Novel biomedical applications and developments in microscopy

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In parallel with developments in microscopy, the biomedical research field has gained three novel approaches: liquid crystal, nanomaterial and optical nanoscopy based applications. LCs are excellent sensing materials that have been used for the assembly of affinity components for biomedical devices. LC based diagnostic biosensors enabled detection of diseases, pathogens, sepsis markers, blood and urine chemicals. At this point, polarizing optical microscopy (POM) takes part as a valuable measurement tool for the identification of LC phases and phase transitions. On the other hand, most of the nanomaterial based improvements are related to fluorescent light microscopy as a result of advances in this field. For biomedical applications, those materials are chemically decorated with peptides or DNA as targeted nanoparticles and used in imaging processes combined with diagnosis or therapy. While LCs and nanomaterials are being improved to bring novel imaging strategies for existing microscopical devices, optical nanoscopy has come into prominence with an extraordinary technical expansion. Overcoming the diffraction limit has enabled fluorescence microscopes to achieve nanoscale resolution in living specimens. Here, the material and technological based knowledge on novel biomedical applications related to the developments in the field microscopy has been summarized.

Keywords: liquid crystals; nanomaterials; nanoscopy; biomedical applications; microscopy

1. Preface

Biomedical research turned its direction to a way in a close relation to the material science and is increasingly targeting more than just the cell as the classical object of microscopy (1). Liquid Crystals (LCs) and designed nanomaterials are good examples of this phenomenon depending on their fascinating physical properties compatible with biological samples, thus, allowing them to be used in medical devices or in biological molecule targeting agents. Although different microscopical techniques have been primary tools for the observation of LCs or nanomaterials, with different perspectives in biomedical research, optical nanoscopy has raised with a prime motto based on observing cells in a living organism with high accuracy and in nanoscale resolution (2). With regard to these rapid and substantial developments in the field of microscopy, the present paper summarizes novel materials, technologies, research and applications in the biomedical field.

1.1 Liquid Crystals and its applications in microscopy

LCs are excellent sensing materials that have molecules oriented in a crystal-like manner with an optical anisotrophy and high sensitivity of alignment to the conditions of surronding immiscible media (3). These properties enable them to produce a rapid and easily-visualised response in the detection of chemical/biochemical events with a label free strategy and without requiring complex instruments (4,5). Therefore, development of LC-based biosensors has become an attractive research subject. Protein-protein, protein-ligand interactions and nucleic acid hybridization events constituted basic principles of the operational mechanism for these sensors since natural or chemically synthesized peptides and DNA are capable to form LC phases in *in vitro* environments (6). Numerous sensors have been designed for the detection of targets such as DNA (7), proteins (8), glucose (9), bile acids (10), organophosphates (11) and heavy metal ions (12). Among these, DNA biosensors have attracted attention since they provide label-free, inexpensive, fast and sensitive way for the detection of pathogens or cancer cells. Khan and associates filled a transmission electron microscopy grid with LCs and coated it with a cationic surfactant. They saturated the LC/aqueous interface with a single stranded DNA probe. The resulting sensor was capable of detecting the genomic DNAs of tested bacterium and fungi with a potential application for other pathogens (13). Popov and associates described a biosensor combining enzyme-linked immunosorbent assay (ELISA) method with LCs for the visual detection of serum antigens specifically and the method could be generalized for any pathogen (5). LC-based commercial biosensor pioneered by Crystal Diagnostics, Ltd. currently being used for the selective identification of microbes (14). In a study carried out by Yoon and associates, designed LC microdroplets have shown potential for use in the *in vitro* detection of mouth epidermal carcinoma cells (15). Another LC microdroplet based sensor utilizing protein-antibody interactions proposed by Ding and associates have shown to be useful for the naked eye detection of SK-BR3 human breast cancer cells (16). On the other hand, one more gripping physical ability of LCs lead to a new potential use of these materials in the medical field. It is the delicate balance between the intermolecular forces which promotes self-assembled structures of LCs to a slight structural re-organization (17). This change can be quite dramatic and very fast thus, the concept has been adequate to be used in various types of actuator devices such as artificial muscles (18) or soft robots (19, 20).

Optical signals of LCs can be easily observed with naked eyes. However, polarizing optical microscopy (POM) is the critical tool for LC based research. POM is supportive for the quantitative measurement of LC signals. It has been also used by researchers to observe LC phases and phase transitions (21, 22). Such a polarization-sensitive optical detection platform has been presented by Nguyen and associates. By using POM, it has shown to be capable for quantitative determination of the optical birefringence of artificial cells containing LCs, whose orientation depends on the immobilized biomolecules (23). Besides being an observation material in microscopy, LCs capability of modulating light provide an important contribution to optics. They can induce controllable amounts of retardation and changes in the polarization state of the incident optical field, therefore take part in applications related to adaptive optics (24). LCs have been used for the development of a new type optical polarizing microscope called the LC-PolScope with more expanded functions that give the possibilities to monitor dynamic structural changes *in vivo* non-invasively (25). Zhou and associates used LC-PolScope to examine the textures of a new type of 'living liquid crystals' (LLC) in which motile bacteria are placed in water-based LC. Bacterial dynamics are dramatically altered and swimming bacteria perturbed the orientational order of the LC thus, LLC supposed to be convenient for biomedical applications (26). As it is utilized in the LC-PolScope, LCs have been progressively used to improve the optical properties of various devices in bio-imaging field including super resolution microscopes (27). Being one of the most fascinating materials to be used in biomedical technologies, LCs' potential applications should not be expected to remain limited with sensors, actuators or adaptive optic devices.

1.2 Nanomaterials and its applications in microscopy

Advances in the imaging techniques and microscopy are important keystones for the developments in biomedical field. Various microscopic techniques have been used as indispensible tools but fluorescence microscopy is advantageous among these techniques because of its consonance with living cells. Thus, most of the nanomaterial based improvements are related to fluorescent light microscopy and the improvement of novel fluorescent nanomaterials.

Nanomaterials possess low cytotoxicity and do not display nonspecific binding with cellular biomacromolecules or unwanted sequestration. They are photostable, can internalize into cells and tissues. For biomedical applications, those materials can be chemically decorated with peptides or DNA as targeted nanoparticles and used in imaging processes combined with diagnosis or therapy (28, 29, 30).

Quantum dots (QDs), fluorescently-doped silicas, carbon NPs, metal nanoclusters and soft nanomaterials such as polymeric NPs, nanogels and micelles represent some of the important nano-sized imaging agents that are used for the visualisation of structures within a cell, tissue or even an organism (31). Among these, QDs are semiconductor nanocrystals ideal for biological imaging. Biological molecules, such as an antibody recognising the target, can be linked to the surface of colloidal QDs, in order to introduce specific functionalities (32,33,34,35). Soft fluorescent nanomaterials also have attracted recent attention because of the advantages they provide such as biocompatibility, high brightness and easy biofunctionalization (31).

Nanomaterial-nanoprobe based research have pioneered improvements in other molecular imaging techniques, such as magnetic resonance (MR), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) (36). Near-infrared (NIR) fluorescence imaging approach, in which the absorbance spectra for all biomolecules reach minima, particularly provides a clear window for *in vivo* optical imaging (37). For cancer patients which constitute one of the primary target group of these applications, nanobiosensors have been designed to identify disease at the earliest stage possible, ideally at the level of a single cell or multiple cells of cancer stages (38). In a recent study, Rizvi and associates showed that near-infrared-emitting quantum dot bioconjugates can be used for localization of the human epidermal growth factor receptor 2 gene (HER2) receptors which is overexpressed in 25%-30% of breast cancers. It is reported to have a potential to be used for targeted therapy as well as image-guided surgery (39). Organic dye-doped nano particles (NPs) and upconversion NPs are the other promising nanoscale tools for diagnostic and optical imaging applications in cancer patients (40).

On the other hand, the recent concept in neuroscience research focused on targeted drug delivery with a principle of reaching therapeutic drug levels inside the brain (41). Blood–brain barrier (BBB) is the greatest impediment for delivering therapeutic levels of drugs via blood circulation in the treatment of central nervous system (CNS) disorders. However multifunctional nanoplatforms combining versatile therapeutic modalities with a variety of imaging options have the potential to diagnose, monitor and treat brain diseases. For this purpose, NPs provide a huge surface area supporting absorbtion, ability to bind or absorb theranostics, ease of cellular permeation and mask the reticuloendothelial system (42). They are often conjugated with ligands such as transferrin, low density lipoprotein and insulin to enhance their uptake at the BBB. Nanoliposomes, nanoemulsions, lipid nanoparticles, nanostructured lipid carriers, micelles and polymeric nanoparticles have been used for this pupose, with several advantages and limitations. Liposomes are attractive among other colloidal carriers depending on their steric stabilization and remote drug loading properties. They can also form complexes with nucleic acids or proteins (43). Airoldi and associates decorated nanoliposomes with ligands for Aβ-peptides which have the ability to target Aβ-peptide aggregates in the Alzheimers' disease. It is reported that, these functionalized nanoliposomes significantly decreased amyloid peptide aggregation *in vitro* (44). In a more recent study, Turjeman and associates introduced two different liposomal nano-drugs to show the superiorities of liposome- based nano-drugs in treating neurodegenerative diseases that involve neuroinflammation. On

an animal model for multiple sclerosis (MS), it was demonstrated that these nano-drugs alleviate the pathology of the disease (45). According to the results of those mentioned studies and others non-mentioned here, nanomaterials promise novel insights in the diagnosis and theraphy of diseases particularly with the support of extraordinary developments in the field of microscopy.

1.3. Optical nanoscopy and its applications

One of the important questions in biomedical research is how specific, nanometer-scale biomolecules are organized into multicomponent micron-scale structural and signaling ensembles inside the cells (46). Among various microscopy techniques, fluorescence microscopy offers the advantage of observing specific cellular components through molecule specific labeling and observing the structures inside a live sample in real time (47). However, optical microscopy lacks the required resolution to visualize these details in nanometer scale. In 2014, Nobel Prize in Chemistry was shared by Betzig, Hell and Moerner, introducing super resolution fluorescence microscopy or "nanoscopy" which has overcome the diffraction limit of light and become a powerful research tool for future biomedical applications (48, 49). One important application is the use of single molecule localisation microscopy (SMLM) as the ultimate single molecules biosensor. Photo-activated localization microscopy (PALM) and *direct* stochastic optical reconstruction microscopy (*d*STORM), based on stochastic activation of individual fluorophores and precise position determination (localization), have been used in different applications depending on their differing superiorities (50). DNA origami studies in this context are particularly attractive since these structures allow the organization of different molecules including proteins, aptamers, or nanoparticles into specified geometries. Therefore, they represent promising scaffolds for molecular computation, artificial molecular machines, molecular assembly lines, nanorobots, and factories (51). Amir and associates has shown that DNA origami can be used to fabricate nanoscale robots that are capable of dynamically interacting with each other in a in a living animal. Cockroaches were used as model organism in the study and DNA origami robots have been used successfully to control a molecule that targets their cells (52). DNA origami nano-rulers have been introduced as reference samples for fluorescence microscopy which also has a potential to be used for counting molecules by providing resolution and sensitivity (49).

Recently, nanoscopy techniques have begun to enter the neurosciences with a significant potential. Stimulated emission depletion (STED) microscopy and SMLM have been useful for studying the molecular composition and structural/functional diversity of presynaptic active zones where neurotransmitter release takes place (53). STED microscopy has reached insofar into a living mouse brain where it provides *in vivo* real time information on the morphological behaviour of neurons potentially reflecting alterations in the connectivity of the neural network (54).

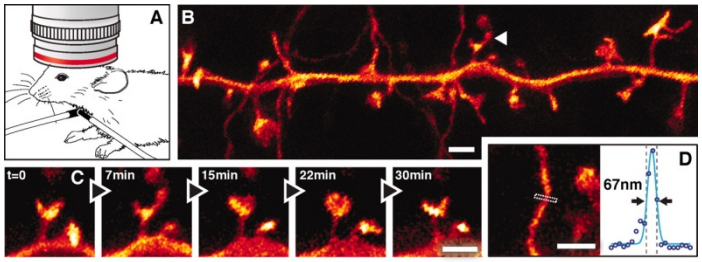

Figure 1. STED nanoscopy in a living mouse brain (from Berning et al., 2012) **A)** Representative drawing of anesthesized mouse under the objective lense **B)** Dendritic process within the molecular layer of somatosensory cortex **C)** Morphologic changes in adult dendritic spines **D)** STED images of structures <70 nm

Other nanoscopy applications include the structural and functional characterization of cytoskeleton (55) and structural dynamics of cells (56). In a recent study of D'Este and associates STED nanoscopy has been used to reveal neuronal cytoskeletal features (57). Molecular details of cholesterol and cytoskeleton modulated lipid interactions has been revealed by Mueller and associates using STED microscopy (58). Since they have been generally too small to approach with conventional diffraction-limited imaging methods, studies on cytoskeletal rearrangements in bacteria and yeast cells have benefited from the advantages of nanoscopy (59). Although nanoscopy based research still need to be improved and extended for clinical applications, it is coming into practice as a great achievement in the field of microscopy.

2. Conclusions

LCs, nanomaterials and nanoscopy are very recent and the most promising tools to be used in biomedical field. As a very fascinating material LCs are currently exploited in various medical devices and their potential applications offer practicability to everyday life. Nanomaterials, from diagnosis to targeted therapies, are used in a broad perspective on different types of cancers and CNS disorders such as Alzheimer's disease, Parkinson's disease and MS. These are the only few pathologies that are claimed to be benefited from the recent advancements in NP-based research. With the emerging help of nanoscopy, those mentioned also present novel insights to structural biology, leading to make real time clinical observations possible with unprecedented detail.

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