Wednesday Morning, November 9, 2022

Smart Multifunctional Materials for Nanomedicine Focus Topic

Room 301 - Session SM-WeM

Smart Multifunctional Materials for Nanomedicine and Theranostics

Moderators: Diego La Mendola, University of Pisa, Italy, François Reniers, Université Libre de Bruxelles, Belgium, Cristina Satriano, University of Catania, Italy

8:40am SM-WeM-3 Plasma-Enabled Switchable Surfaces: Going from Molecules to Bacteria, U. Cvelbar, Martina Modic, Jozef Stefan Institute, Slovenia INVITED

Switchable and smart surfaces have been a goal in the chemistry community for more than a decade. Although much work has been done in converting surface functionalities going from one function to another, like multiple times reported hydrophobic to hydrophilic or reversible, little has been understood beyond surface morphology, surface energies and surface chemistry initiated. The case of switchable surfaces became even more complex when dealing with macromolecules going down to macro objects like viruses or bacteria. Here plasmas offer a unique opportunity to modify surfaces, not only chemically but also on the atomic scale, interfering and modifying or exchanging chemical bonds in materials or their crystal structures. For this reason, this lecture will try to challenge these ideas, address them on a nanoscopic level and present an overview of results ranging from simple surface properties, macromolecule interactions and at the end, effects on viruses and bacteria interacting with these surfaces.

9:20am SM-WeM-5 Cisplatin-Loaded Pd Nanoparticles as Bimodal Theranostic Nanomedicine in the Tumor Treatment, A. Bellissima, G. Scivoletto, L. Cucci, V. Sanfilippo, University of Catania, Italy; A. De Bonis, University of Basilicata, Italy; R. Fiorenza, S. Scirè, University of Catania, Italy; V. Notarstefano, E. Giorgini, Polytechnic University of Marche, Italy; Cristina Satriano, University of Catania, Italy

In this work, we developed a hybrid nano delivery system made of palladium nanoparticles (NPs) and cisplatin (CisPt), an alkylating drug commonly used to treat various types of malignancies. NPs were synthesized via a new green method based on the use of D-glucose and polyvinylpyrrolidone (PVP) as reducing/stabilizing and capping agents, respectively. Plasmonic properties and photocatalytic activity of the Pd NPs, carried out to prove their capability to act as bimodal theranostic nanomedicine, unveiled a plasmon peak at around 274 nm, well matching an optical size of 5 nm for spherical nanoparticles, and significant H₂ evolution. XPS, XRD, and TEM confirmed the chemical composition and morphology of the NPs. The hybrid NP-drug assembly (Pd@CisPt) was characterized by UV-visible spectroscopy to correlate the changes in the plasmonic peak to the interaction of CisPt with the NP surface as by ICP-OES, to quantitatively estimate the drug loading. AFM and DLS measurements confirmed the strong association of the drug to the nanoparticle surface. The SOD-like activity was tested in a cell-free environment to confirm the maintenance in the Pd@CisPt sample of the antioxidant capability of Pd nanoparticles. The Pd@CisPt theranostic nano platform was tested in prostate cancer cells (PC-3 line) in terms of cytotoxicity, to prove the antitumoral action of the developed nanomedicine. Raman microspectroscopy (RMS) with PCA indicated a condition of protein misfolding/unfolding, directly or indirectly due to cisplatin and/or palladium treatment, and DNA damage especially enhanced upon the treatment with PdNPs@CisPt. The MitoSOX assay confirmed an increase in ROS generation, thus proving that oxidative damage is a key factor for the induction of antitumoral action. LSM cell imaging evidenced dynamic processes at the level of subcellular compartments and modulation of intracellular copper ions accumulation. Finally, cell migration studies upon the treatment with Pd@CisPt evidenced an intermediate response between the inhibitory effect by CisPt and the enhanced rate of cell migration for the metal NPs alone, which pointed out the promising potential of the developed theranostic nanomedicine in tissue regeneration.

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9:40am SM-WeM-6 Old Drugs for a Golden Future: Clinically Established Au-Based Complexes...from Repurposing to Potential Application in Nanomedicine, *Tiziano Marzo*, *L. Chiaverini*, *D. La Mendola*, University of Pisa, Department of Pharmacy, Italy

Inorganic compounds played a key role in the pioneering times of modern pharmacology starting from the late 19th century. Gold, Bismuth, Antimony, but also Mercury compounds were widely used to treat a variety of diseases, mostly infectious, such as tuberculosis and syphilis, and a variety of parasitic diseases.^{1,2} Their use in recent time underwent a rapid decline mainly because of concerns on their systemic toxicity. However, some Gold, Platinum or Arsenic compound is still widely used for clinical applications. Among them, antiarthritic compounds such as Auranofin (AF) or Aurothiomalate (ATM) found important role as repurposed drugs for treating cancer or infections.³ Interestingly, the medicinal properties of gold(I) compounds might be even improved using biocompatible nanostructures for their delivery.⁴ In this frame, we proceeded with the preparation of a nanoformulation of Et₃PAuCl, this latter being an AF analogue endowed with promising anticancer properties. The well-known PLGA-PEG nanoparticles (NPs) were chosen for the encapsulation process and functionalized with a biocompatible fluorescent probe for their tracking in cell. After the characterization of the NPs, we performed a few biological tests on the Et₃PAuCl loaded nanoparticles in comparison to the free Et₃PAuCl drug in 2D and 3D (HCT-116) colorectal cancer models. The biological activity and the mechanistic aspects for the anticancer effects of the loaded NPs are comparatively and critically discussed.

1-E. J. Anthony, E. M. Bolitho, H. E. Bridgewater, O. W. L. Carter, J. M. Donnelly, C. Imberti, E. C. Lant, F. Lermyte, R. J. Needham, M. Palau, P. J. Sadler, H. Shi, F.-X. Wang, W.-Y. Zhang and Z. Zhang, Chem. Sci., 2020,11, 12888-12917.

2- D. Cirri, F. Bartoli, A. Pratesi, E. Baglini, E. Barresi, T. Marzo, Biomedicines, 2021, 9(5), 504.

3- T. Marzo, D. La Mendola, Inorganics, 2021, 9, 46.

4- A. Menconi, T. Marzo, L. Massai, A. Pratesi, M. Severi, G. Petroni, L. Antonuzzo, L. Messori, S. Pillozzi, D. Cirri, Biometals, 2021, 34, 867–879.

11:00am SM-WeM-10 Plasmonic Tuning of Go-Based Nanosheets by Plasmonic Noble Metal Nanorods for Self-Cleaning Photothermal Surfaces to Fight Surface Contamination, Vanessa Sanfilippo, T. Pascal, A. Foti, University of Catania, Department of Chemical Sciences, Italy; A. Fraix, S. Petralia, G. Forte, University of Catania, Department of Drug and Health Sciences, Italy; C. Fortuna, University of Catania, Department of Chemical Sciences, Italy; A. Giuffrida, C. Satriano, University of Catania, Department of Chemical Sciences, Italy

In this study, we propose a novel hybrid 2D nanomaterial based on thiolated reduced graphene oxide (rGOSH) sheets and silver (Ag) or gold (Au) nanorods (NRs). The goal is to integrate the enhanced self-cleaning properties (super-hydrophobicity) and the plasmonic tuning of photothermal response of the hybrid nanoplatform (GO-NR), to the intrinsic antibacterial properties of each component (GO, Ag, Au), to inhibit surface contamination. Both experimental and theoretical studies were the design, the synthesis, and focused on the physicochemical/biochemical/cellular characterization of the hybrids. A multitechnique approach by UV-visible spectroscopy (UV-VIS), dynamic light scattering (DLS), Zeta potential (ZP), atomic force microscopy (AFM), and water contact angle (WCA) measurements was used to scrutinize the plasmonic features (including the optical size), the hydrodynamic size and the surface charge, the topography and the surface free energy, respectively. The photothermal properties of the hybrids were examined in solution following the increase of temperature under irradiation with CW laser using a FLIR C3 thermal imaging camera. Proof-of-work in vitro cellular experiments on human prostate cancer cells (PC-3 line) and murine fibroblasts (L929 line) were carried out to test the nanotoxicity of the hybrids as well as their capability to induce oxidative stress by the production of reactive oxygen species (ROS). Intracellular imaging using confocal laser scanning microscopy (LSM) was performed to evaluate the mechanism of interaction and internalization of hybrid systems on the studied cell lines.

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Wednesday Morning, November 9, 2022

11:20am SM-WeM-11 Green Synthesis of Metal Nanoparticles for Wound Healing Applications, *Alice Foti*, *V. Sanfilippo*, University of Catania, Italy; *V. Caruso, R. Inturri, P. Amico, S. Vaccaro*, Fidia Farmaceutici S.p.A., Italy; *C. Satriano*, University of Catania, Italy

Wound healing is a complex process involving multiple cell strains and the related products trying to regenerate and repair the damaged tissue. The multidisciplinary field of nanotechnology that includes, among others, interface and colloid science, molecular biology, and engineering, offers a huge range of applications, including nanomedicine. Metallic nanoparticles (NPs), 1-100 nanometers (nm) in size, have unique physicochemical, optical, and biological properties that gives them a great potential in treating several diseases. Indeed, it is essential to find controlled and reproducible synthetic methods to achieve biocompatibility and the desired therapeutic properties, giving a ground-breaking approach towards the promotion of wound healing. This work focuses on a green synthesis approach to synthesize gold (Au), silver (Ag), and palladium (Pd) NPs, aiming to promote the proliferation of cells in wound healing. Moreover, NPs surface, is capped with hyaluronic acid (HA), known to improve their biocompatibility and promote endothelial regeneration. Physicochemical and biological characterization of the synthesized NPs was assessed with a multi-technique approach. Optical properties were studied with UV-vis spectroscopy; the increase of the hydrodynamic diameter after the conjugation with HA was investigated with dynamic light scattering (DLS) experiments; and the measurement of viscosity was performed with a viscosimeter. Biological tests were assessed on fibroblast (L-929) cells. Particularly, cytotoxicity was inspected via MTT assay; wound healing potential was investigated by in vitro wound scratch assay; sub-cellular interactions were analyzed with confocal microscopy.

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11:40am SM-WeM-12 Nanoparticles Loaded with Histidine Rich Peptides for Wound Healing, *Diego La Mendola*, Università di Pisa, Italy; *L. Chiaverini, T. Marzo*, University of Pisa, Italy

The histidine-proline-rich glycoprotein (HPRG) is a single polypeptide chain protein of 70–75 kDa, with a multidomain structure. In humans, the protein is synthesized in the liver and is present in plasma at relatively high concentrations of 100–150 $\mu g/mL$ (1.5 μ M) [1]. HPRG ability to simultaneously interact with a large number of protein ligands and has been implicated in the regulation of various physiological and pathological processes including the formation of immune complexes, apoptotic/necrotic and pathogen clearance, cell adhesion, antimicrobial activity, anti-/pro-angiogenic activity, coagulation and fibrinolysis. Interestingly, these processes are often associated with sites of tissue injury or tumour growth, where the concentration and distribution of copper and zinc ions is known to vary.

In this work, we tackled the assembling of hybrid platforms made of gold or silver nanoparticles (NPs) functionalized with the peptides belonging to HPRG protein, and rich in histidine residues, in order to achieve a modulation of the angiogenic process in the wound healing treatment in presence/absence of divalent metal ions.

These new systems exploit the synergic effects of different components in the regeneration of damaged tissues.

Nanoparticles were characterized by UV-visible spectroscopy, to scrutinise the peptide binding to Au, by monitoring the changes in the plasmonic peak (AuNP). Atomic force microscopy and dynamic light scattering analyses confirmed a strong association of the peptides to the plasmonic nanoparticles.

Cellular experiments on human foreskin fibroblasts demonstrated the low cytotoxicity of the hybrid assemblies and their high ability to promote wound closure and fibroblasts migration. The activity of Au-GO-ANG were also tested in the presence of copper and/or zinc ions. Besides, cell imaging by confocal microscopy revealed synergic dynamic processes modulated by the different sub-cellular structures (lysosomes, mitochondria, cell cytoskeleton). The obtained results evidence the promising applications of the synthesized nanoparticles for wound care treatment and tissue regeneration.

The authors thank the University of Pisa, "PRA – Progetti di Ricerca di Ateneo" Institutional Research Grants – Project no. PRA_2020_58) and Rating Ateneo 2019-2020 for financial support.

[1] K.M. Priebatsch, M. Kvansakul, I.K.H. Poon, M.D. Hulett, Biomolecules, 2017, 7, 22.

Thursday Evening, November 10, 2022

Smart Multifunctional Materials for Nanomedicine Focus Topic

Room Ballroom A - Session SM-ThP

Smart Multifunctional Materials for Nanomedicine and Theranostics Poster Session

SM-ThP-1 Electrospun Alligned and Randomly Oriented Fibers Using a Novel Collector, *Tessa Gilmore*, *P. Gouma*, The Ohio State University

Electrospinning is a specialized processing technique for the formation of sub-micron diameter fibers of certain materials including polymers. Electrospinning may be used in the medical field, as in cases of drug delivery or tissue engineering. The orientation of the fibers, aligned or random, can affect the application of the non-woven mat due to the difference in properties. For example, aligned fibers have a higher tensile strength and modulus than randomly oriented fibers. Aligned fibers are also better suited for tissue engineering as they have improved cell proliferation and regeneration. Conversely, randomly oriented fibers are better suited for filtration applications as they can maximize separation of unwanted particulates. In this study, a novel collector and experimental setup were used to create both aligned and randomly oriented fibers during the same experiment. A solution of 15 wt% Polyvinylpyrrolidone (PVP) in ethanol was spun, and a hollow casing of fibers that surrounded the top and sides of the collector was observed. Scanning electron microscopic (SEM) images revealed that the casing had uniaxial fibers along the sides and randomly oriented fibers on the top. Additional experiments are being conducted using Cellulose Acetate (CA), which is a popularly used bioplastic. Cellulose Acetate also is known for being difficult to electrospin due to its tendency to crystalize at the extruder tip. However, this novel setup may mitigate this problem.

SM-ThP-2 Effect of Metal-Mediated Oxidative Stress on Lysosomal Damage/Dysfunction, V. Sanfilippo, C. Bonaccorso, L. Cucci, University of Catania, Italy; R. Inturri, Fidia Farmaceutici S.p.A., R&D Unità locale Fidia Research sud, Italy; P. Amico, Fidia Farmaceutici S.p.A., R&D Unità locale Fidia Research sud, Italy; S. Vaccaro, Fidia Farmaceutici S.p.A., R&D Unità locale Fidia Research sud, Italy; Cristina Satriano, University of Catania, Italy

Lysosomes are specialized vesicles within cells that digest large molecules by hydrolytic enzymes; several studies demonstrated that metallic nanoparticles are degraded in the lysosomes with ionic release in the cytosol that induces cell damage. In this work, we reported the synthesis and characterization of plasmonic nanoparticles for subcellular targeting and intracellular imaging of lysosomes. A fluorescent and colorimetric probe (LysoBC1) was designed and synthesized for the dynamic tracking of Cu²⁺ in living cells, to image lysosomal damage. The cytotoxicity and cellular uptake of metallic nanoparticles of silver and gold nanospheres (AgNS, AuNS), both bare and capped with hyaluronan, were scrutinized either on healthy (mouse fibroblast L929 line) or cancerous (human prostate cancer PC3 line) cells. The nanoparticle chemical structure and surface functionalization resulted critical to control the release of toxic species, i.e., $\mathsf{Ag}^{\scriptscriptstyle +}$ ions, and the ROS generation process, which inhibits the antioxidant defense system causing mechanical damage to the cell membrane. The nanoparticles were prepared by chemical reduction methods and characterized by UV-visible spectroscopy and dynamic light scattering analyses, to study the plasmonic properties and the hydrodynamic size, respectively. The toxicity of intracellularly ions, the cellular internalization of the systems and the involvement of lysosomes in the cellular stress induced by the treatment was investigated in terms of cell viability, ROS production, and live cell-confocal imaging.

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Keywords: metallic nanoparticles, hyaluronic acid, ROS production, cell confocal imaging

SM-ThP-3 The Interaction of Neurotrophin-Mimicking Peptides and Artificial Cell Membranes: An Experimental and Theoretical Study, *Vanessa Sanfilippo*, *L. Redigolo*, University of Catania, Department of Chemical Sciences, Italy; *G. Forte*, University of Catania, Department of Drug and Health Sciences, Italy; *C. Satriano*, University of Catania, Department of Chemical Sciences, Italy

In the present work we assembled hybrid peptide-nanomaterial (p-NM) systems to scrutinize their interaction at the biointerface with artificial cell membranes in 3D or in 2D, i.e., with phospholipid small unilamellar vesicles (SUVs) or supported lipid bilayers (SLBs), respectively. The peptide sequences BDNF(1-12), NT3(1-13) and NGF(1-14), encompassing the Nterminal domains respectively of Brain Derived Neurotrophic Factor (BDNF), NeuroTrophin 3 (NT3) and Nerve Growth Factor (NGF), were immobilized by physisorption onto graphene oxide (GO). The optical characterization through UV-Vis and Fluorescence spectroscopies, in terms of FRET (Forster Resonance Energy Transfer) has been made to shed light on the electron transfer processes occurring firstly at the interface between carboxyfluorescein-labelled peptides and GO (quencher of fluorescence) and then between the peptide-functionalized GO sheets (FRET donor) and the lipid membranes dye-labelled with rhodamine (FRET acceptor). The biophysical properties of the artificial cell membrane, before and after the interaction with p-NM systems, were investigated by atomic force microscopy (AFM), in terms of morphology, and by laser scanning confocal microscopy (LSM). In particular, the latter was utilized with the Fluorescence Recovery After Photobleaching (FRAP) technique, to study the average molecular lateral diffusion at the hybrid nanobiointerface. 3D optical characterization has been made through UV-Vis and Fluorescence spectroscopies, also in terms of FRET (Forster Resonance Energy Transfer) to understand the electron transfer processes. The experimental studies were paralleled by computational analyses by molecular dynamics. Cellular experiments were carried out to investigate the interaction with HUVECs cells in terms of cytotoxicity, through MTT assay, and cellular internalization, through LSM, after being treated with the p-NM. Moreover, wound closure experiments and tube formation assays were carried out to investigate the cell-migration effects and the angiogenic response induced by p-NM systems.

SM-ThP-4 CTAB Removal and Graphene Oxide Functionalization of Metallic Nanorods for Theranostic Applications, *Alice Foti*, *V. Sanfilippo*, *P. Tomasella*, University of Catania, Italy; *L. Le Meur*, *T. Bretot*, University of Rennes, France; *C. Satriano*, University of Catania, Italy

Cetyltrimethylammonium bromide (CTAB) is a strong surfactant which plays a fundamental role in several procedures, such the seed-mediated growth of plasmonic nanorods.

In this respect, CTAB is used as growth and stabilizing agent as it forms bilayers on the surface of the nanorods. However, the dissociation of CTAB into CTA⁺ and Br⁻ makes it toxic for cells, therefore the application of nanorods in biomedical fields has some limitations. Herein, we present a strategy to remove CTAB from the metallic nanoparticle surface, followed by the replacement of the ligand with graphene oxide (GO) and reduced-thiolated GO (rGOSH), to obtain nanoparticles with a low or null level of toxicity, thus suitable for theranostic applications.

Plasmonic properties of the different systems were studied by UV-visible spectroscopy and monitored during time. The surface free energy of the CTAB-capped and CTAB-rinsed nanorods were determined by contact angle (CA) measurements and the surface charge was monitored by zeta potential (ZP) measurements. Atomic force microscopy (AFM) and dynamic light scattering (DLS) measurements were performed to investigate for size distributions and hydrodynamic size of the nanoparticles, respectively. The cytotoxicity was studied *in vitro* on different cell lines by means of cell viability detection by nuclear staining of dead/total cells and mitochondrial enzymatic activity (MTT assay). The reactive oxygen species (ROS) production was determined by MitoSOX assay, laser scanning confocal microscopy (LSM) allowed to shed light on the intracellular organelle perturbation.

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Thursday Evening, November 10, 2022

SM-ThP-5 Hydropolymers, Hydrogels and Hydrogel Composites as Lubricants, Nir Kampf, W. Lin, M. Kluzek, Weizmann Institute of Science, Israel; S. Angayarkanni, SRMIST, India; N. Iuster, E. Shimoni, Weizmann Institute of Science, Israel; R. Goldberg, Iipo-sphere, Israel; J. Klein, Weizmann Institute of Science, Israel

About 20% of the world's total energy consumption spent to overcome friction. Friction is also present water-based environments like biological systems, widely in hips and joints.Due to the molecular complexity of the biological systems, the mechanism of lubrication is still not clear. Apart from our efforts to find the major components responsible for the low friction in biological systems, we also try to exploit nature's solution for lubricating interfaces such as cartilage by mimicking nature's strategies of boundary lubrication, which lead to an extreme reduction of friction in aqueous environments. Low frictions failure in joints is correlated with diseases such as osteoarthritis. In my talk, I will present several examples of bio-inspired lubrication by polymers and in polymer networks. We carried out systematic investigations from the molecular to the macroscopic level, demonstrating excellent lubrication by polymer assemblies (1) and hydrogel composites (2), attributed to the hydration lubrication mechanism acting at highly-hydrated boundary layers.

1) Angayarkanni et al., (2019) Langmuir. 35, 48, p. 15469-15480.

2) Lin et al., (2020) Science. 370, 6514, p. 335-338.

SM-ThP-6 Nanocomposites of Gold Nanoparticles-Graphene Oxide and Angiogenin for Wound Care Treatment, L. Chiaverini, T. Marzo, Diego La Mendola, University of Pisa, Italy

Hybrid platforms made of gold nanoparticles (AuNPs), graphene oxide (GO) nanosheets and angiogenin (ANG) protein were prepared to tune angiogenic process in the wound healing treatment.

Nanocomposites were characterized by UV-visible spectroscopy, to scrutinise the ANG binding to Au-GO, by monitoring the changes in the plasmonic peak (AuNP) as well as in the π -> π * transition electronic band (GO), respectively. Atomic force microscopy and dynamic light scattering analyses confirmed a strong association of the protein to nanoparticles/nanosheets. Cellular experiments on human foreskin fibroblasts demonstrated the low cytotoxicity of the nanocomposites and theiractivity in promoting wound closure. Cell imaging by confocal microscopy revealed synergic dynamic processes modulated by the different sub-cellular structures. The obtained results evidence the promising applications of the synthesized multifunctional nanocomposites for wound care treatment.

The authors thank the University of Pisa, "PRA – Progetti di Ricerca di Ateneo" Institutional Research Grants – Project no. PRA_2020_58) and Rating Ateneo 2019-2020 for financial support.

SM-ThP-7 Novel Synthesis of Silver Nanoparticles and Their Antibacterial Activity for Therapeutic Applications, *H. Arshad*, Rutgers, The State University of New Jersey; *Umer Hassan*, Rutgers The State University of New Jersey

Silver nanoparticles (AgNPs) finds many biomedical applications due to their antimicrobial activity. Traditional material synthesis process employed to fabricate these nanoparticles require hazardous chemicals thereby posing a significant personnel and environmental risk. To mitigate this, we developed a novel eco-friendly fabrication process to synthesize silver nanoparticles using plant extracts. Here, we report the utility of Salvadora persica extract as reducing agent for nanoparticle synthesis. Further, we employed sunlight and LED irradiation methods for AgNPs fabrication. Nanoparticles were synthesized within 10 min and were characterized using multiple techniques. UV-Vis. absorbance spectroscopy analysis demonstrated spectral peaks at 450 nm corelative to AgNPs synthesis while X-ray diffraction (P-XRD) pattern depicted nanoparticles crystal structure. Fourier transform infrared spectroscopy (FTIR) demonstrated the role of phytochemicals for AgNPs reduction. Morphological analysis was done using transmission electron microscopy (TEM) and field emission scanning electron microscopy (FE-SEM) which demonstrated nanoparticles spherical shapes and revealed their size of approximately 39.7nm.

Synthesized AgNPs were extensively characterized for their antibacterial activity against Escherichia coli (E. coli) and Staphylococcus epidermidis (S. epidermidis) pathogens. Kirby Bauer antimicrobial assay was used for this analysis, and minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) were calculated. For E. coli MBC and MIC were determined to be 3.0 μ g/mL and 1.5 μ g/mL respectively. However, for S. epidermidis, these values were determined to be 25 μ g/mL and 12.5

 μ g/mL respectively. This study highlights a novel nanoparticles fabrication method and provide their extensive characterization analysis with a focus on their role in antibacterial activity for therapeutic applications. These nanoparticles can be used to design next generation wound dressings or impregnated in surgical masks to provide enhanced antimicrobial protection.

Author Index

— A —

Amico, P.: SM-ThP-2, 3; SM-WeM-11, 2 Angayarkanni, S.: SM-ThP-5, 4 Arshad, H.: SM-ThP-7, 4 — B — Bellissima, A.: SM-WeM-5, 1 Bonaccorso, C.: SM-ThP-2, 3 Bretot, T.: SM-ThP-4, 3 - C -Caruso, V.: SM-WeM-11, 2 Chiaverini, L.: SM-ThP-6, 4; SM-WeM-12, 2; SM-WeM-6, 1 Cucci, L.: SM-ThP-2, 3; SM-WeM-5, 1 Cvelbar, U.: SM-WeM-3, 1 - D -De Bonis, A.: SM-WeM-5, 1 — F — Fiorenza, R.: SM-WeM-5, 1 Forte, G.: SM-ThP-3, 3; SM-WeM-10, 1 Fortuna, C.: SM-WeM-10, 1 Foti, A.: SM-ThP-4, 3; SM-WeM-10, 1; SM-WeM-11, **2** Fraix, A.: SM-WeM-10, 1

Bold page numbers indicate presenter

— G – Gilmore, T.: SM-ThP-1, 3 Giorgini, E.: SM-WeM-5, 1 Giuffrida, A.: SM-WeM-10, 1 Goldberg, R.: SM-ThP-5, 4 Gouma, P.: SM-ThP-1, 3 — Н — Hassan, U.: SM-ThP-7, 4 -1-Inturri, R.: SM-ThP-2, 3; SM-WeM-11, 2 luster, N.: SM-ThP-5, 4 — к — Kampf, N.: SM-ThP-5, 4 Klein, J.: SM-ThP-5, 4 Kluzek, M.: SM-ThP-5, 4 -L-La Mendola, D.: SM-ThP-6, 4; SM-WeM-12, 2; SM-WeM-6, 1 Le Meur, L.: SM-ThP-4, 3 Lin, W.: SM-ThP-5, 4 - M -Marzo, T.: SM-ThP-6, 4; SM-WeM-12, 2; SM-WeM-6, **1**

Modic, M.: SM-WeM-3, 1 — N -Notarstefano, V.: SM-WeM-5, 1 — P — Pascal, T.: SM-WeM-10, 1 Petralia, S.: SM-WeM-10, 1 — R — Redigolo, L.: SM-ThP-3, 3 -s-Sanfilippo, V.: SM-ThP-2, 3; SM-ThP-3, 3; SM-ThP-4, 3; SM-WeM-10, 1; SM-WeM-11, 2; SM-WeM-5, 1 Satriano, C.: SM-ThP-2, 3; SM-ThP-3, 3; SM-ThP-4, 3; SM-WeM-10, 1; SM-WeM-11, 2; SM-WeM-5, 1 Scirè, S.: SM-WeM-5, 1 Scivoletto, G.: SM-WeM-5, 1 Shimoni, E.: SM-ThP-5, 4 — T — Tomasella, P.: SM-ThP-4, 3 -v-Vaccaro, S.: SM-ThP-2, 3; SM-WeM-11, 2