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Carbon nanotube electrodes for retinal implants: A study of structural and functional integration over time

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ABSTRACT

The choice of electrode material is of paramount importance in neural prosthetic devices. Electrodes must be biocompatible yet able to sustain repetitive current injections in a highly corrosive environment. We explored the suitability of carbon nanotube (CNT) electrodes to stimulate retinal ganglion cells (RGCs) in a mouse model of outer retinal degeneration. We investigated morphological changes at the bio-hybrid interface and changes in RGC responses to electrical stimulation following prolonged *in vitro* coupling to CNT electrodes.

We observed gradual remodelling of the inner retina to incorporate CNT assemblies. Electrophysiological recordings demonstrate a progressive increase in coupling between RGCs and the CNT electrodes over three days, characterized by a gradual decrease in stimulation thresholds and increase in cellular recruitment. These results provide novel evidence for time-dependent formation of viable bio-hybrids between CNTs and the retina, demonstrating that CNTs are a promising material for inclusion in retinal prosthetic devices.

1. Introduction

Retinal prostheses aim to restore a degree of vision in patients with photoreceptor degeneration. The principle is either to take advantage of the surviving retinal circuitry or to target retinal ganglion cells (RGCs) directly, the output channels from the eye to the brain, encoding visual scenes into spike trains which are then transmitted to central visual targets via the optic nerve [1,2]. Epi-retinal prostheses consist of micro-electrode arrays (MEAs) apposed to the vitreal side of the retina, providing direct electrical stimulation to the RGC layer [3]. Individual electrodes have to deliver electrical pulses strong enough to elicit action potentials in surrounding RGCs without damaging the electrode material or the target tissue [4].

As the main interface between the prosthetic device and the tissue, electrodes are critically important components of any neuro-prosthetic system. For epi-retinal prosthetic devices, maximal resolution would ideally involve stimulation of individual RGCs, requiring electrode sizes to match those of their target neurons. However, small electrodes require higher charge densities to provide sufficient power to drive cells above firing threshold. If the charge density is too high, it can damage the tissue or the electrode, rendering the system unusable in the long term. Hence, it is important to estimate safe charge density limits based on electrode and tissue properties, allowing the system to function safely over prolonged periods. An optimal epi-retinal stimulation system would thus include very small, capacitive electrodes, located in close proximity to the RGC layer, requiring low amounts of current to depolarise RGCs to threshold. Small electrode dimensions guarantee stimulation localization and capacitive electrodes help avoiding direct charge injection into the tissue and undesired Faradaic reaction.

As charge density is intrinsically related to the effective surface area of the electrode, the geometry of stimulating electrodes strongly affects their charge density limit. As such, materials with large effective surface areas are ideal for efficient stimulation. Materials such as titanium nitride (TiN), iridium oxide (IrOx) and platinum grey are considered as gold standards for neural prosthetics and used respectively by the three main retinal prosthetic projects [5–7]. Carbon nanotubes (CNTs) have attracted much

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attention since their emergence in the field of bioengineering [8] due to their outstanding electrical [9,10], chemical and mechanical properties. Their high surface area [11], remarkable tensile strength [12], biocompatibility [13] and high conductivity [14] make them an alluring candidate material to use in neural prosthetic electrodes [15–17].

Neurodegeneration is characterized by strong glial proliferation, and rejection of epi-retinal electrodes by glial cells can potentially widen the gap between electrodes and their target neurons, thus increasing the amount of charge required to elicit action potentials. The glial population of the retina, consisting of microglia (scattered throughout the inner retinal layers), astrocytes (horizontal syncytium at the nerve fibre layer) and Müller cells (spanning the retina transversely) maintain homeostasis [18,19], provide immunological protection [20,21] and structural support [22,23]. Macroglia (astrocytes and Müller Cells) are also the source of the inner limiting membrane (ILM), a basement membrane providing physical and electrostatic barrier between the vitreous and the retina [24]. Similar to other basement membranes, the ILM is composed of three layers [25]: the *lamina rara interna* (adjacent to glial cell endfeet), the *lamina densa* and the *lamina rara externa* (contiguous with the vitreous humour). These layers have the typical molecular constitution found in basement membranes with laminins, collagen, and other heparan sulfate proteoglycans [26–29]. Integration of epi-retinal electrodes into the ILM is essential to reduce the distance between stimulating electrodes and target neurons.

Understanding the interactions between stimulating electrodes and the complex micro-environment at the vitreo-retinal interface is thus an important step for the successful design of efficient epi-retinal prosthetic devices. In this study, we investigated the response of RGCs, macroglial cells and the ILM of dystrophic retinas (here, we used the Crx knockout mouse model of Leber congenital amaurosis [30,31]) to interfacing with CNT electrodes over up to three days *in vitro*. During this incubation period, we observed a substantial increase in the coupling between RGCs and CNT electrodes, reflected both by electrophysiological and anatomical changes. Signals exhibited a gradual improvement in signal-to-noise ratio and decrease in stimulation thresholds. At the same time, CNT structures became gradually integrated into the ILM, reducing the distance between electrodes and RGCs.

2. Materials and methods

2.1. Device fabrication

We fabricated both MWCNT (Multi-walled CNT) MEAs and isolated MWCNT islands (Fig. 1a) to investigate respectively the electrophysiological and morphological impact of CNT electrodes interfaced with the inner retina. Our CNT assemblies displayed a high surface area characteristic of unaligned CNTs grown by chemical vapour deposition (CVD, Fig. 1b). Active MEAs were used to stimulate cells and to record electrophysiological signals from the RGC layer in intact, live tissue. Passive MEAs were used to study the structural integration of CNT islands (initially just loosely attached to a silicon/silicon dioxide (Si/SiO_2) substrate) into retinal tissue.

The fabrication process for electrically connected CNT MEAs has been described elsewhere [17], and isolated CNT islands were fabricated using a similar process, but involving fewer steps [32]. Briefly, Ni was patterned on Si/SiO_2 substrates by photolithography, and then used as a catalyst to grow CNTs by CVD. For active MEAs, the CNT islands had a 30 μm diameter and a 200 μm pitch (30/200). Both processes require nickel, a toxic metal, to be used as a catalyst for CNT growth. Nickel is effectively embedded in the CNTs and has no adverse effects on neuronal cells, as validated by long term culturing experiments and energy-dispersive x-ray spectroscopy testing [32]. Long-term stability of our CNT electrodes was previously tested extensively in cell cultures [33].

Passive MEAs had two types of islands. 30 μm islands were organised in a central orthogonal lattice and had a 50 μm pitch whilst 100 μm islands were organised in an orthogonal lattice concentric to the 30 μm island lattice (Fig. 1b4) with a 200 μm pitch. Some of the islands in Fig. 1b4 are intentionally displaced to illustrate how easily they can be detached for the formation of functional bio-hybrids (see below). The charge injection limit of the active CNT electrodes was ~2 mC/cm^2 [34]. With large 3D surface areas, CNT islands are more efficiently coupled to biological tissue when hydrophilic. This was achieved through ionisation with Nitrogen plasma for 10 min followed by submersion in de-ionised H_2O for at least 24 h prior to retinal interfacing.

2.2. Retinal isolation

Experimental procedures were approved by the UK Home Office, Animals (Scientific procedures) Act 1986. Cone rod homeobox (Crx) knockout animals were sacrificed by cervical dislocation followed by immediate enucleation. Isolation of the retina was performed in carboxygenated (95% CO_2/5%CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): 118 NaCl, 25$NaHCO_3$, 1NaH_2PO_4, 3KCl, 1$MgCl_2$, 2$CaCl_2$, 10$C_6H_{12}O_6$, 0.5 L-Glutamine (Sigma Aldrich, UK). First, the cornea was pierced with a hypodermic needle (23G gauge, BD Microlance 3, USA). Then, a pair of Vannas scissors (WPI, USA) was used to shear through the cornea, along the ora serrata before removing the lens. The sclera was then peeled off, using two pairs of sharp forceps, exposing the retina. The vitreous humour was teased off using those forceps, taking great care not to pierce the retina or remove the ILM.

2.3. Electrophysiological setup

Isolated retinas were mounted onto MEAs with the RGC layer facing down onto the electrodes and the optic disc outside the MEA active area. To improve coupling between the tissue and the electrodes, a polyester membrane filter (5 μm pores) held the retina in place whilst being weighed down by two stainless steel anchors bearing a framework of parallel glass capillaries. Thirteen retinas were mounted onto CNT MEAs and six onto TiN MEAs (MultiChannel Systems, Reutlingen, Germany).

To preserve physiological conditions, the tissue was perfused with oxygenated aCSF at 1 ml/min over the course of up to 72 h using a peristaltic pump (SCI400, Watson Marlow, UK). During the recording of electrophysiological activity, retinal explants were maintained at 32 °C using a temperature controller (TC02, MultiChannel Systems, Reutlingen, Germany) regulating a metallic plate below the MEA (MEA1060 INV, MultiChannel Systems, Reutlingen, Germany) and an inline heater for the inflow of aCSF (Ph01, MultiChannel Systems, Reutlingen, Germany). To prolong the viability of the tissue, retinas were kept at room temperature overnight [35,36].

Although Crx−/− mice do not develop photoreceptor-mediated vision, melanopsin-induced phototransduction occurs in intrinsically photosensitive RGCs in the presence of blue light [37]. To avoid activation of these cells, all experiments were carried out in complete darkness.

Electrophysiological signals were recorded using 60-channel MEAs interfaced with a computer running the proprietary software MC_Rack (MultiChannel Systems, Reutlingen, Germany) using an MEA1060INV (Multi Channel Systems, Reutlingen, Germany) amplifier via a PCI MCS card.

Recordings were acquired at 25 kHz sampling frequency.