PERSPECTIVE

Visual prostheses, optogenetics, stem cell and gene therapies: splitting the cake

The size of the blind population in 2015 was estimated to be approximately 36 million (Bourne et al., 2017). According to the predictions by Bourne and co-workers, the number of the visually impaired is expected to reach nearly 100 million by 2050. Although some of these diseases can be treated, to date, some other eye conditions such as retinitis pigmentosa (RP), an inherited degenerative condition of the photoreceptors, have no treatment except electrical stimulation of the surviving neurons of the visual system. This therapy, delivered *via* a visual prosthesis, relies on an electrode array, implanted in close proximity to the target neurons, able to deliver a series of electrical impulses that activate these cells thus eliciting a visual sensation (Lewis et al., 2016). These electrodes can be implanted in the retina (three approaches exist: epiretinal, subretinal and suprachoroidal implants), the optic nerve, the lateral geniculate nucleus or the visual cortex. The medical device industry has spotted the opportunity and several companies have already obtained approval for commercialisation of their devices in the US and the European markets. However, the niche for these technologies may be soon occupied by new promising therapies based on a biological approach.

Optogenetic strategy: An adeno-associated virus can be engineered to induce light sensitivity in the surviving retinal neurons by altering their genetic information (Gaub et al., 2015). These viral vectors are loaded with genes that codify light-sensitive proteins and alter the DNA of the retinal neurons to induce their expression. It has been demonstrated that after infection, these neurons exhibit light-gated ion channels in their cell membranes and therefore become activated *via* incident photons in a similar way to the physiological photoreceptors. While this strategy has been demonstrated effective *in vivo* using animal models, its safety is still a question that needs to be further investigated. The main concern of these therapies relies on the potential reaction of the immune system. Although strong immune responses have not been reported in mice or primates in optogenetic experiments involving infection brain and retinal neurons, human immune responses could differ (Busskamp et al., 2012). Another potential limitation relates to the ability of the modified neurons to convey understandable neural messages. The retina codifies visual information in many ways including transition of light through the ON- and OFF-pathways. Reactivation of the retinal circuitry is feasible with this technique, but the neural messages elicited by visual scenes may be substantially different compared to those in the physiological retina. In addition, if the aim was to mimic the natural responses of the retina, this approach should target specific cells. However, it is expected the brain plasticity to compensate for inappropriate encoding (Busskamp et al., 2012). A third drawback of this approach is the poor light sensitivity imparted to the retinal neurons, but at present, some researchers are already working on this limitation, for example, using native light-gated G-protein-coupled receptors instead of microbial opsins (Gaub et al., 2015).

Therapies based on stem cells: The idea underlying this approach is to regenerate the retinal tissue by transplanting stem cells, a type of cells that have the ability to become, in this case, photoreceptors (Nazari et al., 2015). Briefly, this technique consists of replacing the unhealthy retinal tissue by a stem cell engineered one. For example, a recent study by Shirai and co-workers (Shirai et al., 2016) has shown, in a primate model, that a layer of photoreceptors obtained from human embryonic stem cells can form synaptic connections with the remaining retinal neurons. These are promising results as optimal host-graft integration would potentially lead to more natural neural messages being transmitted to higher visual centres in the brain. However, there are relevant technical limitations that need to be addressed before this therapy can reach the bedside, particularly in relation to long-term safety. Immune responses can occur in some types of implants and there is a potential for these cells to form tumours (Nazari et al., 2015). In these lines, several companies have started clinical trials to test their therapies. For instance, jCyte launched in 2017 a phase IIb clinical trial to test the efficacy of 'jCell', an intravitreal injection of allogeneic human retinal progenitor cells able to rescue the degrading photoreceptors during progression of RP. Despite the enormous progress in the laboratory, the scientific community is also facing important ethical challenges, for example, in the use of embryonic-derived stem cells. These concerns may slow down the progression and the development of some of these techniques.

Gene editing therapies: It is now possible to repair the genome of non-dividing cells *in vivo* through the Clustered Regularly Interspaced Short Palindromic Repeat technique (CRISPR). Using electroporation, an RNA-guided Cas9 nuclease can cross the cell membrane and edit the DNA of the target cells (Suzuki et al., 2016). This is of particular relevance in the treatment of RP (Bakondi et al., 2016). However, there are other eye problems such as trauma for which this strategy offers no solution. Furthermore, the existence of numerous ethical concerns on the use of this technique may blur the future application of this therapeutic approach. There are in addition strict regulatory requirements that need to be met before these therapies can be approved for the use in humans. Nevertheless, CRISPR is making a rapid progress as two clinical trials are scheduled in Europe and the USA in 2018. Although these studies are not related to the treatment of visual impairment, they may facilitate approval of further trials to test its use as a therapy for retinal degenerative diseases.

The three emerging therapies described here are promising a different scenario in the treatment of some types of visual impairments, and may replace, in some cases, the use of visual prostheses. However, with a number of challenges yet to be overcome, these biological approaches may not become the mainstream for number of years, and a generation, or perhaps two, of blind people may miss the opportunity of being sighted again. Hence, at present those patients currently suffering from vision loss have no other alternative but visual prosthetics. For those, there are two approved retinal implants, the Argus® II (Second Sight Medical Products, Sylmar, CA, USA) and the Alpha IMS (Retina Implant AG, Reutlingen, Germany) (Lewis et al., 2016); the first, accounting a total of 60 electrodes, is an epiretinal device that relies on an external camera to bypass the degenerated photoreceptors, and the second is a subretinal implant that uses an array of 1,500 microphotodiodes to elicit visual perception. Several implants still remain on the bench but are making important progress towards the bedside, and some other devices such as the epiretinal IRIS® II (Pixium Vision, Paris, France) or the cortical Orion (Second Sight Medical Products) are currently undergoing clinical trials. Although the second type of prostheses requires brain surgery to implant the electrode array on the visual cortex, they can target a wider spectrum of pathologies and therefore may be able to compete with the emerging biological approaches when they reach maturity. However, these devices have some limitations as well and can only provide a rudimentary functional visual perception. Bionic vision is mainly limited by the electrochemical reactions that can occur at the electrode-tissue interface during electrical stimulation (Barriga-Rivera et al., 2017a) and by the interferences created between neighbouring electrode sites (Matteucci et al., 2016). In fact, these interferences, known as crosstalk, can lead to inhibition of the neural activity due to summation of the overlapping electric fields produced when several electrodes are activated concomitantly, as in the case of bright visual scenes

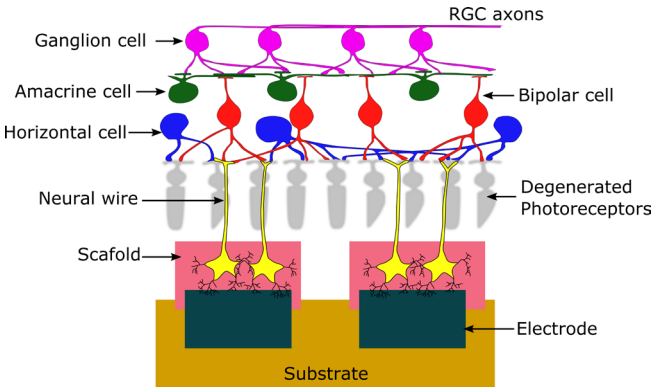

Figure 1 Subretinal electrode array consisting of a group of metallic electrodes coated with a cell-laden material.
The scaffold has to be designed to allow growth of the interfacing cells and to provide sufficient adhesion to the electrodes. Electrical stimulation can be used to either differentiate the cells (in case of neural stem cells) and to stimulate projection of the neuronal axons to form synaptic connection with the remaining retinal neurons. With the photoreceptors degraded by the progression of a degenerative disease, the retinal network typically preserves the horizontal, the amacrine and the retinal ganglion cells (RGCs). The axons of the RGCs form the optic nerve, which must be viable to allow transmission of neural information to higher visual centres in the brain.

(Barriga-Rivera et al., 2017b). Retinal implants have also a limited capacity to elicit physiological neural messages. For example, when a stimulus is delivered, both ON- and OFF-pathways are activated simultaneously resulting in confusing information being sent to the brain. To address these limitations, researchers are directing their efforts in different ways (Barriga-Rivera et al., 2017a): (1) the use of new biomaterials such as conducting polymers or carbon nanotubes among others may help reducing the electrochemical burden of conventional metallic electrodes, (2) by growing neurons on the surface of the electrodes as shown in **Figure 1**, the development of living electrodes may provide an optimal electrode-neuron interface, and (3) the development of new stimulation strategies, particularly those relying on the use of high frequency neurostimulation, can provide a method to selectively activate different cell types.

In a scenario of rapid development and intense competition for restoring sight to the blind the question on whether bionic vision will remain as the main therapy is under debate. The biological approaches are in a strong position to become the gold standard in the treatment of some eye diseases. This would leave a reduced spectrum available for the application of bionic vision technologies. A recent example of success of the gene therapies is Luxturna (Spark Therapeutics, Philadelphia, PA, USA), the first gene therapy approved by the US Food and Drug Administration (FDA) to treat blindness. In particular this solution targets patients with mutations in the *RPE65* gene. This is quite an expensive therapy that has a great potential for causing dangerous side effects, but it also shows clearly the potential of the biological approaches. Among all visual prosthetic devices, cortical implants may have a more exclusive niche as stimulation of the visual cortex can be used to treat almost any type of blindness. Despite the fierce competitors of visual prosthetics, progress in the delivery of bionic vision must continue not only because it will benefit a number of patients with no current alternative, but also because advances in this field can be easily adopted by other forms of neuromodulation therapies, or perhaps, because the combination of visual implants and biological techniques may exploit new synergies, as in the case of organic electrodes (Aregueta-Robles et al., 2014).

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