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Chapter

Application of Nanowires for Retinal Regeneration

Davood Kharaghani, Zahra Tajbakhsh, Phan Duy Nam and Ick Soo Kim

Abstract

Nanowires aim at developing advanced architectures are gaining popularity for damaged neural systems. The retina with a complicated structure is an essential part of our visual nervous system. Any disorder inside retina could lead to blindness due to irregularity in transferring neural signals to the brain. In recent years, the emergence of nanostructures, as well as nanowires, has provided a viable means for enhancing the regeneration of retinal. Nanowires with the ability to sense light and converting it to the electrical signals simulate the extracellular electrical properties, which are the newest nanostructures for the retinal applications. The different structure of nanowires has been examined in vitro, and several others are undergoing in vivo for vision recovery. Among the structures, core-shell nanowires and functionalized nanowires with gold nanoparticles attract the attention for the regeneration of retinal neural systems. Herein, subsequently provide an introduction to the anatomy of the retina, and retinal disorders, the latest progress in the regeneration of retina and vision using nanowires will be reviewed. Also, the different structures, including core-shell and functionalized nanowires with nanoparticles, will be examined. Eventually, the point of view and perspective of applying nanowire in retinal regeneration will be offered.

Keywords: regeneration of retina, nanowires in ophthalmology, nanowires for tissue engineering, nanowires biocompatibility

1. Introduction

The retinal transplantation is limited due to the complex neural network [1]. Retina senses light and converts it into the neural signals and transfers neural signals to the brain and cause visual perception [2, 3]. Nanostructures have evolved as multidisciplinary applications by combination with materials as an advanced architecture to develop functional substitutes for various proposes such as wound dressing [4–6], tissue engineering [7–11], and biomedical applications [12–14]. Neuroscience, related to the retina is one of the most exciting fields where nanostructures with modified properties serve as scaffolds to promote and facilitate the migration and adhesion of the cells [15].

Until recently, it was believed that scaffolds simulated the extracellular matrix (EMC) in the regeneration of retina, and served as a support for cell migration, adhesion, and morphology only [16–18]. Emphasis was on loaded materials and morphology construction to develop biocompatible and biodegradable scaffolds
with appropriate mechanical properties [19–22]. However, the first definition of tissue regeneration is developing scaffolds with acceptable biocompatibility to implant in the host body to repair damaged tissues or organs. Therefore, the electrospun nanofibers with a high ratio of surface-to-volume, tunable porosity, and similarity to natural ECM show the ability to modify the surface functions with different structures for a wide range of tissue regeneration [23]. Nanofibers have emerged as a potential to simulate the ECM in many tissues such as bone [10], nerves [24], and various techniques have been employed to fabricate the nanofibers with excellent properties [25].

As extracellular electrical simulation involves in neuroscience and neural tissue engineering [26–28], attention is focused on nanowires applications for visual nervous system [29–31], brain [32, 33] and cardiac [34]. Nanowires have recognized as widely used nanostructure for the cell microenvironment where the electrodynamic properties [35] have permanently affected cellular functions, such as morphology, adhesion, differentiation, and proliferation [36]. As a consequence, researchers have developed new structures for better electroconductivity, biocompatibility, and cell adhesion [37–39].

Nanowires have been shown that simulate the nerve signals in the retina and transfer between the layers could improve the vision loss by the damaged retina. To explain the recovery of vision with nanowires, which lost by retinal degeneration, we will begin by describing the retinal anatomy, and how various retinal disorders may cause blindness. Then we will investigate the nanowires which could enhance retinal organization with sensing light and converting it to the electrochemical signals with different materials, structures, and properties. Finally, we will discuss the challenges ahead and prospect in the application of nanowires for recovery of vision that lost by retinal destruction.

2. Retina
2.1 Anatomy of the retina

The retina is the innermost multilayered structure of the eye with an approximate thickness of 0.50 mm [40]. Retina first translates light into a biochemical message and then prepared biochemical messages converted into the electrical messages, cause to the visual information with transmitted to the primary visual cortex of the brain via the optic nerve.

The retina is composed of retinal pigment epithelium (RPE) and neuroretina, which is further divided into nine layers. Respectively, the neuroretina layer includes the outer and inner segments of photoreceptors (PL), outer limiting membrane, outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer [41], inner plexiform layer (IPL), ganglion cell layer (GCL), nerve fiber layer (NFL) and inner limiting membrane (ILM) from nearest layer to choroid up to nearest layer to vitreous [40]. This part is consists of five types of neurons: the visual receptors cells (the rods and cones), the horizontal cells, the bipolar cells, the amacrine cells, and the retinal ganglion cells [42].

RPE with a function to absorb light is a monolayer of pigmented hexagonal cells which are denser in the macular area. Mostly, the interaction between RPE and photoreceptors has a significant effect on the ability of photoreceptors to detect light and convert the light into the electrical signals, which caused vision preparation [43]. RPE is separated from the choroid by the Bruch’s membrane. As RPE is located between the outer segments of the photoreceptors and the vascular layer of the choroid, it has a two-directional function. RPE with transports ions, water, and
metabolic products from the subretinal space to the blood and receiving nutrients such as glucose and retinol from the blood to nourish the photoreceptors playing the critical role in the retina layer. However, failure in mentioned functions can lead to retinal degeneration, visual loss, and eventually blindness [43].

PL is the only light-sensitive part of the neuroretina and is composed of outer and inner segments of the rod and cone cells. Cone cells are responsible for color detection and are found in high number in the macula, especially foveal region, whereas rods are more active in the dark and are abundant in the peripheral retina. OLM layer separates PL from the photoreceptor nuclei, and it is not considered as an actual layer. ONL contains the nuclei of photoreceptors, and its thickness varies across the retina with the maximum thickness at the fovea. However, the axons of the photoreceptors cells and their synapses with bipolar and horizontal cells form the OPL. On the other hand, cell bodies of horizontal cells, bipolar cells, amacrine cells, and Muller glial cells are in the INL layer. The INL layer, playing the critical role to transmit inputs signals from IPL to OPL, which is composed of synapses between the bipolar, ganglion, and amacrine cells. The innermost layer of the retina is GCL and located in the place near to the vitreous and contains the cell bodies of ganglion cells and displaced amacrine cells, astrocytes, and Muller cell bodies that their axons converge on the way to the optic disc and form NFL. The latest layer of the retina is ILM, which forms the inner boundary of the retina on the vitreous side. **Figure 1** is showing the retinal layer that divided into nine layers with nine different cell type [40].

The macula is a region inside the retina which contains the highest number of ganglion cells and cause the optimal vision and color perception process [44]. Rods and cones are responsible for the initiation of the scotopic and photopic visual processing, respectively. When the light absorbed by photoreceptors, rod, and cone cells with releasing glutamate as a neurotransmitter cause to transfer the electrical signals from synapse onto the bipolar cells at the OPL layer. Afterward, transferring
the electrical signals from bipolar cells synapse by amacrine and ganglion cells at the IPL to the axons of the ganglion cells lead to the output neurons signals formation as the optic nerve and deliver the visual information to the brain [45].

2.2 Retinal pathology

It is reported that half of the blindness in the world is related to the various retinal damages. Degenerative damages such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP), optic neuropathy such as glaucoma and vascular retinopathy as well as diabetic retinopathy (DR) are the most common retinal diseases and the leading causes of legal blindness [46].

AMD is an age-related retinal problem occur in two forms of dry and wet. The dry form is mainly diagnosed by drusen and extracellular materials deposition such as lipids and proteins which accumulate in Bruch’s membrane, and loss of photoreceptors by geographic atrophy of the macula. However, wet form or neovascular/exudative AMD is diagnosed by choroidal neovascularization (CNV), blood vessel development and hemorrhage/leakage of blood and fluid accumulation into the neural retina which can lead to the RPE detachment. The hallmark of AMD is the degeneration of neurons in the macula, which may result in central vision loss at the early stage of the disease [47].

RP refers as a hereditary and neurodegenerative disease of the retina may appear in different forms. The outcome in all forms is photoreceptor degeneration and subsequently, cell death. Photoreceptor degeneration starts from the periphery of the retina, which progressively decreases the visual field and consequently makes a tunnel vision for the patient. Vision impairments in RP will start with rod photoreceptors and followed by cone photoreceptors degeneration that latter can lead to alteration and abnormalities in the RPE [48].

Glaucoma is the most common optic nerve disease that can lead to blindness. Glaucoma with increasing the intraocular pressure may cause retinal ganglion cells degeneration. However, disorders in the retinal nerve fiber layer and optic nerve proceed during glaucoma and will lead to irreversible vision loss if the treatment is not appropriate [49].

DR as vascular retinopathy is another cause for blindness in the worldwide which divided into type-one and type-two. There is evidence for possible dysfunction of Muller cell during diabetic neuropathy [50]. DR is classified into the proliferative stage with loss of blood supply and non-proliferative stage with altered retinal vascular permeability, intraretinal microaneurysms, and macular edema [51].

There is no definite cure for retinal diseases, and most of them end up with severe visual impairment or blindness. Current medical treatments may help to decrease the progression of the disease and are unable to cure them. Therefore there is a need for novel approaches to target restoring partial vision by preparing the advanced structure as well as nanowires and nanomedicines.

3. Structure and overview of nanowires

Nanostructures could be useful to follow the development of advanced structures for retinal applications where functionality depends on materials properties. Moreover, the combination of scaffolds with existing nanomaterials may improve the required functions [52]. In a recent study, nanowires with various structures have been used for regeneration of retina. The synthesis and fabrication of nanowires, with a narrow range of subtracting such as gold and TiO$_2$ nanoparticles...
combined with polymers as well as poly (e-caprolactone) cast onto anodized aluminum oxide template, have been used for retinal regeneration applications [53–55].

Gallium phosphide is another example of materials have been employed for the regeneration of retinal. Gallium phosphide as multimodel nanowire employed for different geometries such as rod and cone photoreceptor, ganglion cells, and bipolar cells [56]. Also, the same structure coated with poly-L-ornithine showed that nanowires have significant potential on morphology, adhesion, and metabolism of cells in comparison to the flat surface [57, 58].

The nanostructures designs based on silicone, to simulate the retina photoreceptor was improved by nanomaterials coating as well as gold and titanium oxide nanoparticles [59]. Whereas, silicon nanowires have been shown to form spontaneous conjunction with photoreceptor cells. Such nanostructures showed that they could improve the quality of photoreceptor simulation and increase cell adhesion to the nanowires when placed in direct contact with cells [60].

Silicon nanowires coated with gold were shown to be more effective at the simulation of photoreceptors; this is mostly attributed to the higher surface to the area for sensing light and charge transfer [61, 62]. Another type of nanostructures is thin films functionalized with the nanoparticle to sense light. Thine film structures were able to simulate cultured photoreceptors when subjected to direct visible light [50, 63–65].

In addition to sense light, nanowires can use for transferring the electrical signals through the cells such as rode and cone cells [66]. One way for transfer signals between the nanostructures and cells is to simulate solar panels structure in nanosize via materials such as n-type and p-type silicon, which can sense the light and convert the light signals into the electrical signals. In particular, silicon nanowires are useful to sense light and transfer the signals to the internal layer of the retina to recover the eyesight [67, 68].

The architecture of nanowires has been clarified as complex core-shell nanowires with complex chemical profiles. These advanced structures have been

<table>
<thead>
<tr>
<th>Nanowire</th>
<th>Forms</th>
<th>Length (μm)</th>
<th>Modification</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iridium wire</td>
<td>Pillar array electrode</td>
<td>75</td>
<td>Embedded with glass</td>
<td>[66]</td>
</tr>
<tr>
<td>Poly (e-caprolactone)</td>
<td>Nanowire</td>
<td>2.5–27.5</td>
<td>Cast onto anodized aluminum oxide template</td>
<td>[53]</td>
</tr>
<tr>
<td>Poly (e-caprolactone)</td>
<td>Short nanowire</td>
<td>2.5</td>
<td>Electrospinning method</td>
<td>[55]</td>
</tr>
<tr>
<td>Gallium phosphide</td>
<td>Nanowire</td>
<td>0.5–4</td>
<td>Gold nanoparticles</td>
<td>[56]</td>
</tr>
<tr>
<td>Parylene/silicon</td>
<td>Silicon tip</td>
<td>Not reported</td>
<td>Platinum and gold tine film</td>
<td>[59]</td>
</tr>
<tr>
<td>Gallium phosphide</td>
<td>Functionalized nanowire</td>
<td>Not reported</td>
<td>Gold/palladium nanoparticles, nanowires coated with poly-L-ornithine</td>
<td>[57]</td>
</tr>
<tr>
<td>Silicon</td>
<td>Nanowire/microelectrode</td>
<td>20</td>
<td>Coated by polyimide</td>
<td>[62]</td>
</tr>
<tr>
<td>n-type silicon</td>
<td>Nanowire</td>
<td>Not reported</td>
<td>Gold/palladium nanoparticles</td>
<td>[60]</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>Nanowire</td>
<td>Not reported</td>
<td>Gold nanoparticles</td>
<td>[67]</td>
</tr>
<tr>
<td>n-type/p-type silicon</td>
<td>Coaxial nanowire</td>
<td>Not reported</td>
<td>Gold nanoparticles</td>
<td>[69]</td>
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Table 1.
The materials have been used to prepare the nanowires structure for retinal implant applications.
made from \textit{n-type} and \textit{p-type} silicon for making connections between the membranes of live bipolar cells and nanowires to sense the light for the recovery of vision [69]. Table 1 represents the materials have been used as nanowires for vision recovery lost due to retinal disorders.

4. Nanowire based mechanism of retina regeneration

4.1 Extracellular matrix simulation and cell adhesion

Tissue engineering specified as two foremost policies to redevelop the injured tissues or organs such as (1) cell-based; when cells are the critical substance to modify the place before transplanted to the host body, and (2) scaffold-based; when an extracellular matrix (ECM) from biomaterials designed and simulate in vivo structures. Recreating the retina requires effective architecture to mimic the extracellular matrix with physiological and morphological features resembling the in vivo structure [70]. The ECM with the composition of an intricate interweaving of protein provides the appropriate structure for cells grow with vast morphogenesis, which creates the vary forms of tissue and organs. However, the ECM classified into the two main categories of interstitial and pericellular. The interstitial matrices define as matrices that surround cells, whereas; pericellular matrices are with close contact with cells. The obvious example is the basement membrane, which is a type of pericellular matrix, and with providing an anchoring, membrane prevents parenchymal cells to break apart [71]. Also, unique ECM is associate to differentiate, changes in morphology and topography of cells to form the unique tissue and organs [72].

The mature mammalian retina is consists of two basement layer such as Brunch membrane at the interface between retinal pigment epithelium (RPE) membrane and choroid and second is the inner limiting membrane (ILM) at the interface of the neural retina with the vitreous body [73]. Figure 2 is showing the two basement layers in the structure of the retina and their morphogenesis.

Figure 2.
Immunohistochemistry of the 8 weeks gestation human that stained with the immunofluorescent antibody. The yellow line labeled are showing the Bruch's and ILM membrane that continues the same identical membrane folded over inside the eye (A), the scanning electron microscope of the ILM membrane showing that the surface in contact with RPE (Ret) is irregular whereas the vitread surface (Vit) represents the smooth surface (B), and Bruch's membrane showing the composition of aligned collagenous fibers (F) in composition of cell process (C) [74, 75].
Recently, Kharaghani and co-workers [76] reported a study emphasizing the importance of morphogenesis in the three-dimension (3D) nanofibers scaffold structure in guiding the seeded cells for ophthalmic tissue engineering mainly for cornea and retinal applications. The necessities for using scaffolds in ophthalmic tissue engineering are cell adhesion besides to tensile strength, effectiveness on cell morphology, and topography of scaffolds [76]. This report stressed the significance of the underlying ECM in endorsing exclusive micro and nanoenvironments that fosters tissue regeneration. However, it is unfortunate that there is no report about the study of ECM simulation by nanowires or surface chemistry of nanowires that shows cell adhesion, where it is used for retinal regeneration. At the moment, all reports have been used the single nanowire for the regeneration of retina.

In attempts to control the cell adhesion on nanowires, researchers repopulated their strategy to change the surface chemistry nanowires prepared based on titanium, silicon, and zinc [77–79]. However, the in vitro and in vivo researches have been don around the regeneration of retina by nanowires, and results show that cells tended to encompass. Also, it should be happening due to electrical stimulation of place based on the extracellular electrical.

4.2 Extracellular electrical simulation

It is mentioned here earlier that converts light into the neural signals and transfer neural signals to the brain by retina will lead to visual perception. Extracellular electrical involving ion channels has a vital role in nervous systems such as the retina. Photovoltaic polymers such as silk have been shown the great potential to use a connection between the layer of the retina to restore the vision with transfer the electrical signals [80]. The intracellular voltage is one of the unknown metrics that many types of research have been focused on recording signal strength use a nanoscale electrode as well as nanowires. Nanowires have been shown the tremendous potential upon extracellular electrical stimulation of cells to promote cell growth, adhesion, and differentiation. Also, Vodovnik and coworkers showed that the external electrical field had a significant effect on the polarization of cells on the cathodal and anodal side of the electrode [81].

On the other hand, the positive and negative charge should be optimized for the clinical implant to achieve successful results. The cationic polymers and nanoparticles with high concentrations of nitrogen may help to compaction of negative charge DNA and RNA and lead to better gens protection and endosomal escape in addition to high transaction efficiency and stability [2]. Figure 3 is showing how nanowires with simulating the extracellular electric could cause cell adhesion to the nanowires and effect on the recovery of vision after implantation in mice eye.

Among the various nanostructures, gold nanoparticles due to high electroconductivity, biocompatibility, and chemical inertness have been attracting the attention to develop scaffolds for neural systems such as retinal applications. However, it is unfortunate that the development of high-quality gold nanowires faces challenges in the absence of robust methods for synthesis. Nevertheless, the gold nanoparticle with the electrical resistance of 52 Ω is one of inseparable part of scaffolds have been used for retinal applications as reported in Table 1, which gold nanoparticles have embedded with nanowire structures [61].

In an attempt to design an implantable electronic device for regeneration of retina when photoreceptors are damaged, electrically stimulate of retinal neurons, become an essential challenge. Whereas, several retinal prostheses are going on, but none of them have shown the ability to evoke phosphenes in blindness. Refer to retinal anatomy controlling the signal impedance is the most crucial subject to
prepare the appropriate prosthesis due to discernment of the retina as a layered structure with different electrical conductivity for each layer which the inner layers have higher resistivity in comparison to outer layers such as retinal epithelial pigment and membrane [83].

However, among the different research, simulation of photovoltaic materials, as well as silicon and titanium nanowires functionalized by gold nanoparticles that have the ability to receive the light signals and change the light signals to the electrical signals, are attracting the interests [68, 84]. Also, photovoltaic nanowires started the new generation of materials with extracellular electrical simulation and conjunction with the cells that are remaining the main challenges as implants used for retinal regeneration (Table 2).

4.3 Light sensation

Nanowires have been explored extensively as a component of photovoltaic to improve the efficiency of sensing light for retinal applications. The use of single nanowire as photovoltaic nanostructures present the several crucial advantages, which may leverage to produce robust, high efficiency and compatibility with cells. The simple example of sensing light and converting the light signals to the electrical signals are solar cells made from n-type and p-type silicon. Briefly, the p-type silicon produced by materials that have one free place for accepting one extra electron on the outer layer of orbital despite the n-type silicone which produced by elements and has one extra electron in the outer layer of their orbital and electron is not involved in any bonding. However, light absorption by the n-type silicon layer will cause to the movement of an extra electron to the empty orbital of p-type silicon, and this electron movement between the n-type and p-type silicon layers lead to changing the
light signals to the electrical signals [41, 85]. Figure 4 is showing the process of turning light signals to the electrical signals used for regeneration of retinal photoreceptor. Also, sensing the light and electron travels between the n-type, p-type silicon will cause to the preparation of local extracellular electrical simulation, and conjunction between nanowires and cells as explained earlier. However, the gold nanoparticles with one free electron in the outermost layer of orbital play a vital role in improving the sensitivity of light perception in composition with coaxial silicon nanowires [86]. Therefore in most of the nanowires have been produced for the regeneration of retina, gold nanoparticles loaded on the surface of nanowires.

Another example of using the same construction introduced by Tang et al. [67] use titanium dioxide nanowire loaded by gold nanoparticles. Also, they show that titanium dioxide nanowire loaded by gold nanoparticles with size 10 nm have the ability to efficient electron injection into the nanowires and implant as photoreceptor simulation in the rat retina success upon photo-illumination [67].

In the past years, nanowires with various structures but the same proposition for the regeneration of retina, recovery of vision have been developed, and their performance has been investigated. The researchers found that nanowires loaded with gold nanoparticles, showed a robust potential compare with cheapest and devices
for vision recovery lost due to retinal degeneration. Such observation emphasizes that nanowires are starting point in the progress for regeneration and implantable artificial photoreceptors.

5. Challenges and prospects

We believe that future strategies could involve incorporating smart nanostructures to simulate the extracellular matrix in addition to extracellular electric based on the intracellular electric charges to follow the polarization and adhesion of cells in the retina. For example, when the level of light changes, the system would control the transferred electrical signals by implants. Therefore we believe that nanostructures such as carbon nanotubes could serve to control the extracellular electrical charge incorporation nanoparticles such as gold nanoparticles for light sensation with a size of less than 10 nm to control the thermal stability. Figure 5 is showing the retinal cell polarization when external electrical charge

![Regenerative Medicine](image)

**Figure 4.**

Schematic of electron movement and holes toward the n-type and p-type silicon at the neural cell membrane for simulating the light senses. Light with receive to the n-type silicon containing the positive ions causes to the movement of electrons to the p-type silicon layer containing negative ions cause to senses the light and transfer produced faradic current to the cells [65]. (Refer to ACS ChemMatters online archive 2013–2014).

**Figure 5.**

The confocal microscopy image of seeded retinal ganglion cells into the random polypyrrole/graphene/poly (lactic-co-glycolic acid) nanofibers (a), polypyrrole/graphene nanofibers (b), the aligned polypyrrole/graphene/poly (lactic-co-glycolic acid) nanofibers (c), the SEM mapping of loaded gold nanoparticles into the polystyrene/grafted polymer (d) and the energy-dispersive X-ray for loaded gold nanoparticles into the polystyrene/grafted polymer (e) [24, 26].
supplied into the aligned nanofiber [26] and how nanofibers can simulate the extracellular matrix with loading the gold nanoparticles into the nanofibers which done by our team [24].

Finally, we envisage the use of stem cells incorporation of smart nanostructures and instruct the formation and regeneration of retina. Smart nanostructures with the ability to control the neural signals and simulation of the extracellular matrix could potentially circulate inside the retina and repair by cell adhesion, and in conjunction with smart nanostructures followed by transferring the adjusted signals to the brain in order to form clear vision. Therefore, our team has suggested to use the carbon nanowires to prepare the appropriate scaffold that can support both extracellular matrix and extracellular electric for improving the cell adhesion and light sensation.

6. Conclusion

Nanowires have had a substantial impact on retinal applications and still, have great potential to advance therapeutic implants for retinal regeneration. The development of new structures, and their incorporation into the simulation of the extracellular matrix, extracellular electric and light senses may lead to improvement of advanced structure for developing artificial retina. However, challenges still need to be addressed in controlling the local charges and light sensation improvement. It is believed that engineered nanowires with high efficiency will be increasingly used in retinal implantations. In recent years, several in vitro and in vivo reports have indicated the possibility of a significant effect of nanowires on the recovery of vision that lost due to retinal degeneration. Another challenge that must be addressed is the extracellular matrix to create a 3D scaffold, where it did not discuss in reports that have been done for nanowires usage in vision recovery, due to a limitation in synthesizing of nanowires. Also, it is crucial to discover the key factor promoting the assemblies of different layers of the retina and create specific scaffolds for polarization and construction of cells for transferring the neural signals to the brain. Developing nanowires to control the neural signals and guide the cells for polarization will be useful for engineering complex architecture as well as the retina.

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Competing interests

The authors declare no competing interests.
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Regenerative Medicine


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