Isolation and Culture of Human Microvascular endothelium for comparison of the morphological and molecular characteristics of Microvascular endothelial cells under normal gravity against simulated micro gravity

Manjunath S\textsuperscript{1}, Chatterjee S\textsuperscript{2}, Majumder S\textsuperscript{2}, Srinivasan V\textsuperscript{1}, Murugan P\textsuperscript{1} Thamaraikannan P\textsuperscript{1}, Tholcopiyan L\textsuperscript{1}, Abraham S \textsuperscript{1,3}

\textsuperscript{1}Nichi-In Centre for regenerative Medicine, Chennai, India
\textsuperscript{2}Vascular Biology Lab, AU-KBC Research Centre, Anna University, Chennai, India
\textsuperscript{3}Yamanashi University - Faculty of Medicine, Chuo, Japan

Published online on 30 Oct 2010

BACKGROUND

Vascular endothelial cells play a major role in wound healing and also in growth of the tumors. Angiogenesis can be a target for treating diseases that are due to either poor vascularisation or decreased blood supply as in stroke, ulcers, heart disease, etc or abnormal and increased vasculature like in tumours. Application of specific compounds that may inhibit or induce the creation of new blood vessels in the body may help in the treatment of such diseases \cite{1}. \textit{Ex vivo} generation of blood vessels may offer an excellent alternative to the synthetic valves that are being currently used in cardiology. Micro gravity also referred to, as weightlessness is not essentially zero gravity but rather minimal gravity. According to cell type, micro gravity causes variety of changes in proliferation and differentiation of cells while also affecting the migration of cells and cellular functions \cite{2, 3}. Siamwala et al from AUKBC have already studied the effects of microgravity on the microvascular endothelial cells from bovine lung and macrovascular endothelial cells from the bovine pulmonary artery. It was observed that the proliferation and migration of macrovascular endothelial cells were increased in microgravity \cite{4, 5}. Nitric oxide production was also studied and observed that microgravity treatment did not change nitric oxide production by microvascular endothelial cells \cite{4}.

OBJECTIVES

Isolation and Comparison of culture characteristics of Human microvascular endothelium cultured conventionally and in novel nanomaterial scaffold and further study the morphological and molecular characteristics of microvascular endothelial cells under normal gravity against simulated micro gravity.
MATERIALS AND METHODS

The human Omentum samples were obtained using surgical procedures after informed consent. The microvascular endothelial cells were isolated following the protocol described by Scott et al. The isolated cells were seeded in two groups; Group I - Cells in Nano Polymer, Overlaid M199 medium +20%FBS and, Group II - Cells in Conventional M199 medium+20%FBS. The cultures were maintained at 37°C with 5% CO2. The medium was changed every 3 days thereafter. The cells were observed regularly for their morphological characteristics and images were documented. At day 7 and day 13 the cells were harvested and the cells were subjected to two assays to confirm the presence of nitric oxide (NO) in samples, which will in turn confirm the presence of endothelial cells. In the first assay the isolated cell suspension was probed with diaminorhodamine-4M (DAR-4FM). In the second assay, the total nitrite level in the isolated cells suspended media was measured using Griess Assay protocol.

RESULTS:

A 21% increase in number of NO positive cells was observed in the cells cultured on the novel nanopolymer, while a 1.7 fold increase in nitrite production was detected in Group I in comparison to that of Group II.

CONCLUSION

Our data suggests the presence of more NO driven nitrite in the Microvascular endothelial cells cultured in the nanopolymer. In the next phase of the study, the cells thus isolated from both the groups will be subjected to simulated microgravity of 10-3 g in a clinostat at the AUKBC Vascular Biology Lab and the morphological and molecular characteristics of Microvascular endothelial cells will be studied to identify whether the effects of microgravity on Microvascular endothelial cells is organ specific.

REFERENCES


