european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 67/ supplement 1
2023

Proceedings of the workshop NANO23@uniVR

8-9 June 2023

University of Verona
Verona, Italy

ejh

under the auspices of
the University of Pavia, Italy

Published by PAGEPress, Pavia, Italy

Editorial Office:
PAGEPress s.r.l.
via A. Cavagna Sangiuliani 5, 27100 Pavia, Italy
Phone: +39.0382.1549020 - Fax: +39.0382.1727454
E-mail: info@pagepress.org

Printed quarterly by:
Press Up s.r.l.
via E.Q. Visconti, 90, 00193 Roma, Italy
Tel. +39.0761.527351 – Fax +39.0761.527254

Annual Subscriptions
Europe: Euro 250
All other Countries: Euro 300

Subscriptions, cancellations, business correspondence and any enquiries must be sent to PAGEPress Publications, Pavia, Italy.
Cancellations must be received before the end of September to take effect at the end of the same year.

Reg. Tribunale di Pavia n. 289/23.2.1984.

Recognized by the Ministero per i Beni e le Attività Culturali, Italy as a publication of high cultural value.

Associato all'USPI
Unione Stampa Periodica Italiana

european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 67/ supplement 1
2023

ejh

under the auspices of
the University of Pavia, Italy

European Journal of Histochemistry
a journal of functional cytology

The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published till 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is now published under the auspices of the University of Pavia, Italy.
The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Views and Comments, and Book Reviews concerning investigations by histochemical and immunohistochemical methods, and performed with the aid of light, super-resolution and electron microscopy, cytometry and imaging techniques; attention is also given to articles on newly developed or originally applied histochemical and microscopical techniques.

Coverage extends to:
- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

2021 Impact factor: 1.966. ©JCR Clarivate Analytics

Proceedings of the workshop NANO23@uniVR

8-9 June 2023

University of Verona, Italy

Nanoscience is constantly expanding in many fields, such as Biotechnology, Energy- and Environment-nanotech, Nanomedicine. Whatever the nanoconstructs are intended for, information on their structural features is essential to ensure successful application. Therefore, imaging and microscopy techniques represent a fil rouge linking the various fields of the nanotechnological research. The workshop NANO23@uniVR is organized by the PhD Course in Nanosciences and Advanced Technologies of the University of Verona with the aim to create a forum on hot topics of current interest in Nanoscience and Nanotechnology, and to promote an interdisciplinary exchange of knowledge and creativity among researchers.

Scientific Committee

Federica Bortolotti
Department of Diagnostics and Public Health, University of Verona

Mariapina D'Onofrio
Department of Biotechnology, University of Verona

Manuela Malatesta
Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona

Alessandro Romeo
Department of Computer Science, University of Verona

Adolfo Speghini
Department of Biotechnology, University of Verona

Franco Tagliaro
Department of Diagnostics and Public Health, University of Verona

Under the auspices of
the Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM)

table of contents

Proceedings of the workshop NANO23@uniVR

european journal
of histochemistry
ISSN 1121-760X
volume 67/supplement 1
2023

MAIN LECTURES

NANOTECHNOLOGY FOR PHYTOCOMPOUND ADMINISTRATION

E. Esposito[1], M. Sguizzato[1], F. Ferrara[1], A. Bondi[1], W. Pula[1], R. Cortesi[1], M. Drechsler[2], M. Malatesta[3], G. Valacchi[4,5]

[1]Dept. Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy; [2]Bavarian Polymer Institute Keylab Electron and Optical Microscopy, University of Bayreuth, Germany; [3]Dept. Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy; [4]Animal Science Dept., Plants for Human Health Institute, NC Research Campus, NC State University, Kannapolis, USA; [5]Dept. Environmental Sciences and Prevention, University of Ferrara, Italy
E-mail: ese@unife.it

The tremendous potential of nanotechnology in biomedicine is the object of many pharmaceutical researches and clinical studies. Particularly, nanocarriers based on lipid matrix possess interesting features, being biocompatible, suitable for lipophilic drug encapsulation by simple "green" production protocols, thus providing low toxicity profiles[1]. Among the different applications, many efforts are devoted to the nanoencapsulation of phytochemicals. Indeed natural bioactive compounds derived from edible plants can prevent or counteract serious diseases[2,3]. For instance, curcumin, crocin, caffeic acid, and mangiferin are potent antioxidants, representing potential skin cancer chemo-preventive agents, due to their anti-inflammatory and immunomodulatory activity[2-6]. Despite the pharmacological potential of these phytocompounds, their low solubility and stability drawbacks hamper their pharmaceutical use. In this respect, we employed different strategies to encapsulate phytocompounds in lipid based nanostructured liquid systems, such as solid lipid nanoparticles, monoolein based dispersions and ethosomes. The nanoencapsulation of phytocompounds enabled to improve their stability and to prolong their action, promoting their transdermal delivery, suggesting the possibility to treat inflammatory disorders affecting the skin, mainly induced by oxidative stressors, such as cigarette smoke and ozone[2-7].

References

1. *Sajadimajd S et al. Pharmacol Res 2020;151:104584.*
2. *Hallan SS et al. Nanomaterials 2021;11:171.*
3. *Touitou E and Natshe H. Pharmaceutics 2021;13:2129.*
4. *Ferrara F et al. Pharmaceutics 2022;14:1038.*
5. *Sguizzato M et al. Antioxidants 2021;10:768.*
6. *Sguizzato M et al. Pharmaceutics 2021;13:1124.*
7. *Esposito E et al. Int J Mol Sci 2022;23:15112.*

EXPLORING THE CHEMICAL PARAMETERS SPACE FOR THE LOW TEMPERATURE AND SUSTAINABLE SYNTHESIS OF CRYSTALLINE INORGANIC NANOMATERIALS FOR CATALYSIS AND ENERGY CONVERSION

S. Gross[1,2]

[1]Dept. of Chemical Sciences (DiSC), University of Padova, Padova, Italy; [2]Karlsruhe Institute of Technology (KIT), Institute of Chemical Technology and Polymer Chemistry (ITCP), Karlsruhe, Germany
E-mail: silvia.gross@unipd.it

The possibility to orient and steer the chemico-physical and structural evolution of inorganic nanomaterials by tuning the experimental conditions of the synthesis is currently a major endeavour in the field of inorganic synthesis chemistry. In this framework, the resort to unconventional synthesis conditions discloses exciting perspectives in orienting, *inter alia*, the morphogenesis and the final structure of the crystalline materials. Analogously, the paradigms of green and sustainable chemistry are currently catalysing sharply growing interest in all fields of chemistry. In particular inorganic chemistry represents an exciting playground for the design and optimization of sustainable routes and the implementation of green chemistry paradigms to inorganic chemistry represents one of most bewitching developments. In this framework, in these last years, in our group we have explored different low temperature (T< 150°C) and sustainable wet chemistry and colloidal routes, namely *i.* hydrothermal routes, *ii.* miniemulsion, *iii*. microfluidic synthesis and *iv*. combination thereof, to prepare different inorganic functional nanomaterials for heterogeneous catalysis and energy conversion in crystalline form, by exploiting the unconventional experimental conditions disclosed by the different methods, *i.e*. non standard temperature and pressure in the former case, the confined space inside the miniemulsion-generated droplets or in microchannels in the latter ones. The obtained materials ranged from ferrites[1] and manganites[2], to pure and doped metal oxides, sulphides, and halogenides, to metal/metal oxide nanocomposites, to supported metal nanoparticles. The adopted wet chemistry routes ranged from 1) miniemulsions[3] to 2) coprecipitation combined with hydrothermal route to 3) microfluidic[4] and 4) classical colloidal routes. Exciting results could be achieved by the combination of the above mentioned routes[5] and by a systematic exploration of the broad parameters landscape. The structural evolution as a function of time and temperature has been also followed by using different analytical methods. This contribution provides an overview of the *pros* and *cons* of the proposed routes for the obtainment of targeted inorganic colloids, also outlining as the role of a combination of analytical tools can unravel the complex interplay among experimental parameters and microstructure of the materials.

References

1. *Diodati S et al. Nano Res 2014;7:1027-22.*
2. *Minelli A et al. J Mater Chem C 2017;5:3359-71.*
3. *Dolcet P et al. J Mater Chem 2012;22:1620-26.*
4. *Tajoli F et al. ACS Appl Mater Interfaces 2020;12:44074.*
5. *Antonello A et al. Chem Mater 2017;29:985-97.*

URINARY BLADDER AND NANOPARTICLES: TOOLS FOR BASIC AND APPLICATIVE RESEARCH

S. Hudoklin

Institute of Cell Biology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
E-mail: samo.hudoklin@mf.uni-lj.si

The urinary bladder is covered by urothelium, a highly specialised epithelium that maintains the blood-urine barrier. The barrier is formed during urothelial cell differentiation and is characterised by the formation of tight junctions and the synthesis of uroplakin proteins organised in urothelial plaques. In terminally differentiated superficial urothelial cells, urothelial plaques cover the apical plasma membrane and the fusiform vesicles that transport the plaques between the cytoplasm and the apical plasma membrane. However, there are unanswered questions about membrane transport in urothelial cells, *e.g*., are urothelial plaques transported during the normal micturition cycle/is endocytosis increased in non-differentiated or cancer urothelial cells/can we target or kill cancer cells based on their endocytosis properties? Nanoparticles (NPs) are a promising tool to answer these questions. Using ultra-small gold NPs administered to mice *in vivo*, we demonstrated that fusiform vesicles in differentiated cells are not involved in endocytosis. NPs were only found in the endosomes of non-differentiated cells of the normal and neoplastic urothelium[1]. Next, we investigated the rate of endocytosis in *in vitro* models. NPs were found to enter papillary and invasive urothelial neoplasms by micropinocytosis, while uptake into normal cells was largely limited[2]. Hybrid FePt/SiO_2/Au NPs were used to test the potential for photothermal therapy and magnetic resonance imaging (MRI). Results showed a 30% decrease in viability of the cancer cell line compared to a normal urothelial culture and the usability of the NPs as MRI contrast agents[3]. Increased cytotoxicity was obtained after photocatalytic treatment of TiO_2 NPs administered to the cells[4]. In conclusion, we have shown that NPs are an excellent tool for studying membrane trafficking in urothelial cells. Moreover, due to a wide variety of compositions and properties of NPs, they can also be applied in studies of other biological model systems.

References

1. Hudoklin S et al. Int J Nanomed 2013;8:3945-53.
2. Lojk J et al. Histochem Cell Biol 2018;149:45-59.
3. Kostevšek N et al. Nanoscale 2018;10:1308-1321.
4. Imani R et al. Photochem Photobiol Sci 2015;14:583-90.

BIOMIMETIC MAGNETIC NANOPARTICLES AS PLATFORMS TO COMBINE DIRECTED CHEMOTHERAPY AND HYPERTHERMIA

C. Jimenez-Lopez[1], F. Oltolina[2], A. Peigneux[1], Y. Jabalera[1], P. Rodriguez-Jimenez[3], M. Montalban[1], M. Prat[2], M. Maqueda[1], G. Iglesias[3]

[1]Dept. Microbiology, Science School, University of Granada, Granada, Spain; [2]Dept. of Health Sciences, Università del Piemonte Orientale, Novara, Italy; [3]Dept. of Applied Physics and Instituto de Investigación Biosanitaria ibs. GRANADA, NanoMag Laboratory, University of Granada, Granada, Spain
E-mail: cjl@ugr.es

MamC-mediated biomimetic magnetic nanoparticles (BMNPs) composed of Fe_3O_4 are proposed as nanocarriers for targeted chemotherapy due to their novel properties resulting from the exquisite control over the crystal nucleation and growth that MamC, a magnetosome protein from *Magnetococcus marinus* MC-1 exerts. It is of particular interest their size (36 ± 12 nm) and surface properties, having an isoelectric point of 4.4. They are superparamagnetic at room and body temperatures, have a large magnetic moment per particle, mediate hyperthermia and are cytocompatible. Having a negative surface charge at physiological pH, they can be efficiently coupled with charged molecules as antibacterial peptides, such as the circular bacteriocine AS-48 and with antitumoral drugs such as Doxorubicin (DOXO). The release of the electrostatically bonded molecules occurs at acidic pH value, where BMNPs become uncharged, and can be further enhanced with hyperthermia. Our results from experiments performed with the two nanoassemblies show, on one hand, that AS-48-BMNPs, either by itself or combined with magnetic hyperthermia, have a strong local bactericide effect on Gram-positive bacteria (*Enterococcus faecalis, Enterococcus faecium* and *Staphylococcus aureus*) and, also in Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*). These results represent a step forward in the area offering a nanoassembly that is less prone to generate resistances, that may allow the local treatment of infections and, either by itself or combined with magnetic hyperthermia, offers a potent antibacterial effect[1]. On the other hand, DOXO-BMNPs could be directed and concentrated on the target tumor cells by the apposition of a magnet. Moreover, these nanoassemblies respond to an alternating magnetic field acting as magnetic hyperthermia agents and generating a local fever that, on one hand triggers tumoral cells death, on the other, enhances drug release, further intensifying cell death. Indeed, when this nanoformulation is injected *in vivo* in mice model in the tumor site, and hyperthermia is generated, the combined chemo-thermal therapy mediated by these drug-loaded magnetic nanoparticles have a stronger therapeutic benefit compared to that carried out by the chemotherapeutic alone. This nanoformulation and strategy are thus promising tools for translational applications in cancer therapy[2].

References

1. Jabalera Y et al. Int J Biol Macromol 2021;189:206-13.
2. Oltolina F et al. Cancers 2020;12:2564.

INTERFACIAL PROPERTIES IN COMPOSITE NANOSYSTEMS FOR ENERGY HARVESTING

A. Vomiero[1,2]

[1]Dept. of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Venice, Italy; [2]Dept. of Engineering Sciences and Mathematics, Luleå University of Technology, Sweden
E-mail: alberto.vomiero@unive.it

Composite nanostructures can be efficiently applied for Sunlight detection and conversion and, more in general, for energy harvesting and generation of solar fuels. In most of the applied systems, like photodetectors, excitonic solar cells and (photo)-electrochemical cells to produce solar fuels, nanomaterials can play a critical role in boosting photoconversion efficiency by ameliorating the processes of charge photogeneration, exciton dissociation and charge transport. Critical role in such processes is played by the structure and quality of the interface, which needs to be properly assembled to obtain the desired functionality. Several strategies can be pursued to maximize energy harvesting and storage, including broadening of light absorbance to reduce solar light losses, fastening exciton dissociation and charge injection from the photoactive medium to the charge transporting materials, reducing charge recombination during charge transport and collection at the electrodes. In this lecture, a few examples of application of nanocomposites will be discussed, including all-oxide coaxial p-n junction nanowire photodetectors and solar cells, core-shell quantum dot fluorophores for high-efficiency luminescent solar concentrators, composite sulfides for hydrogen generation, and oriented carbon nanotube forest dispersed in polymer matrix as efficient low-temperature thermoelectric composite. Emphasis will be given to the role of interface engineering in improving the efficiency of energy conversion in different systems, spanning from electric power generation from Sunlight, to chemical fuel production, to conversion of heat lost through thermoelectric materials.

References

1. Zhao H et al. Energy Environ Sci 2021;14:396-406.
2. Gong X et al. Nano Energy 2022;101:107617.
3. Solomon G et al. Adv Energy Mater 2021;11:2101324.
4. Akbar K et al. Adv Opt Mater 2021;9:2100532.
5. Liu G et al. J Mater Chem A 2023;11:8950-60.

SESSION NANO@BIOMEDICINE

HOW TO PRESERVE THE PHYSICOCHEMICAL PROPERTIES OF BIODEGRADABLE NANOPARTICLES DURING FREEZE DRYING?

I. Andreana, V. Bincoletto, M. Manzoli, F. Rodà, V. Giarraputo, P. Milla, S. Arpicco, B. Stella

Dept. of Drug Science and Technology, University of Torino, Torino, Italy
E-mail: ilaria.andreana@unito.it

Nanocarriers make possible to overcome the bioavailability hurdles of poorly water-soluble drugs, improve efficacy and prevent significant side effects.[1] However, their long-term stability and storage in aqueous solutions can be challenging. Freeze drying is commonly used in the pharmaceutical field to allow long-term stability and easy storage of nanoparticle formulations.[2] To protect the formulations from possible alterations induced by the freeze-drying process, it is advisable to add some excipients that act as protectants during the process. Saccharides are considered working excipients, acting by replacing the bound water around nanocarriers and creating a viscous matrix which reduces the mobility of nanoparticles (NPs) through the process.[3,4] In this study, we evaluated the applicability of sucrose, trehalose, mannitol and glucose as cryoprotectant agents for poly(lactic-*co*-glycolic) acid (PLGA) NPs and liposomes. Only PLGA NPs with the highest percentage of trehalose and sucrose (10 and 20 % w/w) retained their particle size after resuspension. Similarly, in liposomes, the protective activity of trehalose and sucrose confirmed the ability of high concentration (sugar:lipid ratio of 5:1) of saccharides in retaining particle size. Furthermore, we tested the cryoprotection ability of a polysaccharide, hyaluronic acid (HA) at different molecular weights (4.8 kDa and 14.8 kDa). HA was added to the formulation either as free HA or conjugated with a phospholipid (HA-DPPE) to achieve a more stable association of HA to nanocarriers. We observed a weak cryoprotectant effect of free HA 14.8 kDa on liposomes. On the contrary, all the formulations in the presence of HA-DPPE conjugates could be easily redispersed after freeze-drying with a size variation below 20%. Considering the targeting effect of HA, these results pave the way to the use of HA-DPPE conjugates to formulate actively targeted lyophilized nanocarriers.

References

1. Farokhzad OC et al. ACS Nano 2009;3:16.
2. Trenkenschuh E et al. Eur J Pharm Biopharm 2021;165:345.
3. Guimarães D et al. Front Bioeng Biotechnol 2019;7:424.
4. Fonte P et al. Biomatter 2012;2:329.

NANOTRACERS TARGET ABNORMAL ASSEMBLIES OF THE AMYLOIDOGENIC PROTEIN TAU

C.G. Barracchia[1], F. Parolini[2], A. Volpe[3], D. Gori[3], F. Munari[1], S. Capaldi[2], M. D'Onofrio[1], M. Assfalg[1]

[1]Laboratory of Chemistry of BioMicroMacroMolecules, and [2]Laboratory of Biocrystallography and Nanostructures, Dept. of Biotechnology, University of Verona, Italy; [3]ACZON srl, Monte San Pietro (BO), Italy
E-mail: carlogiorgio.barracchia@univr.it

The abnormal accumulation of misfolded tau protein fibrils in the brain is a hallmark event in many neurodegenerative disorders (NDs)[1,2]. The pathogenesis of these diseases is still unclear and effective treatments are currently lacking[1]. Modulating the dynamics of these aggregation pathways with small molecules, macromolecules, and nanoparticles (NPs) represents a breakthrough in research, providing an opportunity to redirect the formation of neurotoxic aggregates[4-6]. NPs emerged as potential tools for investigating the mechanisms of protein fibrillation and could potentially redirect conformational transitions[3-7]. However, our understanding of how amyloidogenic proteins interact with NPs remains limited despite extensive investigation. In our work[8], we functionalized dye-doped silica NPs with tau protein to give them the capability to interact with protein aggregates or condensates. Therefore, we suggest that NP-tau conjugates could be used as nanotracers for both *in vitro* and in-cell studies, to selectively target and visualize tau assemblies and condensates, enabling selective targeting and visualization of tau assemblies and condensates. This would contribute to the elucidation of molecular mechanisms underlying abnormal protein aggregation.

References

1. Soto C and Pritzkow S. Nat Neurosci 2018;21:1332.
2. Reiss AB et al. Rev Neurosci 2018;29:613.
3. Sonawane SK et al. ACS omega 2019;4:12833.
4. Edward C et al. Curr Alzheimer 2009;6:409.
5. Al. Lira AL et al. Nanoscale 2018;10:3235.
6. Pickhardt M et al. Curr Alzheimer 2015;12:814.
7. Fardnesh A et al. Int J Nanomedicine 2019;14:901.
8. Barracchia CG et al. Bioconjugate Chem 2022;33:1261-8.

THE ANIMAL MODEL IN THE STUDY OF MEDICATION ADHERENCE

S. Baskhanova[1,2], N. Moskaleva[2], S. Appolonova[2], F. Tagliaro[1]

[1]Laboratory of Forensic Toxicology, University of Verona, Verona, Italy; [2]Laboratory of Pharmacokinetics and Metabolic Analysis, Sechenov University, Moscow, Russia
E-mail: sabina.baskhanova@univr.it

A high level of adherence to long-term therapy is one of the necessary conditions for successful pharmacotherapy in the treatment of chronic diseases in patients. According to WHO data, the average rate of adherence to therapy reaches only about 50% in developed countries.[1] Information about accurate adherence extents can help to avoid misinterpreting treatment efficacy and unnecessary intensifying of pharmacological therapy.[2,3] It is well known that hair is a biological tissue capable of storing information about the therapeutical drugs taken for long time.[4] The possibility of investigating the "history" of exposure to medicines by further research on hair analysis will provide a new tool to assesses the extent of adherence to therapy.[5] In the present research, 120 rats were divided into 3 groups. The first group of rats was administrated atorvastatin (0.5; 1.75 and 2.5 mg/kg/day), the second group received atenolol (0.2; 1.2 and 2 mg/kg/day) and the third one - amlodipine (1; 3.5 and 5 mg/kg/day). All medicines were crushed and mixed with aqueous glucose solution and given *per os*. In the end of experiment, rats' furs were shaved by electric shaver and an extract of fur was analysed by using HPLC-MS/MS. As results of the experiment, there was significant strong correlation of the atenolol concentration and the dose administration in the rats' newly regrown hair (r=0.931, p<0.05). The correlation of the dosing administration with amlodipine hair concentration was weaker (r=0.537, p<0.05). The low level of correlation between the levels of amlodipine in hair samples implies that amlodipine may be metabolized into its derivatives. Measuring the content of atenolol and amlodipine in rat hair is a dependable indicator of the dosing regimen. According to the study's findings, hair analysis could prove to be a useful means of tracking patient adherence with atenolol and amlodipine treatment, which would assist clinicians in managing patients with cardiovascular diseases. However, additional research on patients is necessary before implementing this approach in clinical practice.

References

1. Sabate E. World Health Organization, 2003.
2. Brown MT et al. Am J Med Sci 2016;351:387-99.
3. Lam WY and Fresco P. BioMed Res Int 2015;2015:217047.
4. Kintz P et al. Ther Drug Monit 2006;28:442-6.
5. Avataneo V et al. J Pharm Biomed Anal 2019;166:40-51.

HIGHWAY TO BREAST CANCER: PEPTIDE-BASED NANOPLATFORMS FOR DRUG DELIVERY

R. Bellavita[1], M. Piccolo[1], C. Irace[1], A. Falanga[2], S. Galdiero[1]

[1]Dept. of Pharmacy, School of Medicine, University of Naples "Federico II", Naples; [2]Dept. of Agricultural Science, University of Naples "Federico II", Portici, Naples, Italy
E-mail: rosa.bellavita@unina.it

Triple-negative breast cancer (TNBC) is defined by the lack of both estrogen and progesterone receptor as well as human epidermal growth factor receptor 2, and represents the most complex and aggressive subtype of breast cancer. Although chemotherapy poses the cornerstone in TBNC treatment, its use is seriously dissatisfactory due to issues related to formulation and pharmacokinetic properties of drugs. To overcome these limitations, herein we present a self-assembled peptide-based nanofiber as smart platform to achieve a selective on-demand drug release into breast cancer cells.[1] The nanofiber consists of two structured self-assembled peptides featured by the presence of an aliphatic sequence of six alanine residues and a lipid tail (nonadecanoic acid) linked to the amino group of lysine in C-terminal, and by positively and negatively charged amino acid residues.[2] On the external surface, the nanofiber is decorated by *i)* doxorubicin (Dox), as chemotherapeutic, *ii)* the cell-penetrating peptide, named gH625, which increases cellular uptake and promotes endosomal escape of nanofiber into cancer cells,[3] and *iii)* the peptide targeting the epidermal growth factor receptor (EGFR) overexpressed on cancer cells.[4] Dox is covalently bound to the fiber surface through an on-demand strategy that involves the over-expression of matrix metalloproteinase-9 (MMP-9) in cancer cells, by the introduction of the specific

MMP-9 cleavage sequence between Dox and the fiber (Bellavita *et al. manuscript in preparation).* Biophysical assays, including dynamic light scattering, zetametry, and fluorescence assays, were performed for determining the size, charge, and critical micelle concentration of fiber, and its formation was confirmed by transmission electron microscopy. The proteolytic cut for the on-demand drug release was performed in presence of recombinant MMP-9 and *in vitro* using cells overexpressing MMP-9. The cytotoxicity profile of the nanofiber carrying Dox was established on the triple negative cancer cell lines and the internalization pathway of the nanofiber was monitored by fluorescence.

References

1. Del Genio V et al. Pharmaceutics 2022;14:1544.
2. Lombardi L et al. Biomacromolecules 2019;20:1362-74.
3. Ben Djemaa S et al. Biomacromolecules 2019;20:3076-86.
4. Hossein-Nejad-Ariani H et al. Sci Rep 2019;9:2723.

STAT3-LOADED EXTRACELLULAR VESICLES: BASIS OF A NEW POSSIBLE THERAPEUTIC APPROACH TO RESTORE STAT3 SIGNALLING DEFICIENCY

I. Bettin[1], M. Brattini[1], E. Ataie Kachoie[2], S. Capaldi[2], M.A. Thalappil[1], P. Bernardi[1], I. Ferrarini[3] G. Fuhrmann[4], S. Mariotto[1], E. Butturini[1]

[1]Dept. of Neuroscience, Biomedicine and Movement Sciences; [2]Dept. of Biotechnology, and [3]Dept. of Medicine, University of Verona, Verona, Italy; [4]Dept. of Biology, FAU, Erlangen, Germany
E-mail: ilaria.bettin@univr.it

In the last decades, extracellular vesicles (EVs) have been widely proposed as advanced drug delivery systems owing to their peculiar features.[1] Here, we propose the use of EVs as a platform to deliver a functional form of STAT3 to restore its signalling in Autosomal Dominant Hyper-IgE syndrome, a disease characterized by negative mutations in the *stat3* gene.[2] A novel recombinant fusion construct of STAT3 tagged with EGFP was produced using a baculovirus-based expression system and characterized from a biochemical and biophysical point of view. EVs were isolated from RO cells conditioned medium by ultracentrifugation and EGFP-STAT3 was encapsulated using a saponin-assisted method followed by size-exclusion chromatography.[3] The obtained EVs were characterized by Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), immunoblotting and fluorescence detection. Protein encapsulation was assessed by treatment of EVs with Proteinase K, while their internalization in PBMCs was investigated by confocal microscopy. NTA and TEM analyses revealed that EGFP-STAT3 EVs presented a size range of 80-150 nm, which is in accordance with previous reports.[4] CD63, EVs surface marker, as well as STAT3 were detected by immunoblotting, and densitometric analysis of the obtained bands indicates that 2.7 ± 0.9 μg of EGFP-STAT3 were associated with the EVs. Protease treatment showed that most of the protein was protected by the EVs membrane. Confocal microscopy images confirmed that EVs successfully delivered STAT3 into PBMCs. Overall, our data represent an interesting starting point for the development of a new therapeutic strategy to restore STAT3 signalling.

References

1. Rankin-Turner S et al. Adv Drug Deliv Rev 2021;173:479-91.
2. Milner JD et al. Nature 2008;452:773-6.
3. Frank J et al. Sci Rep 2018;8:1-8.
4. Heinrich E et al. Small 2023 (in press).

THE FATTY ACIDS COACERVATION METHOD: AN EASY WAY TO OBTAIN SOLID LIPID NANOPARTICLES FROM SYNTHETIC AND NATURAL SOAPS

A. Bozza[1], A. Marengo[1], E. Marini[1], E. Muntoni[1], C. Dianzani[1], E. Camisassa[2], L. Battaglia[1]

[1]Dept. of Drug Science and Technology, University of Turin, Turin, Italy; [2]Aulina s.r.l., Racconigi (CN), Italy
E-mail: annalisa.bozza@unito.it

Solid lipid nanoparticles (SLNs) are proposed for healthcare-related products due to their high biocompatibility. Within this context, fatty acid coacervation is a feasible, solvent-free and easy to scale-up preparation method, which allows to obtain SLNs made-up of fatty acids, starting from their alkaline soaps, following proton exchange[1]. SLNs from synthetic soaps (*e.g.* stearate, palmitate) have been proposed for: oral administration of insulin in type I diabetes[2]; intranasal administration of drugs in cerebrovascular diseases[3]; and gene delivery in ocular administration[4]. Given that eco-sustainability drives towards the so-called "green" products, natural soaps, obtained by saponification of solid butters and fats, can be exploited to obtain "green" SLNs by the coacervation process[5]. Indeed, the unsaponifiable fraction can confer antioxidant and vaso-protective properties to the SLNs. Moreover, the presence of poli-unsaturated fatty acids (PUFA) in the lipid matrix is beneficial for neurovascular health, whilst the mono-unsaturated ones (*e.g.* oleic) can act as permeation enhancers on skin and mucosas, *i.e.* by promoting nose-to-brain drug delivery. Of note, taking advantage of the short time-to-market of cosmetics, "green" SLNs can also be employed to deliver anti-age active ingredients through the stratum corneum. Such SLNs were characterized with Transmission Electron Microscope (TEM), Scanning Electron Microscope (FE-SEM), Dynamic Light Scattering (DLS) and Differential Scanning Calorimetry (DSC). Fatty acids composition of "green" SLNs was determined with gas chromatography coupled to mass spectrometry (GC-MS). Engineered prototypes based upon "green" SLNs underwent preliminary *in vitro*, *ex vivo* and *in vivo* studies.

References

1. Battaglia L et al. J Microencapsul 2010;27:78-85.
2. Muntoni E et al. Acta Diabetologica 2019;56:1283-92.
3. Muntoni E et al. Colloids Surf B: Biointerfaces 2022;214:112470.
4. Gómez-Aguado I et al. Pharmaceutics 2021;13:1472.
5. Bozza A et al. Italian Patent application 102022000021093.

HYBRID PNIPAM-KERATIN MICROGELS: SYNTHESIS AND RELEASE STUDY FOR BIOMEDICAL APPLICATIONS

E. Buratti[1], F. Longo[1], M. Sguizzato[1], F. Ferrara[1], G. Sotgiu[2], S. Buoso[2], R. Cortesi[1], M. Bertoldo[1]

[1]Dept. of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy; [2]Kerline Srl, Bologna, Italy
E-mail: elena.buratti@unife.it

Hybrid materials are constituted by chemically, functionally and morphologically distinct building blocks from at least two classes of molecules, which include biologically active polymers (polysaccharides, proteins) and nano/microstructures.[1] Recently,

hybrid hydrogels obtained by natural and synthetic polymers have aroused interest in the biomedical field since they allow to create materials with a well-defined structure and bioactive properties. Poly(N-isopropylacrylamide) (PNIPAM) is one of the most used synthetic thermo-responsive polymers, thanks to its lower critical solution temperature around 32°C, close to human body temperature.[2] Proteins are chosen as natural polymer since they are biocompatible, biodegradable and mimic the extracellular environment.[3] Keratin, the major component of epidermis, can be efficiently extracted with a sulfitolysis process[4] and, as a protein, has functional groups that can be charged upon pH variation. The combination of these materials creates an interesting biocompatible and dual stimuli-responsive system. However, so far only few hybrid systems containing both PNIPAM and keratin have been investigated, principally as hydrogel.[5,6] In this study, hybrid microgels were prepared by polymerization of NIPAM in presence of keratin, to incorporate the protein inside the particle. Syntheses carried out at different NIPAM/keratin ratio showed a changing in the size of the particle with respect to pure PNIPAM microgel, indicating that there is an effect due to the presence of the second component. To pursue the application of these systems in the biomedical field, microgels were loaded with active principles, like caffeic acid, and the swelling behavior of the particles was investigated by DLS measurements.

References

1. *Vasile C et al. Molecules 2020;25:1539.*
2. *Sanzari I et al. Sci Rep 2020;10:6126.*
3. *Cilurzo F et al. Pol Adv Tec 2013;24:1025-28.*
4. *Zoccola M et al. In: Dullaart R and Mousques J, editors. Keratin: Structure, properties and applications. Nova Publishers 2012;36-62.*
5. *do Lago GL et al. Eur Polym J 2020;125:109538.*
6. *Zhu Q et al. Int J Pharm 2019;566:342-51.*

EVERYWHERE AND EVERYTHING: A METHOD TO VISUALIZE A375 MELANOMA EXTRACELLULAR VESICLES AND NON-INVASIVE MRI TRACKING IMAGING WITH IRON SUPERPARAMAGNETIC NANOPARTICLES

A.M. Capuzzo[1], A. Negri[1], G. Piccolantonio[2], P. Bontempi[2], P. M.-J. Lievens[3], T. Knickrehm[4], K. Lüdtke-Buzug[4], R. Mariotti[5], P. Marzola[2]

[1]Dept. of Diagnostics and Public Health, [2]Dept. of Engineering for Innovation Medicine, [3]Dept. of Neuroscience, Biomedicine and Movement Sciences, Division of Biology and Genetic, and [5]Dept. of Neuroscience, Biomedicine and Movement Sciences, Division of Anatomy and Histology, University of Verona, Verona, Italy; [4]Institute of Medical Engineering, University of Lübeck, Lübeck, Germany
E-mail: arnaudmartino.capuzzo@univr.it

Most types of cells could secrete Extracellular Vesicles (EVs), which are nanosized vesicles with a diameter of 30-120 nm.[1,2] The membrane integrity of EVs, which allows them to perform their biological functions by transferring their contents to the target cell, is crucial for a cell therapy, considering that EVs are becoming a considerable source of biological agents. Therefore, compared to normal cells, tumour cells may produce and secrete more EVs, which may be used as potential diagnostic biomarkers.[3] The goal of this work is to extract EVs labeled with iron superparamagnetic nanoparticles (NPs) from a well-established melanoma cell line (A375) and use them to demonstrate efficacy in tracking EVs-NPs using magnetic resonance imaging (MRI). This protocol is partially based on previous "passive" EVs labelling[4]: firstly, we label A375 with two NPs, one by the Lübeck group and one commercially available Resovist® as a reference; secondly, we isolate EVs from the A375 cells with ultracentrifugation procedure. After extraction, the EVs-NPs were counted in concentration and the diameter measured by Nanosight. The EVs-NPs obtained were visualized at TEM by single droplet on mesh copper grid with pioloform. To investigate the detectability of EVs-NPs, MR images of agarose gel phantoms containing increasing number of labelled cells were acquired. Preliminary results showed that those labelled using NPs from the Lübeck group have better detectability.

References

1. *Turano E et al. Int J Mol Sci 2023;24:2917.*
2. *Capuzzo et al. IntechOpen 2023. doi: 10.5772/intechopen.110572.*
3. *Zhong Y et al. Front Oncol 2021;11.*
4. *Busato A et al. Curr Protoc Cell Biol 2017;75:3.44.1-15.*

MAGNETIC RESONANCE IMAGING OF ADIPOSE-DERIVED ADULT STEM CELLS LABELLED WITH SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

A.M. Capuzzo[1], I. Scambi[2], R. Mariotti[2], A. Lacavalla[3], T. Knickrehm[4], K. Lüdtke-Buzug[4], P. Marzola[3]

[1]Dept. of Diagnostics and Public Health, [2]Dept. of Neurosciences, Biomedicine and Movement Sciences, and [3]Dept. of Engineering for Innovation Medicine, University of Verona, Verona, Italy; [4]Institute of Medical Engineering, University of Lübeck, Lübeck, Germany
E-mail: arnaudmartino.capuzzo@univr.it

The application of mesenchymal stem cells (MSCs) represents a new promising approach for treating neurodegenerative diseases. Recently, considerable attention has been paid to adipose-derived adult MSC (ADAS), thanks to the easy availability of adipose tissue and to the possibility of autologous cells transplantation.[1] Any possible application of therapies based on ADAS in the clinics cannot occur without elucidation of their homing.[2] Superparamagnetic iron-oxide nanoparticles (NPs) can be used to label and track cells *in vivo* via Magnetic Resonance Imaging (MRI).[3] The accessibility, non-invasiveness, and radiation-free features of MRI make this imaging tool appropriate for translation to the clinics.[4] To label ADAS we used two NPs: L-NP, synthesized by the Lübeck group and Resovist®, commercially available, used as reference. Both NPs were characterized from a biophysical point of view with: Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Nano Sight and MRI. L-NPs sizes determined by DLS (~53.02 nm), TEM (~100 nm) and Nano Sight (~93.9 nm) were distinct from Resovist® (DLS ~157.67 nm, TEM ~70 nm and Nano Sight ~56.7 nm) and were somewhat dependent on the solvent. MRI transversal relaxivity of L-NPs 277.8 mM-1s-1 was higher than Resovist® 156.4 (mM-1s-1). Tests of viability of the labelled cells, by MTT, showed that L-NPs up to 100 μg Fe/mL can be used for cell labelling. To investigate the detectability of ADAS-NPs, MR images of agarose gel phantoms containing increasing number of labelled cells were acquired. Preliminary results showed that as few as 100 labelled cells can be tracked using L-NPs.

References

1. Turano E et al. Int J Mol Sci 2023;24:2917.
2. Capuzzo AM et al. XXVII School of Pure and Applied Biophysics. Extracellular vesicles: from biophysical to translational challenges, Venice, 2023.
3. Busato A et al. Curr Protoc Cell Biol 2017;75:3.44.1-15.
4. Bontempi P. et al. Magn Reson Med 2018;79:459-69.

A NEW OBJECTIVE METHOD TO TEST ON SITE THE FISHERY PRODUCTS FRESHNESS

P. Caricato[1], G. Musile[2], I. Tagliaro[3], F. Bortolotti[2], F. Tagliaro[2]

[1]DG Health and Food Safety, EU Commission, Brussels, Belgium; [2]Dept. of Diagnostic and Public Health, Unit of Forensic Medicine, University of Verona, Verona, Italy; [3]Dept. of Materials Science, University of Milano Bicocca, Milano, Italy
E-mail: paolo.caricato@univr.it

According to EU legislation, random organoleptic controls shall be carried out at all stages of production, processing, and distribution of fishery products. One aim of the controls is to verify compliance with the freshness criteria. When the organoleptic examination gives rise to any doubt as to the freshness of the fishery products, the levels of total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) are tested in the flesh. The current chemical method applied is obsolete and time-consuming. The aim of the research is to find new investigative technologies that could be applied to fishery products for determining their freshness. In particular, after a feasibility study using an instrument method based on capillary electrophoresis,[1] based on two developed and validated low-cost approaches,[2,3] the capability of on-site approaches has been preliminarily tested. The instrumental method based on capillary electrophoresis has been used for the detection of ammonium in vitreous humour of Rainbow trout (*Oncorhynchus mykiss*). In a preliminary way, different chitosan-based membranes have been tested. The developed membrane has been tested at basic pH (activation of the membrane using 10 μL of 6 M NaOH) by pouring the sample containing ammonia (10 μL) directly on the membrane, and by promoting the evaporation of ammonia from a solution (200 μL) by adding a NaOH powder (20 mg) to the sample. The analysis of fresh (day 0) rainbow trout vitreous humour samples by using capillary electrophoresis showed the following ions composition: ammonium (below the limit of quantification, <0.01 mg/L), magnesium, calcium and potassium are the higher, respectively 9.15, 8.25, and 6.40 mg/L. The test for the detection of ammonium cations was repeated after 3 and 10 days showing a concentration of ammonium cations of 0.60 mg/L, and 2.10 mg/L, respectively. A preliminary investigation has been carried out using chitosan-based membranes on ammonia standard solutions over 5 order of magnitude range (0.145 mg/L – 0.145 x 10E5 mg/L). The optimized chitosan film demonstrated both for direct contact and gas diffusion, a color change down to 1.45 mg/L. These preliminary results confirm the possibility to use ammonium as a biomarker for demonstrating the freshness of fishery products.

References

1. Gottardo R et al. Clin Chem Lab.Med 2019;57:504-9.
2. Musile G et al. Forensic Sci Int 2019;295:150-6.
3. Musile G et al. Anal Chim Acta 2019;1083:150-6.

THE ROLE OF MICROSCOPY TO DEVELOP HYALURONIC ACID-BASED NANOPARTICLES FOR THE TREATMENT OF MUSCLE DISEASES

F. Carton[1], M. Repellin[2,3], G. Lollo[3], M. Malatesta[2]

[1]Dep. of Health Sciences, University of Piemonte Orientale 'A. Avogadro', Novara, Italy; [2]Dept. of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona; Italy; [3]University of Lyon, LAGEPP, Villeurbanne, France
E-mail: flavia.carton@uniupo.it

Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder that involves multiple organs and tissues, with particular emphasis on skeletal muscles. Currently, no therapy is available to cure DM1. However, some molecules like pentamidine (PTM) proved to be efficient in mitigating pathological hallmarks of DM1 but exhibit substantial toxicity. To overcome this issue, polymeric hyaluronic-acid-based nanoparticles (HA-NPs) were developed as a therapeutic strategy to efficiently deliver PTM[1]. To obtain HA-NPs suitable for clinical applications, it is mandatory to elucidate their behavior within the biological milieu. In this context, microscopical approaches represent a suitable technique able to characterize, localize and track the intracellular fate of NPs[2]. Firstly, morphological analysis of HA-NPs carried out at transmission electron microscopy (TEM) and Cryogenic TEM (CryoTEM) revealed a monodispersed population of rounded shaped particles. In the aim to precisely visualize the low electron dense HA-NPs at the ultrastructural level, Alcian Blue (AB) staining was applied[3]. Once HA-NPs were unequivocally identified inside muscle cells, TEM analysis revealed that HA-NPs were internalized via endocytosis with a time dependent internalization and a cytoplasmic accumulation. Finally, conventional and confocal microscopy were used to visualize the distribution of NPs in myofibers of explanted muscles maintained in a bioreactor. Results confirmed the behavior observed at TEM for cultured muscle cells. Furthermore, NPs were found to move along the entire myofibers of explanted muscles. The results obtained from this work underline the importance of microscopical technique to investigate the behavior of NPs inside cells and tissues, providing essential information for drug delivery strategies.

References

1. Repellin M et al. Nanomedicine 2023;47:102623.
2. Carton F et al. Eur J Histochem 2022;66:3603.
3. Carton F et al. Eur J Histochem 2019;63:3086.

ADIPOSE TISSUE ENRICHED WITH BIOPOLYMER SCAFFOLDS IN ISCHEMIA/REPERFUSION INJURY

A. Conti, F. Merigo, L. Quintero, R. Ossanna, G. Conti, A. Sbarbati

Dept. of Diagnostic and Public Health, University of Verona, Verona, Italy
E-mail: anita.conti@univr.it

Ischemia-reperfusion injury (IR) is a complex process that induces muscle functionality loss, atrophy, and necrosis.[1] The stromal vascular fraction (SVF) is a heterogeneous collection of cells contained within the adipose tissue.[2] SVF could promote muscle fiber regeneration. To deliver SVF, it has been proposed the use of

injectable hyaluronic acid (HA) that works as a cell carrier.[2] In this work, we combined the micro fragments enriched with SVF with a self-crosslinked low molecular weight HA to understand their synergetic role in an IR mouse model. The IR was induced through the tourniquet clamping of the quadriceps of mice. Reperfusion was induced by tourniquet remotion after 3 h. 24 h after the IR induction, animals were intramuscularly injected with SVF mixed with HA (SVF-HA). Animals treated with HA and saline were used as control. All animals were *in vivo* monitored by magnetic resonance imaging (MRI) at different time points. The motor performance of animals was monitored with Rotarod Test. After 18 days from IR, animals were sacrificed for histological examination. At 7 dpi, animals of the SVF-HA group showed a reduction of signal intensity, and their T2 relaxation time (T2) of the muscle tissue was 29 ± 0.5 ms, which is comparable with the T2 of contralateral muscular tissue. These suggest a reduction of edematous overflow and swelling. Contrarily, the T2 at 7dpi of control groups were 84 ± 2 and 90 ± 5 ms, respectively, which remained elevated throughout the experiment. The evaluation of vascular regeneration showed similar results. Indeed, DCE-MRI analysis revealed a complete recovery of muscular tissue perfusion after 14 dpi for the SVF-HA group, while the controls remained in a damaged state. Finally, the histological examination of SVF-HA treated animals exhibited well-defined and organized fiber morphology, like contralateral healthy muscular tissue. On the contrary, control groups presented fibers degeneration, characterized by atrophy, inflammation, and fibrotic areas. Our findings show that connective tissue micro-fragments enriched with SVF mixed with HA induce higher muscle homeostasis and perfusion restoration in contrast to control groups.

References

1. Kalogeris T et al. Int Rev Cell Mol Biol 2012;298:229-317.
2. Huang G et al. Drug Deliv 2018;25.1:766-72.
3. Hemshekhar M et al. Int J Biol Macromol 2016;86:917-28.

MODELING COMPLEX NANOSYSTEMS FOR DRUG DELIVERY, TARGETING AND IMAGING

C. Di Valentin[1,2,3], P. Siani[1], G. Frigerio[1], E. Donadoni[1], S. Motta[1]

[1]Dip. di Scienza dei Materiali, [2]BioNanoMedicine Center NANOMIB, and [3]NanoQlab, University of Milano-Bicocca, Milano, Italy
E-mail: cristiana.divalentin@unimib.it

In this poster, I will present an overview on our multiscale computational activity devoted to nanohybrid systems based on transition metal oxide nanoparticles (*i.e.* TiO_2) and graphene-based materials for biomedical applications. Atomic models of realistic size (2-4 nm, *i.e.* 800-4000 atoms) are used to simulate, at a quantum mechanical level of theory in combination with molecular mechanics and classical molecular dynamics, the structural and electronic properties of the nanosystems, their interaction with light and with the aqueous environment. Surface functionalization with stabilizing polymers or functionalizing molecular species for drug delivery, targeting and imaging is also investigated and discussed.

Acknowledgments: *The project has received funding from the European Research Council (ERC) under the European Union's HORIZON 2020 research and innovation programme (ERC Grant Agreement No [647020]) and from the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to Prof. Cristiana Di Valentin CUP H43C22000830006 of University of Milano Bicocca, Italy.*

References

1. Motta S et al. Nanoscale 2021;13:13000.
2. Siani P and Di Valentin C. Nanoscale 2022;14:5121.
3. Siani P et al. Colloid & Interface Sci 2022;627:126.
4. Donadoni E et al. Nanoscale 2022;14:12099.
5. Motta S et al. Nanoscale 2023; doi:10.1039/D3NR00397C.

MULTI-SCALE MODELING OF FOLIC ACID-FUNCTIONALIZED TiO_2 NANOPARTICLES FOR ACTIVE TARGETING OF TUMOR CELLS

E. Donadoni[1], P. Siani[1], G. Frigerio[1], C. Di Valentin[1,2]

[1]Dip. di Scienza dei Materiali, and [2]BioNanoMedicine Center NANOMIB, University of Milano-Bicocca, Milano, Italy
E-mail: e.donadoni@campus.unimib.it

Strategies based on the active targeting of tumor cells are emerging as smart and efficient nanomedical procedures.[1] Folic acid (FA) is a vitamin and a well-established tumor targeting agent because of its strong affinity for the folate receptor (FR), which is an overexpressed protein on the cell membranes of the tumor cells.[2] FA can be successfully anchored to several nanocarriers, including inorganic nanoparticles (NPs) based on transition metal oxides.[3] Among them, TiO_2 is extremely interesting because of its excellent photoabsorption and photocatalytic properties, which can be exploited in photodynamic therapy.[4] However, it is not yet clear in which respects direct anchoring of FA to the NP or the use of spacers, based on polyethylene glycol (PEG) chains, are different and whether one approach is better than the other. In this work, we combine Quantum Mechanics (QM) and classical Molecular Dynamics (MD) to design and optimize the FA functionalization on bare and PEGylated TiO_2 models and to study the dynamical behavior of the resulting nanoconjugates in a pure water environment and in physiological conditions. We observe that they are chemically stable, even under the effect of increasing temperature (up to 500 K). Using the results from long MD simulations (100 ns) and from free energy calculations, we determine how the density of FA molecules on the TiO_2 NP and the presence of PEG spacers impact on the actual exposure of the ligands, especially by affecting the extent of FA–FA intermolecular interactions, which are detrimental for the targeting ability of FA towards the folate receptor. This analysis provides a solid and rational basis for experimentalists to define the optimal FA density and the more appropriate mode of anchoring to the carrier, according to the final purpose of the nanoconjugate.[5]

References

1. Chen W et al. Angew Chem Int Ed 2021;60:5626-43.
2. Leamon CP and Low PS. Drug Discovery Today 2001;6:44-51.
3. Rajkumar S and Prabaharan M. Colloids Surf B 2020;186:110701.
4. Liang X et al. J Photochem Photobiol B 2021;215:112122.
5. Donadoni E et al. Nanoscale 2022;14:12099-116.

TOWARDS THE DESIGN OF NANO-PLATFORMS TO TARGET INTRACELLULAR PATHOGENS

S.C. Gaglio[1], Y. Jabalera[2], M. Montalbán-López[2], A.C. Millán-Placer[3,4], M. Lázaro-Callejón[5], M. Maqueda[2], M. Paz Carrasco-Jimenez[6], A. Laso[6], J. A Aínsa[3], G. R. Iglesias[5], M. Perduca[1], C. Jiménez López[2]

[1]Biocrystallography and Nanostructure Laboratory, Dept. of Biotechnology, University of Verona, Verona, Italy; [2]Dept. of Microbiology, Faculty of Sciences, University of Granada, Granada, Spain; [3]Departamento de Microbiología, Pediatría, Radiología y Salud Publica (Facultad de Medicina) & BIFI, Universidad de Zaragoza, Zaragoza, Spain; [4]CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain; [5]Dept. of Applied Physics and Instituto de Investigación Biosanitaria ibs.Granada, NanoMag Laboratory, and [6]Dept. of Biochemistry and Molecular Biology I, University of Granada, Granada, Spain
E-mail: salvatorecalogero.gaglio@univr.it

The World Health Organization (WHO) is warning about the increase in the failure of common antibacterial therapies due to the development and selection of multidrug-resistant (MDR) bacteria, mainly due to the generalized and/or extended drug administration. Among the strategies employed to avoid the development of multidrug-resistant condition, directed chemotherapy combined with local therapies (*e.g.*, magnetic hyperthermia) has gained great interest. Following this philosophy Jabalera *et al*. designed AS48-BMNPS nano-assembly showing a strong bactericidal effect not only on Gram-positive bacteria, but also against drug resistant strain, like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, when the antimicrobial activity of the peptide was combined with magnetic hyperthermia. The therapeutic approach becomes harder when the disease is caused by an intracellular pathogen (*Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella enterica* ...) due to the difficulties to reach the bacterium: thus, improving the cellular uptake of the nanocarrier is crucial for the success of the treatment. In this regard, AS-48-BMNPs was embedded with the FDA-approved Poly lactic-co-glycolic acid (PLGA) and the nanoplatform was also optimized in terms of size, colloidal stability, and hyperthermia activity (either magnetic or photothermal). We highlighted the potential of PLGA[AS-48-BMNPs] in the treatment and prevention of infection caused by intracellular pathogens, since the nanoplatform were internalized in THP-1 cells without affecting cell viability. Furthermore, the AS-48 slow-release pattern enabled by PLGA cover, could be an advantage in improving therapy efficacy providing sustained release. This could be the first step toward a new approach in the treatment of diseases caused by intracellular pathogenic species, even if belonging to MDR strains, using a safe nanomaterial.

A FLUID DYNAMIC SYSTEM TO TEST NANOVESICLES ON HUMAN SKIN EXPLANTS

A. Galvan, L. Calderan

Dept. of Neurosciences, Biomedicine and Movement Sciences, Section of Anatomy and Histology, University of Verona, Verona, Italy
E-mail: andrea.galvan@univr.it

Nowadays nanotechnologies are becoming increasingly more attractive due to their high potential in different fields, such as chemistry, energy and medicine. In the latter, for example, the production of nanoparticles could help in the delivery of drugs to their site of action in a more efficient way, increasing their bioavailability bypassing biological barriers, such as skin. In this study, the ability of vesicular nanocarriers to penetrate human skin was tested taking advantage from an *in vitro* model based on human skin explants preserved in an innovative fluid dynamic system[1]. Three types of nanovesicles were evaluated: ethosomes based on phosphatidylcholine (PC) 0.9% w/w (mean diameters = 200 nm) and transethosomes containing polysorbate 80 0.3% w/w and PC 0.9% w/w (mean diameters = 146 nm) or PC 2,7% w/w (mean diameters = 350 nm). Each formulation was applied topically on human skin explants maintained in a bioreactor for 24 h, time frame in which tissue integrity is optimally preserved[1,2]. The efficacy to penetrate into the deep layers of skin was tested with a transmission electron microscopy analysis. Results demonstrated that all the three types of nanovesicles succeeded in penetrating the skin while preserving their structural integrity, but reaching different depth levels. In detail, the highest penetration capability was seen in ethosomes, which reached basal keratinocytes and even the dermis, while PC 0.9% transethosomes were found until keratinocytes and the PC 2.7% ones in the corneocytes only. The ability to reach different skin layers likely depends on the physical-chemical characteristics of each nanovesicle. Thus, our dynamic skin model not only proved its suitability to act as a biological barrier *in vitro*, but provided original insights into the efficacy of ethosomes and transethosomes as nanovectors for topical administration.

References

1. Galvan A et al. Int J Mol Sci 2023;24:6284.
2. Cappellozza E et al. Microsc Microanal 2021;27:923-34.

TRANSMISSION ELECTRON MICROSCOPY METHODS TO REVEAL NANOPARTICLES INSIDE CELLS

C.R. Inguscio, M. Malatesta

Dept. of Neurosciences, Biomedicine and Movement Sciences, Section of Anatomy and Histology, University of Verona, Verona, Italy
E-mail: chiararita.inguscio@univr.it

Nanomedical research requires that new nanoproducts are investigated for their interactions with the biological environment. In fact, their safety and efficacy strictly depend on the structural and functional relationships established with cells, tissues and organs, whatever the scope of the nanoconstruct type (drug delivery systems, contrast agents, scaffold components, etc.). To this aim, transmission electron microscopy (TEM) plays a primary role due to its high resolution (about 0.2 nm in conventional TEM) that makes clearly visible the fine morphology of the nanoparticles as well as of the cellular components. Nanoparticles containing metal atoms such as gold or iron are markedly electron dense, thus being very evident in the cells as dark structures; on the contrary, nanoconstructs containing atoms of low mass (as it occurs with organic materials such as lipids or PLGA) show an electron density similar to many cellular organelles, thus being hardly recognizable[1]. To overcome this problem, in our laboratory we have been applying various methods to make unequivocally recognizable low-contrast nanoconstructs at TEM. Lipid-based nanoparticles were made electron dense by fixing the cells with osmium tetroxide, which binds the double carbon-to-carbon bonds induc-

ing the formation of a dark reduction product[2]. Hyaluronic acid-based nanoparticles were revealed at TEM by adapting the Alcian blue staining set up in the Sixties of the last century to visualize glycosaminoglycans in tissue sections[3]. Fluorescent or fluorescently-labelled nanoparticles were made visible at TEM by the photo-oxidation of diaminobenzidine, a procedure converting the fluorescent signal into electron dense precipitates[4]. The study of appropriate methods to make different kinds of nanoconstructs unequivocally identifiable in the intracellular environment at TEM therefore represents a basic, although little known, area of research in nanomedicine.

References

1. Malatesta M. Int J Mol Sci 2021;22:12789.
2. Costanzo M. et al. Int J Mol Sci 2021;22:5341.
3. Carton F et al. Eur J Histochem 2019;63:3086.
4. Malatesta M et al. Eur J Histochem 2012;56:20.

ETHOSOMES AND TRANSETHOSOME FOR VITAMIN D3 DELIVERY: AN *IN VITRO* STUDY

M.A. Lacavalla[1], E. Esposito[2], M. Sguizzato[2], C. Zancanaro[1], M. Malatesta[1]

[1]Dept. of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy; [2]Dept. of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy
E-mail: mariaassunta.lacavalla@univr.it

Vitamin D (VD) plays an important role on human health and its deficiency has been correlated to many disorders such as dermatological syndromes, systemic infections, cancer[1], and sarcopenia[2]. An adequate dietary supplementation of VD can reduce its lack. Moreover, VD topical administration could be a smart strategy to cure skin disease or increase muscular function especially in patients suffering from malnutrition, aging, hepatic or renal disorders. In this context, we studied the suitability of lipid-based nanosystems, named ethosomes (ET) and transethosomes (TET), as nanocarriers to deliver cholecalciferol (VD3). A formulative study has been performed to test the influence of pharmaceutically acceptable ionic and non-ionic surfactants in the preparation of different TET. The biocompatibility of ET and TET was evaluated on cultured keratinocytes, fibroblasts, and muscle cells, respectively representative of skin, connective tissue, and skeletal muscle tissue. Then, observations at bright field, fluorescence and transmission electron microscopy were conducted to assess nanocarriers internalization, intracellular fate, and the potential alterations of cellular organelles. Both ET and TET showed physicochemical features suitable for transdermal penetration and efficient VD3 loading; furthermore, they were internalized by the three cell types, although they followed two distinct intracellular fate: ET remained for long period inside the cytoplasm without provoking subcellular changes, while TET were rapidly degraded inducing lipid accumulation. These preliminary results set the stage for *in vivo* investigations aimed at testing vitamin D transdermal administration as a novel strategy to address skin pathological condition and/or age-related muscular diseases.

References

1. Lehmann B et al. Exp Dermatol 2004;13;11-55.
2. Dawson-Hughes B. J Steroid Biochem Mol Biol 2017;173:313-6.

CYTOTOXIC INVESTIGATION OVER SILVER AND GOLD NANOPARTICLES OBTAINED BY CHEMICAL AND BIOGENIC SYNTHESIS

M. Leoncini[1], S. Tinello,[1,5], C. Mazzariol[1,4], M. Mognato[2], S. Gross[1,3]

[1]Dept. of Chemical Sciences (DiSC), and [2]Dept. of Biology, University of Padova, Padova, Italy; [3]Institute of Chemical Technology and Polymer Chemistry (ITCP), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany; [4]Institute for Inorganic and Analytical Chemistry, Justus-Liebig-University Giessen, Giessen, Germany; [5]Laboratory for Multifunctional Materials, Departments of Materials, ETH Zürich, Zürich, Switzerland.
E-mail: martina.leoncini@studenti.unipd.it

Nowadays, silver (Ag) and gold (Au) nanoparticles (NPs) are mainly synthesized through classical chemical syntheses which results in more energy-consuming processes compared to a biogenic approach which is considered a cheaper, low temperature, safer and environmentally friendly method for obtaining inorganic nanomaterials.[1,2] The aim of the present work is to study and compare classical colloidal chemical (*i.e.* Turkevich method) and biogenic synthesis methodologies for obtaining Ag and Au NPs with particular regard to their cytotoxicity. Currently, these inorganic nanomaterials are widely studied for their chemical-physical and functional properties that make them ideal candidates for applications in numerous fields of considerable technological interest such as the biomedical one.[1] There is a growing interest in developing environmentally benign procedures for the synthesis of these metallic NPs; a promising approach to achieve this objective is to exploit the array of biological resources in nature. The biogenic agent of choice in this work is the black tea infusion, a widely consumed beverage throughout the world with health benefits, such as antioxidant one. The black tea is composed of different organic molecules which are well-known to be effective reducing and stabilizing agents in NPs synthesis.[3] In view of potential applications in the biomedical field, the syntheses were optimized in order to obtain high NPs concentrations and to ensure the NPs suspension stability over time. The NPs suspensions obtained were characterized with different analytical techniques such as UV-Vis, DLS, Z-potential and TEM. Both Ag and Au NPs, synthesized through biogenic reduction at room temperature, resulted in being smaller, more instable and morphologically non-uniform than the chemical ones obtained with sodium citrate dihydrate at relatively high temperature. Furthermore, considering their possible application in the biomedical field, the cytotoxicity of Ag and Au NPs produced by the two routes was tested in human lung carcinoma epithelial cells (A549), since their main absorption route is through the respiratory system. The different responses of the cells against the Ag and Au NPs synthesized through chemical and biogenic approaches were investigated. From the observation of any morphological alterations of the treated cells and from cell viability assays it was possible to establish the effective cytotoxic action of Ag and Au NPs. Biogenic Ag NPs resulted to be more cytotoxic than the chemical prepared ones because their toxic effect occurred at lower concentrations. Unlike Ag NPs, both chemical and biogenic Au NPs showed instead a high biocompatibility without any statistical evidence of cytotoxicity effect. Based on these observations, the tea infusion compounds also seem not to contribute to the toxic effect of NPs; a wider set of investigations is required to confirm these results.

References

1. Baig N et al. Mater Adv 2021;2:1821.
2. Thakkar KN et al. Nanomed: Nanotechnol Biol Med 2010;6:257-62.
3. Begum NA et al. Colloids Surf B 2009;1:113-8.

COATING NATURE DIRECTS THE CELLULAR UPTAKE OF GOLD NANOPARTICLES

F. Luchi, A. Eguskiza Bilbao, R. Ossanna, G. Climani, R. Fiammengo

[1]Laboratory of Organic Chemistry and Nanobiointeractions, Dept. of Biotechnology, University of Verona, Italy.
E-mail: federico.luchi@univr.it

Nanoparticles (NPs) that are introduced into a biological medium interact with the biomolecules that are present, in particular with proteins, leading to the formation of the so-called protein corona. The presence of the protein corona may alter the characteristics of the NPs and consequently influence their cellular uptake.[1] This work focuses on the comparison between 13 nm diameter spherical gold nanoparticles[2] (AuNPs) coated with different hydrophilic polymers and how these coatings modulate cellular internalization. The synthetized polymeric coatings consist of thiols with a C11-long alkyl chain and a hydrophilic part characterized by polyethylene glycol (PEG) or polyglycerol (PG) units with neutral or negative charge. While in literature a number of studies deal with PEG-based coatings,[3] there is only scarce information about PG-based coatings. In this contribution, we describe the impact of a progressive and gradual variation of the coating structure on nanoparticle internalization by immune system cells. We demonstrate that the increase of surface disorder and the reduction of surface charge result in improved stealth properties. We also demonstrate that PG-based coatings may be a valuable alternative to PEG. These AuNPs show properties that can be exploited in therapeutic and diagnostic application fields.

References

1. Mishra RK et al. Int J Biol Macromol 2021;175:1-18.
2. Turkevich J et al. Discussions of the Faraday Society 1951;11:55-75.
3. Maus L et al. ACS Nano 2010;4:6617-28.

EFFECTIVENESS OF BARRIER CREAMS AGAINST HUMAN SKIN PENETRATION OF Ni NANOPARTICLES

G.C. Magnano[1,2], M. Crosera[1], F. Larese Filon[2], D. Voinovich[1]

[1]Dept. of Chemical and Pharmaceutical Sciences, and [2]Clinical Unit of Occupational Medicine, University of Trieste, Trieste, Italy
E-mail: gmagnano@units.it

Nickel nanoparticles (NiNPs) have increasingly attracted researchers' attention for their unique physical and chemical properties such as small size effect, surface effect, quantum size effect, and macroscopic quantum tunneling effect of nanomaterials.[1] Owing to these characteristics, they are widely used in in several fields including modern metallurgy, bioengineering, and medicine. The broad application of NiNPs represents a new type of environmental[2] and occupational exposure. Unintended exposure to human skin may occur through the manufacture and removal of used NPs, mainly in an occupational setting. As nanoparticles, NiNPs may easily permeate and penetrate through the skin compared to bulk material leading to various health problems.[3] In fact, such growth of the areas of application is actually accompanied with an increase of exposure to Ni, thus an intensification of the negative effects, the most frequent being the allergic contact dermatitis. Further, it is important to consider that an exposure (or accidental contamination) to NiNPs can bring to a potential local and/or systemic health risk.[3] According to the International Agency for Research on Cancer (IARC) Ni compounds are classified as carcinogenic to humans (Group 1) and metallic Ni is classified as possibly carcinogenic to humans (Group 2B).[4] To reduce the Ni cutaneous penetration, barrier creams (BC) are applied on the skin surface. There is little information, however, on the efficiency of such commercial protective creams on decreasing Ni cutaneous penetration. For this reason, the objective of the current study was to investigate the protective role of one commercially available formulation for Ni (Nik-L-Block™ containing a chelating agent) and one moisturizing cream (Ceramol 311 basic cream without chelating agent), following exposure to NiNPs, using *in vitro* Franz cells, as well as the cytotoxicity of NiNPs in primary human dermal fibroblasts was studied. Our results demonstrated that although both tested formulations can decrease Ni accumulation in the skin (4.13 ± 1.74 $\mu g/cm^2$ for Nik-L-Block™ and 7.14 ± 1.46 $\mu g/cm^2$ for Ceramol 311 basic cream); there are significant differences between the two creams ($p = 0.004$). Based on the experimental evidence, we therefore conclude that the composition of such formulations has an imperative role for dermal uptake of Ni. Finally, NiNPs showed no cytotoxic effect on cultured human dermal fibroblasts after 24 and 72 h.

References

1. Flauraud V et al. Nat Nanotechnol 2017;12:73-80.
2. Lippmann M et al. Environ Health Perspect 2006;114:1662-9.
3. Stefaniak A et al. Environ Sci Process Impacts 2014;16:341.
4. IARC Monogr Eval Carcinog Risk Hum100C 2012;169-218.

BIOGENIC SYNTHESIS OF ZnS AND ZnO NANOPARTICLES BY MICROALGAE EXTRACTS FROM *NANNOCHLOROPSIS GADITANA*

C. Maucieri[1], S. Tinello[1,4], C. Mazzariol[1,5], G. Perin[2], T. Morosinotto[2], M.M. Natile[1,6], S. Gross[1,3]

[1]Dept. of Chemical Sciences (DiSC), and [2]Dept. of Biology, University of Padova, Padova, Italy; [3]Institute of Chemical Technology and Polymer Chemistry (ITCP), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany; [4]Laboratory for Multifunctional Materials, Dept. of Materials, ETH Zürich, Zurich, Switzerland; [5]Institute for Inorganic and Analytical Chemistry, Justus-Liebig-University Giessen, Giessen, Germany; [6]Institute of Condensed Matter Chemistry and Technologies for Energy (ICMATE), National Research Council (CNR), Padova, Italy
E-mail: corrado.maucieri@studenti.unipd.it

In recent years, many studies have been focused on the development of new methods for the synthesis of nanoparticles compliant with the principles advocated by Green Chemistry,[1] which has the ultimate goal of avoiding or reducing the use of toxic substances, preferring instead the use of renewable resources and minimizing waste production. For this reason, several research groups have sought to replace the classical chemical syntheses with more environmentally friendly and sustainable techniques. Within this context, biogenic synthesis is an excellent solution as it is simple, sustainable, cost-effective and environmentally friendly.[2] Biogenic synthesis involves the use of a bioactive component, typically enzymes, microorganisms, or plant extracts, which promotes the reduction and/or stabilization of inorganic nanoparticles and can additionally act as a "green scaffold", promoting the formation of

nanoparticles with different morphologies. The main goal of this project is the biogenic synthesis of zinc oxide (ZnO) and zinc sulfide (ZnS) nanoparticles obtained through the exploitation of the microalgae extract from *Nannochloropsis gaditana*, which serves as a bioactive component. Several studies report that algae are among the best candidates in the production of nanomaterials,[3,4] as they are easy to cultivate, abundant in nature, and, unlike bacteria and fungi, in general, do not produce toxins. However, there are still critical experimental and theoretical issues to be overcome as, for example, to unveil the exact mechanisms involved in the biogenic synthesis of metal oxides. The synthesized micro- and nanostructures were characterized through different analytical tools from the structural, morphological, and functional points of view. Furthermore, Eu^{3+}-doped ZnO and ZnS nanoparticles obtained by biogenic synthesis were also considered in order to modulate the luminescence properties of the NPs through the formation of new energy levels in the forbidden band. The fluorescence properties were investigated in order to evaluate thei potential use of the Eu-doped nanoparticles in the field of optical bio-imaging.

References

1. Anastas PT and Warner JC. Green Chemistry: Theory and Practice, New York, 1998.
2. Ingle A et al. Biogenic Synthesis of Nanoparticles and Their Role in the Management, in Microbial Nanotechnology, CRC Press, 2020;135-62.
3. Fawcett D et al. Journal of Nanoscience 2017; doi.org/10.1155/2017/8013850.
4. Makarov VV et al. Acta Naturae 2014;6:35-44.

HYALURONAN-CHOLESTEROL NANOGELS FOR THE ENHANCEMENT OF THE OCULAR DELIVERY OF THERAPEUTICS

C. Migliorini[1], L. Paoletti[1], N. Zoratto[1], L. Forcina[2], A. Musarò[2], R. Matassa[3], G. Familiari[3], L. Mosca[4], M. Mattei[5], C. Di Meo[1], P. Matricardi[1]

[1]Dept. of Drug Chemistry and Technologies, [2]DAHFMO-Unit of Histology and Medical Embryology, [3]Dept. of Anatomical, Histological, Forensic and Orthopaedic Sciences, Section of Human Anatomy, and [4]Dept. of Biochemical Sciences "A. Rossi Fanelli", Sapienza University of Rome, Rome, Italy; [5]Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy
E-mail: migliorini.1746848@studenti.uniroma1.it

The anatomy and physiology of the eye have always been a limit to the local delivery of therapeutics; cornea represents the main mechanical and chemical barrier limiting the diffusion of both hydrophilic and hydrophobic drugs. Thus, the use of nanocarriers able to encapsulate therapeutics appears as an attractive strategy to facilitate the permeation and enhance ocular drug delivery[1,2]. Among all the polymeric nanocarriers, polysaccharide-based nanogels (NHs) offer several advantages, such as biocompatibility, biodegradability and mucoadhesive property[3,4]. Since its abundance in the eyes, hyaluronic acid (HA) represents a good candidate for the preparation of NHs[5]. On this basis, an HA's amphiphilic derivative, obtained by grafting the polymeric backbone with cholesterol moieties (HA-CH), was used to obtain self-assembled NHs able to load both hydrophobic (dexamethasone and piroxicam) and hydrophilic (tobramycin and diclofenac) drugs. *Ex vivo* studies by fluorescence microscopy and in-tube analyses with mucin showed that HA-CH NHs can interact with corneal components, being retained on porcine corneas, but they weren't able to penetrate the stroma. Furthermore, e*x vivo* transcorneal permeation experiments were performed to assess the capability of such NHs to behave as permeation enhancers, showing that NHs formulations can improve the ocular bioavailability of the instilled drugs by increasing their preocular retention time (hydrophobic drugs) or facilitating their permeation (hydrophilic drugs).

References

1. Mun EA et al. Mol Pharmaceutics 2014;11:3556.
2. Cuggino JC et al. J Control Release 2019;307:221.
3. Ilka R et al. Int J Biol Macromol 2018;109:955.
4. Grimaudo MA. Int J Pharm 2020;576:118986.
5. Zoratto N et al. Pharmaceutics 2021;13:1781.

OPTICAL THERMOMETRY USING LANTHANIDE ACTIVATED FLUORIDE NANOMATERIALS

E. Milan, C. Cressoni, F. Dalla Nese, E. Radicchi, A. Speghini

[1]NRG, Dept. of Biotechnology, University of Verona, and INSTM, RU Verona, Verona, Italy
E-mail: adolfo.speghini@univr.it

Nanoparticles (NPs) doped with lanthanide ions (Ln^{3+}) have many applications in nanomedicine as sensors and diagnostic probes, thanks to their strong luminescence.[1,2] In this communication, we describe fluoride-based nanomaterials, as KY_3F_{10}, properly activated with Ln^{3+} ions (*e.g.* Yb^{3+}, Nd^{3+}, Tm^{3+} and Er^{3+}), which show strong emissions in the optical range, upon visible or Near Infrared (NIR) laser excitation. Their preparation in colloidal form exploits a "green chemistry" method, involving a microwave (MW) assisted hydrothermal technique and biocompatible capping agents, with excellent colloidal chemical stability of the resulting water-based dispersions, also due to coordination of the capping molecules on the NPs surface. Core@shell structured NPs, with different Ln^{3+} doping between the core and shell, have been also prepared with the same method and show enhanced luminescent efficiency in colloidal water dispersions. The optical thermometry of the colloidal NPs exploiting luminescence bands within the biological windows will be considered. Ratiometric methods for temperature measurements will be described, using emissions due to the same or different lanthanide ions.

References

1. Cressoni C et al. ACS Appl Mater Interfaces 2023;15:12171-88.
2. Cortelletti P et al. Nanoscale 2018;10:2568-76.

INFLUENCE OF THE SYNTHESIS CONDITIONS ON THE PARTICLE SIZE AND LUMINESCENCE OF LANTHANIDE ACTIVATED FLUORIDE-BASED NANOMATERIALS

E. Milan[1], N. Da Roit[2], C. Cressoni[1], F. Dalla Nese[1], E. Radicchi[1], N. Fernandez Delgado[3], M. Herrera Collado[3], A. Speghini[1]

[1]Nanomaterials Research Group, Dept. of Biotechnology, University of Verona, and INSTM, RU of Verona, Verona, Italy; [2]Institut fuer Katalyseforschung und technologie (IKFT), Eggenstein-Leopoldshafen, Germany; [3]Dept. of Materials Science and Metallurgic Engineering and Inorganic Chemistry, University of Cadiz, Puerto Real, Càdiz, Spain
E-mail: emil.milan@univr.it

Lanthanide (Ln) activated fluoride nanoparticles are one of the

most interesting nanomaterials (NMs) for application in nanomedicine, as theranostic tools.[1-3] The synthesis conditions strongly affect their chemico-physical properties, so that understanding how these conditions influence the obtained NMs permits an efficient tuning of their features.[4] In this study, we investigated Ln-doped alkaline earth fluorides (as CaF_2 and SrF_2) NMs synthesized with a microwave assisted hydrothermal method, adding biocompatible, hydrophilic capping agents such as citrate and glutamate. We found that a proper selection of synthesis conditions (*e.g.* temperature and reaction time, reagents concentration, capping moiety) allows to tune the NMs size, which also reflects on their luminescent properties. In particular, preliminary results demonstrated that it is possible to finely tune the NMs size over tenths of nanometers on varying the reaction temperature and time and the reagents concentration, while their surface charge can be modulated by using different capping agents. Finally, the interesting luminescence features found for the investigated NMs can be enhanced by growing a shell around the particle core.

References

1. Cortelletti P et al. Nanoscale 2018;10:2568.
2. Lucchini G et al. Nanoscale Adv 2019;1:757.
3. Cressoni C et al. ACS Appl Mater Interfaces 2023;15:12171-88.
4. Przybylska D et al. Sci Rep-Uk 2019;9:8669.

CIRCULARLY POLARIZED ACTIVITY FROM TWO PHOTON EXCITABLE EUROPIUM AND SAMARIUM CHIRAL BIOPROBES

S. Mizzoni[1,2], S. Ruggieri[1], A. Sickinger[2], F. Riobé[2], L. Guy[2], M. Roux[2], G. Micouin[2], A. Banyasz[2], O. Maury[2], B. Baguenard[3], A. Bensalah-Ledoux[3], S. Guy[3], A. Grichine[4], X.-N. Nguyen[5], A. Cimarelli[5], M. Sanadar[6], A. Melchior[6], F. Piccinelli[1]

[1]Luminescent Materials Laboratory, DB, University of Verona, and INSTM, UdR Verona, Verona, Italy; [2]Univ. Lyon, ENS de Lyon, CNRS UMR 5182, Laboratoire de Chimie, Lyon, France; [3]Univ. Lyon, Institut Lumière Matière, UMR 5306 CNRS–Université Claude Bernard, Lyon 1, Villeurbanne Cedex, France; [4]Institute for Advanced Biosciences, University Grenoble Alpes, Inserm U1209, CNRS, UMR 5309, La Tronche, France; [5]Centre International de Recherche en Infectiologie (CIRI), Univ. Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France; [6]Dip. Politecnico di Ingegneria e Architettura, Laboratorio di Tecnologie Chimiche, University of Udine, Udine, Italy
E-mail: silvia.mizzoni@univr.it

In this contribution[1], we synthesized and spectroscopically characterized in polar protic solvents (water, methanol) two couples of cationic enantiomeric complexes [(R,R)-[LnL]Cl and (S,S)-[LnL]Cl, with Ln = Sm and Eu and L = N, N'-bis(2-pyridylmethyl)- 1,2-(R,R or S,S)- cyclohexanediamine functionalized at sp3 N with the picolinate antennae[2]]. Both complexes are highly stable in aqueous solution (logK = 20.13 for the EuL species chosen as representative) and only one main species is present at physiological pH (7.4). The complexes exhibit a good CPL activity, in particular for the magnetic dipole (MD) allowed transitions, [$^4G_{5/2} \rightarrow {}^6H_{5/2}$ (564 nm) of Sm(III) and $^5D_0 \rightarrow {}^7F_1$ (593 nm) of Eu(III)]. Since the luminescence of both Sm(III) and Eu(III) complexes can be sensitized upon 1P and 2P excitation of the chromophoric antenna, they can be considered promising candidates as NIR-to-RED in cellulo chiroptical bioprobes. In fact, preliminary biphotonic imaging experiments on (S,S)-[EuL]Cl complex reveal that it can be easily internalized in two different cell lines (293T cancer cells and THP-1 macrophages).

References

1. Mizzoni S et al. J Mater Chem C 2023;11:4188-202.
2. Bunzl J-CG et al. Chem Soc Rev 2005;34:1048-107.

THE USE OF ZEBRAFISH EARLY LIFE STAGES TO INVESTIGATE THE METABOLISM AND TOXIC EFFECTS OF NINE NOVEL PSYCHOACTIVE SUBSTANCES WITH OPIOID EFFECTS

M. Murari[1], S. Pesavento[1], F. Greco[2], A. Vettori[2], F. Tagliaro[1], R. Gottardo[1]

[1]Unit of Forensic Medicine, Dept. of Diagnostics and Public Health, and [2]Dept. of Biotechnology, University of Verona, Verona, Italy
E-mail: matilde.murari@univr.it

The use of novel psychoactive substances (NPS) has been dramatically increasing worldwide and, among them, synthetic opioids are one of the fastest growing groups. Cinnamylpiperazines and 2-benzylbenzimidazoles represent two of the most relevant subclasses.[1] However, data on their toxicity and metabolism are still limited. Zebrafish larvae have been suggested as an alternative animal model to perform an initial toxicity assessment of new drugs. The aim of the present study was to evaluate toxicity and metabolic pathways of AP-237, 2-methyl AP-237, isotonitazene, flunitazene, etodesnitazene, metonitazene, metodesnitazene, N-pyrrolidino etonitazene, butonitazene by using zebrafish larvae. The cellular toxicity was assessed at the fourth day post-fertilization with Acridine Orange staining. Possible morphological defects were evaluated after 24 h of exposure to 1 μM concentration of each drug. After these assays, larvae underwent extraction to carry out a metabolic analysis with a Vanquish UPLC coupled with an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer. Scattered spots of apoptotic cells were higher in larvae exposed to drugs compared to the control. Overall, the morphological assay showed some deformities in exposed larvae. However, no significant difference in mortality between treatment and control groups was observed. By using LC-HRMS with full-scan mode acquisition several metabolites, mainly produced through monohydroxylation, N-dealkylation, and O-dealkylation, were identified. In the present study, the toxicological effects of the studied synthetic opioids were assessed using zebrafish embryos. Morphological defects, as well as cell apoptosis were observed. In addition, the study allowed for the identification of biotransformations consistent with those reported in literature, suggesting the possibility of using these metabolites as biomarkers of synthetic opioids use.

Reference

1. United Nations Office on Drugs and Crime (UNODC). (2020). UNODC Early Warning Advisory (EWA) on New Psychoactive Substances (NPS). https://www.unodc.org/LSS/Home/NPS.

IRON-BORON NANOPARTICLES AS DIAGNOSTIC AGENT IN MRI AND THERAPEUTIC AGENT IN PHOTOTHERMAL AND MAGNETIC FLUID HYPERTHERMIA

A. Negri[1], M. Bissoli[2], V. Amendola[2], P. Marzola[3]

[1]Dept. of Diagnostic and Public Health, and [3]Dept. of Engineering for Innovation Medicine, University of Verona, Verona, Italy; [2]Dept. of Chemical Sciences, University of Padova, Padova, Italy
E-mail: alessandro.negri@univr.it

Hyperthermia, a limited increase in tumor tissue temperature up to a maximum of 45°C, is considered a promising therapeutic approach thanks to the capability of localized treatment with negligible systemic toxicity. Among different hyperthermia methods, photothermal therapy (PTT) and magnetic fluid hyperthermia (MFH) have attracted extensive research attention in the last decades. In this work, we investigated innovative Iron-Boron (Fe-B) nanoparticles (NPs) as PTT and MFH agents. Such NPs can convert near-infrared (NIR) light and magnetic energy into heat. Here we report preliminary results of Fe-B NPs as multimodal therapeutic agents and as diagnostic agents in MRI. Fe-B NPs are synthetized as reported in.[1] Since penetration-depth of NIR light is limited to a few mm, human melanoma A375 cells were chosen for experiments. Without 785 nm laser irradiation, Fe-B NPs are safe up to 50 μgFe/mL when incubated for 24 and 48 h with cells. At this concentration, when irradiated for 10 min at 200 mW, the cell viability decreases to 53%. Fe-B NPs have good characteristics as magnetic fluid agents for MFH (SAR = 40.46 W/g; f = 524.7 kHz and H = 15.9 kA/m). Moreover, Fe-B NPs can act as contrast agents in MRI since their transversal relaxivity resulted in an r_2 = 60.28 $mM^{-1}s^{-1}$ (comparable to Endorem®). The performances of Fe-B NPs as PTT agents, combined with the SAR and relaxivity values, open the way to their use in bimodal therapy guided by tomographic imaging.

Reference

1. Torresan V et al. Adv Healthc Mater 2021;10:e2001632.

SYSTEMIC ADMINISTRATION OF DOPED FERRITE NANOPARTICLES FOR MAGNETIC FLUID HYPERTHERMIA ANTITUMOR TREATMENT

A. Negri[1], F. Vurro[2#], M. Gerosa[2], M. Muccilli[2], G. Piccolantonio[3], A.M. Capuzzo[1], E. Milan[4], E. Forlin[5], F. Gherlinzoni[6], G. Morana[6], M. Gottardi[6], P. Matteazzi[5,6], A. Speghini[4], P. Marzola[3]

[1]Dept. of Diagnostics and Public Health, [2]Dept. of Computer Science, [3]Dept. of Engineering for Innovation Medicine, and [4]Dept. of Biotechnology, University of Verona, Verona, Italy; [5]M.B.N. Nanomaterialia S.p.A., Treviso, Italy; [6]Foundation for Nanotheranostics Research in Cancer Therapy, RNC, Treviso, Italy.
[#]Current affiliation: Division of Experimental Oncology, Urological Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy.
E-mail: alessandro.negri@univr.it

The delivery of an effective quantity of nanoparticles (NPs) to tumors, with homogeneous distribution throughout the tissue, remains one of the main limitations to the clinical applications of nanotechnologies. We have previously demonstrated the efficacy of doped ferrite (G-M55) nanoparticles as Magnetic Fluid Hyperthermia (MFH) agents when injected intratumorally in an experimental model of breast cancer.[1,2] However, the presence within the tumor tissue of regions that remained free of NPs strongly limited the efficacy of the treatment. We have therefore tested the possibility of systemic administration through intravenous injection. Two experiments were performed with high dosage administration of G-M55 and (in one of the two experiments) with concomitant administration of liposomes to limit the liver uptake. In both experiments, the iron content within the tumor tissue was evaluated *in vivo* via Magnetic Resonance Imaging (MRI), and *ex vivo* via Prussian Blue (PB) staining. The presence of relevant amounts of iron was detected by PB staining, while MRI data showed only a slight alteration in Signal Intensity (SI) and quantitative T_2 mapping. Moreover, the iron content was evaluated by PB staining in other organs, such as in liver, spleen, and kidneys. Hematoxilin and Eosin staining revealed that the morphology of such organs is not altered, hence demonstrating the safety of i.v. administration of high dosages of G-M55.

References

1. Gerosa M et al. Nanotheranostics 2021;5:333-47.
2. Vurro F et al. Cancers 2022;14:5150.

ADIPOSE TISSUE-DERIVED PRODUCTS FOR REGENERATIVE MEDICINE APPLICATIONS

R. Ossanna, L. A. Quintero Sierra, A. Conti, G. Conti, A. Sbarbati

[1]Dept. of Neuroscience, Biomedicine and Movement, Human Anatomy and Histology Section, University of Verona, Verona, Italy
E-mail: riccardo.ossanna@univr.it

Adipose tissue-derive products are widely used in reconstructive surgery and regenerative medicine applications.[1] Several studies demonstrate that the tissue regeneration effects of Mechanically-Digested Stromal Vascular Fraction (MD-SVF), are attributable to its rich content of adipose-derived stem cells (ASCs). ASCs are adult mesenchymal stem cells easily isolable from adipose tissue allowing autologous cell transplantation.[1] ASCs module the inadequate healing responses which lead to tissue degeneration, such as chronic inflammation, hypermetabolic responses and fibrosis.[2] Moreover, ASCs stimulate extracellular matrix production, new collagen deposition and early revascularisation.[3] Their biological effect is due to the self-renewal property, immunosuppressive potential, and ability to differentiate into different mesodermal cell lineages, such as adipocytes, osteocytes and chondrocytes.[4] A novel stem cell niche, called multilineage-differentiating stress-enduring cells (Muse cells), is recently emerging. These cells are of particular interest because they are pluripotent and are found in adult human mesenchymal tissues.[5] One of their most significant features is that they can produce representative cells of the three germ layers. Furthermore, they can be easily isolated from the large population of mesenchymal stem cells. Adipose tissue was mechanically digested using a novel Nanofat system to obtain the MD-SVF. It was *in vitro* characterised in terms of ASCs content, multi-lineage differentiation ability and FACS analysis. Furthermore, a neuronal differentiation of the Nanofat product was performed in order to investigate the possible MD-SVF application in the central nervous system regeneration. Our results showed that the Nanofat-derived ASCs can grow in colonies and

show high differentiation capability into adipocytes, osteocytes, and chondrocytes. Moreover, immunophenotyping analysis revealed the expression of Muse cells antigen, making this Nanofat enriched of pluripotent stem cell, increasing its potential in regenerative medicine. In addition, Nanofat extracted stem cells were able to *in vitro* differentiate into neuronal-like cells.

References

1. Dai R et al. Stem Cells Int 2016;2016:1-19.
2. Kotani T et al. Sci Rep 2017;7:14608.
3. Kalinina N et al. Stem Cell Res Ther 2015;6:221.
4. Paliwal S et al. J Biomed Sci 2018;25:31.
5. Sato T et al. Cell Transplant 2020;29:963689720923574.

HYALURONAN-ESTRADIOL NANOGELS AS POTENTIAL DRUG CARRIERS TO TARGET ER+ BREAST CANCER CELL LINE

L. Paoletti[1], N. Zoratto[1], L. Masuelli[2], R. Bei[3], P. Matricardi[1], M. Nalli[1], C. Di Meo[1]

[1]Dept. of Drug Chemistry and Technologies, and [2]Dept. of Experimental Medicine, Sapienza University of Rome, Rome, Italy; [3]Dept. of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy
E-mail: luca.paoletti@uniroma1.it

The delivery of bioactive molecules to the target site has attracted increasing attention over the past three decades as a turning point in the treatment of several diseases. In fact, the disadvantages of the traditional drug use could be overcome by controlling and targeting the delivery of bioactive molecules using nano-drug delivery systems[1]. Polysaccharide-based nanoparticles offer benefits in terms of high loading efficiency, rapid drug release rates and good targeting ability through the possibility of easy functionalization of the polymeric backbones[2,3]. Finally, small molecules, peptides, proteins, and nucleic acids can be loaded into nanoparticle systems, which can be functionalized on the surface to actively deliver the cargo to cells that have receptors capable of selectively recognizing the ligand[4]. In this light, an innovative hyaluronan-based nano-delivery system is proposed for the active targeting towards ER+ breast cancer. Hyaluronic acid (HA), an endogenous and bioactive anionic polysaccharide, is functionalized with estradiol (ES), a sexual hormone involved in the development of some hormone-dependent tumors, to give an amphiphilic derivative (HA-ES) able to spontaneously self-assemble in water to form soft nanoparticles or nanogels (ES-NHs). ES-NHs ability to entrap hydrophobic molecules has been also investigated, by loading curcumin and docetaxel, both able to inhibit the growth of ER+ breast cancer. The formulations are studied for their capability to inhibit the growth of the MCF-7 cell line, thus evaluating their efficacy and potential as a selective drug delivery system, and internalization assays are performed to see the distribution of the nanosystems inside the cells.

References

1. Mazdaei M et al. Br J Pharm 2022;7:1-13.
2. Plucinski A et al. J Mater Chem B 2021;9:7030-62.
3. Zoratto N et al. Pharmaceutics 2021;13:1781.
4. Sanità G et al. Front Mol Biosci 2020;7:587012.

A POINT-OF-NEED DEVICE FOR DETECTING CYANIDE IN CADAVERIC BLOOD: INTEGRATION OF GAS DIFFUSIVE AND PAPER-BASED TECHNOLOGIES

S. Pesavento, G. Musile, F. Bortolotti

[1]Unit of Forensic Medicine, Dept. of Diagnostic and Public Health, University of Verona, Verona, Italy
E-mail: sara.pesavento_01@univr.it

Cyanide is a potent and short-acting toxicant, which may enter the bloodstream by inhalation, ingestion and skin-absorption.[1] Accidental deaths (*e.g.* fires involving plastic materials), suicides or homicides related to this poison are frequently encountered in clinical and forensic practice. The determination of cyanide levels in biological fluids could be performed by using different chromatographic methods, which require complex instrumentation and trained personnel.[2] For this reason, instrument-free techniques, involving colorimetric detection, have been recently proposed. However, these methods could not be carried out without sample pre-treatments, due to the complex nature of blood and its deep red color.[3] The aim of present study was the development of a custom-made device, suitable for a rapid and direct detection of cyanide in cadaveric blood and based on the integration of gas diffusion approach with paper-based analytical devices (pad). Briefly, the device consists of a glass vial with a 'modified cap', which holds a circular piece of pad with a hydrophilic and a hydrophobic part. The analytical procedure could be summarized in three steps. Firstly, 200 μL of cadaveric blood are transferred into the glass vial containing trifluoroacetic acid (25 μL), which converts cyanide into volatile hydrogen cyanide (above 26 °C) that reaches the 'modified cap'. Secondly, 10 μL of ninhydrin solution (20 g/L in sodium carbonate 20 g/L) are placed on the hydrophilic part of the pad, forming a droplet, where cyanide, if present, reacts with ninhydrin. The reaction produces a color change (from yellow to red) of the droplet. Afterwards, a picture was taken by means of a smartphone camera and quantitatively evaluated in terms of red, green and blue components by using a free application (ImageJ). The analytical approach was optimized and validated in terms of selectivity, sensitivity, trueness and imprecision. Moreover, LOD and LLOQ were calculated as 2.4 and 4.8 μM, respectively. Finally, the method was tested by analyzing real and spiked cadaveric blood samples (n=12) by using both the point-of-need device and a gas chromatographic method, obtaining consistent results.

References

1. Baselt RC. Disposition of toxic drugs and chemicals in man, 11th ed., Biomedical publications, 2018.
2. Frison G et al. Rapid Commun Mass Spectrom 2006;20:2932-8.
3. Jackson R et al. Anal Chim Acta 2017;960:18-39.

A NEW APPROACH FOR IDENTIFYING THE *POST-MORTEM* FORMATION OF ETHANOL: DEVELOPMENT AND VALIDATION OF A SALT-ASSISTED HS-GC-FID METHOD FOR DETERMINING ETHANOL IN THE VITREOUS HUMOR

N. Pigaiani[1,2], G. Musile[1], E. Pasetto[1], M. Ballotari[1], F. Bortolotti[1], F. Tagliaro[1]

[1]Unit of Forensic Medicine, Dept. of Diagnostics and Public Health, University of Verona, Verona, Italy; [2]Division of Forensics, Dept. of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA
E-mail: nicola.pigaiani@univr.it

Alcohol is the psychoactive compound most frequently detected in postmortem specimens in cases of violent and accidental deaths. The presence of concentration limit thresholds established by law requires that the diagnostic procedures show the maximum accuracy of both the pre-analytical and analytical phases. In this regard, it is necessary to consider that *post-mortem* fermentation phenomena can influence the quantification of ethanol in cadaveric blood. To overcome this problem, some authors have proposed determining alcohol in the vitreous humor (VH), which is less affected by putrefactive phenomena than blood and has a comparable diagnostic window. The present work aimed to verify the capability of an already validated diagnostic approach to determine ethanol in *post-mortem* VH by salted-assisted HS-GC-FID[1] in identifying *post-mortem* ethanol formation. The method was based on the 1:9 dilution of VH with a solution of 2.5 mol/L K_2CO_3 and 0.0012 mol/L tert-butanol (internal standard). After 1 min of incubation, part of the specimen, evaporated in the headspace (2000 μL), was injected into the chromatographic system and analyzed in isothermal mode (40ºC), with a chromatographic time of 1.6 min. The total run time was 2.6 min. The method was applied to 100 samples of VH. The resulting alcohol concentrations were compared with that identified in the corresponding blood samples. Most of the samples (81.3%) showed a good correlation (R2 = 0.9846) between the two matrices with an average difference of 0.25%. In the remaining samples, the blood alcohol concentrations were significantly higher than the VH alcohol concentration (1300%). All these cases showed the presence of n-propanol in blood samples, confirming a *post-mortem* production of ethanol. The validated salted-assisted HS-GC-FID method for determining ethanol in VH was a supplementary diagnostic approach in *post-mortem* cases, suitable to identify ethanol production by putrefactive phenomena.

Reference

1. Musile G et al. J Anal Toxicol 2022;46:e274-79.

NIOGELS FOR POLYPHENOLS SKIN APPLICATION

M. Sguizzato[1], P. Mariani[2], A. Baldisserotto[3], L. Montesi[3], M. Drechsler[4], R. Cortesi[1]

[1]Dept. Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy; [2]Dept. Life and Environmental Sciences, Polytechnic University of Marche, Italy; [3]Dept. Life Sciences and Biotechnology, University of Ferrara, Italy; [4]Bavarian Polymer Institute Keylab Electron and Optical Microscopy, University of Bayreuth, Germany
E-mail: sgzmdl@unife.it

Gallic acid (GA) and ferulic acid (FA) are phenolic compounds of natural origin possessing several properties, such as anti-inflammatory and antioxidant, interesting for the treatment of dermatological disorders[1,2]. However, due to their scarce water solubility and stability, their encapsulation in delivery nanosystems could represent a suitable strategy to develop effective topical formulations[3]. With this aim, GA and FA have been embedded in niosomes of different compositions, in order to evaluate how the vesicular systems affect the release of the active ingredient and to find out the most suitable formulation for skin application[4]. Niosomes were produced through the thin-layer hydration method, alternatively using borate buffer or a micellar solution of poloxamer 188 as aqueous phase. They were then characterized in terms of morphology, size and encapsulation stability. Xanthan gum and poloxamer 407 were selected as thickening agents to obtain niosomal gels (niogels) with a certain grade of viscosity, and their spreadability and adhesiveness properties have been investigated. The *in vitro* diffusion of drugs, studied by mean of Franz cells, showed the main role of the poloxamer micellar hydration phase in governing the drug release and of the niogels in ensuring the controlled diffusion of polyphenols[5]. Finally, the *in vivo* irritation test confirmed the safeness of niogels after application onto the skin.

References

1. Marino T et al. J Phys Chem B 2014;118:10380-9.
2. Yang J et al. LWT 2021;146:111411.
3. Sguizzato M et al. Int J Mol Sci 2021;22:8319.
4. Sguizzato M et al. Gels 2023 9:107.
5. Franzè S. Eur J Pharm Sci 2019;130:27-35.

MECHANISTIC INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS IN NANOMEDICINE RESEARCH

P. Siani[1], C. Di Valentin[1,2]

[1]Dip. di Scienza dei Materiali, and [2]BioNanoMedicine Center NANOMIB, University of Milano-Bicocca, Milano, Italy
E-mail: paulo.siani@unimib.it

Molecular dynamics (MD) simulation techniques have been in the spotlight of recent nanomedicine research, becoming an indispensable tool for unveiling complex molecular mechanisms that are sometimes unreachable by experimental methods. Here, I will exemplify how MD simulations can complement existing experimental knowledge or provide new mechanistic insights into relevant aspects of nanoscale devices designed for nanomedicine. Through some case studies - from how thermodynamic variables (*e.g.*, pH and ionic strength) affect the protein corona formation onto organic-functionalized nanoparticles[1] to the impact of lipid composition in the permeation process of anti-tumoral drugs in membranes[2] - this presentation will address how classical MD simulations can be helpful bridging the simulated microscopic behaviour to their corresponding macroscopic manifestation.

References

1. Siani P and Di Valentin C. Nanoscale 2022;14:5121-37.
2. Siani P et al. BBA-Biomembranes 2022;1864:183763.

CAN TISSUE DEPARAFFINIZATION INFLUENCE THE EXTRACTED DNA FOR FORENSIC PURPOSES?

G. Soldati, S. Turrina, C. Saccardo, D. Raniero, D. De Leo

Dept. of Diagnostics and Public Health, University of Verona, Italy
E-mail: giulia.soldati_02@univr.it

In forensic genetics, sometimes the biological specimen available for DNA extraction is biopsy tissue taken in life from deceased subjects and then fixed in formalin and embedded in paraffin (FFPE). Degradation and chemical alteration of DNA caused by treatment of tissue with formalin and paraffin is an event reported in the literature, even if the influence of exposure time and intensity of the phenomenon are still uncertain. This results in obtaining

DNA of degraded quality, the quantity of which is for sure affected by paraffin hindering its release from the tissues in the lysis step. Therefore, pretreatment of the biological sample, *i.e.* deparaffinization, may assume a relevant role in the subsequent DNA extraction and amplification steps. In this study, five different tissue deparaffinization protocols were compared to determine which was the most appropriate for the aim, exploiting two tissue samples (lung and kidney), FFPE over the next 24 h, taken during autopsies on two male cadavers. The deparaffinization protocols involved the use of the standard procedure with xylene and 100% ethanol and four methods in which paraffin solubilizing solvents were used, *i.e.* chloroform and white mineral oil. Then, DNA extraction was performed by employing the QIAamp DNA FFPE Tissue Kit, modifying the procedure only in the post-lysis step, in which the provided treatment at 90°C for 1 h was omitted, proceeding with incubation at 70°C for 24 h, after addition of Tris 1M to the lysate. Extracted DNA was quantified and normalized to 1 ng/μL and then submitted for amplification with two forensic kits. Amplicons were genotyped in capillary electrophoresis and fragment analysis was conducted with the GeneMapper ID-X v1.6 software. The two panels of Short Tandem Repeats yielded reproducible, albeit partial, genetic profiles, referable to 12/13 loci (molecular weight <300 bp), and showed no significant differences correlated with the adopted deparaffinization procedure. Therefore, while being aware that the study needs to implement the number of samples, it seems reasonable to assume that deparaffinization can also be carried out by procedures other than the standard one, using solvents with low toxic characteristics.

FUNCTIONALIZATION OF NANODIAMONDS WITH HYALURONIC ACID: A STUDY FOR THEIR POTENTIAL APPLICATIONS IN RADIOSENSITIZATION AND SELECTIVE TUMOR DETECTION

S. Sturari[1,2], V. Varzi[1,2], P. Aprà[1,2], G. Tomagra[3], V. Monti[1,2], V. Bincoletto[3], B. Zurletti[3], E. A. Durisi[1,2], S. Arpicco[3], C. Riganti[4], P. Olivero[1,2], F. Picollo[1,2]

[1]Physics Dept., [3]Dept. of Drug Science and Technology, and [4]Dept. of Oncology, University of Torino, Torino, Italy; [2]National Institute of Nuclear Physics, sect. Torino, Torino, Italy
E-mail: sofia.sturari@unito.it

Due to their high biocompatibility and tunable surface chemistry, nanodiamonds (NDs) are widely studied in the biomedical field, with a particular focus in radiosensitization and tumors detection. NDs with hydrogen-terminated surface (HNDs) emerged as potential radiosensitizers, *i.e.*, agents capable to increase the sensitivity of tumour tissues to ionizing radiation. As a result of their negative electron affinity, HNDs were indeed reported to enhance, *via* electronic emission, the production of free radicals, such as hydroxyl radicals (•OH). On the other hand, NDs can host lattice defects called *color centers*: these are characterized by fluorescence properties,[1] which can be exploited for visualizing tumor tissues, in order to facilitate their detection and their complete surgical removal. In these two contexts, surface functionalization with hyaluronic acid (HA) confers to the NDs specific characteristics, enabling their effective applicability. Being highly hydrophilic, HA can be used to avoid NDs aggregation in aqueous media, which would hamper their employment in the water-rich cellular environment. Moreover, in the latter application, decoration of NDs surface with HA is required to target selected types of tumors, such as bladder carcinoma, featured by over-expression of specific HA receptor.[2] Here, we investigated the creation of •OH from irradiated HA-functionalized HNDs (HA-HNDs) in aqueous solution, with the goal of assessing the possibility of using them as radiosensitizers. To this aim, we exploited the hydroxylation reaction of terephthalic acid (TPA), yielding the fluorescent 2-hydroxyterephthalic acid (HTPA), which allows the indirect determination of •OH concentration through fluorescence spectroscopy. Our data show that HTPA fluorescence is not increased neither by HNDs nor by HA-HNDs, whereas it decreases when only HA is present. These results, joined with insights from Dynamic Light Scattering measurements, suggest that the observed behavior could be attributed to interparticle agglomeration in the case of HNDs, probably hindering electronic emission from the nanoparticles. At the same time, for HA-HNDs this could be caused by the free radicals scavenging action of HA. In parallel, we also optimized NDs fluorescence through ion implantation and thermal treatments for their future use as selective probes for bladder cancer.

References

1. Picollo F et al. Sci Rep 2018;8:2221.
2. Arpicco S et al. Front Chem 2020;8:578008.

CONTINUOUS-FLOW CRYSTALLIZATION OF SURFACTANT-FREE DOPED ZINC SULFIDE NANOPARTICLES FOR OPTICAL BIOIMAGING

F. Tajoli[1], P. Dolcet[2], M. Mognato[3], M. Maggini[1], T. Carofiglio[1], A. Speghini[4], S. Gross[1,2]

[1]Dept. of Chemical Sciences (DiSC), University of Padova, Padova, Italy; [2]Karlsruhe Institute of Technology (KIT), Institute of Chemical Technology and Polymer Chemistry (ITCP), Karlsruhe, Germany; [3]Dept. of Biology, University of Padova, Padova, Italy; [4]Dept. of Biotechnology, University of Verona, Verona, Italy
E-mail: francesca.tajoli@unipd.it

In recent years, microfluidic reactors have become highly attractive devices for synthetizing inorganic nanoparticles (NPs) of high quality. Indeed, microfluidic setups allow a precise control over the final product, in term of size, size distribution and composition, mainly thanks to i) the achievement of homogeneous reaction mixtures within millisecond time scale and ii) the tight and rapid control of the reaction temperature.[1] Within this framework, the room temperature, controlled crystallization of ZnS NPs with an average size of 5 nm and doped with luminescent ions (such as Mn^{2+}, Eu^{3+} and Nd^{3+}) was achieved. Notably, under microfluidic conditions, small and monodispersed NPs were obtained without the use of any ligand and/or surfactant to control nuclei growth and the final size of the NPs. The synthesized nanomaterials were characterized from the structural (XRD, XAS at lanthanide L3 edges), morphological (TEM) and compositional (XPS, ICP-MS) points of view, giving complementary information on the materials' features. In view of potential applications in the field of optical bioimaging, the optical emission properties of the doped nanoparticles were assessed. Furthermore, *in vitro* cytotoxicity experiments were carried out, showing no negative effect and evidencing the appeal of the synthesized materials for potential applications in the optical bioimaging field.[2]

References

1. Nightingale AM and DeMello JC. Adv Mater 2013;25:1806.
2. Tajoli F et al. ACS Appl Mater Interfaces 2020;12:44074.

BIOMIMETIC NANOBINDERS BUILT ON BIORESPONSIVE PRODRUGS FOR IMMUNE CHEMOTHERAPY AGAINST TRIPLE-NEGATIVE BREAST CANCER

M. Tubertini[1], C. Milani[2], C. Martini[1], L. Menili[2], M.L. Navacchia[1], M. Bartolini[3], D. Tedesco[1], E. Martella[1], A. Guerrini[1], C. Ferroni[1], F. Moret[2], G. Varchi[1]

[1]Institute for Organic Synthesis and Photoreactivity, National Research Council of Italy, Bologna, Italy; [2]Dept. of Biology, University of Padova, Padova, Italy; [3]Dept. of Pharmacy & Biotechnology University of Bologna, Bologna, Italy
E-mail: mtubertini@uninsubria.it

Despite recent advances in cancer therapy, several aggressive solid tumors, including triple-negative breast cancer (TNBC) still lack effective treatment options.[1] Over the last decades, immunotherapy has gained increasing attention due to its ability to reverse tumor-mediated immunosuppression and provide long-term immunological memory. Indoleamine 2,3-dioxygenase-1 (IDO1) is an immunosuppressive enzyme, which is over-expressed in TNBC patients and represents an appealing target to restore immunosurveillance. Certain antineoplastic drugs have been shown to induce immunogenic cells death (ICD), boosting the host immune response and eradicating primary tumor growth.[2] Based on these premises, combining an IDO1 inhibitor (NLG919) with an ICD inducer (paclitaxel, PTX) could synergistically enhance their antitumor effect. Despite promising, this approach poses several issues in terms of dosing and control of the pharmacokinetic profiles of each drug to achieve the optimal synergetic effect. To this end, we explored the concept of carrier-free drug delivery systems, harnessing endogenous human serum albumin (HSA) to transport the drugs at the tumor site selectively.[3] This work describes the design, synthesis, and *in vitro* characterization of novel non-covalent HSA-binding amphiphilic prodrugs of PTX and NLG919, promoting the spontaneous formation of carrier-free nanosystems in water, which selectively release the parent drugs under the reductive tumor microenvironment conditions. The *in vitro* investigations on 2D- and 3D-TNBC models (MDA-MB-468 and MDA-MB-231 cells) revealed that the proposed nanosystems promote tumor cell killing, inhibit IDO1, and efficiently induce ICD.

References

1. *Ensenyat-Mendez M et al. Front Oncol 2021;21:681476.*
2. *Wang YJ et al. Genes Dis 2018;5:194-203.*
3. *Zhang Y et al. Biomaterials 2018;183:243-57.*

ULTRASMALL GOLD NANOPARTICLES: CANDIDATES TO MITIGATE AMYLOIDOGENIC PROTEIN AGGREGATION

G. Viola, C.G. Barracchia, R. Tira, F. Munari, M. D'Onofrio, M. Assfalg

Dept. of Biotechnology, University of Verona, Italy
E-mail: michael.assfalg@univr.it

Ultrasmall nanoparticles (usNPs), defined as particles with core size in the range of 1–3 nm, have drawn increasing attention in recent years due to their distinctive physicochemical properties and unique biological behavior. They show great promise for applications in biosensing, drug delivery, and cellular imaging.[1,2] Moreover, usNP could be also used as therapeutic tools in a great variety of diseases. An emerging field of interest concerns the amyloidogenic intrinsically disordered proteins (IDPs). These proteins are found to undergo aberrant aggregation under defined conditions and are associated with neurodegenerative diseases: *e.g.* the protein tau is involved in Alzheimer's disease.[3] Elucidating the interactions of fibrillogenic proteins with NPs and the associated conformational rearrangements could provide the molecular basis for developing new treatments.[4] In our work,[5] we focused on the synthesis of ultrasmall gold nanoparticles (usGNPs)[6], their characterization, and their interaction with tau, using diverse techniques. To shed light into the association between tau and usGNP, we obtained thermodynamic information through isothermal titration calorimetry (ITC). We then studied in detail the protein regions involved in the interaction by site-resolved nuclear magnetic resonance (NMR) experiments. The analysis of NMR spectra showed that the lysine residues are the most involved, supporting the electrostatic character of the interaction. Finally, based on transmission electron microscopy images, we found that usGNP influenced aggregation and, at the higher concentrations, they were able to inhibit the formation of the fibrils.

References

1. *Kim BH et al. Chem Mater 2014;26:59-71.*
2. *Shang L et al. Nano Today 2011;6:401-18.*
3. *Chiti F and Dobson CM. Annu Rev Biochem 2017;86:27-68.*
4. *D'Onofrio M et al. Molecules 2020;25:5625.*
5. *Viola G et al. 2022;22:8875-82.*
6. *Shang L et al. Small 2011;7:2614-20.*

INTERACTION OF ULTRASMALL NANOPARTICLES WITH CONDENSATES OF AN INTRINSICALLY DISORDERED PROTEIN

G. Viola[1], F. Floriani[1], C.G. Barracchia[1], F. Munari[1], M. D'Onofrio[1], M. Assfalg[1]

[1]Lab. of Biomacromolecules, University of Verona, Verona, Italy.
E-mail: giovanna.viola@univr.it

Liquid-liquid phase separation (LLPS) of biopolymers to form condensates is a widespread phenomenon in underlying the formation of membraneless organelles (MLOs) in eukaryotic cells (also known as biomolecular condensates or droplets).[1,2] Condensation, occur upon weak multivalent interactions, such as heterotypic electrostatic interactions between oppositely charged polyelectrolytes, or homotypic interactions involving repetitive low-complexity sequences and multiple interacting sites.[1,3] MLOs are enriched in intrinsically disordered proteins, (IDPs) which play an important role in supporting cellular functions. Beyond this, they are related with neurodegenerative diseases and their structural plasticity allows to undergo various multivalent homo- and heterotypic interactions. In this scenario, the ability to target and control condensation can help uncover elusive physiological mechanisms and solve pathological outcomes. In this respect, nanoparticles (NPs) represent attractive condensate-targeting agents, due to their unique material properties and modes of interaction with biomolecules. Such particles generally exhibit high biocompatibility and low toxicity, efficient renal clearance, improved tumor distribution, and good cell penetration.[4,5] In our work, we elucidated the interaction between ultrasmall gold NPs (usGNPs) and diverse types of condensates of the microtubule-associated protein tau, an intrinsically disordered macromolecule. The usGNPs were found to concentrate into condensed liquid droplets, consistent with the formation of dynamic client (NP) - scaffold (tau) interactions, and

were observable thanks to their intrinsic luminescence. Our findings suggest that usGNPs could be used to interrogate phase separation, and control the formation and dissolution of tau condensates.

References

1. Banani SF et al. Cell Biol 2017;18:285-98.
2. Boeynaems S et al. Trend Cell Biol 2018;28:420-35.
3. Brangwynne CP et al. Nat Phys 2015;11:899-904.
4. Liu J et al. J Am Chem Soc 2013;135:4978-81.
5. Sokolova V et al. Biomater 2020;111:349-62.

HYALURONAN-CHOLESTEROL NANOGELS: NOVEL NANO-DELIVERY SYSTEMS FOR TOPICAL TRANSDERMAL DELIVERY OF BETAMETHASONE IN POTENTIAL PSORIASIS TREATMENT

J. Wang[1], L. Paoletti[1], C. Migliorini[1], L. Forcina[2], A. Musarò[2], C. Di Meo[1], P. Matricardi[1]

[1]Dept. of Drug Chemistry and Technologies, and [2]DAHFMO-Unit of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy
E-mail: ju.wang@uniroma1.it

Psoriasis is an inflammatory skin disease affecting about 2~4% of the worldwide population, and betamethasone (BM) is commonly used glucocorticoid in its treatment[1]. However, the current treatment options are limited due to the inherent skin barriers and poor bioavailability of BM in conventional formulations. Polymeric nanogels (NHs) is a promising strategy in the improvement of cutaneous drug delivery[2]. Among various polymeric carriers, cholesterol (CH) grafted hyaluronic acid (HA) is an excellent candidate as both components occur naturally in the skin[3]. Developing HA-CH NHs based formulation for BM encapsulation. Evaluating the skin permeability both *in vitro* and *ex vivo*, as well as the potential advantages for psoriasis therapy. Amphiphilic HA-CH was synthesized by grafting the CH moieties onto polymeric backbone, giving spontaneously self-assembly ability in aqueous environment. BM-loaded NHs were incorporated into Carbopol based hydrogel to improve rheological properties for topical applications. The properties of nano-systems, such as size, ζ potential, encapsulation efficiency (EE%), rheological properties, and *in vitro* drug release profiles were investigated. Both *in vitro* (Strat-M® membrane) and *ex vivo* (pig ear skin) permeation capacities were evaluated by Franz diffusion cells. Further skin permeation and deposition was studied by confocal fluorescence microscope. HA-CH NHs showed high EE% (apparent solubility of BM improved up to 9-fold), small size (~190 nm) and good stability. Besides, Carbopol based gel system exhibited excellent rheological properties for skin application. The *in vitro* sustained drug release lasted for over 8 h. The obtained results revealed that the NHs system can effectively promote skin permeation and retention in the deeper layers of epidermis and dermis, making it advantageous for topical BM delivery. Overall, we have developed a novel HA-CH NHs system for effective BM loading and skin delivery, which shows promise in the psoriatic topical treatment.

References

1. Rendon A et al. Int J Mol Sci 2019;20:1475.
2. Cuggino JC et al. J Control Release 2019;307:221-46.
3. Zoratto N et al. Pharmaceutics 2021;13:1781.

SESSION NANO@ENERGY

THIN FILM SOLAR CELLS

E. Artegiani, N. Torabi, S. Olavil Karayi, I. Anefnaf, M. Mukhtar, S. Zanetti, A. Romeo

LAPS-Laboratory for Photovoltaics and Solid State Physics, Dept. of Computer Science, University of Verona, Verona, Italy
E-mail: elisa.artegiani@univr.it

The photovoltaic market has always been dominated by the first generation of photovoltaics, *i.e.* devices that exploit the properties of mono or polycrystalline silicon. The second PV generation concerns thin film devices; it has been developed to produce panels cheaper than the first generation ones, making use of alternative semiconductors such as CdTe, CIGS, CTZS, Sb_2Se_3, etc. In fact, high temperatures and long production times are required for the production of silicon. Furthermore, the obligation to work in the complete absence of oxygen and the complexity of cutting and assembling the silicon wafers make this technology intrinsically complicated and expensive. On the other hand, it is possible to manufacture thin-film devices with new methods, significantly reducing production costs. Thin film means a layer of thickness between few tens of angstroms and some microns deposited on a supporting substrate. The deposition of multiple thin layers on a polymeric, glass or metallic substrate creates the thin film technology. For this reason, a smaller amount of semiconductor material is needed (at least a hundred times less). Moreover, since the layers can be deposited straight on a large substrate, these devices are more suitable for large scale production than the first-generation ones: the fabrication process is simpler and needs less energy. While crystalline silicon cells are assembled to constitute a panel, thin film cells are obtained from the layers through laser cut; in that way, a single production line is needed and the production is faster.[1] Our laboratory is well-established in the study of CdTe technology, which has so far achieved the greatest use in large-scale production among thin-films. This is because CdTe is a very robust and chemically stable material, moreover it is a simple binary compound, which evaporates congruently, and for this reason it can be deposited with a large variety of methods. The only disadvantage of this technology is that it contains the word "cadmium", which gives people a bad perception. In reality, CdTe is a very stable compound and numerous scientific papers demonstrate the very low environmental impact of this technology. Even non-friendly reports show that the possibility for broken modules to have high cadmium leakage in the soil is very unlikely. As well as no emission of cadmium can occur in case of residential fires. In addition, the complete recyclability of the CdTe modules makes this great technology absolutely clean.[2]

References

1. Bosio A and Romeo A. Thin film solar cells, current status and future trends (Energy Science, Engineering and Technology). New York, Nova Science Publishers, 2011.
2. Romeo A and Artegiani E. Energies 2021;14:1684.

INTEGRATING LAB- AND INDUSTRY-SCALE BATTERY PRODUCTION: CONTINUOUS HYDROTHERMAL SYNTHESIS OF NMC-LIKE CATHODES

F. Barbon[1], L. Campagnaro[1], D. Mosconi[2], M. Bersani[2], D. Versaci[3], S. Bodoardo[3], S. Gross[1,4], A. Martucci[5]

[1]Dip. di Scienze Chimiche, University of Padova, Padova, Italy; [2]Particular Materials srl, Cadoneghe (PD), Italy; [3]Dip. di Scienza Applicata e Tecnologia, Politecnico di Torino, Torino, Italy, [4]Institute for Chemical Technology and Polymer Chemistry (ITCP), Karlsruhe Institute of Technology (KIT), Germany; [5]Dip. di Ingegneria Industriale, University of Padova, Padova, Italy
E-mail: federico.barbontudenti.unipd.it

Bridging the academic world and the industry production represents a challenge and requires a great amount of time. This is a crucial point in the battery field, where the fast-growing demand in term of both production volumes and technological advancement faces the hindered passage from the latest research founding with their successive implementation at the industry-scale.[1] In this context, continuous hydrothermal flow synthesis (CHFS) has proven to be a reliable, up-scaled, water-based technique to promote the transition towards the next generation synthetic approaches, and it has been implemented only recently for cathode materials. Here, a feed with the metal salt precursor is mixed with a stream of supercritical water, causing the hydrolysis and subsequent dehydration and formation of the product (oxides, hydroxides, phosphates etc.).[2] NMC ($LiNi_{1-x-y}Mn_yCo_xO_2$) is a widely employed compound at the industry level for electric vehicles. The synthesis consists of two steps: first, the hydroxide precursors are synthesized and then calcinated to yield the oxides. With the proposed approach, the hydroxide precursor can be made in continuous, within seconds, and with a tunable composition. Different compositions were optimized both structurally and morphologically and the initial electrochemical tests show promising results, comparable with similar literature.[3] The capacity is 140 mAh g^{-1} and 120 mAh g^{-1} at C/10 for NMC111 and 622 respectively, with 1C=155 mA g^{-1}, ca. 4.5 mg cm^{-2} loading, voltage window 2.7-4.3 V, and at 2C the NMC111 still retains over 60% of the initial value (ca. 90 mAh g^{-1}). Long term testing of the half cells, also employing higher mass loadings, is ongoing.

References

1. Ye N et al. Ceram Int 2018;44: 4521.
2. Darr JA et al. Chem Rev 2017;117:11125.
3. Commandeur D et al. J Materiomics 2022;8:437.

ENERGY TRANSFER PROCESS IN $TbAl_3(BO_3)_4$ HUNTITE-TYPE MATERIALS UNDOPED AND SINGLY DOPED WITH Eu^{3+}

L. Ceccon, S. Ruggeri, M. Bettinelli, F. Piccinelli

Luminescent Materials Laboratory, DB, University of Verona, and INSTM, UdR Verona, Verona, Italy
E-mail: leonardo.ceccon@univr.it

In this work, we studied the $Tb^{3+} \rightarrow Eu^{3+}$ energy transfer process in a series of trigonal huntite-type $TbAl_3(BO_3)_4$ crystals doped with Eu^{3+} ion, which have been synthesized by crystal growth reaction technique[1]. The samples have been characterized by powder X-diffraction, room temperature luminescence spectra and excited states emission decay. The optical spectroscopy of crystals and powders of neat $TbAl_3(BO_3)_4$ have been already reported by Kellendonk and Blasse[2]. The experimental results show that excitation of the Tb^{3+} ions gives rise to the Eu^{3+} emission from $^5D_0$ level, and therefore non-radiative energy transfer involving the two ions is operative. Analysis of the experimental data indicates that the excitation of the Tb^{3+} donor ion gives rise to a diffusion-limited migration process of the excitation energy towards the Eu^{3+} acceptor ions. Upon increasing the Eu^{3+} acceptor concentration, the efficiencies of energy transfer (η_{ET}) increase from 17% (Eu = 1%) to 80% (Eu = 20%) and the mainly green emission intensity and the decay time of the $^5D_4$ level decrease, whilst the Eu^{3+} red emission from $^5D_0$ becomes dominant. The Tb-Tb distance in the crystal structure (5.9 Å), even if smaller than Rc[3] (≈ 7.85 Å), is prohibitive for transfer by exchange interaction and could account for the relatively slow Tb-Tb energy migration observed in this host.

References

1. Bartl MH et al. Spectrochim Acta A 2001;57:1981-90.
2. Kellendonk F and Blasse G. J Phys Chem Solids 1982;43:481-90.
3. Blasse G and Grabmaier BC. Luminescent Materials, Springer Verlag, Berlin Heidelberg;1994, Chap. 5.

PREPARATION AND PROPERTIES OF Tm^{3+}-DOPED $NaBiF_4$ BLUE-EMITTING PHOSPHORS

X. Hu

Luminescent Materials Laboratory, Dept. of Biotechnology, University of Verona, and INSTM, UdR Verona, Verona, Italy
E-mail: xiaowu.hu@univr.it

A novel blue-emitting phosphor of Tm^{3+}-doped $NaBiF_4$ has been synthesized *via* a facile reaction at room temperature.[1] XRD, TEM, EDX were utilized to characterize the products, and the fluorescence properties were measured *via* fluorescence spectrometer. XRD exhibited hexagonal phase of $NaBiF_4$ (JCPDS No. 41-0796) with a high crystallization degree,[2] and TEM displayed a well-distributed spherical-like morphology of average size 36 nm. Also, the dopant Tm was verified to be present in the products *via* EDX spectra. The fluorescence spectrum showed the products could emit bright blue light that the characteristic peak situated at ~ 451 nm corresponded to the transitions of Tm^{3+} ions from $^1D_2 \rightarrow {}^3F_4$ under the excitation of 357 nm.[3-5] Moreover, the optimal doping concentration of Tm^{3+} ions was 50 mol% and the concentration quenching was proved as electric dipole-dipole interactions. The color coordinate of the product (x = 0.5) was determined to be (0.1354, 0.0602), and the color purity was about 92.4%. Besides, the crystal field environment around the Tm^{3+} ions was further explored *via* the Judd-Ofelt theory.[6] The products were promising candidates for blue-emitting phosphors in the fields of white light-emitting diodes, ceramics as well as display devices.

References

1. Du P et al. Chem Eng J 2018;337:91-100.
2. Du P. RSC Adv 2018;8:26676-81.
3. Venkatachalaiah KN et al. Spectrochim Acta-Part A Mol Biomol Spectrosc 2017;184:89-100.
4. Yamamoto H et al. J Lumin 2000;87-89:1079-82.
5. T. Jiang T et al. J Fluor Chem 2012;140:70-5.
6. Darshan GP et al. Dye Pigm 2016;134:227-33.

DEVELOPMENT OF HIGH EFFICIENCY SECOND GENERATION THIN FILM PHOTOVOLTAIC CdTe CELL MANUFACTURING SYSTEM

M. Mukhtar, E. Artegiani, A. Romeo

LAPS-Laboratory for Photovoltaics and solid state physics, Dept. of Computer Science, University of Verona, Verona, Italy
E-mail: mariyam.mukhtar@univr.it

CdTe is a very robust and chemically stable material; it can be deposited with a large variety of methods making it very much ideal for production on large area. For this reason, it is now the only thin film technology in the first 10 top producers in the world. For a very long period of time, CdTe solar cell has been prepared in superstrate configuration by depositing subsequently on glass substrate: transparent conductive oxide, CdS layer, CdTe as absorber layer, and finally the back contact.[1] The strong improvement in efficiency was obtained by a new redesign of the CdTe solar cell device and by this reaching an efficiency of 22.3%.[2] This was obtained by a complete redesign of the device by introducing 1) development of a stable and efficient back contact that works as well as back reflector, 2) band gap tuning by the introduction of $CdSe_xTe_{1-x}$ in the CdTe absorber, 3) removal of the CdS buffer layer.[3] In our laboratory we fabricate CdTe thin film solar cells by thermal evaporation technique in superstate configuration. In this work we will describe the fabrication process and the achievements that have been obtained in the lab. We are using a commercial FTO/TO as front contact, on that the $CdSe_xTe_{1-x}$ layer can be fabricated both by selenization of a thin CdTe layer, or by mixing of a CdSe and CdTe layer, then the absorber gradually changes from $CdSe_xTe_{1-x}$ to CdTe. The absorber must be treated by the $CdCl_2$ activation treatment which favours its re-crystallization, passivation of the grain boundaries and also the formation of above mentioned $CdSe_xTe_{1-x}$/CdTe layers mixing. The back contact consists of a Cu-Au bi-layer. Copper can be introduced in small quantities by its thermal evaporation by dropping a $CuCl_2$ containing solution on the top of the absorber, followed by annealing in air at 190°C, noticeably improving the stability of the devices.[4]

References

1. Romeo A and Artegiani E. Energies 2021;14:1684.
2. First Solar Q1'23 Earnings Call 2023.
3. Artegiani E et al. Sol Energy Mater Sol Cells 2021;226:111081.
4. Artegiani E et al. Prog Photovolt 2019;27:706-15.

ANALYSIS OF CdSe AS ALTERNATIVE BUFFER LAYER FOR Sb_2Se_3 SOLAR CELLS

N. Torabi[1], E. Artegiani[1], A. Gasparotto[2], F. Piccinelli[3], M. Bertoncello[4], M. Meneghini[4], A. Romeo[1]

[1]LAPS-Laboratory for Photovoltaics and solid state physics, Dept. of Computer Science, University of Verona, Verona, Italy; [2]Dept. of Physics and Astronomy "G. Galilei", University of Padova, Padova, Italy; [3]Dept. of Biotechnology, University of Verona, Verona, Italy; [4]Dept of Information Engineering, University of Padova, Padova, Italy
E-mail: alessandro.romeo@univr.it

In the conventional CdS/Sb_2Se_3 superstrate configuration devices, the high concentration gradient leads to excessive selenium diffusion into the CdS and sulfur diffusion into the Sb_2Se_3, resulting in high defect density at the junction. Ensuing selenium vacancies in Sb_2Se_3 thin films can reduce the photogenerated charge carrier lifetime by increasing the recombination rate. For this reason, the control of an additional amount of Se can be a path to improve the efficiency of these devices. In this sense, the introduction of an alternative buffer layer, like CdSe, could minimize this detrimental effect. The impact of CdSe on the growth of Sb_2Se_3 in superstrate configuration and the performance of the resulting devices is analysed and compared with the standard CdS/Sb_2Se_3 structure. We have demonstrated that by applying CdSe as a buffer layer we are able to improve the external quantum efficiency across the entire light spectrum. This results in an increased average current density of the devices by up to 2 mA/cm^2 compared to CdS/Sb_2Se_3. This despite CdSe has a narrower band gap, which is a good hint that the improvement is not given by a larger transparency. Additionally, we observed an improvement in the fill factor, while the V_{oc} slightly decreased due to the narrower band gap of CdSe compared to CdS. Finally, $CdSe/Sb_2Se_3$ samples exhibit excellent stability during accelerated stability tests and no diffusion is observed in long time stress at high temperatures.

SUSTAINABLE ENCAPSULATION OF PHASE CHANGE MATERIALS FOR SMART BUILDING APPLICATIONS

P. Vaccari[1,2], A. Speghini[2]

[1]Nanomnia S.r.l, Verona, Italy; [2]NRG, Dept. of Biotechnology, University of Verona, and INSTM, RU of Verona, Verona, Italy
E-mail: pietro.vaccari@univr.it

The idea of using passive insulation systems for buildings has found increasingly interest in the last years, as the energy demand for indoor air conditioning accounts for about 28% of global consumption.[1] In this contest, a reduction of energy demand would be reflected in lower consumption of fossil fuels, thus contributing to decrease of greenhouse gas emissions.[2] Phase Change Materials (PCMs), particular organic based (fatty acids, esters, and paraffin), are promising tools for this type of application, as they can absorb relevant amounts of thermal energy during their phase transition (solid-liquid). Incorporation of PCMs within concrete or gypsum boards can mitigate the thermal fluctuations to which buildings are usually subjected. Use of PCMs in this context presents some issues, as reduced heat transfer efficiency, corrosivity, and possible migration during their phase state transition.[1,3] To overcome these problems, encapsulation is a promising methodology, creating a coating around the thermal insulating material, and preventing its interaction with the surrounding environment and thus avoiding possible liquid diffusion. In the present investigation, to encapsulate organic PCMs, an emulsion formulation was used to create a fine dispersion in an aqueous environment using different kinds of additives, in particular chitosan and alginate as encapsulant polymers. The prepared beads were obtained using coagulation baths with different crosslinkers, namely calcium chloride for alginate and sodium tripolyphosphate for chitosan.[4,5] These obtained beads were incorporated into a low-density concrete mixture and into gypsum blend and some chemico-physical characterization were performed. Specimens incorporating PCM encapsulating beads were analyzed by Differential Scanning Calorimetry to evaluate their thermal features.

References

1. Tyagi VV et al. Renewable Sustainable Energy Rev 2011;15:1373-91.
2. Akeiber H et al. Renewable Sustainable Energy Rev 2016;60:1470-97.
3. Khan Z et al. Energy Convers Manag 2016;115:132-58.
4. Bajpai SK et al. React Funct Polym 2004;59:129-40.
5. Jóźwiak T et al. React Funct Polym 2017;114:58-74.

SYNTHESIS OF METAL OXOCLUSTER-BASED HYBRID NANOPARTICLES AS CATALYSTS FOR OXIDATION REACTIONS OF ENVIRONMENTAL INTEREST

D. Vendrame[1], G. Bragaggia[1], S. Gross[1,2], M. Carraro[1]

[1]Dept. of Chemical Sciences (DiSC), University of Padova, Padova, Italy; [2]Karlsruhe Institute of Technology (KIT), Institute of Chemical Technology and Polymer Chemistry (ITCP), Karlsruhe, Germany
E-mail: davide.vendrame@phd.unipd.it

The design and catalytic applications of novel oxocluster-based hybrid nanoparticles is the focus of the present work. The synthesised hybrid materials are characterized by covalent incorporation of structurally well defined oxoclusters of group 4 metals [$Zr_4O_2(OMc)_{12}$,[1] $Ti_6O_4(O^iPr)_8(OMc)_8$,[2] $Ti_4O_2(O^iPr)_6(OMc)_6$,[2] $Ti_4Zr_4O_6(OBu)_4(OMc)_{16}$,[3] $Ti_2Zr_4O_4(OBu)_2(OMc)_{14}$[3]] (OMc: methacrylate) in an organic matrix based on polymethylmethacrylate. The presence of polymerizable groups (C=C) in the oxocluster structure allowed the formation of strong chemical bonds between the organic and inorganic counterparts, protecting the guest species (oxocluster) toward hydrolysis and avoiding possible leaching and/or migration phenomena. The free radical copolymerization between the oxocluster and methyl methacrylate was carried out in the confined space of miniemulsion droplets, allowing the control of the size distribution of the resulting hybrid particles. The polymerization was performed under UV light using a proper photoinitiator. Hybrid organic-inorganic nanoparticles were obtained with a spherical morphology and dimensions similar to those reported in a previous study.[4] The obtained hybrid nanoparticles were tested as heterogeneous catalysts for the oxidation of methyl p-tolil sulphide, benzyl alcohol and cyclooctene. H_2O_2 was selected as oxidizing agent due to the already known activation of this molecule by Zr oxoclusters.[5] The higher conversion values were obtained with the monometallic hybrids, in particular with those based on $Ti_4O_2(O^iPr)_6(OMc)_6$. After the catalytic trials, the catalysts were separated from the reaction mixture to assess the retention of the oxocluster structures, which are particularly prone to hydrolysis. The absence of species related to the hydrolysis of the oxoclusters was confirmed by infrared (IR) and Raman analysis, confirming the successful incorporation of the oxoclusters in the polymer and demonstrating the role of the polymer matrix in their stabilisation.

References

1. Trimmel G et al. App Organometal Chem 2001;15:401-6.
2. Moraru B et al. Chem Mater 2002;14:2732-40.
3. Moraru B et al. J Inorg Chem 2001;2001:1295-1301.
4. Benedetti C et al. ACS Appl Mater Interfaces 2016; 8:26275-84.
5. Faccioli F et al. Eur J Inorg Chem 2015;2015:210-25.

SESSION NANO@ENVIRONMENT

GOLD NANOMATERIALS: EXPLOITING PLASMONICS FOR SURFACE ENHANCED RAMAN SCATTERING APPLICATIONS

S. Cerra[1], C. Romeo[2], E. Milan[3], T. A. Salamone[1], E. Radicchi[3], I. Fratoddi[1], A. Speghini[3]

[1]Dept. of Chemistry, "La Sapienza" University, Rome, Italy; [2]Dept. of Industrial Engineering, Trento University, Trento, Italy; [3]NRG, Dept. of Biotechnology, University of Verona, Verona, Italy
E-mail: sara.cerra@uniroma1.it

Plasmonic gold nanomaterials (AuNMs) are considered one of the breakthroughs in the fields of nanomedicine[1], biotechnology[2], optoelectronics[3] and sensing.[4] For these applications, it is of crucial importance to tailor the AuNMs properties by controlling their structural parameters (*i.e.*, size and shape) as well as the chemical nature of its stabilizing ligand shell, which is of particular relevance when dealing with colloidal dispersions. Different synthetic methods have been studied to take control over these parameters through careful choice of the reaction conditions during synthesis or by post-synthetic modifications. In this work, we synthesized and studied the surface properties of AuNMs with different morphologies: gold nanobipyramids (AuNBPs) and gold nanoparticles (AuNPs). Cetyltrimethylammonium bromide (CTAB) and cetyltrimethylammonium chloride (CTAC)-functionalized plasmonic nanostructures were obtained through bottom-up approaches, starting from $HAuCl_4$ as a gold precursor. Due to the high affinity between gold and sulfur, surface functionalization can be finely tuned using molecules with thiol moiety. To this aim, a ligand exchange reaction was carried out on surfactant-capped gold nanostructures to obtain thiol-functionalized AuNBPs and AuNPs. In view of surface enhanced Raman scattering (SERS) applications, three different aromatic thiols were chosen: thiosalicylic acid (TSA), 4-mercaptophenylboronic acid (4MPBA), and 4-mercaptobenzoic acid (4MBA). Investigation of the colloidal properties and on the extent of the thiol-exchange using different spectroscopic and electron microscopy techniques were carried out.

References

1. Cerra S et al. Mater Sci Eng C 2020;117:111337.
2. Chronopoulou L et al. Int J Biol Macromol 2020;146:790-7.
3. Grigorian S et al. Synth Met 2022;283:116973.
4. Cerra S et al. Coll Surf B 2021;203:111727.

GOLD-DECORATED BIOMORPHIC NANOSTRUCTURED TITANIA FOR POLLUTANTS PHOTODEGRADATION

P. Maugeri[1], E. Milan[1], F. Dalla Nese,[1] A. Di Michele[2], A. Speghini[1], E. Radicchi[1]

[1]Nanomaterials Research Group, Dept. of Biotechnology, University of Verona, and INSTM, RU Verona, Verona, Italy; [2]Dept. of Physics and Geology, University of Perugia, Perugia, Italy
E-mail: eros.radicchi@univr.it

Titanium oxide (TiO_2), a low toxic and largely available semiconductor material, is one of the most investigated materials, especial-

ly in nanosized form, for photodegradation of pollutant species.[1] This material features a wide band gap of ~ 3.1 eV,[2] capable of acting as a photocatalyzer for several reactions under ultraviolet (UV) light through a mechanism involving radical species as reaction intermediates. However, to extend its applicability range and enhance its performance, it is desirable to optimize TiO_2 depositions in order to: 1) increase the catalytic active area, and therefore the photodegradation yield and 2) increase the absorption of radiation in the visible range, to better harvest the solar radiation. To these aims, we followed a modified sol-gel synthesis[3] using natural organic structures (*e.g.*, diatom earths) as templating agents, to obtain a so-called "biomorphic" structure, while decorating the TiO_2 surface with Au nanostars[4] to exploit plasmonic surface resonance effects. The synthesized material is characterized by powder X-ray diffraction (XRD) and scanning electron microscope combined with energy dispersive X-ray spectroscopy (SEM-EDX), while preliminary studies on its photodegradation capability have been carried out using a dye, namely Rhodamine B, following its decomposition under a visible LED lamp through absorption spectroscopy.

References

1. Kumar R. Adv Sustainable Syst 2023;2300033.
2. Carp O et al. Prog Solid State Ch 2004;32:33-177.
3. Zhang Q et al. J Ocean Univ China 2012;11:507-10.
4. Zheng F et al. Int J Mol Sci 2022;23:13741.

CAN THE SURFACE OF AN ARTWORK BE A SOURCE OF INFORMATION ABOUT CLIMATIC CHANGES?

S. Mazzocato, C. Daffara

Dept. of Computer Science, University of Verona, Verona, Italy
E-mail: sara.mazzocato@univr.it

Can surfaces be interpreted as archives of information, especially when they are subjected to different spatio-temporal processes as a result of interaction with environmental or anthropic factors? In order to investigate this question, we have used the optical scanning microprofilometer to acquire different surfaces of artistic interest subjected to climatic alterations, performing a multiscale analysis[1]. By its intrinsic nature, a surface is a complex superposition of scales and information: different length scales (spatial wavelengths) of overlapping stochastic signals are reproduced in the digitized surface according to the properties of the instrument, with the shortest spatial structure determined by the sampling resolution and the longest by the sampling length. Here, the focus is on a small-scale surface analyzed with a multiscale approach using the workflow of surface metrology *i.e.*, the surface topography is analyzed using quantitative descriptors of the ISO standard (amplitude, spatial and hybrid parameters), beyond the simple inspection of the surface morphology. The surface signals are divided into components of different bandwidths along the scan length *i.e.*, texture is separated from form, and then roughness (irregularities on smaller scales) and waviness (more wider variations) are studied. Inspection of the variation of texture features with scale is performed with two different types of multiscale analysis: scale inspection and signal separation. The first part aims to study the variation of roughness in sub-regions, evaluating the behavior of the stochastic signals as the evaluation length varies. In the second part, roughness separation is performed on the entire sample by means of Gaussian filtering with different cutoff values: this procedure allows the study of roughness parameters in surface components at a limited scale. The key aspect is to understand the most relevant scales on which the process occurs by studying the in-band and scale-limited signals.

Reference

1. Daffara C et al. Eur Phys J Plus 2022;137:430.

INNOVATIVE MATERIAL FOR SOLAR FUEL PRODUCTION IN VISIBLE LIGHT

L. Sarasino[1,2], A. Naldoni[1], M.C. Paganini[1]

[1]Dept. of Chemistry, University of Turin, Turin, Italy; [2]Dept. of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy
E-mail: lorenzo.sarasino@unito.it

The fight against global warming and the production of greenhouse gasses is one of the most pressing challenges which we need to take action on as soon as possible. Therefore, it is urgent to develop advanced technologies that are capable of decreasing the CO_2 concentration in the atmosphere and achieving the conversion of CO_2 into useful low-carbon fuels. Cuprous oxide (Cu_2O) has been identified in the last years as one of the most promising nanomaterials for CO_2 photo-reduction in visible light[1]. It's also reported that the product selectivity of the photo-reduction toward the most reduced species (CH_3OH, CH_4, C_2H_6, etc…) is enhanced by the morphology of the nanoparticles of Cu_2O.[2] Unfortunately, the material is prone to photo-corrosion due to dismutation events that occur after the photo-formation of the electron-hole couple. Here controlled-geometry nanoparticles of Cu_2O are prepared and the photo-stability issue is faced throughout the formation of the heterojunction of the Cu_2O nanoparticles with different metal oxide nanoparticles, looking for the best solution. The materials have been tested in a flow gas-solid photoreactor under a solar-simulator lamp to emulate the real conditions of the application.

References

1. Rej S et al. J Mater Chem A 2021;9:5915.
2. Li C-F et al. Small 2023;19(19):e2207875.

.

NANOTECHNOLOGY FOR BIOCATALYSIS: CELLULASE IMMOBILIZATION ON MAM-C MEDIATED BIOMIMETIC MAGNETIC NANOPARTICLES (BMNPs)

G. Zanella[1], S. C. Gaglio[1], F. Battista[2], F. Perozeni[3], D. Bolzonella[2], M. Ballottari[3], M. Perduca[1]

[1]Biocrystallography and Nanostructure Laboratory, [2]Laboratory of Environmental Chemical Engineering and Bioprocesses, and [3]Solar Energy Bio-exploitation Laboratory, Dept. of Biotechnology, University of Verona, Verona, Italy
E-mail: giorgia.zanella@univr.it

Immobilization of enzymes has been extensively required in a wide variety of industrial applications to ensure functionality and the potential of enzyme recycling after use. Specifically, enzyme immobilization on magnetic nanoparticles in order to recycle and apply circular economy, can offer an efficient support to recovery and concentration, with increasing stability and amplify activity in different physico-chemical conditions. Our intention is to couple cellulase from *Aspergillus niger* on Mam-C mediated biomimetic magnetic nanoparticles (BMNPs)[1] using two different immobilization protocols[2]: electrostatic interactions and covalent bond. The immobilization protocols are optimized varying enzyme quantity, reaction temperature and time. The enzymatic activity of the nanocompounds will be tested in a first attempt against a less complex substrate, as lactose, and then against cellulose. Under optimal condition, 2 mg of BMNPs are used as a support for 1.5 mg of cellulase; the total amount of enzyme is completely immobilized with both interaction (EDC/NHS protocol for the formation of the covalet interaction and electrostatic bond sealed using glutaraldehyde). The immobilization of the enzyme do not alter its functionality, whereas, the enzyme stability is increased if compared to the free enzyme upon storage at 4 or 20 °C. The results suggest the great potential of these nanoassemblies in bioindustry applications using a magnetic recovery and their possible innovation approaches in this fields.

References

1. Valverde-Tercedor C et al. Appl Microbiol Biotechnol 2015;99:5109-21.
2. Salem K et al. ACS Sustain Chem Eng 2021;9:4054-63.