

FIBRIN CLOT FOR REPAIR OF THE CANINE MENISCUS DEFECT

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ABSTRACT

A fibrin clot contains numerous growth factors to accelerate tissue healing. The present study aimed to investigate the therapeutic effect of fibrin clot either from bone marrow or peripheral blood on meniscal defects at the avascular area of the anterior part of the canine medial meniscus of the stifle joint. Twelve dogs were divided randomly into 2 groups: the bone marrow blood group (6) and the peripheral blood group (6). A defect was created at the avascular area of the medial meniscus near the anterior horn, then a fibrin clot was implanted. All animals were euthanized at 3 months post-implantation. Medial meniscal samples were collected, examined, and stained by H&E. Grossly, both treated groups revealed complete filling of the meniscal defect with newly formed tissue resembling the surrounding tissue. Histologically, the repaired tissue contains chondrocytes embedded in unarranged, oriented collagen bundles surrounded by fibrous connective tissue. The use of fibrin clots from different sources might represent the potential method for repair of avascular meniscal defects in large animal models.

Keywords: Fibrin clot; Meniscal injury; Repair; Large animal model.

INTRODUCTION

The meniscus, a crescent cartilaginous structure found in stifle joints, is primarily responsible for transmitting load throughout the joint (Lee *et al.*, 2006). Additionally, menisci aid in articular cartilage preservation, stability, lubrication, and shock absorption (Ralphs and Whitney,

2002). Meniscal tears accounted for around 15% of stifle joint injuries (Baek *et al.*, 2016). The medial meniscus is more likely to tear than the lateral meniscus. (Ralphs and Whitney, 2002).

Red-red tears, red-white tears, and white-white tears are the three primary categories of meniscal tears. The most challenging to treat are white-white tears, which occur in the inner third area without a blood supply (Noyes and Barber-Westin, 2012). Due to the tissue's poor ability to mend, meniscectomy, the removal of injured tissue was carried out in a significant proportion

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of instances with meniscal tears. It is thought to be the most prevalent treatment for meniscal injury (Cook *et al.*, 2006). When meniscal tissue is lost, the knee's capacity to bear weight, absorb shock, maintain stability, lubricate, and nourish can all be seriously threatened. These disruptions cause osteoarthritis by compromising the protection of the articular cartilage of the femoral and tibial condyles (Andersson-Molina *et al.*, 2002; Cicuttini *et al.*, 2002).

Recently, fibrin clot is considered one of the usable options for tissue regeneration due to its unique biological properties, such as containing several growth factors, especially platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF β) in case of peripheral blood, and in addition to abundant mesenchymal stem cells in the case of bone marrow blood (Shoji *et al.*, 2017).

Up to now, there is no *in vivo* study demonstrating the therapeutic effect of fibrin clots from different sources on meniscal lesions, especially at the avascular zone of the canine as a large animal model. For this, the study aimed to compare the fibrin clot to peripheral and bone marrow blood to repair meniscal lesions in a canine model.

MATERIALS AND METHODS

Following institutional rules for the care and use of experimental animals, this controlled laboratory study was carried out with ethical approval from the Faculty of Medicine, Assiut University (04-2025-300598).

Animal used

A total of 12 dogs (10 males, 2 bitches) weighing 10-15 Kg, aged 1-2 years, were used in the present study. The experiment was performed on stray dogs that were used for educational purposes for undergraduate

students of our faculty. These animals were given a deworming protocol and kept in clean individual boxes. All dogs were divided randomly into two groups: Bone Marrow Blood (BM) (5 males, 1 bitch) and Peripheral Blood (PB) (5 males, 1 bitch).

Preparation of fibrin clot

All procedures were performed under general anesthesia using intramuscular injection of a combination [of ketamine (5mg/kg) and xylazine (1mg/kg)]. Fibrin clots were prepared from peripheral blood and bone marrow blood. In brief, 10 ml of bone marrow and peripheral blood were collected in a sterilized beaker from the iliac crest and cephalic vein, respectively, which were agitated by a glass rod in a rotatory movement until a fibrin clot formed on the tip of the glass rod and washed with normal saline (Desai *et al.*, 2021; Kinoshita *et al.*, 2023).

Induction of acute meniscal lesions

The medial parapatellar approach was used for performing arthrotomy with lateral dislocation of the patella and full extension of the leg to expose the medial meniscus. A part of the inner aspect (white-white tears) of the medial meniscus was removed. Then, the treatment option of fibrin clot from bone marrow or peripheral blood was performed under sterile conditions.

Implantation of fibrin clot into the defect lesion

The fibrin clot was applied to the defect area of the medial meniscus of the knee (stifle), then the fibrin clot was fixed with medial synovium by using absorbable suture material (Vicryl) size 4/0, using an interrupted horizontal mattress. Then the joint capsule was routinely closed by using absorbable suture material and a simple interrupted pattern.

Macroscopic & histological evaluation of the meniscus

Three months after implantation of the fibrin clot, adult dogs were euthanized, then

medial meniscal tissue samples were collected and fixed in 10% neutral buffered formalin for 24 hours. These samples were dehydrated in graded alcohols, embedded in paraffin, and then cut into 5 µm sections, which were stained with H&E stain.

RESULTS

On gross inspection, the meniscal lesions showed a good degree of healing in all animals in either the PB or BM treated groups. In addition, the colour of the repaired tissue was somewhat like the surrounding one. Also, we could not easily identify the defect borders (Fig. 1). Also, we did not notice any gross osteoarthritic changes either in the medial femoral condyle or tibial plateau (data not shown). The normal meniscus was filled with normal fibrocartilage tissue that appeared as a combination of hyaline cartilage through normal compacted oriented collagen bundles with normally arranged meniscal cells in between and fibrous connective tissue (Fig. 2). In the PB- and BM-treated groups, the defect was filled with quite normal fibrocartilage tissue, with less

compacted and differentially oriented collagen bundles and fewer meniscal cells randomly arranged between them, and not completely organized fibrous connective tissue (Fig. 3 & 4).



Fig. 1: Representative photos for gross findings of the menisci after implantation of fibrin clot from bone marrow (BM) and peripheral blood (PB).

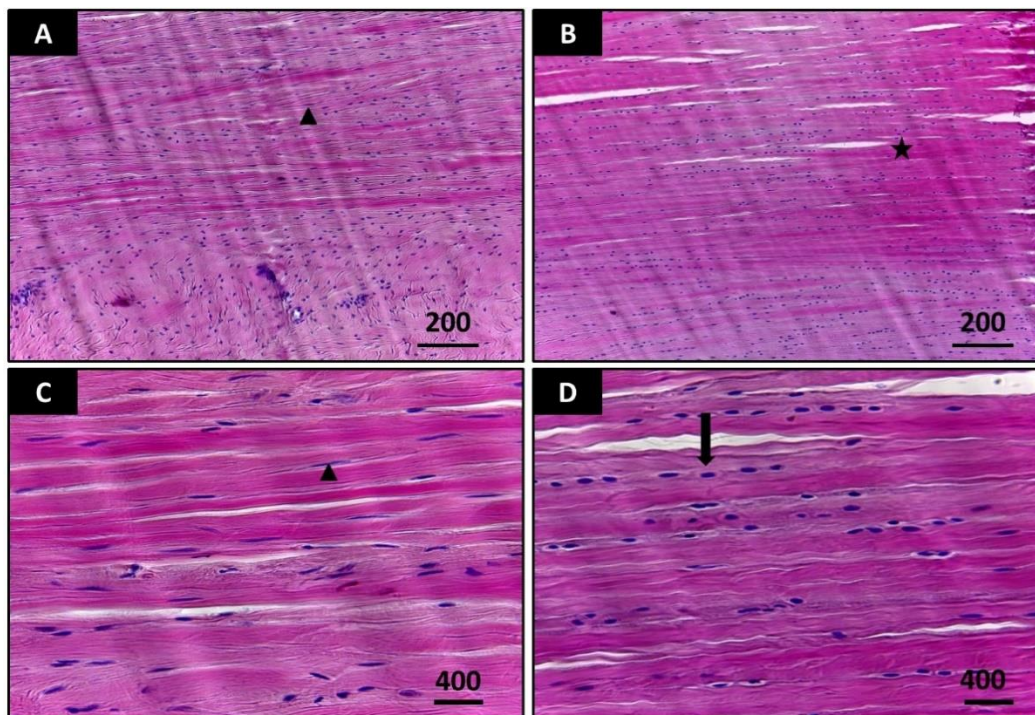


Fig. 2: Normal meniscal tissue of canine shows arranged collagenous bundles (5-point star), chondrocytes in lacunae (down arrow), and dense connective tissue (isosceles triangle). (H&E stain).

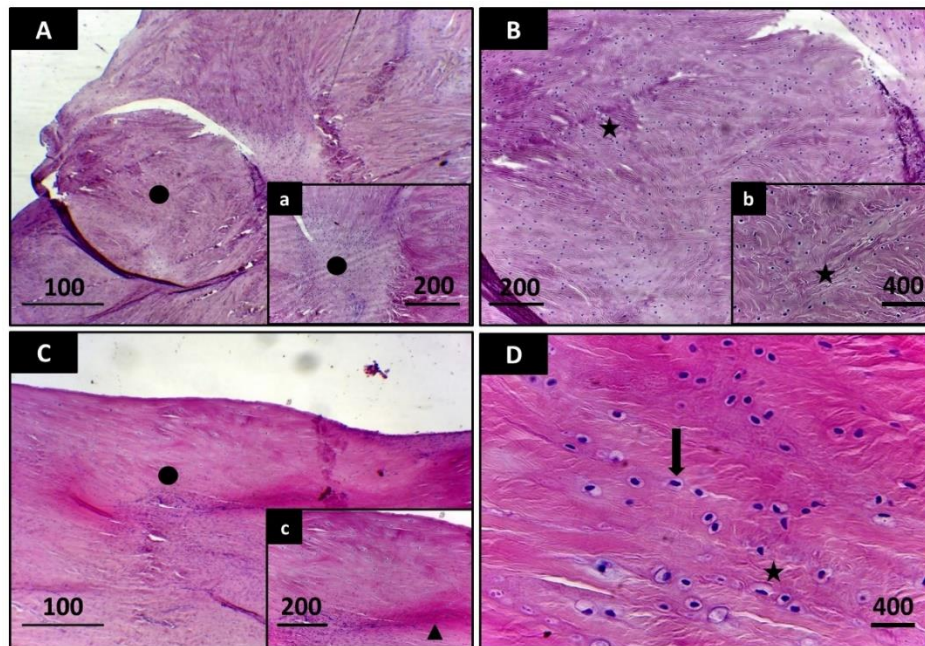


Fig. 3: PB PB-treated group is showing a complete full defect (oval) surrounded by fibrous connective tissue (isosceles triangle), differently oriented collagen bundles (5-point star) and chondrocytes (down arrow). (H&E stain).

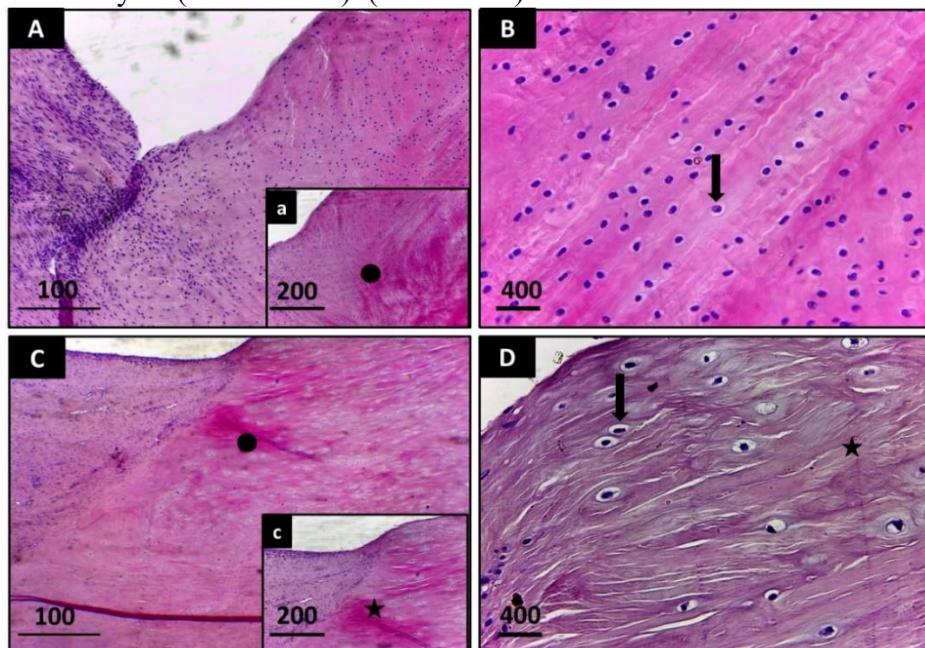


Fig. 4: BM BM-treated group shows a complete full defect (oval), and chondrocytes in lacunae (down arrow) randomly distributed among different oriented collagen bundles (5-point star). (H&E stain).

DISCUSSION

Several studies were performed on the fibrin clots either from peripheral blood or bone marrow blood alone for musculoskeletal tissue regeneration, focusing on the concentration of the growth

factors in fibrin clots. However, the previous study of exogenous fibrin clot and cultured marrow cells did not enhance meniscal healing in the goat as a large animal model (Port *et al.*, 1996). The present study investigated the regenerative effect of fibrin clot, either from bone

marrow or peripheral blood, on meniscal defects at the avascular inner area in the canine as a large animal model, which revealed complete filling of the meniscal defects with newly formed tissue resembling the native one in the treated groups, with fibrin clot either from PB or BM. Histologically, fibrocartilage tissue was noticed, resulting from fibrous connective tissue proliferation, except with less compacted and less organized collagen bundles, fewer meniscal cell arrangements in between, compared to normal ones.

Animal models play a critical role in helping translate fundamental scientific discoveries into useful clinical applications that eventually improve human health. Meniscal damage and repair biological mechanisms have been studied in both small and large animal models (Arnoczky *et al.*, 2010; Deponti *et al.*, 2015; Janusz *et al.*, 2002). Generally, large animal models seem to be more appropriate for meniscal biological research than small animals (Krupkova *et al.*, 2018). A recent study investigated the effectiveness of fibrin clot from bone marrow and peripheral blood in a rabbit as a small animal model, which revealed meniscal healing resulting from bone marrow fibrin clot was superior to from peripheral blood fibrin clot (Kinoshita *et al.*, 2023).

We must take into consideration that fibrin clot has several advantages, such as easy accessibility, no need for extra surgery, autogenic source to avoid disease transmission, and contains several growth factors and mesenchymal stem cells. Numerous growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), stromal cell-derived factor-1 (SDF-1), and hepatocyte growth factor (HGF), were detected in the bone marrow blood at higher levels than the peripheral blood (Shoji *et al.*, 2017). These

growth factors are necessary for the proliferation and differentiation of fibroblasts and osteoblasts, which are vital for the regeneration of different tissues, as well as for angiogenesis through the activation of endothelial progenitor cells (Li *et al.*, 2006). Moreover, IGF-1 has also been shown to decrease the expression of inflammatory genes and promote the growth of mesenchymal stromal cells. However, it was shown that the combination of HGF and mesenchymal stromal cells enhanced the therapeutic effects (Kim *et al.*, 2014; Zhang *et al.*, 2014).

Potential limitations were recorded in the present study: firstly, long-term evaluation of the meniscal repair; secondly, osteoarthritic changes of the medial condyle and tibial plateau correlated with the meniscus. In addition to an estimation of the growth factors, such as VEGF and bFGF, in the fibrin clot will be evaluated in further studies. The fibrin clot seems to serve as a scaffold for the reparative process, as well as a chemotactic and mitogenic stimulation for reparative cells (Arnoczky *et al.*, 1988).

CONCLUSION

An exogenous fibrin clot's capacity to promote and facilitate a reparative response in the meniscus's avascular region could be a good technique for the repair of avascular meniscal injury in large animal models.

REFERENCES

- Andersson-Molina, H.; Karlsson, H. and Rockborn, P. (2002): Arthroscopic partial and total meniscectomy: a long-term follow-up study with matched controls. *Arthroscopy*, 18(2): 183-189.
- Arnoczky, S.P.; Cook, J.L.; Carter, T. and Turner, A.S. (2010): Translational models for studying meniscal repair

- and replacement: what they can and cannot tell us. *Tissue Eng. Part B Rev.*, 16: 31-39.
- Arnoczky, S.P.; Warren, R.F. and Spivak, J.M. (1988):* Meniscal repair using an exogenous fibrin clot. An experimental study in dogs. *J. Bone Joint Surg. Am.*, 70(8): 1209-1217.
- Baek, J.; Sovani, S.; Glembotski, N.E.; Du, J.; Jin, S.; Grogan, S.P. and D'Lima, D.D. (2016):* Repair of avascular meniscus tears with electrospun collagen scaffolds seeded with human cells. *Tissue Eng. Part A*, 22(5-6): 436-448.
- Cicuttini, F.M.; Forbes, A.; Yuanyuan, W.; Rush, G. and Stuckey, S.L. (2002):* Rate of knee cartilage loss after partial meniscectomy. *The Journal of Rheumatology*, 29(9): 1954-1956.
- Cook, J.L.; Fox, D.B.; Malaviya, P.; Tomlinson, J.L.; Kuroki, K.; Cook, C.R. and Kladakis, S. (2006):* Long-term outcome for large meniscal defects treated with small intestinal submucosa in a dog model. *The American Journal of Sports Medicine*, 34(1): 32-42.
- Deponti, D.; Di Giancamillo, A.; Scotti, C.; Peretti, G.M. and Martin, I. (2015):* Animal models for meniscus repair and regeneration. *J. Tissue Eng. Regen. Med.*, 9(5): 512-527.
- Desai, T.; Babu, S.S.; Lal, J.V.; Kaushik, Y.S.; Lukose, A.M.; Sandesh, G.M. and Amaravathi, R.S. (2021):* Fibrin clot augmented repair of longitudinal tear of medial meniscus. *Arthrosc. Tech.*, 10(11): e2449-e2455.
- Janusz, M.J.; Bendele, A.M.; Brown, K.K.; Taiwo, Y.O., Hsieh, L. and Heitmeyer, S.A. (2002):* Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. *Osteoarthritis Cartilage*, 10: 785-791.
- Kim, M.D.; Kim, S.S.; Cha, H.Y.; Jang, S.H.; Chang, D.Y.; Kim, W.; Suh-Kim, H. and Lee, J.H. (2014):* Therapeutic effect of hepatocyte growth factor-secreting mesenchymal stem cells in a rat model of liver fibrosis. *Exp. Mol. Med.*, 46: e110.
- Kinoshita, T.; Hashimoto, Y.; Orita, K.; Iida, K.; Takahashi, S. and Nakamura, H. (2023):* Bone marrow-derived fibrin clots stimulate healing of a knee meniscal defect in a rabbit model. *Arthroscopy*, 39(7):1662-1670.
- Krupkova, O.; Smolders, L.; Wuertz-Kozak, K.; Cook, J. and Pozzi, A. (2018):* The Pathobiology of the Meniscus: A Comparison Between the Human and Dog. *Front. Vet. Sci.*, 5: 73.
- Lee, S.J.; Aadalen, K.J.; Malaviya, P.; Lorenz, E.P.; Hayden, J.K.; Farr, J.; Kang, R.W. and Cole, B.J. (2006):* Tibiofemoral contact mechanics after serial medial meniscectomies in the human cadaveric knee. *The American Journal of Sports Medicine*, 34(8): 1334-1344.
- Li, Y.; Titterton, J.; Song, Y.; Reger, R. and Delafontaine, P. (2006):* Differential effects of IGF-1 and VEGF on migration, proliferation and differentiation of bone marrow-derived mesenchymal stem cells. *Circulation*, 114(18): 84-85.
- Noyes, F.R. and Barber-Westin, S.D. (2012):* Management of meniscus tears that extend into the avascular region. *Clinics in Sports Medicine*, 31(1): 65-90.
- Port, J.; Jackson, D.W.; Lee, T.Q. and Simon, T.M. (1996):* Meniscal repair supplemented with exogenous fibrin clot and autogenous cultured marrow cells in the goat model. *The American Journal of Sports Medicine*, 24(4): 547-555.
- Ralphy, S.C. and Whitney, W.O. (2002):* Arthroscopic evaluation of menisci in dogs with cranial cruciate ligament injuries: 100 cases (1999–2000). *Journal of the American Veterinary Medical Association*, 221(11): 1601-1604.
- Shoji, T.; Nakasa, T.; Yoshizuka, M.; Yamasaki, T.; Yasunaga, Y.; Adachi,*

- N. and Ochi, M. (2017): Comparison of fibrin clots derived from peripheral blood and bone marrow. Connect. Tissue Res., 58(2): 208-214.*
- Zhang, L.; Zhang, L.; Lan, X.; Xu, M.; Mao, Z.; Lv, H.; Yao, Q. and Tang, P. (2014): Improvement in angiogenesis and osteogenesis with modified cannulated screws combined with VEGF/PLGA/fibrin glue in femoral neck fractures. J. Mater. Sci. Mater. Med., 25(4): 1165-1172.*

استخدام جلطة الفيبرين لإصلاح عيب الغضروف الهلالي للكلاب

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يحتوي جلطة فيبرين على العديد من عوامل النمو لتسريع إلتئام الأنسجة. وكانت هذه الدراسة للتحقيق في التأثير العلاجي لتجلط الفيبرين إما من نخاع العظام أو الدم الطرفي على العيب الخاص بالغضروف الهلالي في الجزء الأمامي من medial meniscus. تم تقسيم ١٢ كلب بشكل عشوائي إلى مجموعتين: مجموعة دم نخاع العظام (٦) وفئة الدم الطرفي (٦). تم استحداث عيب في الغضروف الهلالي للكلاب، ثم تم زرع جلطة الفيبرين. تم إعدام جميع الحيوانات بعد ٣ شهور من زراعة جلطة الفيبرين. تم جمع عينات الغضروف الهلالي وفحصه. كشفت المجموعتان المعالجتان عن ملء كامل للعيب بالأنسجة الجديدة التي تشبه المحيطة بها. أيضا تحتوي الأنسجة المعاد ترميمها على الكريات chondrocytes في حزم الكولاجين غير المنظمة المحيطة بالأنسجة الضامة الليفية. لذلك قد يمثل استخدام جلطة الفيبرين من المصادر المختلفة طريقة محتملة لإصلاح العيوب الخاصة بالغضاريف الهلالية الخاص بالحيوانات الكبيرة.