

A comparison of fetal bovine serum extracellular vesicle (FBS-EV) depletion protocols and their effect on umbilical cord mesenchymal stem cells (UC-MSCs)

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EPSRC/MRC DTC in Regenerative Medicine
The James Richardson Studentship, Institute of
Orthopaedics Ltd., Oswestry
ISTM and ACORN funding, Keele University

Aims & Objectives

- To compare the efficiency of FBS-EV depletion protocols.
- To determine how the resulting EV collection media effects cell phenotype of primary UC-MSCs.

Summary

- UF was the most efficient FBS-EV protocol, but completely eradicated protein.
- Less FBS contribution slowed cell growth, but was only significant for SF.
- MSC phenotype was unaffected.
- Less FBS contribution decreased the immunomodulatory potential of UC-MSCs.

What are EVs?

- Extracellular vesicles (EVs) are small membrane bound particles that are capable of recapitulating the abilities of their parental cells¹.
- EV research aims to utilise these particles as an alternative to cell therapy.

Fetal Bovine Serum (FBS)

- Pros:** Provides proteins, lipids and growth factors needed to support cell growth².
- Cons:** Contaminates cell culture with FBS-EVs (or associated protein or RNA cargo) that skews downstream analysis of EVs of interest².

Serum Free (SF)

- Pros:** Minimal source of FBS contamination.
- MSC phenotype was retained (>95% positive markers, <2% negative markers).
- Cons:** Base media only provides amino acids, vitamins and sugars, impacting growth² (C).
- Immunomodulatory potential, assessed by IDO, impacted (D).

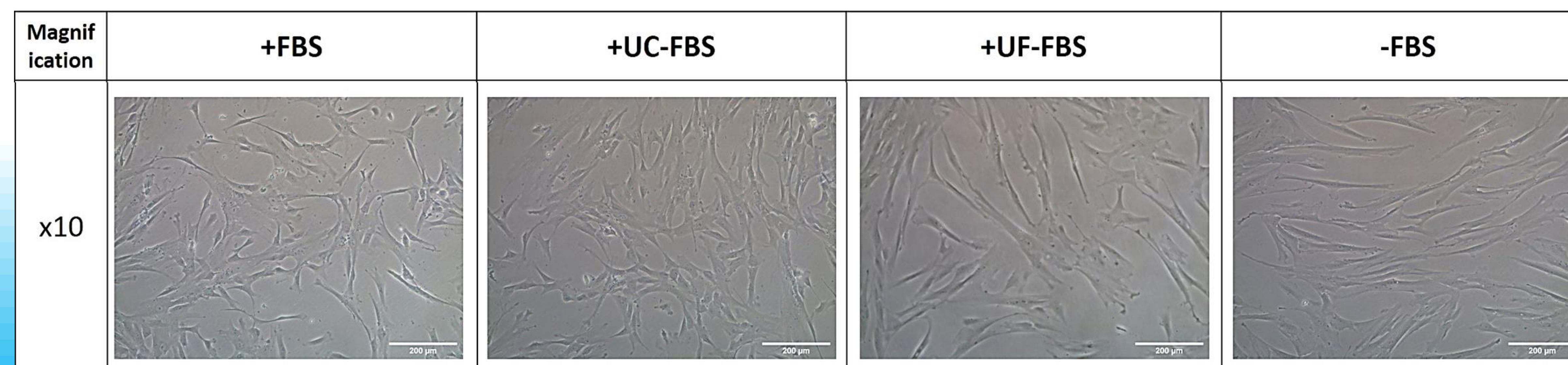
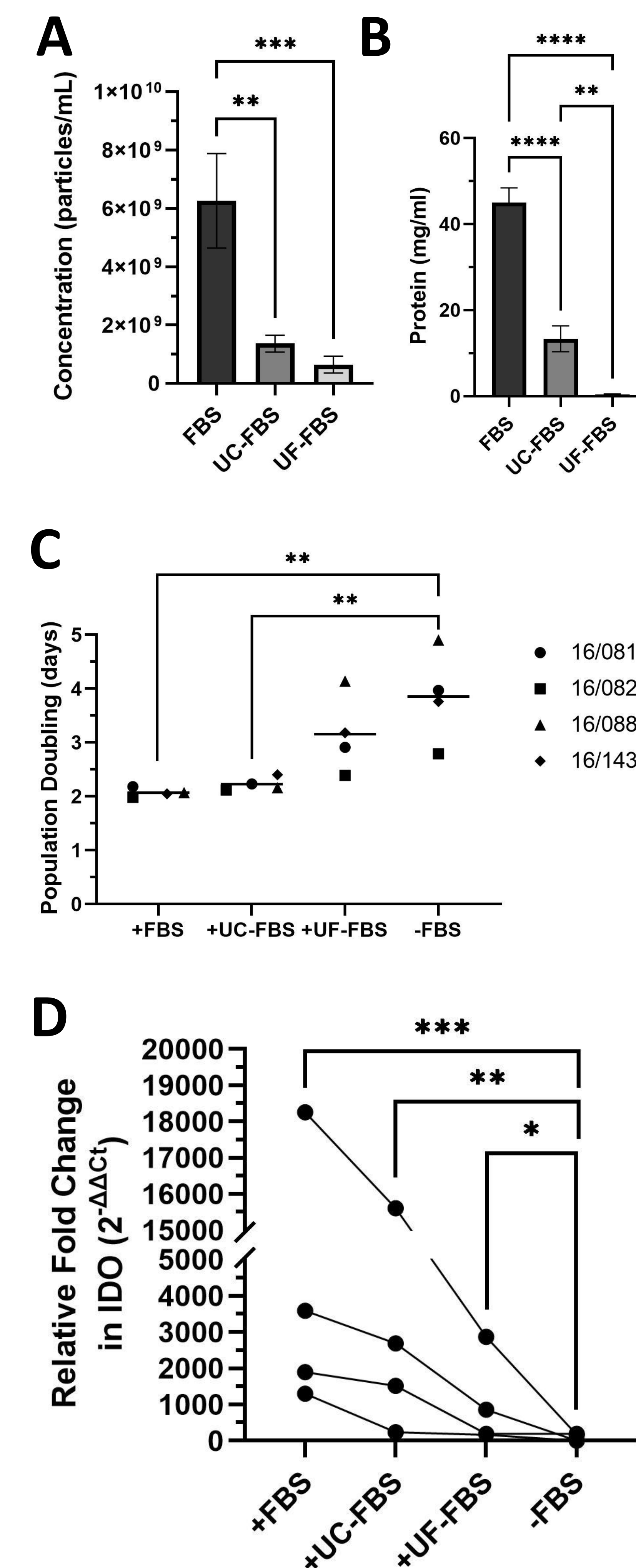
Ultracentrifugation (UC)

- Protocol:** Prolonged UC (>18-hours) at 120,000g³.
- Pros:** Retained 29.58% of proteins that support cell growth (B).
- Immunomodulatory potential, assessed by IDO, retained (D).
- Cons:** Depleted 78.28% particles (A,C).



Ultrafiltration (UF)

- Protocol:** Amicon ultrafilter, 100kDa, spin for 55min at 3,000g³.
- Pros:** Depleted 89.78% particles (A).
- Cons:** >99% protein removed, impacting cell growth (B,C).
- Immunomodulatory potential, assessed by IDO, impacted (D).



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