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EFFICACY OF BIOSCAFFOLD FOR TREATMENT OF FEMORAL ARTICULAR CARTILAGE DEFECTS IN RABBITS MODELS

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Abstract

The ultimate goal of this study was to evaluate the functional effectiveness of acellular xenograft urinary bladder submucosa powder implantation on critical size defect 3 mm in depth and 3 mm in diameter for articular cartilage repair in rabbits. For this purpose, twenty four healthy rabbits were used. They were randomly divided into two equal groups (n=12), before proceeding with the surgical procedures, the rabbits were generally anesthetized and aseptic conditions were established. All animals will be carried out to arthrotomy of stifle joint, with making full thickness articular cartilage defect 3 mm in diameter and 3 mm in depth were created in the right medial femoral condyle. In control group, the defect was treated with 1 ml phosphate buffer saline (PBS). While, the scaffold group, was treated by implantation of 0.05 mg bovine urinary bladder sub mucosa powder in side cartilage defect. Each group was further divided into two equal subgroups (n=6) categorized according to the post operation periods. The outcome was assessed clinically, macroscopically and histologically at 4th and 8th weeks post-surgery. The clinical outcome of the operated articular cartilage defect demonstrated no infection and no rejection also all rabbits showed lameness degree but gradually improved in the scaffold group with significant ($p \leq 0.05$) differences compared to control group at 8th weeks post-operative. In contrast, most of animals from the control group showed minor lameness. However, the average time to re-covery of normal ambulation of scaffold group was significantly ($P \leq 0.05$) shorter than that of control group. The results gross morphology showed in treated group the articular cartilage defect were filled with a glistening white tissue was very similar to normal after 8th weeks postoperative, lead to significant ($P \leq 0.05$) improvement in cartilage defects compared to control group. The histopathological examinations demonstrated that significantly better filling with hyaline like cartilage of the defect relative to the surface of normal adjacent cartilage, better integration of repair tissue with surrounding articular cartilage and matrix staining with safranin O fast green was also better on the scaffold group, than in those with untreated group. However, the gap filled with mature fibrous connective tissue no obvious cartilaginous extracellular matrix was identified by safranin O staining in control group at eight weeks after surgery. In conclusion; depending on all parameters used for this study, we demonstrated that scaffold group obtained a comparative advantage in quality and quantity of hyaline cartilage formatting in a period at 8th postoperative week because the bovine urinary bladder submucosa powder showed both mechanical and chemical support to the articular cartilage regeneration.

Key words: articular cartilage ,rabbits ,urinary bladder cartilage ,scaffold

Introduction

Articular cartilage plays an important role in the function of diarthrodial joints, providing frictionless motion between the joint surfaces and distributing the mechanical stresses (Zylinska *et al.,* 2018). Articular cartilage injuries present a unique and challenging medical problem due to the tissues lack of regenerative ability (Sutherland *et al.,* 2015). The reduced vascularity, limited cell population and dense extracellular matrix (ECM) inhibit cartilage regeneration. Untreated cartilage defects due to osteoarthritis or injury can lead to swelling, joint pain and further degeneration of the tissue and eventually the need for a total joint replacement (Benders *et al.,* 2016). There are three main goals to achieve for cartilage trauma treatment by restoration of the joints motion, pain relief and elimination and delay of the onset of osteoarthritis (Widuchowski *et al.,* 2017).

The goal of cartilage regeneration and repair is to produce fully integrated tissue at both the articular surface and the subchondral bone that has mechanical and chemical properties similar to native cartilage (Yokoo *et al.,* 2014). Many current surgical cartilage defect

treatments such as autologous chondrocyte implantation (ACI), microfracture, osteochondral transplantation

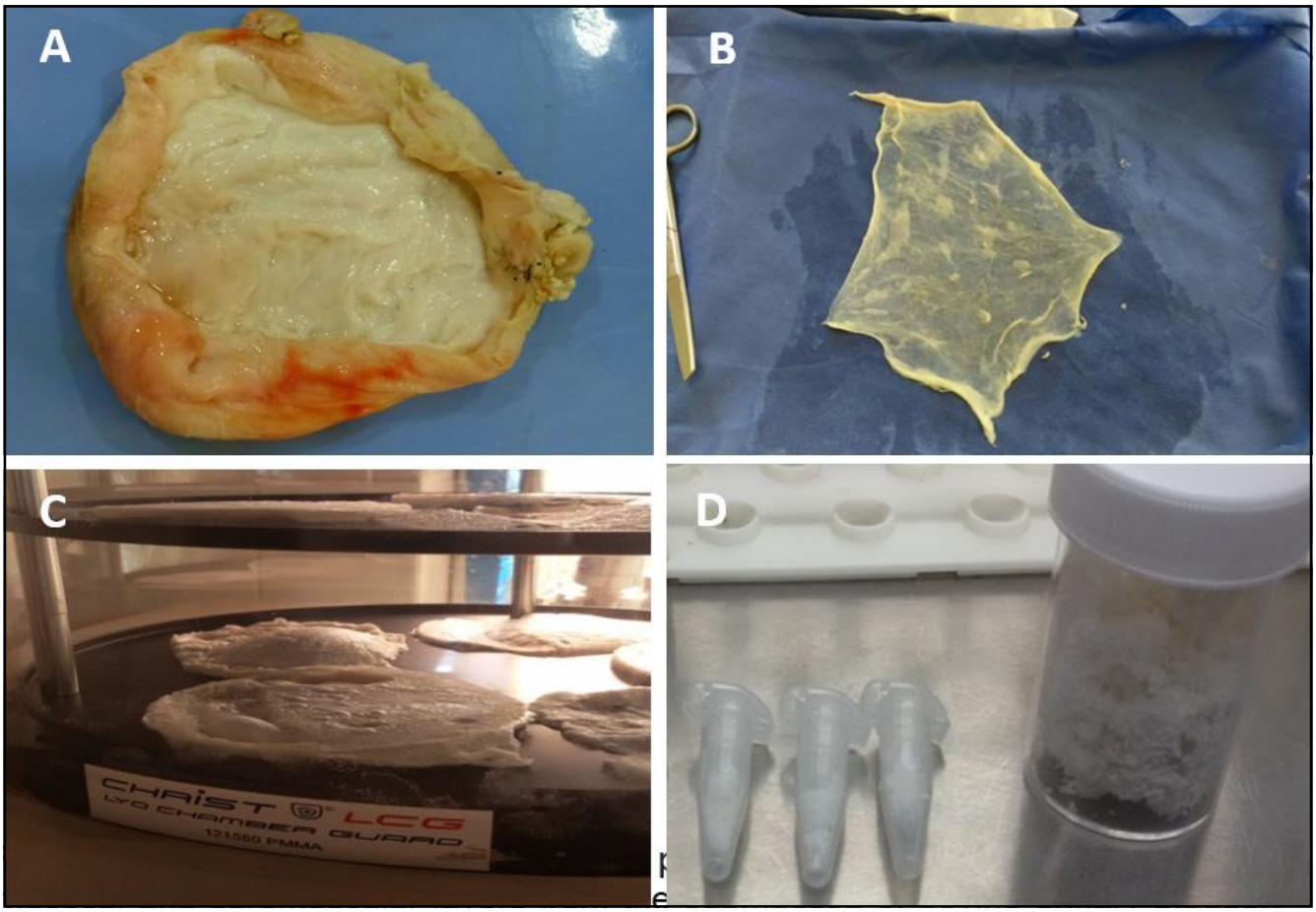


Fig. 3.1: Shows steps of UBM-powder preparation A. Mechanical separation of mucosal and seromuscular layers from the submucosa of bovine urinary bladder. B. Prepared urinary bladder sheet. C. Lyophilizing the urinary bladder sheet D. UBM powder.

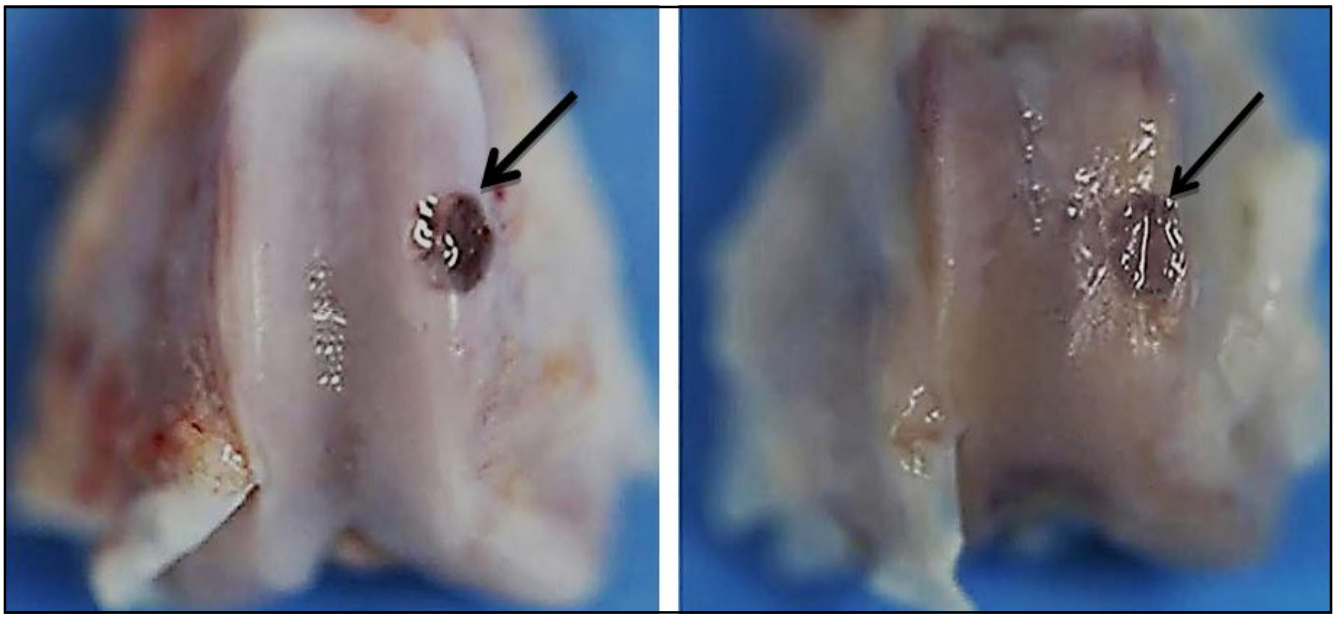


Fig. 4.1: Photographs articular cartilage defect at 4th weeks PO shows, in control group A. the defect area large depression in the central areas (yellow arrow). In scaffold group B. the defect were filled with a glistening white tissue resembled articular cartilage (yellow arrow).

(mosaicplasty) and current allograft implants usually do not produce fully integrated tissues, tissues with native mechanical strength, or tissues with the same composition as native articular cartilage (Hunziker, 2002 and Anraku *et al.,* 2014).

De Franceschi *et al.,* (2005) suggested that implanted autologous chondrocytes seeded on a type I collagen scaffold into full-thickness defects in the weight-bearing surface of the medial femoral condyle of rabbits investigators reported a significantly higher presence of type II collagen and proteoglycan production in the chondrocyte seeded scaffold group compared to control.

Conflicting findings generated by recent studies that employed ECM scaffolds as techniques for cartilage repair have encouraged the initiation of the current study using a method with a novel identity in cartilage repair

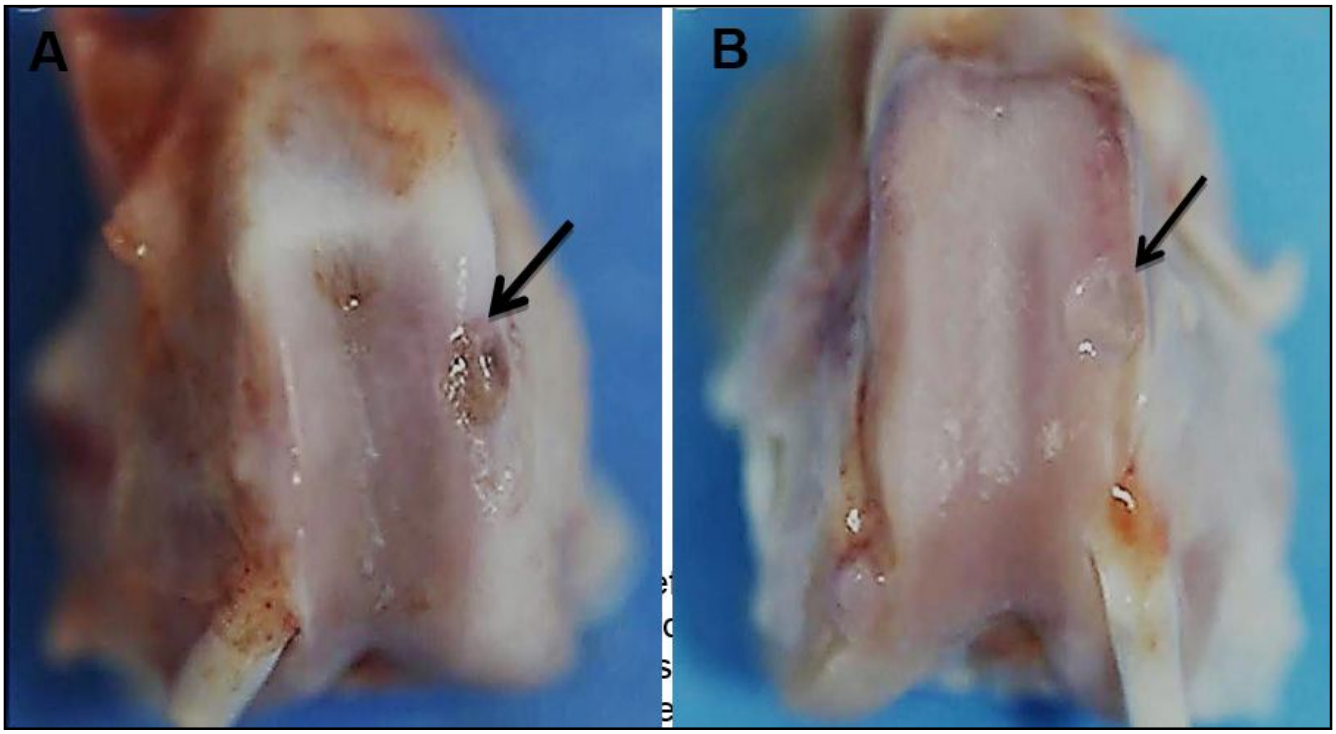


Fig. 4.2: Photographs articular cartilage defect at 8th weeks PO shows, in control group A. partially had dark red color filled defect area with central depression and it was still irregular, rough uneven tissue (yellow arrow). In scaffold group B. defect area had white color visually the graft appeared integrated well with the adjacent cartilage (black arrow).

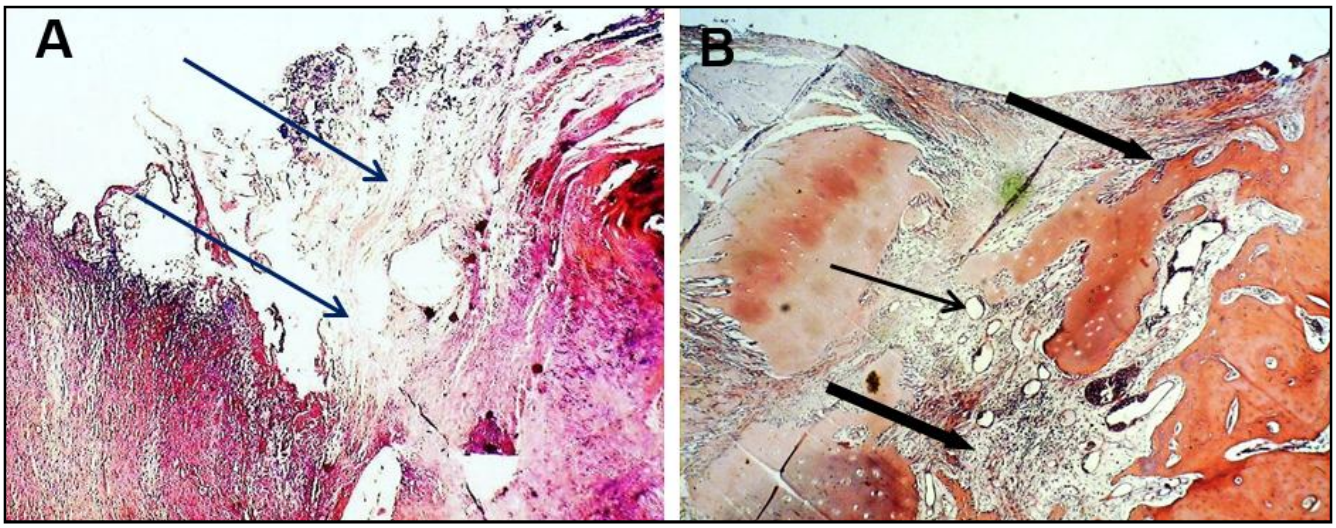
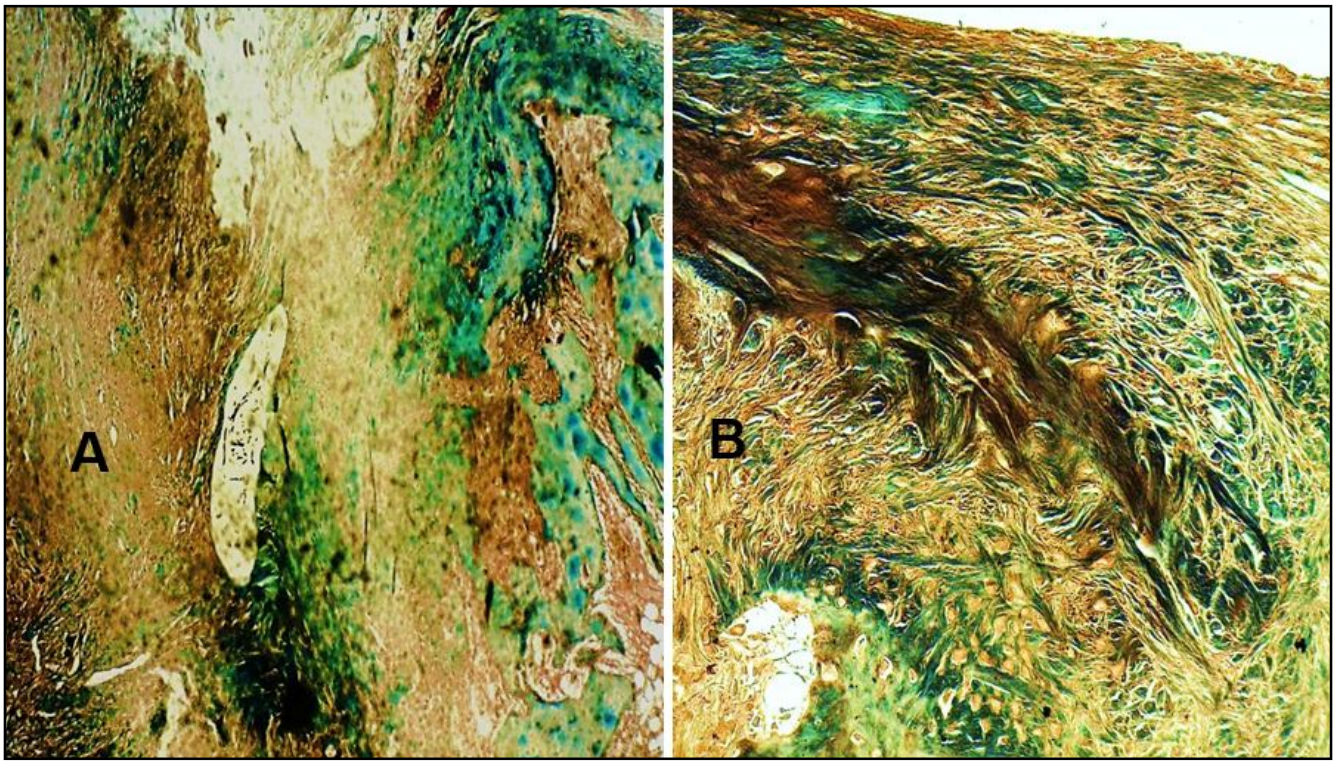


Fig. 4.4: Longitudinal section at the site of articular cartilage defects at 4th weeks PO. In control group showed, A. severe loss of safranine O staining, the loss of articular cartilage (thick arrow) multifocal decrease in chondrocytes (thin arrow) (Safranin O stain 100X) B. No obvious cartilaginous extracellular matrix and irregular surface was observed, reaching beyond the level of the adjacent cartilage (thick arrows) (Safranin O stain 200X).

that introduced lyophilized urinary bladder matrix as powder.

Materials and Methods

Twenty four apparently healthy adult local breed rabbits, weighing (1-2) kg, were recruited for this study.



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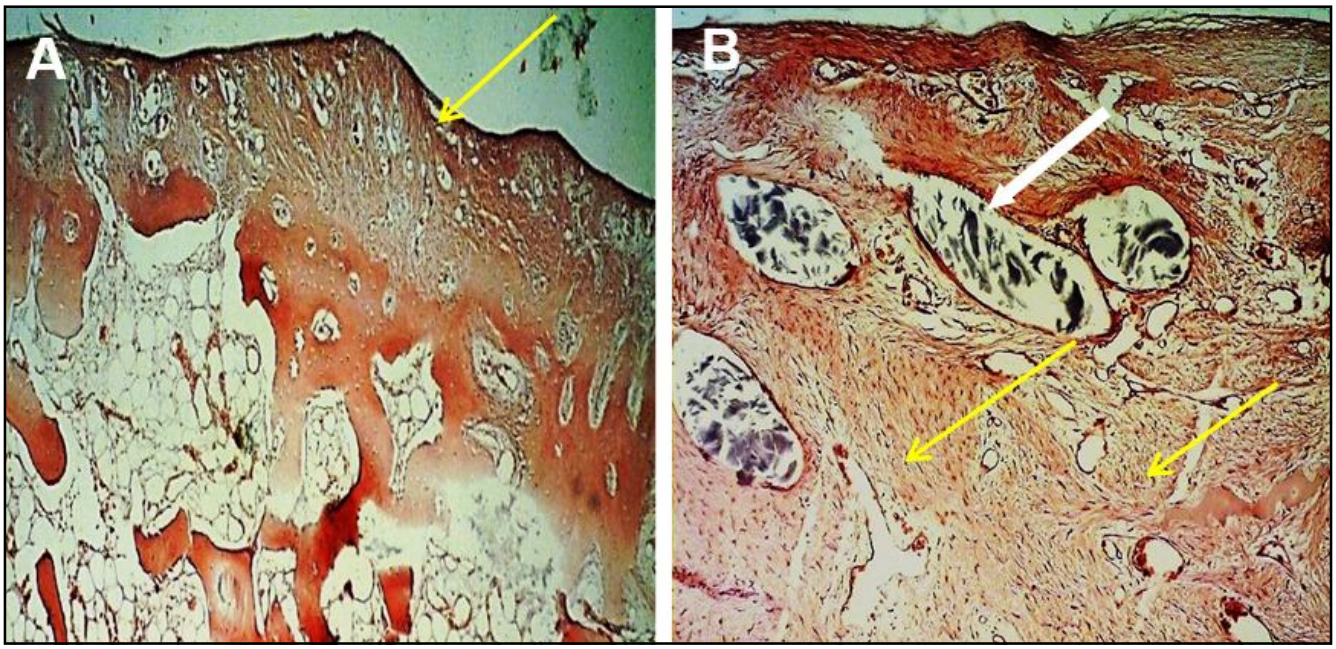

Fig. 4.5: Longitudinal section at the site of articular cartilage defects at 4th weeks PO. In scaffold group, A. the defect was mostly filled with fibrocartilage (thick arrow) with individual, scattered chondrocytes and the surface was irregular (thin arrow). B. show border between the scaffold and walls of the defect area (thick arrow) infiltrated with few mononuclear cells (thin arrow) (H&E, × 100).

They were evaluated to be healthy based on physical examination.. A defect was created on the medial femoral condyle in right stifle joints approximately (3 mm in diameter and 3 mm deep was created centrally in the medial condyle by careful drilling). These defects were allocated randomly, depending on the method of the treatment. In control group; articular cartilage defect was treated with 1 ml phosphate buffer saline (PBS) and in scaffold group; articular cartilage defect was treated by implantation of 0.05 mg acellular urinary bladder matrix

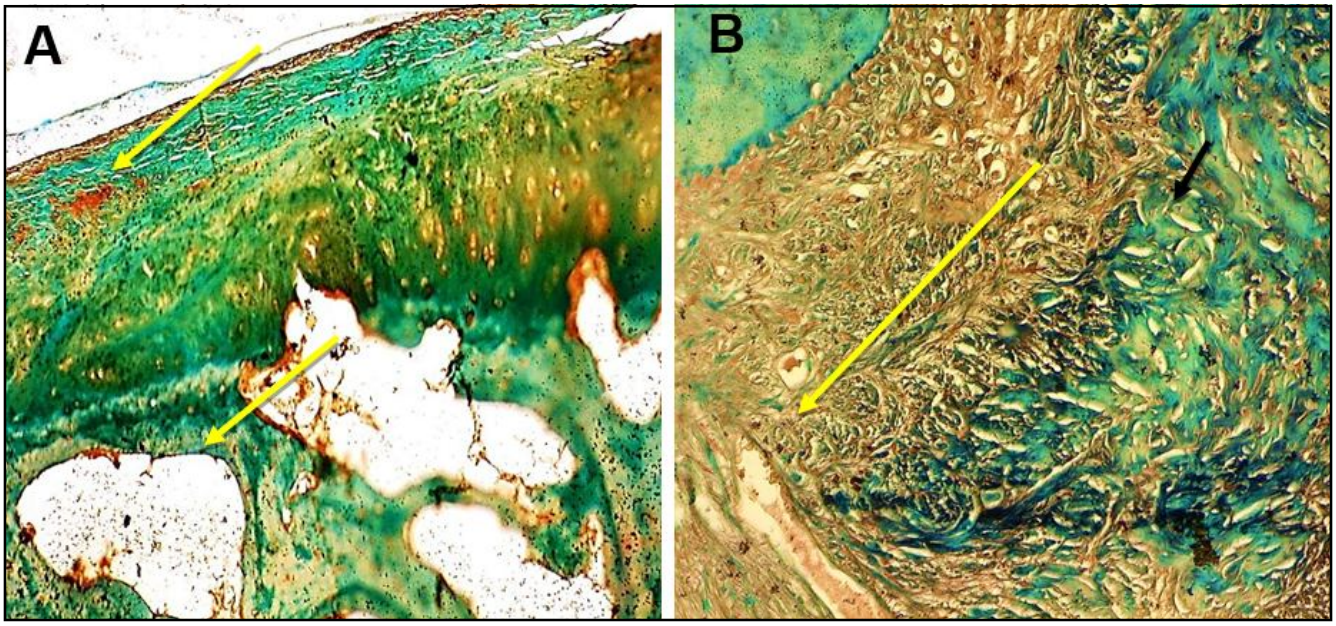

Fig. 4.6: Longitudinal section at the site of articular cartilage defects at 4th weeks PO. In scaffold group, A. positive safranin O proliferation of extracellular matrix extended into granulation tissue filled the gap (thin arrows) B. Proliferation of hyaline like cartilage element in granulation tissue that attachment to the native cartilage (thin arrows) (Safranin O stain x200).

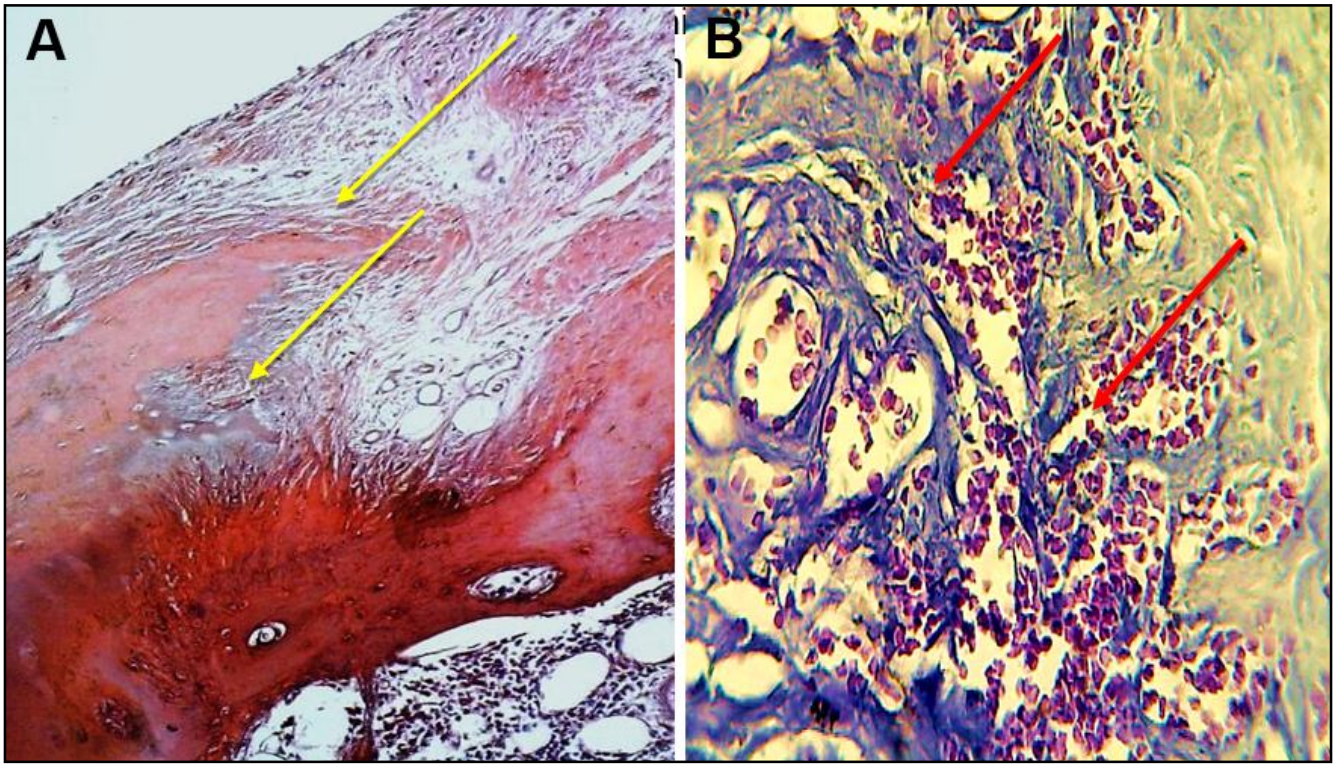

Fig. 4.7: Longitudinal section at the site of articular cartilage defects at 8th weeks PO. In control group, A. gap filled with mature fibrous connective tissue extended from intact cartilage (thin arrows) (H&E, ×100). B. vascular granulation tissue with mononuclear aggregation and no cartilage tissue regeneration was apparent in defect area (thin arrows) (H&E, × 400).

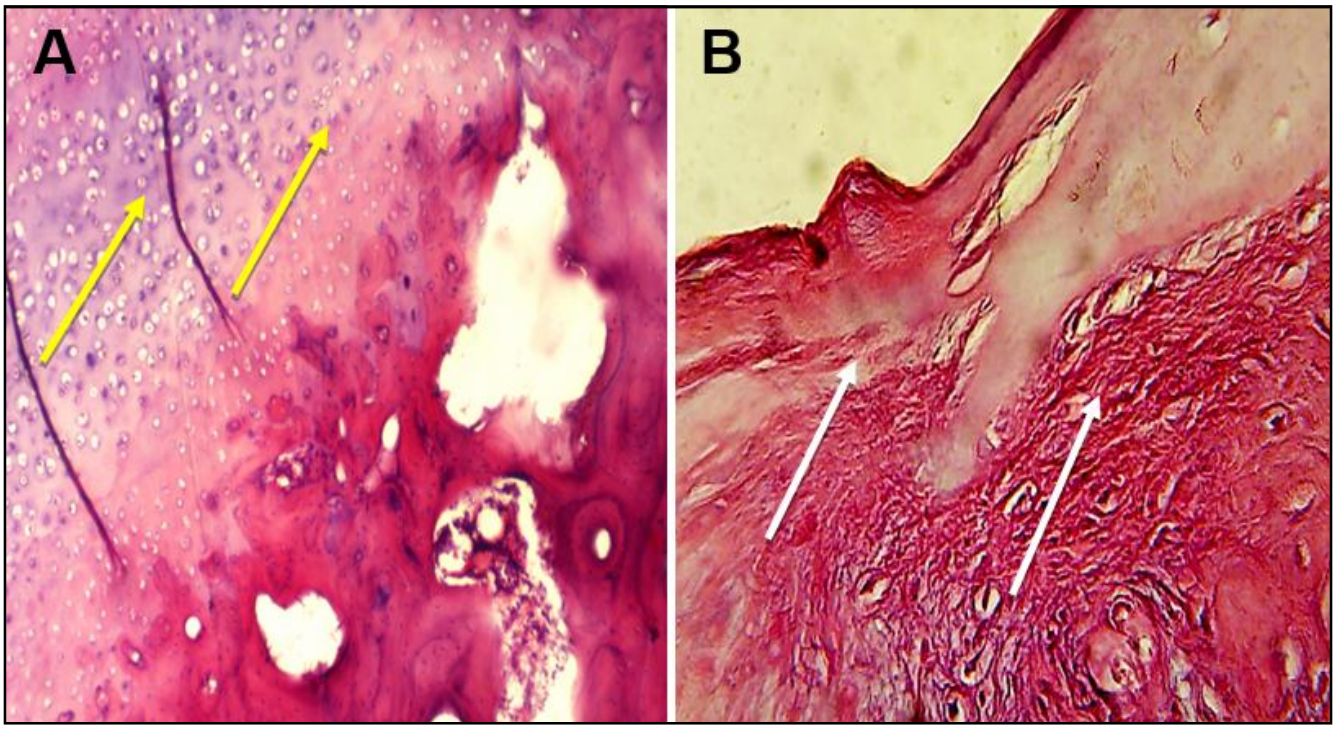

Fig. 4.9: Longitudinal section at the site of articular cartilage defects at 8th weeks PO. In scaffold group, A. hyaline like cartilage filled the gap (thin arrows) (H&E, × 100). B. hyaline cartilage filled the gap characterized by proliferation of chondrocytes and fibroblast produced collagen (thin arrows) (H&E, × 400).

powder (UBM).

The clinical follow-up was evaluated from the first week to 8th week after surgical operation. The animals of each group were sacrificed on two periods of time after 4th and 8th weeks post operation for evaluation of resultant repair tissue by macroscopical and histopathological examinations.

Fabrication of powder from Bovine UBM

Bovine UBM delaminated and decellularized with mixture of per acetic acid (0.1%) and ethanol (4%) as a protocol mention by Eberli *et al.,* (2011).

The decellularized UBM sheets were then lyophilized and comminuted to a particulate as protocol described by Freytes *et al.,* (2008).

Then collected powder has been sterilized at 60°C in oven at 16 hrs later kept in a sterile container until use.

Results

Results are shown in Figures and Table below.

Discussion

The articular cartilage grows and a repair was slowly, this is because the chondrocytes are bound in a small space called lacunae and thus they cannot migrate to the damaged areas (Hunziker, 2002; Knutsen *et al*., 2012). Besides, as there are no blood vessels and chondrocytes are supplied by diffusion with the help of pumping action generated by compression of the cartilage (Temenoff and Mikos, 2002). Therefore, if cartilage lesions are left untreated, they can lead to debilitating joint pain, joint dysfunction and osteoarthritis (Steadman *et al*., 2014). These disorders can be partially repaired through cartilage replacement therapy or other surgical interventions. But these treatments are often less than satisfactory and seldom renovate full function or return the tissue to its native normal state (Redman *et al*., 2005).

References

Anraku, Y., H. Mizuta, A. Sei, S. Kudo and Y. Hiraki (2014). The chondrogenic repair response of undifferentiated mesenchymal cells in rat full-thickness articular cartilage defects. *Osteoarthritis and cartilage,* **16(8):** 961-964.

Benders, K.E., P.R. Van Weeren, S.F. Badylak and J. Malda (2016). Extracellular matrix scaffolds for cartilage and bone regeneration. *Trends in biotechnology,* **31(3):** 169-176.þ

De Franceschi, L., B. Grigolo, L. Roseti, A. Facchini and R. Giardino (2005). Transplantation of chondrocytes seeded on collagen based scaffold in cartilage defects in rabbits. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials and The Australian Society for Biomaterials and the Korean Society for Biomaterials,* **75(3):** 612-622.þ

Eberli, D., A. Atala and J.J. Yoo (2011). One and four layer

acellular bladder matrix for fascial tissue reconstruction. *J. Mater. Sci. Mater. Med.,* **22(3):** 741-751.þ

Freytes, D.O., J. Martin, S.S. Velankar, A.S. Lee and S.F. Badylak (2008). Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. *Biomaterials,* **29(11):** 1630–7.

Hunziker, E.B. (2002). Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis and cartilage,* **10(6):** 432-463.þ

Redman, S.N., S.F. Oldfield and C.W. Archer (2005). Current strategies for articular cartilage repair. *Eur. Cell Mater,* **9(23-32):** 23-32.þ

Sutherland, A.J., G.L. Converse, R.A. Hopkins and M.S. Detamore (2015). The bioactivity of cartilage extracellular matrix in articular cartilage regeneration. *Advanced healthcare materials,* **4(1):** 29-39.þ

Steadman, J.R., K.K. Briggs, J.J. Rodrigo, M.S. Kocher, T.J. Gill and W.G. Rodkey (2014). Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy: *The Journal of Arthroscopic and Related Surgery,* **19(5):** 477-484.þ

Temenoff, J.S. and A.G. Mikos (2000). Tissue engineering for regeneration of articular cartilage. *Biomaterials,* **21(5):** 431-440.þ

Widuchowski, W., W. Tomaszewski, J. Widuchowski and A. Czamara (2017). Current concepts in the treatment of cartilage lesions with special regard to the knee joint. *Ortopedia, traumatologia, rehabilitacja,* **13(4):** 327-341.þ

Yokoo, N., T. Saito, M. Uesugi, N. Kobayashi and T. Koshino (2014). Repair of articular cartilage defect by autologous transplantation of basic fibroblast growth factor gene–transduced chondrocytes with adeno associated virus vector. *Arthritis and Rheumatism: Official Journal of the American College of Rheumatology,* **52(1):** 164-170.þ

Zylinska, B., P. Silmanowicz, A.L. Sobczynska-rak and T. Szponder (2018). Treatment of articular cartilage defects: focus on tissue engineering. *In vivo,* **32(6):** 1289-1300.þ