

Neural Interfaces – Abstracts

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Carbon based nanomaterials for active neuronal interfacing: impact on signaling and regrowth

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In neuroscience, a relevant source of innovation in the design of nanomaterials has emerged from the vast research area of neural electrodes and interfaces: the need of overcoming the challenges posed, for example, by adapting the mechanical properties and the shape of planar electrodes to the brain or spinal cord, has been translated into applications and developments of new materials. Studying nanomaterials for implementing neural interface performance has long suggested that physical and chemical features of the electrode per se are crucial factors to control the long-term tissue response. In neural interface engineering, the use of carbon nanotubes has opened an additional area of development. This new class of materials can improve exploring fundamental biological phenomena as well as contribute to biomedical and clinical applications. I will present our results concerning multi-walled carbon nanotubes (MWCNTs) interfacing neurons, I will describe the effects of such materials on neuron signaling when cell maturation, axon growth and synapse formation are driven by the nanomaterials.

CMOS-based multielectrode arrays for high-resolution electrophysiology in networks and brain circuits

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Brain functions and pathological dysfunctions emerge over time by involving a complex dynamics of intracellular and intercellular signalling interactions occurring within large networks of heterogeneous types of connected and variable spiking neurons. While techniques for perturbing neuronal networks by acting at molecular or cellular scales have remarkably progressed over recent years, resolving and monitoring the dynamical changes of these signals from a large number of single neurons and, possibly, at multiple scales simultaneously remains a fundamental challenge. Our approach is based on CMOS-circuit design and micro-/nano-technologies to realize large arrays of closely spaced electrodes providing high-resolution electrical recordings in vitro and in vivo and to study neuro-electrode couplings for enhanced interfacing capabilities. During this seminar, I will present and discuss these technologies and the experimental capabilities achieved so far on cell culture models and brain circuits. As it will be presented, our planar and implantable CMOS-devices provide dense arrays of several thousands of simultaneously recording microelectrodes and allow studying at high-resolution network responses and spontaneous activity under conditions of induced neurodegeneration, genetic manipulation or during artificial/natural inputs integration. In parallel, we are exploring the potential of on-chip plasmonic 3D nanostructures that we have recently demonstrated for chemical spectroscopy, cell poration and nanofluidic intracellular interfacing.

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Neurons on Nanotopographies

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Topography, the physical characteristics of an environment, is one of the most prominent stimuli neurons can encounter in the body. Many aspects of neurons and neuronal behavior are affected by the size, shape, and pattern of the physical features of the environment. A recent increase in the use of nanometric topographies, due to improved fabrication techniques, has resulted in new findings on neuronal behavior and development. Factors such as neuron adhesion, neurite alignment, and even the rate of neurite formation have all been highlighted through nanotopographies as complex phenomena that are driven by intricate intracellular mechanisms.

The translation of physical cues is a biologically complex process thought to begin with recognition by membrane receptors as well as physical, cell-to-surface interactions, but the internal biological pathways that follow are still unclear. In this respect, nanotopography would be a more suitable platform on which to study receptor interfaces than microtopography because of the subcellular topographical features that are relevant in scale to the receptor activity. Ultimately, the characterization of this unknown network of pathways will unveil many aspects of the behavior and intracellular processes of neurons, and play an important role in the manipulation of neuronal development for applications in neural circuits, neuroregenerative medicine and prostheses, and much more.

Probing the interface between the cell membrane and the nanoscale electrode

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The rapidly evolving field of nanotechnology creates new frontiers for probing neuronal cells. Recently, we and other groups show that vertical nanopillars protruding from a flat surface support cell survival and can be used as subcellular sensors to probe biological processes in live cells. In particular, nanopillar electrode has been shown to record cellular electric activities with significantly enhanced signal-to-noise ratio. The interaction between the cell membrane and the recording electrode is crucial for such sensors. We are interested in exploring nanotechnology and novel materials to improve the membrane-surface interactions. Vertical nanopillars deform the plasma membrane inwards and induce membrane curvature when the cell engulfs them, leading to a reduction of the membrane-substrate gap distance. The reduced gap distance results in a higher sealing resistance for the electrode and better signal detection for electrophysiological measurement in cells. We also found that the high membrane curvature induced by vertical nanopillars significantly affects the distribution of curvature-sensitive proteins and stimulates several cellular processes in live cells. Our studies show a strong interplay between biological cells and nano-featured surfaces, which is an essential consideration for future development of interfacing devices.

Mesh electronics: A paradigm for tissue/neuron like brain probes

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Nanoscale materials enable unique opportunities at the interface between the physical and life sciences, for example, by integrating nanoelectronic devices with cells and/or tissue to make possible bidirectional communication at the length scales relevant to biological function. In this presentation, I will overview a new paradigm for seamlessly merging nanoelectronic arrays and circuits with the brain in three-dimensions (3D). First, the design consideration of matching structural, mechanical and topological characteristics of neural probes and brain tissue will be discussed, thus leading to the new concept of tissue-like mesh electronics. Second, quantitative time-dependent histology studies demonstrating the absence of a tissue immune response on at least a year time-scale, as well as interpenetration of neurons and neurofilaments through the open mesh electronics structures will be presented. Third, uniquely stable electrophysiology data demonstrating the capability to track and stably record from the same single neurons and neural circuits for more than a year will be described. Finally, I will describe several current directions of research, including studies that push the limits of the electronics design paradigm as well as work focused on fundamental brain science problems, including aging and vision. Finally, the prospects for future advances of these nanoelectronic tools for overcoming complex challenges in neuroscience through the development of precision electronic therapeutics and brain-machine interfaces will be discussed.

Gap junctional proteins and the formation of coupled neural circuits

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Gap Junctions (GJ) perform a variety of important functions in intercellular communication. In early development, they may function as important general adhesive structures that gradually become more specific to tissues and, among neurons, to particular circuits where they function as electrical synapses that can be bi-directional or rectifying, and subject to plasticity. In addition, hemichannels comprised of gap junctional proteins may function as mediators of local extracellular communication by releasing, for example, intracellular ATP. Examples from our recent studies on the medicinal leech nervous system will be discussed.

Revealing New Physiology with Brain In Vitro Models

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Micro-engineered cell culture models, so-called Organs-on-Chips have emerged as a new tool to recapitulate human physiology and drug responses. Multiple studies and research programs have shown that Organ-on-Chips can recapitulate the multicellular architectures, vascular-parenchymal tissue interfaces, chemical gradients, mechanical cues, and vascular perfusion of the body can produce levels of tissue and organ functionality, as well as mimicry of human disease states, which are not possible with conventional 2D or 3D culture systems. Here we exploit the micro-engineering technology in a novel system-level approach to disintegrate the functions and coupling of neurovascular unit in to its individual cellular compartments while keeping the paracellular metabolic coupling. Using individual, fluidically connected, chip units we created a system modeling influx and efflux functions of the brain vasculature and interaction with the brain parenchyma. Based on proteomic and metabolic assessment we would conclude that this coupled system mimicked the effect of intravascular administration of the psychoactive drug methamphetamine observed *in vivo*. Moreover, this method revealed an additional metabolic role of the of the brain endothelium to neural cell metabolism. In addition to the well established function of metabolic transport the brain endothelium secretes metabolites that are being directly utilized by neurons. This contribution isnot possible to evaluate in conventional *in vitro* or *in vivo* measurements.

In-vivo optogenetic neural interfaces

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Optogenetics is now an established technique in neuroscience, allowing optical control over specific neurons. However, delivering spatiotemporal patterns of light to neural circuits, either over large areas or at depth in tissue, is still a challenge. Techniques such as multiphoton microscopy have allowed depths up to ~1mm to be perturbed [1], but delivering light to depths beyond this requires insertion of a device, such as an optical fibre [2] or microfabricated structures containing waveguides [3] or miniature light sources such as microLEDs [4-5].

Here we report on the efforts to use microLEDs, formed from gallium nitride (GaN) based materials, to provide spatiotemporal control over neural circuits, at depth, during *in vivo* experiments.

More specifically, we show *in vivo* optogenetic control over neurons in the mouse cortex [6] and highlight work on membrane LED devices capable of being transfer printed to non-native substrates with either improved thermal characteristics (diamond membranes) or providing sophisticated control (CMOS circuitry).

The microLED devices have also been integrated with glass optrode arrays in the style of the well-known Utah arrays [7]. This has the potential to allow 181 independent optical sites that are controlled electrically and so able to be packaged into a miniature system for studies in the non-human primate.

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Photovoltaic Restoration of Sight in Retinal Degeneration

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Retinal degenerative diseases lead to blindness due to loss of the “image capturing” photoreceptors, while neurons in the “image-processing” inner retinal layers are relatively well preserved. Information can be reintroduced into the visual system using electrical stimulation of the surviving inner retinal neurons. Some electronic retinal prosthetic systems have been already approved for clinical use, but they provide low resolution and wired retinal arrays require very difficult implantation procedures.

We developed a completely wireless photovoltaic subretinal prosthesis which converts light into pulsed electric current, stimulating the nearby inner retinal neurons. Visual information is projected onto the retina by video goggles using pulsed near-infrared (~880nm) light. This design avoids the use of bulky electronics and wiring, thereby greatly reducing the surgical complexity. Optical activation of the photovoltaic pixels allows scaling the implants to thousands of electrodes, and multiple modules can be tiled under the retina to expand the visual field.

We found that similarly to normal vision, retinal response to prosthetic stimulation exhibits flicker fusion at high frequencies (>20 Hz), adaptation to static images, antagonistic center-surround organization, and non-linear summation of subunits in the RGCs receptive fields, providing much higher spatial resolution than the average size of receptive fields. Such implants elicited responses to both onset and offset of light, with approximately 1/6th of the natural contrast sensitivity. Photovoltaic arrays with 55 μ m pixels implanted in blind rats restored a grating visual acuity up to a pixel pitch. If these results translate to human retina, such implants could restore visual acuity up to 20/200. Higher resolution arrays may be enabled by 3-dimensional electro-neural interfaces, which are currently being tested.

Ease of implantation and tiling of these wireless modules to cover a large visual field, combined with high resolution, opens the door to highly functional restoration of sight.

ALS as a spatiotemporal mis-localization disease

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Amyotrophic Lateral Sclerosis (ALS) is a devastating, rapidly progressing neurodegenerative disease that affects lower and upper motor neurons. Increased neuronal activity and mitochondrial defects were suggested to play a key role in this neurodegenerative process. Here, we hypothesize that ALS motor neurons possess a unique spatial metabolic demand which facilitates rapid and fatal motor neuron degeneration. High energy demand and mitochondrial usage in the neuromuscular junction, away from the cell body, may be a source for cellular vulnerability which facilitates neurodegeneration in the presence of prolonged mitochondrial stress. We use different mouse ALS disease models to characterize motor neurons synaptic metabolic demand and compare it to other neuronal subtypes. Furthermore, we try to understand if this process leads to neurodegeneration via inhibition of axonal and synaptic local protein synthesis. There are currently no available therapies for ALS patients, and our study may contribute for the understating of disease mechanisms and future drug development.

Nanowires: a promising material for designing neural implants

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Semiconductor nanowires have been increasingly used in a broad range of bio-applications. In this talk, the work undertaken in our lab towards the use of nanowires for neural interface applications will be reviewed. We have shown that patterns of vertical nanowires can guide and sort axons^{1,2}. We have also shown that PNS and CNS neurons thrive when cultured on vertical arrays of nanowires^{3–5}, whereas the growth of glial cells on such arrays is limited compared to when cultured on flat substrates^{4,5}. Phase holographic microscopy live cell imaging shows that the proliferation and motility of cells cultured on nanowire arrays are greatly affected by the nanowires^{6,7}, which may explain the different effects of nanowires on neurons and glial cells. Taken together, these results suggest that arrays of semiconductor nanowires are promising nanomaterials for designing neural interfaces that support neurons steadily over time while limiting the formation of a glial scar.

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From 2D to 3D platforms for directing neurons and neural growth

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Controlling cell navigation, organization and growth has great importance in tissue engineering and regeneration, for a wide range of tissues. In this talk I will present our recent studies of magnetic-based manipulations for nerve regeneration and for controlled drug delivery.

Neurons rely on physical topographical cues. Techniques to control cell growth include bio-mimetic scaffolds, nano-fibrous constructs, structured gels, etc., offering a mechanical guide to the regenerating cells. We have developed a novel approach of injectable hydrogels combined with magnetic nanoparticles (MNPs), to be incorporated directly into the injured site. We show that gel fiber structure can be aligned in situ dynamically and remotely in response to an external magnetic field. Neurons embedded within the aligned gel demonstrated polarized growth pattern. We show a directed and effective neuronal regeneration for neurons embedded in the aligned gels in vitro and ex-vivo. This platform is now examined as a novel method to direct neuronal growth and to bridge gaps efficiently post trauma. In addition, we functionalized the MNPs by coating them with nerve growth factor, presenting a 'smart' delivery system of biomolecules, together with integral guidance cues. The enrichment of the gel platform with biomolecules conjugated MNPs promoted differentiation and elongation.

As physical mechanical forces play a key role in neuronal morphogenesis, we used magnetic nanoparticles (MNPs) as mediators to apply forces locally on neurons throughout their migration and organization. Following incubation, the MNPs accumulated in the cells, turning the cells sensitive to magnetic stimulation. Applying magnetic fields with controlled magnetic flux densities led to pre-designed cellular movement and to organized networks. Growing neurons loaded with MNPs under magnetic fields has affected the pattern of dendritic trees. With this method we could control drug distribution and delivery as well. Our study presents an emerging magneto-chemical approach for promoting tissue regeneration.

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Title: Large scale functional connectivity mapping in cortical circuits

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Brain activity is generated and propagated via a large repertoire of transmembrane currents with different time scales. These currents shape the electric potential as measured with multi-electrode arrays. Despite the wealth of information embedded in these potentials and the abundant usage of extracellular probes, only limited information is usually extracted: time stamps of action potentials in single neurons and local field potential. Here we focus on slow potentials, indicative of synaptic currents, to examine whether morphological properties of single neurons can be also extracted. We utilized the unique anoxia resistance of turtle brains to record from isolated eye-brain preparations in vitro. Under these conditions we could investigate intact circuits receiving realistic sensory drive, while benefiting from the high stability, accessibility and ease of manipulation of in vitro approaches. By spike-triggering the LFP recorded from large-scale electrode arrays (up to 1024 channels), we could determine the identity of the neurons (excitatory vs. inhibitory) and estimate the spatial distribution of their functional axonal projections in cortex. We found that these projections showed a strong bias towards the lateral zone of cortex. Interestingly, such a bias could explain the consistent observation of medially propagating waves in this cortex.

Abstracts for Posters

1. Innervation of an engineered muscle graft for reconstruction of muscle defects

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Autologous muscle flaps are commonly used to reconstruct defects that involve muscle impairment. In order to maintain viability and functionality of these flaps, they must be properly vascularized and innervated. Tissue engineered muscles could potentially replace autologous muscle tissue, but still require establishment of sufficient innervation to ensure functionality. In this study, we explored the possibility of innervating engineered muscle grafts transplanted to an abdominal wall defect in mice, by transferring the native femoral nerve to the graft. Six weeks post-transplantation, nerve conduction studies and electromyography demonstrated increased innervation in engineered grafts that were neurotized with the femoral nerve, as compared to non-neurotized grafts. Histological assessments revealed axonal penetration and formation of neuromuscular junctions within the grafts. The innervation process described in this study may advance the fabrication of a fully functional engineered muscle graft that will be of utility in clinical settings.

2. Single layer graphene promotes neuronal activity by regulating potassium ion channels in interfaced neural networks

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Graphene, with its peculiar bi-dimensional crystal arrangement of pure carbon atoms, is catching the eye of the research community with its extraordinary physicochemical properties. In particular, single layer graphene (SLG) potential applications in biology and (nano)medicine have been deeply investigated during last years. Although preliminary reports have shown that graphene based materials can be safely interfaced with neuronal cells, to date an exhaustive functional study of neuronal networks developed interfaced with SLG is missing. For the first time, we show that uncoated SLG is not only fully biocompatible but, surprisingly, induces in cultured neurons an increased network synaptic activity, presumably by altering the availability of extracellular K^+ ions. The homeostatic changes observed in SLG-interfaced cells, as well as the increased network activity, were not observed when neurons were interfaced neither on a many-layers graphene (MLG), nor onto conductive thin gold substrates, suggesting a highly material specificity of this adaptive interaction. In particular, combining material characterization, electrophysiological patch-clamp recordings and neuronal network simulations, we propose a model in which the peculiar interaction of SLG with the ionic species present in solution shifts a significant fraction of phasically firing neurons towards a tonic phenotype, and these changes ultimately reflect as an increase in firing activity of the entire network.

3. A Quest for the Cortical Representation of Subjective Surprise With a Virtual Reality Neurofeedback Platform

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It is well known that responses to external stimuli are context dependent. Specifically, when a rare event occurs, it elicits the P300 event-related potential (ERP), while when a stimulus repeats itself responses are attenuated. Context dependency can be modelled by conditional probabilities of future events. Arguably, these are the measures that give rise to the experience of expectation and surprise. These expectations, or conditional probabilities, should depend on both the memory capacity of subjects and on their goals. Using the Information Bottleneck method developed by Tishby et al., a trial-by-trial subjective surprise signal can be calculated, taking into account the subject's memory resources and goals. This calculated surprise signal can then be tested against physiological data.

We examine the above hypothesis in the framework of an auditory oddball experiment. Our results indicate a correlation between the trial-by-trial measure of subjective surprise and an EEG metric based on the P300 component. Moreover, a platform we developed containing a virtual reality game combined with EEG measurements, allows us to have an ERP-based neurofeedback in which the subject is getting feedback within the game on his current surprise-related EEG feature.

4. Extended LFP study reveals the dorsal STN as the source of Beta oscillations

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Enhanced beta oscillations in the Subthalamic nucleus (STN) are being studied as a signature for closed loop deep brain stimulation (DBS) in Parkinson's disease (PD). Despite the assumed emergence of beta oscillations from dorsal STN, no Local Field Potentials (LFP) recordings were conducted outside of the STN borders, due to limitation of lead configuration. Therefore, the possibility of extra-STN beta activity recorded due to volume conducted is yet to be excluded.

The aim of this study was to investigate the characteristics of intra and extra STN LFPs in PD using the novel 8 ring contact DBS leads to better locate the source of these oscillations.

LFPs were recorded intra-operatively from 11 DBS leads (BS Corp.) implanted in 8 PD patients. Phase, amplitude and phase-amplitude coupling (PAC) of the LFP signal were extracted and analyzed for each contact pair.

Beta oscillatory power, phase and PAC between beta and broadband gamma bands were all maximal at dorsal STN contacts, compared to ventral STN and other subcortical areas. These contacts were correlated with the contacts clinically used for DBS in these patients. PD severity of motor symptoms (bradykinesia and rigidity) was significantly correlated with the beta power at the dorsal STN, while tremor severity was correlated with alpha power.

Our findings support the dorsal STN as the primary source of beta oscillations in the STN region, and argue against the hypothesis that STN beta activity represents volume-conducted cortical activity.

5. Optimization of drying and membrane staining techniques for studying cell interface with 3D models

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Keywords: Retinal Pigment Epithelium Cells; Photoreceptor Precursor Cells; Cell membrane; cell interface; Scanning Electron Microscopy, Focus Ion Beam Electron Microscopy; fixation; drying; staining; 3D scaffolds; 3D models

Abstract

Introduction

Studying the neural-surface or neural-electrode interface by SEM/FIB is of great importance for various applications. However, during the preparation of the cells for imaging, significant artifacts can be caused by morphological changes occurring during cell drying and fixation processes. The aim of the current research is to optimize drying and membrane staining techniques of cells in order to study the neuron-electrode or neuron-surface interface.

Materials and Methods

A Retinal pigmented epithelium (ARPE) and photoreceptor precursors (PRP) cells were cultured on glass for 24hrs and the effect of 4 different drying techniques (CPD- critical point drying machine, HMDS, Resin and OTOTO air dry) and four different staining techniques (Osmium, Osmium+salts, OTOTO, osmium + uranyl) on the cell morphology was investigated. The cells of choice possess fine protrusions, which can be used for evaluating the drying and fixation effect on cellular structures. Scanning electron microscope (SEM) imaging was used to measure the preservation of the cell-surface interface by analyzing the percentage of broken protrusions. In addition, Focus Ion beam (FIB) and SEM imaging were used to evaluate the cellular membrane structure contrast, for both the upper and the lower membrane, for the investigated staining techniques. Furthermore, as a cellular scaffold we used 3D SU-8 wells fabricated by photolithography combined with 3D two-photon based nano-printing by (Nanoscribe GmbH, Germany).

Results and Discussion

The percentage of broken cellular extensions in the resin drying technique (22.57%,13.3) and OTOTO air dry (27.74%, 14) were significantly lower as compared with CPD (59.73%,10.3) and HMDS (61.42%,13.7) (mean, STDEV). Furthermore, the inner cellular structures contrast was enhanced by the resin drying and OTOTO staining accompanied with a higher membrane contrast and with the lowest shrinkage (less artifacts).

Conclusion

We optimized the fixation and staining protocols and studied cells interface with different surfaces including 3D surfaces and structures. Our results reveal that the resin and OTOTO air dry technique significantly preserve cellular structure as compared with CPD or HMDS techniques. The OTOTO staining yields the most detailed images of the cell, enables the visualization of cell organelles and membrane. Finally, this study highlights that a proper drying and fixation method is vital for the preservation of natural cellular morphology which is of great importance for the study of cell-surface interface.

6. Brain–islets interface: implication for Alzheimer’s disease and obesity

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Background. Alzheimer’s disease (AD) dementia is often associated with brain insulin resistance and peripheral metabolic dysfunctions. Recently, we showed a high efficacy of insulin delivery to the brain using intracranially implanted pancreatic islets encapsulated in alginate hydrogel (Bloch et al., *J Tissue Eng Regen Med.* 2017). In the current study, we studied the effect of intracranially grafted islets on cognitive and peripheral metabolic dysfunctions in rats with sporadic AD.

Methods. AD-like dementia associated with obesity was induced in inbred Lewis rats using a single intracerebroventricular injection of streptozotocin (icv-STZ). Two months after icv-STZ, a small number of syngeneic islets (100 islets/recipient) was implanted in the cranial subarachnoid cavity of icv-STZ rats. Cognitive functions and peripheral metabolism were studied in all tested groups.

Results. Icv-STZ treatment induced increased food intake, accelerated body weight gain, and elevated levels of insulin and leptin in the blood without alteration of peripheral glucose homeostasis. Two and six months after islet transplantation, the spatial learning and memory of transplanted icv-STZ rats were significantly improved compared to sham-operated icv-STZ rats. Grafted islets reduced body weight gain, food consumption, peripheral insulin resistance and hyperleptinemia. In addition, grafted islets reversed deficiency of glucose transporter-2 in the brain of icv-STZ rats, but did not affect expression of brain insulin receptor.

Conclusions. Intracranial islet transplantation attenuates cognitive decline and obesity-related metabolic dysfunctions in rats with sporadic AD. Efficient and metabolically regulated islet-based insulin delivery to the brain provides a novel approach for therapy of AD associated with peripheral metabolic dysfunctions.

7. Excitation/Inhibition ratios determine neuronal network activity

Nirit Sukenik

Elisha Moses lab, Weizmann institute

Background

Neural cultures exhibit a typical synchronized spontaneous network activity, which can typically be modified mainly using chemical blockers. We show experimentally how to modify the network activity by controlling its excitatory/inhibitory (E/I) balance. Labelled hippocampal neurons are sorted using fluorescence activated cell sorter (FACS) and seeded in a range of ratios, to reconstruct into unique neural cultures based on changing the E/I ratios.

Results

We trace out the inter burst interval (IBI) values of whole network bursts for the various mixes of E/I neuronal cultures as a function of inhibitory percent. Surprisingly, we find that it follows a U shape trend with significantly higher values at the edge values. Networks with little or no inhibitory neurons are active in a very distinctive, reproducible manner characterized by low bursting rates, long duration and large amplitude bursts, in agreement with spontaneous activity of control cultures blocked with bicuculline (inhibition blocker). We used a range of pharmacological agents to investigate how sensitive the system is to inhibition blocking and follow particular behaviors according to the culture inhibitory percent.

Conclusion

Our system enables us to characterize cultures based on specific cell ensemble properties, such as varying amounts of inhibitory and excitatory inputs. The importance of E/I ratios can be investigated in full, and is found to influence a variety of network behaviors. The abundance of theoretical models for this system now enables the study of open theoretical questions and provides a precise comparison to our experimental results.

8. Nisim Perets

Dani Offen lab

Abstract: Exosomes are small Nano-particles that are naturally used for molecular communication between numerous cell types. Mesenchymal stem cells (MSC) have been shown to have therapeutic and migration abilities in the brain. Here we show that exosomes derived from Mesenchymal stem cells (MSC-exo) present homing and migration abilities to areas of damages in the brain when given by intranasal administration. Furthermore, MSC-exo treatment in a model of Autism (BTBR) resulted in significant behavioural improvement of social interaction and repetitive behaviour. And in Model of Alzheimer (5xFAD) resulted in significant improvement in learning and memory.

Altogether, MSC-exo can be used for non-invasive, targeted treatment to neurological and psychiatric disorders.

9. Optical Imaging Characterization of Visual Cortical Responses to Natural and Prosthetic Retinal Stimulation

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Abstract

Retinitis Pigmentosa (RP) and Age-related Macular Degeneration (AMD) are two common degenerative diseases of the outer retina leading to blindness. The inner retinal layer in these diseases are relatively preserved presenting a good candidate for vision restoration technologies through electrical stimulation of the surviving cells.

Interestingly, the implantation process presents a unique study model whereby a localized retinal degeneration zone is induced by the implant as a result of photoreceptors separation from the RPE layer, which is manifested by a cortical non responsive zone to visible light termed lesion projection zone (LPZ). Implantation in a normally sighted animal will therefore enable the study of combined input of prosthetic and natural information and their integration in the visual cortex. This ability is of high importance for understanding prosthetic vision in AMD patients, where the central vision is damaged while the peripheral vision is preserved

While previous studies used visual evoked potential (VEP) to assess visual performance of natural and electrical stimulations, we aim to study the activation patterns at a higher spatial resolution using voltage sensitive dye imaging (VSDI). VSDI enables the visualization of the responsive visual cortical zones at a high spatial resolution thus facilitating the study of cortical natural vision induced by regions of healthy retina and prosthetic vision induced by the retinal implant. Towards this end, we constructed a unique slit-lamp based projection system for stimulating the retina which combines both visible light, for the stimulation of natural vision, and a NIR light source for stimulating the retinal implant.

This study is an important step towards the understanding of visual cortex processes following prosthetic retinal activation and the integration of natural and prosthetic vision.

10. Modulation of scar tissue formation in injured nervous tissue cultivated on surface-engineered coralline scaffolds

Orly Weiss, Ariel University

Biological scaffolds provide a supportive environment for tissue generation and may be strong candidates for treating damage repair in the nervous system. Scaffolds made of coral skeletons promote bone regeneration and provide nutritional support for neurons *in vitro*. This study characterizes the structural reaction of an injured nerve tissue to contact with coralline scaffolds of distinct topologies, as a prerequisite test of its usage for nervous tissue repair *in vivo*. Hippocampal slices from postnatal rats were cultivated on two distinct shaped coralline scaffolds: 1. micro-rough surface, porous, complex 3D architecture - made of intact skeleton pieces (ISP); 2. Macro-rough surface, non-porous, planar - a powder-like scaffold made of grained skeleton (GS). Slices strongly adhered to the surface on both scaffolds. On ISP, slices deformed into complex 3D structures by engulfing the outer surface of the scaffold without penetrating the pores, yet, preserving their coherence. By contrast, on GS, slices were planar but broken into interconnected small segments of tissue. Both scaffolds induced formation of reactive astrocytes. However, whereas on GS these cells tightened into a single thin stripe at the slice's periphery, on ISP, they dispersed globally, forming meshes having inter-cell distances spanning up to dozens of microns.

Hence, implantation of scaffolds of pre-designed roughness and porosity can provide a control over the coherence and shape of nerve and scar tissues in site of injury, opening a route for cell invasion, thus assisting in damage repair following brain wounds.

11. *In vivo Neuroimaging of Exosomes using Gold*

Nanoparticles

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Exosomes, extracellular vesicles that participate in cell-to-cell communication, are evolving as effective therapeutic tools for various pathologies. These extracellular vesicles can bypass biological barriers such as the blood-brain barrier, and can function as powerful nanocarriers for drugs, proteins and gene therapeutics. However, to promote exosomes' therapy development, especially for brain pathologies, a better understanding of their mechanism of action, trafficking, pharmacokinetics and bio-distribution is needed. In this research, we established a new method for non-invasive *in-vivo* neuroimaging of mesenchymal stem cell (MSC)-derived exosomes, based on computed tomography (CT) imaging with glucose-coated gold nanoparticle (GNP) labeling. We demonstrated that the exosomes were efficiently and directly labeled with GNPs, via an energy-dependent mechanism. Additionally, we found the optimal parameters for exosome labeling and neuroimaging, wherein 5 nm GNPs enhanced labeling, and intranasal administration produced superior brain accumulation. We applied our technique in a mouse model of focal ischemia. Imaging and tracking of intranasally-administered GNP-labeled exosomes revealed specific accumulation and prolonged presence at the lesion area, up to 24 hrs. We propose that this novel exosome labeling and *in-vivo* neuroimaging technique can serve as a general platform for brain theranostics.

12. Graphene Oxide Flakes Selectively Target and Down-regulate Excitatory Hippocampal Synapses *in Vivo*

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Nano-materials, tailored as delivery vehicles, may significantly help in targeting neurotransmission and manipulate neuronal functions against severe pathologies. Graphene is a highly versatile two-dimensional nanomaterial widely adopted in many domains of science and technology, including biomedical applications. In neurology, graphene represents a promising tool for neuronal implants or bio-devices, with potential applications that range from neurooncology to neuroregeneration. In cultured neurons, small graphene oxide nanosheets (s-GO) were reported to directly and selectively size down glutamatergic activity without affecting cell viability. Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS) that mediates neuronal differentiation, migration, synaptic maintenance and transmission and growing evidence suggest that an abnormal release of glutamate may lead to neuronal excitotoxicity, neurodegeneration and even neurological disorders, including pain. Localized targeting and fine-tuning of the glutamatergic system are attractive targets in neuroscience. Here, we describe, by patch-clamp electrophysiology, the ability of s-GO flakes to impair synaptic release in excitatory synapses of both cultured neurons and hippocampal acute slices, by interfering with the probability of vesicle release leading to a down regulation of the glutamatergic activity. We further investigated whether such materials similarly affect glutamatergic transmission *in vivo*, by injecting s-GO in the dentate gyrus of the hippocampus of juvenile rats. We recorded neuronal activity from *ex-vivo* hippocampal slices isolated 48 and 72h after s-GO injections. Our results demonstrate that s-GOs target and down-regulate glutamatergic synapses *in vivo* and illustrate the potential of these nanosheets to be engineered as specific glutamate transmission modulators.

13. Engineering Personalized Neuronal Tissue Implants for Regenerative Medicine

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Tissue engineering has evolved as a promising strategy for regenerating diseased or injured organs. In this approach, cells are engineered in the lab as 3-dimensional constructs using biomaterials, promoting their assembly into a functioning tissue. Despite incremental improvements in the field of tissue engineering, no technology is currently available for producing completely personalized implants where both the cells and the scaffolding material are generated from the same patient, and thus do not provoke an immune response that may lead to implant rejection. In our study, fatty tissues are extracted from patients, and while the cells are reprogrammed to become induced pluripotent stem cells, the extracellular matrix is processed into a thermoresponsive personalized hydrogel. In this approach, we can develop different neuronal tissues that can tackle different neuronal diseases. Our developed technology promotes efficient cell differentiation within the hydrogel, and allows to generate functional cortical, spinal cord, and dopaminergic tissue implants. After generating and characterizing the personalized implants we show the ability of different neuronal implants to mend spinal cord injuries and to support the growth of dopaminergic neurons, respectively. We show that these implants have spontaneous electrical activity, respond to stimuli and can secrete tissue specific factors. This bioengineering approach may pave the way to personalized implants for regenerative medicine by minimizing the risk of immune rejection.

14. Electrophysiological characterization of Human Embryonic Stem cells-derived photoreceptor precursors

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Purpose: Cell replacement therapy, specifically transplantation of photoreceptor precursors (PRP), offers an innovative and promising approach for vision restoration in patients suffering from outer retinal degenerative diseases, such as retinitis pigmentosa (RP) and age related macular degeneration. Notwithstanding their great importance, little is known about their electrophysiological characteristics. The aim of the current research is to fully characterize the electrophysiological activity of PRP, and investigate their expression pattern of different voltage-gated ion channels.

Methods: Human embryonic stem cells (hESC line H9) were differentiated into PRP using eIWR 1, SAG and CHIR 99021 for up to 90 days. Whole-cell recordings of 30- 60- and 90-days old PRPs were performed for electrophysiological characterization. In addition, qPCR analysis was performed on PRP at these 3 time-points to characterize the expression pattern of the following voltage-gated ion channels: KCNV2, HCN1, SCN2A, CACNA1F, TMEM16B and the calcium pump PMCA. Finally, to characterize the electrically induced calcium currents we employed calcium imaging (rhod2) to visualize intracellular calcium dynamics in response to electrical activation.

Results: Voltage clamp recordings revealed that PRP express both voltage-gated sodium channels and voltage-gated potassium channels, as evident from the acquired current traces. Moreover, the voltage-dependent inward sodium and outward potassium currents were blocked by tetrodotoxin (TTX) and tetraethylammonium (TEA), respectively. These results are further supported by qPCR analysis which revealed significant and continuous increase in expression of voltage-gated channels from day 24 to 60 and 90, simultaneously with increase in CRX expression. Calcium imaging demonstrated our ability to visualize calcium currents induced by voltage clamping the cell membrane. An increase (approximately 3%) in intracellular calcium could be observed.

Conclusions: The full characterization of PRPs is an important step in devising cell therapy based vision restoration treatment strategies for patients suffering from degenerative diseases of the outer retina

15. Roy Maimon

Tel Aviv University

Axon degeneration and disruption of neuromuscular junctions (NMJs) are key events in Amyotrophic Lateral Sclerosis (ALS) pathology. Although the disease's etiology is not fully understood, it is thought to involve a non-cell-autonomous mechanism and alterations in RNA metabolism. Here, we identified reduced levels of miR-126-5p in pre-symptomatic ALS models, and an increase in its targets: axon destabilizing type-3 Semaphorins and their co-receptor Neuropilins. Utilizing compartmentalized *in vitro* co-cultures, we demonstrate that myocytes expressing diverse ALS-causing mutations promote axon degeneration and NMJ dysfunction, which were blocked by applying Neuropilin1 (NRP1) antibody. Finally, overexpressing miR-126-5p is sufficient to rescue axon degeneration and NMJ disruption both *in vitro* and *in vivo*. Thus, we demonstrate a novel mechanism underlying ALS pathology, in which alterations in miR126-5p facilitate a non-cell-autonomous mechanism of motor neuron degeneration in ALS.

16. 3D implantable nanostructures: Directing axonal navigation towards successful bridging of spinal segments

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Neural interfaces as prosthetic devices such as implantable stimulating electrodes, brain-machine interface demonstrate assistance in potential CNS function rehabilitation. However, biocompatibility of such materials pose serious questions to functional effectiveness and their practical use *in vivo*. In this work, we demonstrate use of freestanding self-assembly of nanotubes in the form of three-dimensional carbon nano-fibers scaffold (3DCNF) as neural implants that could effectively reconstruct neural growth *in vitro*, as well as improve biocompatibility and functional recovery when implanted into CNS injury site. By means of immunohistochemistry and confocal imagery, we show that these conductive 3D devices can guide formation of neural webs *in vitro* into a dense 3D hybrid of CNT and neurite networks. To understand if this fiber regrowth morphology shaped by 3D structure support neuronal reconnection, we performed simultaneous electrophysiological recordings of local field potential to reveal interactions between segregated spinal cord segments. We further assessed *in vivo* adaptability of such 3D devices in a physiological environment, in brain as well as spinal cord. When implanted into a rodent model of spinal cord injury, 3D CNF mediate functional locomotor performance (assessed by behavioral studies i.e. BBB locomotor rating, ladder rung test and foot print analysis) towards an improved function persisting over a course of 8 weeks. Our results show that 3D artificial scaffolds, by providing a neural friendly environment can offer potential use in future *in vivo* interfaces development.

17. Topaz Altman

Tel Aviv University

Amyotrophic Lateral Sclerosis (ALS) is a devastating, rapidly progressing neurodegenerative disease that affects lower and upper motor neurons. Increased neuronal activity and mitochondrial defects were suggested to play a key role in this neurodegenerative process. Here, we hypothesize that ALS motor neurons possess a unique spatial metabolic demand which facilitates rapid and fatal motor neuron degeneration. High energy demand and mitochondrial usage in the neuromuscular junction, away from the cell body, may be a source for cellular vulnerability which facilitates neurodegeneration in the presence of prolonged mitochondrial stress. We use different mouse ALS disease models to characterize motor neurons synaptic metabolic demand and compare it to other neuronal subtypes. Furthermore, we try to understand if this process leads to neurodegeneration via inhibition of axonal and synaptic local protein synthesis. There are currently no available therapies for ALS patients, and our study may contribute for the understating of disease mechanisms and future drug development.

18. Coral Skeleton Topography Induces Neural Elongation and Activation

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In recent years, several biodegradable materials were explored as scaffolds for tissue engineering and regenerative medicine. Topography modification of these scaffolds was shown to influence their bioactivity and regenerative capacity. In this regard, coral skeleton was shown to promote neuronal regeneration processes. However, neural response to different coral scaffold's topography was not yet examined. In this study, we assessed the response of dissociated neural cells to distinct coral scaffold topographies. We distinguished between three types of topography: smooth, ragged and ridged surfaces. We observed that, with the increase of the surface curvatures, astrocytes' expression of glial fibrillary acidic protein was 10-fold higher compared to control. Moreover, astrocytes developed thin edged processes, which were found to be 20% longer when compared to control. Interestingly, we also showed that dendrites' expression of microtubule-associated protein 2 was also increased in response to ridged coral surface. Thus, we suggest that the topography induces an increase in neuronal cells activation and expansion. These effects of the coral scaffold's topography on neural cell behavior might play a key role in applications for neuronal tissue restoration.

19. Development of Vascularized 3D stem cells Derived Neural Constructs

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Engineering of neural implants has a myriad of applications, spanning from basic science research, through disease models and drug treatment examination, to treatment of neural disorders. Even though neural tissue engineering has taken its first clinical steps, many challenges are still to be addressed to obtain functional, clinically-applicable viable neural implants. Human stem cell derived neural cells holds considerable promise as a cell based therapy and for research due to their proliferation capabilities and relevance as human central nervous system (CNS) model. Pre-vascularization of engineered neural tissue is crucial for its capability to enhance construct-host integration following implantation, and possibly improving the functional attributes of the neural network, through interplay between neural and vascular networks. In this work, we develop a method to engineer a vascularized neural construct using induced stem cell derived neural cells. Here, we present how utilizing different supporting cells and cell environments modulate the vascular-neural construct properties.

20. Fluorescent Metal doped carbon dots for Neuronal Manipulations

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Sonochemistry has been used to develop a one-pot synthesis of carbon dots (C-dots) and doped C-dots based materials. It is demonstrated how various experimental parameters, such as the sonication time, the temperature and the power of sonication affect the size of the C-dots (2-10 nm) and their fluorescence properties. The highest measured quantum yield of emission was ~16-44%. Similarly, we synthesized C-dots doped with metals (Ga, In, Sn) and non-metals (N, P, S) for various physical, chemical, and biomedical applications. The synthesized C-dots were coated on polythene, Si-wafer, glass substrate, and activated carbon (AC). The Ga doped in C-dots (Ga@C-dots) and used for antimicrobial activity against free-living *P. aeruginosa* bacteria. Ga@C-dots was reflected full inhibition of the bacterial growth at the much lower concentration of Ga within Ga@C-dots. The Ga-doped C-dots on Ga nanoparticles (Ga@C-dots@Ga NPs), which is deposited on a glass substrate for neural growth. SH-SY5Y cells were plated on the substrates. The effect of the Ga@C-dots@Ga NPs on the development of the neurites during the initiation and elongation growth phases were studied and compared with C-dots, Ga@C-dots and Ga NPs. We observed that cells grown on a Ga@C-dots@Ga-coated substrate exhibit a 97% increase in the number of branches originating from the soma. Furthermore, synthesized the N doped C-dots (N@CDs) by hydrothermally reacting an aqueous solution of Bovine Serum Albumin (BSA) with high quantum yield (44 %), high photostability, colloidal stability, and high functionalization efficiency. The N@CDs have demonstrated a non-toxic and long-lasting effect when applied for imaging human cells (Osteosarcoma cell, U2OS) at the nuclear level of the cells. Furthermore, N@CDs are nontoxic to the selected cell line in our preliminary evaluation. However, only a few studies have been focused on assessing the fluorescence of N@CDs during *in-vivo* exposure. Strong fluorescence emission from Zebra Fish (ZF) embryos and larvae successfully confirms the distribution of N@CDs in ZF. The retention of N@CDs is very stable, long lasting and with no detectable toxicity. The presence of strong fluorescence at the yolk sac, especially in vicinity to the intestine, suggests that a high content of N@CDs entered the digestive system. This indicates that, N@CDs may have potential live cell imaging applications in elucidating different aspects of lipoprotein and nutritional biology in a ZF yolk lipid transport and metabolism model. Moreover, we have also fabricated low-cost, composites of NCDs with γ -Fe₂O₃ (NCDs/ γ -Fe₂O₃), which is based on a hydrothermal cum co-precipitation method. The product is a fine powder of particles having an average diameter of 9 ± 3 nm. The physical and chemical properties of NCDs/ γ -Fe₂O₃ were studied, as-well-as superconducting quantum interference device (SQUID) and Mossbauer analysis of the magnetic properties of these nanocomposites. The interaction of NCDs/ γ -Fe₂O₃ nanocomposites with neuron-like cells was examined showing efficient uptake and low toxicity. Our research demonstrates the use of the nanocomposites for imaging and for controlling the cellular motility. The NCDs/ γ -Fe₂O₃ nanocomposites are promising due to their biocompatibility, photo stability, and potential selective affinity, paving the way for multifunctional biomedical applications.

22. THE ROLE OF MgSO₄ AS AN NMDA RECEPTOR BLOCKER IN THE PROTECTION AGAINST CNS-OXYGEN TOXICITY IN RATS

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Introduction: Combat divers, using closed-circuit breathing apparatus, run the risk of developing central nervous system oxygen toxicity (CNS-OT). This may lead to seizures underwater and eventually drowning. Evidence have accumulated to suggest that CNS-OT is related to increased production of reactive oxygen and nitrogen species. Hyperactivation of N-Methyl-D-Aspartate receptors (NMDARs) contribute to the production of these reactive molecules as well. Mg⁺² is a natural blocker of NMDARs in both the peripheral and central nervous system. Magnesium Sulfate (MgSO₄) is an accepted treatment for prevention of pre-eclampsia related seizures by blocking NMDARs in skeletal muscles. However, it has also been suggested that MgSO₄ affects NMDAR activation in the CNS. A previous study in our laboratory demonstrated that MgSO₄ significantly prolongs latency to electro-encephalo-graphic (EEG) manifestation of CNS-OT in rats. MgSO₄ may therefore provide protection against CNS-OT by blocking NMDARs. Electrophysiological studies in rat brain slices showed postsynaptic excitability of NMDAR at hyperbaric pressure (HP), due to reduction in Mg²⁺ voltage depended inhibition efficiency of the NMDAR.

Purpose: Examining the protective effect of MgSO₄ against CNS-OT via its role as an NMDAR blocker under HP, using EEG-telemetry system in rats.

Results: EEG signals and seizures were recorded in living-rats by a telemetry system under HP. Relative to control group, the latencies to CNS-OT significantly increased in a MgSO₄ treated group and significantly decreased in a Mg⁺²-deficient group.

Conclusions: Our data suggests that MgSO₄ may be used as a protective agent against CNS-OT and that NMDARs are probably involved in this mechanism.

24. **Temporally precise single-cell-resolution Optogenetics**

Shemesh OA., Tanese D, Zampini V, Linghu C, Piatkevich K, Ronzitti E, Papagiakoumou E, Emiliani V and Boyden ES.

Optogenetic control of individual neurons with high temporal precision within intact mammalian brain circuitry would enable powerful explorations of how neural circuits operate. Two-photon computer-generated holography enables precise sculpting of light and could in principle enable simultaneous illumination of many neurons in a network, with the requisite temporal precision to simulate accurate neural codes. We designed a high-efficacy soma-targeted opsin, finding that fusing the N-terminal 150 residues of kainate receptor subunit 2 (KA2) to the recently discovered high-photocurrent channelrhodopsin CoChR restricted expression of this opsin primarily to the cell body of mammalian cortical neurons. In combination with two-photon holographic stimulation, we found that this somatic CoChR (soCoChR) enabled photostimulation of individual cells in mouse cortical brain slices with single-cell resolution and <1-ms temporal precision. We used soCoChR to perform connectivity mapping on intact cortical circuits.

25. Micro-patterned devices for magnetic organization of neural networks

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Bar Ilan University

Positioning neurons and guidance of nerve cell processes is important for interfacing the nervous system for neuro-electronic devices and for therapeutic applications. Guidance and positioning can be used to lure regenerating axons to form predesigned structures of neural networks and to pattern synaptic contacts. Many studies focus on achieving cell and axonal guidance by using a variety of chemical and physical modifications. A recent innovative and promising approach to achieve site specific targeting in vitro and in vivo is to form complexes of cells interacting with magnetic nanoparticles (MNPs). Due to their magnetic properties, MNPs experience force in inhomogeneous magnetic fields and hence can be manipulated through such fields. By incorporating MNPs within neuronal cells, cells can be guided and controlled by external magnetic field gradients. In the present study, we used such nano-complexes in order to locate cells at specific sites, promote neuronal growth and affect growth orientation. Neuronal cells were incubated with iron oxide nanoparticles and turned sensitive to magnetic stimulation with no cytotoxic effect. We setup several profiles of magnetic tips for cell positioning. Moreover, based on theoretically modeled magnetic fluxes, we designed and fabricated micro-patterned substrates consisting of arrays of magnetic pads and stripes that can be magnetized selectively. We investigated cell motility and network organization of MNPs-loaded cells on these substrates along their differentiation process. MNPs-loaded cells were plated atop the micro-patterned substrates and showed high affinity to the patterns, adhering and clustering at magnetic pad sites. The majority of cell somas were found on the magnetic stripes and neurites were seen to align according to stripes orientation. Molecular and morphological measurements are performed to evaluate viability and network formation. Our study presents an emerging magneto-chemical method for the manipulation of neuronal migration and growth opening new directions in non-invasive neuronal repair.

26. **Membrane-targeted delivery of nitrogen vacancy nanodiamonds**

Shemesh OA., Gupta I, Gaathon O, Wassie A, Foy C, Chang JB, Walcott A, Kodandaramaiah S, Wickersham I, Englund D and Boyden ES.

Nanodiamonds containing nitrogen vacancy (NV) color centers have emerged as promising fluorescent sensors for nanoscale magnetometry, thermometry, electrometry, and pH sensing. The low cytotoxicity and high brightness of NV-nanodiamonds (NVNDs) has spurred interest in life science applications. However, a central challenge for many NVND uses, such as neural voltage imaging, is the development of stable delivery methods of NVNDs to cellular membranes. In this paper, we propose and demonstrate the use of micelles to deliver NVNDs with hydrophobic surface termination to neural membranes. Our approach consists of silanization functionalization of NVNDs with octyl chains, encapsulation in polyethylene glycol (PEG)-conjugated phosphoethanolamine (PEG-PE) micelles, and delivery to primary hippocampal neurons. Optical (single and multi-photon) and electron microscopy reveal monodisperse NVNDs on the membranes of neurons, with retention of >90% of the silanized NVNDs (Si-NVNDs) on the membrane for >6 hours. In contrast, control studies with the starting material, hydroxyl-functionalized NVNDs (OH-NVNDs), produce agglomerates on the cell membranes that are rapidly endocytosed. Propidium-iodide staining revealed no increased cell death of neurons during 12 hours of testing, for NVNDs of either functionalization. We anticipate that this method of micelle-mediated delivery of fluorescent nanodiamonds will enable new sensing and imaging applications on cellular surfaces.

28. Photolithography & 3D Nano-printing Combination for Hybrid Retinal Implant For Vision Restoration Implant

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Purpose:

Current electronic retinal prosthesis for sight restoration have shown relatively success, however are limited by inherent limitations decrease its resolution. Our lab develops a novel approach, a hybrid retinal implant, composed of high-resolution 3D well-like electrode array integrated with glutamatergic neurons. Herein we present the development and optimization process of our implant, fabricated through a combined process of conventional photolithography and 3D nano-printing techniques, which enables optimization of the implant for better integration in the retina.

Methods:

Gold electrodes in SU8 well-like scaffold were fabricated by sequences of photolithography followed by a bi-layer lift-off process. Different process parameters together with undercut profile and release methods were investigated in order to optimize the fabrication process.

3D structures, such as micro-well configuration or pillars were printed from IP-DIP and Ormocomp polymers on top of the SU8 implant by Nanoscribe GmbH 3D printer, based on two-photon IR laser polymerization. In order to adjust the Z-axis of this printer to print accurately on the SU8 surface, we develop a novel method based on a simple stairs-designed structures, while printing on gold surfaces was optimized by adjusting laser intensity parameters and careful design of voxels printing sequence. The implant's height, electrode profile and morphology and 3D nano-printings were investigated by profilometer measurement, SEM and FIB microscopes.

Results:

3D SU8 implant with micro-well-like structure and gold electrodes was fabricated by sequences of photolithography followed by a bi-layer lift-off process. Exposed AZ1505 together with LOR10B were used to pattern electrodes shape in a bi-layer process which was followed by sputtering Cr/Au and a lift-off process. Better adhesion between the SU8 and gold was achieved by using Cr and O₂ plasma treatment, where Ni layer was used as a sacrificial layer to release the SU8 implant by HN03 21% wet etching. 3D nano-structures of IP-DIP and Ormocomp polymers were printed on top of the SU8 implant with diverse shapes and combinations at high accuracy and repeatability.

Conclusions: In this study we present a novel combined approach to fabricate a hybrid retinal implant utilizing conventional photolithography together with 3D two-photon printing, which allows for fabrication of complex implantable device aimed at sight restoration.

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