NMRC, Cooperative Basic Research Grant 2013-New Investigator Grant: *'Role of Mesenchymal Stem Cell Secretome in Cartilage Regeneration and Potential for Development of Novel Cell-Free Therapeutics'* 7/2014 – 7/2017, $150,000 + $30,000 IRC. Toh Wei Seong (PI)

Summary of project progress

In this study, we have evaluated the efficacy of MSC exosomes in our established osteochondral defect model in adult immunocompetent Sprague Dawley (SD) rats. In each animal, one defect was treated with 100µg exosomes and the contralateral defect treated with phosphate-buffered saline (PBS). Intra-articular injections of MSC exosomes or PBS were administered weekly for up to 12 weeks. Analyses were performed by gross examination, histology, and scoring at 2, 6 and 12 weeks. Hematoxylin and eosin (HE), Safranin-O (Saf-O), and immunohistochemical staining for type II, I, VI and X collagens were performed.

Our *in vivo* findings provided strong evidence of enhanced cartilage repair with MSC exosome treatment. At early time-points, exosome-treated defects showed enhanced cellular infiltration and neotissue formation compared to the control defects. Analysis of proliferative cell nuclear antigen (PCNA) immunoreactivity further showed significantly higher numbers of PCNA-positive cells in the reparative tissue in animals treated with MSC exosomes. By 12 weeks, exosome-treated defects displayed complete restoration of hyaline cartilage and underlying subchondral bone (Figure 1). In contrast, only fibrous/non-cartilaginous tissues with minimal matrix deposition were found in the control defects. The regenerated cartilage layer showed hyaline matrix deposition with high levels of type II collagen and glycosaminoglycan (GAG) and low level of type I collagen. Additionally, there was pericellular localization of type VI collagen, similar as the native control. Macroscopic and histological scores were significantly improved by exosome treatment. Importantly, no adverse tissue reaction was observed in all the animals treated. Taken together, our results for the first time showed that exosomes are the principal mediator underlying the therapeutic effects of MSCs in cartilage repair. This work entitled: "Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration." has been published in the field's leading journal *Osteoarthritis and Cartilage*. (IF: 4.535)

As with all cell-based therapies, there exist operational and logistical costs and challenges in maintaining the vitality and viability of the cells needed for transplantation. Our study provides a strong basis for future use of human MSC exosomes as a safe, ready-to-use and 'cell-free' therapeutic for cartilage repair in patients. With the high potential for clinical translation, the paper entitled: "Human mesenchymal stem cell-derived exosomes promote orderly cartilage regeneration in an immunocompetent rat osteochondral defect model" has been awarded the Young Investigator Award in the 22nd annual meeting of ISCT (International Society for Cellular Therapy) held in Singapore (May 25-28, 2016).

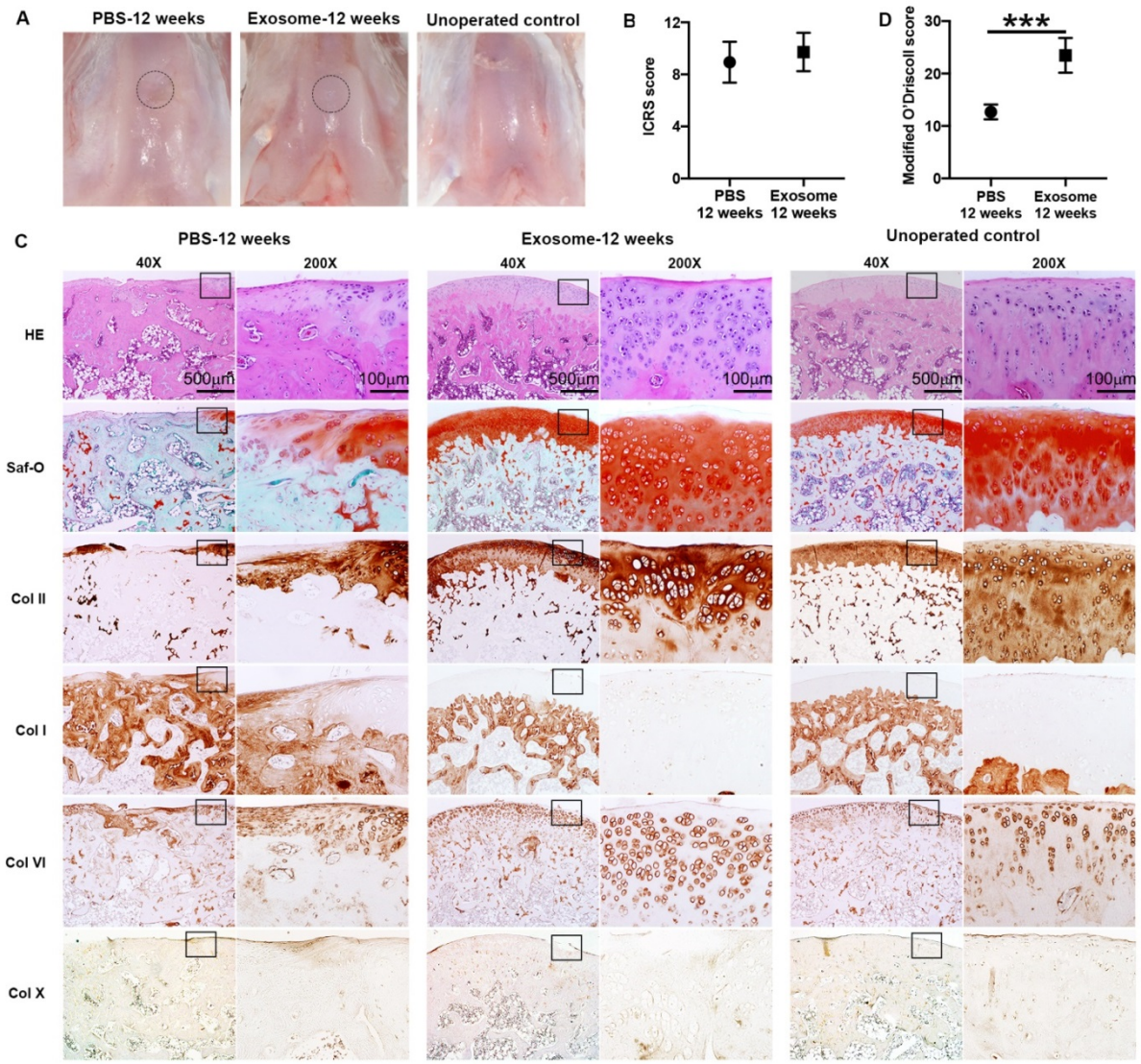

Figure 1: *In vivo* cartilage repair at 12 weeks post-surgery. Exosome-treated defects were compared against the contralateral control defects treated with PBS and unoperated native control. (A) Representative gross morphologies. (B) ICRS macroscopic scores. Values represent the mean ± 95% CI. P-values were determined by unpaired two-tailed Student t test (n = 6 per group). (C) Staining results of HE and Saf-O, and immunohistochemical staining for type II, I, VI and X collagens. (D) Modified O'Driscoll histologic scores for cartilage repair at 12 weeks. Values represent means ± 95% CI. P-values were determined by unpaired two-tailed Student t test (n = 6 per group). By the end of 12 weeks, exosome-treated group showed a new cartilage layer with good surface regularity and structural integration. The regenerated neocartilage layer appeared hyaline with comparable matrix staining to the age-matched native cartilage. There was also complete subchondral bone regeneration. Histologic scoring showed significant difference in the repair of osteochondral defects between the groups (P < 0.001).

Invited talks

- Toh WS. Mesenchymal Stem Cell Exosomes – A Novel Cell-free Therapy for Cartilage Repair. Department of Biomedical Engineering, College of Engineering, Peking University, Beijing, China (Oct 17, 2016).
- Toh WS. Mesenchymal Stem Cell Exosomes – The *Next Generation* Therapy for Cartilage Repair. 4th Asian Cartilage Repair Society (ACRS) Congress 2016 and 1st ICRS-China Scientific Meeting, Beijing, China (Oct 14-17, 2016).
- Toh WS. MSC-EVs – A 'Cell-Free' Therapeutic for Cartilage Regeneration. Singapore-France EV 2015 Meeting, Matrix, Biopolis, Singapore (Nov 11, 2015).
- Toh WS. Cartilage Regeneration with MSC-EVs. International Society for Extracellular Vesicles (ISEV) 2015 Workshop: Application of Therapeutic EVs in the Patient, Matrix, Biopolis, Singapore (Oct 19-20, 2015). *A focus meeting with selected 50 participants in the field.*
- Toh WS. From Stem Cells to their Secretome in Cartilage Repair. Regenerative Orthopedics: Regenerative Challenges in Ageing Population, 1st Aarhus Regenerative Orthopedics Symposium (AROS) Aarhus University, Denmark (Aug 13-15, 2015). *A focus meeting with selected 50 participants in the field*.

*Conference presentations (*corresponding)*

- Zhang S, Lai RC, Hui JH, Lee EH, Lim SK, Toh WS* The multifaceted role of mesenchymal stem cell exosomes in cartilage regeneration. Cold Spring Harbor Asia Conference 2016: Biology & Function of Extracellular Vesicles: Exosomes, Microvesicles & Beyond, Suzhou, China. (Dec 12-16, 2016) (Oral)
- Zhang S, Chu WC, Lai RC, Lim SK, Lee EH, Hui JH, Toh WS*^ Mesenchymal stem cell-derived extracellular vesicles: An off-the-shelf and cell-free therapeutic for cartilage repair. The 16th International Conference on Biomedical Engineering (ICBME) Singapore. (Dec 7-10, 2016) (Oral) ^presenting author
- Zhang S, Chu WC, Lai RC, Lim SK, Lee EH, Hui JH, Toh WS* Injectable mesenchymal stem cell exosome therapy for repair of full-thickness cartilage defects – A pre-clinical study. International Cartilage Repair Society (ICRS) 2016 – 13th World Congress, Naples, Italy. (Sep 24-27, 2016) (Oral) Abstract 7910
- Zhang S, Chu WC, Lai RC, Lim SK, Hui JH, Lee EH, Toh WS* Novel cell-inspired nanotherapeutics for cartilage regeneration. TERMIS Asia Pacific 2016, Taipei, Taiwan. (Sep 3-6, 2016) (Oral)
- Zhang S, Chu WC, Lai RC, Hui JH, Lee EH, Lim SK, Toh WS* Human mesenchymal stem cell-derived exosomes promote orderly cartilage regeneration in an immunocompetent rat osteochondral defect model. International Society for Cellular Therapy (ISCT) 2016 Annual Meeting, Suntec Singapore, Singapore. (May 25-28, 2016) *Cytotherapy* 18(6) Supplement: S13. (Oral) Abstract 21 (Awarded Young Investigator Award)
- Zhang S, Wong JS, Lai RC, Hui JH, Lee EH, Lim SK, Toh WS* Stem cell-based nanomedicine for cartilage repair and regeneration. 7th Stem Cell Society Singapore (SCSS) Symposium 2015, Biopolis, Singapore. (Nov 17-18, 2015) (Oral) Abstract S15

*Publications (*corresponding)*

- Toh WS*, Lai RC, Hui JH, Lim SK* (2016) MSC exosome as a cell-free therapy for cartilage regeneration: Implications for osteoarthritis treatment. *Seminars in Cell and Developmental Biology* doi: 10.1016/j.semcdb.2016.11.008. *(In press)* IF: 5.181; Too early for citation analysis
- Zhang S, Chu WC, Lai RC, Lim SK, Hui JH, Toh WS* (2016) Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. *Osteoarthritis and Cartilage* 24(12): 2135-2140. IF: 4.535; Times cited: 4
- Zhang S, Yap AU, Toh WS* (2015) Stem cells for temporomandibular joint repair and regeneration. *Stem Cell Reviews and Reports* 11(5): 728-742. IF: 3.111; Times cited: 3
- Toh WS*, Foldager CB, Pei M, Hui JH (2014) Advances in mesenchymal stem cell-based strategies for cartilage repair and regeneration. *Stem Cell Reviews and Reports* 10(5): 686-696. IF: 3.111; Times cited: 20