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Articular Cartilage Regeneration with Autologous Peripheral Blood Stem Cells and Adjuvant Hyaluronic Acid: An Animal Study in Sheep Model

Khay-Yong Saw (Presenter)
MB ChB, MCh Orth, FRCS(Edin)
Kuala Lumpur Sports Medicine Centre (KLSMC), MALAYSIA

Caroline Siew-Yoke Jee BEng, PhD, KLSMC, MALAYSIA
Alisha Ramlan BSc, KLSMC, MALAYSIA
Razana Ali MBBS MPATH, UPM, MALAYSIA
Hui Cheng Chen DVM, MVM, DVSc, MMCVS, UPM, MALAYSIA
Nurul Hayah Khairuddin DVM, PhD, UPM, MALAYSIA
Amal Dawam MSc, KLSMC, MALAYSIA
Yan Chang Saw MBChB, NHS Grampian, UK

Disclosure Information

- Conflict of Interest:
  - The authors declare no conflicts of interest related to this study.
- Funding:
  - This study was supported by TRB Chemedica Malaysia.
- Ethical Approval:
  - Ethics approval was obtained from Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (reference number: AUP-R014/2022). All procedures were conducted in accordance with the Animal Welfare Act 2015, IACUC Policy and Code of Practice for the Care and Use of Animals for Scientific Purposes

Introduction

- K.A.R.T.® (KLSMC Articular Cartilage Regeneration Technology) is a regenerative approach that integrates subchondral drilling, intra-articular peripheral blood stem cell (PBSC) and hyaluronic acid (HA) injections, and biomechanical stimulation to promote articular cartilage repair. This method facilitates endogenous and exogenous stem cell recruitment, extracellular matrix formation, and chondrogenesis, demonstrating efficacy in published clinical studies. [1-9]
- Sheep are a preferred large animal model for articular cartilage research due to their knee joint similarity to humans, suitable cartilage healing response, and weight-bearing biomechanics. Their joint size allows precise surgical procedures, and they are easy to manage for long-term studies. [10-11]

Purpose

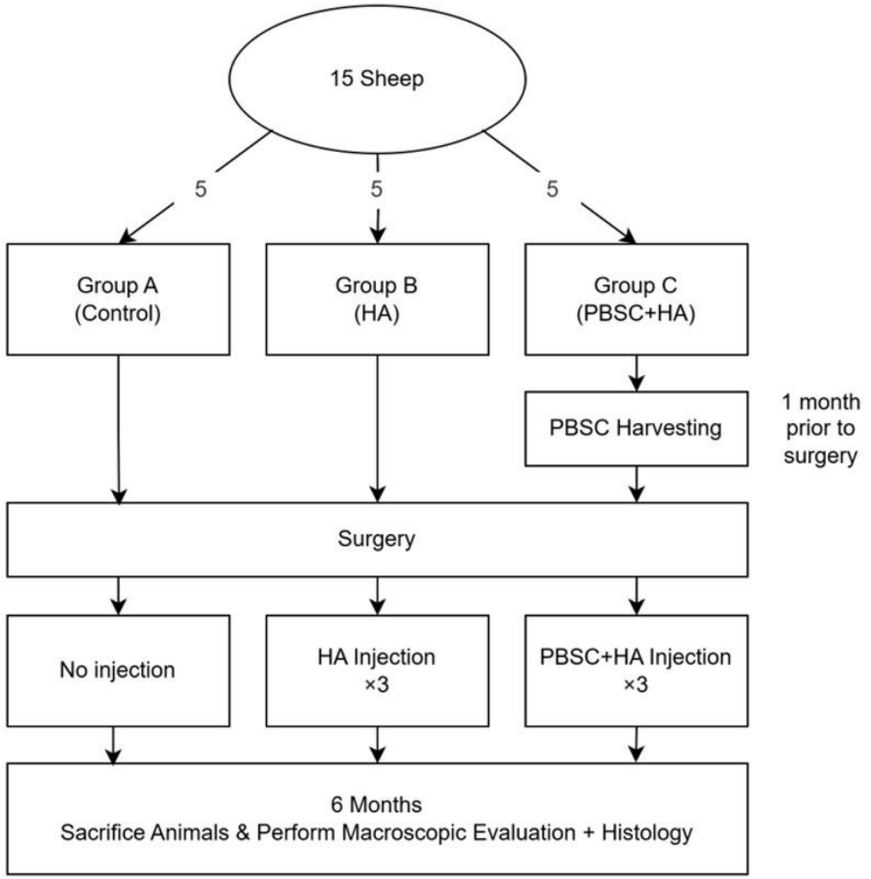
- The purpose of this study was to determine histologically whether intra-articular injections of autologous PBSC and adjuvant HA after subchondral drilling resulted in better articular cartilage regeneration in an animal model.

Animals

- 15 male mixed-breed sheep (aged 12–36 months, and weighing 36–52 kg).
- 5 sheep were assigned to each of the 3 groups: Control (A), HA-treated (B), and PBSC+HA-treated (C).

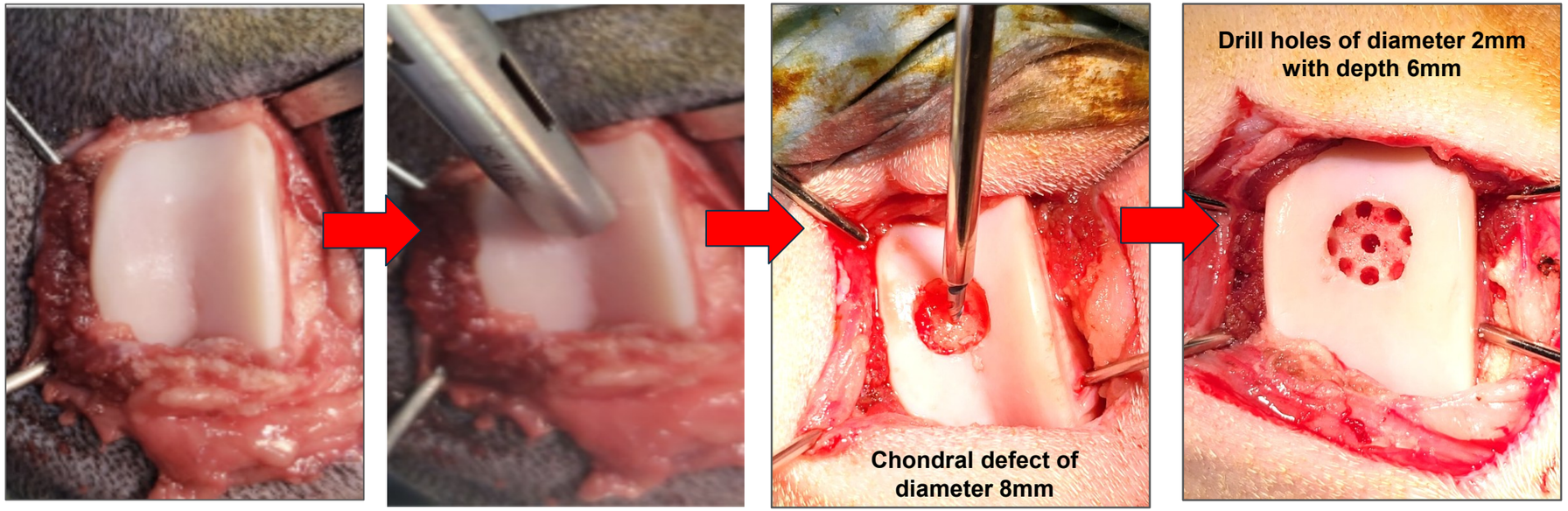
Methods - PBSC Collection

- Group C sheep received G-CSF injections (5 mg/kg) for three days before PBSC collection via apheresis on Day 4.
- Blood was drawn through a catheter in the jugular vein and processed using the Spectra Optia Apheresis Machine.
- PBSC collection was done one month before surgery and cryopreserved in liquid nitrogen for later use.
- Flow cytometry showed an average cell count of 51.69 ± 29.21 x 10^6 cells/mL with 99.11% viability.

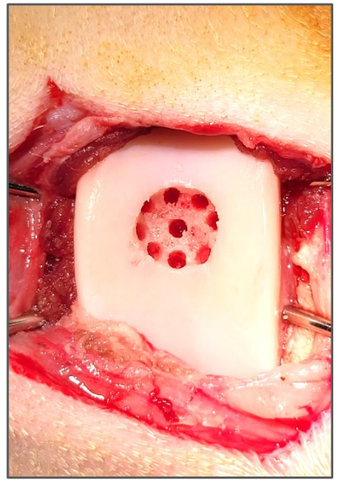


Methods - Surgery

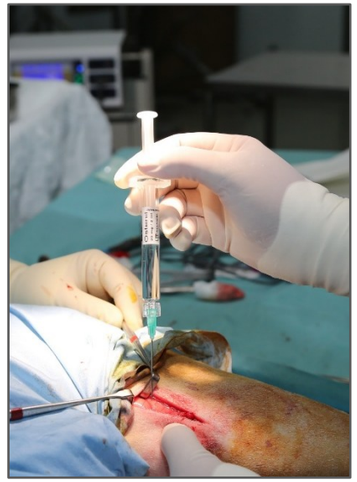
- Surgeries were performed under general anesthesia for all animals.
- An 8-mm full-thickness cartilage defect was created in the left stifle joint, followed by subchondral drilling.



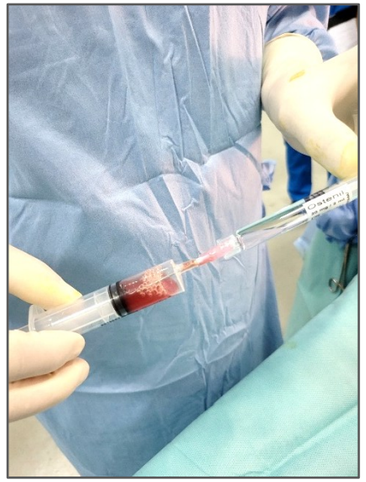
Methods - Injections



Group A (Control):
No injections



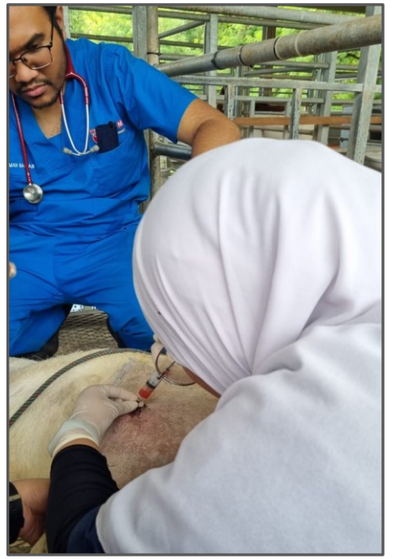
Group B (HA):
2mls Ostenil



Group C (PBSC+HA):
2mls PBSC + 2mls Ostenil

Group A received no injections, Group B received 2 mL HA injections, and Group C received 2 mL PBSC mixed with 2 mL HA.

Injections were given on surgery day, then on Day 7 and Day 14 post-surgery.



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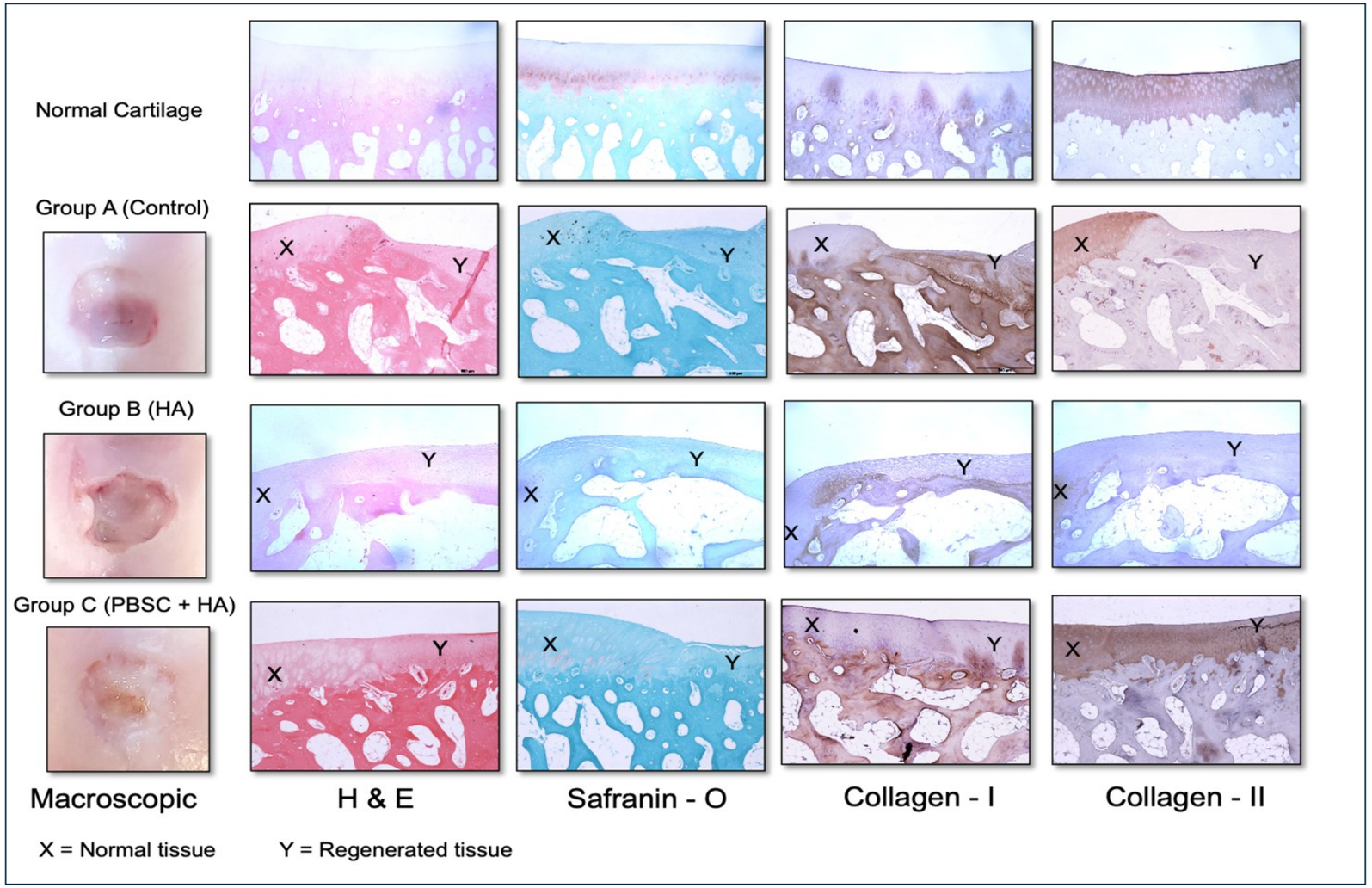
Histological Analysis

- Tissue samples were evaluated by 2 independent-blinded histopathologists
- Animals were scarified six months after surgery, the joints were collected for evaluation.
- Tissue samples were stained with H&E, safranin-O, collagen type I, and collagen type II,
- Histology evaluation and grading using the ICRS II system (max score: 1,400). [12]
- Statistical analysis was performed using SPSS, with significance set at $p < 0.05$.

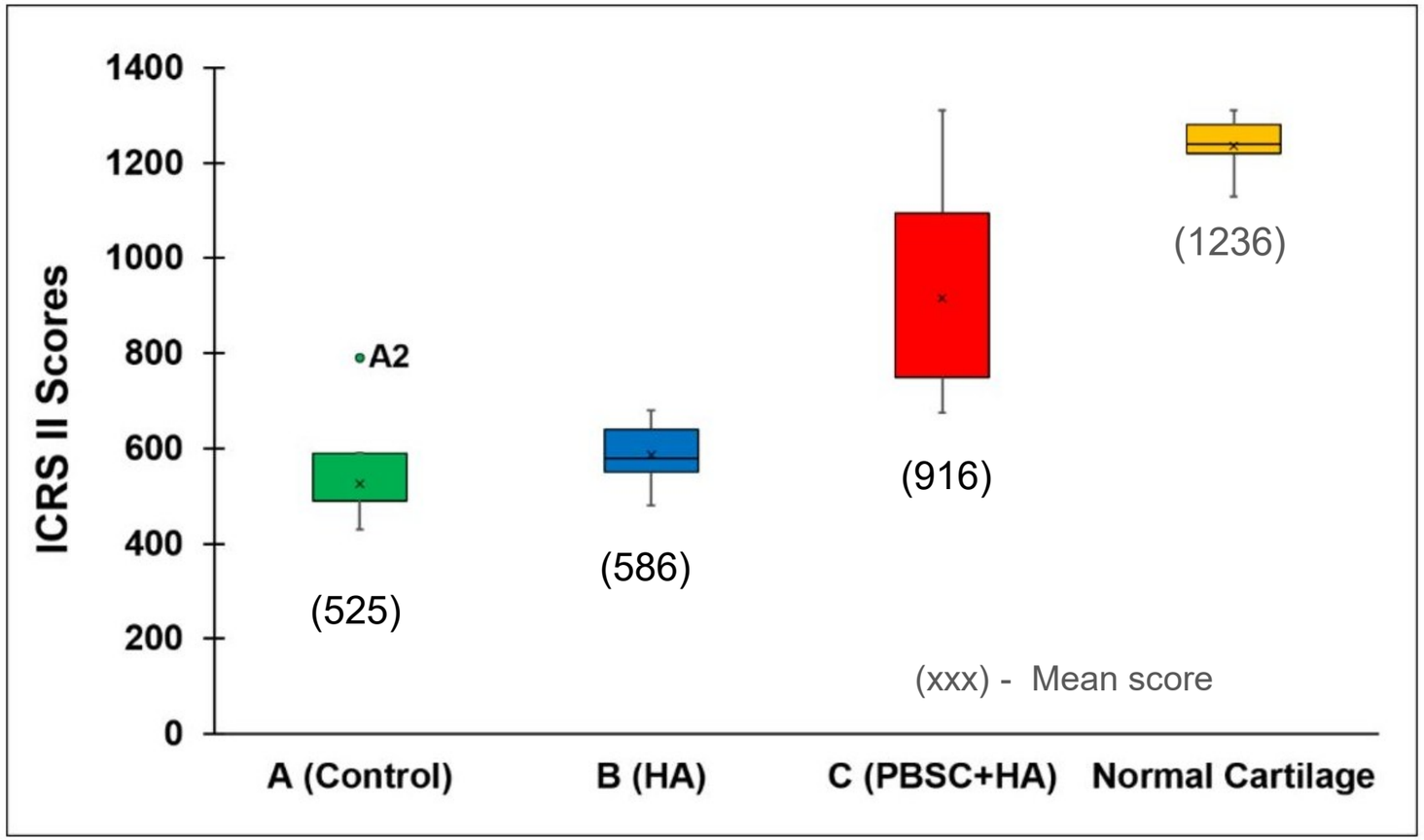
Results

- All animals in the study survived through the study.
- Groups A and B showed fibrous repair tissue over the defects.
- Group C (PBSC+HA) demonstrated better cartilage and bone regeneration, with tissue morphology closely resembling normal cartilage.
- Specimen with the highest ICRS II score, showed safranin-O and collagen I & II staining patterns similar to normal cartilage.
- No signs of inflammation or degeneration were observed in any group.

Histology comparison



ICRS II Histological Scores



- Higher ICRS II scores indicate better regenerated cartilage.
- Group C showed a statistically significant difference compared to:
  - Group A (p=0.014)
  - Group B (p=0.016)
- Group C showed no statistically significant difference compared to normal cartilage (p=0.093).

Discussion

- Macroscopic and histological analysis confirmed better proteoglycan content, round chondrocytes, smooth surface architecture, and minimal calcification in Group C, while Groups A and B had fibrous tissue with increased vascularization.
- Tidemark formation and type II collagen presence were stronger in Group C, confirming better-quality cartilage.
- While sheep serve as a strong model for cartilage research, study limitations include a small sample size, breed variation, and a six-month follow-up, with longer studies needed to observe full regeneration.
- Despite this, the study successfully established a PBSC-based approach for musculoskeletal regeneration, supporting future research on cell bio-distribution and long-term repair strategies.

Conclusions

Post-operative intra-articular injections of autologous PBSC and adjuvant HA after subchondral drilling resulted in better articular cartilage regeneration as assessed histologically by ICRS II in a sheep model.

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More information

Kuala Lumpur Sports Medicine Centre
www.klsmc.com

Email: enquiry@klsmc.com

Website: www.klsmc.com