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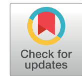
Review

Retinal organotypic culture – A candidate for research on retinas



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ABSTRACT

Reliable disease models are essential for investigations on mechanisms and therapies. The Retinal organotypic culture can maintain the architecture and cellular connections within the tissue *in vitro*. The system is a refined retinal experiment platform. It narrows the gap between cell line studies and *in vivo* models and is flexible enough for sophisticated experimental procedures. It greatly reduces the consumption of time or resources. Retinas of many species in various development stages have been used for diverse explorations based on their morphologies and physical characteristics. But the culture time course and the viability of the cultured tissue restrict the utility of the system. Recently, researchers have made increasing attempts to improve the culture conditions and applications of this systems for retina experiments *in vitro*. Accordingly, there is a great need for a comprehensive summary of these systems for researchers seeking proper *in vitro* models. In this review, we clarify several key points for the culture procedure and summarize its utility in retinal research.

1. Introduction

The retina is a delicate, organized tissue consisting of layers of different cells. Cellular homeostasis and interactions are critical for visual signal transduction. Numerous diseases and factors could affect the homeostasis of the retina resulting in vision impairment. Dissociated retinal cells,such as retinal pigment epithelium cells (hRPE19) (Liu et al., 2016), retinal cone photoreceptor cells (661 w) (Imamura et al., 2017), retinal microvascular endothelial cells (REC) (Xie et al., 2017), retinoblastoma cell lines (Y79,WERI-RB24 and WERI-RB27) (Reid et al., 1974; Sery et al., 1990; Surgucheva et al., 2013), and the retinas of living animals have long served as experimental platforms *in vitro* and *in vivo*. However, dissociated cells that are isolated from the microenvironment of the retina and cultured as monolayer cells provide information on only a single type of cellular reaction to conditional experiments, and animal models of many retinal diseases are either time-consuming or expensive. Therefore, an experimental platform that can highly imitate the retinal environment while being convenient to manipulate is required.

Retina organotypic culture (ROC) were first developed in the 1930s (Tansley, 1933) and can culture the entire retina *in vitro* in a limited time with maintenance of the architecture and cellular interactions of the tissue. This makes it a desirable replacement for dissociated cells in experimental explorations *in vitro*. Meanwhile, as some experimental procedures, such as drug delivery (Miguel et al., 2012) and transplanted cell tracing (Bray et al., 2014), are easier to operate *in vitro*, the culture system could act as a complement for *in vivo* explorations. Furthermore, it can even reduce the suffering (Valdés et al., 2016) and consumption of experimental animals because most of the operation can be carried out *in vitro* and one piece of retina can be divided into parts (Taylor et al., 2016a) for different conditional groups. Currently, retinal organotypic culture has been enhanced and widely used for many explorations on retina development, neurodegeneration and neuroprotection, cell transplantation and pharmacological studies.

Organotypic cultures of the central nervous system have been widely used for neurobiological studies on cell proliferation and death *in vitro* (Lossi et al., 2009), there is an increasing need for a comprehensive review of the retinal organotypic culture for researchers seeking proper *in vitro* models for the retina experiments. This paper is focused on evaluating current knowledge on the constitution and application of these systems. Key points and the application potential for future investigations are also discussed.

2. Establishment of the culture system

Retinas have been isolated and cultured since the 1930s (Tansley, 1933), and many studies have focused on searching for an appropriate culture mode (Di Lauro et al., 2016; Pinzon-Duarte et al., 2000),

Abbreviations: ROC, Retina organotypic culture; RPE, retinal pigment epithelium; OLM, outer limiting membrane; OS, outer segment; RGCs, retinal ganglion cells; ONL, outer segment layer; DMEM, Dulbecco's Modified Eagle Medium; PDE6, phosphodiesterase-6; MEF2, myocyte enhancer factor 2; BMSCs, bone marrow-derived mesenchymal stem cells; IRBP, retinoid-binging protein-specific

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identifying (Ogilvie et al., 1999) and stabilizing (Taylor et al., 2014; Taylor et al., 2013b) tissue viability, and exploring its potential as an experimental platform *in vitro*.

2.1. The culture modes in use

Two types of ROC modes are generally in use: the neuroretina culture (Taylor et al., 2013b) and the intact retina culture with the neuroretina adhered to the retinal pigment epithelium (RPE) layer (Pinzon-Duarte et al., 2000). Generally, the anterior segment was removed by a sharp incision in the pars plana and cut around it. After carefully removal of the vitreous, the neuroretina can be detached from the RPE layer mechanically. As retina is soft, filmy and membranous tissue, paintbrushes (Fernandez-Bueno et al., 2012; Johnson and Martin, 2008) or filter papers (Wang et al., 2011) were used to isolate or transport the tissue. Each of these methods has been applied for gentle explantation with minimal damage. Protease treatment before isolation enhances the possibility of separating the entire retina, with the neuroretina and RPE layer adhered, from the sclera (Pinzon-Duarte et al., 2000; von Toerne et al., 2014). Recently, attempts have also been made to co-culture the neuroretina with separated monolayer RPE cells (Di Lauro et al., 2016; Kolomeyer et al., 2011).

RPE cells are vital for maintaining retinal homeostasis (Kolomeyer et al., 2011; Mack et al., 2003) in both immature and mature retina. In an immature retina culture, the RPE layer has an effect on the complete development of the outer retina, including photoreceptor migration, formation of the outer limiting membrane (OLM), and outer segment (OS) disk-folding; however, the RPE layer does not affect the inner retina (Mack et al., 2003; Pinzon-Duarte et al., 2000). Meanwhile, in a mature retina culture with an intact RPE layer, better preservation of the tissue structure and cellular organization with less photoreceptor loss and lower reactive gliosis levels are observed (Di Lauro et al., 2016; Kaempf et al., 2008b; Kolomeyer et al., 2011).

2.2. Species and development stages

Retinas of various species have long been in use for experimental research, and attempts have also been made to culture them *in vitro* (Table 1). Fishes, whose optic nerve can survive and regenerate their axons after optic damage (Kato et al., 2013), are ideal for research on optic nerve axons regeneration (Ogai et al., 2014). Sharing a similar neuronal structure with humans makes the retinas of pigs appropriate for exploring the mechanisms of retinal diseases (Engelsberg et al., 2005; Hendrickson and Hicks, 2002). The retinas of murine animals have long been used for many explorations of retinal diseases, especially those affecting rod cells (Arroba et al., 2011; Pinzon-Guzman et al., 2015). Lately, ROC of human retinas have been used in several studies (Engelsberg et al., 2008; Fernandez-Bueno et al., 2012; Niyadurupola et al., 2011; Osborne et al., 2016). Certain cell markers and their expression patterns in the macular and para-macular areas have been identified to establish a human ROC system and make it a candidate for experiments on disease model constitutions and therapeutic explorations.

The cell viability of an immature retina exceeds that of a mature retina during culturing. Retinas explanted from embryos (Engelsberg et al., 2008; Engelsberg et al., 2005; Ghosh et al., 2012; Thangaraj et al., 2011) and neonatal animals (Ogilvie et al., 1999; Pinzon-Duarte et al., 2000) remain immature and maintain programs of cell differentiation, lamination and synaptogenesis during culturing *in vitro*, which are comparable with those *in vivo*, despite an incompletely developed OS and thinner lamination in the absence of the RPE (Ogilvie et al., 1999). However, attention should be paid to the disturbance of green cone visual pigment differentiation if isolation occurs prior to its initiation (Caffe et al., 2001a), and vascularization of an immature retina could occur in cultured retinas from neonatal animals (Curatola et al., 2005; Rezzola et al., 2014). In addition, gene transcription does not stop, as fluorescence dyeing and mRNA of specific proteins are still detectable after days of culturing (Caffe et al., 2001a; Caffe et al., 1993; Osborne et al., 2016). However, some studies have demonstrated that prolonged cultures do not show further development beyond 15 days (Pinzon-Duarte et al., 2000), and the retina becomes thinner with obvious folding and a rosette appearance (Ogilvie et al., 1999).

The explantation procedure requires axotomy of ganglion cell axons and, in many cases, separation of the outer segment layer (ONL) from the RPE layer, which may affect cell viability to some extent. Mature retinas in culture are characterized by neuron degeneration and reactive gliosis that significantly limit the culture time (Fernandez-Bueno et al., 2012; Johnson and Martin, 2008; Müller et al., 2017; Osborne et al., 2016). Neuronal degeneration initiates as early as 2 or 3 days post division with significant loss of photoreceptors, second neurons and the RGCs, indicating a progressively thinner lamination. Meanwhile, Müller cells spanning the entire retina as a scaffold of retinal neurons can turn into reactive gliosis during culturing, which results in disorganized lamination and cytoarchitecture. Therefore, methods aiming to preserve the tissue organization and prolong the culture time course have been explored recently and will be discussed in the next subsection.

The viability of cultured retinas may vary between development stages or donation ages. A better cytoarchitecture (Ferrer-Martin et al., 2014) and less rosette formation (Pinzon-Duarte et al., 2000) were observed in the retina from late postnatal (murine, P10) rather than neonatal (murine, P3) or adult (murine, P60) retinas. The expression of cell markers characterizing cells in the outer retina, such as arrestin and rhodopsin, varies between postnatal donation days, while that of the inner retina remains comparable (Caffe et al., 2001a). In addition, in the mature retina of humans, the age of the donation would not influence cell viability in the cultured tissue (Osborne et al., 2016). More

Table 1
Retinal organotypic culture of different species and developing stages.

Species	Development stage	Comments	Reference
Chicken	Immature	Observation of the development of embryonic retina	Thangaraj et al. (2011)
Fish	Mature	Observation on axon regeneration	Ogai et al. (2014)
Human	Immature	Observation of the development in embryonic retinal culture	Engelsberg et al. (2008)
	Mature	Establishment of the retina culture mode and analysis the neuron pattern in macular area	Niyadurupola et al., (2011); Osborne et al. (2016)
Murine	Immature	Observation of development of the neuron or vascular plexus; gene manipulation in the system	Curatola et al. (2005); Caffe et al. (2001a); Ghosh et al. (2009); Wang et al. (2002)
	Mature	Observation on the RGCs degeneration and neurotropic effects of exogenous factors; Coculture with stem cells	Johnson and Martin (2008); Subrizi et al. (2012); Johnson et al. (2016)
Porcine	Immature	Observation of the development in embryonic retinal culture	Engelsberg et al. (2005)
	Mature	Optimization of culture conditions for the mature retina	Taylor et al. (2014); Taylor et al. (2013b)
Rabbit	Immature	Observation of the development of neonatal retina, especially the cone photoreceptors	Huang et al. (2000)
	Mature	Gene manipulation	Koizumi et al. (2007)

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