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Probing safety of nanoparticles by outlining sea urchin sensing and signaling cascades

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A B S T R A C T

Among currently identified issues presenting risks and benefits to human and ocean health, engineered nanoparticles (ENP) represent a priority. Predictions of their economic and social impact appear extraordinary, but their release in the environment at an uncontrollable rate is in striking contrast with the extremely limited number of studies on environmental impact, especially on the marine environment. The sea urchin has a remarkable sensing environmental system whose function and diversity came into focus during the recent years, after sea urchin genome sequencing. The complex immune system may be the basis wherefore sea urchins can adapt to a dynamic environment and survive even in hazardous conditions both in the adult and in the embryonic life. This review is aimed at discussing the literature in nanotoxicological/ecotoxicological studies with a focus on stress and innate immune signaling in sea urchins. In addition, here we introduce our current development of in vitro-driven probes that could be used to dissect ENP aftermaths, suggesting their future use in immune-nanotoxicology.

1. Introduction

In the course of the last decades, nano-objects and their aggregates and agglomerates (ISO/TR 13014: Nanotechnologies-Guidance on physicochemical characterization of engineered nanoscale materials for toxicologic assessment, 2012) have emerged as promising new nanomaterials with applications in biomedicine, engineering, telecommunications, electronics, transportation and manufacturing technologies.

Predictions of their economic and social impact appear extraordinary, for example, the nanotechnology economy was worth a trillion dollars at the end of 2015. The expenditures, however, are in striking contrast with the extremely limited nature of the health impact studies of engineered nanoparticles (ENP). The European Marine Board recently published a position paper on linking seas and human health as a strategic research priority for Europe (Fleming et al., 2014). Among currently identified issues presenting risks and benefits to human and ocean health, anthropogenic chemicals, marine plastics/litter, and ENP in principle represent a priority. Under realistic environmental conditions, ENP can influence the environment in three ways: by a direct interaction with any organism, by a change in bioavailability of different toxins/nutrients or by an indirect impact on the ecosystem (Christian et al., 2008). As far as reproductive, developmental and immunological functions are central to any life cycle, the full understanding and extrapolation of the effects of ENP on these functions can be helpful to predict and mitigate the potential long-term risk related to their spreading in the environment on keystone species, biodiversity, ecosystems and human health. In this context, the enormous diversity of life in the sea offers a rich selection of organisms, especially invertebrates, with specialized adaptations that enable researchers to learn more about the conserved molecular signaling pathways involved in protection, robustness, resistance and plasticity toward exposure to contaminants (Bodnar, 2016). Among marine invertebrates, sea urchins provide an attractive and alternative *proxy to a human non-mammalian model* for exploring the safety/toxicity of ENP and uncovering fundamental molecular and regulatory mechanisms that control the functioning and reproduction of living organisms. This review will discuss in detail the literature related to the state-of-the-art methods and tools developed/being developed to contribute to highlighting the major strength of sea urchins to serve as a model in nanotoxicological/ecotoxicological studies with a focus on stress and innate immune signaling.

2. The sea urchin as a model organism in biology

The sea urchin (class Echinoidea, phylum Echinodermata) is a small and spiny animal, living in all oceans across the world, generally in the shallows and tide pools of ocean environments. It is a pivotal

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component of sub-tidal marine ecology and an important fishery resource in several areas of the world. This echinoderm is a very successful marine invertebrate, with two life stages: (i) an early and brief developmental stage (planktonic) and (ii) a remarkably long-lived adult stage (epi-benthonic) (i.e. *Strongylocentrotid* species can live to over a century) producing millions of gametes each year. Part of the sea urchin's value as a model organism is due to advantageous technical and biological features such as (i) ease and responsiveness to experimental manipulation; (ii) a good understanding of gene functions and developmental Gene Regulatory Networks (GRN); (iii) a close genetic relationship to humans; (iv) a discrete sensitivity to low concentrations of contaminants and a discriminatory capability.

2.1. Impact on developmental biology field

The sea urchin has a long and uninterrupted history as a model organism in developmental biology research, contributing to understanding both the important aspects of development and differentiation, and the origins of cellular and molecular biology (Ernst, 2011; Martik et al., 2016; McClay, 2016; Peter, 2017). Most recently, sea urchins were employed as a model organism in the field of toxicology and environmental toxicology due to their sensitivity towards various pollutants, especially during their embryonic life stages (Morroni et al., 2016). Sea urchin development is fairly straightforward, quick and easy to observe and manipulate. In fact, since the first half of the 19th century Derbes, Dufosse and von Baër had taken advantage of the transparency of sea urchin eggs to study fertilization, reproduction and embryonic development (Briggs and Wessel, 2006). In the 1980s, the sea urchin embryo became the focus of cis-regulatory analyses of spatial-temporal embryonic gene expression, and an unlimited expansion of molecular investigations of the signaling interactions, the signaling cascades, and the developmental GRN have taken place (Peter and Davidson, 2011). Models of developmental GRNs were based on the idea that complex patterns self-organize naturally when “simple” patterns lose their stability and were generated by functional experimental perturbations of the normal developmental program (Su et al., 2009; Poustka et al., 2007; Pinsino et al., 2011). Currently, the sea urchin embryo endomesoderm GRN is the most nearly completed, validated and useful, among the GRNs available from other organisms (Peter et al., 2012). Due to the fact that GRNs have the potential of providing a causal understanding of how upstream specification controls downstream events, there is a high interest in extending this type of genomic network analysis to other developmental and cellular models combining both experimental and theoretical approaches.

2.2. Promising impact in the field of immunology

Despite immune cell behavior and the recognition of self/non-self that was first established in echinoderms in the 19th century (Hirano, 2016) no attempts in the description of an immune GRN have been made so far. A very promising perspective to the genomic approach in studies of the sea urchin immune response was opened ten years ago, by the full sequence release of the *Strongylocentrotus purpuratus* sea urchin genome (Purple Sea Urchin) that revealed the close genetic relationship between sea urchins and humans (Sea Urchin Genome Sequencing Consortium, 2006). A similar effort has been made by a core group of European laboratories forming a consortium for the sequencing, assembly and annotation of the *P. lividus* genome (Genoscope project running). Phylogenetically, the sea urchin *P. lividus* is a sister species to *S. purpuratus*, sharing a high percentage of homology (80–90%) of their proteins. About 23,000 annotated *S. purpuratus* genes comprise many previously thought to be vertebrate innovations, including for example sensory and genetic diseases genes. The sea urchin occupies a strategic phylogenetic position since it represents an "evolutionary link" between invertebrates and vertebrates. Thus, following the publication of the sea urchin genome, a new prospect seems to be opened in biological research: the use of the sea urchin immune cells as a tool to uncover underlying molecular and regulatory mechanisms of immune response. Aside from its phylogenetic position, the morphology and the life history of the sea urchin are different in comparison to the traditional models used in immunity studies (e.g. flies, worms, rodents). As already mentioned, the sea urchin is a long-lived marine invertebrate with a biphasic ontogeny exhibiting resistance to diseases, an extensive innate immune system as well as other set of genes involved in defense, last features shared with other marine invertebrates such as bivalve mollusks (Zhang et al., 2012; Murgarella et al., 2016). The largest expansion is noticed in the several classes of host sensory genes, collectively named *pattern recognition molecules* that provide a remarkable adaptive capacity to the environmental changes both in the adult and in the embryonic life of the sea urchin (Rast et al., 2006; Smith, 2010). Besides, sea urchins grow indeterminately and do not lose metabolic functions or fertility over time, and, to the best of our knowledge, only rarely they suffer from cancer, immune diseases or age-related diseases (Bodnar, 2009). Bearing in mind its resistance and plasticity, the sea urchin appears as a promising tool to assess the immunotoxicity of chemical compounds (Pinsino and Matranga, 2015) as well as to study resistance to immune and age-related diseases, respecting the 3Rs criteria (reduction, refinement, and replacement of animal experiments) of EU Agency for Alternative Approaches for Animal Testing (EPAA). The discovery of sea urchin key protective molecules as well as of the other promising marine invertebrates could be very beneficial to medical advances and could have large implications for the improvement of human health.

3. Sea urchin as an emerging nanosafety/nanotoxicity probing model

In recent years, due to rising of awareness for ENP use, the first articles covering this research topic tried to emphasize the impact of ENP on different aspects of embryonic and adult tissues of different sea urchin species (Fig. 1). Most of the experiments were focused on the ENP impact on the embryonic development of the sea urchin, performing biological analyses, approached by using traditional toxicological testing: dose- and time-dependent assessment of morphological changes and viability, analysis of the impact on embryonic behavior and analysis of the trace amount of ENP accumulated in tissues. Sea urchin embryos exposed to metal -oxide, metal, carbon-based and polystyrene ENP, showed countless malformations in shape and size depending on the type, experimental condition and ENP concentration and accumulation in embryonic tissues (Fairbairn et al.,

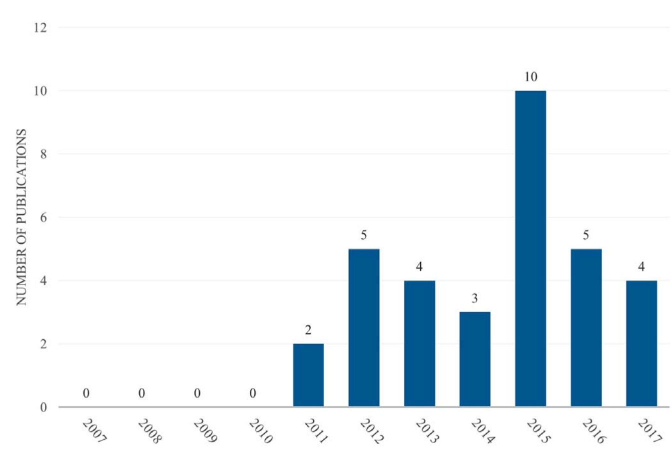

Fig. 1. An Overview of the main available publications focusing on the impact of ENP on the sea urchin, from 2007 to present. Querying PubMed by using the search criteria ‘nanoparticles’ and ‘sea urchin’ reveals that 33 reports have been published on this topic in the last ten years of which 29 come from Europe, and the number has grown only during the previous five years.