Effects of Magnesium on endothelial barrier functions

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Introduction:

Magnesium (Mg) based alloy is a very promising biodegradable material for cardiovascular stent application. Stent material will directly contact with blood and vascular tissue upon deposition. During the degradation process, stent will be degraded into metal ions and released to surrounding tissue, which could affect the metabolism and function of local tissues, especially endothelial cells. Since Mg is the major component of the Mg-based alloys, it is important to know how high concentration of Mg ion affects endothelial cell functions. Endothelial cells are the most inner layer of blood vessel which plays important roles in hemostasis, immune response, and molecular transportation. It has been shown by our previous study that a high amount of Mg ion can disrupt the endothelial cell-cell connection and stimulate stress fiber formation. In this study, our goal is to test how Mg changes the expression and structure of the endothelial junction molecules and its barrier functions.

Materials and Methods:

Human coronary aorta endothelial cell (HCAEC) was cultured in endothelial cell culture media (ECM). Cells were treated with ECM supplemented with different concentration of Mg^{2+} (0 mM, 5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM). Endothelial cell attachment will be measured by suspending cell pellet in ECM supplemented with Mg ion solution and the number of attached cells at different time period will be counted. Endothelial cells will be cultured by ECM supplemented with Mg ion for 2 weeks and long-term endothelial layer integrity will be imaged by a phase contrast microscope. Trans-well permeability assay will be used to study the endothelial barrier function. Cadherin-5, Platelet endothelial cell adhesion molecule 1(PECAM1), occluding, and Junction adhesion molecule -3(JAM3) will be stained by their corresponding antibody and imaged. Endothelial cells will be lysed and junction protein will be extracted and used for western blot. Total RNA of endothelial cell after treated with Mg ion will be extracted and used for q-PCR.

Results:

Mg ion affects both the short-term and long-term endothelial integrity on a dose-dependent manner. The junction proteins expressions are differentially altered after treatment with Mg ion as revealed by Western blot and q-PCR. Mg also introduced cytoskeletal reorganizations (Fig. 1). In addition, the permeability of the endothelial monolayer is changed as well with the presence of Mg ion. The underlying molecular mechanisms including MAPK and Roc signaling pathways are also explored.

Fig.1

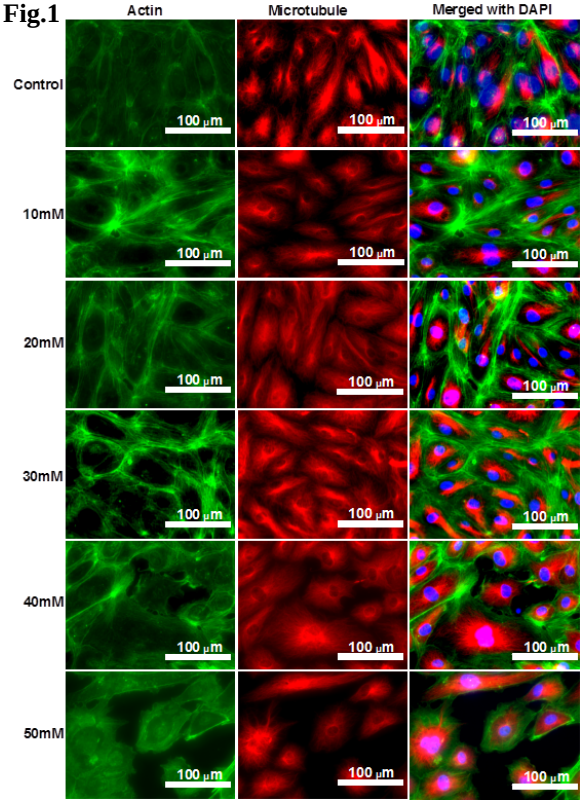

Fig.1 Fluorescent images of HCAECs after treated with different concentrations of $MgCl_2$ for 24 h. Cell nucleus (Blue) was stained by Slow-fade Gold anti-fade Reagent with DAPI. Microtubule (Red) was stained by mouse anti-β tubulin followed by Alexa Fluor 546 rabbit anti-mouse IgG. Microfilament (Green) was stained by Actin Green 488 Ready Probes Reagent.

Conclusion

Mg ion at low concentration may be beneficial to endothelial cell growth, migration and the integrity of barrier functions, but not at high concentrations. Information in this study provides useful guidance for future Mg-based implant design.