Scientific contribution

Short Term Effect of Plant Hybridosomes on Growth of *Phaeodactylum Tricornutum* Culture

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Abstract:
Microalgae are in focus of extensive study due to their abundance and important role in equilibration of the global ecosystem. Living organisms communicate through nano-sized membrane-enclosed particles which are continuously shed by cells but can also be fabricated artificially. In this work we examined the effect of hybridosomes composed from soyabean lecithin, aqueous solution containing substances from spruce needles and glycerol and hybridosomes composed from soyabean lecithin, ultraclean water and hemp oil on the number density of microalgae *Phaeodactylum tricornutum* in culture. We measured the number density of microalgae by flow cytometry and the number density and hydrodynamic diameter of small particles in the samples by interferometric light microscopy. We observed considerable increase of the number density of microalgae with respect to control (untreated) samples after three days, which was connected to the amount of the material added. Addition of membrane-enclosed particles had a favourable effect on the microalgae growth. Microalgae proved a convenient system for in vitro studies of the effects of substances.

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1. Introduction

1.1. Small Cellular Particles (SCPs) and hybridosomes

Cells shed in their exterior SCPs (such as extracellular vesicles (EVs), lipoproteins, antibody complexes, viruses) that can move more or less freely in the surrounding medium and transport a variety of cargo to adjacent or remote cells (Herman et al., 2021, Lenzini et al., 2020). Extensive studies and empirical knowledge indicates that these tiny particles may have a great impact on living systems and mediate interactions between different life domains and kingdoms. Recent reports on the role of SCP-mediated horizontal transfer of bioactive proteins, lipids and nucleic acids (Fischer et al., 2016) in gene regulation, local phenotypic adaptation and immune evasion render SCPs as ideal candidates to serve as biomarkers, nano-sized drug-delivery vehicles, and mediators for a variety of therapeutics in oncology, immunotherapy and regenerative medicine (Yates et al., 2022, Armingol et al., 2021, Jin et al., 2021, Combarnous et al., 2020, Fais et al., 2016). Furthermore, this so-called theranostic "all-in-one approach" has potential in the field of personalised medicine, as it enables the detection and monitoring of a disease in individual patients, possibly in early clinical stages, as well as in targeted drug delivery to the required.

Recent research indicates that SCPs may play a role in the larger communication systems of multicellular organisms within endocrine, paracrine and angiocrine systems (Raposo et al., 2021), suggesting that they may be powerful and versatile tools for vaccination strategies due to their immunogenic properties, natural adjuvanticity, uptake by mammalian cells, and potential for genetic engineering (Akuma et al., 2019, van der Pol et al., 2015). Interaction between virions and SCPs have been suggested to regulate virion production, and their secretion (Mammadova et al., 2021, Badierah et al., 2021), and SCPs were found to transport different types of RNA (messenger RNAs, microRNAs, ribosomal RNAs, transfer RNAs, small RNAs and long non-coding RNAs) (Turchinovich et al., 2019; Shao et al., 2018) and DNA (Elzanowska et al., 2021). It was found that SCPs transfer short RNAs from plant to pathogen cells and trigger host-induced gene silencing, a mechanism that allows the regulation of gene expression of the invading pathogen or parasite (Cai et al., 2018). Increasing evidence suggests that beneficial abilities of mesenchymal stem cells can be attributed to their paracrine secretion of SCPs and that administration of small particles that contain a mixture of proteins, lipids, and nucleic acids, resembling the secretome of mesenchymal stem cells, can mimic most of the effects of the parental cells (Herman et al., 2021). Furthermore, SCPs can be efficiently delivered to the respiratory and neural systems through inhalation thereby offering the advantage of non-invasive and repeated administration (Frolich, 2021).

1.2. Nanoalgosomes – SCPs from natural sources

We have recently considered microalgae as a promising source of deliverosomes. Microalgae constitute a rich reservoir of bioactive metabolites such as pigments, polyunsaturated fatty acids, antioxidants or antimicrobial compounds, which are being increasingly exploited in commercial ventures (Cuellar-Bermudez et al., 2015). Many microalgae species are suitable for growth in industrial scale photobioreactors and are seen as highly productive crops when compared with terrestrial plants (Khan et al., 2018). As microalgae cells have high growth rates, they can be cultured on non-arable land under controlled environmental conditions (Koller et al., 2012), so that large scale production of SCPs with algal technologies seems feasible (Paganini et al., 2019). Their natural and sustainable origin grants them a likely greater societal acceptance (with reduced sensitive ethical questions). SCPs called nanoalgosomes were isolated from cultured media of microalgae Tetraselmis chuii and characterized (Adamo et al., 2021). Nanoalgosomes did not show significant toxicity on the tumorigenic MDA-MB-231 breast cancer, non

tumorigenic 1–7 HB2 and Hep G2 cell lines over time and at different concentrations in a specific dose- and time-dependent manner (Adamo et al., 2021). The genotoxicity assay showed no DNA damage or apoptotic events and cultured cells were able to uptake nanoalgosomes (Adamo et al., 2021). However, the yield of the nanolagosomes in Tetraselmis chuii and in particular, their protein content was rather low which indicates that harvesting of the nanoalgosomes should be boosted to reach a scale production required for therapeutic use.

1.3. SCPs from plants

The hemp plant contains an enormous variety of chemicals (ElSohly and Slade, 2005). Recent studies report more than 1200 different compounds, out of which there are about 140 terpens, about 50 flavonoids and more than different cannabinoids/ phytocannabinoids (Andre et al., 2016). The term cannabinoids represents a group of C21 or C22 terpenophenolic compounds found in Cannabis sativa L (Mechoulam and Gaoni, 1967). Hemp plants contain levels of psychoactive molecules under regulatory levels so the products are considered safe to use as food, food supplements, or cosmetics. Cannabinoids are highly hydrophobic lipids, almost insoluble in water (Guzman, 2003) but show good solubility in different organic solvents, such as methanol and ethanol (Smith and Vaughan, 1977). Of these, cannabidiol (CBD) in low doses exhibited anti-oxidant and neuroprotectant (Hayakawa et al., 2010; Hampson et al., 1998,), anti-inflammatory (Burstein, 2015), anti-emetic (Parker et al., 2011) and antipsychotic (Zuardi et al., 2012) effects.

Spruce bark is known to be a rich source of terpenes, but also of polyphenols, resin acids, flavonoids, stilbenes and stilbene glucosides, lignin, holocellulose, β-sitosterol and methyl dehydroabietate, with some of these substances having antibacterial and antioxidant properties (Jeran et al., 2022). Polyphenols are compounds with one or more hydroxyl groups attached to the benzene ring, which give them the ability to capture free radicals, moreover it gives them a stronger acidic character in comparison to other alcohol groups and therefore antioxidant properties (Nisca et al., 2021). Needles contain mono- and sesquiterpenes, fatty acids, phenolic compounds, stilbene glucosides, waxes and carbohydrates as well as long-chain alcohols, e. g. nonacosan-10-ol, which has superhydrophobic properties (Bukhanko et al., 2020). Jokinen and Sipponen (2016) reported that a complex mixture of spruce resin acids lignans showed antimicrobial, wound healing, and skin regeneration effects.

1.4. Liposomes

Above the critical micelle concentration, lipid molecules aggregate and form larger structures such as micelles, inverted micelles, or bilayers (Lombardo, 2015). The propensity for the membrane depends on the shape of the constituents (Israelachvili, 2015; Iglič et al., 2015). The technique for bilayer vesicle production can influence the final properties of hbridosomes, such as size, lamellarity, and encapsulation efficiency (Pattni et al., 2015). The most common methods to prepare lipid vesicles are thin film hydration, reverse phase evaporation and solvent injection (Karn et al., 2013; Meure et al., 2008), and electroformation (Drab et al., 2021). In order to be up-taken by cells, deliverosomes should be small (i.e. submicron-sized). Larger vesicles therefore require additional processing to reduce their size, such as sonication, extrusion, microfluidization, high-pressure homogenization, or shear force-induced homogenization (Guimaraes et al., 2021; Wagner and Vorauer-Uhl, 2011). In extrusion, the vesicles pass several times (extrusion cycles) through a membrane of defined pore size, to render the size distribution more uniform (Meure et al., 2008; Olson et al., 1979).

1.5. Hybridosomes

Formation of hybridosomes by processing material from natural sources and lipids is expected to capture the substances of the natural sources with health-beneficial effects, increase the yield and render material with better controlled properties.

In this work we report on preparation of three types of small particles: liposomes composed of lecithin, water and glycerol, and two types of hybridosomes where natural compounds from hemp and spruce were added. We characterized these particles with respect to number density and size. To assess their effect *in vivo* we added these particles to the culture of microalgae *Phaeodactylum tricornutum* and followed their number density and the number density of small particles in the conditioned media for 6 days.

2. Methods

2.1. Cultivation of microalgae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A was from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scot-land). The culture was grown in mineral water Radenska Naturelle (Ca 59, Mg 20, Na 6.9, K 0.7, HCO3- 280, Cl 5.0 SO_4 1.1, F<0.2 in borosilicate glass bottle. Edible salt (Droga, Portorož, Slovenia) was added to the mineral water in proprortion 22 g of salt per one litre of mineral water. Salt was previously sterilized by heating over 80 °C in the microwave oven. Mineral water with added salt was supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Al-drich, USA) in proportion 20 mL of F/2 per one litre of mineral water with added salt. Culture was grown in a room at 18 °C with natural light. The aliquots for the experiment were taken at Day 18 after inoculation of microalgae into the bottle.

2.2. Design of the experiment with microalgae

Microalgae culture were aliquoted into Petri dishes, in triplicates for each added compound (hybridosomes from spruce needles and hybridosomes from hemp oil) and for controls (liposomes from soya lecithin and untreated samples). Each type of samples was kept in a separate box. The number density of microalgae, the number density of SPs in the culture and the number denisty of hybridosomes/liposomes were measured before the addition of the compounds to the microalgae. 2 mL of culture was put in each Petri dish. At Day 0 we added 20 µL of dissolved hybridosomes or liposomes to the samples. The boxes were placed in a room at 18 °C and exposed to natural light. At days 1,2,3 and 6 we gently mixed the samples by circular motion of the boxes and took 200 µL of the conditioned media for measurements by FCM and ILM. We have replenished the volume of the samples by ultraclean water with added salt and f/2.

2.3. Preparation of spruce needle homogenate

Branches were cut from the *Picea abies* tree and used immediately. Branches were immersed into 1.5 L of water at 30 °C with 10 mL of sodium hypochlorite (NaClO, 0.1 %) for 1 hour. The branches were rinsed with water. The needles were cut off from the branches. 50.0 g of wet needles were immersed in 300 mL of ultraclean water and stirred for 1 minute in KOIOS 850W Smoothie Bullet Blender (KOIOS, Neweg, USA). The homogenate was filtered through 0.5 mm nylon net cloth to remove larger particles.

2.4. Isolation of SCPs from spruce needle homogenate

SCPs were isolated by differential centrifugation, using a protocol widely used for the isolation of small extracellular vesicles (Mantille et al., 2022). Briefly, the cells and larger particles were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge

tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA). The pellet was resuspended in 50 µL of ultraclean water.

2.5. Preparation of hybridosomes

Hbridosomes were prepared by mixing appropriate proportions of liophylized soya lecithin granules with ultraclean water/supernatant of isolation of SCPs from spruce needle homogenate and oily glycerol/hemp oil, at room temperature. Three samples were prepared. Sample A contained 33 weight % of soya granules, 33 % of ultraclean water and 33% of glycerol; sample B contained 33 weight % of soya granules, 33% of supernatant of centrifugation of homogenate from spruce needles at 300g I and 33% of glycerol, and sample C contained 33% of soya granules, 33% ultraclean water and 33% of hemp oil. Soyabean lecithin granules were placed into the falcon tubes. Water or supernatant was added and the suspension was left at room temperature for 1 hour. Glycerol or hemp oil was added and the samples were mixed mechanically (manually) with metallic stick until the ingredients formed a uniform cream-line consistence. The samples were kept at room temperature.

2.6. Flow Cytometry (FCM)

The microalgae number densities were measured by flow cytometry by a MACSQuant Analyzer flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany) and the related software. The following instrument settings were employed: FSC: 458 V; SSC: 467 V with a trigger set to 1.48, B3: 300 V; R1: 360 V. Particles were detected from the forward (FSC) and side scatter parameter (SSC). Samples were mixed by pipetting before measurement and 20,000 events per well were acquired. We have also measured number densities of hybridosomes for which the samples were diluted 1000 × with ultraclean water.

2.7. Interferometric Light Microscopy (ILM)

The average hydrodynamic diameter (Dh) and the number density of small particles in the conditioned media and in hybridosome/liposome preparations were determined by interferometric light microscopy using Videodrop (Myriade, Paris, France). The conditioned media were measured undiluted while the hybridosome/liposome preparations were diluted 1000 ×. Signals of the media (salted mineral water supplemented by F/2) were under the detection limit. The threshold value 4.2 was used. 7 µL of sample was placed between cover glasses and illuminated by 2W blue LED light. The light scattered on the particle was imaged by a bright-field microscope objective and allowed to interfere with the incoming light. The image was recorded by a complementary metal–oxide–semiconductor high resolution high speed camera. The obtained pattern that includes contrasting black and white spots was recognized as a particle and its position in the sample was assessed. Number density of the particles is the number of the detected particles within the detected volume (e.g. 15 pL). Dh was determined by tracking the position of the imaged particle within the recorded movie. It was assumed that particles undergo Brownian motion due to collisions with surrounding particles. The diffusion coefficient D of the motion of the particle is taken to be proportional to the mean square displacement d of the particle between two consecutive frames taken in the time interval Δt, $\langle d2(\Delta t)\rangle = \langle 4D\ \Delta t\rangle$ while Dh was estimated by assuming that the particles were spherical and using the Stokes-Einstein relation $Dh = kT/3\pi\eta D$. Each particle that was included in the analysis was tracked and processed indi-

vidually and the respective incident light signal was sub-tracted from each image. Processing of the images and of the movies was performed by using the associated software QVIR 2.6.0 (Myriade, Paris, France).

2.8. Statistical analysis

All measurements were performed in triplicates and presented by the average values and standard deviations. Correlations between variables were assessed by the Pearson correlation coefficient and the respective probability. The value $p = 0.05$ was taken as a threshold for statistical significance.

3. Results

Figures 1A shows microalgae *Phaeodactylum tricornutum*. The cells are in fusiform shape. **Figures 1B** shows their SCPs in conditioned media. Numerous SCPs are relatively homogeneous in shape and size (smaller than 100 nm in diameter). The mechanism of formation of these SCPs remains obscure. **Figure 1C** shows shows cryo-TEM image of liposomes composed of soya lecithin, water and glycerol and **Figure 1D** shows cryo-TEM images of hybridosomes composed of soya lecithin, supernatant of 300 g sedimentation of homogenate from spruce needles and glycerol. It can be seen by comparing Figures 1E and 1F that the particles are of similar shapes and sizes.

To observe the effect of hybridosomes on the growth of microalgae, we initially planted 2 mL of culture in each Petri dish and each day we subtracted 200 µL of the culture for measurement by FCM, light microscopy observation and ILM. To maintain the same volume of the sample, 200 µL of mineral water with added salt and f/2 was replenished to each Petri dish. The number densities of hybridosomes and liposomes in the added suspensions are given in Table 1.

Table 1. Number density and hydrodynamic diameter D_h of hybridosomes and liposomes in the suspension that was added to microalgae cultures

Sample	Number density by FCM (/µL)	Number density by ILM (/µL)	D_h (nm)
Hemp hybridosomes	114 ± 21	$(5.47 \pm 10) \times 10^5$	224 ± 13
Spruce hybridosomes	46 ± 5	$(2.01 \pm 11) \times 10^5$	249 ± 10
Liposomes	240 ± 13	$(3.89 \pm 10) \times 10^5$	363 ± 9

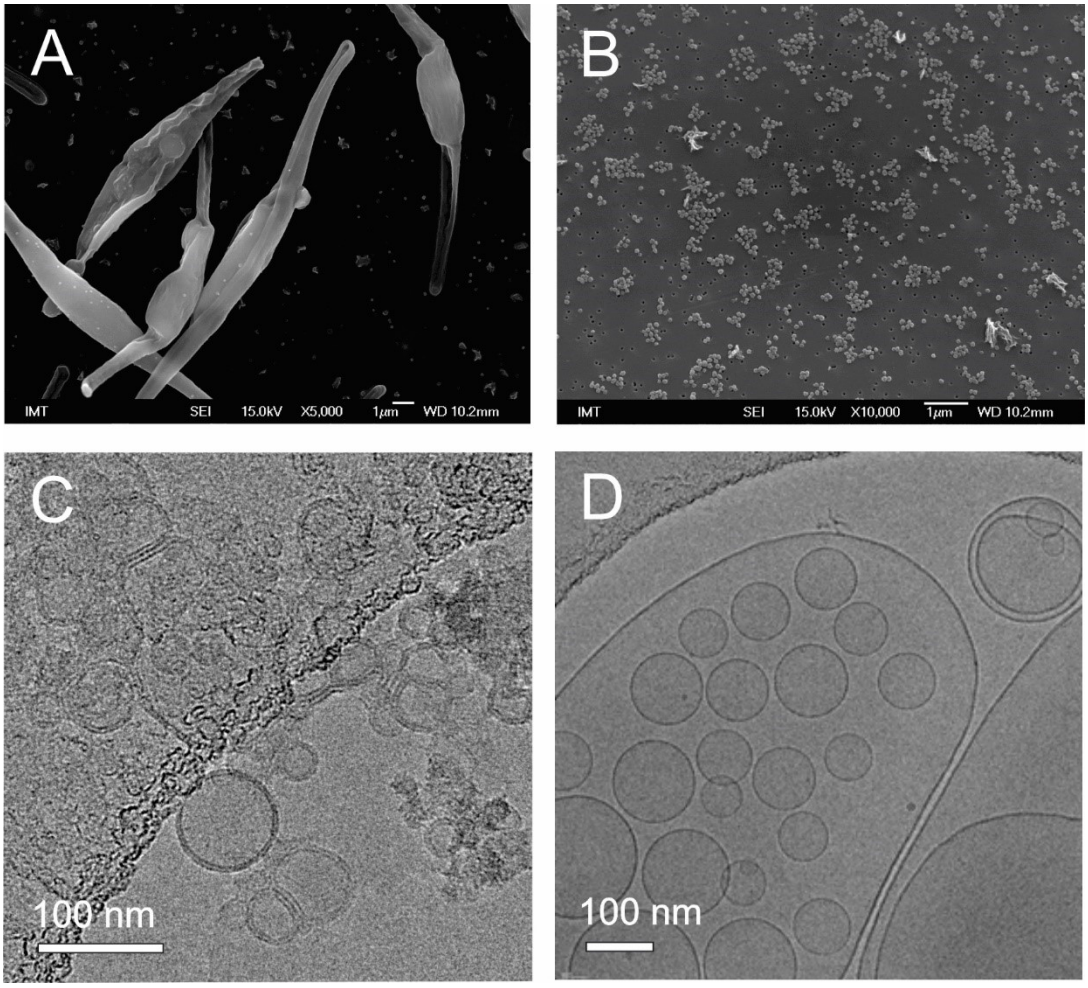

Figure 1. A: SEM images of Microalgae *Phaeodactylum tricornutum*, **B**: SEM image of SCPs in conditioned media of *Phaeodactylum tricornutum*, **C:** Cryo-TEM image of hybridosomes composed of lecithin, supernatant after 300 g centrifugation of spruce needles homogenate and glycerol, **D**: Cryo-TEM image of liposomes composed of soya lecithin, water and glycerol. A,B: From C: From Jeran et al., (2023). D: From Romolo et al., (2022).

Addition of liposomes to the culture of *Phaeodactylum tricornutum* caused a short-term increase of the number density of microalgae (Figure 2A) as it can be seen in **Figure 2A** that the number density of microalgae has increased considerably with respect to the baseline the first two days after the addition of hybridosomes to the culture. Hybridosomes of bolth types also showed increase after two days while the number density remained more or less constant in the control samples (**Figure 2A**). In the following days, the number densties of the microalgae in all the samples were unified. A possible reason for this is the presence of small microorganisms in the samples which were visible under the light microscope. A fibrous network with dilatations was noted, however, these organisms did not swim. Number density of small particles was considerably higher in the samples with added liposomes and hybridosomes than in the control samples (**Figure 2B**). The number densities of SPs remained constant during the experiment (green curve in **Figure 2B**).

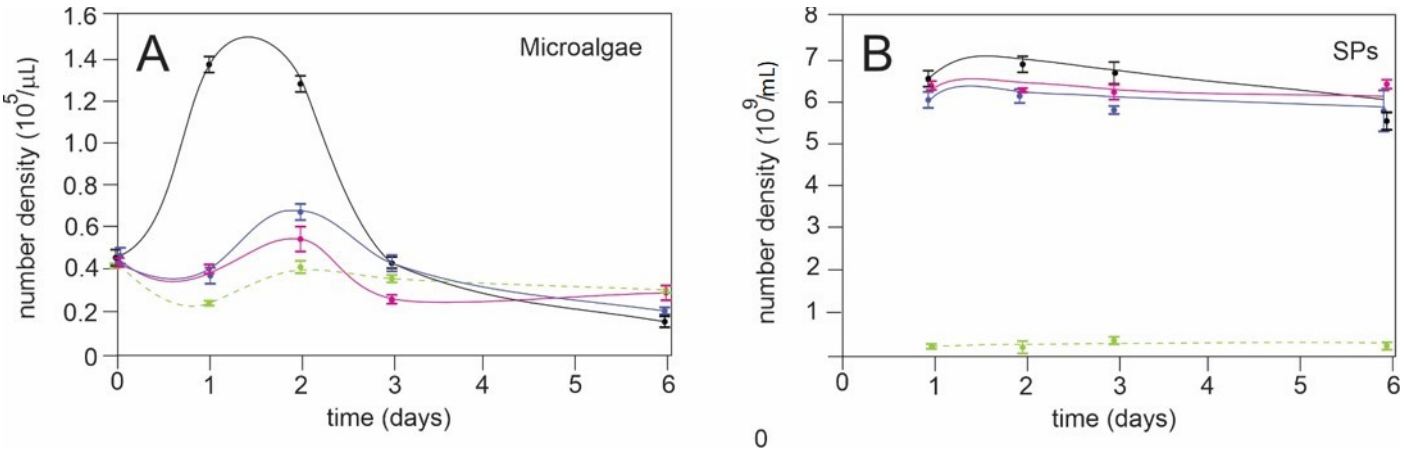

Figure 2. Number density of microalgae (A) and small particles (SPs) (B) in dependence on time. Magenta: samples with added hemp hybridosomes, blue: sample with added spruce hybridosomes, black: samples with added liposomes, green: control samples.

Average hydrodynamic diameters of small particles in the samples were different (**Figure 3**). In the amples with more numerous particles the particles had larger D_h. The Pearson coefficient of the correlation taking into account 20 samples was 0.62 with probability 0.0046.

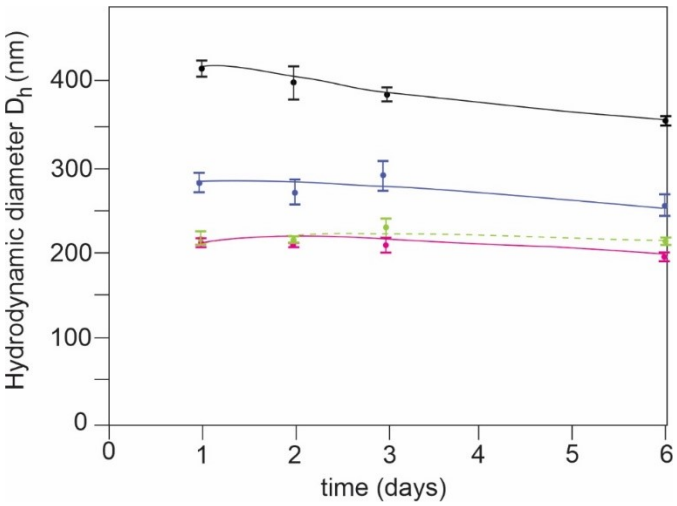

Figure 3. Hydrodynamic diameter of small particles (SPs) in the samples of microalgae with added hybridosomes. Magenta: samples with added hemp hybridosomes, blue: sample with added spruce hybridosomes, black: samples with added liposomes, green: control samples.

4. Discussion

We have observed the effect of added liposomes and hybidosomes on the growth of *Phaeodactylum tricornutum* in culture. We found that the addition of hybridosomes promoted the growth of microalgae in the first two days. The results indicate that the effect was larger if the initial number density of the added particles was larger (**Figure 2**). Microalgae were found a convenient culture to study the in-vivo effects as the test samples showed considerable difference with respect to the control (untreated) samples in the interval of one day. The microalgae growth was hindered after two days day as they and the accompanying microorganisms have became overcrowded in the Petri dishes. In the next experiments, larger dishes should be provided to allow for the expansion of all organisms in the samples

and therefore longer observation time. Microorganisms are naturally present in the microalgae samples and it is indicated that the optimal way of coexistence should be sought. Better characterization of the material added is also necessary. Liposomes and hybridosomes are kept at room temperature in the state of a creamy substance. This substance is not readily dissolved in aqueous media and therefore the dosage of the material is subject to large errors.

Due to their small size and heterogeneous composition of samples, and the transient identity of some types of SCPs, their harvesting and assessment remains a significant challenge. Presently, integration of different methods is recommended (Thery et al., 2018), however, new technically advanced solutions are urgently needed. The most commonly used method for EV harvesting involves differential centrifugation (Kirbas et al., 2019; Thery et al., 2006), which can be followed by gradient ultracentrifugation for example on continuous or discrete sucrose or iodixanol gradient (Iwai et al., 2016). As this technique is time consuming and is of limited capacity, alternative techniques have also been proposed. Ultrafiltration, field-flow fractionation, dialysis, size exclusion chromatography (SEC), microchip-based techniques and precipitation-based methods, alone or in combination with ultra-centrifugation-based methods. Immunoaffinity-based isolations are used to harvest SCPs with particular surface protein compositions (Beekman et al., 2019). Recently, a number of commercial kits have been made available. Ion exchange chromatography (Kosanović et al., 2018) is intended to the fast and cost effective SCP capturing from large volumes of diluted suspensions. However, different isolation methods were found to lead to different EV preparations (Freitas et al., 2019, Tian et al., 2019, Skotland et al., 2017) reflecting the fact that the techniques applied are invasive enough to transform them to such an extent that identification of their original nature is obscured. As they are very small, SCPs are hidden within the organisms or cell assemblies. They are observed in isolates and not in their natural environment. Commonly used SCP imaging methods are scanning and transmission electron microscopy (SEM and TEM, respectively), cryo TEM and atomic force microscopy (AFM) which require invasive preparation procedures. Physico-chemical techniques widely used in EV research that measure the properties, including morphology and particle size distribution are flow cytometry, light scattering, fluorescence microscopy with analysis of Brownian motion (nanoparticle tracking analysis), assesment of zeta potential and tunable resistive pulse sensing. EVs contain genetic material (different types of RNA, in particular microRNA, and DNA), proteins, lipids and other small molecules (Hartjes et al., 2019) that are analyzed by HPLC-MS/MS-based shotgun workflows that typically lead to the identification from several hundred to several thousands of units with interpretation depending on the data banks. Proteins in SCP samples separated by gel electrophoresis are assessed by Western Blot or specific immunosorbent assays. Different harvesting methods yield SCPs and SCP sub-fractions of variable homogeneity and purity that is often inadequate for downstream structural and functional analysis, resulting in SCP databases of poor data quality. Different extraction methods are applied to prepare samples for different high resolution molecular profiling studies of specific molecular classes (omics) (Pocsfalvi et al., 2016a, 2016b) with troublesome integration of the data. Orthogonal single-particle platforms with diverging results cannot be valued one above the other as there is presently no reference method outlined (Arab et al., 2021). The existing methods to harvest, identify and characterise SCPs urgently need improvement to achieve analytical level and adequate repeatability which calls for new approaches to SCP structural and functional assessment and interpretation of the data. Interference light microscopy is a recently developed methods and there are not many reports published on its use for assessment of SCPs. To our best knowledge, it has hitherto been applied to marine and river microorganisms (Boccara et al., 2016, Roose-Amsaleg, 2017), viruses (Turkki et al., 2021) and extracellular vesicles isolated from blood plasma (Sabbagh et al., 2021). Recently, the methods was applied to different types of SPs (extracellular vesicles isolated from suspension above in vitro aged washed erythrocytes, extracellular vesicles isolated from

plasma, small cellular particles isolated from conditioned culture media of *Phaeodactylum tricornutum* and of Tetraselmis chuii, small cellular particles isolated from spruce needle homogenate, liposomes made from soya lecithin, water and glycerol and hybridosomes made from soya lecithin, supernatant form isolation of SCPs from spruce needle homogenate and glycerol (Romolo et al., 2022). The statistically significant positive correlation between the number density and size of SCPs could be a consequence of the presence of fibrous microorganisms observed in the samples. Namely, the fibrils hinder the movement of the particles and in samples with high number density the motion of the particles deviates from Brownian motion. In samples with higher number densities the displacements recorded by the video are therefore smaller due to hindered motion and hence, the diffusion coefficient also smaller. As the hydrodynamic diameter is calculated from the Stokes-Einstein equation in which D_h is inversely proportional to the diffusion coefficient, D_h can be is overestimated in dense samples. The presence of fibrous microorganisms additionally contributes to this effect. With more studies presented by using the interference light microscopy, these relations will expectedly become clearer, however, it is indicated that positive correlation between the size and number density of particles detected by interferometric light microscopy may indicate direct interactions between particles in the samples.

Recently, it was shown that CBD and its metabolite, 7-OH-CBD (but not congeneric cannabinoid) potently blocked SARS-CoV-2 replication in lung epithelial cells (Nguyen et al., 2022). After cellular infection, CBD and 7-OH-CBD inhibited viral gene expression and reversed some effects of SARS-CoV-2 on host gene transcription, induced interferon expression and up-regulated its antiviral signaling pathway (Nguyen et al., 2022). A cohort of human patients previously taking CBD had significantly lower SARS-CoV-2 infection incidence (up to an order of magnitude) relative to matched pairs or the general population, which implicated CBD and 7-OH-CBD as potential preventative agents and therapeutic treatments for SARS-CoV-2 at early stages of infection (Nguyen et al., 2022). Since almost 80 % of the human body consists of water, this correlates with cannabinoid low absorption rate and bioavailability, greatly hindering clinical use (Lipinski, 2002). The fabrication of membrane-enclosed nano-sized cannabinoid products is therefore of significant importance.

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Conflicts of Interest: The authors declare no conflict of interest.

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