Research

Standardization of Procedures: the Nanostructurome Pipeline

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Abstract

Large scale ecological features are driven by the interactions between organisms at all life scales, in particular the microorganisms living in the air, soil, and water. If we want to understand how an ecosystem will respond to natural and human-induced changes, we need to understand the state of the unseen majority – the microorganisms. Understanding of a system means characterising the function of its components and their interactions. In the presently acknowledged view, an ecosystem is considered as an organization of molecules (genes, mRNAs, proteins, and metabolites) into complex molecular pathways (such as gluconeogenesis and tricarboxylic acid cycle). It is thought that interacting molecules form functional modules (such as groups of molecules involved in the same biological process), which then drive larger scale biological processes. Supported by the omic-level high-throughput data acquisition and collections, comprehensive models of the interactions among biomolecules are sought. Since the discovery of DNA molecule as a powerful carrier of the information, solutions at the molecular level have been in focus so far. But for understanding of some essential cellular processes downscaling from micro to molecular level is too big to overcome in a single step. Apparently, the substance (and with it the information) is exchanged between cells within nano-sized extracellular particles (EPs) and as this level is at the core of interaction between the microorganisms, it could be the lever to shift the state of the ecosystem. By creating a pipeline of standardized complementary methods for characterization of samples containing EPs we are approaching the largely unknown landscape of a "nanostructurome" that will in the future become increasingly filled with knowledge on sub-micron cellular structures that convey matter and information between cells.

Keywords: **I**nteractome; Extracellular particles; Standardization; Reproducibility, reliability; Standard operating procedures; Nanostructurome; Pipeline

1. Introduction

Interacting molecules form functional modules which drive large scale biological processes. Interactome studies, then, give us the tools to prepare for and confront the significant challenges that society is grappling with, such as disease prevention and cure, hunger relief, population growth, and climate change. Researchers are seeking comprehensive models of the interactions among biomolecules, with omic-technology providing support.The problem of different interactive relationships between genes considering their unique intrinsic properties and defects is incorporated in cellular interactomes. Narrowing the focus, a metagenomics approach was developed to study the plasmidome (the overall plasmid content in a given environment) which indicate that gene mobility between different phyla is still fairly common in microbially rich environments. The interactome network of protein–protein interactions aims to capture the molecular interactions that underlie organismal complexity. However, the basic principles of how such interactome networks respond to environmental unpredictability and change during evolution are largely unknown. Nanoparticles and nanotechnology offer a fascinating and evolving field of study with many practical applications. At the same time, due to their extremely small size, the study of nanoparticles requires a multidisciplinary platform that integrates data from different sophisticated and complementary technologies.

The progress of technology generates a huge array of data, which poses new challenges in quality control, reproducibility, management and sharing of the experimental results. Optimized and standardized procedures are essential for generating, validating, integrating, analyzing, storing and reusing the wide variety of data available as well as to increase research performance and reduce related cost and time (Liguori and Kisslinger, 2021; Hollmann et al., 2022). Optimization of multivariable processes can be achieved by varying one specific factor at a time, as in the traditional OFAT method, or implementing statistical approaches and quality management tools, such as Design of Experiments (DoE), for a more powerful analysis. DoE has been used in many different fields to identify the effect of simultaneous variations in variable process factors, the interaction between the factors and ultimately the optimal setting that maximizes process output. (Mancinelli et al., 2015, 2021; Xu et al., 2020; Schade and Middendorf, 2021; Rampado and Peer, 2023). Following optimization, information on the optimal factor combination(s) to use, the critical process parameters to monitor, as well as the required equipment and professional competence needed can be formalized in quality assurance tools such as guidelines and standard operating procedures or SOPs (Digilio et al., 2016; Hollmann et al., 2020; Liguori and Kisslinger, 2022). These tools are fundamental to guarantee uniformity in the performance of a specific function or process, which is the basis for data reproducibility.

2. Standard Operating Procedures

Based on the existing literature on the definition of SOPs (Hattemer-Apostel, 2001; Gough and Hamrell, 2010; Akyar, 2012; Hollmann et al., 2020) and our direct experience in dealing with standardization issues in multicentric national and international consortia (Bongiovanni et al., 2015; Liguori and Kisslinger, 2021), we here summarise the main characteristics and purposes of a SOP.

SOPs are detailed step-by-step written instructions for performing a procedure to achieve a predetermined specification and quality end result. In addition, SOPs also contain key information on the materials, equipment and software/hardware required, health and safety warnings, cautions and instructions for the management of records, data and waste, as well as the roles, competencies and responsibilities of all personnel involved. This information, which nevertheless has a major impact on the performance of the procedure, is sometimes left out, whereas SOPs provide an overview of all the key aspects involved in the process and thus allow it to be kept under control.

The purpose of SOPs is to ensure that all operators perform tasks in the same way, which is a necessary condition for obtaining the expected output from the process. Standardized procedures guide workers and reduce the possibility of missed steps or other errors that affect the performance of the process and the quality of the results. SOPs are obviously useful in meeting compliance requirements in regulatory environments where a Quality Management System (QMS) and/or Good Manufacturer Practice (GMP) is in place (e.g. manufacturing and biotechnology industries). However, SOPs are just as useful in non-regulated research to work in a consistent and efficient manner, improve resource management, mitigate health and safety risks, and ultimately support overall research performance and technology transfer. Moreover, SOPs are useful for training new personnel and ensuring appropriate transfer of skills and knowledge, which is particularly relevant in research environments, where staff turnover is typically high. In the context of multicentre projects and consortia, especially the one developing cutting-edge technologies to address current scientific challenges, the identification and sharing of agreed SOPs is essential for data reproducibility, analysis and integration.

3. The Importance of Micro and Nanostructures in Pursuing the One Health Principle

The health maintenance faces many challenges. Societal need for effective disease prevention, treatment, and rehabilitation has been recognized by scientists from diverse fields such as physics, chemistry, biology, and interdisciplinary sciences who are striving to unravel the underlying mechanisms at the cellular level. It is acknowledged that when living cells are exposed to stressful conditions, the secreted cellular substance provides valuable information for other cells for coping with destabilizing stress events. Secreted small (nano-sized) extracellular particles EPs (e.g. extracellular vesicles, lipoproteins and antibody complexes) can transport cargo to neighboring or distant cells (Herman et al., 2021, Lenzini et al., 2020) and thereby mediate and trigger inter- and intracellular communication. It was found that this communication is relevant to biofilm formation, antibiotic resistance, and toxin delivery (Woith et al., 2019). Entering in the process as active players, scientists envisage nanoparticulate platforms as potential carriers of beneficial substances such as vaccines, regenerative material and therapeutics for various diseases (Armingol et al., 2021, Jin et al., 2021, Combarnous et al., 2020, Fais et al., 2016). Nanoparticles are very small particles, generally in the range of 1 to 100 nanometres, that have special physical and chemical properties compared to bulk materials due to their minute size. Nanoparticles, are used in a wide range of applications, including electronics, energy, environmentl applications and medicine, where they play a key role in therapeutic drug-delivery (Joudeh and Linke, 2022). Nanovesicles enclosed by artificial membranes (liposomes) have been considered as substance carriers already for decades. Recently, research has focused also on development of cell-based drug carriers that better mimic the complexity of the natural membrane, however, their survival, migration, function, and toxicity were found to be of concern (Hermann et al., 2021). In addition to lipid nanoparticles and liposomes, extracellular vesicles (EVs) produced by cells have emerged as promising natural nanovectors for drug delivery in several pathological contexts, including cancer, due to their high biocompatibility, safety, targeting capabilities and ability to cross body barriers (Mantile et al., 2020; Herrmann et al., 2021). Finally, bioinspired nanoparticles, which combine the advantages of both synthetic and natural nanoparticles, have also been produced to enhance drug delivery performance (Liu et al., 2023; Tripathi et al., 2023).

Nowadays, it is of great interest to find solutions for promotion of health and search for alternative bioinspired materials that are abundant, easily accessible and fulfill environmental friendliness and sustainability. Considering ocean as a vast reservoir of living organisms, the research includes the possibilities provided by marine microalgae. Microalgae are the primary source in the food chain and by performing photosynthesis, they also contribute to the formulation of the atmosphere. Besides being acknowledged for their important ecological role, they are being considered for various human uses (Balsanti and Gualitieri 2006, Rosales-Mendoza 2016).

Microalgal EPs (so called nanoalgalosomes) (Picciotto et al., 2021, Adamo et al., 2021) were isolated, imaged by electronic microscopy and characterized according to the guidelines of the International Society for Extracellular Vesicles (ISEV) (Thery et al., 2018). They were found non-toxic and able to transmit the substance to eukaryotic cells (Adamo et al., 2021). As the study (Adamo et al., 2021) was the first systematic aproach to microalgal EPs, further research is necessary to consider also other microalgae species that could be subjected to different mechanisms of formation of SCPs and yield higher quality and quantity EPs (Božič et al., 2022). Preliminary results indicate that the mechanisms of EP formation can be profoundly different in different microalgae species.

Plants provide the core basis for life on Earth and they are the single most important pillar of human nutrition, but healthy plants are not something that we can take for granted. Plants, which make up 80 percent of the food we eat, and produce 98 percent of the oxygen we breathe, are under constant and increasing threat from pests and diseases. Numerous plant pathogens, including bacteria, fungi, and nematodes, are responsible for many plant diseases, which reduce the yield and quality of agricultural production worldwide every year (Fisher et al., 2012; Savary et al., 2019).

The global plant biotechnology market is projected to expand from usd 51.73 billion in 2025 to reach usd 76.79 billion by 2030 (AGI 9348, 2025). Demand for genetically modified seeds is growing as for the needs in India and China which drives the growth of the plant biotechnology market and also induced also a huge shift in terms of innovative products and patent registrations (AGI 9248, 2025). Global players are investing in R&D to manufacture products that are economical and suitable for use with fertilizers and pesticides. The top plant biotechnology market companies are BASF SE (Germany), Corteva Agroscience (USA), Bayer AG (Germany) and Syngenta AG (Switzerland) (AGI 9348, 2025). The outlined factors for growth of the plant biotechnology market are growing demand for high value crops and use of plant growth regulators to combat climatic changes. Protecting plant health can help end hunger, reduce poverty, protect the environment, and boost economic development (UN news, 2019).

Exploring the interaction between plants and microorganisms is conducive to plant disease control and agricultural production. Namely, plants and pathogens secrete multitudes of molecules into the extracellular environment for cross-border communication, which is crucial to plant defense and pathogen virulence (Delaunois et al., 2014; Toruno et al., 2016). As key resources for life on the planet (energy, nutrients, water, soil) are becoming ever more subject to climate and ecological pressures, European Commission has specified nature based solutions as likely means for understanding and managing the urban ecosystem's equilibrium.

Biostimulants are substances with growth-promoting activities on plants, mostly related to nutrient uptake and stress tolerance. We consider EPs as a major way to achieve communication between plants and microorganisms to enhance crop resilience, productivity and quality. EPs can be up-taken by other cells and therefore mediate intercellular interaction by transferring proteins, lipids, nucleic acids and other molecules to neighbouring or distant cells. By changing the functioning of the recipient cells, EPs play an important role in many physiological and pathophysiological processes (Welsh et al., 2024). Communities of microorganisms managed or functionally enhanced through a greater understanding of EPs communication have several additional extraordinary advantages, key among them being scalability and cost. While urban farming, and bioremediation often require large-scale investment and equipment, EPs are cheaper and easier to scale, including to the individual household level, allowing easier adaptation of local communities and neighbourhoods to climate change system and global changes soon to be impacting urban areas, especially in less-well developed economic areas. One can distinguish between macro and micro remediation methods and contexts, and also along the social-ecological, natural and technical axes differentiating resilience management at city and household scales, while all scales are governed by the mechanisms at the cellular level. Since EP field is a relatively new rapidly developing field the key mechanisms are not yet fully understood and the methods for EP harvesting and characterization and extraction of the compounds are not yet being fully explored. Furthermore, interpretation of the results on new substances in connection with other parameters of the medium urgently need improvements and clarifications.

4. The Nanostructurome

Nanostructurome contains knowledge on nanostructures, in this particular case those that are relevant in living systems. It focuses on investigation how matter and information are transported at the cellular level in the form of EPs or EVs whose size is in the nanometre range. There are many challenges in harvesting, characterization and application of EPs and presently, it is recommended that the samples are assessed by orthogonal methods (Welsh et al., 2024). Part III of the 12th Proceedings of the Socratic Lectures is devoted to the efforts to assemble and coordinate processing of samples containing EPs to develop a standardized pipeline and an integrated platform for the physico-chemical, molecular and functional study of EPs. The Part III of the 12th Proceedings of the Socratic Lectures presents the first 9 standardized procedures developed inside the Nanostructurome pipeline, spanning from biomass production (Cepec et al., Danilović Luković et al), biophysical analysis (Romolo and Kralj-Iglič), physico-chemical analysis (Heath et al, Bar and Lavrič, Hočevar et al) to molecular and functional analysis (Kovačevskij et al, Michelini et al, D'Antonio et al). Cepec et al. and Danilović Luković et al address biomass production and processing, using microalgae as natural source of EPs. Other SOPs are dedicated to analytical methods such as Interferometric Light Microscopy (Romolo and Kralj Iglic, 2025), Liquid Chromatography coupled to Mass Spectrometry (Heath et al, 2025), NMR spectroscopy (Hocevar et al., 2025) Thermal Analysis (Bar and Lavrič, 2025; Cerc and Lavrencic, 2025) and Next Generation Sequencing (Kovachevikj et al., 2025). Finally, two SOPs focus on biological aspects, such as the quantification of growth and inflammatory factors in extracellular medium (Michelini et al., 2025) and a wound-healing functional assay to determine the effect of nanoparticles on cell migration (D'Antonio et al., 2025). All SOPs are written according to specific guidelines and have the same structure and content. The present SOPs together with those in progress will identify a standardized procedural pipeline for the production, physicochemical, molecular and functional analysis of both unpurified and purified EPs.

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