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What's up in nanomedicine?

The publications discussed here are selected exemplarily among many other excellent publications. This also applies for the mentioned conferences or events. We very much appreciate your feedback on the column, suggestions for topics to be argued about, or other related information. As was already stated in the first version of this column, its purpose is to present novel and significant developments within the multidisciplinary field of nanomedicine or significant developments in other scientific areas.

Publications digest

Nanoparticle albumin-bound paclitaxel

Paclitaxel (PTX) is a cytostatic agent, which is used for the treatment of various cancers, for example ovarian cancer. The current used PTX in Taxol® exerts various side-effects, such as aggregation of erythrocytes, peripheral neuropathy, severe anaphylactoid hypersensitivity reactions and cardiovascular events (1, 2). Taxol® has the two excipients Cremophor EL (CrEL) and ethanol. CrEL is a vehicle used for various poorly-water soluble drugs and it forms micelles in an aqueous environment. It consists of polyoxyethylated castor oil and one of the main components is the fatty acid esters polyethyleneglycol ricinoleate. Even though this medication is already used for many years, it was not clear from the literature whether the side-effects are due to Taxol®-PTX or the excipient Cremophor EL (CrEL). Therefore, Vader and coworkers (3) investigated the effects of CrEL-PTX on red blood cells (RBCs) and compared the results with RBCs exposed to the novel nanoparticle albumin-bound paclitaxel (NABP). In NABP, the carrier material CrEL is replaced by human serum albumin. The investigators performed their research as follows; RBCs were exposed for 24 h at 37°C to Taxol®, CrEL/EtOH, EtOH or the NABP. The used concentrations where similar as the peak concentrations used during treatments. After 24 h, the supernatants of Taxol® and CrEL/EtOH were colored dark red to purple, which indicates hemolysis. This was not the case with the other incubations with EtOH or NABP alone. From this it is concluded that CrEL caused the damaging effects. To confirm with another method that the RBCs were damaged, they investigated the exposure of phosphatidylserine (PS), which is normally present at the inner leaflet of the RBC. When the RBC is aging or damaged, this phospholipid is exposed on the surface of the RBC (4). Vader and coworkers determined that the induced PS exposure was <1% for RBCs stored at 4°C for 24 h, whereas the exposure was 4±1.4% for RBSc stored at 37°C. The cells were exposed to Taxol® and its carrier CrEL/EtOH (in separate vials) for 24 h at 37°C. An exposure of 100% of PS was induced at both conditions. In addition, when the RBCs were exposed to EtOH and NABP the PS exposure was not induced (3.5±1.0% and 4.3±1.0%, respectively). Incubation with concentrations measured in patients after 24 h after infusion, performed similar results. The authors indicated that the effect on the RBCs could be a long-lasting effect. Another interesting part of this investigation was done with human umbilical vein endothelial cells (HUVECs). It is already known that PS-exposing RBCs are capable to adhere to vascular endothelium (5). This could have damaging effects when the vasculatures are obstructed by these cells. Vader and coworkers (3) therefore investigated this adhesion with the RBCs, incubated with CrEL and with and without the opsonin lactadherin. Note, an opsonin is a molecule that marks an antigen (in this case PS) to enhance phagocytosis. In the presence of lactadherin, only the CrEL/EtOH and Taxol® treated RBCs showed adherence and/or phagocytosis. When lactadherin was absent, no adherence could be shown for all different incubated RBCs. From this it is concluded that this interaction might be lactadherin and probably PS-dependent. Stimulation and damage of RBCs leads to the formation of microparticles and ectosomes (herein after referred to as MVs). MVs are composed of phospholipid bilayer vesicles

of <1 μm in diameter (6). After exposure of Taxol®, CrEL, EtOH or NABP the MVs were isolated and characterized. The results were in agreement with the earlier investigations, only Taxol® and the excipients had an increased MV formation; and only *these* MVs contained flotillin-1, a protein associated with lipid rafts. The size of these MVs ranges from 50 to 300 nm. With this, the authors showed for the first time that an excipient is involved in MV formation. From previous investigations, it is known that MV numbers are elevated in cardiovascular diseases that are characterized by endothelial dysfunction (7). Vader et al. (3) studied the uptake of MVs by HUVECs and showed that the fluorescently labeled MVs were readily digested and *without* the presence of lactadherin. Both the MVs of Taxol® and CrEL incubated RBCs showed this uptake and they seemed to be trapped in intracellular vesicles and accumulated in the perinuclear region.

Another promising recent publication of Ko and coworkers (8) concerning NABP showed that this medication could also be useful for the second line treatment of metastatic urothelial carcinoma for which no standard treatment exists (8). With the current treatments of this type of cancer, a response of <20% is provided with no survival benefit. Ko and coworkers (8) presented that NABP was well tolerated in patient populations with pretreated advanced urothelial cancer with an encouraging tumor response. The authors suggested conducting a further study on this topic.

As final remark, I would like to emphasize that the work of Vader et al. (3) as well as the work of Ko et al. (8) are exiting examples of positive developments towards the use of nanomedicines. They should be brought into general public to promote nanotechnology for medical purposes and also to decrease the public skeptic feelings towards nanotechnology in general.

New self-reporting contrast and delivery agent

Core-shell polymeric nanoparticles are attractive candidates to use as contrast agents but also as in-cell delivery platforms in nanomedicine (9, 10). Incorporation of contrast and bioactive media in such core-shell polymeric nanoparticles has been achieved in various ways (like crosslinking) and at various locations in the particle (such as the surface). However, use of the core-shell interface as a labeling location has been limited. According to Robin and coworkers (11), this position offers the advantage being shielded from the environment and having a distinct spatially separated location from the (loaded) interior of the scaffold. The investigators presented a novel chromophore-bridged block copolymer system (11). The used highly emissive dithiomaleimide (DTM) can be incorporated into polymeric scaffolds without effecting or disrupting the self-assembly process. The versatility of DTM for protein and polymer labeling is presented in another recent publication of this group (12). The incorporation of the DTM unit at the core-shell interface was performed by using ring-opening polymerization (13) and reversible addition-fragmentation chain transfer polymerization (14). The result is a y-shaped amphiphilic copolymer from which, using direct dissolution methods, micelles can be achieved by self-assembly. Dynamic light scattering, atomic force microscopy and transmission microscopy showed that the particle size was 23 nm, 19–27 nm and 19.1±2.1 nm, respectively. The emission of the micelles was compared with a synthesized small molecule containing DTM. Both have similar UV-vis and excitation spectra with an excitation maximum near 403 nm. The influence of concentration on the emission was studied and the micelles provided relatively flat emission profiles over 3 orders of magnitude of concentration. This is in contrast with the small molecule, which responds like most self-quenching fluorophores. To confirm the supramolecular state of the micelles, the emission anisotropy was compared to that of the small molecule. The emission anisotropy of the small molecule remained low and uniform regardless of concentration, in contrast to the polymer, which showed a local maximum indicative of a micellar structure. From these results, Robin and coworkers (11) concluded that the emission and anisotropy trends provide a method for distinguishing between supramolecular conditions and other states. This was also confirmed by the profiles provided when solution state time correlated single photon counting was performed. For nanomedical applications, the viability of the nanoparticles to function as self-reporting agents is important. Therefore, micelles and the solvent-state components were investigated by fluorescence lifetime microscopy (FLIM). The two different states, dilute polymer and micelles, were cast onto glass. Excitation was done at 405 nm with a diode laser and 450 nm long-pass collection of the signal. The dilute system generated large droplets, whereas the micelles resulted in a more uniform film. The authors related the emission decay profiles of the two castings directly to the supramolecular state, with longer emission decays for the micellar sample. This could be seen even without formal emission fitting. Thus, from these results they concluded that future work with DTM functional nanoparticles does not necessarily require an extensive and time-consuming analysis of the decay spectra. As a proof of principle, they

successfully performed FLIM of the micelles in rat hippocampal tissue. A cross section of living rat hippocampus cells were incubated to 0.1 mL of 10 mg/mL polymer micelles in a phosphate buffered solution for 1 h and fixed with ethanol. By performing FLIM, micelles and degradation products were observed in three forms in the tissue. To conclude, with the system presented here it is possible to perform cellular imaging where the intensity is truly an indication of particle density and emission lifetime can be used to track degradation. The system also enables the use of the fluorescence resonance energy transfer signal to or from the DTM to track molecules moving across the core-shell interface. This work offers many possibilities for nanomedicine applications.

Upcoming events

The **NanoFar,** was proposed by leading European academic teams with the purpose to work together on the integrative approaches to nanomedicine. It is an innovative harmonized joint Doctorate program of excellence for the most talented EU and non-EU students. Each year an **autumn school** is organized by one of the partner universities and this year it will take place from 21 to 25 of October at the Universidad de Santiago de Compostela, Spain. It is open for all PhD students who wish to improve their knowledge on nanomedicine. Topics of sessions are for example: oral delivery, theranostics and imaging, transversal skills and metabolic diseases. For further reading see also: http://www.erasmusmundus-nanofar.eu/nanofar-autumn-schools/autumn-school-2013/.

Worth to mention is that this school is taking place in collaboration with: (1) the European Technology Platform on Nanomedicine (www.etp-nanomedicine.eu), (2) the COST TD1004 Network on "Theranostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-guided Drug Delivery (www.cost.eu) and (3) the Trans-Int FP7 EU consortium on "New Oral Nanomedicines: Transporting Therapeutic Macromolecules across the intestinal barrier (www.trans-int.eu).

The European Technology Platform for Nanomedicine (ETPN) and the EU funded consortium **Nanomed 2020** are for the first time organizing a **Nanomedicine Award**. The ceremony will take place during the 19th **BIO-Europe** conference from November 4 to 6 in Vienna, Austria (www.ebdgroup.com/bioeurope/index.php). The BIO-Europe conference is the largest partnering conference in Europe for the global biotechnology industry. Here you will meet pharmaceutical companies, private investors including venture capital and private equity, companies developing companion diagnostics and others. The Nanomedicine Award has the goal to promote excellent new projects in nanomedicine to the pharma/biotech community. The candidate projects should be diagnostic and therapeutic approaches which meet criteria such as: (1) are not feasible without nanotechnology, (2) are placed within the category nanotherapy in regenerative medicine or into the category nanodiagnostic or nanotherapy in general and (3) address the unmet medical need shared by many. The prizes to be won are for example a full ticket including Partnering for BioEurope 2013, one year of free membership at ETPN, a presentation during the Nanomedicine Panel Discussion of the BioEurope 2013, as well as a presentation during the **CLINAM conference in 2014** (www.clinam.org). The first prize will be to receive media coverage across Europe as well as becoming visible for EU decision-makers. The call is open until the September 7th. For more details, see http://nanomedicine-award.com/prizes.

Then finally, **the ETP Nanomedicine General Assembly 2013** will take place in Grenoble on 1st and 2nd October and will give the opportunity to get detailed insights into the concrete implementation of ETPN's recommendations for nanomedicine under the coming Research Framework Programme Horizon 2020. This event aims also at catching the context in which the field is now evolving with the presence of high-level representatives of the European Commission and national authorities. The General Assembly preliminary program and the online registration form are publically available on the ETPN Website under: www.etp-nanomedicine.eu/ga2013.

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