Doctoral School in Life and Health Sciences

PhD in Medical Physiopathology

Investigating the pharmacological properties of bioproducts from kidney stem/progenitor cells

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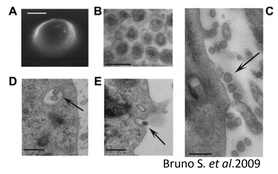
Introduction

Acute Renal Injury (AKI) is characterized by an abrupt decrease in kidney function. Mainly caused by an ischemic or nephrotoxic insult, AKI may lead to the development of total renal failure. Hence, the development of novel therapies ameliorating AKI appears to be of great importance. A big research is being carried out on the potential role of microvesicles (MVs,) as cell bioproducts, in kidney damage restore. MVs are small vesicles, released by cells carrying membrane and cytoplasmic constituents. It has been suggested that MVs derived from both embryonic and adult stem cells can be used as a shuttle of genetic information between cells [1-4]. Our group has demonstrated that MVs derived from adult human MSCs contribute to kidney repair in glycerol-and ischemia-reperfusion-induced AKI [5]. In addition, CD133+ cells in human renal tissue have been identified as potential kidney stem and progenitor cells (KSPCs) [6-8].

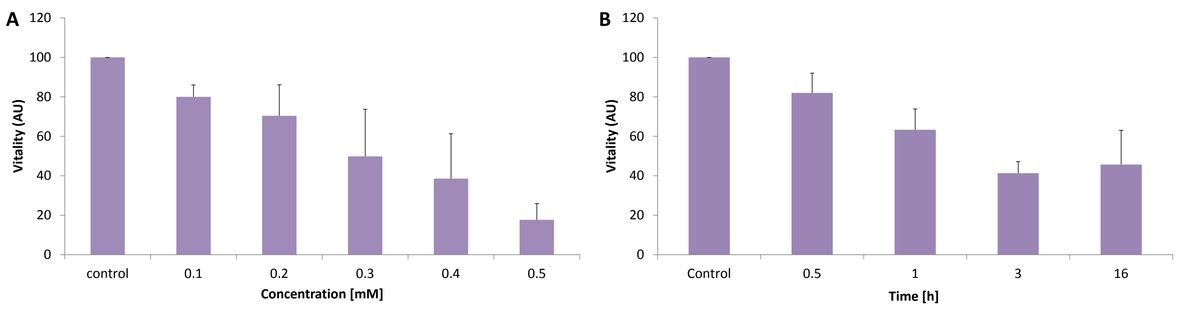


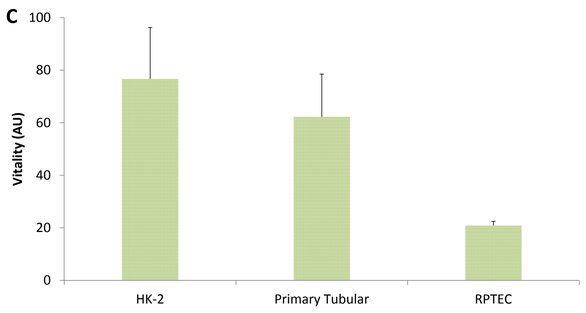
Bruno S. *et al.*2009

Aim

In this project, we aim to evaluate the potential role of MVs derived from CD133+ KSPCs and urine, as a therapeutic renoprotective "drug" for AKI damage restore in adult human podocytes and tubular cell lines. We plan to set up two in vitro AKI models. In order to mimic the hypoxia/re-oxygenation damage occurring during ischemia cells were exposed to Hydrogen Peroxide. In addition, to induce the nephrotoxic damage, cells were treated with cisplatin.

Set up of ischemic and nephrotoxic damage

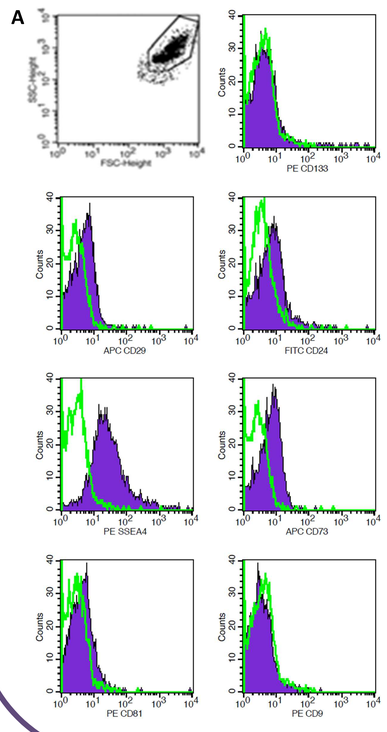


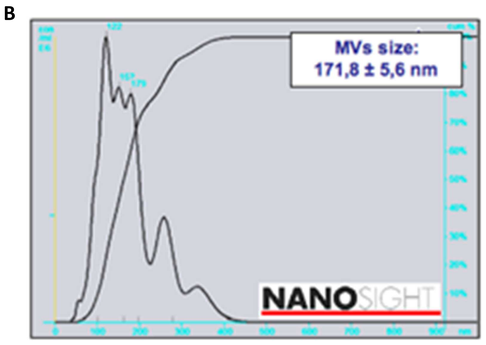


Primary tubular cells were exposed to different concentrations of Hydrogen Peroxide for 16 hours and cell vitality was evaluated by MTT (**A).** The cellular damage was found to be dose dependent. Concentrations over 0.3mM were considered highly toxic for the cells, therefore 0.25mM of Hydrogen Peroxide was chosen for the subsequent evaluation of timing (**B).** The toxicity of Hydrogen Peroxide was found to be time-dependent for the first three hours of incubation, when the damage was comparable to the one of 16 hours incubation. Cell exposure in 0.25mM of Hydrogen Peroxide for 3 hours was chosen as a reproducible damage for the subsequent experiments on primary tubular cells.

HK-2 (Human Kidney-2), RPTEC (Renal Proximal Tubule Epithelial Cells, from Lonza) and Human Primary Tubular epithelial cells isolated from biopsies, were exposed to cisplatin 5 μg/ml for 48 hours and the subsequent toxic effect was evaluated with MTT assay (**C).**

Characterization of MVs derived from CD133+ kidney progenitor cells

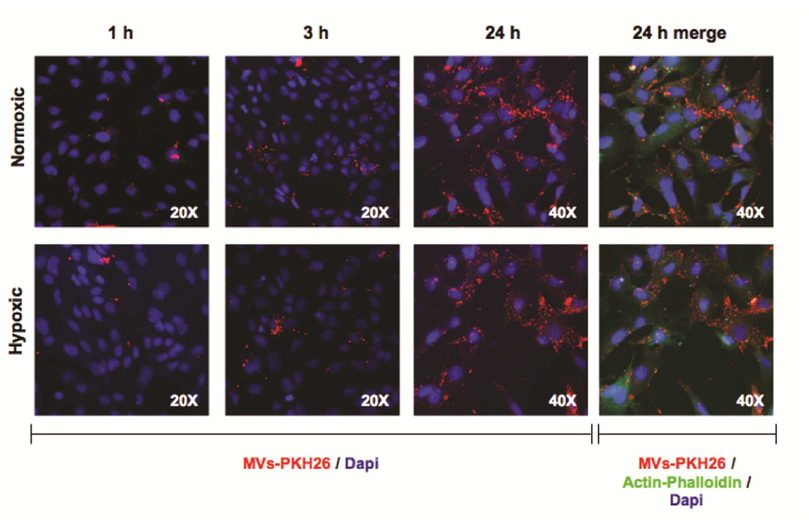




Cytofluorimetric characterization of CD133+ kidney progenitor-derived microvesicles (MVs). Representative FACS analyses of MVs showed high expression of the stem cell marker SSEA4. Among markers expressed by CD133+ cells, only CD29, CD73 and CD24 were expressed whereas no expression of CD133 was observed (**A**). In addition, the exosome marker CD81 but not CD9 was expressed at low level.

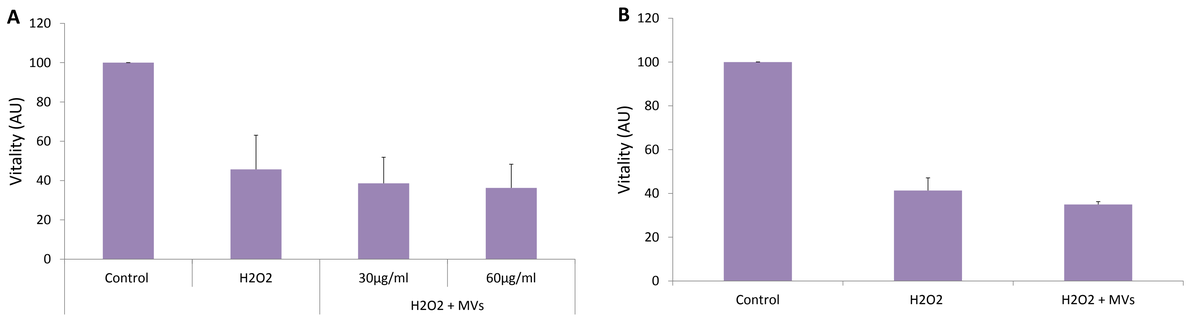
MV size analysis by direct measurement with Nanosight showed an average MV-diameter of 171nm (**B**)

Uptake of MVs by tubular epithelial cells

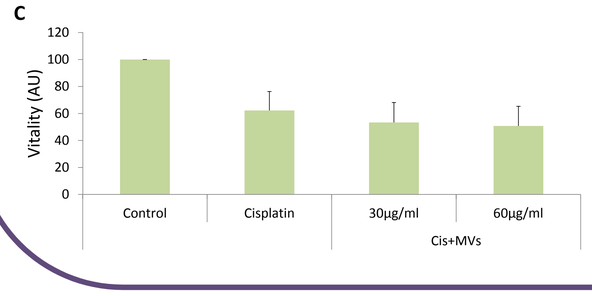


Incorporation assay was performed for the evaluation of CD133+ cell-derived MVs uptake from tubular epithelial cells. MVs were obtained both under normoxic and hypoxic [1% O2] conditions and labeled with PKH26 dye. Renal tubular epithelial cells were incubated for 1,3 and 24 hours respectively at 37 °C with PKH26 labeled MVs. DAPI dye was added for nuclear staining, while Phalloidin was used for the label of actin filaments. The incorporation of MVs was followed at different time points (1,3 and 24 h) after which cells were fixed in paraformaldehyde 3.5%. As shown by confocal microscopy images, the intenalization of labeled MVs in tubular cells started after 3 hours, while the peak of their uptake was observed after 24 hours of incubation. No difference in the uptake was observed between the normoxic and hypoxic MVs.

Effect of MVs on damaged Primary Tubular Cells



Primary Tubular cells, isolated from three different biopsies, were treated with Hydrogen Peroxide (0.25mM) and MVs derived from three different adult CD133+ progenitor cell lines for 16 hours. No damage recovery was observed in the presence of MVs in any of the chosen concentrations (30 and 60μg/ml) (**A)**. Since no effect of MVs was observed, we decided to pre-treat the cells 24 hours with MVs (60μg/ml) before exposing them to Hydrogen Peroxide (0.25mM) for 3 hours (**B)**. Even in this case MVs did not seem to repair the damage



Primary Tubular cells were chosen for the evaluation of MV effect on toxic damage caused by cisplatin. Cells treated with MVs and cisplatin (5μg/ml) for 48 hours did not exhibit any difference in vitality comparing to the ones exposed only to the drug only (**C)**.

References

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Conclusion

- CD133+ cells release MVs uptaken by renal tubular epithelial cells in normoxic and hypoxic conditions.
- Tubular epithelial cell damage is not affected by CD133+ cell derived MVs
- Further studies are needed to be done for the evaluation of MVs function