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Acellular regenerative medicine

EXTRACELLULAR VESICLES: AREAS OF INTEREST

What area are you working in?

- Basic research 31.9%
- Therapy manufacturing 25.5%
- Disease modeling 14.9%
- Drug discovery or testing 14.9%
- Other 12.8%

Are you currently researching, developing or manufacturing extracellular vesicles (EVs)?

Yes	No, but I plan to	No, and I don't plan to
59.3%	33.3%	7.4%

If yes, at what stage(s) are your current EV research projects?

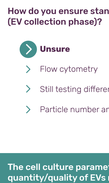
- Discovery/basic research studies 45.0%
- Preclinical 27.5%
- Early-phase clinical trial 10.0%
- Marketed product 7.5%
- Other 7.5%
- Late-phase clinical trial 2.5%

KEY APPROACHES AND TECHNIQUES

The top five cell types used as an EV source included:

1. Mesenchymal stem cells
2. Induced pluripotent stem cells
3. Fibroadipocytes
4. Cord tissue (Wharton's jelly)
5. Skin fibroblasts

Cell culture systems used to perform the upstream part of the workflow included:



- Adherent cell culture in flasks 70.4%
- Suspension cell culture in shaker flasks 7.4%
- Suspension cell culture in bioreactor 3.7%
- Adherent cell culture on microcarriers in bioreactor 3.7%
- Other 14.8%

The most common techniques used to characterize EVs included:

- Flow cytometry 27.1%
- Electron microscopy 22.0%
- Other (e.g., 'not yet done') 16.9%
- ELISA/western blotting 13.5%
- RNA profiling 11.9%
- Potency assay 6.8%
- Mass spectrometry 1.8%

What EV production range is most interest (quantity of EV/batch)?

To be determined

- The highest possible
- 10^9 particles/ml
- 1 x 10^{12}/µl
- Liters or billions/ml of EV
- Not settled on EV number but production from ~10 L of conditioned media

QUALITY CONTROL

How do you ensure standardization of your upstream process (EV collection phase)?

Unsure

- Flow cytometry
- Still testing different methods
- Particle number and content
- Characterization of EVs for size and known surface markers (e.g., CD63, CD81, TSG101, etc.)
- Standardization of the cell culturing including several quality parameters (e.g., for cells: viability, cell phenotype, sterility; for the environment: CO_2 levels, temperature, pH)

The cell culture parameters that seemed to impact the quantity/quality of EVs most included:

- Cell culture medium composition 40.7%
- Age of the cells 29.6%
- Other (e.g., 'not sure yet') 22.3%
- Cell culture system (static vs dynamic) 3.7%
- Oxygen tension 3.7%

WHAT CHALLENGES ARISE FROM WORKING WITH EVS?

The main challenges faced when producing or using EVs included:

- Lack of standardization 25.0%
- Isolation and purification 17.2%
- Regulatory limitations (no specific regulation of EV-based therapeutics) 15.6%
- Reproducibility 14.0%
- Long processing times 12.5%
- Lack of knowledge regarding EV biological function 12.5%
- Storage temperatures 1.6%
- Administration route 1.6%

Why haven't we seen many acellular therapies come to market thus far?

- Lack of standardized techniques for characterization of EVs
- Fixation on iPS cell therapeutics
- Lack of reproducibility (e.g., batch-to-batch variability)
- Insufficiently developed field of research
- Manufacturing scale-up challenges
- Unclear safety and efficacy

THE FUTURE OF ACELLULAR REGENERATIVE MEDICINE

In the next 5 years, the use of extracellular vesicles will...

- Increase 92.6%
- Decrease 3.7%
- Stay the same 3.7%

Will acellular therapies become a viable treatment option for patients?

- Yes 77.8%
- No 7.4%
- Unsure 14.8%

DEMOGRAPHIC OF RESPONDENTS

Location

Scientific area:

Academic	50.0%
Hospital | Clinic	19.2%
Pharma | Biotech	11.5%
Government | NGO	7.8%
CRO | CDMO	3.9%
Vendor | Manufacturer	3.8%
Other	3.8%

Job title

Researcher | Scientist	61.5%
Clinician | Surgeon	15.4%
Managing Director | Senior management	11.5%
Student	7.7%
Quality assurance	3.9%

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This infographic was created as part of the RegMedNet Spotlight on acellular regenerative medicine in association with Eppendorf.