Extracellular vesicles in cardiovascular diseases, are they Jedi or Sith?

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Table 2

Technique	Examples and types	Advantages	Disadvantages	Suggested choice for	References
Differential centrifugation	- sequential centrifugation with or without gravity size filtration	- most widely used - separates EV size-based subpopulations - processes large sample volume (20~200ml)	- >3hrs - requires ultracentrifuge	- large sample volume (e.g. conditioned cell media) - no prior knowledge of EV markers - large scale EV isolation	(Théry *et al.*, 2001a)
Density gradient separation	- sucrose density gradient - iodixanol (Optiprep™) density gradient - PureExo exosome isolation kit™	- high EV purity	- >10hrs - requires ultracentrifuge - low yields - small sample volumes (0.5-3ml) - doesn't necessarily fractionate EV subpopulations as they share density	- purification of EV isolates - small sample volume	(Raposo *et al.*, 1996; Théry *et al.*, 2001a; Théry *et al.*, 2001b; Marzesco *et al.*, 2005; Van Deun *et al.*, 2014; Zonneveld *et al.*, 2014; Keerthikumar *et al.*, 2015)
Precipitation	- polyethylene glycol - acetate - ammonium sulphate - Total Exosome Isolation™ - Exoquick™ - Exo-spin™	- commercial kits available - fast - no specialized equipment - simple operation	- low purity (co-precipitation of non-vesicular material) - possible interference of precipitation reagents with downstream applications	- small volume (e.g. limited biological samples) - fast processing time	(Lee *et al.*, 2012; Musante *et al.*, 2012; Brownlee *et al.*, 2014)
Microfluidic Devices	- sieving - trapping - immunological separation - nanoshearing	- simple operation - single-step isolation - on-chip EV characterization - easier standardization and higher reproducibility	- currently not readily available - small sample volume (<500ul) - to date has only been applied to EXOs and MVs	- small volume (e.g. limited biological samples)	(Chen *et al.*, 2010; Davies *et al.*, 2012; Wang *et al.*, 2013; He *et al.*, 2014; Kanwar *et al.*, 2014; Vaidyanathan *et al.*, 2014)
Affinity Capture	- antibody coated magnetic beads - antibody coated latex beads - heparin affinity	- relatively high EV purity - some commercial kits available - simple operation	- biased by the choice of affinity reagent (only EVs with the corresponding ligand will be purified) - 0.5-3ml sample volume - >2hrs - difficulty in recovering EVs after capture for other applications	- small volume (e.g. limited biological samples) with known EV markers	(Wubbolts *et al.*, 2003; Caby, 2005; Kim *et al.*, 2012; Balaj *et al.*, 2015)
Size-Exclusion Chromatography	- Sepharose gel - Sephadex gel - Bio-gel A™ - Izon qEV™ column	- ~15mins - inexpensive - no specialized equipment required - simple operation	- 0.5-1.5ml sample volume - doesn't fractionate EV subpopulations as they are all excluded from the gels - further dilutes EVs from the sample	- purification of EV isolates - small volume (e.g. limited biological samples) - no need for prior assumption of EV markers - fast processing time	(Ogawa *et al.*, 2008; Sokolova *et al.*, 2011; Böing *et al.*, 2014; Hong *et al.*, 2014)