The Effect of Intraosseous Graft Length on Tendon-bone Healing in Anterior Cruciate Ligament Reconstruction Using Flexor Tendon

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Abstract

The current study was performed to understand the relationship between graft length placed within the bone tunnel and intraosseous graft healing in anterior cruciate ligament reconstruction. Twenty-four adult beagle dogs were divided into two groups of 12 animals each. In each animal, anterior cruciate ligament reconstruction using a 4-mm diameter autogenous flexor tendon graft was done in the left knee. In Groups I and II, the graft having a length of 15 mm and 5 mm, respectively, was placed within the tibial tunnel. The proximal end of the graft was placed through the over-the-top route in all animals. In each group, 5 animals were sacrificed immediately after surgery, and the remaining 7 were sacrificed at 6 weeks postoperatively. Biomechanical and histologic evaluations were performed. In pull-out testing, the ultimate failure load and the linear stiffness of the graft-tibia complex harvested at 6 weeks were significantly greater than those harvested at the time-zero period. There were no significant differences in those parameters between Groups I and II at 6 weeks. In each group, the perpendicular collagen fibers connecting the tendon to the bone tunnel wall were observed only in the narrow area located close to the intraarticular tunnel outlet. In conclusion, excessively long placement of the flexor graft within the bone tunnel does not result in an additional increase of anchoring strength and stiffness of the graft in anterior cruciate ligament reconstruction.

Keywords: Anterior cruciate ligament reconstruction, Intraosseous graft length, Flexor tendon graft, Anchoring strength, Intraosseous graft healing;

INTRODUCTION

A firm attachment of a tendon graft to the bone is a significant factor for the success in anterior cruciate ligament (ACL) reconstruction. However, much remains unclear about healing of the flexor tendon graft within the bone tunnel. In the previous literature, a number of histologic studies have suggested that collagen fiber continuity between the graft and the bone is progressively reestablished [2, 3, 5, 6, 10, 11, 16, 19]. Biomechanically, Rodeo et al. [15] developed an extraarticular tendon graft model using a canine extensor tendon, and reported that the degree of the re-establishment of collagen-fiber continuity between the graft and the bony wall is correlated with an increase in the anchoring strength of the graft within the bone tunnel. Grana et al. [6] performed pullout testing of the a flexor tendon graft from the bone tunnel using a rabbit ACL reconstruction model. Tomita et al. [18] used a canine ACL reconstruction model with a flexor tendon graft, and reported that the pullout strength of the graft from the bone tunnel is equivalent to that of the bone-patellar tendon-bone (BTB) graft at 6 weeks. Recently, the authors clarified that the graft-tunnel diameter disparity within 2 mm does not affect the anchoring strength of the flexor tendon within the bone tunnel [22]. Moreover, Greis et al. [7] reported about the relationship between the intraosseous graft length and graft healing within the extraarticular bone tunnel. They demonstrated that the length of tendon placed within a bone tunnel influences tendon pull-out strength at 6 weeks in canine extraarticular tendon graft model. In clinical ACL reconstruction, it has been commonly believed that a decrease of the intraosseous graft length may result in reduction of the anchoring strength of the graft within the bone tunnel. On the other hand, surgeons have sometimes experienced to implant a relatively short end of the graft within the bone tunnel in their clinical practice. Interestingly, poor clinical results have not been reported in the instance of a shortened

graft portion. Therefore, we can assume that the anchoring strength of the flexor tendon graft is not affected by the length of the graft placed within the bone tunnel in the current intraarticular ACL reconstruction surgery, if the length is longer than a certain minimal but adequate length.

We hypothesized that the anchoring strength and stiffness of the flexor tendon graft having a whole length of 50 mm are equivalent between the two implantation conditions: The first condition is placing the end portion with a length of 5 mm within a bone tunnel, and the second condition is placing the end portion with a length of 15 mm. The purpose of this study is to test this hypothesis.

MATERIALS AND METHODS

Study Design

The present study was performed with 24 healthy, adult beagle dogs weighing 10.9 ± 0.6 (a mean ± standard deviation) kg. Animal experimentation was carried out under the Role and Regulation of the Animal Care and Use Committee, Hokkaido University, Graduate School of Medicine. The dogs were divided into two study groups of 12 animals each. In each group, ACL reconstruction was performed in the left knee, after the ACL was resected. A tibial tunnel having a diameter of 4 mm was made through the anatomical attachment of the ACL. The distal end of the doubled flexor tendon graft having a diameter of 4 mm was grafted within the tibial tunnel. In Group I, a 15-mm long portion of the graft was placed within the tunnel, while a 5-mm long portion of the graft was placed within the tunnel in Group II (Fig. 1a and b). In each knee, the proximal end of the graft was routed through over-the-top of the lateral femoral condyle. We tethered each end of the graft with polyester sutures to a screw post inserted into the bone. A minimal tension was manually applied to the graft,

although the tension was not measured. In each group, 5 animals were sacrificed immediately after the surgery, and the remaining 7 were sacrificed at 6 weeks after the operation. The 6-week period was chosen for sacrifice because of the following reason. In the literature [2, 3, 6, 18, 22], the anchoring properties were commonly examined at 3, 6, 12, 24, and approximately 48 weeks, and in our previous studies using the same canine ACL reconstruction model as used in the present study [18, 22], the flexor tendon graft could not be pulled out from the tunnel at 12 weeks, while the pull-out strength was extremely low at 3 weeks. In each group, 5 animals were sacrificed by a lethal injection of thiamylal sodium immediately after the operation, and biomechanically evaluated. The remaining 7 animals sacrificed at 6 weeks were used for biomechanical (n = 5) and histological (n = 2) evaluations.

Surgical Procedure and Postoperative Treatment

Surgery was performed under anesthesia induced by the intramuscular administration of ketamine hydrochloride (10 mg/kg) followed by the intravenous injection of pentobarbital (25 mg/kg). Each animal was endotracheally intubated and was fixed in a supine position on an operating table. Using a sterile technique, a median skin incision was made in the left knee, and then the ACL was exposed and resected through a medial parapatellar approach. The transverse ligament was incised from the infrapatellar fat pad to allow for full visualization of the tibial attachment of the ACL.

In each group, the flexor digitorum superficialis tendon having a length of 10 cm was harvested from the left hind-limb through a longitudinal incision made at the posteromedial aspect of the distal lower leg. The tendon was then sharply trimmed parallel to the fiber orientation so that the doubled tendon could be passed through a

4-mm diameter hole. At the looped end of the doubled tendon, a nonabsorbable polyester suture (Number-1 Ticron, Davis & Geck, New Jersey) was passed through the loop. At the free end of the graft, the same suture was firmly attached in a Whip-stitch fashion. Then, the cross-sectional area of the middle portion of each graft was measured with an area micrometer, as described in our previous studies [8, 9]. Briefly, the middle portion was placed in the rectangular slot of the micrometer and the plunger was inserted in the slot. The thickness of the specimen was measured while a constant pressure of 0.12 MPa was applied to the plunger. The cross-sectional area of the middle portion was calculated by multiplying the slot width by the measured thickness. The cross-sectional area of the graft in Groups I and II was 9.42 ± 0.952 mm^2 and 10.15 ± 0.28 mm^2, respectively. There were no significant differences between the two groups ($p = 0.1402$).

The anteromedial surface of the tibia was exposed, and the periosteum was elevated. In each group, a bone tunnel having a diameter of 4 mm was drilled in the tibia through the tibial insertion of the resected ACL to the exposed anteromedial surface of the tibia. Then, a small incision was made in the proximal posterolateral part of the joint capsule. A sharp curette was inserted from the incision to over-the-top of the lateral femoral condyle, and the cortical bone at this portion was then curetted so that a bony "trough" was created along the over-the-top route to enhance the adhesion between the graft and the bone, and to position the graft close to the original femoral insertion. On each graft, a line was drawn with a clinically available marker pen (Vismark, VISCOT, New Jersey) at a position 15 or 5 mm to the looped end. For each graft, the end portion having a length of 15 mm and 5 mm was placed in the tibial tunnel in Group I and in Group II, respectively, using the line as a landmark. The other end portion of each graft was routed through the trough created in the lateral femoral

condyle. We tethered each end of the graft with the suture to a screw inserted into the bone, manually applying an unmeasured degree of low tension with the knee flexed at 45° (Fig. 2). The surgical wound was irrigated with a physiologic saline solution containing antibiotics and closed with 3-0 nylon sutures. Each animal was immobilized postoperatively, and allowed unrestricted daily activity in a cage (70 cm in width, 68 cm in height, and 70 cm in depth).

Biomechanical Testing

A knee specimen with the femur having a length of 45 mm and the tibia having a length of 60 mm was removed from an animal after sacrifice. Each specimen that was used for biomechanical testing was wrapped in gauze moistened with physiologic saline solution and then wrapped in an air-tight polychlorovinylidene film. Each specimen was stored at -32°C until the time of testing, and thawed overnight at 4°C prior to mechanical testing. All soft tissues other than the graft were carefully dissected. Then the cross-sectional area of the graft was measured with the area micrometer in the same manner as previously described [8, 9]. The femur and the tibia were separately cast in rectangular aluminum tubes of 20 x 20 x 50 mm using polymethylmethacrylate resin. With the use of a set of specially designed grips, the prepared femur-graft-tibia specimen was attached to a conventional tensile tester (PMT-250W, Orientec, Tokyo, Japan) so that the tibia was positioned to allow for tensile loading aligned with the long axis of the bone tunnel with the knee flexed at 45° (Fig. 3). For the specimens harvested from the animals sacrificed at 6 weeks in each group, the suture tethering the graft to the tibia was cut, while the suture fixing the graft to the femur was not resected, in order to determine the anchoring strength of the graft within the tibial bone tunnel. For the specimens harvested from the animals

sacrificed immediately after the operation in each group, no sutures were cut to determine the initial strength of the femur-graft-tibia complex as the time-0 control value. Prior to tensile testing, the specimen was preconditioned with a static preload of 0.5 N for 5 min, followed by 10 cycles of loading and unloading with a strain of 0.5% at a cross head speed of 20 mm/min [20]. Following the preconditioning, pull-out tests were performed at the same rate as used in the previous study [20], and failure modes were observed. The specimen was kept moistened throughout the test period with a physiological saline solution spray. Load-deformation curves were drawn with an X-Y recorder (X-Y-T Recorder 3023, Yokogawa, Tokyo, Japan). From the load-deformation relationship, the ultimate load and the linear stiffness were obtained.

Histologic Observation

Each graft-tibia specimen harvested for histological observation was fixed in a 10% buffered formalin solution. After the specimen was decalcified, it was cast in a paraffin block. The specimens were sectioned parallel to the longitudinal axis of the bone tunnel. We obtained 10 sections that closely showed the diameter of the tunnel in each specimen. They were stained with hematoxylin and eosin. In all the sections, the tendon-bone interface was observed with a light microscope.

Statistical Analysis

All measured data are shown as the mean and the standard deviation. For assessment of the influence of time and treatment on the ultimate load and the linear stiffness, the two-way analysis of variance (ANOVA) was performed. The Fisher's protected-least-significant-difference test was applied for post-hoc multiple comparisons. To compare the cross-sectional area between Groups I and II at the time

of surgery, the Student's t-test was used. The significance limit was set at $p = 0.05$ for each test.

RESULTS

Gross observations

At the time of sacrifice, we did not find any tears in the graft or any apparent degenerative changes on the articular cartilage and the menisci in each specimen. The intraarticular portion of the graft was enveloped by granulation tissue. The granulation tissue appeared to be thicker on the anterior aspect than on the posterior aspect.

Mechanical Evaluations

As for the failure modes, all 5 femur-graft-tibia complexes examined immediately after reconstruction failed at the suture-tendon junction, in which the free ends of the graft were tethered to a screw with the Whip-stitch suture technique. At 6 weeks, the graft mid-substance was torn in 2 out of the 5 specimens in both the groups, while the graft was pulled out from the tibial tunnel in the remaining grafts. No grafts failed at the femoral insertion in all pull-out tests.

Concerning the linear stiffness of the femur-graft-tibia complex (Fig. 4a), the ANOVA demonstrated a significant difference ($p < 0.0001$). The post-hoc test showed that the linear stiffness measured at 6 weeks was significantly greater than that measured at the time-zero control in each group ($p < 0.0001$). However, there were no significant differences between Groups I and II at each period ($p = 0.9831$ at 0 weeks and 0.7754 at 6 weeks).

Regarding the ultimate load to failure of the graft-tibia complex (Fig. 4b), the two-way ANOVA demonstrated that there were significant differences among the

groups ($P < 0.0001$). The post-hoc test showed that the ultimate load measured at 6 weeks was significantly greater than that measured at the time-zero control in each group ($p = 0.0010$ in Group I and $p < 0.0001$ in Group II). However, there were no significant differences between Groups I and II at each period ($p = 0.9009$ at 0 weeks and 0.2107 at 6 weeks). In the specimens in which the graft was pulled out from the tibial tunnel, the ultimate load averaged 183 ± 25 N in Group I (n=3) and 224 ± 93 N in Group II (n=3). In the specimens that failed in mid-substance the ultimate load averaged 238 ± 17 N in Group I (n=2) and 299 ± 159 N in Group II (n=2). The latter value was greater than the former value in each group, although we could not perform any statistical comparisons because of the small sample size.

Histological Observations

In Group I, new bone formation was observed along with the wall of the tunnel and in the vacant space within the tunnel (Fig. 5a). The space between the flexor tendon and the bone (tendon-bone gap) was filled with granulation tissue rich in fibroblasts and vessels in both the groups. In the granulation tissue, the perpendicular collagen fibers connecting the tendon to the bone, which resembled Sharpey's fibers, appeared in each group. These collagen fibers were more densely observed in a narrow area located close to the intraarticular tunnel outlet than in an area located far from the outlet (Fig. 5b and c).

In Group II, the new bone formation was observed similarly to be seen in Group I (Fig. 6a). The density of the collagen fibers in this proximal 5-mm area appeared to be almost identical between Groups I and II (Fig. 6b). The tibial end of the graft was connected by the generated collagen fibers to the newly formed bone that filled in a vacant space in the tunnel (Fig. 6c).

DISCUSSION

This study demonstrated that there were no significant differences in the anchoring strength and stiffness of the flexor tendon graft having a whole length of 50 mm between the 15-mm placement group and the 5-mm placement group at the 6-week period after implantation in canine intraarticular ACL reconstruction. These strength and the stiffness values were significantly greater than the maximum load of the stiffness of the femur-graft-tibia complex measured immediately after reconstruction. The histological observations in this study showed that abundant formation of the perpendicular collagen fibers connecting the graft to the bone were observed in a narrow area located close to the intraarticular tunnel outlet in both the 5-mm placement group and the 15-mm placement group. The collagen fiber density in this portion appeared to be identical between the two groups. Moreover, in the 5-mm placement group, the tibial end of the graft was connected by numerous perpendicular collagen fibers like the Sharpey's fibers to the newly formed bone that filled in a vacant space in the tunnel. These histological findings supported the similarity in the anchoring strength and stiffness between the two groups. This study implied that the anchoring strength of the flexor tendon graft is not affected by the length of the graft placed within the bone tunnel, if the length is longer than a certain minimal but adequate length.

There are some limitations in this study. The first limitation is that we used a canine model. Therefore, we did not completely mimic the standard ACL reconstruction procedure performed in human patients. For example, we used the flexor digitorum superficialis tendon instead of the hamstring tendon because the canine hamstring tendons are too thin to use as a single-strand graft in ACL

reconstruction and too short to use as a doubled graft. The femoral side of the tendon was placed in the trough created along the over-the-top route which is not commonly used, although the over-the-top route has commonly been used for the femoral side to minimize variability of the graft position at the femoral site in the canine model [1, 12, 13, 18, 22, 23]. We adjusted the initial graft tension until the positive Lachman test was obliterated. This tensioning technique was not quantitative, but it has been used in previous animal investigations of ACL reconstruction and in clinical practice [1, 6, 12, 13, 17, 18, 22]. In addition, well-controlled rehabilitation could not be applied postoperatively. We have to recognize that these factors may have affected the results in this study.

The second limitation of this study is that we selected the relatively low cross-head speed of 20 mm/min for the loading of the grafts, based on the results of our previous studies in a canine model [8, 9, 18, 22]. Woo et al. [21] and Danto and Woo [4] showed that there was little effect of strain rate on the failure mode or the mechanical properties of the ligament. In contrast, Noyes et al. [14] reported that the rate of loading had a significant effect on the type of failure. Therefore, the strain rate for mechanical tests may slightly influence our biomechanical results. The third limitation is that, in the present study, the graft was not always pulled out from the tunnel in pull-out testing. Therefore, we could not determine the pull-out strength of the graft from the bone tunnel. From a clinical viewpoint, however, it is important to determine the weakest site is in the graft-bone complex, the graft-bone tunnel interface or the intraarticular tendon site, because the lowest strength holds the key to success in ACL reconstruction.

The fourth limitation is that we focused on graft healing within the tibial bone tunnel. Therefore, we could not refer to graft healing within the femoral tunnel in the

present study. There were no graft failures at the femoral insertion. This result may be caused by the following facts. First, during pull-out testing, we did not cut the sutures tethering the graft to the femur. Second, the femoral end of the graft was flexed approximately 90° to the direction of the tensile load at the over-the-top portion of the femoral condyle. Therefore, we cannot directly refer to graft healing within the femoral tunnel.

The fifth limitation of this canine model study is related to study design. Because of the small sample size for histologic observation, we could not attempt quantitative assessment of histologic observations. Therefore, histologic findings in this study were based on subjective assessment. We did not obtain sufficient power for statistical comparisons on biomechanical parameters because of the small sample size. In addition, because we compared only two groups in terms of the intraosseous graft length in the present study, we could not determine what was a minimal length for the graft to be anchored within the bone tunnel.

In the previous literature, Rodeo et al. [15] and Greis et al. [7] reported that the pull-out strength of the tendon from the bone tunnel had a tendency to increase progressively with the extension of the intraosseous graft length. However, they grafted the extensor tendon into the tibia in an extraarticular condition. Concerning the failure modes in mechanical testing, all tendons were pulled out from the bone tunnel at 6 or 8 weeks in their studies, whereas the graft mid-substance was torn in 2 out of the 5 specimens at 6 weeks in our present study. A possible reason why their results conflicted with those of our study is considered to be in the difference of the experimental models. Namely, the biomechanical behaviors of the flexor tendon grafted in the intraarticular ACL reconstruction model may be different from those in the extraarticularly grafted extensor tendon. In our previous study concerning the effect

of the diameter disparity between the graft and the tunnel [22], the biomechanical behaviors of the flexor tendon grafted in the intraarticular ~~and~~ ACL reconstruction model were obviously different from those in the extraarticularly grafted extensor tendon reported by Rodeo et al [15] and Greis et al. [7]. This fact supports the above-described considerations in the present study.

This study revealed that perpendicular collagen fibers connecting the flexor tendon graft to the bone were denser in the proximal portion of the bone tunnel than in the distal portion. Biomechanical and biological environment for the graft were obviously different between the two portions. For example, the flexor tendon graft has flexion-extension motion at the outlet of the proximal portion. In contrast, the graft located in the distal portion is considered to be exposed in tensile force. Therefore, biomechanical environment for the graft may be different between the two portions. In addition, if the graft is firmly fixed at the proximal portion in the bone tunnel, the distal part of the graft may be shielded from stress. Such stress deterioration may affect the healing process of the distal part of the graft. In this study, however, we could not clarify what mechanisms affected the histological results of this study.

Regarding the clinical relevance, this study implied that excessively long placement of the flexor graft within the bone tunnel does not result in an additional increase of the anchoring strength and stiffness of the graft in ACL reconstruction. Therefore, orthopaedic surgeons need not to place an excessively long portion of the flexor tendon graft within the bone tunnel in order to increase the anchoring strength in ACL reconstruction. Although the minimal but appropriate length for the graft to be fixed with an acceptable strength was not clarified in this study, we believe that this information is of value for orthopaedic surgeons to improve ACL reconstruction procedures in the near future.

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LEGENDS OF FIGURES

Fig. 1 Surgical procedures in Groups I (A) and II (B). The intraosseous graft length in each group is shown in each picture.

Fig. 2 Graft fixation procedure. Each end of the graft was tethered with polyester sutures to a screw inserted to the bone. The distal end was placed in the tibial tunnel, and the proximal end was routed through over-the-top of the femoral condyle.

Fig. 3 For pull-out testing, the femur-graft-tibia complex was mounted on a tensile tester to allow for tensile loading aligned with the long axis of the bone tunnel. For the 6-week specimens, the tests were performed after sutures tethering the graft to the tibia were cut in each study group.

Fig. 4 The linear stiffness (a) and the ultimate load (b) of the graft-tibia complex in pull-out testing.

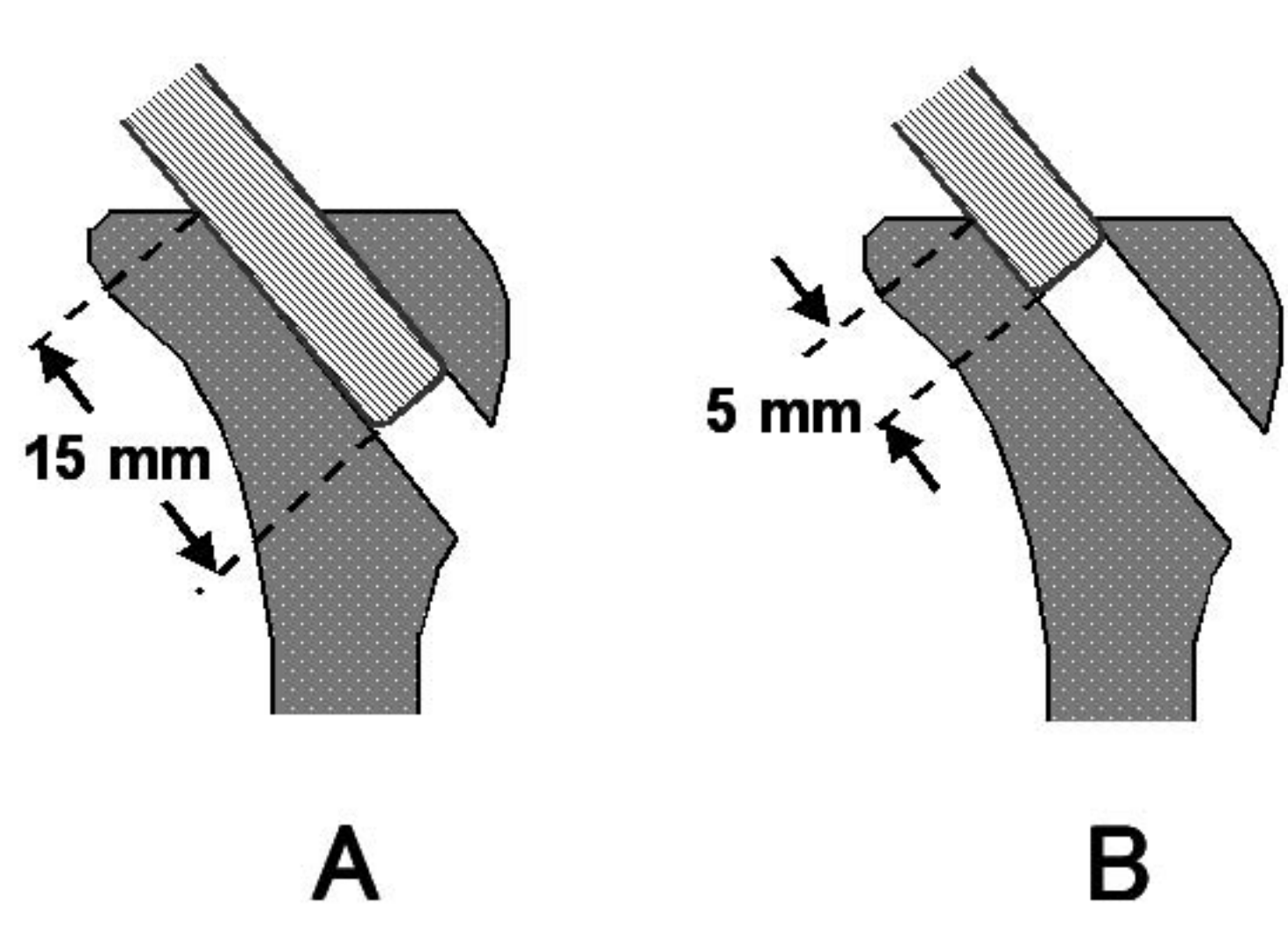
Fig. 5 Histological observations (hematoxylin and eosin stain) in Group I.
a (original magnification, ×5): The space between the tendon-bone gap was filled with granulation tissue. Squares B and C in this picture are magnified as shown as pictures B and C in this figure.
b and c (original magnification, ×50): The perpendicular collagen fibers connecting the tendon to the bone, which resembled Sharpey's fibers, appeared in the granulation tissue. These collagen fibers were more densely observed in a narrow area located close to the intraarticular tunnel outlet (B) than in an area located far from the outlet (C). In these pictures, G means tendon-bone gap and NB shows newly formed bone.

Fig. 6 Histological observations (hematoxylin and eosin stain) in Group II. In these pictures, G means tendon-bone gap and NB shows newly formed bone.

a (original magnification, ×5): The new bone formation was similarly to be seen in Group I. Squares B and C in this picture are magnified as shown as pictures B and C in this figure.

b (original magnification, ×50): The density of the collagen fibers in this proximal 5-mm area appeared to be almost identical between Groups I and II.

c (original magnification, ×50): The tibial end of the graft was connected by the generated collagen fibers to the newly formed bone that filled in a vacant space in the tunnel.


15 mm
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A
B

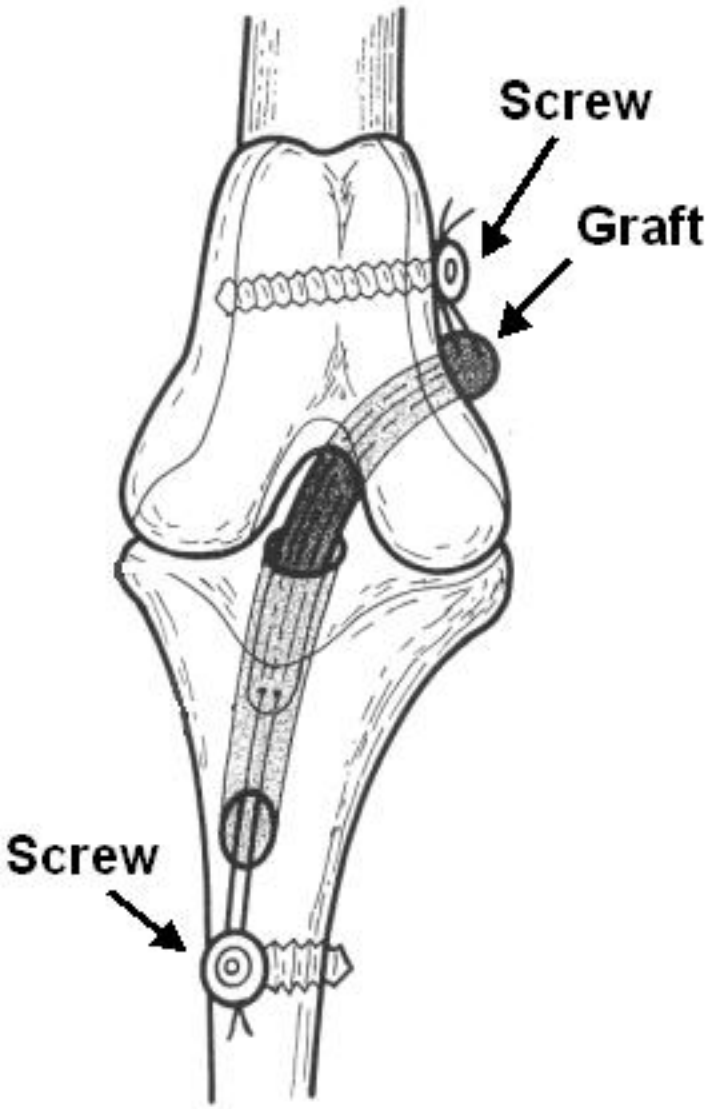


Figure 2

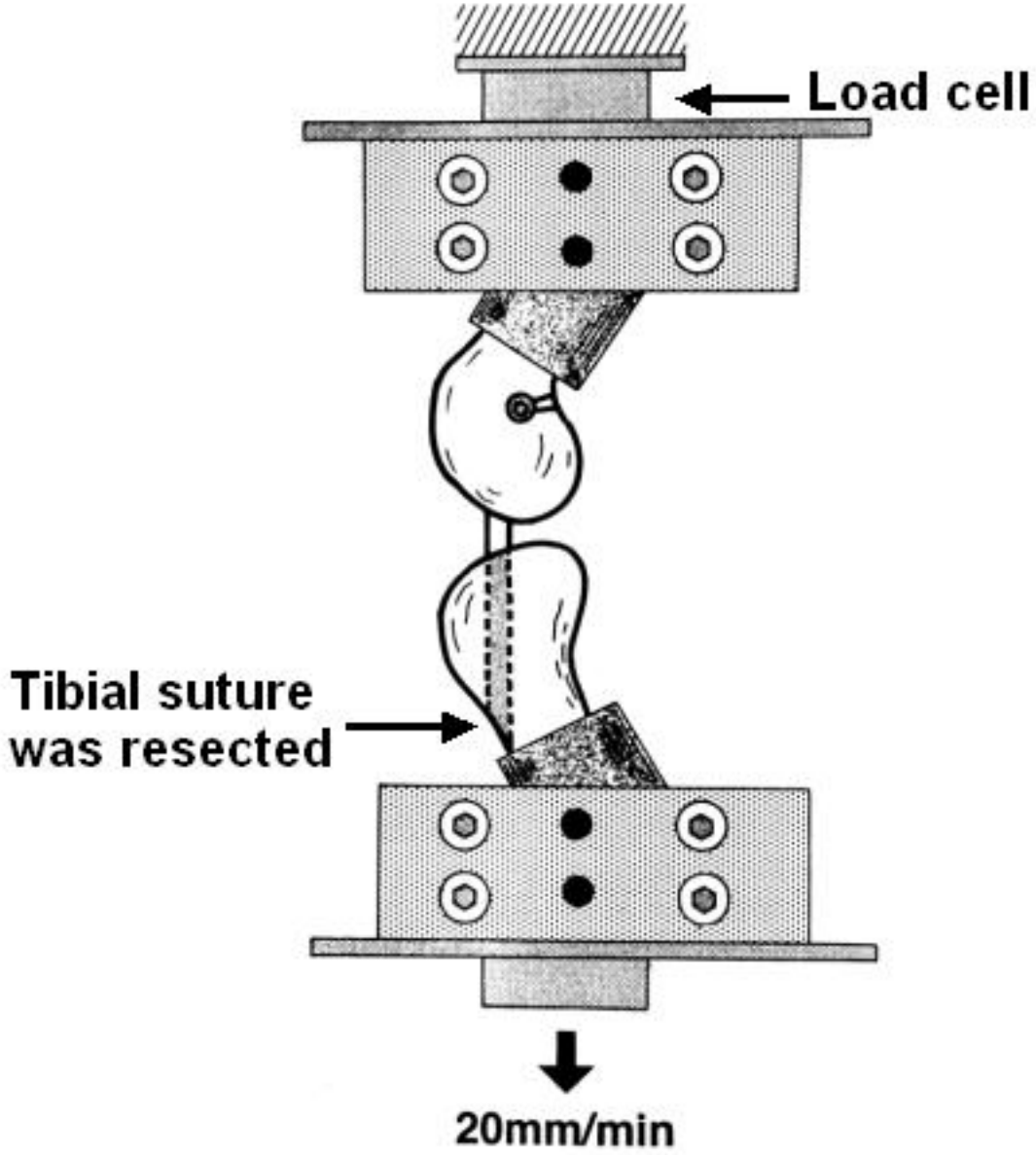


Figure 3

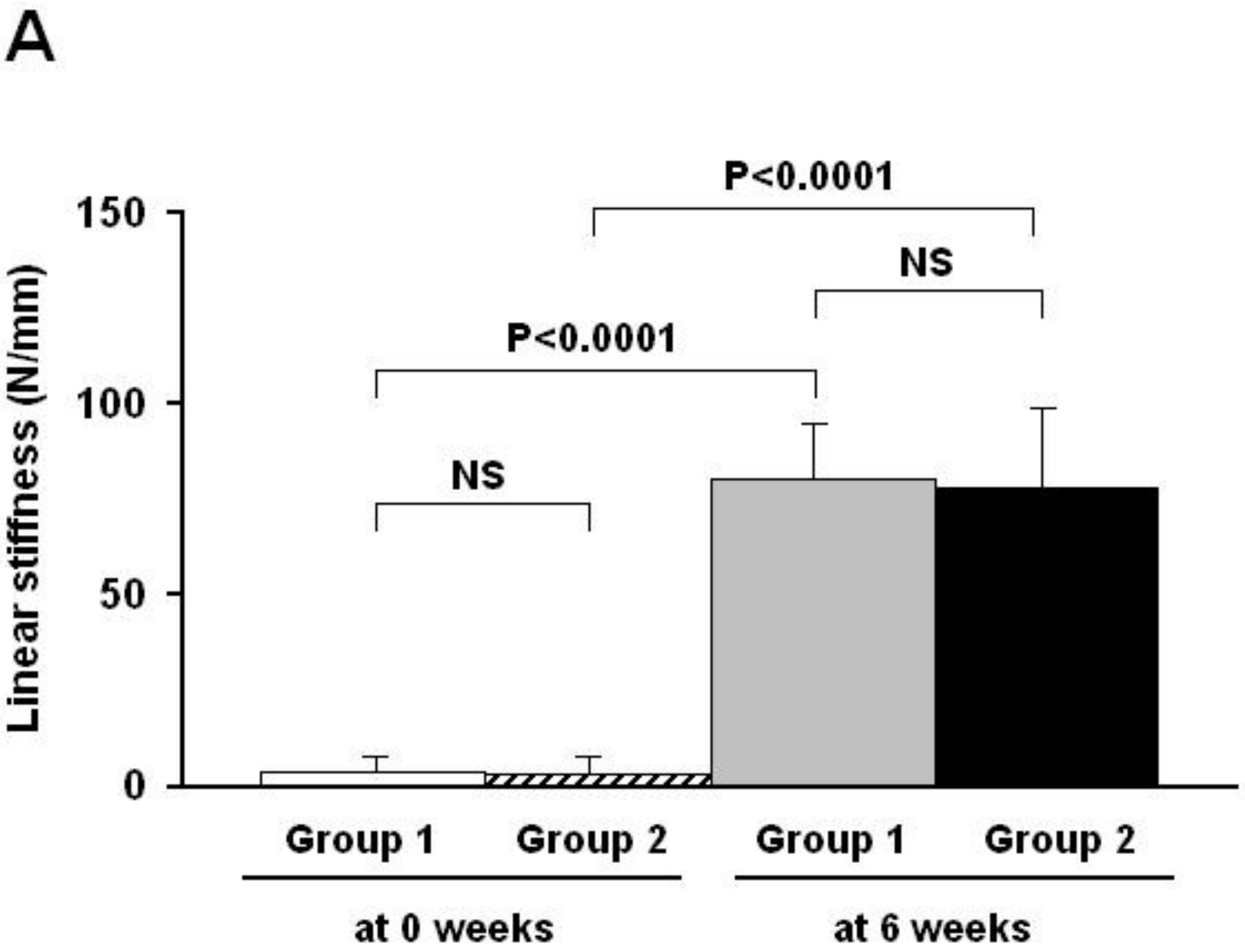

A
P<0.0001
NS
P<0.0001
NS
150
100
50
0
Linear stiffness (N/mm)
Group 1
Group 2
Group 1
Group 2
at 0 weeks
at 6 weeks

Figure 4A

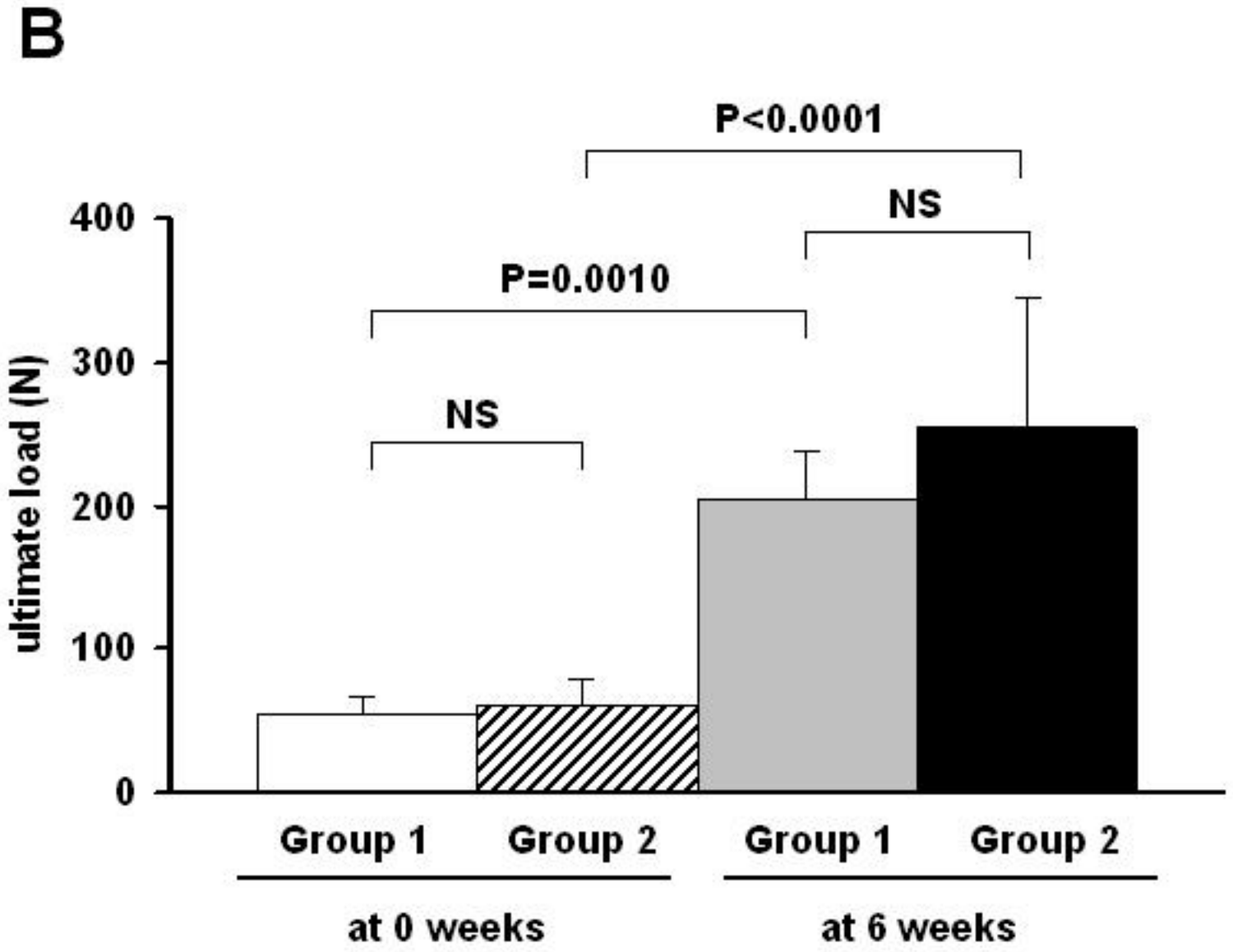
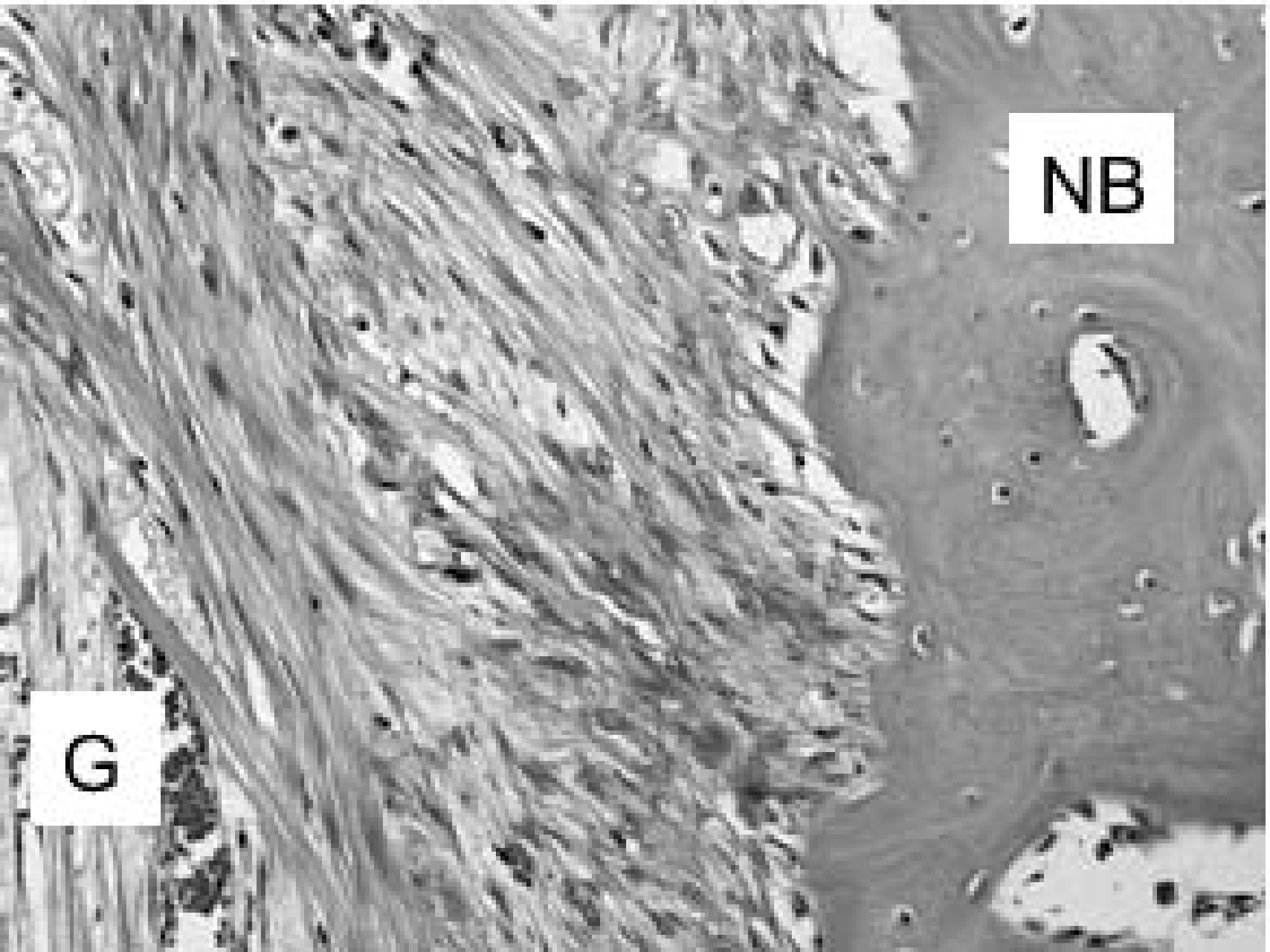

B
P<0.0001
NS
P=0.0010
NS
400
300
200
100
0
ultimate load (N)
Group 1
Group 2
Group 1
Group 2
at 0 weeks
at 6 weeks

Figure 4B

B
C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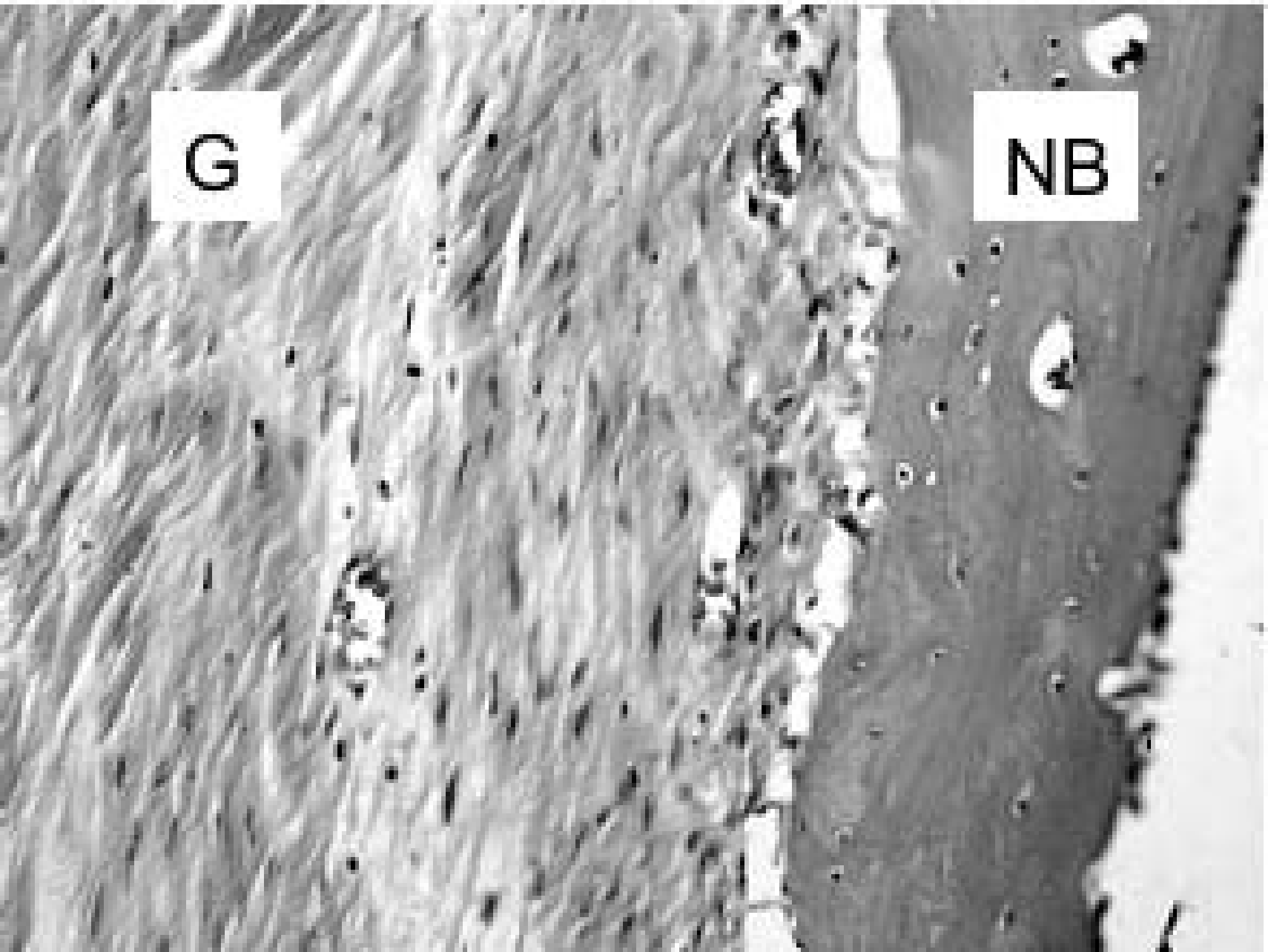
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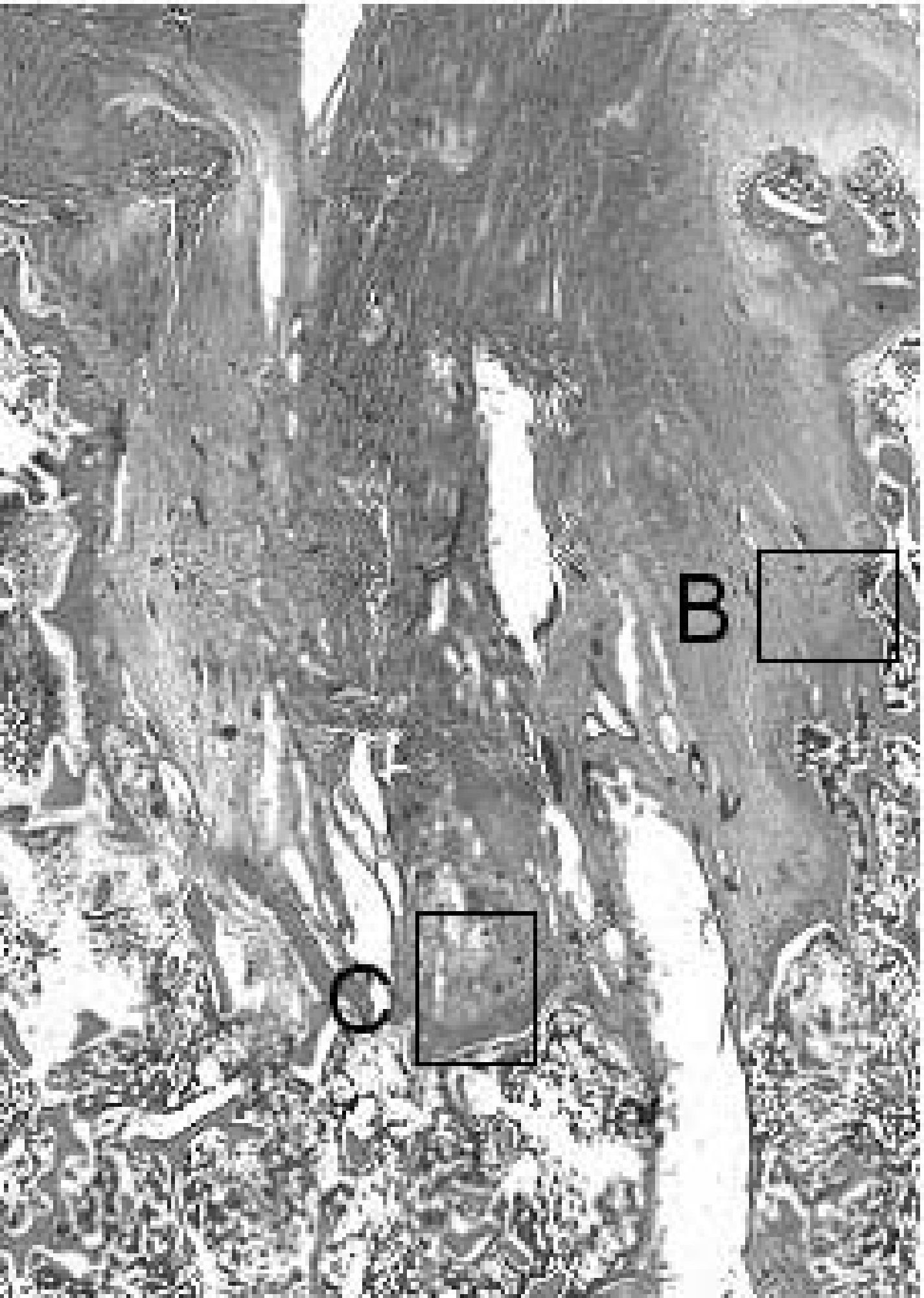
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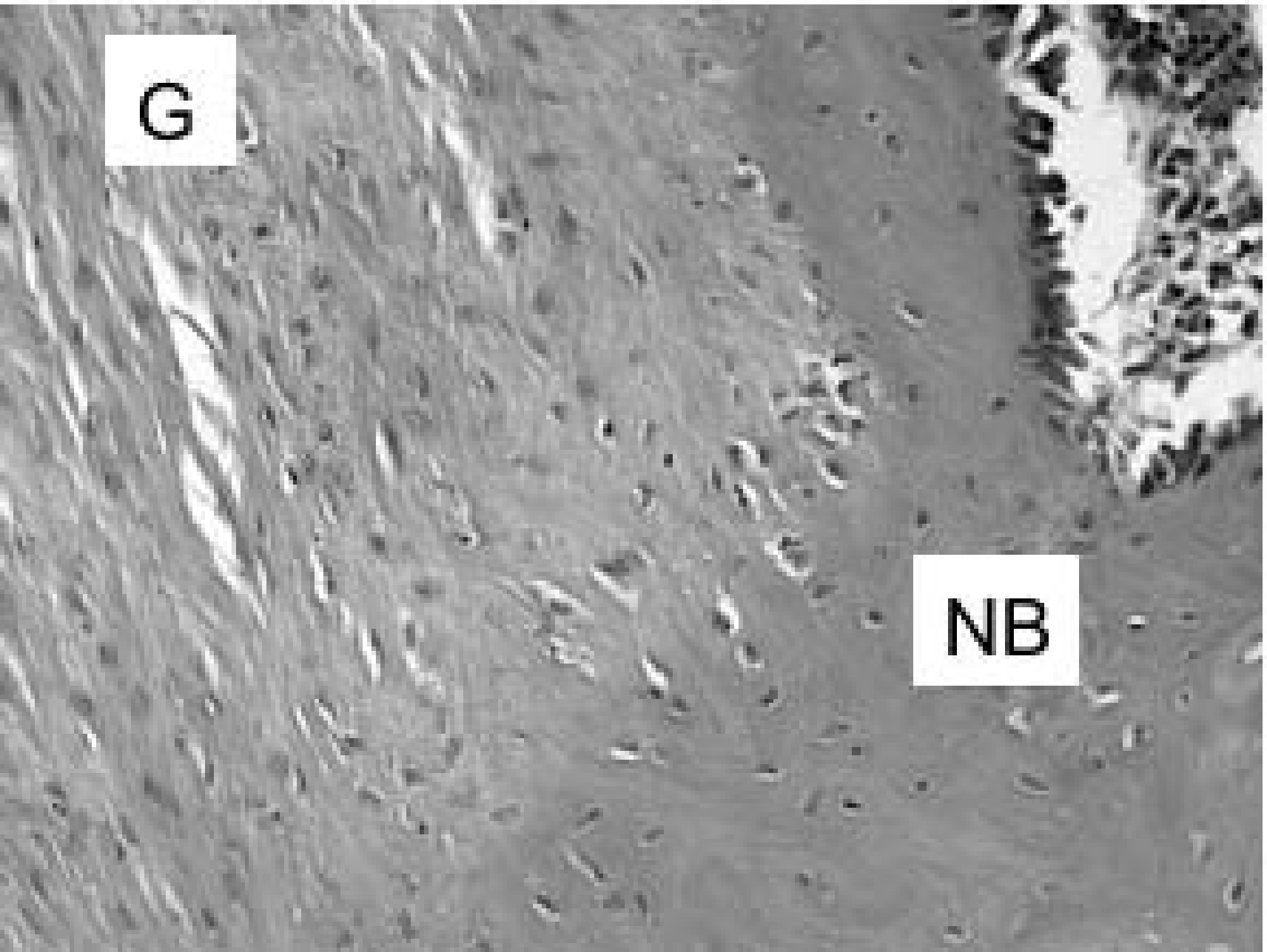
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