DEVELOPMENT AND OPTIMIZATION OF MYOCARDIAL TISSUE CULTURE IN OVO

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Introduction

Characterization of cells in terms of their usefulness in regenerative medicine is based on research on their potential for differentiation and proliferation in vivo. The chorioallantoic membrane (CAM) of chicken embryo is an optimal environment for growing xenografts taken from other individuals or even species. In research conducted so far human stem cells were implanted directly into different parts of the embryo (eg. neural tube) as well as systemically through injections to veins and arteries of CAM [1]. It was also demonstrated their integration into the organism in the late stages of embryo development [2]. CAM as a biological model with natural immunodeficiency has already become a useful tool for breeding and researching many cancers, with no restrictions on the species [3-5]. The CAM model was also used for implantation of tissue fragments. In this system, however, the survival of transplanted tissues is dependent on rapid neovascularization, the failure of which results in death of the transplant. Transplantation of adult tissues did not lead to revascularization of their fragments probably due to lack of ability to stimulate host angiogenesis as it is for tumour tissue [6]. The positive effect augurs a use in place of the adult tissue, cells or tissues of a progenitor character. Both adult and embryonic stem cells provide an excellent tool for cell therapy. They owe the ability to differentiate into somatic cell lines [7]. The control of this differentiation is mainly achieved by direct stimulation of their surface receptors with appropriate transcription factors [8,9]. Previous studies on the chicken embryo CAM model and broiler model in vivo carried out with nanoparticles of colloidal silver showed their strong proangiogenic effects due to increased expression of angiogenic factors (VEGFA, FGF2) [10]. Their influence on the morphology of chick embryos was also reported [11]. The advantage in the case of xenografts implantation is also the proven antiseptic effect of silver.

Excellent blood supply, oxygen access and optimal physico-chemical conditions allow for effective cell culture, which can be differentiated and transformed into tissue. Progenitor cells implanted on the chorioallantoic membrane of the chicken embryo at different stages of development allow to determine which degree of differentiation of implants is optimal for these applications. The aim of the experiment is to determine the influence of in ovo culture conditions, on the size, structure and morphology of the resulting fragments of tissue. The use of nanocolloid of silver as a proangiogenic factor in relation to the cardiac progenitor cells implanted on the CAM could have a positive effect on vascularisation of obtained tissue fragments, which in turn would improve their growth and survival in ovo.

Materials and Methods

The model organism was CAM of a chick embryo and chicken embryos bred under standard conditions until the 18th day of embryonic development. Cardiac progenitor cells were collected at 6th and 18th ED. On each of these days eggs were opened and the embryos were sacrificed. Hearts were immediately isolated, tripsinised, homogenised and mixed with the culture medium to neutralise the enzyme. They were cultured in vitro to check their reaction to the Ag nanoparticles in medium or implanted as a primary cultures on the chorioallantoic membrane of chicken embryo at 8th ED with and without the addition of Ag nanoparticles as pro-angiogenic factor, which were administered in the vicinity of the implanted cells at the time of implantation. The size and morphology of the grown tissue fragments were assessed by macroscopic images from the binocular immediately after their isolation.

Results and Discussion

Preliminary experiment with the nanocolloidal Ag has shown that in a limited range of concentrations (2 and 10 ppm) of the silver nanoparticles, in the short term they stimulate cells' metabolism. In the lower concentration they cause formation of three-dimensional structures and prolong the life of cardiac progenitor cells in culture. As transcriptionally active they can potentially initiate self-renewal of cells or stimulate differentiation of pluri/ multipotent cells. Morphology of primary cells in culture differs depending on the cells' age. In turn, studies carried out on cardiac progenitor cells from chicken embryos (ED 6 and 18) after implantation on CAM of another embryo confirmed the formation of small tissue fragments within the chorioallantoic membrane. Cells collected on day 6th of embryo development after implantation in ovo migrate further from the injection site than 18 ED cells. Cells derived from 6th ED remain alive until the end of the culture in ovo.

Conclusions

Heart-derived progenitor cells taken from the embryo of chicken at 6th and 18th embryonic development days after implantation on chorioallantoic membrane form in ovo vascularised spatial structures. The 15 µg Ag nanoparticles supplementation at cells' implantation time results in more orderly cell proliferation towards heart-like structures.

Acknowledgments

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