



Does platelet-rich plasma enhance microfracture treatment for chronic focal chondral defects? An *in-vivo* study performed in a rat model

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Objective: The purpose of the present study was to compare the effectiveness of platelet-rich plasma (PRP) + microfracture and microfracture treatments in the healing of chronic focal chondral defects.

Methods: The study included 57 adult male Sprague-Dawley rats. Forty-two rats were divided into three groups of 14 rats with a chondral defect (control, microfracture only, PRP+microfracture). The remaining 15 rats were used to produce the PRP preparation. The rats were then euthanized at 3 and 6 weeks after treatment and examined. Histological analysis using the modified Pineda scoring system and immunohistochemical staining for Type 2 collagen were performed.

Results: At both time intervals, control group histological scores (Week 3: 8.8±1.2, Week 6: 8.5±0.7) were higher than microfracture (Week 3: 6.8±1.0, Week 6: 7.1±0.6) and PRP+microfracture (Week 3: 6.4±1.3, Week 6: 5.7±1.2) scores (p<0.05). The microfracture group score was higher at Week 6 than the PRP+microfracture group (p<0.05). The degree of Type 2 collagen staining was higher at Week 6 in the PRP+microfracture group and was unique in showing staining at the cell membrane.

Conclusion: The addition of PRP application to microfracture treatment appears to enhance cartilage healing in chronic focal chondral defects.

Key words: Cartilage repair; microfracture; platelet-rich plasma.

Microfracture is currently accepted as the primary first-line treatment of local contained cartilage defects at the knee as it is a relatively simple one-stage procedure with high effectiveness and low morbidity. Additionally, second-line, more costly procedures such as autologous chondrocyte implantation have been demonstrated to be effective after failed microfracture treatment.^[1,2] However, healing fibro-

cartilaginous tissue resulting from the microfracture technique deteriorates over time^[3,4] possibly explaining the significant reduction in clinical satisfaction outcomes at longer follow-up times relative to the short-term high satisfaction ratings, especially in the athletic population.^[5-7]

Platelet-rich plasma (PRP) is a rich source of autologous growth factors, having a platelet concentration

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above the baseline (whole blood) and including platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), fibroblastic growth factor (FGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and epithelial cell growth factor (EGF).^[8] These growth factors are secreted from α -granules upon platelet activation and delivered to the injury site to facilitate healing.^[9-11]

Platelet-rich plasma used autogenously poses no risk of transmissible disease and provides a simple, low-cost, minimally-invasive way to apply many growth factors. It has begun to be used in various fields of medicine including orthopedics and sports medicine.^[12] Different results have been reported in various clinical and animal studies for fracture healing, nonunion treatment, Achilles tendon repairs, treatment of chronic tendinosis conditions, cartilage healing, and rotator cuff repair.^[11-16] Most recently, an *in vivo* animal study reported the beneficial effect of local PRP applied with a scaffold on the healing of focal osteochondral defects.^[16]

The purpose of the present study was to evaluate the effect of combining a PRP injection with microfracture on the healing of chronic focal chondral defects in a rat model. We hypothesized that the co-administration of PRP alongside microfracture treatment will enhance the cartilage repair and will lead to increased Type 2 collagen expression (specific for hyaline cartilage and chondrogenic differentiation) compared to treatment with microfracture alone.^[2]

Materials and methods

Fifty-seven adult male Sprague-Dawley rats with a mean body weight of 324 (range: 300 to 360) g were used. The study was approved by the institutional animal board and ethics committee. Three rats were housed per cage at 21°C in a 12-hour light-and-dark cycle and given food and water *ad libitum*. Forty-two rats were included in the study and underwent left knee surgery. The remaining 15 rats were used to collect blood and produce PRP.

Rats were divided into three groups of 14 (PRP+microfracture, microfracture only, and control groups). Left knees were shaven and cleaned with antiseptic solution under ketamine and xylazine anesthesia. With the knees in the flexed position, an arthrotomy was made through the medial parapatellar incision. An area of 3×3 mm was marked with a pen on the weight-bearing area of the femoral condyle. Then, an oblique cut was made in the cartilage with a no. 11 blade until a full-thickness defect was created and the subchondral bone was exposed.^[17] The capsule was repaired with absorbable sutures and the skin was closed with nonab-

sorbable sutures. There was no postoperative immobilization and the rats were allowed to move freely.

One month postoperatively, an arthrotomy was repeated through the previous incision and the defect area was reached. A 1.5-mm wide and 2-mm deep hole was created using a K-wire in the microfracture group. In the PRP+microfracture group, 300 μ l of PRP was applied to the microfracture hole: 150 μ l after the microfracture and 150 μ l after the layers were closed. In the control and microfracture only groups, 300 μ l of saline solution was applied. Layers were closed in an anatomical manner and again there was no postoperative immobilization.

The three groups were further divided into 2 groups of 7 rats each. At Week 3, one subgroup from each of the three treatment and control groups were euthanized and underwent histological analysis. The remaining rats were sacrificed at Week 6 for the same purpose.

Histological analysis was performed by the investigator (A.K., Histology and Embryology Specialist) who was blinded to the study groups. Tissues were fixed with 10% formalin and decalcified in EDTA. Decalcified tissue was embedded in paraffin and longitudinal serial sections of 5- μ m thick were taken from the core area of the defect site at the femoral condyle. Hematoxylin and eosin (H&E) and toluidine blue stains were used. For immunohistochemical evaluation, sections were mounted on poly-L-lysine-coated glass slides and immersed in 0.3% H₂O₂ to block endogenous peroxidase activity. The sections were then incubated with a mouse monoclonal antibody directed against Type 2 collagen (Santa Cruz Biotechnology Inc., Heidelberg, Germany).

Table 1. Modified Pineda scoring system^[18] for histological analysis.

Filling of defect as a percentage	Points
125	1
100	0
75	1
50	2
25	3
Reconstitution of osteochondral junction	Points
Yes	0
Almost	1
Not close	2
Cell morphology	Points
Normal	0
Mostly hyaline and fibrocartilage	1
Mostly fibrocartilage	2
Some fibrocartilage but mostly non-chondrocytic cells	3
Non-chondrocytic cells only	4

Defect area healing was evaluated for cellular morphology, reconstitution of the osteochondral junction and percentage filling of the defect. These components were evaluated and scored using the modified Pineda scoring system (Table 1).^[18]

For the PRP preparation, whole blood was collected from 15 male Sprague-Dawley rats. The rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg). Approximately 5 ml of whole blood was collected from the major vessels of each rat. The rats were then killed using an intracardiac overdose of pentobarbital. The anticoagulated blood was centrifuged at 220 G for 20 min. The supernatant was again centrifuged at 480 G for 20 min to form a pellet of rat platelets. The platelet number was counted in the first specimen and found to be 13.8×10^9 platelets/L.^[19]

Mann-Whitney U testing was used to compare histological scores between the groups at two different time periods (Week 3 and Week 6). Differences of $p < 0.05$ were considered significant. Data are presented as mean \pm SD.

Results

There were no postoperative complications. The defect areas were easily detected on each specimen and macroscopically and microscopically evaluated.

In the Week 3 control group, there was a dense connective tissue response at the defect area (Fig. 1a). A connective tissue response was less marked in the Week 3 microfracture group and no cartilage cells were seen in the defect area (Fig. 1b). In the Week 3 PRP+microfracture group, advancement of cartilage cells from the intact cartilage rim into the defect area was observed (Fig. 1c). There was obvious blood congestion at the vessels in all Week 3 groups.

In the Week 6 control group, defect healing was similar to that seen in the Week 3 control group (Fig. 2a), although the degree of connective tissue response seemed to be less in comparison. In the Week 6 microfracture group, a small number of cartilage cells were observed at the defect area (Fig. 2b). In the Week 6 PRP+microfracture group, an eosinophilic stained thin layer containing cartilage cells was observed at the defect area (Fig. 2c). In one specimen, total defect area

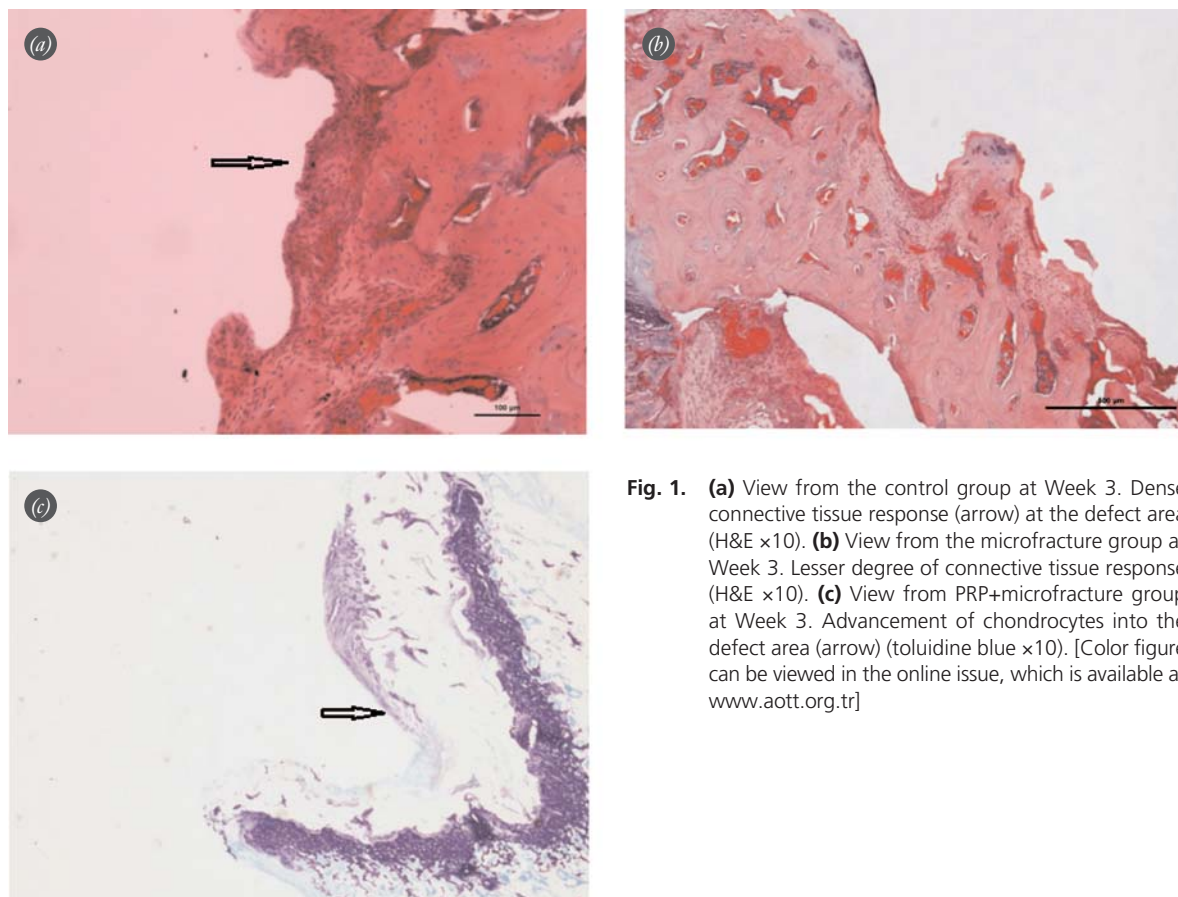


Fig. 1. (a) View from the control group at Week 3. Dense connective tissue response (arrow) at the defect area (H&E $\times 10$). (b) View from the microfracture group at Week 3. Lesser degree of connective tissue response (H&E $\times 10$). (c) View from PRP+microfracture group at Week 3. Advancement of chondrocytes into the defect area (arrow) (toluidine blue $\times 10$). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

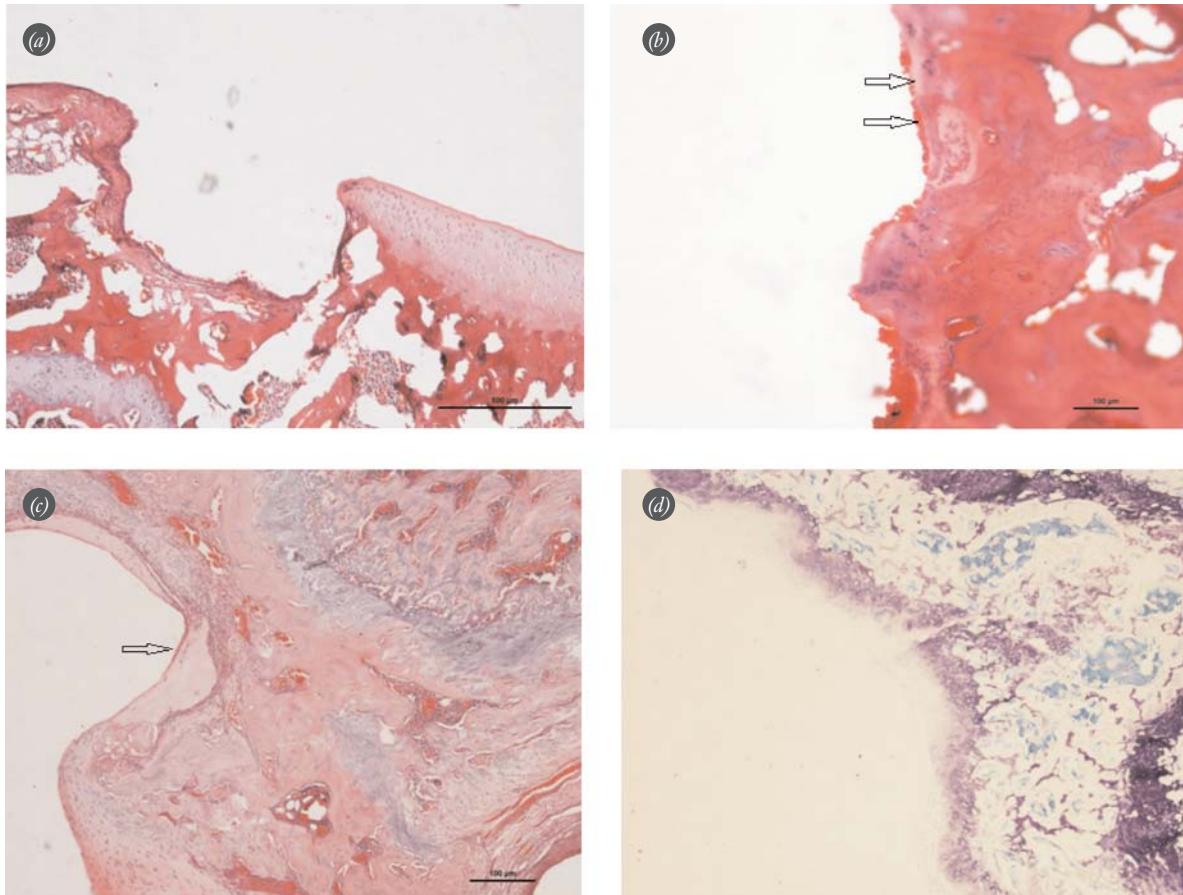


Fig. 2. (a) View from the control group at Week 6 (H&E $\times 10$). (b) View from the microfracture group at Week 6. Thin layer of chondrocytes at the defect area (arrows) (H&E $\times 10$). (c) View from the PRP+microfracture group at Week 6. Eosinophilic-stained thin layer of cartilage cells (arrow) (H&E $\times 10$). (d) View from the PRP+microfracture group at Week 6. Total closure of the defect with a layer of chondrocytes (H&E $\times 10$). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

closure with a thin layer of cartilage cells was observed (Fig. 2d).

Type 2 collagen staining was observed in a few cells at the defect area in the Week 3 and Week 6 control groups (Fig. 3a). A similar pattern and amount of staining was also observed in the Week 3 and Week 6 microfracture groups (Fig. 3b). An increased tendency of staining in the Week 3 PRP+microfracture group (Fig. 3c) was noted, which became marked in the Week 6 PRP+microfracture group. Here, an increased number of cells stained with Type 2 collagen and staining at the cell membrane was noted (Fig. 3d). This differed from the other groups, which showed an intracellular staining pattern instead.

Histological scores in the control group at both 3 weeks and 6 weeks were higher than in the microfracture groups (Week 3: 8.8 ± 1.2 , Week 6: 8.5 ± 0.7 vs Week 3: 6.8 ± 1.0 , $p=0.01$, Week 6: 7.1 ± 0.6 , $p=0.01$, respectively) and in the PRP+microfracture groups (Week 3: 6.4 ± 1.3 $p=0.01$, Week 6: 5.7 ± 1.2 $p=0.002$) ($p < 0.05$).

The Week 6 microfracture group score was higher than the Week 6 PRP+microfracture group score ($p=0.03$).

Discussion

A relatively small number of trials, a short period of follow-up and the heterogeneity of outcome measures make it difficult to favor one technique of articular cartilage repair over the other. Microfracture treatment requires little preoperative planning and does not rule out the option of future, more complex procedures such as autologous chondrocyte implantation or osteochondral autograft transfer. Fibrocartilage formed at the defect area is the predominant reason for the decline in satisfaction with microfracture in clinical outcomes.^[5-7] Recent cartilage treatment modalities such as those mentioned above aim to reproduce or re-implant hyaline cartilage at the defect area.

Platelet-rich plasma's beneficial effects have been proven in the orthopaedic literature in both *in vivo* and

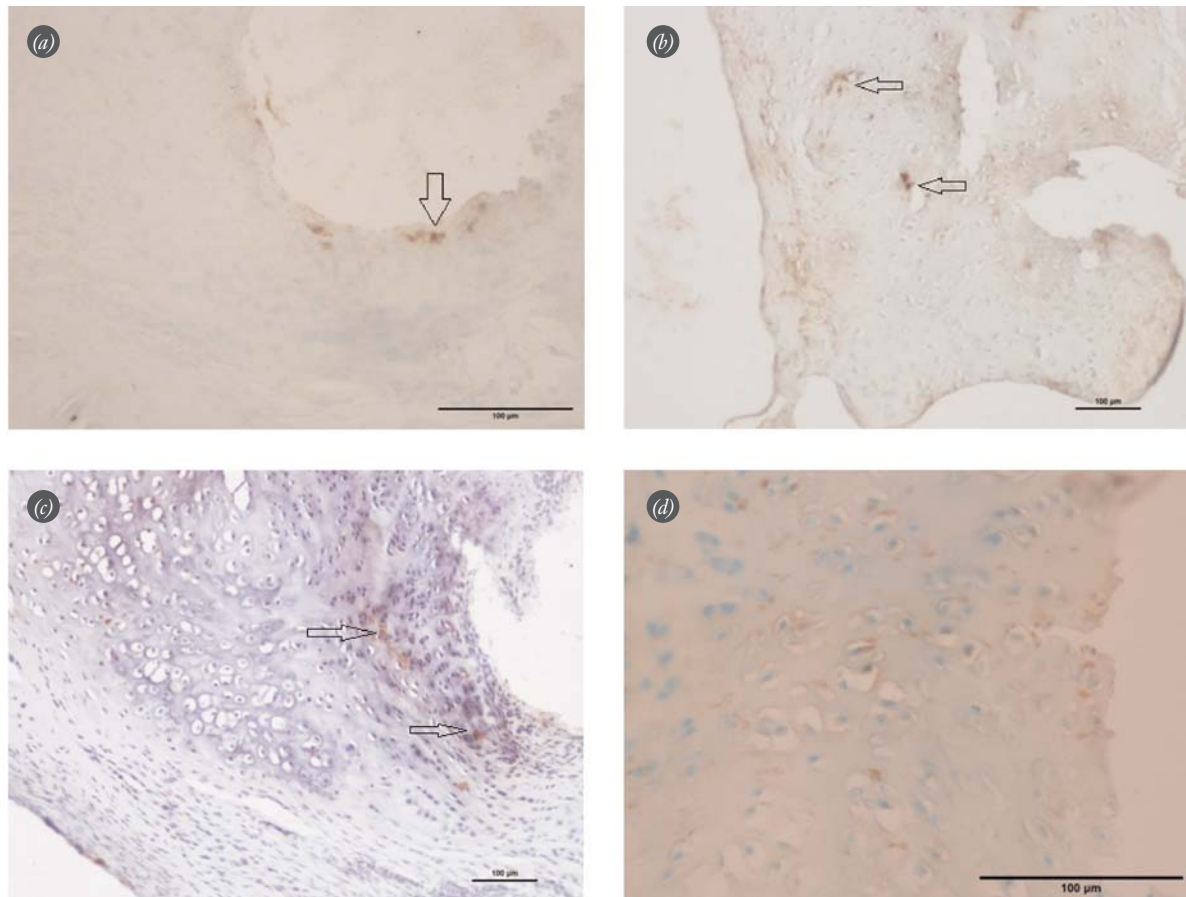


Fig. 3. (a) View from the control group. Immunohistochemical staining for Type 2 collagen (arrow) (H&E x10). (b) View from the microfracture group. Immunohistochemical staining for Type 2 collagen (arrows) (H&E x10). (c) View from the PRP+microfracture group at Week 3. Immunohistochemical staining for type 2 collagen (arrows) (H&E x10). (d) View from the PRP+microfracture group at Week 6. Immunohistochemical staining for Type 2 collagen (H&E x10). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

clinical studies. Positive outcomes have been reported in the treatment of various tendinosis conditions, nonunion and rotator cuff tears.^[11-16] We found a few *in vivo* studies in the literature demonstrating the effectiveness of PRP in combination with microfracture in chronic chondral defect treatment in a sheep model. In these studies, histological scores for PRP gel or PRP liquid injection were higher than in the microfracture only group.^[20-22] However, in contrast to the present study, there was no sham group and Type 2 collagen immunohistochemical staining for detection of hyaline chondrogenic differentiation^[20-22] was not undertaken, although this was a common feature in other *in vivo* cartilage studies.^[18,23]

Increased expression of Type 2 collagen at the cartilage cell membrane in the defect area at the 6th week following PRP+microfracture can be explained by increased cellular activity, extracellular substance synthesis and increased migration and stimulation of sub-

chondral progenitor cells. This has been most recently reported in an *in vitro* study performed on human subchondral progenitor cells.^[24]

Limitations of the present study include the fact that our animal model does not fully correspond to the chronic focal chondral defects seen clinically. As previously outlined in the literature, no lasting effect of either growth factors or cytokines resulting from the original injury was noted one month post injury in chronic focal chondral defects in rat models, while pluripotent cells induced from the bone marrow interacted with growth factors induced both from the marrow and from the PRP.^[18,25] Supporting this is the fact that Type 2 collagen stained cartilage cells at the defect area were seen in higher concentrations in PRP+microfracture groups compared to control groups in the present study.

A second possible limitation is that we did not identify which components of PRP are responsible for the improvements we found or provide any information on

the bioavailability or duration of PRP activity at the repair site. This could be possible with the addition of another group that was only treated with PRP without microfracture.

Follow-up of the present study was relatively short. However, the healing potential of rats is so rapid that the present study was nonetheless able to report the difference between the groups. Additionally, a search of the literature provides various follow-up recommendations ranging from 40 days to 72 weeks.^[17,18,23,26,27] A rat model of full-thickness articular cartilage defects that can be accurately and reproducibly sized is still undergoing research^[28] and most reported techniques use blade or drill bits to create osteochondral defects.^[17,18,26,27] However, a punch biopsy may be preferable to create standardized circular defects that could be more appropriate for cartilage healing assessment.^[29]

We used the Pineda histological scoring system that has previously been used in rat models^[18,23] instead of more recent popular systems like O'Driscoll scoring or International Cartilage Repair Society scoring that have mostly been used in larger animal models such as sheep or rabbits.^[22,30] Additional quantitative scoring of immunohistochemical staining or biomechanical evaluation of the healed cartilage could be made that would further lend weight to the hypothesis. However, we could not find any study in a rat model from which to adapt a quantitative scoring system or biomechanical testing.^[18,22,23,27,28]

In conclusion, this study suggests that in a rat chronic focal chondral defect model, PRP application in addition to microfracture may result in a better cartilage healing and increased Type 2 collagen expression than microfracture alone. Further studies will be necessary to continue to evaluate improved cartilage healing mechanisms.

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Conflicts of Interest: No conflicts declared.

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