1.3.18 Nanotechnologies for the nervous system

The nervous system (NS) is in some ways the most complex and fascinating organ of the human body. Unfortunately, NS pathologies that lead to tissue loss are dramatically difficult to treat because of the negligible regenerative potential of the central nervous system (CNS) on one side, and of the very slow and ineffective repair mechanisms of peripheral nervous system (PNS) on the other. The interest of our research activity in this field is to develop biocompatible nanostructured materials to help the heal of the PNS and cure and study CNS diseases. In particular, at the moment our attention is focused on nanoparticles for Globoid Cell Leukodystrophy (or Krabbe disease; OMIM #245200), on textured surfaces for helping nerve regeneration and on nanotechnological methods for studying Ube3A-related neurodevelopmental disorders (e.g. Angelman Syndrome, OMIM #105830).

Brain-targeted enzyme-loaded nanoparticles: A breach through the blood-brain barrier for enzyme replacement therapy in Krabbe disease

Lysosomal storage disorders (LSDs) result from an enzyme deficiency within lysosomes. The systemic administration of the missing enzyme, however, is not effective in the case of LSDs with central nervous system (CNS)-involvement. An enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) functionalized with brain targeting peptides (Ang2, g7 or Tf2) has been demonstrated for Krabbe disease, a neurodegenerative LSD caused by galactosylceramidase (GALC) deficiency [1]. We first synthesized and characterized Ang2-, g7- and Tf2-targeted GALC CLEA NPs. We studied NP cell trafficking and capability to reinstate enzymatic activity in vitro (Fig. 1a,b). Then, we successfully test our formulations in the twitcher mouse. We reported enzymatic activity measurements in the nervous system and in accumulation districts upon intraperitoneal injections, demonstrating activity recovery in the brain up to the unaffected mice level (Fig. 1c). Together, these results open new therapeutic perspectives for all LSDs with major CNS-involvement.

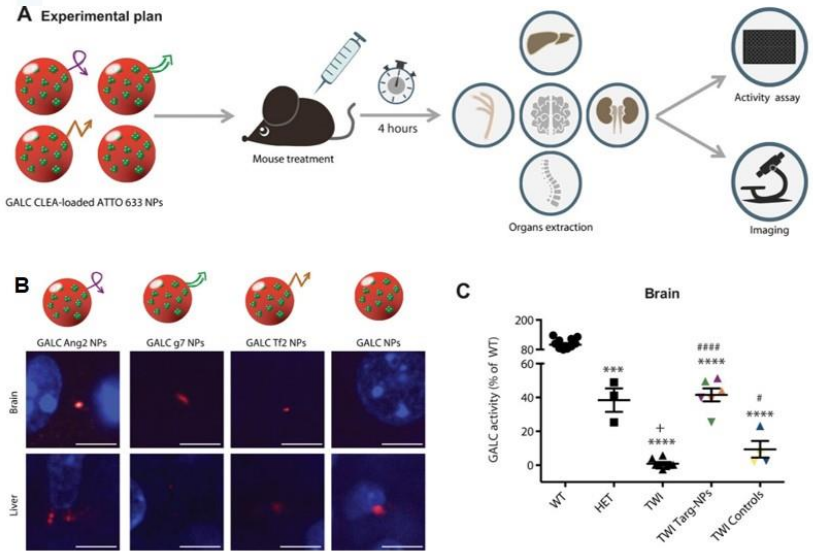

Figure 1. Brain-targeted enzyme-loaded nanoparticles for Krabbe disease

Schwann cell contact guidance versus boundary interaction in functional wound healing along nano and micro-structured membranes

Peripheral nerve transection is often encountered after trauma and can lead to long-term/permanent loss of sensor/motor functionality. The effect of pure contact interaction of several nano/micro-structured substrates on glial Schwann cells (SCs) was previously studied in view of their possible use for nerve-repair applications, for example anisotropic hierarchical rippled nanotopographies [2]. Recently we tested the ability of grating (GR)-patterned elastomeric membranes (i.e. grooved substrates) to control and direct SC shaping and migration, with the aim to evaluate these geometries for nerve-regeneration applications [3]. Poly(dimethylsiloxane) (PDMS) GRs were developed with different lateral periods (ranging from 1 to 20 μm) and depths (from 0.35 to 2.5 μm) (Fig. 2, left), leading to two distinct cell-substrate interaction regimes: contact guidance (GR period < cell body diameter, for T1, T4) and boundary guidance (GR period ≥ cell body diameter, for T20). We examined the response of primary SCs to GRs in terms of cell morphology, actin cytoskeleton organization, single and collective migration, and cell-cell interaction. Specifically, T20 performed best among all tested GRs, showing the best SC elongation and alignment, actin organization and single-cell directional migration. Conversely, wound-healing experiments demonstrated that contact-guidance can be more effective in driving collective SC migration than boundary guidance and indeed only T4 could significantly improve wound-closure speed (Fig. 2, center).

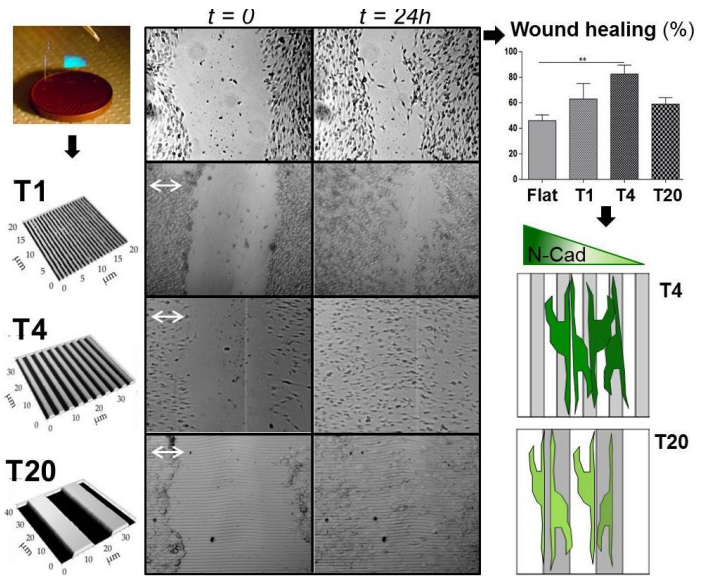

Figure 2. *left)* PDMS micro-grooved membranes were developed with lateral period of 1 (T1), 4 (T4) and 20 (T20) μm; z -scales are 350 nm,850 nm, and 2.5 μm, respectively. *center)* Bright-field images of SC collective migration on the substrates, immediately after the scratch (t = 0) and at t = 24 h; white arrows = GR direction, 200 μm. *right)* Wound closure (%) at t = 24 h: ** P<0.01 T4 vs. FLAT, Dunnett's' test. The collective migration performances are linked to the properties of the SC monolayers generated on the different GRs: SCs on large-period GRs (T20) are characterized by the downregulation of N-Cadherin, a protein that mediates cell-cell adhesion.

We linked this behavior to the properties of the SC monolayers generated on the different GRs by studying the expression of the neural cell-adhesion molecule cadherin (N-cadherin), a protein that mediates cell-cell adhesion, and triggers intracellular signaling cascades to promote migration. SCs on large-period GRs are

characterized by N-cadherin downregulation and enhanced single-cell scattering with respect to SCs on small-period GRs, indicating a less compact monolayer in the boundary guidance regime (Fig. 2, right). Overall T4 (4 μm period, 0.85 μm depth) emerged as the most effective topography in tuning SC directional orientation and migration. Our results provide information of the impact of specific topographical elements that can be exploited for tissue engineering applications and for the production of new devices enhancing peripheral-nerve regeneration.

The role of ubiquitin ligase E3A in polarized contact guidance and rescue strategies in UBE3A-deficient hippocampal neurons

Although neuronal extracellular sensing is emerging as crucial for brain wiring and therefore plasticity, little is known about these processes in neurodevelopmental disorders. Ubiquitin protein ligase E3A (UBE3A) plays a key role in neurodevelopment. Lack of UBE3A leads to Angelman Syndrome (AS), while its increase is among the most prevalent genetic causes of autism (e.g. Dup15q syndrome). By using microgrooved substrates that can induce specific directional stimuli in cells, we previously found deficient topographical contact guidance in AS neurons, which was linked to a dysregulated activation of the focal adhesion pathway [4].

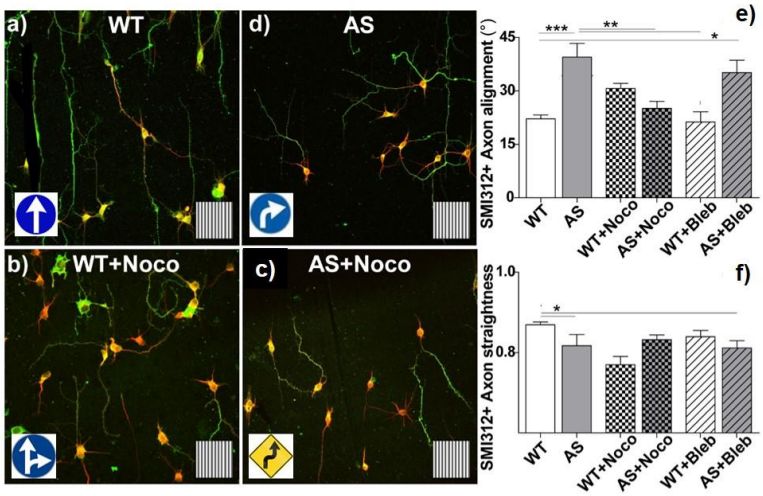

Figure 3. Confocal images of WT (**a-b**) and AS (**c-d**) neurons (div4), untreated (**a**, **d**) or treated from early div2 with nocodazole 40 nM (**b**, **c**); GRs pattern side= 60μm. **e-f**) Axonal morphological parameters of WT (*white*) and AS (*grey*) neurons on GRs in the presence of Noco (*squared*) and Blebbistatin 25μM (*striped*): axon alignment (**e**), and straightness (**f**). */*** Bonferroni test; n ≥ 3, at least 15 neurons/sample.

Here, we studied axon and dendrite contact guidance of Wild-Type, AS and UBE3A-overexpressing neurons (Dup15q autism model) on micrograting (GRs) substrates, with the aim to clarify the role of UBE3A in neuronal guidance [5]. We found that loss of axonal contact guidance is specific for AS neurons while UBE3A overexpression does not affect neuronal directional polarization along GRs. Deficits at the level of axonal branching, growth cone orientation and actin fiber content, focal adhesion effectors and proteins were observed in AS neurons. We tested different rescue strategies for restoring correct topographical guidance in

AS neurons on GRs, by either UBE3A protein re-expression or by pharmacological treatments acting on cytoskeleton contractility. Nocodazole, a drug that depolymerizes microtubules and increases cell contractility, rescued AS axonal alignment to the gratings by partially restoring focal adhesion pathway activation. Surprisingly, UBE3A re-expression only resulted in partial rescue of the phenotype. Overall, cytoskeleton dynamics emerge as important partners in UBE3A-mediated guidance responses. These results support the view that UBE3A-related deficits in early neuronal morphogenesis may lead to defective neuronal connectivity and plasticity.

References

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