OPTIMIZATION OF UMBILICAL CORD MESENCHYMAL STROMAL CELL-DERIVED EVS PRODUCTION CONDITIONS USING EVS-FREE FETAL BOVINE SERUM AND VEGETAL PEPTONE

C.LEZIN[1], B.LORENZINI[1], S.SOAVE[1], S.GOULINET[1], H.LOGTARI[1], AC.PONSEN[1], G.UZAN[1], P.MAUDUIT[1]

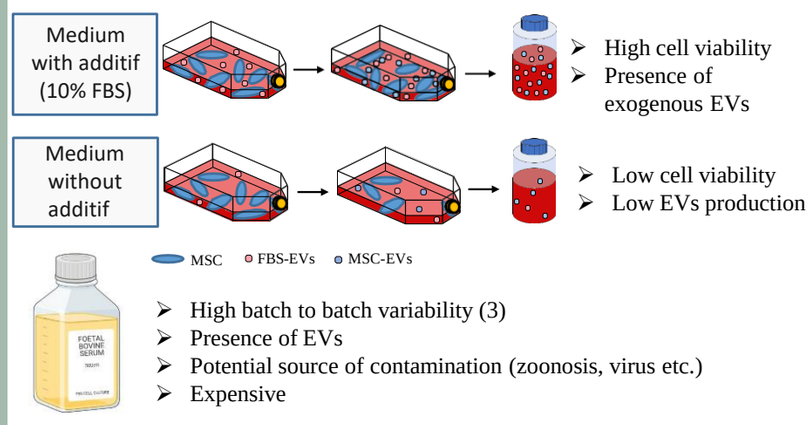
[1]Inserm U1197, Interactions between stem cells and their niches in physiology, tumors & tissue repair, Paris-Saclay University, France

Abstract

Mesenchymal stromal cells (MSCs) has been identified in the 60s and since then, they stole the spotlight, especially in the field of cellular therapy. MSCs are able to communicate with their microenvironment through both juxtracrine and paracrine pathways. Numerous studies show that beneficial effects may mainly be due to the secretome and mostly the extracellular vesicles (EVs) cells derived(1). This opens the door to a new approach which can be named: acellular therapy. MSCs-derived EVs are classically obtained either in a chemically defined media or in a starved condition. **Hence, we focus on the determination of better EVs production conditions compatible with therapeutic applications**. **Our approach is to work both on the media composition and on the culture platform.** We first produce EVs-depleted fetal bovine serum (FBS) through tangential flow filtration(2). We combine this EVs-free FBS with the use of **wheat peptone as coating** while cultivating the MSCs. Our in-house method led us to recover an increased EVs amount compared to more classical methods. Besides, EVs produced with our method exhibit better efficiency on two functional assays: a migration assay and a wound healing assay. Our technique may be useful for upscaling EVs production process which is a mandatory step for therapeutic EVs production.

Key word :MSC / peptone/ TFF/ EV production/ coating

Problematic



Objectives

Optimize the production of EVs by MSC using peptone which has the capacity to :

- To attach the cells to the surface and stimulate cell growth and biomolecules synthesis.
- To reduce the utilization of FBS (4)
- To be a less expensive alternative to FBS

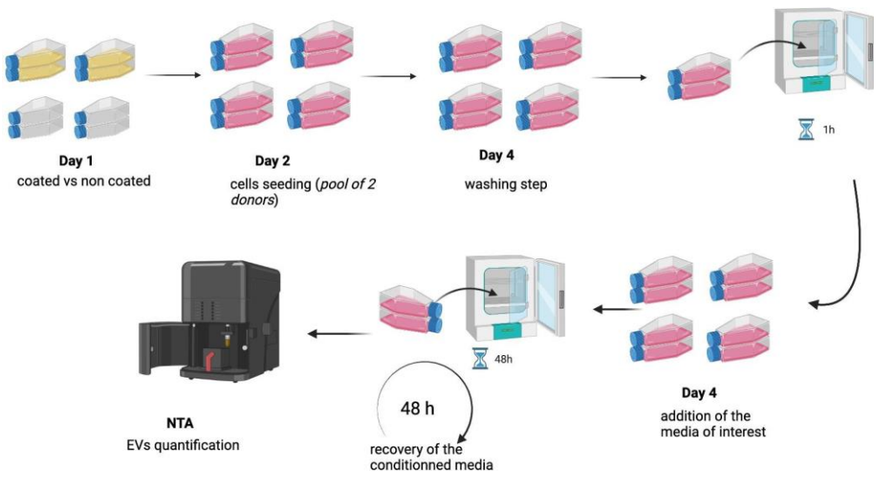
Comparison between the EVs produced in the presence/absence of peptone.

Experiments

A/EVs Production:

Medium used for the production of EVs:

- **Medium 1: MEM No-Coating**
- **Medium 2: MEM coating with wheat peptone (C-CELL W106)**
- **Medium 3: MEM + FBS 5% EVs free + No-Coating**
- **Medium 4: MEM + FBS 5% EVs free + Coating with C-CELL W106**



B/MSCs Characterization:

FACS: Using the markers:

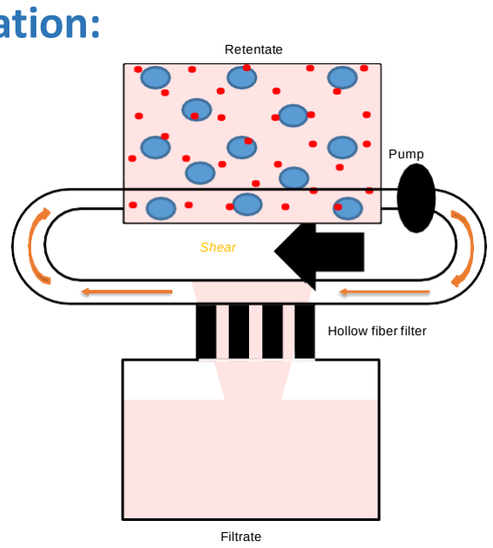
	CD31 FITC	CT34 PE	CD45 FITC	CD73 APC	CD90 FITC	CD105 PE	CD144 PE	CD309 PE
Control	-	-	-	+	+	+	-	-

C/EVs Concentration/Purification:

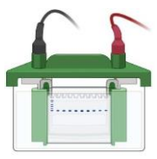
Using Tangential Flow Filtration (TFF)

- EVs
- Other molecules

The medium passes through the filter. EVs will be concentrated in the retentate while the other (smaller) molecules will be in the filtrate. Then our EVs will go under diafiltration to complete our concentration/purification process.



D/EVs Characterization:

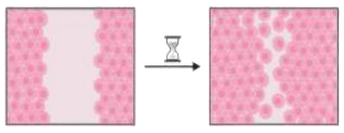


- Transmembrane proteins associated to EVs: CD9 – CD63 – CD81
- Cytosolic protein recovered in EVs: ALIX
- Endoplasmic reticulum protein : Calnexin

E/EVs Functionalities:

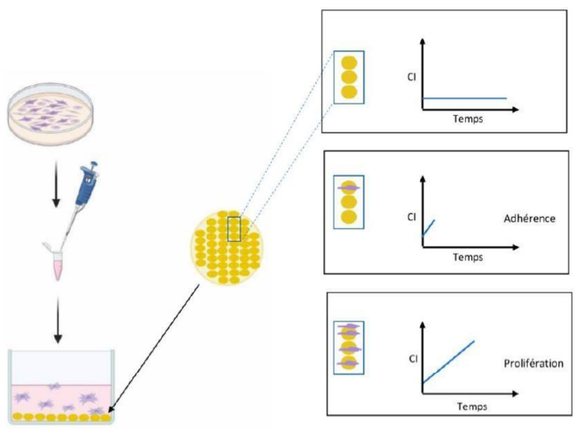
1/Wound healing (HaCaT: keratinocytes)

These assays involve creating a scratch or a gap in a cell monolayer and monitoring the movement and proliferation of cells to close the wound over time.



2/Migration assay (ECFC)

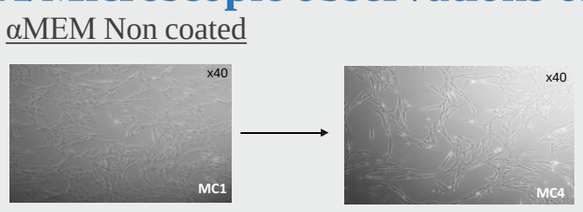
Migration assay using xCELLigence is a method for **studying cell migration in real-time**. xCELLigence measures changes in electrical impedance across microelectrodes integrated in the culture plates. These impedance measurements provide continuous and quantitative information about cell migration.



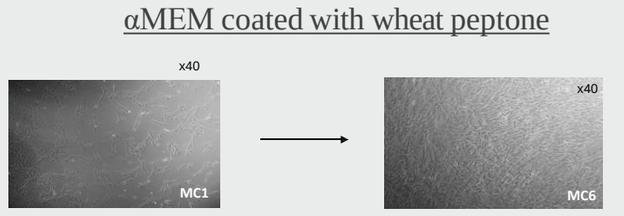
Results

A/Microscopic observations of MSCs:

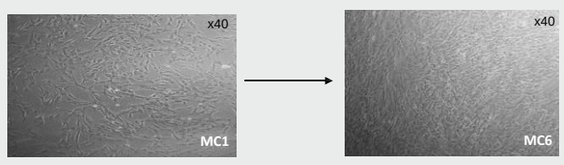
αMEM Non coated



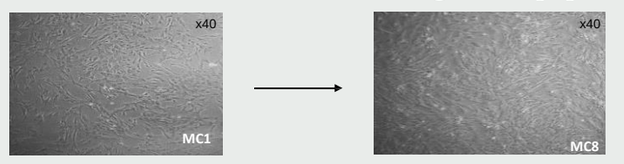
αMEM coated with wheat peptone



αMEM FBS EV free 5% Non coated



αMEM FBS EV free 5% + Coating wheat peptone



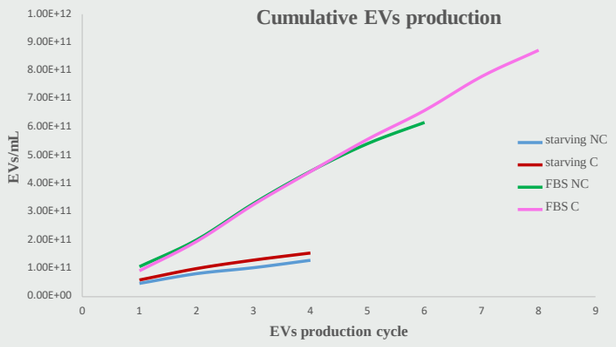
B/MSCs Characterization:

FACS results

	CD31 FITC	CD34 PE	CD45 FITC	CD73 APC	CD90 FITC	CD105 PE	CD144 PE	CD309 PE
Control	-	-	-	+	+	+	-	-
αMEM NC	-	-	-	+	+	+	-	-
αMEM C	-	-	-	+	+	+	-	-
FBS – NC	-	-	-	+	+	+	-	-
FBS - C	-	-	-	+	+	+	-	-

C/EVs Quantification:

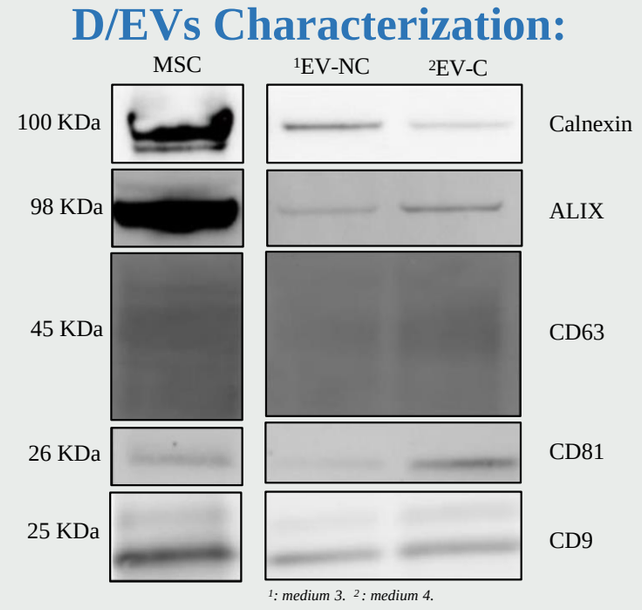
Using NTA technic (NS300)



C/ EVs Concentration/Purification:

	Before TFF (EVs/mL)	After TFF (EVs/mL)
αMEM No Coating (MC1 4)	9,00E+08	1,20E+10
αMEM With Coating (MC1 4)	1,10E+09	1,20E+10
αMEM+FBS 5%+No Coating (MC1 4)	4,00E+09	4,00E+10
αMEM+FBS 5%+Coating (MC1 4)	4,20E+09	5,20E+10
αMEM+FBS 5%+No Coating (MC5-6)	5,00E+09	2,70E+10
αMEM+FBS 5%+Coating (MC5-8)	4,40E+09	4,00E+10

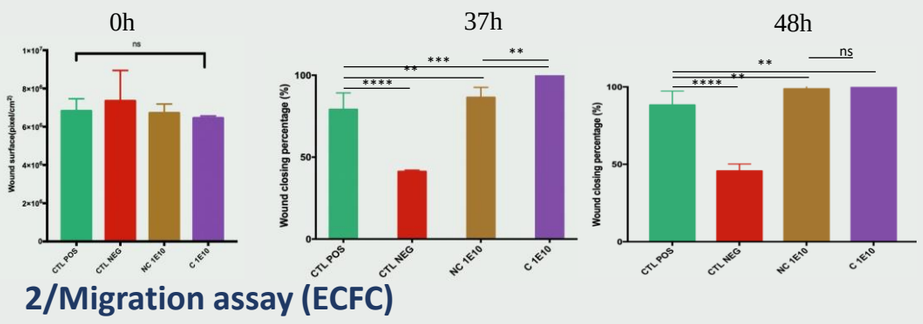
D/EVs Characterization:



[1]: medium 3. [2]: medium 4.

D/EVs Functionalities

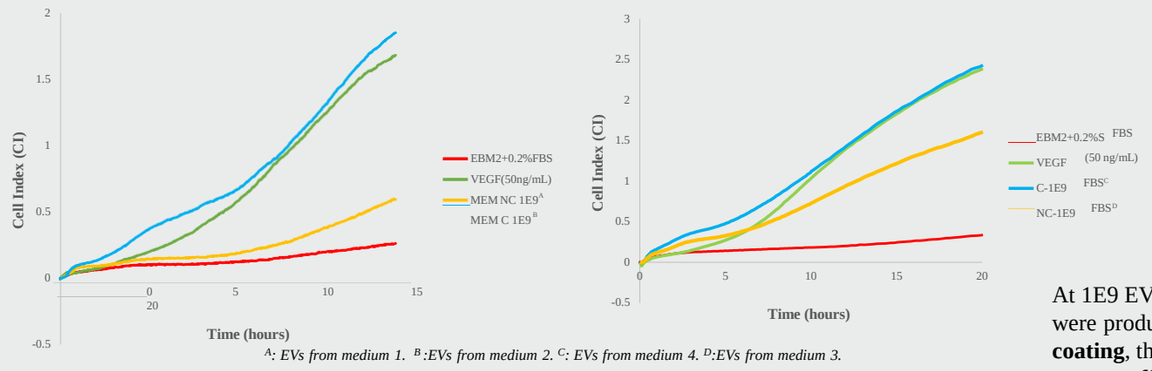
1/Wound Healing assay (HaCaT)



Wound closing percentage			
	0h	37h	48h
[1]CTL POS	0%	79.1% ± 7.1%	88.3% ± 6.4%
[2]CTL NEG	0%	41.2% ± 0.6%	45.6% ± 3.2%
[3]NC 1E10	0%	86,4% ± 5.7%	98.7% ± 1.6%
[4]C 1E10	0%	100%	100%

[1]: 5% FBS(-). [2]:0.2% FBS(-). [3]: EVs from medium 3. [4]:EVs from medium 4.

2/Migration assay (ECFC)



[A]: EVs from medium 1. [B]:EVs from medium 2. [C]: EVs from medium 4. [D]:EVs from medium 3.

At 1E9 EVs/mL, every time they were produced in presence of our **coating**, the EVs displayed a **greater effect** on ECFC migration.

Discussion

- The presence of FBS in the medium (*a*MEM) helps the cells to produce more EVs.
- The **coating with wheat peptone** stimulates the cells to produce more EVs and to stand a **longer period** on the flask.
- The characteristics of MSC and EV have not changed with wheat peptone (C-CELL W106) coating, moreover it improves the functionalities that has been showed on our two models (*i.e.*, migration & wound healing)

References

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