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Organoid technology for retinal repair

Sílvia Llonch[a,1], Madalena Carido[a,b,1], Marius Ader[a,*]

[a] CRTD/Center for Regenerative Therapies Dresden, Center for Molecular and Cellular Bioengineering (CMCB), Technische Universität Dresden, Fetscherstraße 105, 01307 Dresden, Germany
[b] German Center for Neurodegenerative Diseases Dresden (DZNE), Arnoldstraße 18, 01307 Dresden, Germany

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ABSTRACT

A major cause for vision impairment and blindness in industrialized countries is the loss of the light-sensing retinal tissue in the eye. Photoreceptor damage is one of the main characteristics found in retinal degeneration diseases, such as Retinitis Pigmentosa or age-related macular degeneration. The lack of effective therapies to stop photoreceptor loss together with the absence of significant intrinsic regeneration in the human retina converts such degenerative diseases into permanent conditions that are currently irreversible. Cell replacement by means of photoreceptor transplantation has been proposed as a potential approach to tackle cell loss in the retina. Since the first attempt of photoreceptor transplantation in humans, about twenty years ago, several research groups have focused in the development and improvement of technologies necessary to bring cell transplantation for retinal degeneration diseases to reality. Progress in recent years in the generation of human tissue derived from pluripotent stem cells (PSCs) has significantly improved our tools to study human development and disease in the dish. Particularly the availability of 3D culture systems for the generation of PSC-derived organoids, including the human retina, has dramatically increased access to human material for basic and medical research. In this review, we focus on important milestones towards the generation of transplantable photoreceptor precursors from PSC-derived retinal organoids and discuss recent pre-clinical transplantation studies using organoid-derived photoreceptors in context to related *in vivo* work using primary photoreceptors as donor material. Additionally, we summarize remaining challenges for developing photoreceptor transplantation towards clinical application.

1. Introduction

Degenerative diseases of the retina affecting the light sensing photoreceptor cells represent one of the main causes for disability in industrialized societies. As the mammalian retina, including human, does not show significant regenerative capacity the loss of photoreceptors in conditions like Retinitis pigmentosa (RP) or age-related macular degeneration (AMD) remains permanent, leading to vision impairment and eventually blindness. Though diverse therapeutic concepts are currently investigated, no definite cure has been established up to date.

Cell transplantation approaches for the replacement of lost photoreceptors have been investigated over the last three decades in pre-clinical animal models of retinal degeneration (reviewed by Santos-Ferreira et al. (2017)). Starting with primary retinal cells isolated during development as donor material, several studies provided evidence for successful survival and differentiation after grafting. Particularly, young post-mitotic photoreceptors termed photoreceptor precursors, showed the best transplantation success, including restoration of some functionality as observed by vision-based tests (Barber et al., 2013; Pearson et al., 2012; Santos-Ferreira et al., 2015; Singh et al., 2013). The described improvement of visual activity in retinal degeneration mouse models after photoreceptor precursor transplantation might be obtained by two separate mechanisms: either by supporting remaining dysfunctional photoreceptors of the host *via* cytoplasmic material transfer from the healthy donor cells (Ortin-Martinez et al., 2016; Pearson et al., 2016; Santos-Ferreira et al., 2016a; Singh et al., 2016) or by replacement of lost photoreceptors in late stage retinal degeneration models (Singh et al., 2013).

The majority of these preclinical studies were performed using primary photoreceptors isolated from the young, postnatal mouse retina. Aiming to use a similar donor cell population in the clinic would cause major challenges associated with donor material propagation and logistics besides ethical concerns, as the corresponding time period in humans would require the isolation of donor retina material from foetuses within the second trimester of pregnancy. Furthermore, as mice are nocturnal animals their retinas are dominated by rod

* Corresponding author.
E-mail address: marius.ader@tu-dresden.de (M. Ader).
[1] These authors contributed equally to this work.

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photoreceptors and only contain small amounts of cones (*i.e.* 3% of all photoreceptors), the photoreceptor sub-type providing high acuity daylight vision in humans, making pre-clinical transplantation studies for improving day-light vision challenging due to shortness of donor material (Santos-Ferreira et al., 2015). Therefore, an *in vitro* expandable cell source for the production of high numbers of transplantable rod or cone photoreceptor precursors represents an essential prerequisite on the path towards clinical application.

With the generation of human embryonic (hESC; Thomson et al., 1998) and induced pluripotent (hiPSC; Takahashi et al., 2007) stem cells a major breakthrough for the virtually unlimited propagation of donor cells was made, as pluripotent stem cells (PSCs) can massively be expanded *in vitro* and have the potential to differentiate, in principle, into any distinct cell-type of the body. Furthermore, the identification of culture systems for the production of 3D tissue organoids from PSCs, thereby reflecting a developmental environment more closely related to the *in vivo* situation, now allows the generation of specific cell-types in high precision and amounts as it was demonstrated in self-organizing organoids from the gut, liver, kidney or brain (reviewed by Ader and Tanaka (2014) and Lancaster and Knoblich (2014)). Besides gaining insights to early stages of human development, organoid technology also offers the possibility to perform high throughput drug screening and, combined with patient derived iPSCs and gene editing technology, it allows human disease modeling and repair (Fig. 1).

The ability to generate retinal cells from hPSCs has been a great advance towards the generation of clinically relevant cell populations, specifically for cell replacement therapies in the eye. The last 10+ years have witnessed great progress in the field, with several studies reporting the ability of hESCs and hiPSCs to follow a stepwise differentiation process that results in the generation of anterior neural tissue, eye field, retinal (progenitor) cells and, finally, differentiated cell types (Lowe et al., 2016; Meyer et al., 2009; Nakano et al., 2012; Zhong et al., 2014). Particularly seminal work from the Sasai lab that showed the generation of 3D retinal organoids that closely follow *in vivo* retinogenesis (Eiraku et al., 2011; Nakano et al., 2012) has been of tremendous importance for the production and availability of *in vitro* generated photoreceptors. Work performed in recent years has led to an accumulation of knowledge about factors that are required for and/or enhance retinal specification. This body of evidence has been incorporated into protocols developed by several groups, thereby further optimizing efficiency and robustness of retinal organoid production. In this review, we will summarize current protocols used for the generation of retinal organoids from mouse and human PSCs with the aim to produce transplantable photoreceptors for cell-based therapeutic approaches and discuss remaining challenges and roadblocks towards clinical application.

2. Generation of retinal organoids from pluripotent stem cells (PSC)

2.1. Mouse ESC-derived retinal organoids

Most of the currently used protocols for the production of self-organizing 3D retinal organoids from pluripotent stem cells are based on the seminal work of Yoshiki Sasai and his team (Eiraku et al., 2011, Eiraku and Sasai, 2011). Starting with mouse ESCs the protocol allows to mimic retinal development *in vitro* and has set the basis for multiple studies with different purposes: from optimization of the protocol for the derivation of transplantable rod photoreceptors and retinal sheets to developmental studies and disease modeling (see Fig. 2 for detailed comparison of the different protocols of mESC-derived retinal organoids).

Retinal organoid formation in Sasai's protocol is initiated by quick reaggregation of a defined number of dissociated mESCs (*i.e.* 3000cells/well) in 96 well plates under serum-free floating conditions that results in the formation of embryoid-body-like aggregates (Fig. 2). Addition of extracellular matrix (ECM) components (Matrigel) leads to the formation of a rigid continuous neuroepithelia that evaginate and express eyefield transcription factors like Rx and Pax6 within a week of culture, thus representing optic vesicle-like structures. Interestingly, within the next three days of differentiation around 60% of the optic vesicle-like structures undergo a dynamic

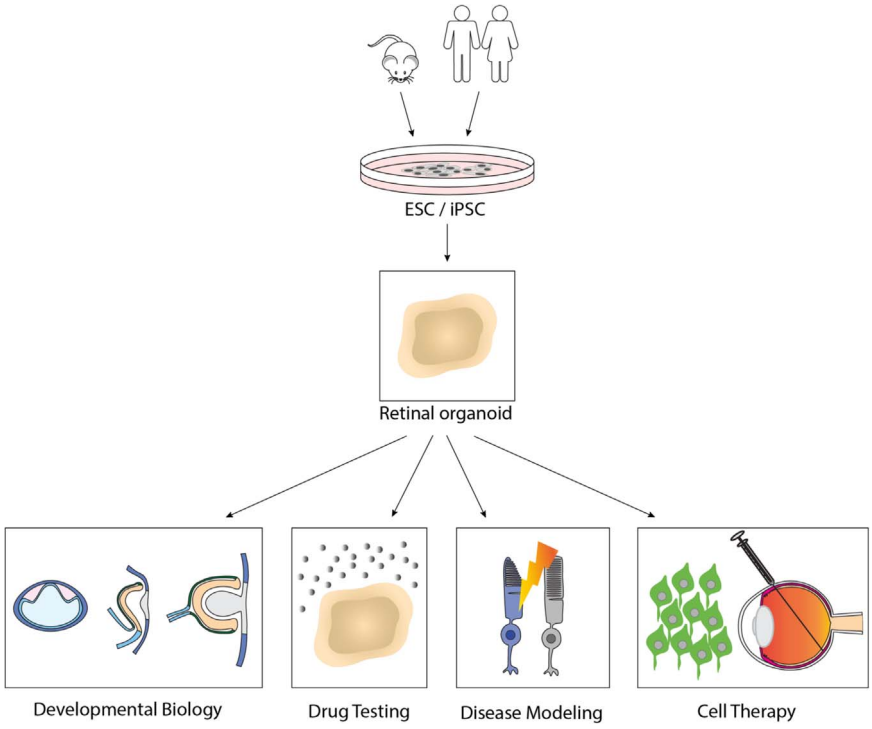

Fig. 1. Summary of potential applications of retinal organoids. Pluripotent stem cells can be used to generate retinal organoids for several applications: from analysis of required factors during retinal development and retinogenesis, to high throughput compound screening, and disease modeling *in vitro*. Another major application is the generation of photoreceptors for cell therapies, the focus of this review.