8 Transplantation of Retinal Pigment Epithelial Cells

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8.1 Introduction

Transplantations of retinal pigment epithelial (RPE) cells into the subretinal space in animal models of age-related macular degeneration (ARMD) have demonstrated that degenerative processes can be prevented, and much hope is pinned on this therapeutic approach. During the past 2 decades, intra- or subretinal transplantation of retinal cells or tissues was extensively studied using various animal models. The most common animal used is the Royal College of Surgeons (RCS) rat, which develops postpartal RPE degeneration. Successful RPE transplantation is based on gaining functional cells as a sheet, in suspension or after multiplication. Maintenance of differentiated morphology and of typical functions of the RPE cells during serial passaging is – besides propagation of the cells – the main task of successful cell culturing in order to provide functional RPE cell transplants. This requires the adaptation and optimization of culture methods with regard to the ocular material available. After transplantation, the cells should be capable of phagocytosing shed rod outer segments, supplying the photoreceptor cells with nutrients, and participating in the retinoid cycle. This implies that function and differentiation of the cells are maintained during cell culture, e.g., measurement of membrane currents is to date the most sensitive method of evaluating the differentiation status of a cell. Based upon this experience, we compared standard culture media with our optimized growth medium F99<sub>RPE</sub>.

Even if the anatomic success of RPE cell transplantation has been demonstrated, there are still problems to overcome, e.g., cell adhesion on Bruch’s membrane, cell density and polarity at the transplant site, and integration of the cells into the recipient tissue. Moreover, long-term survival of the graft is impeded when xenotransplantation is performed. Immunologic processes that influence graft survival are not fully understood, in particular the role of the immune privilege of the subretinal space. This chapter presents an overview of the different attempts and results of RPE transplantation projects and discusses possibilities and limitations.

8.2 RPE Cell Culture

8.2.1 Culture Methods

In the past three decades, several reports on the in vitro cultivation of RPE cells have been published.
Albert et al. (1972) described the in vitro behavior of explants of the choroid in culture. Since then different methods of isolating the cells and different culture conditions have been examined. Success of culturing is influenced by – among other things – postmortem time, and with increasing postmortem time the number of viable cells is considerably reduced. Therefore, the aim of many studies was the optimization of cell culture methods to obtain as many viable cells as possible out of a specimen with longer post mortem times and to cover their specific needs for nutrients to help isolated cells start proliferation. Previous studies demonstrated that the choice of enzyme for isolating cells has a major influence on starting primary cell cultures of RPE. The methods described for the isolation of RPE cells include repeated trypsinization of choroidal sheets (Baumgartner et al. 1989) or of the eyecups (Flood et al. 1980) and the use of other enzymes such as pronase (Zavazava et al. 1991) or dispase (Pfeffer 1991). While trypsin, a nonselective protease, cleaves proteins, including membrane proteins, rapidly and aggressively and thus reduces viability of the cells, mild proteases like dispase or pronase have been shown to be less effective in isolating a sufficient number of cells. Our own studies demonstrated that a mixture of collagenases is most effective in isolating high cell numbers from donor eyes with long postmortem times without reducing their viability (Sobottka Ventura et al. 1996).

The method described here was established at our laboratory and was designed especially for donor eyes with postmortem times longer than 24 h. After enucleation, the corneoscleral disc is dissected according to standardized methods established for corneal organ culture, as described by Bohnke (1991). Subsequently, RPE cells are isolated according to the method of Baumgartner et al. (1989) modified by Sobottka Ventura et al. (1996): the choroidal sheets are carefully prepared off the sclera with scissors and forceps and incubated in 2 ml of a collagenase solution for 16 h in a humidified atmosphere of 5% CO₂ and 95% air at 37°C to loosen RPE cells. This technique yields sufficient viable RPE cells to establish cell cultures for transplantation studies, HLA typing, and other experiments involving RPE cells. Nevertheless, multiplication of the cells after the successful establishment of primary cultures is, in most cases, required for further studies.

Collagenase solution consists of a 1+1 mixture of collagenase types I A and IV in basal medium F99 at a final concentration of 0.5 mg collagenase/ml. Basal medium F99 consists of a 1+1 mixture of Medium 199 and Ham's F12.

A varying number of standard media have been used to culture RPE cells, including RPMI 1640, EMEM, DMEM, Ham's F10 (Albert et al. 1972; Baumgartner et al. 1989; Flood et al. 1980; Ho and Del Priore 1997; Mannagh et al. 1972; Zavazava et al. 1991). None of these culture media or methods was designed specially or proven for its growth-promoting efficiency. In contrast, a very detailed study to evaluate the growth behavior of RPE cells was presented by Pfeffer (1991). He developed a special culture medium for RPE cells which can be used in two modifications: depending on the calcium concentration, the medium can be used to promote either proliferation or differentiation of the cells. One reason that this very well designed medium was not used by other groups may be its detailed and more complicated recipe. Nevertheless, our own studies demonstrated the importance of defining the nutrient needs of highly differentiated human cells (Engelmann et al. 1988; Engelmann and Friedl 1989). Prevention of deadaptation or dedifferentiation of primary cells during cell culture is possible by definition of the nutritional environment, thus preventing loss of typical characteristics and functions. Therefore, the aim of our studies was to design a refined medium for RPE cells followed by evaluation of special functions (membrane currents) and characteristics (MHC antigen expression).

The proceedings at our laboratory involve the use of a specially designed RPE growth medium called F99ₐₕₐₜ, which was developed by Sobottka Ventura et al. (1996) specifically to cover the nutrient needs of adult human RPE cells isolated after long postmortem times. F99ₐₜ is based on F99, which is more suitable for culturing RPE cells than other basal media. It is supplemented with 10% FCS, 1 mM sodium pyruvate, 1 μ lg insulin/ml, antibiotics, and – most importantly – 15% choroid-conditioned medium. Conditioned medium is produced by incubating the remnants of the choroidal sheets in F99 plus 1% FCS for 4 days after collagenase treatment. The content of the conditioned medium is unknown, but its beneficial effect was proven (Sobottka Ventura et al. 1996; Valtink et al. 1999). It is likely that hormones and growth factors secreted by residual cells during the conditioning process are responsible for growth promotion (MacDonald 1994). This has also been described for other cell types (Astaldi 1983; Hoshi and McKeehan 1984). For further information on the topic of culture media and medium design, see Ham and McKeehan (1979). Isolated RPE cells are cultured with F99ₐₜ until the cultures become confluent and are monitored by phase con-