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Validated and reproducible protocol for culturing single-eye porcine retinal pigment epithelium cells

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Abstract: The study of retinal diseases and the development of treatments for conditions such as age-related macular degeneration (AMD) requires reliable and reproducible cell culture models. Porcine retinal pigment epithelium (RPE) cells have been widely used due to their similarities to human RPE cells, making them an ideal model for retinal studies. However, developing a standardized and reproducible protocol for culturing these cells remains a challenge. This article presents a validated protocol for cultivating single-eye porcine RPE cell cultures, ensuring consistency and reproducibility across experiments. The protocol addresses key considerations, including tissue isolation, cell maintenance, and characterization of the cultured cells, providing a useful resource for researchers in retinal biology and ophthalmology.

Keywords: Porcine RPE cells, retinal pigment epithelium, cell culture, phagocytosis, barrier function, immunocytochemistry, retinal research, standardized protocol, retinal diseases.

Introduction: Retinal pigment epithelium (RPE) cells play a crucial role in the health and functionality of the retina. These cells are involved in several essential functions, such as phagocytosis of photoreceptor outer segments, secretion of growth factors, and maintaining the blood-retinal barrier. Defects in RPE cell function are implicated in a variety of retinal diseases, including age-related macular degeneration (AMD), diabetic retinopathy, and retinal dystrophies.

Porcine RPE cells have been extensively used as an in vitro model for human retinal diseases due to their anatomical and functional similarities to human RPE. The use of porcine models is advantageous because of their size and ease of handling compared to other species, such as primates. However, despite their widespread use, the lack of standardized and reproducible protocols for isolating and culturing porcine RPE cells has led to inconsistencies in experimental results.

The goal of this study is to provide a validated and reproducible protocol for culturing single-eye porcine RPE cells, focusing on tissue isolation, cell culture conditions, and characterization methods. By establishing a standardized protocol, this study aims to reduce variability between experiments and enhance the reliability of porcine RPE models for retinal research.

Retinal diseases, particularly those affecting the retinal pigment epithelium (RPE), are a significant cause of visual impairment and blindness worldwide. The RPE is a monolayer of specialized cells located between the retina and the choroid. These cells play a crucial role in maintaining retinal homeostasis by performing essential functions, such as the phagocytosis of photoreceptor outer segments, secretion of trophic factors, absorption of light, and forming part of the blood-retinal barrier. Dysfunction of the RPE is implicated in a wide range of retinal disorders, including age-related macular degeneration (AMD), diabetic retinopathy, and inherited retinal diseases like retinitis pigmentosa. Understanding the biology of RPE

cells, as well as their role in disease progression, is fundamental for developing new therapeutic strategies for retinal diseases.

To study RPE cell function and the mechanisms underlying retinal diseases, researchers often use in vitro cell culture models. The development of reliable and reproducible protocols for culturing RPE cells is essential for obtaining consistent results across laboratories and ensuring the reproducibility of experiments. Among various animal models, the use of porcine RPE cells is particularly valuable due to their close anatomical and functional similarity to human RPE cells. This makes porcine RPE an excellent model for studying human retinal diseases, drug testing, and understanding cellular responses to treatments, such as gene therapy and stem cell-based interventions.

While the porcine model offers several advantages, the isolation and culture of porcine RPE cells have not been standardized across research laboratories. Variability in culture conditions, tissue sources, and methodologies often leads to inconsistent outcomes and limits the reproducibility of experimental results. The lack of a validated, reproducible protocol for isolating and culturing porcine RPE cells further complicates the comparison of results between studies and can slow progress in retinal research.

To address this gap, the current study presents a validated protocol for isolating and culturing single-eye porcine RPE cells with a focus on improving reproducibility and consistency. The protocol detailed in this article was developed to standardize several key steps, such as tissue dissection, cell isolation, and culture conditions, with an emphasis on maintaining cell viability, functionality, and differentiation.

The primary objectives of this work were to:

1. Establish a reproducible method for isolating RPE cells from porcine eyes.

2. Validate culture conditions that allow for optimal cell proliferation and differentiation into functional RPE cells.

3. Characterize the isolated cells to confirm their identity and functionality using markers and functional assays commonly associated with RPE cells.

4. Provide a standardized protocol that can be readily adopted by other research groups studying RPE biology and retinal diseases.

Given the relevance of RPE cells in retinal diseases and the increasing need for reliable in vitro models to investigate these conditions, this study offers an essential resource for the scientific community. By ensuring consistency and reproducibility in the cultivation of porcine RPE cells, the protocol described here will facilitate more accurate and comparable results across retinal research efforts. Additionally, this work may contribute to the development of new treatments for retinal diseases, such as gene therapy, stem cell-based therapies, and pharmacological interventions.

In the following sections, we present the materials and methods used to isolate and culture the single-eye porcine RPE cells, followed by results that demonstrate the effectiveness of this protocol in producing functional and reproducible RPE cell cultures.

METHODS

1. Isolation of Porcine Retinal Tissue

Porcine eyes were obtained from a local abattoir within two hours post-mortem. Each eye was carefully dissected, and the retina was separated from the underlying choroid by gentle mechanical dissection under sterile conditions. The retinal pigment epithelium (RPE) layer was isolated from the retina using a blunt scraping technique. The isolated RPE sheet was transferred to a sterile petri dish containing RPE culture medium for further processing.

2. Cell Culture Conditions

After the RPE layer was separated, it was minced into small pieces, and the tissue was enzymatically dissociated with a 0.25% trypsin solution for 30 minutes at 37°C. The dissociation process was carefully monitored under a microscope to prevent over-digestion. Following enzymatic digestion, the cell suspension was filtered through a 70 µm cell strainer to remove clumps.

Cells were plated in six-well culture plates at a density of 1×10^5 cells/well in a complete RPE culture medium consisting of Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and a mixture of antibiotics. The culture medium was changed every 2-3 days, and cells were maintained in a humidified incubator at 37°C with 5% CO2. The cells were allowed to proliferate for up to 7-10 days before being used for downstream experiments.

3. Characterization of Cultured RPE Cells

To confirm the successful isolation and culture of porcine RPE cells, several characterization techniques were employed. First, the cells were assessed for the expression of RPE-specific markers, including bestrophin, pax6, and ZO-1, through immunocytochemistry. Additionally, the morphology of the cells was observed under phase-contrast microscopy, and confluence was monitored over the culture period.

The functional integrity of the RPE cells was assessed by measuring transepithelial resistance (TER) across

the monolayer, which reflects the barrier function of the RPE. Higher TER values indicate healthy, differentiated RPE cells with intact junctional complexes. Further, phagocytosis assays were conducted to evaluate the cells' ability to ingest and process photoreceptor outer segment-like particles, a key function of RPE cells.

4. Statistical Analysis

All experiments were conducted in triplicate, and data are presented as mean ± standard deviation. Statistical significance was determined using a one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons. P-values less than 0.05 were considered statistically significant.

RESULTS

The protocol successfully isolated single-eye porcine RPE cells with high yield and viability. Upon plating, the RPE cells adhered to the culture surface and exhibited characteristic polygonal morphology within 24 hours. Cells proliferated rapidly, forming a monolayer by day 4-5 of culture, and reached full confluence by day 7. The cells displayed typical RPE features, including pigmentation and the formation of tight junctions.

Immunocytochemical analysis revealed the expression of RPE-specific markers such as bestrophin and pax6, confirming the identity of the cultured cells as RPE. Additionally, the cells stained positively for ZO-1, indicating the formation of tight junctions, a hallmark of RPE cell differentiation.

The functional assays demonstrated that the cultured RPE cells retained their ability to perform phagocytosis of photoreceptor outer segment-like particles, confirming their functional activity. Transepithelial resistance (TER) measurements showed a significant increase in resistance over time, indicating that the cells were forming an intact monolayer with functional barrier properties.

DISCUSSION

This study presents a validated and reproducible protocol for the isolation and culture of single-eye porcine RPE cells. The approach described in this article ensures high cell yield, robust proliferation, and successful differentiation into functional RPE cells, making it an ideal model for retinal research.

The results of immunocytochemistry and functional assays confirmed that the cultured RPE cells maintained key physiological characteristics, such as the expression of RPE-specific markers and the ability to perform phagocytosis. Furthermore, the increase in transepithelial resistance over time suggests that the cells established a functional monolayer, which is critical for studying the blood-retinal barrier and other RPE functions.

This protocol has several advantages over existing methods, including its reproducibility, simplicity, and ability to generate large numbers of cells for experimentation. By providing a reliable method for culturing porcine RPE cells, this study paves the way for future research into retinal diseases, drug screening, and regenerative therapies for retinal degenerative conditions.

However, it is important to note that this protocol was performed using porcine eyes obtained from a single source. Variations in tissue quality and donor age could influence the outcome of the cultures. Future studies should evaluate the reproducibility of the protocol across different sources of porcine tissue and investigate potential variations in RPE cell behavior under different experimental conditions.

Additionally, the long-term viability and functionality of cultured RPE cells, particularly for use in transplantation or retinal regenerative applications, should be further explored.

The present study highlights the development and validation of a reproducible protocol for culturing single-eye porcine retinal pigment epithelium (RPE) cells, providing a reliable model for retinal research. The successful isolation and cultivation of porcine RPE cells is an essential first step toward studying retinal diseases and testing potential therapeutic strategies. This section will discuss the key findings, potential challenges, and opportunities for future research using this protocol, including its application in drug screening, disease modeling, and regenerative therapies.

1. Cell Isolation and Culture Protocol

One of the primary goals of this study was to establish a reproducible method for isolating porcine RPE cells from retinal tissue. This protocol utilizes a combination of mechanical dissection and enzymatic digestion to isolate the RPE monolayer from the underlying choroid. The tissue was carefully dissected, ensuring minimal contamination from surrounding structures, and dissociated using trypsin, which allowed for the effective separation of individual cells while maintaining high cell viability.

The results showed that the protocol successfully yielded a consistent number of viable cells, with high purity and minimal contamination from other retinal cell types. This high degree of reproducibility is important for researchers aiming to obtain a reliable source of RPE cells for their experiments. Additionally, the use of a standard RPE culture medium, which includes Dulbecco's Modified Eagle Medium (DMEM)

supplemented with fetal bovine serum (FBS) and antibiotics, ensures that the isolated cells are maintained in an environment conducive to their growth and differentiation.

However, one important aspect that researchers must consider when using this protocol is the potential variability in tissue quality depending on the donor. While the tissue used in this study was sourced from a single abattoir, different sources of porcine eyes or variations in tissue age could lead to slight differences in cell yield or viability. For instance, younger animals might provide cells with better proliferative potential, while older animals may have cells that are less robust. To address this variability, future studies could explore the impact of age and source of the tissue on cell culture outcomes.

2. Characterization of Cultured RPE Cells

The success of this protocol was further demonstrated through the characterization of the cultured cells. The RPE cells maintained characteristic features, including a polygonal morphology, which is typical of RPE cells in vivo. These cells also expressed RPE-specific markers such as bestrophin, pax6, and ZO-1, confirming their identity as RPE cells. Bestrophin is a well-known marker of differentiated RPE cells and is involved in chloride channel regulation, whereas pax6 is a transcription factor associated with RPE development, and ZO-1 is a protein involved in tight junction formation.

The expression of these markers indicates that the isolated cells retained their molecular identity and were able to differentiate into functional RPE cells. This finding is significant because it confirms that the isolated porcine RPE cells could be used for downstream experiments requiring a differentiated and functional RPE model. These cells could be useful in studying disease processes such as macular degeneration, as well as for testing new drugs or interventions that aim to restore RPE function.

Furthermore, the phagocytosis assay demonstrated that the cultured RPE cells were capable of ingesting photoreceptor outer segment-like particles, an essential function of RPE cells in vivo. The ability to perform this key function is critical for using these cells in disease modeling, as defects in RPE phagocytosis are central to several retinal diseases, including AMD. The presence of functional RPE cells in vitro provides a valuable platform for studying how RPE dysfunction contributes to retinal disease progression.

The transepithelial resistance (TER) measurements indicated that the cultured RPE cells formed a monolayer with functional barrier properties. TER is commonly used as an indicator of the integrity of tight junctions between RPE cells, and the increase in TER over time suggests that the cells were establishing tight junctions and becoming increasingly differentiated. This result is particularly important when studying the blood-retinal barrier, which plays a crucial role in maintaining retinal homeostasis and preventing the entry of harmful substances into the retina.

3. Challenges and Limitations

Although this protocol was successful in producing a consistent, high-quality RPE cell culture, several challenges remain that could affect the broad adoption of this protocol. One significant challenge is the time-sensitive nature of the tissue dissection. Since the porcine eyes were obtained from a local abattoir, there is always a limited window of time in which the tissue must be processed to prevent degradation or loss of cell viability. The need for fresh tissue means that the protocol is best suited for use in laboratories with easy access to porcine tissue sources or for collaborative efforts with abattoirs or suppliers who can provide high-quality tissue on demand.

Another challenge is the variability in cell growth depending on the donor's health and age. Although the cells cultured in this study showed good proliferative potential, researchers must be aware that age, tissue quality, and even differences in harvesting techniques can affect the results. Developing protocols that account for such variability and improving standardization for different tissue sources could help address these issues.

Additionally, while this protocol was able to generate functional RPE cells for experimental purposes, long-term culture of RPE cells in vitro remains a challenge. RPE cells are known to undergo senescence after prolonged culture, and their function may deteriorate over time. Future work may focus on extending the longevity of these cultures, either through genetic manipulation or by modifying the culture medium to better support long-term cell viability. Moreover, the use of 3D culture models or organoids could help replicate the native architecture and function of RPE cells more closely than conventional 2D monolayers.

4. Implications for Future Research

This validated protocol opens up new avenues for retinal research, particularly in the study of retinal diseases and the development of therapies. Retinal degeneration models, such as those for AMD or diabetic retinopathy, could benefit from the use of porcine RPE cells, as they provide a more accurate representation of human retinal biology compared to other models, such as mouse or human cell lines.

Furthermore, the reproducibility of this protocol makes it an excellent platform for drug screening and gene

therapy studies aimed at restoring RPE function. Given the role of RPE cells in retinal diseases, the ability to culture these cells in vitro allows researchers to test potential therapeutic interventions targeting RPE cells, such as gene editing or stem cell-based therapies. Additionally, the use of porcine RPE cells could facilitate the development of personalized medicine for retinal diseases, as patient-specific models can be generated using induced pluripotent stem cells (iPSCs) differentiated into RPE cells, providing a platform for testing drug responses or gene therapies tailored to individual patients.

Finally, this protocol could also serve as a foundation for studying RPE cell transplantation as a potential therapy for retinal degenerative diseases. RPE transplantation has shown promise in preclinical and clinical studies as a strategy to restore vision, and this protocol could be used to better understand the biological mechanisms underlying successful RPE transplantation and engraftment.

5. Future Directions

Looking ahead, further refinement of the protocol could focus on optimizing the culture conditions to improve the long-term viability and functional preservation of the RPE cells. Developing methods to maintain the cells in a more native-like state for longer periods would be beneficial for studying chronic diseases and for long-term therapeutic interventions.

Additionally, exploring the use of 3D culture systems and bioreactor technologies could allow researchers to recreate the three-dimensional architecture of the retina, providing a more physiologically relevant model for studying RPE cell behavior and function in health and disease. Furthermore, genetic modification techniques, such as CRISPR-Cas9, could be applied to these cultures to study the impact of specific genes on RPE cell function and disease progression, potentially leading to new insights into the molecular mechanisms of retinal diseases.

In conclusion, this study provides a robust, validated, and reproducible protocol for culturing porcine RPE cells that can be used in a wide variety of retinal research applications. By providing a standardized approach, this protocol will enhance the consistency and reproducibility of experiments across laboratories and enable new avenues of research into retinal diseases, therapeutic interventions, and regenerative medicine.

CONCLUSION

In conclusion, this article provides a validated and reproducible protocol for culturing single-eye porcine RPE cells, which can serve as a valuable resource for researchers investigating retinal diseases and therapies. The protocol ensures the isolation of high-quality RPE cells with preserved morphology, functionality, and barrier properties, making it suitable for a wide range of retinal studies. By providing a standardized and reliable methodology, this work contributes to advancing retinal research and improving experimental consistency across laboratories.

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