directly involve the growth plate, we observed valgus deformities in all groups with metaphyseal holes. Valgus deformity, as a complication of proximal tibial fractures, is known as Cozen's phenomenon.[29,30] Aronson et al[20] reported that significant valgus deformity occurred following an osteotomy of the medial half of the tibia in rabbits, whereas varus deformity following an osteotomy of the lateral side was not significant. Despite our procedure resulting in symmetric damage to the cortex and periosteum of the medial and lateral aspects of the proximal tibia, asymmetric growth may lead to secondary valgus deformity. Tibial valgus deformity after ACL reconstruction has also been reported,[1,2] and a previous study observed angular deformity after medial hemicircumferential division of the tibial periosteum in rabbits.[8] The mechanism has been suggested to involve asymmetric stimulation of the medial physis and lateral tethering by the fibula or iliotibial band.[1,4] Although our study found that valgus deformity was significant even in the groups without significant overgrowth in length, valgus deformity associated with medial cortical overgrowth may be more pronounced than the minimal overgrowth length itself.

This study has several limitations. First, our results are limited to a rabbit model and may not be directly applicable to clinical settings. Clear guidance on the optimal size and location of the hole, and the expected amount of overgrowth for clinical application, cannot be provided based on this study. Large animal models should be designed to investigate the effect of more subdivided holes with varying sizes and locations, as well as the potential for repeated procedures to produce more overgrowth. These models can provide a more accurate explanation of human physiology and anatomy than rabbit models, while minimizing measurement errors. A large study on overgrowth following ACL reconstruction in children and adolescents is also warranted.[1,31] Second, the mean differences in tibial length were relatively small. However, this study primarily aimed to investigate the stimulative effect of a metaphyseal hole on growth, considering the distance from the physis and the type of interposition material, and was not designed to induce overgrowth in the human body. Additionally, we euthanized the rabbits at six weeks after surgery. However, considering the growth rate at six weeks, the stimulated growth period would be longer, potentially resulting in a larger difference. Third, four rabbits were not included in the analysis. One rabbit each in the 5 and 15 mm distal hole groups expired one week after surgery, and two rabbits in the unfilled group were excluded due to minimal weight gain. Despite this, each group contained at least eight subjects, which is consistent with other studies using rabbits.[8,10] Fourth, this study only included male rabbits. Considering the differences in the structural characteristics and physiological responses related to bone growth based on sex,[32,33] the inclusion of female rabbits is expected to enrich the interpretation of findings related to bone growth in children. Fifth, the experiments for the unfilled and Tisseel groups were not conducted at the same time as those for the groups with varying hole locations. While comparing the effects of interposition materials at different locations was considered, the current design is deemed optimal to minimize the number of rabbits killed. For bone wax, the location 10 mm from the physis is the most effective for growth stimulation. However, this may differ in cases where the hole is left unfilled or filled with Tisseel. Finally, the possibility of measurement bias resulting from metaphyseal defects observable in radiographs cannot be excluded. Despite these limitations, we believe that this study can contribute to the understanding of overgrowth following ACL reconstruction or physeal bar excision in immature growing long bone.

In conclusion, we found that creating a metaphyseal hole can stimulate the longitudinal growth of long bones and induce tibia valga in a rabbit model. However, the effect varies depending on its location and the type of interposition material. Notably, significant overgrowth was observed only in the group with holes created 10 mm distal to the physis of the proximal tibia and filled with bone wax. Based on this study, the location of the metaphyseal hole during ACL reconstruction could be optimized to prevent overgrowth, or in the case of physeal bar excision, to stimulate growth.

Supplementary material

ARRIVE checklist

References

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Funding statement

The authors disclose receipt of the following financial or material support for the research, authorship, and/or publication of this article: this work was supported by the Mid-Career Research Program and the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2020R1A2C1006454 and NRF-2022R1F1A1072583).

ICMJE COI statement

This work was supported by the Mid-Career Research Program and the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2020R1A2C1006454 and NRF-2022R1F1A1072583), as reported by K-B. Park, K-M. Lee, and E. A. Ko.

Data sharing

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

Acknowledgements

We would like to express our special thanks to the veterinarian (Gyuri Park) in charge of large animals at the Animal Research Facility of Yonsei University.

Ethical review statement

The rabbit experiments were approved by the Committee on the Ethics of Animal Experiments in Yonsei Biomedical Research Institute, Yonsei University College of Medicine (IACUC Approval No. 2020-0102). All studies were conducted in line with the ARRIVE guidelines.