

## BACKGROUND

**Amniotic epithelial cells (AEC)** are **embryonic-like cells** with multipotent differentiation potential and immunomodulation properties. For this reason, AECs are a promising source of cells in preventing xenogeneic acute graft-versus-host (aGVHD) disease in transplantation. Inter-individual variability and different isolation protocols cause **differences in isolation yield, plastic adhesion and proliferation ability**, compromising reproducibility among centres and further applications. **Definition of isolation success** is the ability of cells to adhere to plastic surfaces which **takes one week**. High standards of reproducibility/reliability must be established.

## METHODS

### State of Art Analysis

Selector<sup>®</sup> is a new analytical instrument that exploits the **NEGA-DF technology**, as a tool for **quality control on stem cells isolation**. Selector<sup>®</sup> **separates cells in a label-free mode**, based only on their **intrinsic physical characteristics**.

### Method

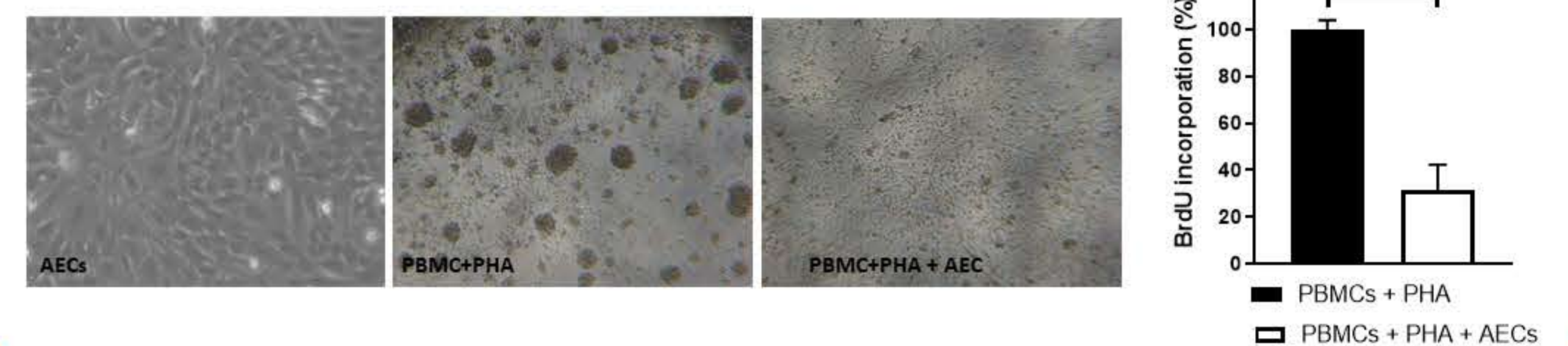
Amniotic membranes were processed by using **two concentration of trypsin (0.1 and 0.25%)**. Freshly isolated cells analysed by Selector<sup>®</sup>

**Geometrical analysis, Sorting & collection for biological evaluation:** adhesion, proliferation, immunomodulation ability

## RESULTS

AECs were cultured with PHA-activated PBMC to test their immunomodulation ability:

- AECs attenuate immune response *in vitro*
- PBMC proliferation statistically drop to 30%



### R1: QC OF ISOLATION PROTOCOL: successful vs unsuccessful

#### SUCCESSFUL isolation protocol of AECs

- **Bimodal** curve composed of two distinct peaks;
- Sorting of two sub-populations, F1 and F2
  - Sorted F1 cells do not adhere to plastic surface
  - Sorted **F2 cells** were the only one **alive, adherent** and **proliferative**.

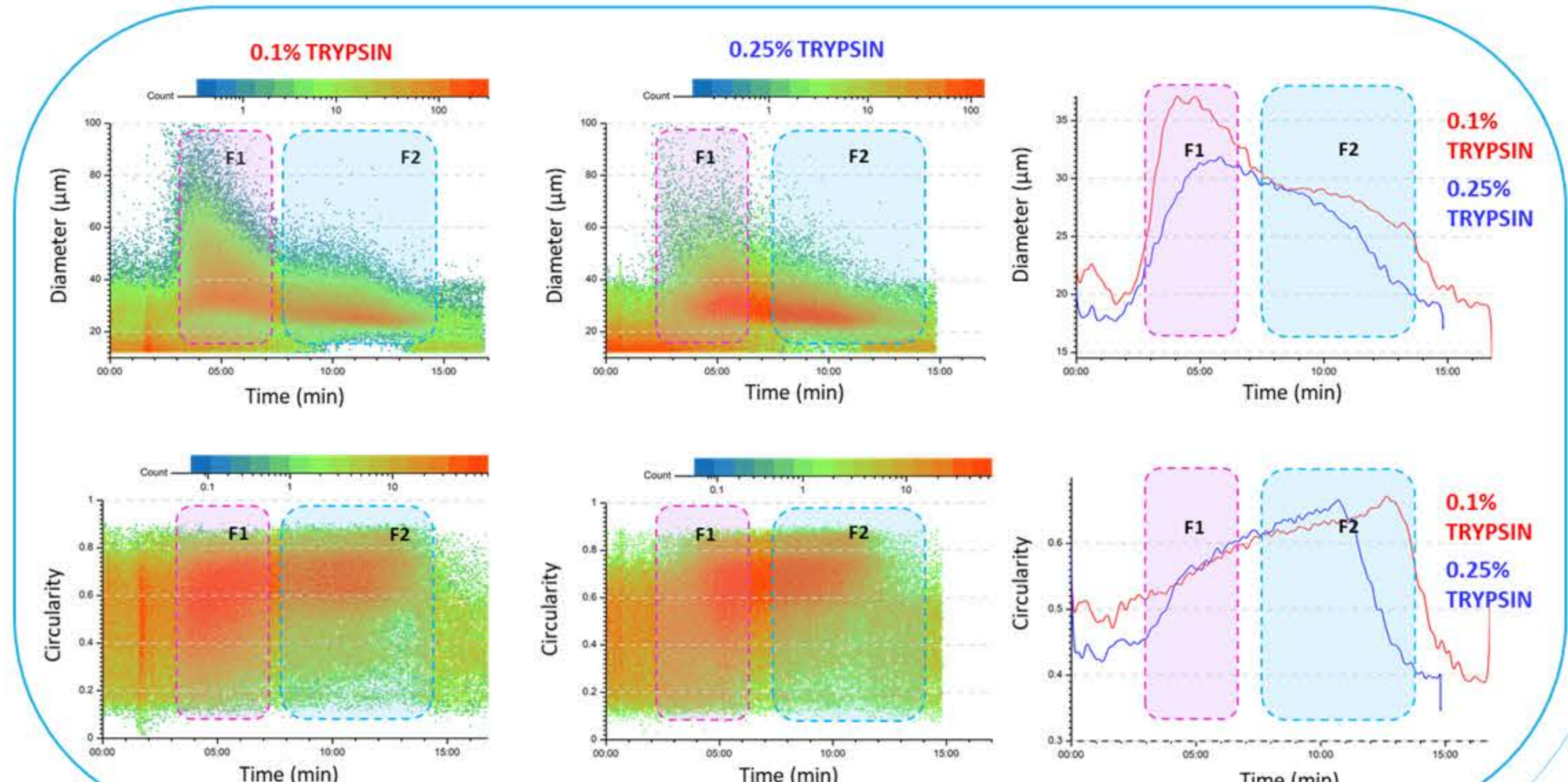
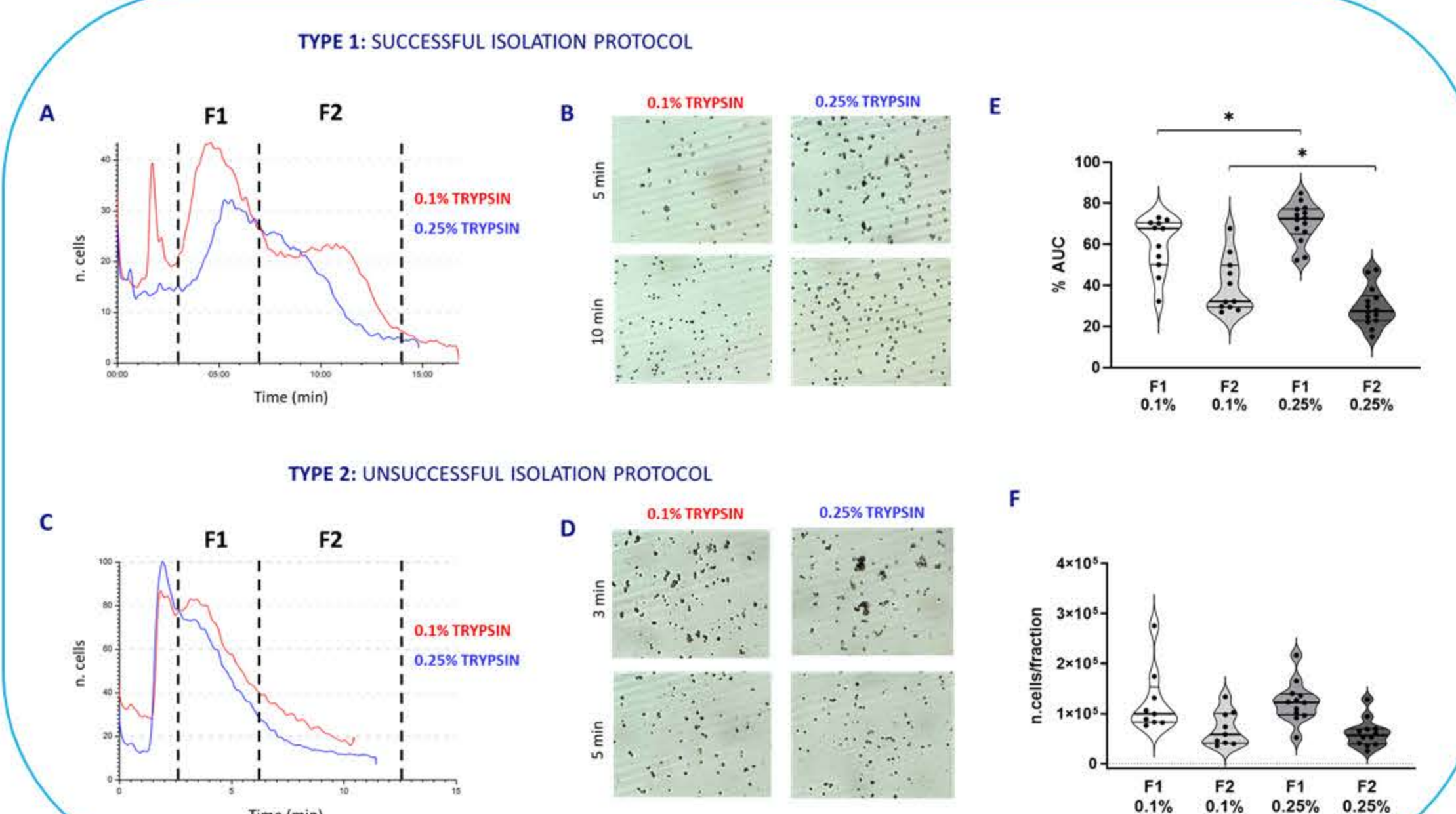
#### UNSUCCESSFUL isolation protocol of AECs

- **Monomodal** curve composed of one main peak
- Sorting of two sub-populations, F1 and F2
  - Sorted F1 cells do not adhere to plastic surface
  - Sorted F2 cells were very **few** and **poor adherent features** and very **low proliferate ability**

**AREA UNDER THE CURVE (% AUC)** predicts cell distribution between subpopulations → Predictive data on **quality and quantity of best stem cells** to sort

### R2: EVALUATION OF PRE-SORTING PROCEDURES: time dependent cell morphological parameter of sorted cells

- 0.25% AECs are homogenous in their distribution between F1 and F2, with F2 cells less abundant but vital, adherent and proliferative.
- 0.1% AECs were differently distributed between two fractions with bigger cells in F1.



## CONCLUSIONS

- **Fast & easy** definition of successful protocol (20 min vs 1 week), avoiding waste of time, material and labor
- **Time dependent morphometric analysis** for new cell population characterization
- Analysis of subpopulations and ability to **sort most vital** cell component
- **Predictive outcome** as a tool for laboratories and cell banks that isolate and cryopreserve fetal annex stem cells for research and future clinical applications, in particular as immunomodulatory mediators in transplantation.