Functional fluorescent materials and migration dynamics of neural progenitor cells

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SUMMARY

Microscopy is the science of examining objects that are too small to be perceived by the human eye. Since microscopes became a popular tool in biological research in the 17^{th} century, this instrument has experienced an immense technological evolution. Particularly, fluorescence microscopy established as a highly valuable standard method in scientific research, giving rise to the development of an enormous range of fluorescent markers and materials. As cells are mainly composed of water and are translucent, fluorescent tagging allows to visualize and differentiate the different cellular structures. The establishment of an incubator stage opened the way to investigate living cells over days or weeks while the development of fast-scanning microscopes enabled fast imaging. Together with the possibility of time-lapse microscopy, whereby the software is programmed to automatically take images in regular time intervals over a set period of time, allowed to gain deeper insights into the dynamics of living cells and their interactions with fluorescent materials.

In this thesis, time-lapse fluorescent microscopy plays a pivotal role in investigating functional materials within living cells as well as the migratory behaviour of neural progenitor cells. The first part of the thesis focuses on two different functional nanomaterials, whereas the second part explores fluorescent labelling of neural progenitor cells and cell dynamics within different *in vitro* systems.

Fluorescent nanomaterials present an exceptionally practical tool to label cells and their organelles. Within the past decades high research effort has been put into developing materials that have a strong and robust fluorescence, that are easily taken up by cells and do not influence cellular processes or health. Further research effort went into designing functional fluorescent materials, that do not solely label an existing structure but respond to the processes inside cells and offer a variability in their response. For example, recent biochemical developments enabled to use the fluorescent signal as a voltage sensor within neurons, functioning as an indicator for neural activity. Along with these developments, chapter II introduces a fascinating hybrid material consisting of a combination of DNA molecules and silver clusters, called DNA-encapsulated silver nanoclusters (Ag-DNA). Ag-DNA show unique optical properties which are tuneable with the selection of the DNA sequence and length, as well as show high sensitivity to its environmental conditions. In other publications, these nanomaterials have been used for metal ions detection *in vitro* or DNA mismatch sensing. In Chapter II, we employ Ag-DNA in living cells and show how the selection of the DNA template influences the resulting structures and yields Ag-DNA constructs that perform different functions within cells. Specifically, we present three different Ag-DNA constructs that show different fluorescence responses upon internalization in cells, including characteristic excitation and emission shifts. Furthermore, by varying the DNA sequence and lenght, it is possible to tune the cytotoxicity of these nanomaterials, adding the possibility of anti-cancer or anti-bacterial applications.

Another category of fluorescent materials is introduced in Chapter III. This chapter focuses on polymersomes that are artifical vesicles consisting of a lipophilic membrane and an aqueous interior which can be loaded with different molecular entities. In our experiments, polymersomes labelled with two different fluorescent dyes are analyzed within living cancer cells. Time-lapse microscopy is employed to observe the internalization process and the fate of the polymersomes inside living cells over the period of 2 hours and 86 hours, respectively. We find that the cells rapidly internalize the dual-labelled nanovesicles and distribute them equally among daughter cells during division. Further, the fluorescence signal remains stable for up to 3 days thus showing the potential of these nanomaterials for long-term bioimaging applications.

The second part of the thesis turns its focus to the multipotent stem cells of the central nervous system – the neural progenitor cells (NPCs). Their multipotency and regenerative capacity makes them an outstanding candidate for the treatment of neurodegenerative diseases and injuries of the central nervous system. We employ the murine neural progenitor cell line C17.2 for our experiments. In chapter IV, we first confirm the neural progenitor state of undifferentiated C17.2 by using morphological analysis and fluorescent immunolabelling. By differentiating the cells into neurons and astrocytes, and using the same analysis methods, we also confirm the presence of their multipotency. Second, several fluorescent dyes and genetic markers are tested with the focus to label the nucleus and cell body or cytoskeleton of C17.2 cells. Finally, a viral dual-transduction is successfully performed and a stable cell line generated with a labelled nucleus and cytoskeleton. This fluorescent C17.2 line is employed in chapter V for the analysis of cellular dynamics.

Despite the numerous publications of studies showing the regenerative capability of neural progenitor cells in disease and injury animal models, basic insights into the migratory behaviour of these cells are missing. In chapter V we introduce different minimalistic *in vitro* systems that are used to produce preliminary results on neural progenitor cells dynamics. More specifically, we coat substrates with extracellular matrix proteins in a plain as well as in a patterned way and observe the behaviour of C17.2 cells under the different conditions via fluorescence time-lapse microscopy. We look at the general statistical values of their migration on plain surfaces and on confined patterns. We calculate the average times that C17.2 cells move into one direction before changing, as well as their average velocities and diffusion values on plain surfaces and on patterned lines of different widths. Furthermore, we find that the NPCs follow a persistent random motion model, whereby they show persistence on short time scales but random motion on long time scales. Additionally, visual analysis of these cells within different patterned environments yielded insights into a set of recurrent behaviours including their frequent turns of direction to explore their environment and their competition for space. The preliminary results can be used for initial modeling purposes focused on understanding how NPCs migrate *in vivo*. All introduced experimental set-ups can be employed in future studies to perform more detail analysis and higher complexity like chemical guidance gradients can be added to mimic the *in vivo* situation closer.

The research presented in this thesis covers different topics that are all joined by the employed investigative tool of spinning disc confocal fluorescent microscopy and the application of fluorescent materials and markers with living cell systems. The presented

results provide new information on how the selection of a DNA template yields Ag-DNA constructs with different functionalities within living cells. It shows the possibility of these materials to be used as stable fluorescent labels for cells, as intracellular sensors, or have the purpose of causing cell death. The observations on the second investigated fluorescent material, dual-fluorescent polymersomes, contribute to our understanding of the short- and long-term fate of these nanovesicles within living cells allowing their application as fluorescent trackers in other biological system. The established stably fluorescent NPCs enable other researchers to use this reporter-cell line for other studies. Lastly, the presented minimalistic *in vitro* systems and the acquired first insights into the dynamics and behaviour of neural progenitor cells set a starting point for further and more detailed analysis of these cells that are crucial for future regenerative treatments of the central nervous system.