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Research Article

Therapeutic Benefit of Intra-articular Administration of Deciduous Teeth Stem Cells in Rabbit Model of Osteoarthritis

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Abstract

Background and Objective: Stem cell therapy is a new treatment option for osteoarthritis. A various applications of stem cell therapy have been used for cartilage regeneration in arthritis patient. The objective of this study was to examine therapeutic effects of puppy deciduous teeth stem cells for the treatment of osteoarthritis using a rabbit model. **Methodology:** In this study, rabbits were subjected to perform osteoarthritis. The anterior cruciate ligament of the stifle joint was transected over 3 months in order to generate the knee osteoarthritis. Puppy deciduous teeth stem cells were intra-articularly introduced in a test group after surgically induced arthritis. Histological characteristic of stifle joint was observed at the end of each experiment for obtaining the therapeutic effects regarding the stem cell treatment. **Results:** Results showed an increased number of chondrocytes and the cartilaginous thickening of articular cartilage layer in the puppy deciduous teeth stem cells-injected group. **Conclusion:** This finding will open a marked discussion for future possibility in using deciduous teeth stem cells as an alternative procedure for osteoarthritis treatment.

Key words: Dental pulp stem cells, osteoarthritis, stifle joint, rabbit

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Osteoarthritis (OA) is the leading cause of chronic pain, caused by inflammation of articular cartilage, subchondral bone, synovium and fluid of joints[1,2]. Clinical appearances included pain, lameness, limited mobility of joint and disability. Diagnosing can be done through different procedures such as orthopedic examinations, radiographic imaging (x-ray) and Magnetic Resonance Imaging (MRI). The x-ray is a common effective method but MRI established better pictures of soft tissue and cartilage, however MRI is more expensive than other tests. Non-steroidal anti-inflammatory drugs (NSAIDs) provide the basis of pharmacological treatment of pain from osteoarthritis. However, NSAIDs can cause adverse effects such as gastric ulcers, bleeding and abdominal pain[3-5]. Stem cell transplantation becomes widely studied for therapeutic approaches in the field of regenerative medicine as discussed elsewhere[6-10]. Mesenchymal Stem Cells (MSCs) can be isolated from a variety of organs and tissues, such as bone marrow, brain, skin, hair follicle, skeletal muscle and dental pulp. However, it is still unclear whether therapeutic effects are the result of differentiation of stem cells into specialized cell types or preserving their self-renewal function[11,12]. However, the clinical use of several stem cells has been controversial and limited due to the ethical concerns[13]. Recently, dental-tissue-derived stem cells such as Dental Pulp Stem Cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) have been suggested as a novel alternative resource for cell therapies and tissue engineering. These dental-tissue-derived stem cells Have Mesenchymal Stem Cell (MSC) qualities, including the capacity for self-renewal and multilineage differentiation potential. Dental MSC like stem cells are not only derived from a very approachable tissue resource but are also able to supply enough cells for clinical application[14]. The objective of this study was to determine whether puppy deciduous teeth stem cells (pDSCs) could be used as alternative treatment for cartilage repair in chronic osteoarthritis.

MATERIALS AND METHODS

Animals: The study was conducted in New Zealand white rabbits and approved (ACKU 03759) by the Ethical Committee for Animal Experiments, Kasetsart University, Thailand. Rabbits were randomly divided into two experimental groups, consist of group 1 (control): Rabbits given PBS alone without stem cell administration (n = 4), group 2: Rabbits given pDSCs (1×10^6) injected intra-articularly in week 2 (10-14 days) and week 4 (24-28 days) after ACLT induced osteoarthritis (n = 4). Clinical evaluation consisted of physical examination and complete blood cell counts were performed. Animals were anesthetized with isoflurane (5% induction and 2% maintenance) intubated and connected to a ventilator. Ventilation was done with a tidal volume of 50 mL, at frequency of 36 bpm. Joint approach was performed along the para-patellar and joint capsule was cut at the level of medial patellar. Surgical approach for Anterior Cruciate Ligament Transection (ACLT) to induce osteoarthritis of the stifle joint was illustrated in Fig. 1. The procedure included the following steps: (1) The stifle was approached through an anterior patellar longitudinal incision, then (2) Medial parapatellar arthrotomy, the patellar was then retracted laterally and (3) Anterior cruciate ligament was cut to dislocate the joint to produce arthritis. Animals were maintained for 3 months for being established as osteoarthritis models.

Cells transplantation: Stem cells from puppy deciduous teeth were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA.) supplemented with 10% fetal bovine serum (Invitrogen, Gaithersburg, MD, USA.) and 1% penicillin/streptomycin at 37°C, 5% CO_2. Cells were harvested and collected from the culture at 80% confluency via trypsin-EDTA treatment. The pDSCs at passages 1, 2 and 3 were characterized by intracellular flow cytometry (Santa Cruze Biotechnology, CA, USA.) as illustrated by previous study[15]. The MSCs adhered to plastic culture dishes and formed fibroblast-like colonies, the phenotype of puppy deciduous teeth stem cells (pDSCS) at passages 5 and 10 represented in Fig. 2. The classical dental stem cells markers (Stro1) was detected on pDSCs and the histograms of flow cytometry analysis of cells surface marker of Stro1 were analysed as shown in Fig. 2. All transplantation techniques were performed under aseptic conditions. Approximately 1.0×10^6 (pDSC) cells were administered intra-articularly into rabbit stifle joint at 2 and 4 weeks after ACLT induced osteoarthritis.

Histological examination: Stifle joints of male rabbits treated with pDSCs for 1 and 2 months were incised. Tissues containing the distal end of femur and the proximal end of tibia were thoroughly cleaned to remove muscles prior to immerge in formaldehyde fixative buffer. Stifle joints with bones were decalcified in the buffer for several days or until they became soft. Tissues were finally embedded in paraffin blocks. Sections of stifle joint were cut and stained with H and E.

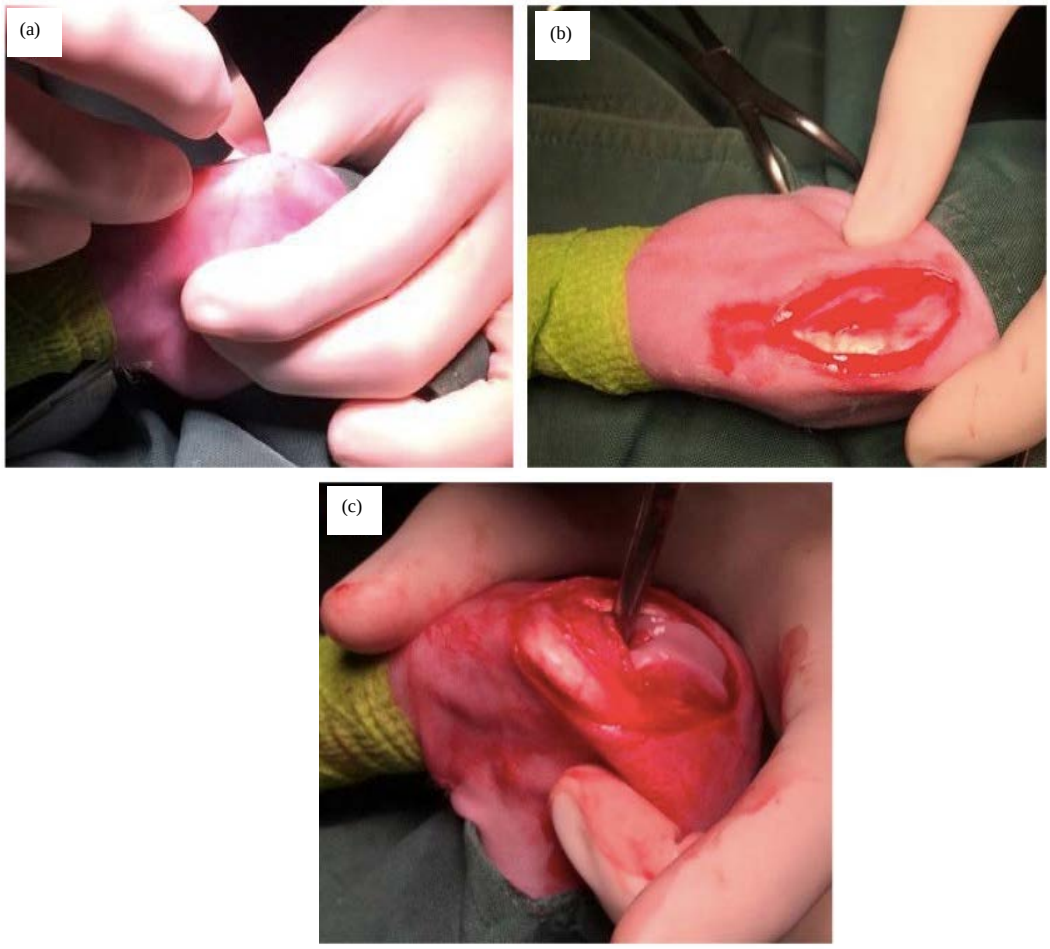

Fig. 1(a-c): Anterior Cruciate Ligament Transection (ACLT) to induce arthritis of the stifle joint of rabbit was created in the right stifle joint. The surgical technique was modified from that reported by Singh[19]

H and E staining of the stifle joint: Histological observation of the stifle joint sections was assessed following H and E staining. Meniscus and ligaments, including cruciate ligaments and collateral ligaments, appeared normal on both sides of the stifle joints. Good condition of the bones was judged from length, width and compactness of the distal femur and the proximal end of tibia. Thicknesses of articular cartilage layer measured from the distal end of femur were 200-250 mm (1 month old treatment) and 250-300 mm (2 months old treatment) for the control groups and 250-350 mm (1 month old treatment) and 250-350 mm (2 months old treatment) for the PBS-treated group, respectively. In the pDSCs-treated group, a 400-500 mm (1 month old treatment) and 500-600 mm (2 months old treatment) thicknesses of articular cartilage layer were observed. Layers of articular cartilage as appeared in paraffin sections were arranged as the followings: superficial tangential layer, middle transitional layer, deep radial layer and calcified cartilage layer. The latter positioned above subchondral bone and cancellous bone, respectively. Deep radial layer is the thickest layer of articular cartilage found in all sample. However, a whole thickness of radial layer in the pDSCs-treated group (200-300 mm) is almost 3 times larger than the control group (70-100 mm) and the PBS-treated group. There were an increased number of chondrocytes and matrices reside in radial layer of the pDSCs group. The cancellous bone of all sample contained a highly vascularized spongy bone. Nevertheless, there was a dense collagenous compartment in spongy bone of the pDSCs-treated group. Strips of dense collagen also occupied at the periphery of chondrocytes in the 2 months old group.

RESULTS

Morphological finding: The appearance of normal articular cartilage from the rabbit stifle joint was white and clear as

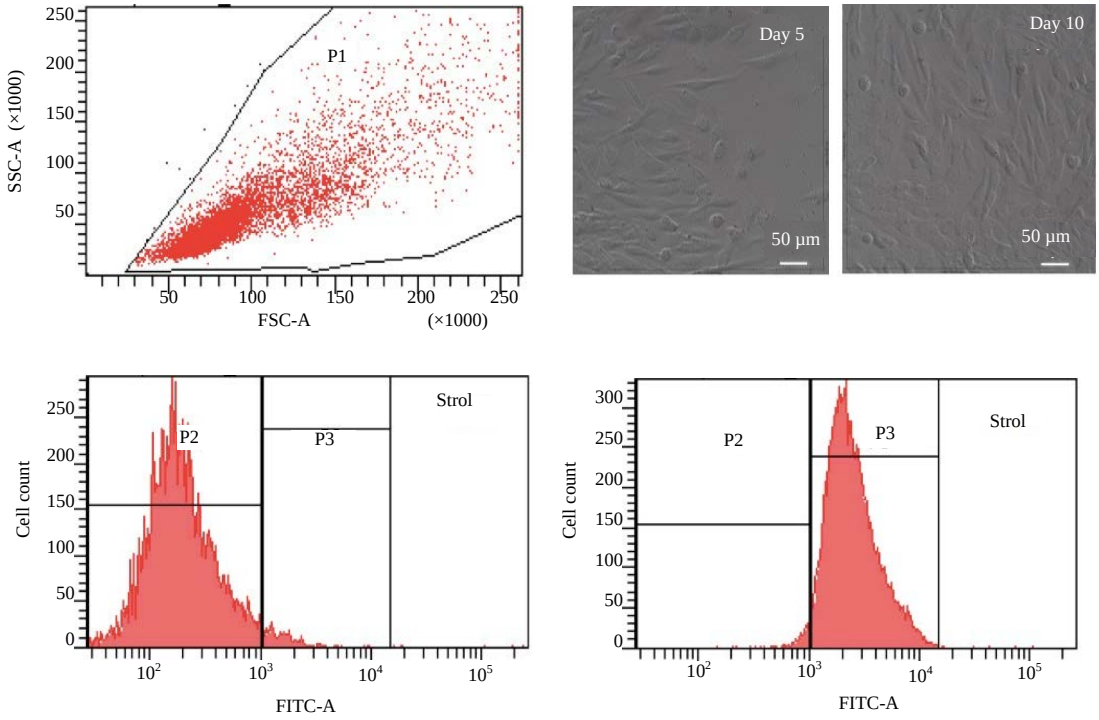

Fig. 2: Upper panel showed flow cytometry profile and phase contrast microscopy of 5 and 10 days post initial isolation of from puppy deciduous teeth. Cells at passages 1 were seed in plastic culture dish. Lower panel showed flow cytometry profile of deciduous stem cells at passages 2 and 3 that expressed Stro1 stem cells marker

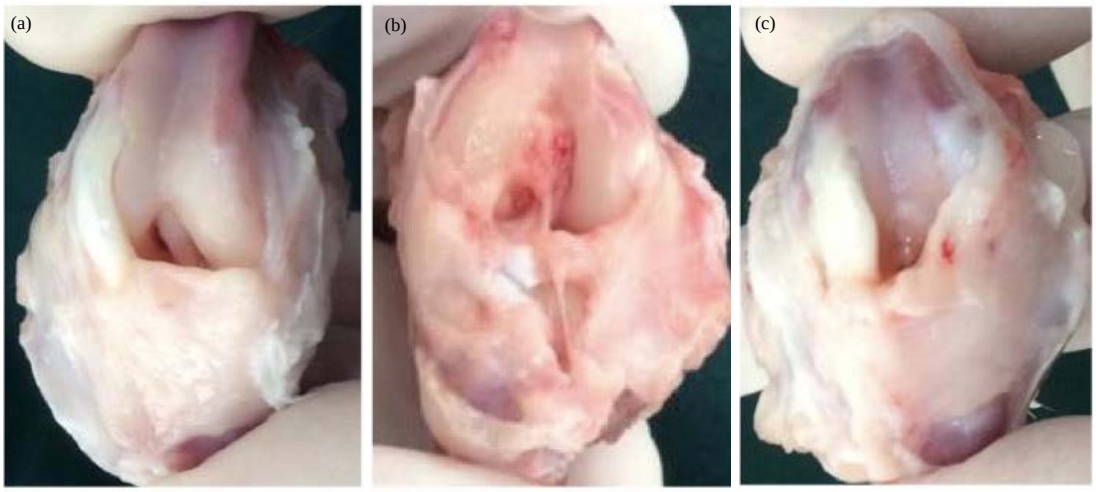

Fig. 3(a-c): Morphology images of femoral condyles in the intact cruciate ligament (a) Normal, at 5 months post surgically ACLT induced osteoarthritis, (b) PBS and (c) pDSCs-treated joint (pDSCs)

shown in Fig. 3. The joint in control group (PBS) was appeared rough, pale and yellowish of cartilaginous tissue after ACLT induced arthritis. However, pDSCs-treated joint (pDSCs) was similar in morphology with the normal rabbit stifle joint (Normal).

Histological finding: Paraffin sections of rabbit stifle joints representing articular cartilage at the distal end of femur were shown in Fig. 4 and 5. Noticeably, H and E staining showed an increasing number of chondrocytes and the thickness of radial layer in 1 and 2 months pDSCs-treated sample compared to

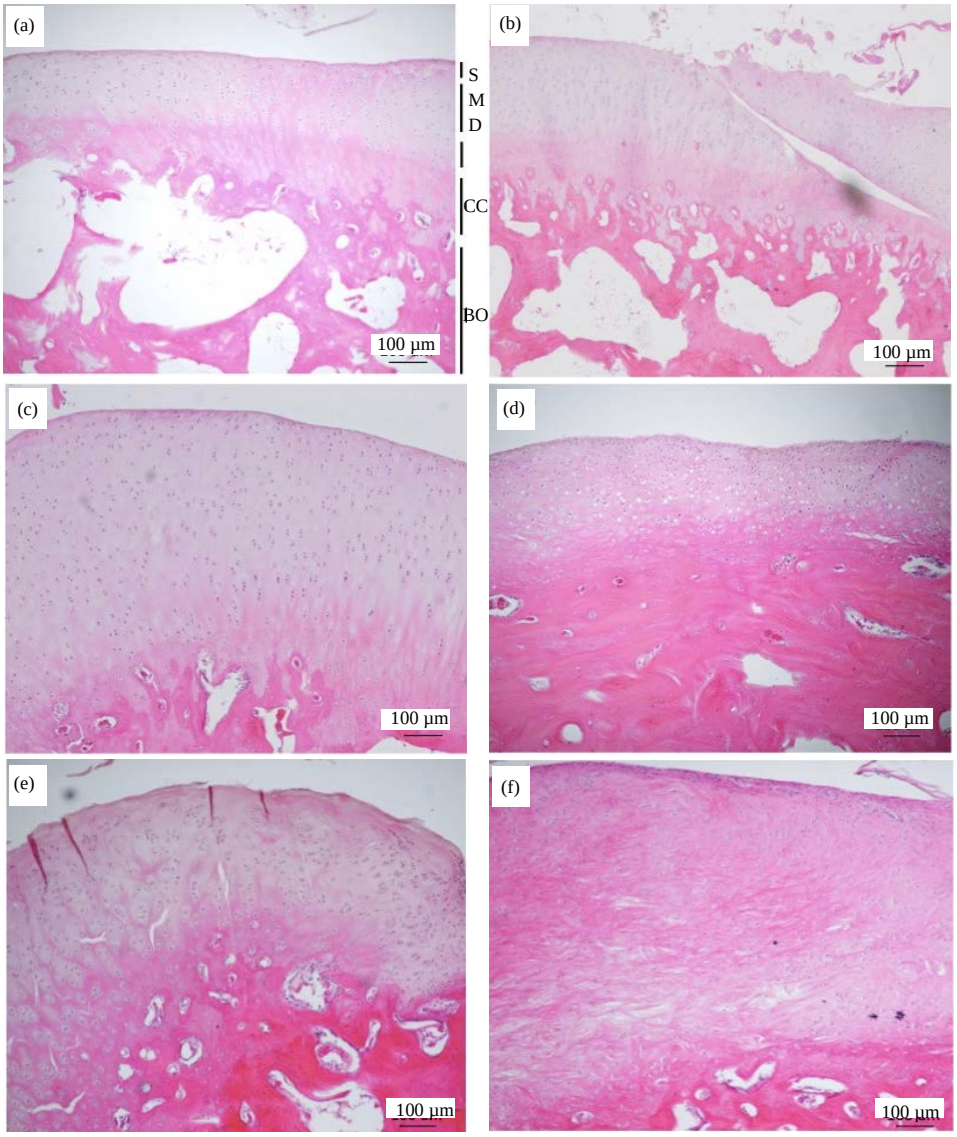

Fig. 4(a-f): H and E staining of rabbit stifle joints showed articular cartilage at the distal end of femur. Articular cartilage layers were characterized as superficial tangential layer (S), middle transitional layer (M), deep radial layer (D), calcified cartilage (CC) and cancellous bone (BO), respectively. Note an increasing number of chondrocytes and the thickness of radial layer were noticeable both in 1 and 2 months (c and f) pDSCs-treated sample compared to (a and b) Control and (b and e) 1 and 2 months PBS-treated group, respectively. Large bundles of collagen fibers were reside in radial layer as chondrocytes proliferated. a-d: Control group, b-e: PBS-treated group and c-f: pDSCs-treated group

control and PBS-treated group. The amount of large bundles of collagen fibers in radial layer as chondrocytes proliferated and the normal articular pattern were obtained in 1 and 2 months pDSCs-treated group when compared with control.

Radiographic examination: Six weeks after ACLT-induced osteoarthritis, the antero-posterior and lateral radiographic images of stifle joint were performed as shown in Fig. 6. No significant changes were seen in the radiological sign of osteoarthritis in animals treated with 1×10^6 pDSCs compared with control animals.

DISCUSSION

The present study demonstrates therapeutic effects of stem cells from puppy deciduous teeth (pDSCs) in rabbit

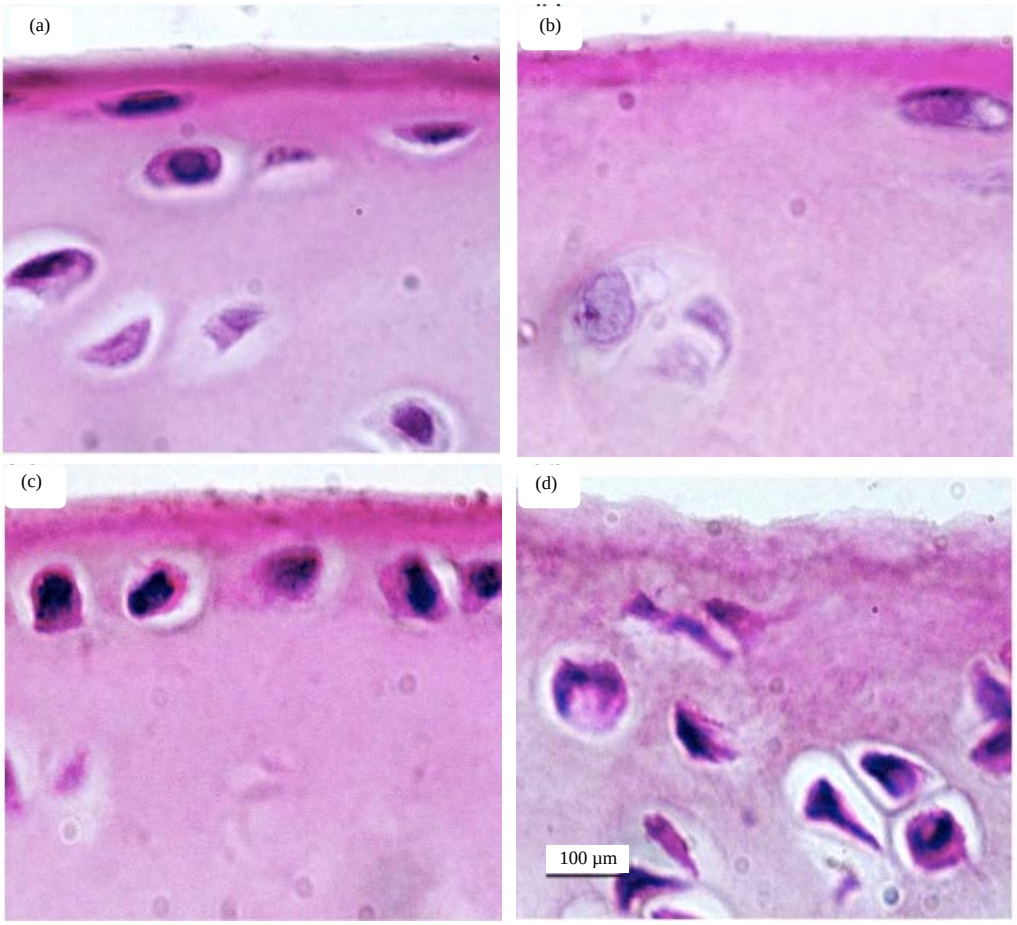

Fig. 5(a-d): H and E images of normal articular cartilage at the distal end of femur, showing orientation of chondrocytes in tangential layers (a) Compared with 5 months post surgically ACLT induced osteoarthritis in (b) Numbers of chondrocytes formation in (c and d) pDSCs-treated joint

model. The ACLT surgery was used to induce osteoarthritis of the stifle joint and 1×10^6 of pDSCs were locally injected into the joint space of rabbit. Compared with the control group, pDSCs group noticeably restore the joint cartilage such as, the number of chondrocytes, the alignment of chondrocytes and the thickness of the radial layers. Although, immunohistochemistry examination to quantitative scoring of each collagen type to indicate the amount of collagen content has not been investigated in this study, there is a positive tendency towards increasing in number of collagen content in H and E staining. For this study, the puppy deciduous teeth stem cell movement and homing ability have not been investigated. However, after pDSCs were injected, they will be in the synovial space for about 48 h to release the chemotactic agents and then absorbed into the systemic circulation. In addition, previous report suggested that dental stem cells can migrate to the damaged tissue, growth factors and paracrine factors such as SDF-1, HGF and VGEF from dental pulp stem cells might be the keys that involved as chemotactic and homing of stem cells to the damaged tissue/cells[16]. Paracrine effects are a possible mechanism that enhances neovascularization, reduces inflammation and is involved in the cartilage remodeling. Many studies have identified the paracrine and growth factors that may help to repair the cartilage tissue such as TGF, VEGF, FGF, IGF and SDF[17,18]. These growth factors would induce the chondrocytes proliferation. Understanding the paracrine mechanism of pDSCs for regenerative therapy requires further studies and a long term following up is needed to support pDSCs therapeutic action[19-21]. In this study, the effects of pDSCs for the limb function improvement and pain relief were not investigated. However, previously published data showed the potential treatment of pDSCs for limb function and pain relief in dogs with chronic osteoarthritis. Intra-articular injections of

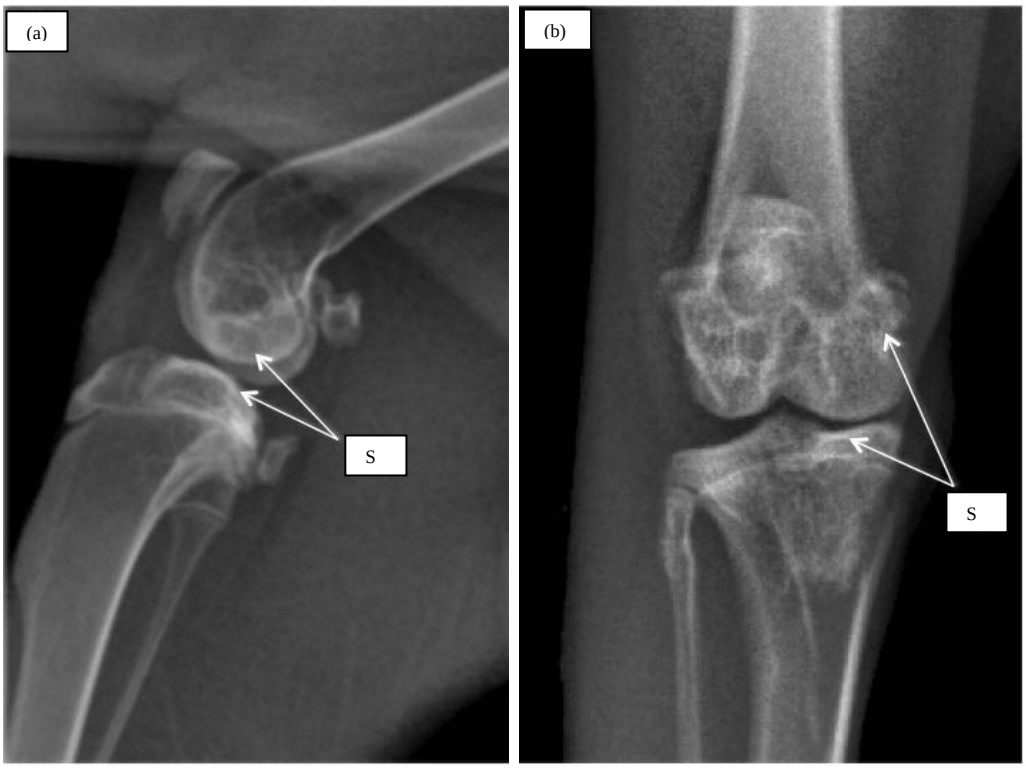

Fig. 6(a-b): Radiographic images of rabbit stifle joint at 5 months post surgically ACLT induced osteoarthritis, showing arthritis sign such as subchondral bone sclerosis (S: white arrow)

allogeneic pDSCs demonstrated statistically significant improvement in lameness and functional ability in arthritis dogs[22]. Results from this study seem to support that pDSCs therapy provide a benefit for the treatment of osteoarthritis.

CONCLUSION

In the present study, the use of pDSCs allows to undertake successful to improve the cartilage of osteoarthritis in the rabbit model. Multi-injections might be required to achieve the therapeutic effects. However, a further study is required for ascertaining of osteoarthritis treatment and for pain reduction. This finding opens an alternative approach for the treatment of osteoarthritis in animals.

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