Cell secretome based drug substances in regenerative medicine: when regulatory affairs meet basic science

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"Regenerative medicine" or "tissue regeneration" had become to an inspired term recently since it has been realized in the last decade that many organs such as the skin display morphological plasticity and possess healing capacity instead of being a post-mitotic terminally developed organ (1,2).

In the light of these findings researchers as well as clinicians have turned their therapy aims from a passive reduction of tissue damage towards an active improvement of tissue regeneration and function. Whereas for almost two decades cell based therapies were thought to be the philosophers' stone to enhance tissue regeneration this concept began to totter after the work of Gnecchi *et al.* in 2005 (3). His group was one of the first showing that paracrine factors released from mesenchymal stem cells are able to promote tissue regeneration. In the following years subsequent work from all over the world supported the hypothesis that rather the paracrine factors than the cells were indeed responsible for the beneficial effects (4-8).

The cell-secretome consists of all factors actively or passively released from cells and contains among others soluble proteins (e.g., cytokines, chemokines and growth factors), lipids, free nucleic acids and extracellular vesicles (EV). The latter can be further subdivided mainly based on their size, density, surface markers and origin into apoptotic bodies, microparticles and exosomes. Especially exosomes have recently come into focus of research based on their high capacity to interact with target cells and their ability to selectively modify cell signalling (9).

One of the most comprehensive article summarizing the role of EV in the field of tissue regeneration and regenerative medicine was recently published by Silva and co-workers (10). The authors explained the principles of EV formation and built a bridge between preclinical work and putative application of EV in the clinics. The authors summarized the major effect of EV on immune cell activity modulation as well as their putative role in extracellular matrix remodelling, both of which linked to tissue repair. Finally, the authors provide an excellent overview of the current literature of EV as therapeutic drug-delivery vehicles. This is of special interest for both clinically orientated scientists as well as scientifically interested clinicians for the following reasons: (I) EV can be seen as a natural "off-the-cell" products released from almost all cell types *in vitro* and *in vivo* (11); (II) modified EV loaded with drugs such as chemotherapeutics, growth factors or tumour antigens might serve as targeted therapies or immune modulatory therapies in several clinical indication. When applying autologous EV the risk of pathogen transmission and immunological intolerances can be strongly reduced. In our opinion this is of special significance in case of any clinical testing of exosomes in phase I or II studies. Especially if allogeneic products are used the authorities require two different virus clearance steps, such as high dose ionizing radiation or methylene blue inactivation,

before approving any clinical testing (7). Currently EV-based therapeutics are classified as biologicals and their active substance determined their pharmaceutical classification (12). It remains to be elucidated whether this mandatory pathogen reduction steps during purification and storage or EV might affect morphology, vesicular integrity, interaction with target cells and finally the biological activity.

A further issue that has to be considered before planning any clinical application is that the production of significant amounts of EV is technically extremely challenging, time consuming and therefore expensive. Commercially available EV purification kits are not suitable to produce enough EV for a routine clinical application. On the other hand ultracentrifugation methods have the drawback that a production according to good manufacturing practice (GMP) guidelines (e.g., viral free environment) would be difficult to establish and costly. Centrifugation steps require several manual processing steps (e.g., removing the supernatant during repetitive centrifugation steps), which have to be conducted in a GMP facility.

Beside the technical production hurdles, probably one of the most important aspects one has to consider is the mode of action (MoA) or mechanism of action (MoA) of EV in the field of regenerative medicine. In principle the MoA describes the interaction of a drug with the organism/cell and its biological or molecular effects. As summarized by Silva *et al.* and others EV display multiple biological effects depending on the one hand on their donor cells and how these cells are stressed an on the other hand on the target cells, their mode of application (i.v., i.p., s.c., topical) and duration of application (13). Although individual groups have highlighted the role of selected EV molecules for their regenerative potential (14,15) it still remains a miracle whether a single component alone or the interaction of different factors present in EV are responsible for their biological effectiveness (12). EV contain distinct classes of molecules such as functional mRNAs, small RNAs, such as microRNAs (miRNAs), lipids and proteins, which alone or in combination are able to promote tissue regeneration. miRNAs and mRNAs can be transferred from donor to recipients cells, thus regulating signalling pathways (16). In addition, selected lipid classes, such as ceramides, have been shown to modulate cell death pathways and induce cell death in an oligodendroglioma cell line (17). The regenerative capacity of EV is furthermore attributed to the cell type from which they are released. It remains unclear which donor cell source provides the most potent EV subtypes in the field of regenerative medicine. Beside stem cell derived EV, peripheral blood mononuclear cell (PBMC) derived or endothelial derived cell types can promote tissue regeneration and cytoprotection (7,18). The combination of pleiotropic EV components that are different between cell and cell type exacerbate a clear description of the EV's role in the field of regenerative medicine. As compared to the whole cell secretome, also EV contain a mixture of proteins, lipids, an regulatory RNAs which probably orchestrate their biological activity by the interaction with each other and with target cell molecules. In our opinion the identification of a single molecule, which is responsible for the distinct regenerative aspects is rather unlikely.

The same may be the case for secretome based therapies. While the beneficial effects of these therapies are increasingly investigated the exact bioactive components still remain unclear in large areas. It seems more reasonable for us that pleiotropic factors of EV or of secretome based therapies should be seen as a whole bioactive drug ready to use instead of trying to find one singe responsible molecule.

Therefore, further research should increasingly address questions concerning the development of a production workflow according to the GMP guidelines and stability and potency aspects of proven biological active EV to move forward to a clinical application, as the authorities will demand these data before approving any clinical testing.

Outlook

Taken together, the number of peer-reviewed articles focusing on the use of EV in regenerative medicine has increased exponentially in the last years. EV contain diverse factors with pleiotropic different biological activities, in part supporting tissue regeneration. Depending on the cell type, experimental setup, and purification method EV consist of different components with different biological effects. However, instead of focusing on the identification of "the" single active factor we—as clinically oriented researchers—should increasingly face regulatory demands and consider or incorporate them into our experimental design. The establishment of production protocols according to the GMP guidelines including product characterization, preclinical proof of efficacy, production characterization, stability assays and the identification of the MoA will facilitate the entry of EV into the clinics. In our opinion a close cooperation between the authorities and researchers might be the key to success.

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Footnote

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References

1. Beltrami AP, Urbanek K, Kajstura J, et al. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 2001;344:1750-7.
2. Bergmann O, Zdunek S, Felker A, et al. Dynamics of Cell Generation and Turnover in the Human Heart. Cell 2015;161:1566-75.
3. Gnecchi M, He H, Liang OD, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367-8.
4. Ankersmit HJ, Hoetzenecker K, Dietl W, et al. Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium. Eur J Clin Invest 2009;39:445-56.
5. Lichtenauer M, Mildner M, Baumgartner A, et al. Intravenous and intramyocardial injection of apoptotic white blood cell suspensions prevents ventricular remodelling by increasing elastin expression in cardiac scar tissue after myocardial infarction. Basic Res Cardiol 2011;106:645-55.
6. Lichtenauer M, Mildner M, Hoetzenecker K, et al. Secretome of apoptotic peripheral blood cells (APOSEC) confers cytoprotection to cardiomyocytes and inhibits tissue remodelling after acute myocardial infarction: a preclinical study. Basic Res Cardiol 2011;106:1283-97.
7. Beer L, Mildner M, Gyöngyösi M, et al. Peripheral blood mononuclear cell secretome for tissue repair. Apoptosis 2016;21:1336-53.
8. Korf-Klingebiel M, Reboll MR, Klede S, et al. Myeloid-derived growth factor (C19orf10) mediates cardiac repair following myocardial infarction. Nat Med 2015;21:140-9.
9. Kourembanas S. Exosomes: vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. Annu Rev Physiol 2015;77:13-27.
10. Silva AM, Teixeira JH, Almeida MI, et al. Extracellular Vesicles: Immunomodulatory messengers in the context of tissue repair/regeneration. Eur J Pharm Sci 2017;98:86-95.
11. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014;30:255-89.
12. Lener T, Gimona M, Aigner L, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 2015;4:30087.
13. De Jong OG, Van Balkom BW, Schiffelers RM, et al. Extracellular vesicles: potential roles in regenerative medicine. Front Immunol 2014;5:608.
14. Beer L, Zimmermann M, Mitterbauer A, et al. Analysis of the Secretome of Apoptotic Peripheral Blood Mononuclear Cells: Impact of Released Proteins and Exosomes for Tissue Regeneration. Sci Rep 2015;5:16662.
15. Nakamura Y, Miyaki S, Ishitobi H, et al. Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. FEBS Lett 2015;589:1257-65.
16. Valadi H, Ekström K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654-9.
17. Podbielska M, Szulc ZM, Kurowska E, et al. Cytokine-induced release of ceramide-enriched exosomes as a mediator of cell death signaling in an oligodendroglioma cell line. J Lipid Res 2016;57:2028-39.
18. Osteikoetxea X, Németh A, Sódar BW, et al. Extracellular vesicles in cardiovascular disease: are they Jedi or Sith? J Physiol 2016;594:2881-94.

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