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New insights into shear-sensitivity in dinoflagellate microalgae

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HIGHLIGHTS

- Resistance of cells to acute hydrodynamic forces is evaluated in a flow chamber.
- Local EDR is simulated with computational fluid dynamic (CFD).
- Microalgae can be shear-sensitive but not fragile.
- Mechanosensing processes may be initiated with single short and low EDRs.
- Microalgae may use membrane fluidity as primary trigger of mechanosensing.

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ABSTRACT

A modification of a flow contraction device was used to subject shear-sensitive microalgae to well-defined hydrodynamic forces. The aim of the study was to elucidate if the inhibition of shear-induced growth commonly observed in dinoflagellate microalgae is in effect due to cell fragility that results in cell breakage even at low levels of turbulence. The microalgae assayed did not show any cell breakage even at energy dissipation rates (EDR) around 10^{12} W m^{-3}, implausible in culture devices. Conversely, animal cells, tested for comparison purposes, showed high physical cell damage at average EDR levels of 10^{7} W m^{-3}. Besides, very short exposures to high levels of EDR promoted variations in the membrane fluidity of the microalgae assayed, which might trigger mechanosensory cellular mechanisms. Average EDR values of only about $4{\cdot}10^{5}$ W m^{-3} increased cell membrane fluidity in microalgae whereas, in animal cells, they did not.

1. Introduction

Marine dinoflagellates are fragile microalgae, frequently associated with the production of bioactives that have biotechnological significance (Gallardo-Rodríguez et al., 2012b; García Camacho et al., 2007) and recently considered as potential candidates for biofuel production (Chen et al., 2012). However, as is the case with animal cells, the bioreactor culturing of marine dinoflagellate microalgae continues to be a bioprocess whose development is significantly limited by shear-sensitivity (Gallardo-Rodríguez et al., 2012b), unlike other robust microalgae, which easily grow in large-scale photobioreactors.

Work showing progress in understanding shear sensitivity in microalgae has recently been published (Gallardo-Rodríguez et al., 2012a; López-Rosales et al., 2015; Michels et al., 2010; Rodríguez et al., 2009) but the reasons have still not been definitively established. The shear sensitivity of macro- and microalgae is strongly species-dependent (Rorrer and Cheney, 2004; Sobczuk et al., 2006). Among microalgae, dinoflagellates have been shown to be the most sensitive, even though marked differences between species still exists (Gallardo-Rodríguez et al., 2012b). The damage threshold for the energy dissipation rate (EDR) has been reported to range from less than 1 cm^2/s^3 ($\sim$0.1 W/m^3) for marine species (Garcia-Camacho et al., 2014) to almost 10^6 cm^2/s^3 ($\sim 10^5$ W/m^3) for freshwater nontoxic species (Hu et al., 2007).

Adverse responses to flow in dinoflagellates are wide-ranging, and comprehensively reviewed elsewhere (Gallardo-Rodríguez et al., 2012b). One of the most interesting cellular responses observed is the membrane fluidity increase as a function of the EDR experienced by the cells. Membrane fluidization was observed under lethal agitation regime in a time-scale of minutes. This effect was totally reversible in a time-scale of hours once the agitation was stopped (Gallardo-Rodríguez et al., 2012a). As a result,

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bioprocesses involving fragile microalgae require a better understanding of the effect of acute hydrodynamic events present in photobioreactors.

It is well known that in typical bioreactors, such as stirred tanks or bubble columns, cells are exposed to an inhomogeneous EDR field. Local EDR values could be up to two orders of magnitude higher in the impeller regions or at the liquid surface where bubbles break (Ståhl Wernersson and Trägårdh, 1999; Zhou and Kresta, 1996a, 1996b). Such high EDR values have been estimated to contribute around 70% of the total power introduced into a typical bioreactor. Extensional flow has been demonstrated to be more damaging to animal cells than shear flow at the same energy dissipation rate (EDR) (Garcia-Briones and Chalmers, 1994; Hu et al., 2011). Most laboratory studies on cell damage from hydrodynamic shear stress have been carried out in Couette flows or viscometers/rheometers (García Camacho et al., 2007; Michels et al., 2010). It has been claimed that more accurate representations of the complex hydrodynamic forces in a bioreactor have been obtained by pumping cells through capillary tubes. Microfluidic channels have successfully been used to subject animal cells to intense EDR exposure (Clay and Koelling, 1997; Ma et al., 2002; Mollet et al., 2007).

In this work we used a previously reported modification of a microfluidic flow contraction device (Mollet et al., 2007) to evaluate the resistance of the dinoflagellate microalgae *Protoceratium reticulatum* and *Karlodinium veneficum* to acute hydrodynamic forces. Characterizing the fluid flow and corresponding energy dissipation rates in this microfluidic chamber was addressed with computational fluid dynamic (CFD) software. By comparing cell fragility and changes in membrane fluidity between dinoflagellates and animal cells we were able to postulate both differences and similarities in cell damage mechanisms depending on the cell lines.

2. Methods

2.1. Cells and culture conditions

The microorganisms used in this study were microalgae and animal cells in exponential growth phase. The microalgae were *P. reticulatum* (strains GG1AM and CCMP3241) and *K. veneficum* (strain K10), belonging to the Division Dynophyta, Class Dynophyceae. The thecate dinoflagellate *P. reticulatum* is a producer of Yessotoxins and the athecate *K. veneficum* produces Karlotoxins. Strains GG1AM and K10 were obtained from the Culture Collection of Harmful Microalgae of IEO, Vigo, Spain. Strain CCMP3241 was obtained from the NCMA culture collection of the Bigelow Laboratory for Ocean Sciences, Maine, USA. All of them were grown in L1 medium (Guillard and Hargraves, 1993) in static Erlenmeyer flasks at 100 μE m^{-2} s^{-1} under a 12:12 h light–dark cycle at a temperature of 18 °C.

Regarding animal cells, mouse T-lymphocytes (line EL-4) were grown in RPMI-10% FBS medium (Sigma–Aldrich, Ref. R8758), in 25 cm^2 T-flasks at 37 °C in 5% atmosphere. Insect cells of *Spodoptera exigua* (line SE-UCR) were grown in TNM-FH-10% FBS medium (Sigma-Aldrich, Ref. T3285) in 25 cm^2 T-flasks placed in an incubator at 27 °C.

2.2. Contractional flow device

A well-defined hydrodynamic stress on the cells was applied in a modification of a previously-reported flow contraction device (Mollet et al., 2007). This device is commonly referred to in the literature as the «torture chamber» (TC) (Godoy-Silva et al., 2009; Ma et al., 2002; Mollet et al., 2007). A scheme of the TC and characteristic dimensions are displayed in Fig. 1. Details of the TC throat and channel are shown in Table 1.

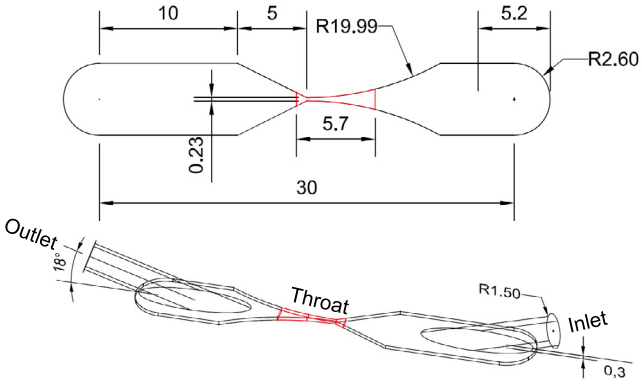

Fig. 1. A top-view scheme (a) and a perspective design of the flow contraction device (b). Dimensions are given in mm. The area traced in red corresponds to the throat-influenced region, where the mesh was finer for the numerical simulations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Physical properties and geometric characteristic used for the simulations in the torture camera.

Property or characteristic	Symbol	Value
Thickness of the stainless steel sheet (channel width)	w	304 μm = 3.04×10^{-4} m
Throat		
Throat spacing	b	222 μm = 2.22×10^{-4} m
Aspect ratio	w/b	0.73
Hydraulic diameter	D_h	257 μm = 2.57×10^{-4} m
Fluid		
Chemical identity		Water
Working temperature	θ	20 °C for dinoflagellates 27 °C or 37 °C for animal cells
Density (at θ)	ρ	1030 kg/m^3 for dinoflagellates 1010.5 kg/m^3 for animal cells
Viscosity (at θ)	μ	1.3×10^{-3} Pa s for dinoflagellates 1.26×10^{-3} Pa s for animal cells

2.3. Experiments in the contractional flow device

Cells from late exponential growth phase cultures were used in all the experiments. The final cell concentration used was $\sim 2 \times 10^5$ cells/ml (microalgae and animal cells). Two aliquots were withdrawn from each cell suspension sample. One aliquot was retained as a control to quantify any background level of released lactate dehydrogenase (LDH) in the supernatant. The second aliquot was ultrasonicated to completely disrupt the cells for a total LDH measurement. The remaining suspension (approx. 25 mL) was pumped in an open loop through the TC using a peristaltic pump (Gilson, Minipuls 3 R1-R2). Therefore, cells experienced a single passage through the TC in each assay. The volumetric flow rate of each experiment was fixed varying the pump rotor speed and the tube diameter (ranging from 3.18 to 8 mm) accordingly. The last 2 ml of effluent from the TC were collected for cell damage analysis. Previous assays with no TC had allowed us to rule out any significant effect on cells from any of the piping system that circulated the culture sample through the TC. Experiments were carried out in triplicate.