Effects of bovine milk-derived extracellular vesicles on a 3D intestinal stromal scaffold

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INTRODUCTION

Milk constitutes an essential element of the diet, due to its nutritional value. It contains nanoscale entities known as extracellular vesicles (EVs), which play key roles in intercellular communication. Specifically, milk-derived EVs (MEVs) may influence intestinal homeostasis, by mitigating inflammatory responses, modulating gut microbiota composition, and contributing to epithelial integrity preservation and restoration. Currently, there is no information regarding their impact on intestinal connective tissue. We tested the hypothesis that MEVs support proliferation of fibroblasts (the major constituent of the connective tissue) in an *in vitro* three-dimensional (3D) intestinal stromal compartment.

METHODS

We generated decellularized intestinal biological scaffolds from organs collected at the local slaughterhouse, applying a protocol consisting of freeze–thaw cycles followed by sequential incubations with 1% SDS (12 h), 1% Triton X-100 (12 h), and 2% deoxycholate (12 h) [1]. We then investigated bovine MEV effects on porcine artificial gut stromal compartment by exposing intestinal biological scaffolds repopulated with intestinal stromal cells to different MEV concentrations ($10*^{6}$,$10*^{8}$,$10*^{10}$ particles/mL). The experimental groups were:

a) Intestinal biological scaffold pre-treated with MEVs + untreated intestinal stromal cells;
b) Untreated intestinal biological scaffold + intestinal stromal cells exposed to MEVs;
c) Untreated intestinal biological scaffold + intestinal stromal cells without MEVs (CTR FBS);
d) Untreated intestinal biological scaffold + intestinal stromal cells without MEVs and FBS (CTR w/o FBS).

«Group a»

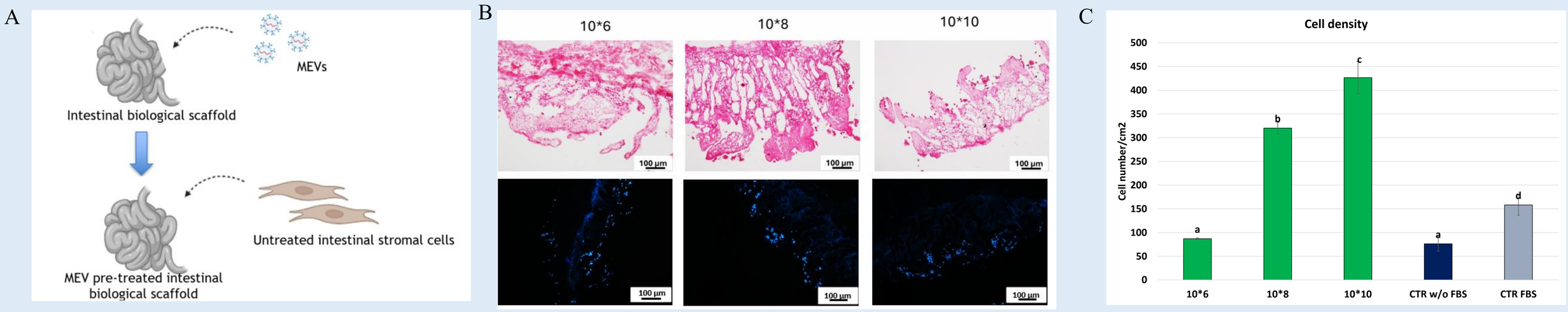


Figure 1. A) Schematic representation of the experimental design. B) Representative images of hematoxylin and eosin (upper panel) and DAPI stainings (lower panel) (scale bar 100 µm). C) Cell density values.

«Group b»

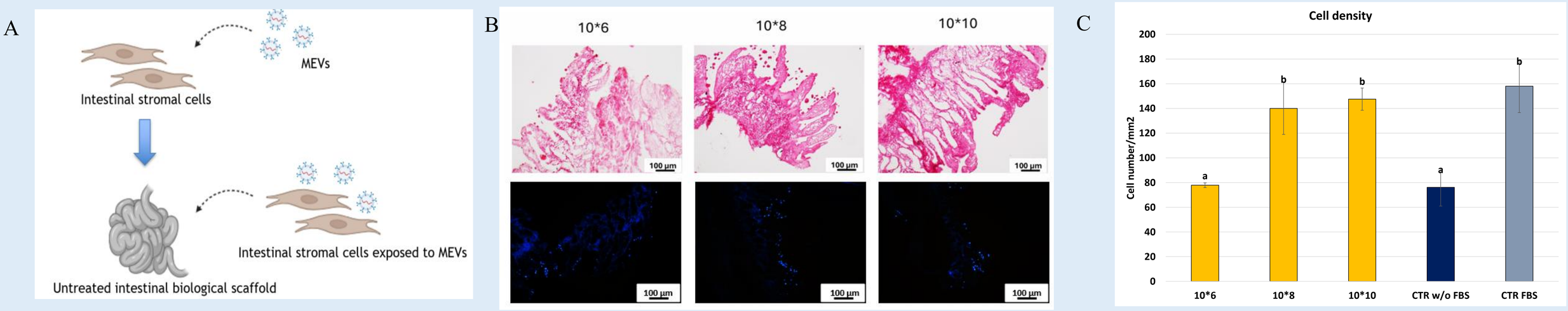


Figure 2. A) Schematic representation of the experimental design. B) Hematoxylin and eosin (upper panel) and DAPI stainings (lower panel) (scale bar 100 µm). C) Cell density values.

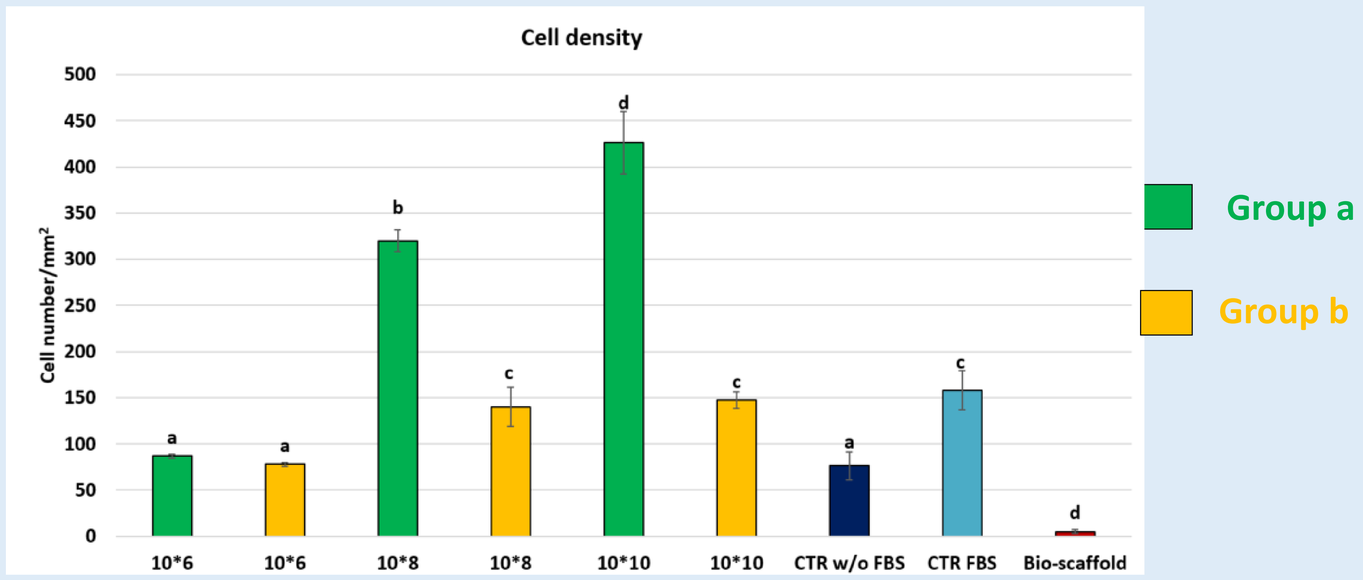


Figure 3. Cell density of all experimental groups. [a,b,c,d] Different superscripts indicate $p<0.005$

RESULTS

MEVs induced a dose-dependent increase in stromal fibroblast proliferation. Treatment with 10^{6} particles/mL did not significantly alter proliferation compared to CTR w/o FBS. In contrast, 10^{8} and 10^{10} particles/mL applied directly to stromal cells restored proliferation to levels comparable with CTR FBS. Notably, pre-conditioning the ECM scaffolds with 10^{8} or 10^{10} particles/mL MEVs further enhanced proliferation, yielding significantly higher values than CTR FBS.

CONCLUSIONS

Altogether the results suggest that MEVs encourage proliferation of intestinal fibroblasts in an in vitro three-dimensional (3D) intestinal stromal compartment and support their possible use as in vitro enriching supplements, to reduce/replace FBS.

REFERENCE

1. Arcuri S. et al., 2024, "Use of decellularized bio-scaffolds for the generation of a porcine artificial intestine". DOI: 10.3390/mps7050076

AKNOWLEDGMENTS: This research was supported by HORIZON-WIDERA-2021 project n#101079349 (OH-Boost).

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN

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