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Mesenchymal Stromal Cell Exosomes Enhance Primary Repair Of Rabbit Anterior Cruciate Ligament

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Disclosures

- Dr Sai Kiang Lim holds founder shares in Paracrine Therapeutics.

Background

- ACL tears are one of the **most common** injuries in the knee
  - Incidence of ACL ruptures: 30 to 78 per 100,000 person-years[1-7]
- ACL tears has **historically been managed with primary repair**
  - High failure rates of primary repair in anterior cruciate ligament (ACL) tears → widespread abandonment + **transition towards reconstruction**[8-19]
- While **ACL reconstruction is still the current gold standard** for treatment[8,20,21], this is not without drawbacks and **suboptimal outcomes**[22-30] **continue to persist** despite advancements in surgical techniques[8,20]
  - Graft rupture rates [22-24]
  - Reoperation[25-28]
  - Return to pre-injury level of sporting activity[29,30]

- Societal and economic impact of ACL tears (Mather RC 3rd et al, JBJS 2013)[31]
  - Lifetime burden of ACL tears in the U.S.
    - $17.7 billion annually with structured rehabilitation alone, **$7.6 billion annually with ACL reconstruction**
- **Revision rate of ACL reconstruction: 3.14%** (Liukkonen et al. OJSM 2022)[32]
  - Metanalysis involving 52,878 patients, median patient age 28 years (range, 15-57 years)
- **Higher risk in paediatric and adolescent populations** with ACL reconstruction
  - **Graft failure rate 9.6%** (54 out of 561 ACL reconstructions) (Ho et al. J Pediatr Orthop 2018)[33]
  - **Growth disturbance**
    - **2% risk** of growth disturbance following surgery (International Olympic Committee consensus, Arden et al. Br J Sports Med 2018, Frosch et al. Arthroscopy 2010)[34,35]
    - Rate of growth disturbance with **physeal-sparing techniques not better than transphyseal techniques** (Longo et al. JBJS 2017)[36]
  - **ACL reconstruction rates have increased 29 fold** over the last 20 years (Nogaro et al. JBJS 2020)[37]

Background

- Led to shift towards biology preservation in the effort towards improving outcomes[38-42] and a **renewed interest in primary repair.**[8,20]
- Biologically augmented ACL repair has gained significant interest in recent years
    - Murray et al's **bridge-enhanced ACL repair (BEAR) technique** utilizing a bioinductive scaffold with suture repair achieved **results similar to ACL reconstruction** with hamstring allograft graft **in human studies**[43,44]
- Mesenchymal stem/stromal cells (MSCs) are the current stem cell-of-choice for regenerative medicine applications.[45]
    - Limited by constraints of cell-based therapy[46-48]
        - Low yield
        - Aging and heterogeneity of cells harvested
        - Poor survival of cells following transplantation
- Although use of MSCs for tissue repair was first predicated on their differentiation potential, it is now accepted that these cells mediate tissue repair through paracrine factors instead of their differentiation potential to replace injured/diseased cells.[49,50]
- **Exosomes identified as the principal mediator underpinning the biological effects of MSCs in tissue repair.**[51,52]
    - Implicated in biochemical and cellular processes such as communication, immunomodulation, structure and mechanics, metabolism, tissue repair and regeneration.[53]

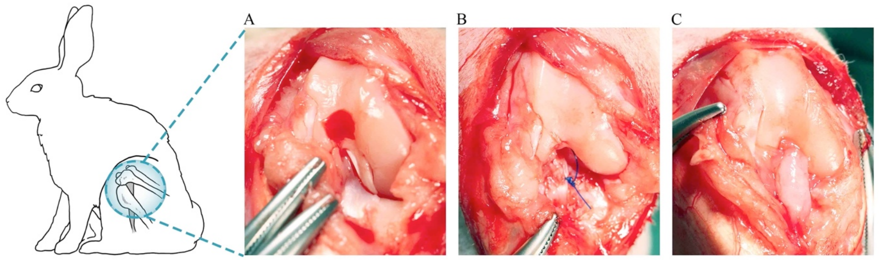
Hypothesis: MSC exosomes delivered using a fibrin sealant can enhance primary ACL repair

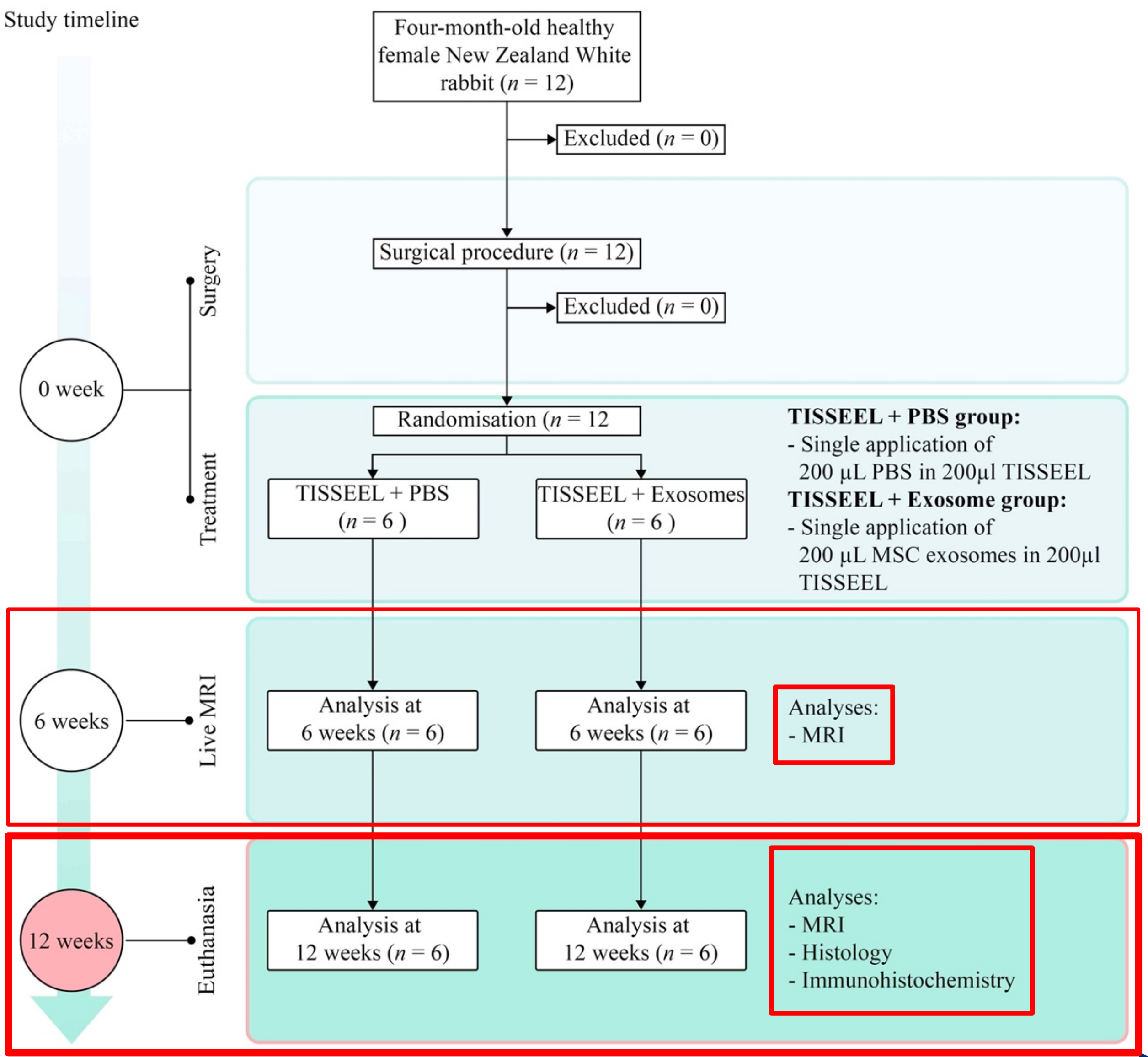
Study Objective: To investigate the efficacy of MSC exosomes for primary ACL repair in a rabbit model

Method

- 12 rabbits randomly allocated to 2 treatment groups (n=6 per group)
  - **Group 1: Fibrin sealant and MSC exosomes (TISSEEL+Exosomes)**
  - **Group 2 (control): Fibrin sealant and phosphate buffered saline (TISSEEL+PBS)**

    *PBS used as a vehicle control as PBS is the solution used to prepare and dilute exosomes
- ACL tears surgically created in unilateral knee joint of each rabbit
  - Open arthrotomy
  - Medial parapatellar approach
- ACL tears repaired with simple interrupted Nylon sutures (Ethilon®, Ethicon)





Method

To assess for the **degree and quality of healing**, various assessments were performed:

- Radiological assessment via MRI
  - Anterior Cruciate Ligament Osteoarthritis Score (ACLOAS) MRI grading system to classify ACL healing (BJSM 2022[54], Osteoarthritis Cartilage 2014[55])
    - 0=Normal ligament with hypointense signal and regular thickness and continuity.
    - 1=Thickened ligament and/or high intraligamentous signal with normal course and continuity.
    - 2=Thinned or elongated but continuous ligament.
    - 3=Absent ligament or complete discontinuity.

    ****0 considered intact, 1 or 2 considered partial healing, 3 considered torn**
- Histological assessment
  - For general morphology and degree of ligamentous integrity restoration
  - For quality of ACL repair through immunohistochemical staining for types I and III collagen

In vitro work

Cell cultures utilizing rabbit ACL cells also performed **to investigate cellular processes mediated by MSC exosomes** during ACL repair

- Cell metabolic activity, total DNA content, cell migration in response to treatment, and amount of collagen deposition were measured at 4, 24, 48 and 72h post-treatment.
- **Gene expression analysis**
  - Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) was performed to examine the genes associated with proliferation, migration, and matrix synthesis.

Results

<u>5 out of 6 rabbits in TISSEEL+Exosomes group showed sustained ACL healing on MRI</u>
from 6 to 12 weeks

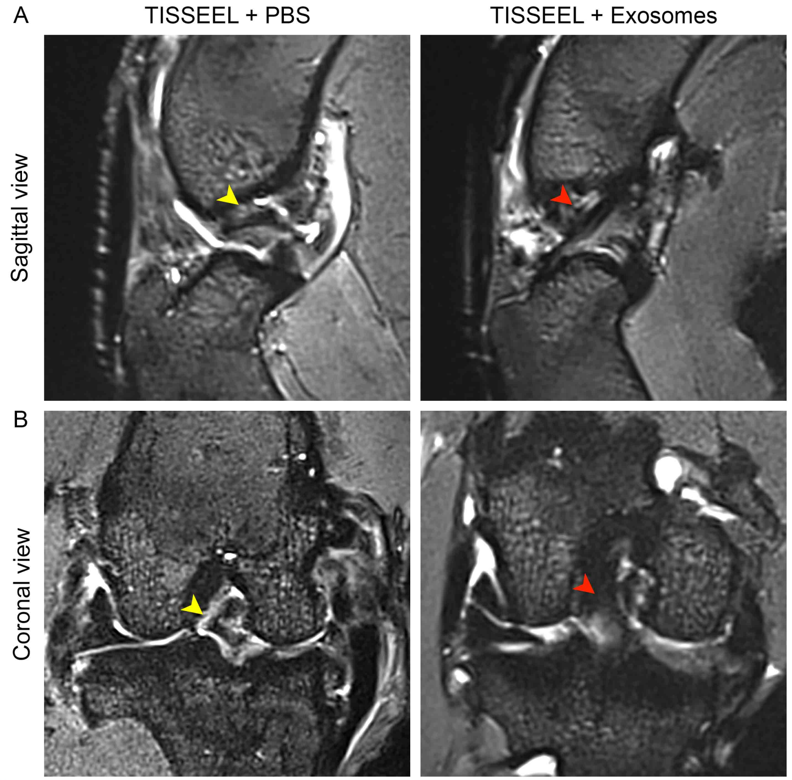
- Compared to only 1 of the 6 rabbits in TISSEL+PBS group at both timepoints

TISSEEL+Exosomes group

- Correlated well with complete **<u>morphological restoration of ligamentous integrity</u>** and **<u>rich deposition of predominantly type I collagen</u>**, similar to the native ACL.

TISSEEL+PBS group

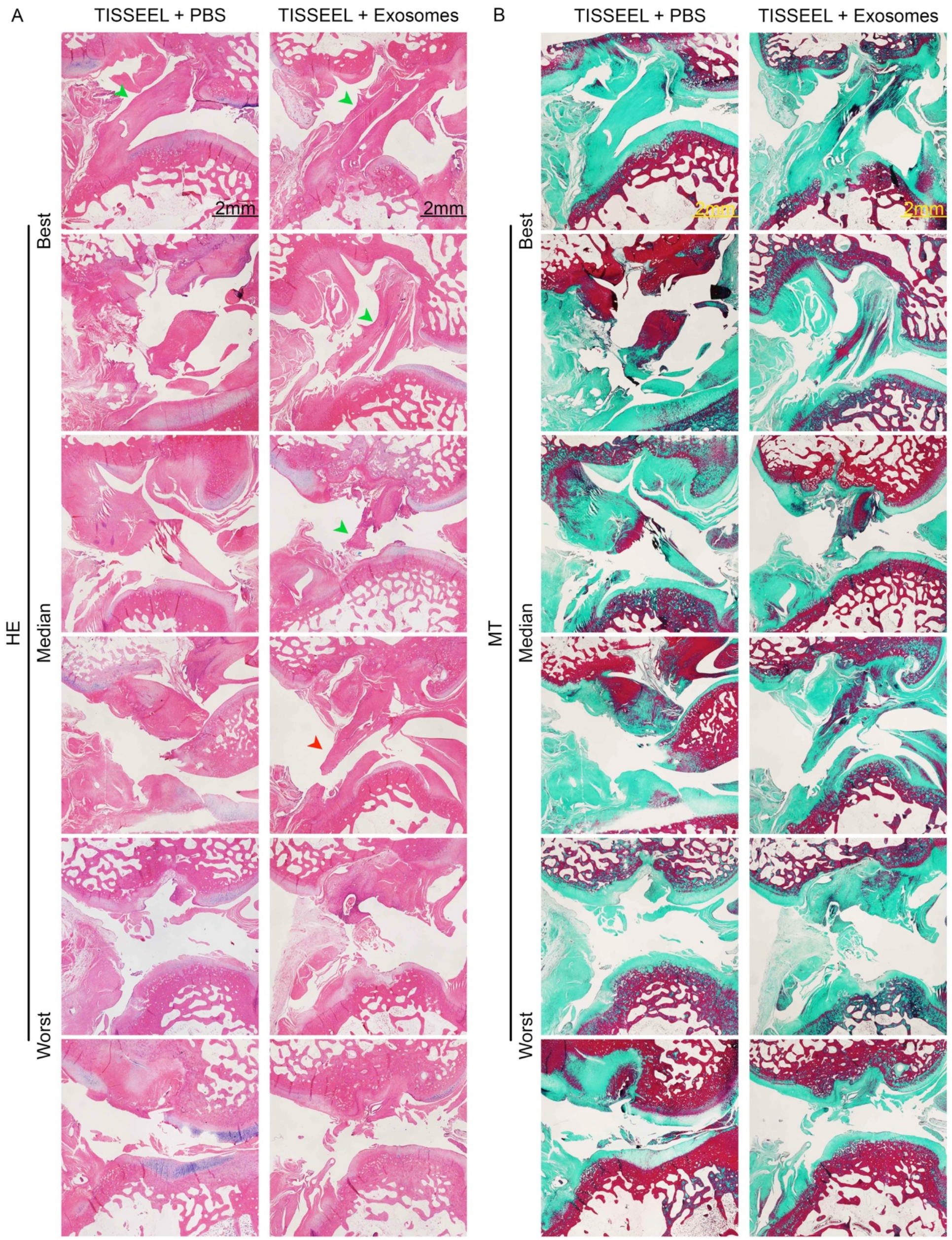
- **<u>Mainly type III collagen</u>** with ligamentous integrity restored to some degree



Groups		TISSEEL + PBS				TISSEEL + Exosomes		
Ranking	Rabbit ID	6 weeks MRI	12 weeks MRI	12 weeks Histology	Rabbit ID	6 weeks MRI	12 weeks MRI	12 weeks Histology
1	1076	Intact	Intact	Intact	1043	Intact	Intact	Intact
2	1203	Torn	Torn	Torn	0907	Intact	Intact	Intact
3	1062	Torn	Torn	Torn	1032	Intact	Intact	Intact
4	1104	Torn	Torn	Torn	0977	Partial	Partial	Partial
5	1105	Torn	Torn	Torn	1020	Partial	Partial	Torn
6	1114	Torn	Torn	Torn	0941	Torn	Torn	Torn
	Torn	5/6	5/6	5/6	Torn	1/6	1/6	2/6
Counts	Partial	0/6	0/6	0/6	Partial	2/6	2/6	1/6
	Intact	1/6	1/6	1/6	Intact	3/6	3/6	3/6

H&E

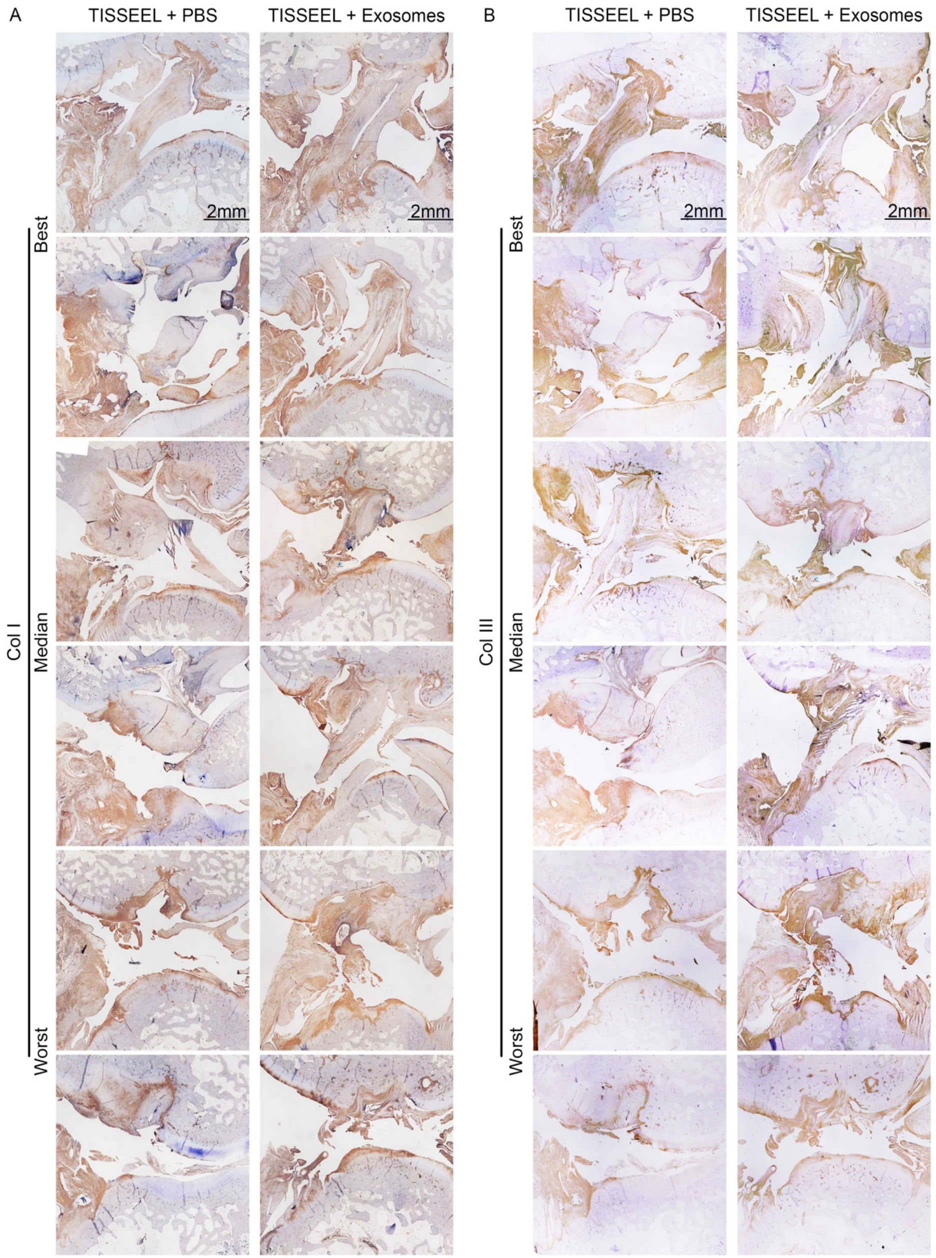
MT



Immunohistochemical staining

Type I collagen (Col I)

Type III collagen (Col III)



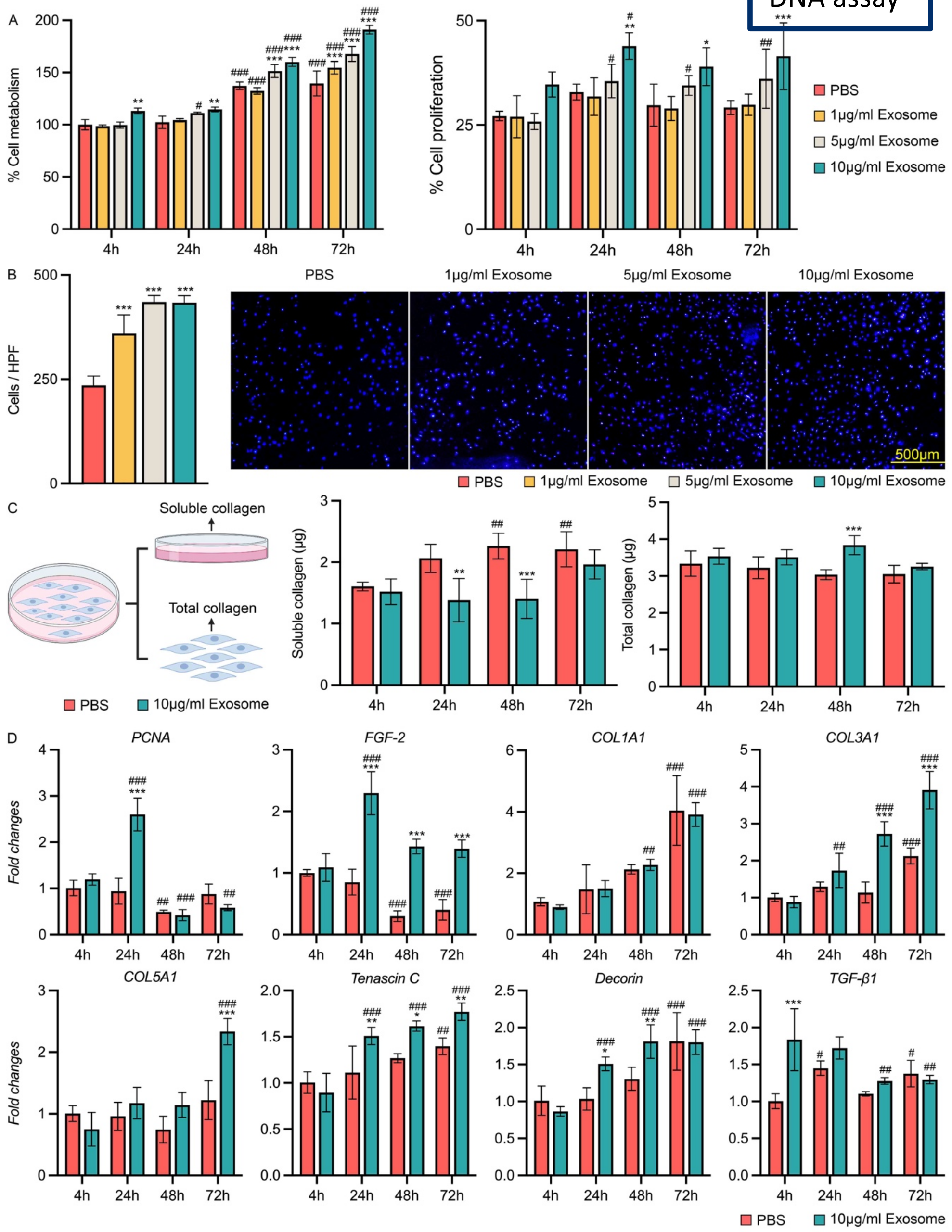
MTS metabolic essay

Transwell migration assay

Collagen measurement

Quantitative RT-PCR analysis

DNA assay



- Using ACL cell cultures, we demonstrated that **MSC exosomes dose-dependently enhanced cell metabolic activity** (p=0.002), **proliferation** (p<0.001), and **migration** (p<0.001).

- Exosome treatment also **suppressed ACL matrix degradation and enhanced collagen synthesis** at 48h post-treatment (p=0.015).

- **Supported by increased gene expression associated with proliferation** (PCNA, FGF-2; p<0.001), **migration** (FGF-2; p<0.001), **and matrix synthesis** (COL3A1, COL5A1, TGF-β1; p<0.001, Tenascin C, Decorin; p<0.01) with exosome treatment compared to control.

Strengths

1. Provides proof-of concept for the role of MSC exosomes in augmenting healing in ACL primary repair

- First study utilizing exosomes as a biological strategy to improve healing rates in mid substance ACL tear repairs
- Holds potential as a cell-free MSC therapeutic to augment healing in ACL primary repair

2. Works well in combination with a simple internal suture construct

- Focused study on the MSC exosomes as a biological solution to aid healing achieved
  - Simple internal suture construct provided minimum mechanical stabilization required for approximation of ACL tear [simple interrupted Nylon sutures (Ethilon®, Ethicon)]
- Easily reproducible and potentially translatable setup
  - Avoids confounding factors arising from technical difficulties with complex internal suture strut constructs → Reduces issues with learning curve
  - No specific minimum stump length requirement
  - Can potentially be used with in conjunction with arthroscopic or MIS approaches

Limitations

1. Difference in intra-articular environment and ACL condition compared to in vivo conditions with acute pivot shift injury

- Transection of the ACL performed with sharp division using a blade in view of small size of rabbit knees
  - Does not replicate in vivo conditions of pivot shift injury causing ACL tear
    - Knee does not go through phase of acute knee swelling with significant inflammation and hemarthrosis
    - ACL does not go through phase of plastic deformation before tear
  - Accurately achieves an isolated mid substance tear without introducing new confounding factors → ACL healing can be studied in isolation

2. Relatively short time-to-harvest post treatment

- 12-week time-to-harvest sufficient in showing a difference in healing rates between the MSC exosomes treatment group against the control
  - Proof of concept
- Future studies with a longer time-to-harvest period to allow further healing may be useful in assessing the full reparative potential of the MSC exosomes
  - Will also be useful to assess for longevity of the regenerated ACL when compared against our data at 12 weeks

Conclusion

MSC exosomes with fibrin sealant biologically enhance ACL primary repair, possibly by augmenting ACL cell functions.

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