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RESEARCH ARTICLE

A human kidney and liver organoid-based multi-organ-on-a-chip model to study the therapeutic effects and biodistribution of mesenchymal stromal cell-derived extracellular vesicles

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Introduction: Extracellular vesicles (EVs)

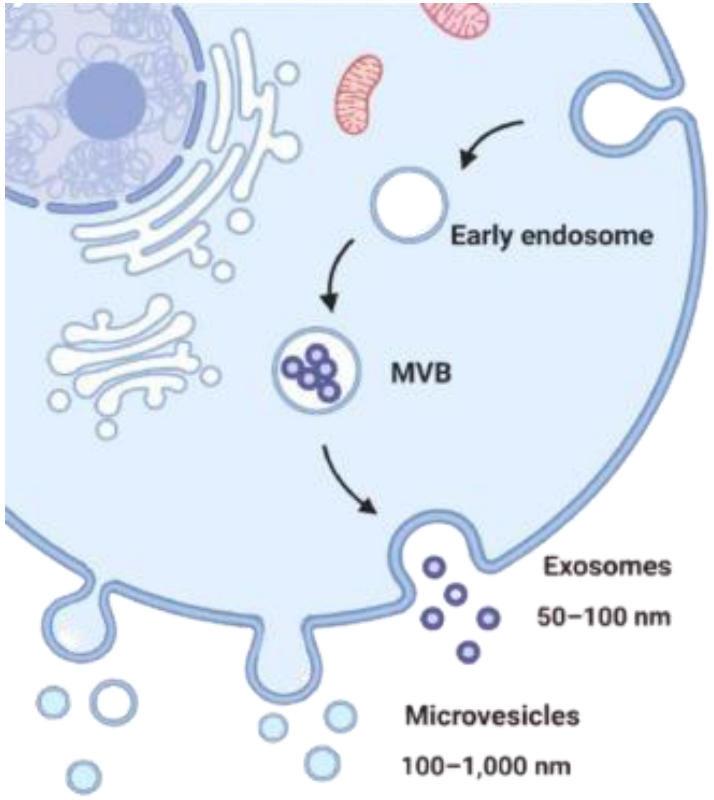
Extracellular vesicles (EVs)

- A subcellular structure with a lipid bilayer similar to a cell membrane
- Commonly classified as exosomes and microvesicles
- Essential signaling mediators in various (patho-) physiological process.

Mesenchymal stromal cell (MSC)-derived small EVs (sEVs):

- Can promote therapeutic activities through paracrine effects
- The variable beneficial effects of MSC-derived sEVs have been reported (tissue regeneration and immune modulation, …).

→ **To enable required therapeutic actions, it is crucial for sEVs to localize to the target site.**



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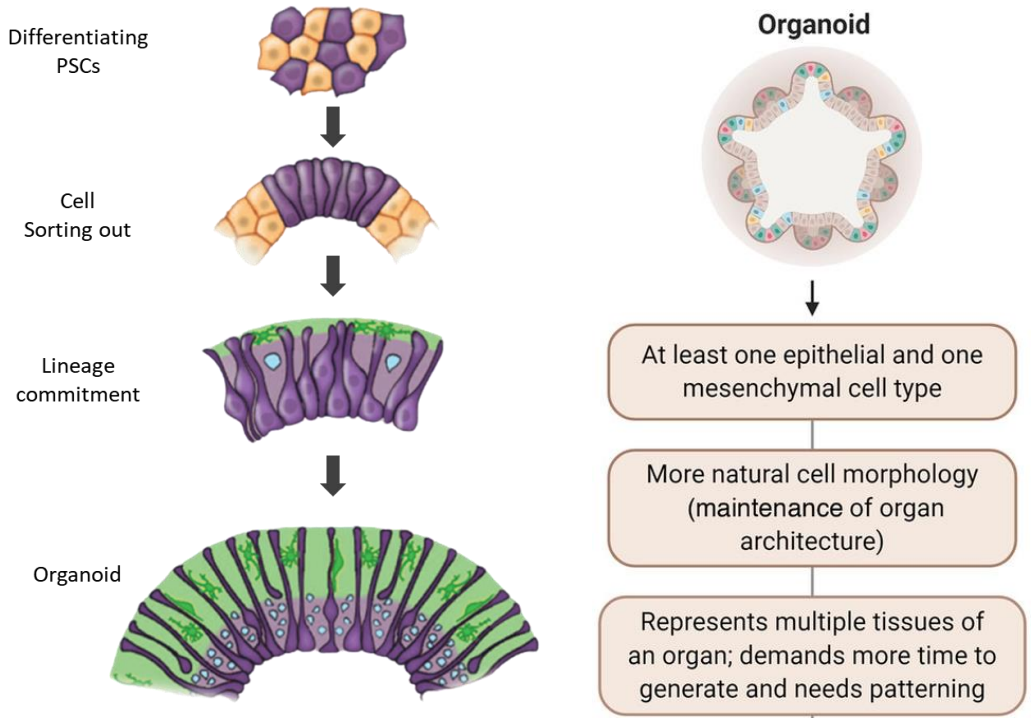
▪ MVB: Multivesicular bodies

Introduction: Organoid

Organoid

- Organoids have a specific tissue-like 3D structure derived from pluripotent stem cells or isolated organ progenitors that differentiate to from an organ like tissue.



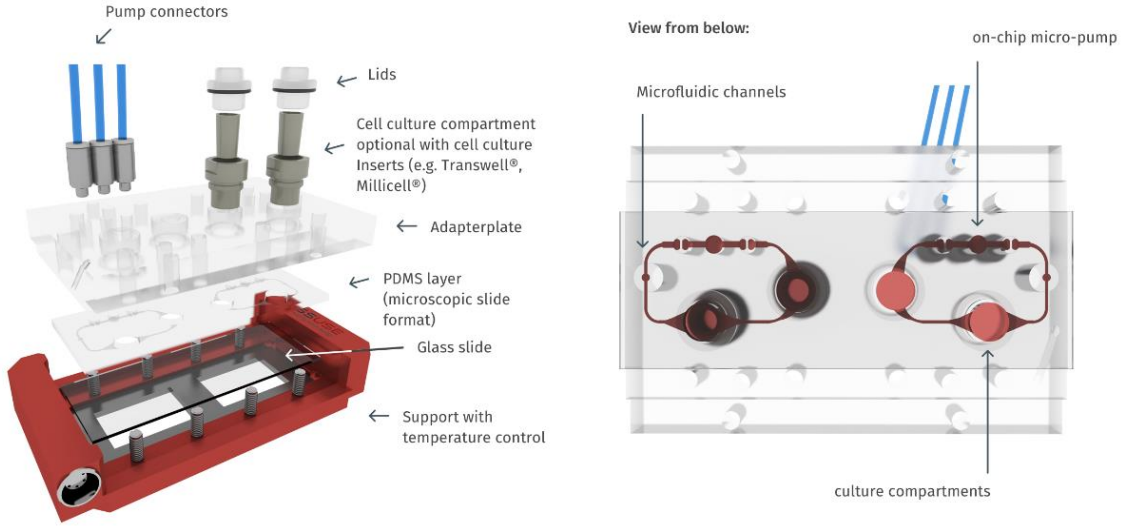
Multi-organ-on-a-chip (MOC) model

- Combining multiple (organoid-based) organ models in a single microfluidic circuit
- Allow the analysis of biodistribution, efficacy and potential undesired (off-target) side effects of EVs.

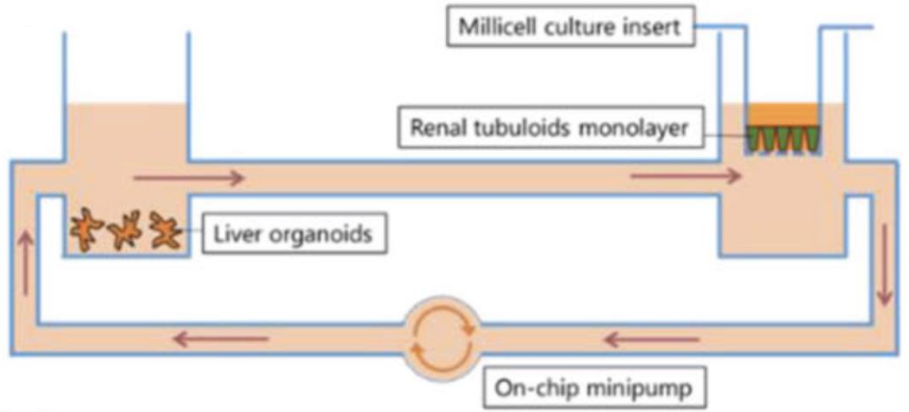
Brief experimental design

Human organoid-based multi-organ-on-a-chip (MOC) model

- **Kidney tubuoloids**
  - ✓ Human adult stem cell-derived organoid
  - ✓ To generate a functional tubular epithelium separating a blood and a urine compartment, renal tubuloids are cultured on a semi-permeable membrane.

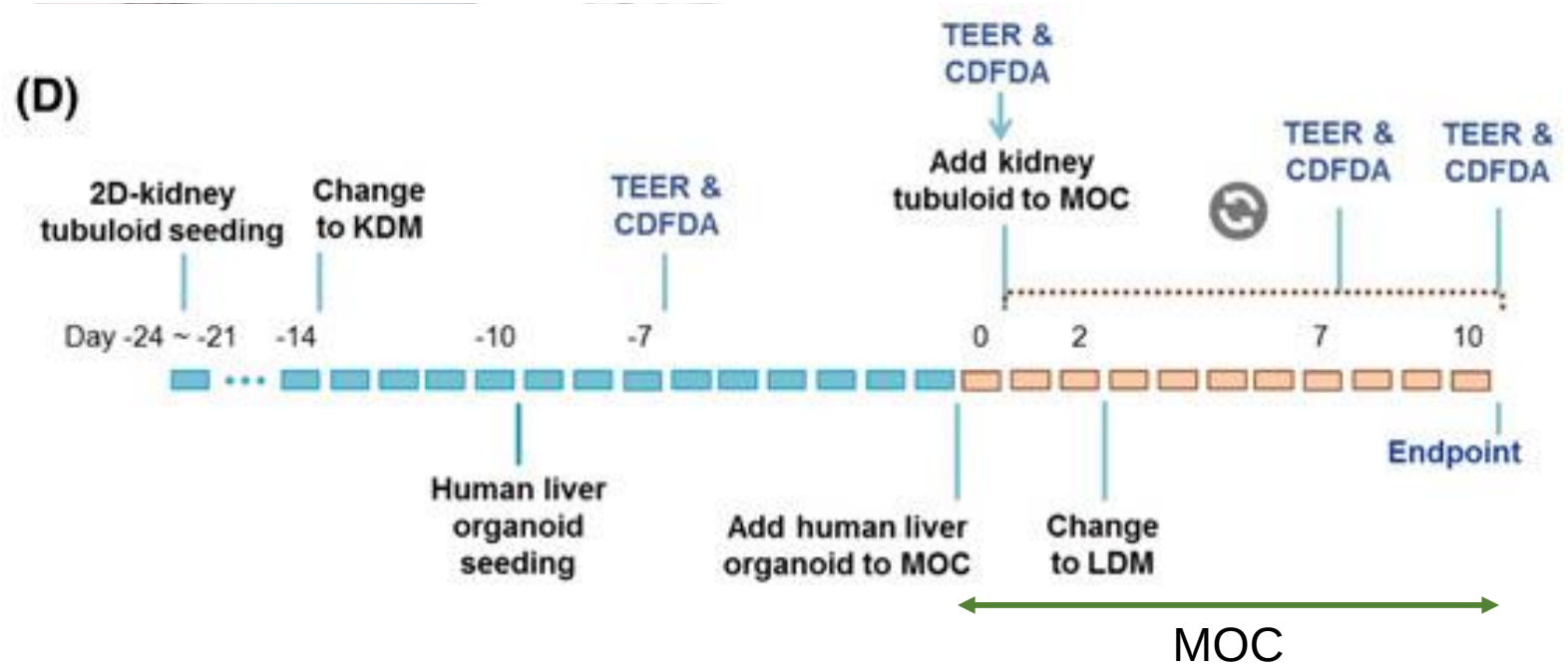


- **Liver organoids**
  - ✓ Representing a crucial organ for drug metabolism in general
  - ✓ A major site for EV accumulation



→ These organoids are combined via a microcirculatory system to study the regenerative potential and organ distribution of MSC-sEVs in a model for acute renal injury.

Brief experimental design

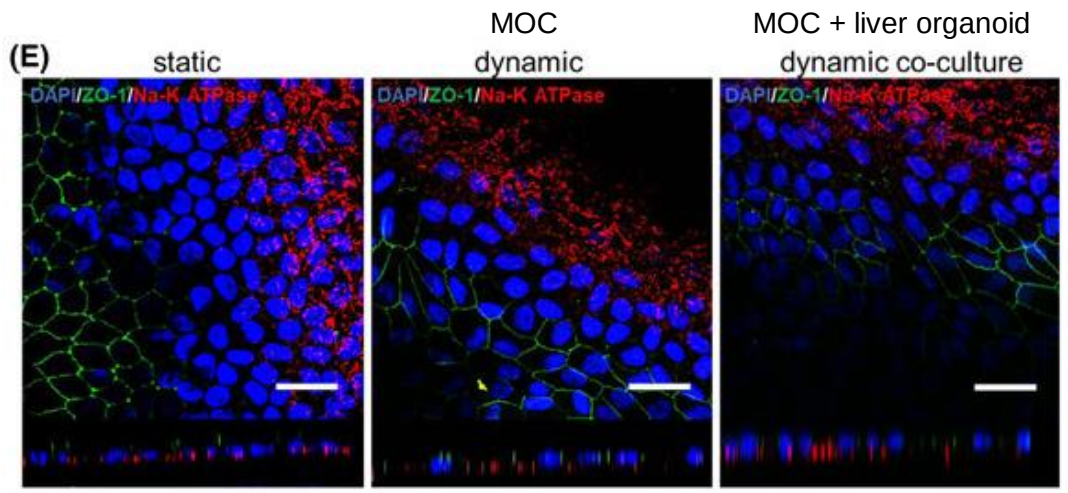


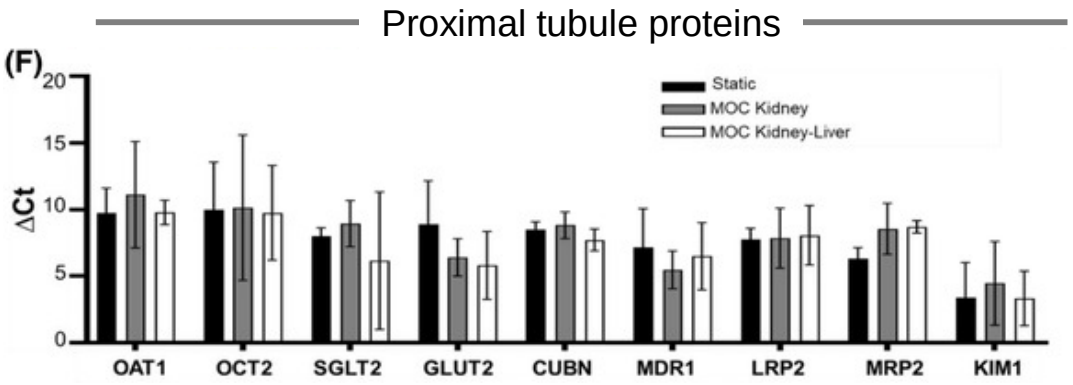
- **Trans-epithelial electric resistance (TEER) analysis**
  - ✓ To confirm the barrier integrity of the cell-layer

- **CDFDA transport assay**
  - ✓ To determine transmembrane transport functionality of 2D-cultured renal tubuloids

- KDM: Kidney organoid differentiation medium
- LDM: Liver organoid differentiation medium

Characterization of 2D-cultured renal tubuloids

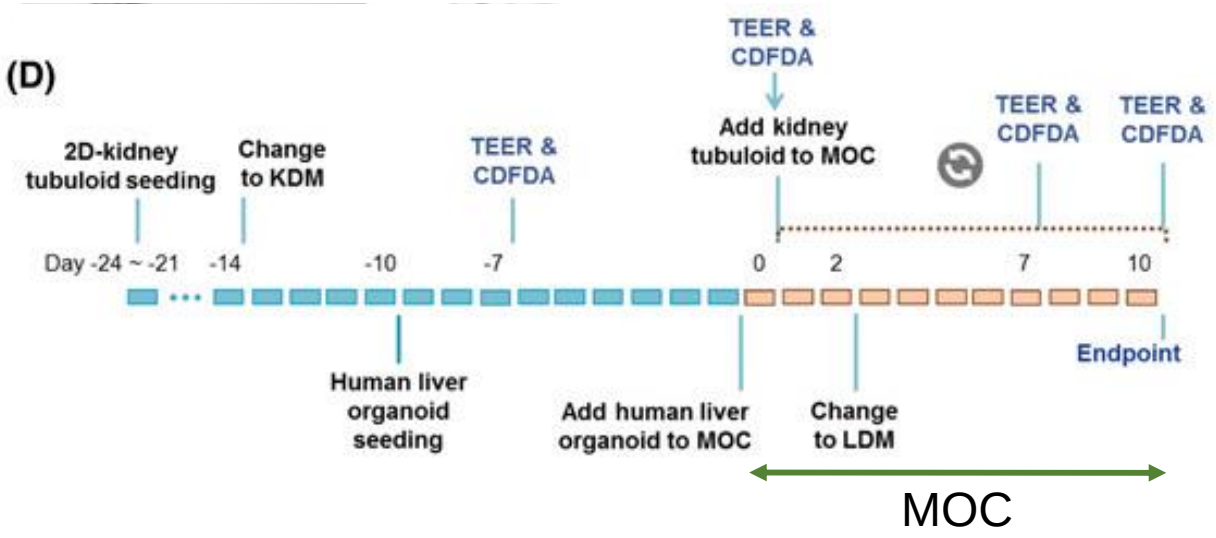


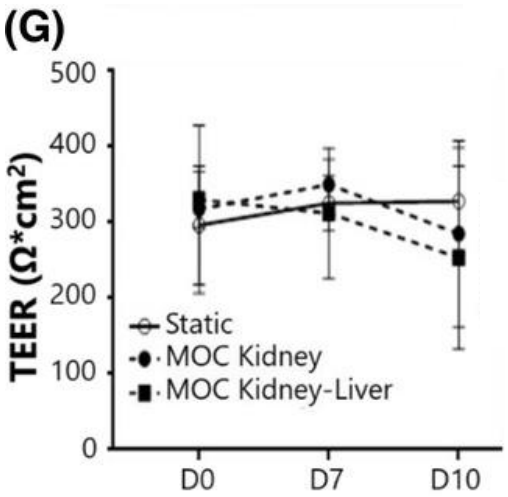


- Polarisation of the kidney tubuloid monolayer grown under static conditions was shown.
  - ✓ Basolateral expression of the Na+/K+-ATPase
  - ✓ Apical localization of ZO-1
- The ZO-1 staining area appears stretched in the dynamic MOC conditions.
  → The renal cells adapt their morphology in response to flow.
- Several proximal tubule proteins were equally expressed at day 10 after the differentiation in static, dynamic, and co-culture conditions.

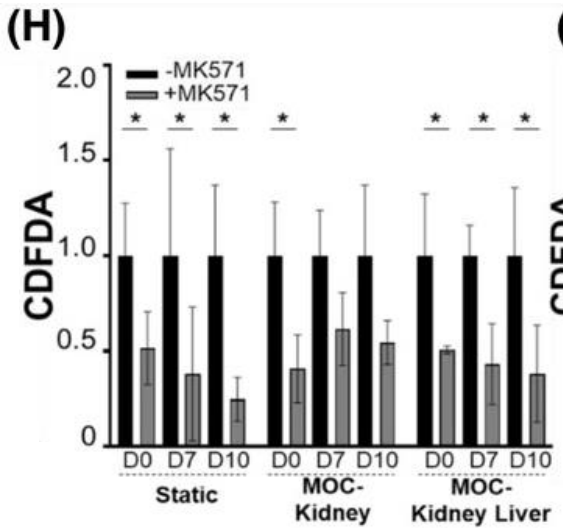
- ZO-1: Tight junction marker

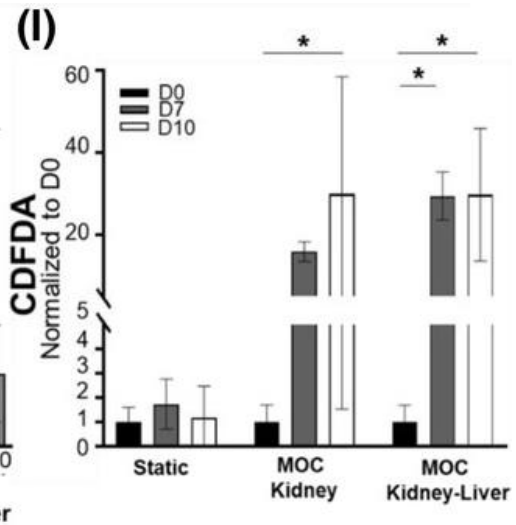
Characterization of 2D-cultured renal tubuloids





- The barrier integrity of the cell-layer remained stable in static and dynamic (co-)culture condition.

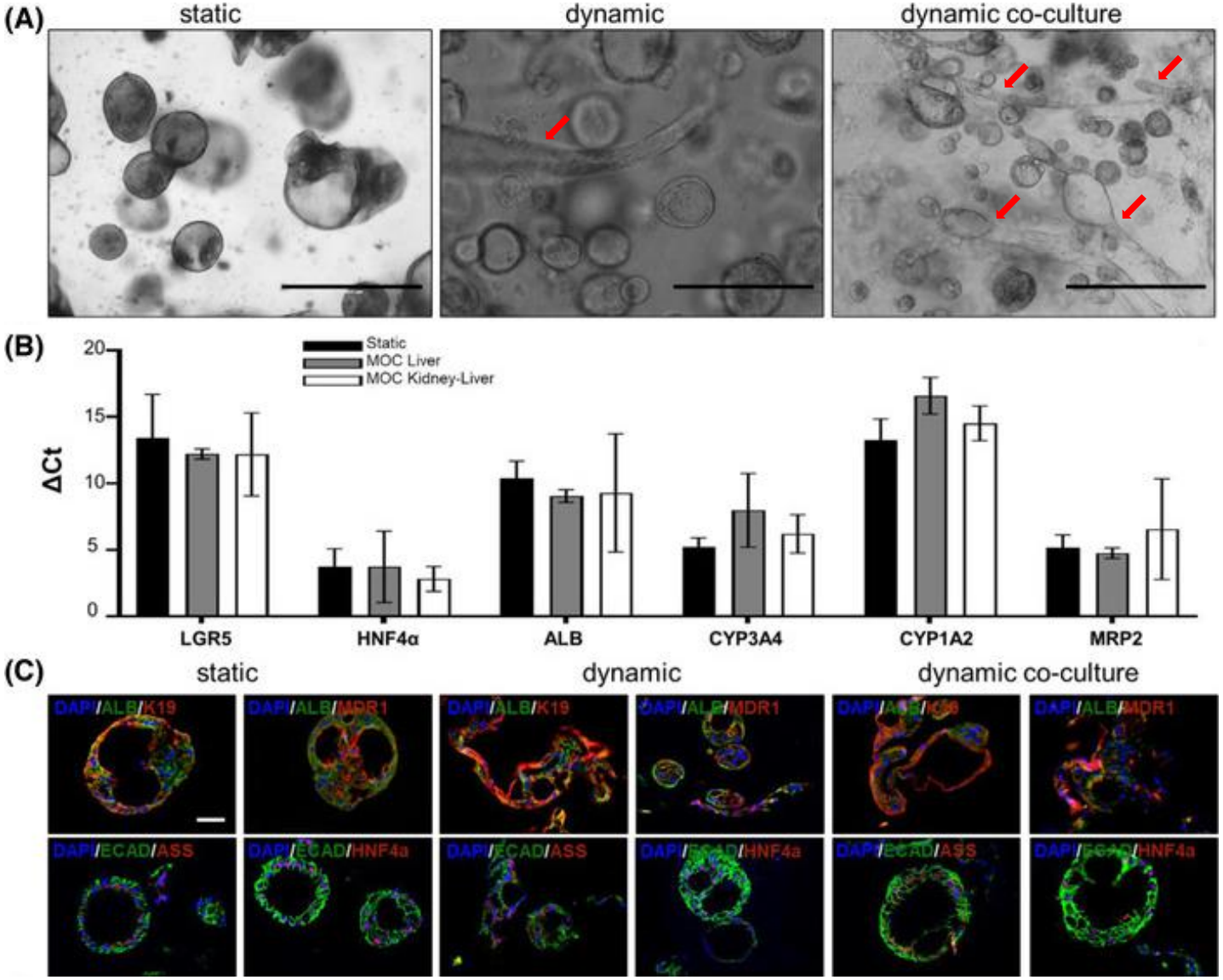




- MK571: A selective inhibitor of CDFDA
- Although the relative contribution of MRP2 remains equal to static conditions, dynamic culturing conditions resulted in significant increase of absolute CDFDA transport at day10.

→ **2D-cultured renal tubuloids has generated a leak-tight epithelial barrier after the differentiation and retained barrier function in static and dynamic culture conditions.**

Liver organoids in static and dynamic (co-)culture

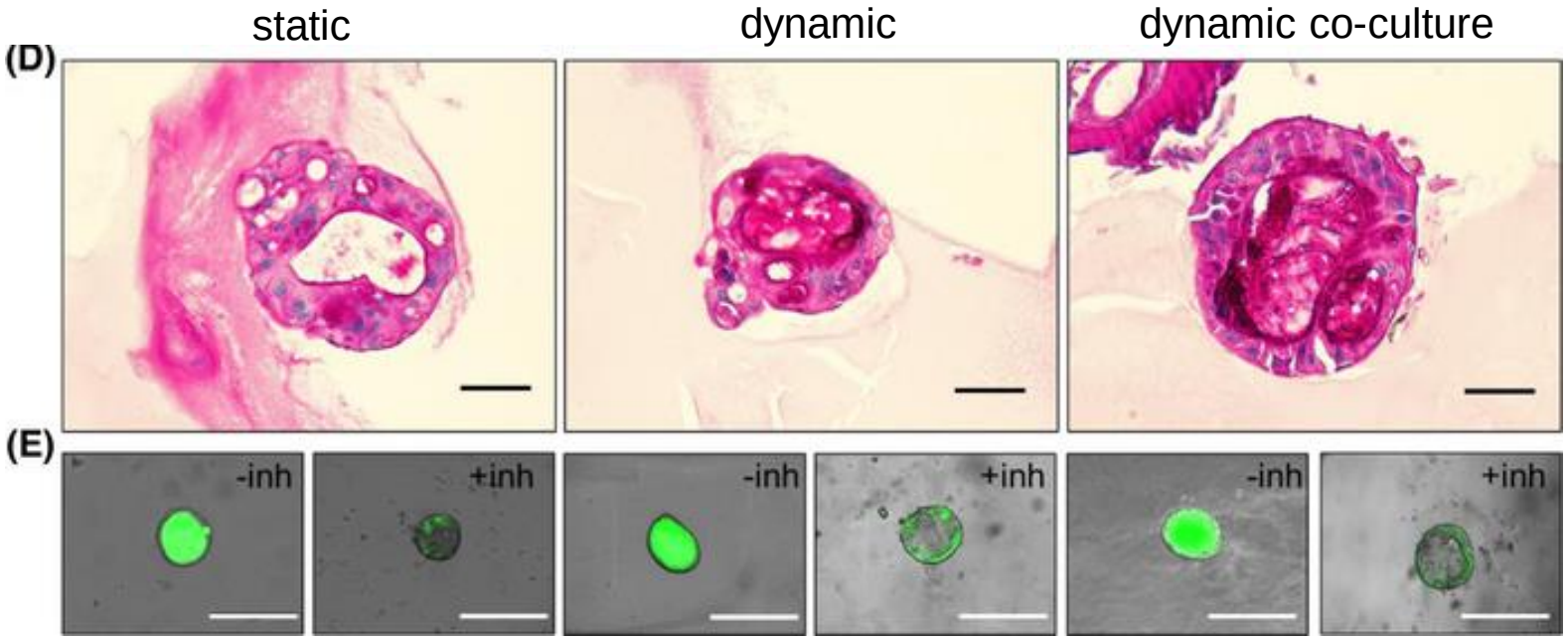


- After transfer to chips and exposure to flow, alone or in co-culture with kidney tubuloids, liver organoid showed the more circular-like shape, but also obtained a tubular-like shape.

- There are no significantly differences of stemness and hepatocyte-related marker in static and dynamic (co-) culture conditions.

- LGR5: Stemness marker
- HNF4a, ALB, CYP3A4, CYP1A2, MRP2, MDR1, ASS : Hepatocyte marker
- KRT19: Cytoskeleton marker
- ECAD (E-cadherin): Membrane marker

Liver organoids in static and dynamic (co-)culture



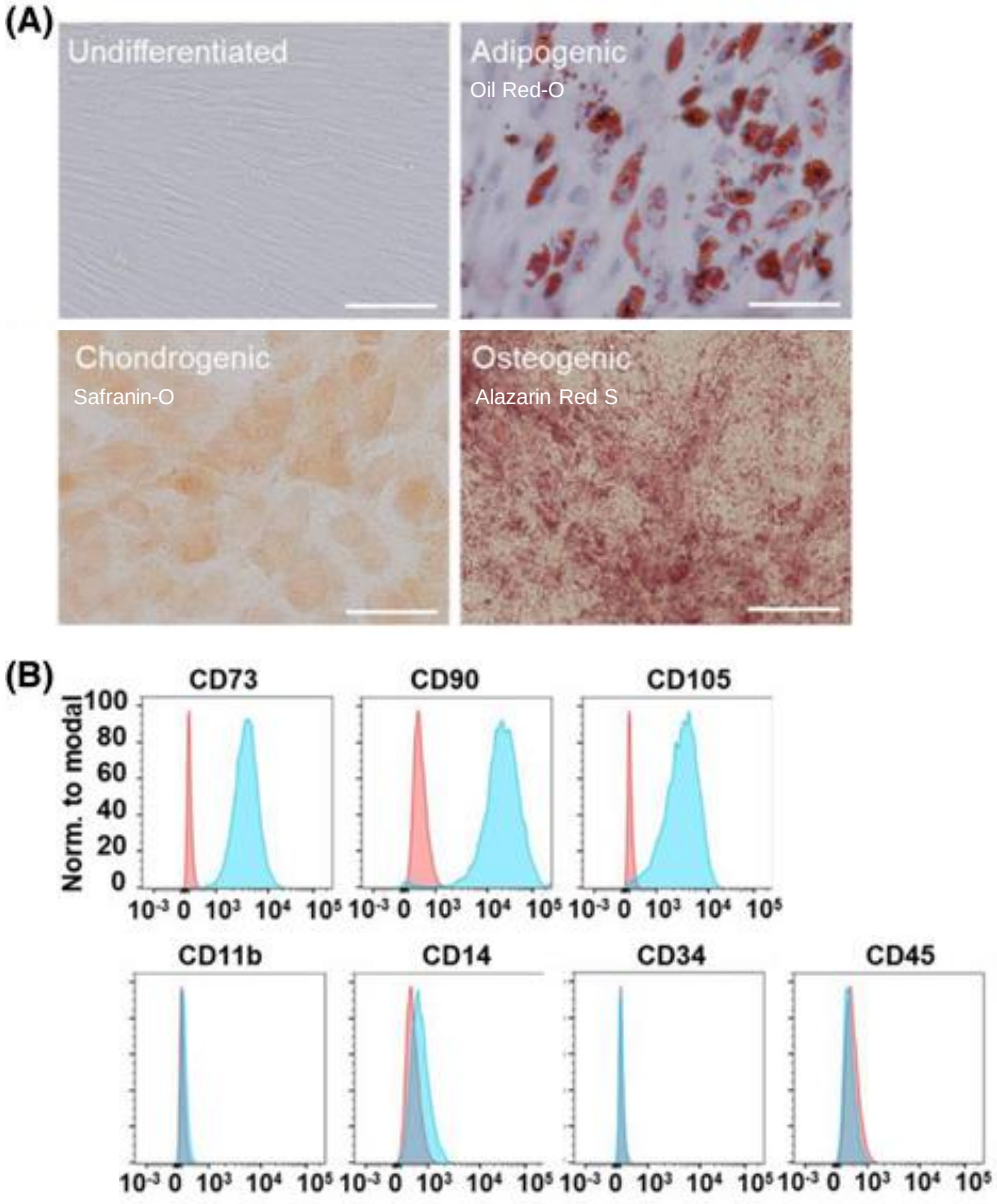
→ Glycogen storage ability

→ Transmembrane transport activity

- When the organoids were pre-treated with the competitive inhibitor of MDRI (Verapamil), the fluorescent accumulation was not found in the lumen of organoids.

→ Hepatic function has been acquired by differentiated liver organoids in both static and dynamic cultivation.

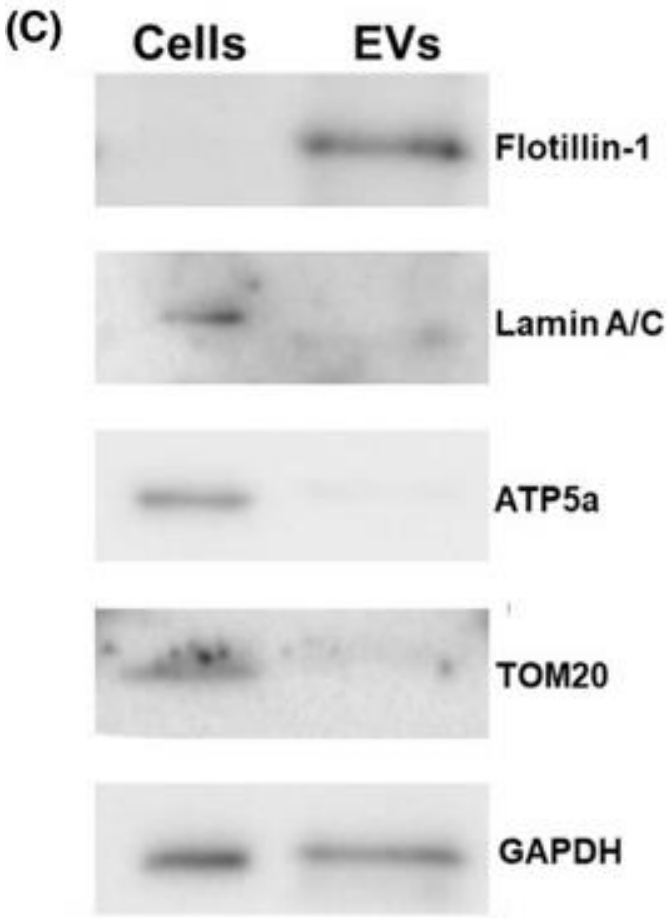
MSC and EV characterization



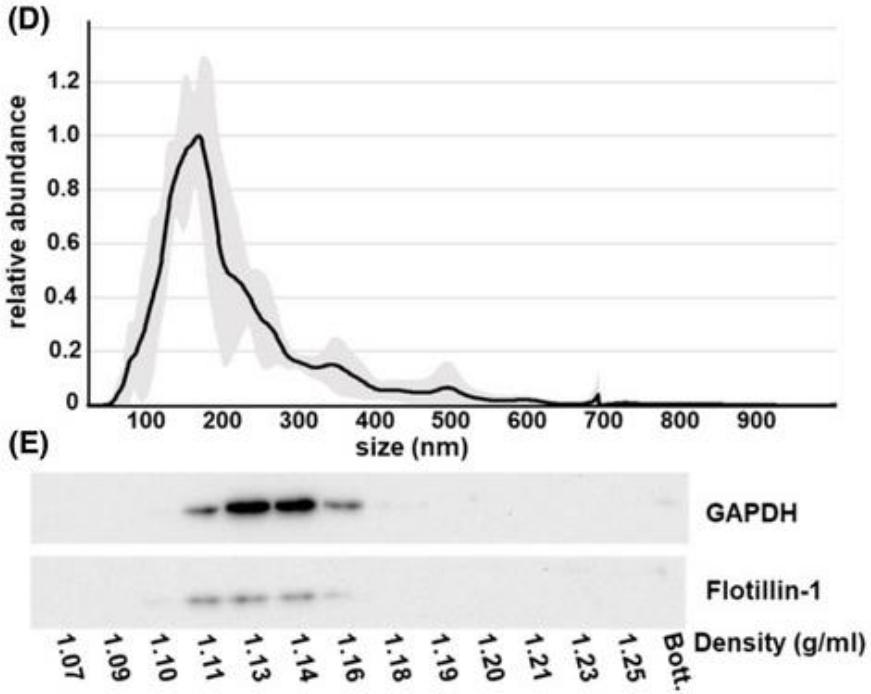
- Human bone marrow-derived MSC
- Tri-lineage differentiation capacity of MSC was confirmed.
  → MSC that were used as a source for EVs are capable of differentiation towards adipocytes, chondrobasts and osteoblasts.

- CD73/90/105 were expressed.
- CD11b/14/34/45 cannot be detected.

MSC and EV characterization



- sEVs were investigated for potential contamination by cellular debris through immunoblotting for Lamin A/C, ATP5a, TOM20.

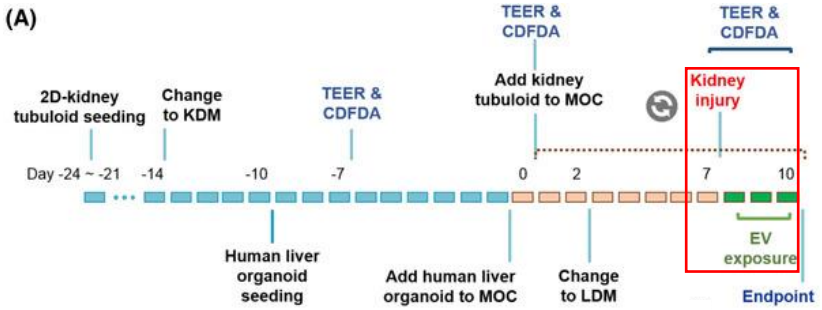


- Nanoparticle tracking analysis was employed to assess EV size:
  - ✓ Average size of 345 nm
  - ✓ A modal size of 149 nm

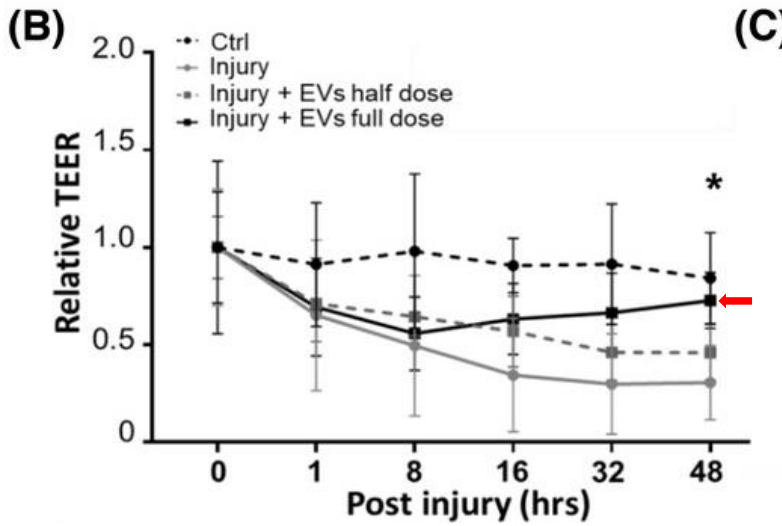
- Isolated sEVs have a density of 1.13-1.14 g/mL.

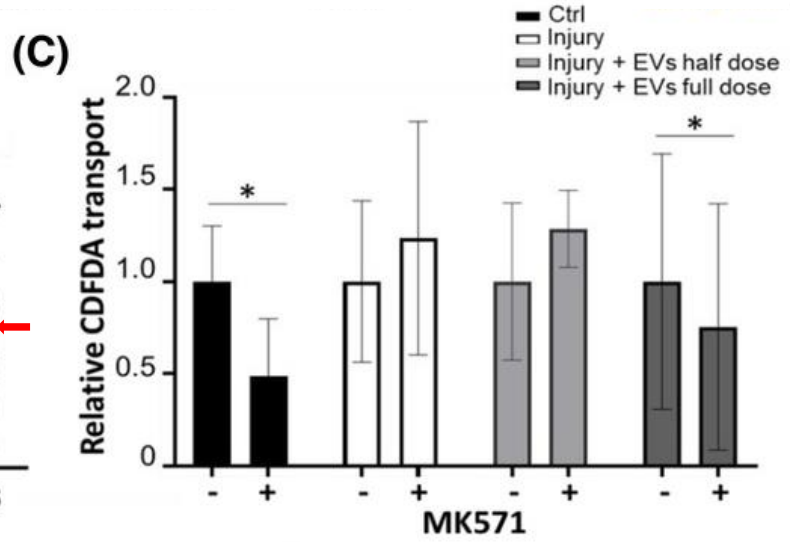
- Flotillin-1: sEV-resident protein
- Lamin A/C: Nuclear marker
- ATP5a: Mitochondrial marker; ATP synthase lipid-binding protein
- TOM20: Translocase of outer mitochondrial membrane 20

MSC-derived sEV therapeutic effects and biodistribution



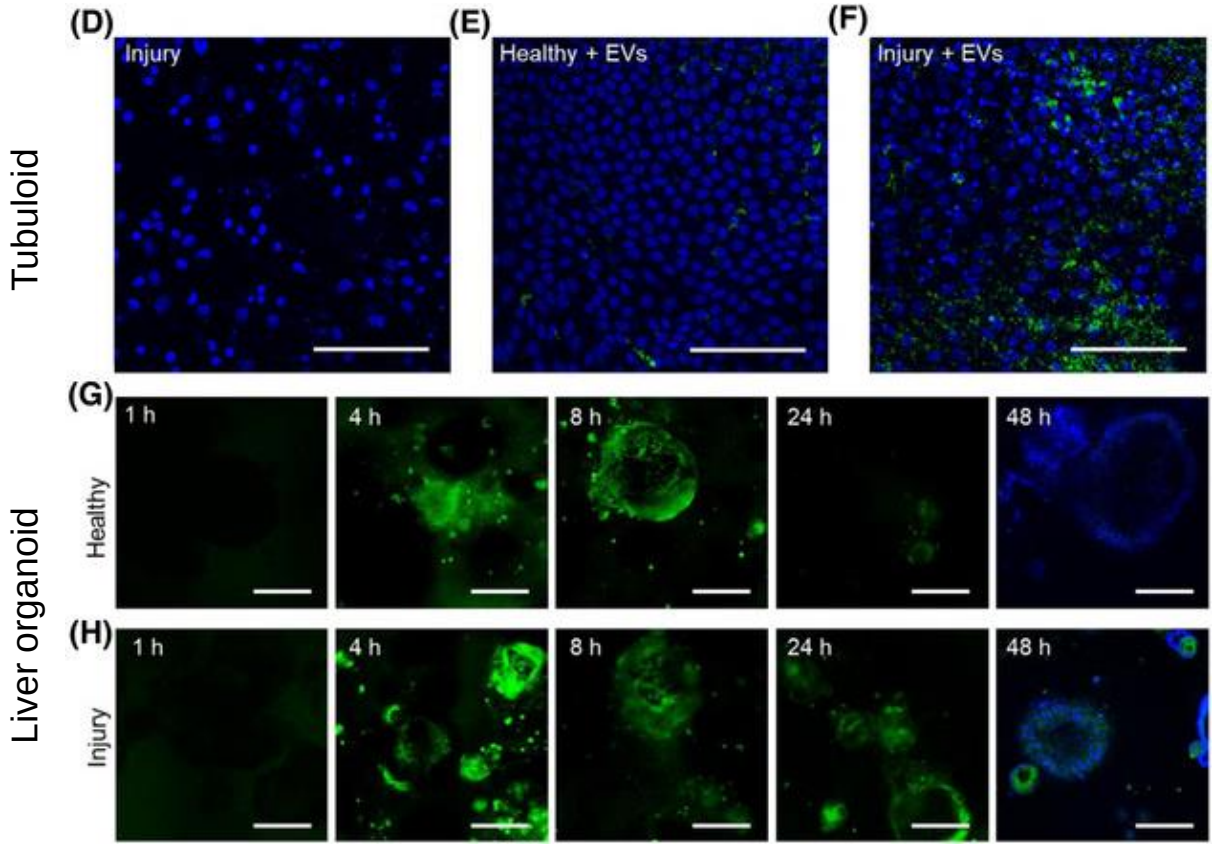
- To model acute kidney injury, renal tubuloids were exposed to H_2O_2 for 1 h.





- Injured tubuloids treated with full dose showed significant recovery of the barrier function.

MSC-derived sEV therapeutic effects and biodistribution



- MSC-sEVs were fluorescently labelled with PKH67.
- When EVs were administrated, the accumulation of sEVs on tubuloids in injury condition was significantly higher compared to that in normal conditions.
- After 24 and 48 h, sEVs retention in liver organoids was detectable compared to the sEV signal in those combined with healthy control.

- PKH67: a dye commonly used to monitor spatial distribution of sEVs in vitro and in vivo

Summary

- They were established an in vitro human organoid-based circulatory model.
- After induction of kidney injury, MSC-sEVs accumulate at the site of injury and reverse the impairment in kidney epithelial integrity and transport function.
- MSC-sEVs also localize to the liver and that is more pronounced after injury.