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## Application of poly(2-hydroxyethyl methacrylate) hydrogel disks for the immobilization of three different microalgal species

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Ela Eroglu,^{a*}[®] Risa S Wijihastuti,^b Elizabeth Grenik,^a Ashiwin Vadiveloo,^b Navid R Moheimani^b and Xia Lou^{a*}



#### Abstract

BACKGROUND: Algal growth on solid surfaces confers the advantage of combining the algal harvesting and bioprocessing steps at a single stage, in addition to the easier handling of the immobilized cells that occupy a reduced amount of space. The current work employed the application of macroporous poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel disks as a water-insoluble, non-toxic and recyclable immobilization matrix for different microalgal strains (*Nannochloropsis* sp., *Dunaliella salina*, and *Botryococcus braunii*) that offer value-added products for various commercial applications.

RESULTS: The study demonstrated the effect of variations in the surface characteristics of the algal strains and hydrogel surfaces on the immobilization efficiencies. Gelatin was further used to modify PHEMA hydrogels to achieve higher bioaffinity and surface hydrophilicity. The results showed that highly salt-tolerant microalgal cells (*Dunaliella salina, Nannochloropsis* sp.) had significantly higher tendencies to attach on the gelatin-modified PHEMA hydrogel compared with the freshwater *B. braunii* colonies; embedded within an extracellular matrix mainly made of hydrophobic components, which displayed better attachment to the unmodified PHEMA hydrogels.

CONCLUSION: The proposed PHEMA hydrogels are easily-manufactured and highly durable materials with the hydrogel disks still retaining their integrity after several years when in contact with a liquid. PHEMA disks also have the benefits of having adjustable porosities by changing the composition of the polymerization mixture, and modifiable surface properties by simply binding various synthetic or natural molecules on their surfaces, which can bring several new opportunities for harvesting various microalgal cells with different surface morphologies and chemical compositions.

Supporting information may be found in the online version of this article.

Keywords: microalgae; PHEMA hydrogels; immobilization; cellular harvesting; algal biofilm

#### INTRODUCTION

Immobilization of cells has various benefits over their free-cell suspensions such as easier handling, occupying a smaller space, single-stage cellular harvesting, enhancing the efficiency of wastewater treatment, and permitting the retention and recycling of algal biomass for high-value-added bioproduct generation in further stages.¹⁻⁵ Cells can be immobilized using different techniques including their entrapment within three-dimensional gel matrices that can be made of either synthetic or natural polymers, attachment onto the surface of solid supports, and confinement within liquid-liquid emulsions or semi-permeable membranes; the two former methods are the most conventional techniques.^{1-3,6-8} Challenges to find a suitable matrix include surfaces with adequate porosity that allow the diffusion of nutrients to the cells, while permitting the removal of cellular waste or by-products through their environment.^{1,9} Entrapment of microalgal cells within insoluble materials faces some difficulties

on the transfer of light and nutrients, which reflects in lower cell viabilities and slower growth rates compared with their free-cell suspensions.^{3,5,10,11} This is mainly due to the slower diffusion rates of the ions and/or light that need to reach the algal cells after passing through the mostly-spherical entrapment material, which typically has a volume to surface ratio larger than thin films.^{3,9,12} Another important point is to use an insoluble

^{*} Correspondence to: E Eroglu or X Lou, Curtin University, Department of Chemical Engineering, Perth, WA 6845, Australia. E-mail: ela.eroglu@curtin.edu.au (Eroglu); x.lou@curtin.edu.au (Lou)

a Department of Chemical Engineering, Curtin University, Perth, Western Australia, Australia

b Algae R&D Centre, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia

matrix that keeps its integrity without being degraded throughout the process. In the light of these requirements, thin films of macroporous poly(2-hydroxyethyl methacrylate (PHEMA) and gelatin-modified PHEMA hydrogel were investigated as novel immobilization matrices for the biofilm growth of three different species of green microalgae: (i) Botryococcus braunii strain BOT-22, (ii) Nannochloropsis sp. (MUR 267), (iii) Dunaliella salina (MUR 8). These three species of microalgae were chosen as they are currently targeted for mass production for biofuel generation (B. braunii and Nannochloropsis),¹³⁻¹⁵ high value pigments such as carotenoids (D. salina),^{13,16-18} or high value fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Nannochloropsis).^{13,19} Biofilm growth on solid surfaces can potentially reduce the overall cost of mass production by reducing the associated costs of a dewatering stage, while providing a more efficient harvesting step with retention of the high-value-added algal biomass for product generations.^{1,3,4}

PHEMA is made of crosslinked polymers of 2-hydroxyethyl methacrylate (HEMA). Due to its swelling properties within water, it is classified as a type of hydrogel material that can sustain its three-dimensional structure.^{20,21} This highly hydrophilic material has various advantages including its stability and inertness under varying environmental conditions such as temperature and pH; ease of fabrication and alteration of its physical form into any desired shape; and permitting the incorporation of various natural or inorganic molecules into its structure that change its mechanical/chemical assets and its biocompatibility.^{20,22,23} Its high biological tolerance permits various applications including vision improvements in the form of intraocular and contact lenses;^{24,25} tissue engineering;^{23,26} dental implants;^{27,28} breast prosthesis^{29,30} or nasal cartilage replacements³¹ in plastic surgeries; and controlled drug delivery systems.³²⁻³⁴ PHEMA hydrogels were also used as an immobilization matrix for various enzymes and biomolecules for enhancing bioreactor applications.^{35–37} Although the utilization of PHEMA for intraocular and contact lenses mainly involves the use of nonporous and transparent PHEMA hydrogels,^{24,25} there is also a significant interest in the fabrication of macroporous PHEMA hydrogels for other biological applications such as the delivery of drugs at higher drug loading capacities^{34,38} and ability to transfer large biomolecules including growth factors and proteins.³³ One of the cost-effective ways to generate macroporous PHEMA hydrogels is the polymerization of HEMA monomers in the presence of a free radical initiator to activate a HEMA molecule that will continuously attach onto another one under a chain reaction until termination of the HEMA supply; a crosslinking agent that forms an insoluble network by connecting the PHEMA chains together; and a diluent.^{38–41} The concentration of the diluent used during the polymerization process determines the porosity of the generated hydrogel. When water is used as the diluent, optically transparent and nonporous hydrogels are produced if the concentration of water in the monomer mixture is kept below the critical limits (cited variously between 40 and 50 wt%), whereas exceeding those limits would result opaque hydrogels with macroporous morphology as the excess water induces phase separation during the polymerization process.^{20,34,42} One of the main advantages of the solution polymerization processes is to allow alteration of the pore sizes and structures by simply changing the concentration ratios of the components within the mixture; i.e. [HEMA]:[solvent] or [initiator]:[crosslinking agent].³⁹ Applying suitable mixture concentrations at the beginning of the free radical processes allows one to produce a stable macroporous material with opaque and

spongy characteristics as the presence of hydrophilic groups in their structure lets them absorb water.^{21,39} In the current study, gelatin is used for the generation of a modified PHEMA hydrogel with greater surface hydrophilicity and more importantly stronger bioaffinity, which allows us to compare its bioactivity with unmodified PHEMA hydrogels at different porosities and light transparencies. Gelatin has been known as a surface modifier to PHEMA due to its biocompatibility, low cost and its collagen-based structure.²³ It has been largely used in various biological applications – mostly with mammalian cells – by improving the cellular attachment on culture plates,^{43,44} which has also been applied for several microalgal processes such as long-term preservation of microalgae by embedding the cultures on a gelatin based matrix⁴⁵ and encapsulation of microalgal oil within a gelatin–gum Arabic complex.⁴⁶

#### **MATERIALS AND METHODS**

## Preparation of PHEMA hydrogels and gelatin-modified PHEMA hydrogels

A previously reported method has been applied to cast the PHEMA hydrogel disks used in this study.^{27,34,38} In brief, HEMA and water were well mixed in a beaker followed by the addition of crosslinking agent (ethylene glycol dimethacrylate = EDMA) and initiators (ammonium persulfate = APS & N,N,N',N'-tetramethylethylene diamine = TEMED), according to the chemical composition listed in Table S1 and S2 in File S1, Supporting material. The solution was then distributed into a 24-well tissue culture plate to obtain 1 mL solution per well. Polymerization was carried out at room temperature for 3 h, followed by 50°C for 24 h. Following polymerization, the disks were removed from the mould and immersed in deionized water for 4 weeks with daily water exchange to remove residual monomers and oligomers. For the synthesis of PHEMA-gelatin disks, 1 wt% gelatin in water was used instead of pure water in the preparation. Then it followed the same polymerization procedure as stated above for the PHEMA hydrogels. After polymerization, the disks were swollen in 0.5 wt% glutaraldehyde solution at room temperature and 170 rpm (orbital shaker) for 16 h to allow crosslinking of gelatin with glutaraldehyde. The disks were rinsed with deionized water three times at 170 rpm for 10 min and further purified with daily water exchange for 4 weeks. It should be noted that all hydrogels can be cast into polymer sheets and cut into any geometry necessary.⁴⁷ In this study, the polymer sheets were cut into disks of two different sizes with diameters of 8 mm and 17 mm, having a constant thickness of around 1 mm.

#### Microalgal strains and culture conditions

Race B *Botryococcus braunii* (BOT-22), *Nannochloropsis sp.* (MUR 267) and *Dunaliella salina* (MUR 8) were used as the microalgal species for this study. *Botryococcus braunii* (BOT-22) was obtained from The Network of Asia Oceania Algal Culture Collections (AOACC), Japan. *Botryococcus braunii* culture was maintained in modified AF-6 medium⁴⁸ at a pH around 6.4. The marine Eustigmatophyceae, *Nannochloropsis* sp. (MUR 267) and the Chlorophyceae, *Dunaliella salina* (MUR 8) used in this study were obtained from Murdoch University Algae culture collection. *Nannochloropsis* sp. was grown in F/2 medium with a salinity of 3.5%, while *Dunaliella salina* was grown in F medium with a salinity of 7% as formulated by Guillard.⁴⁹ Both F and F/2 media were made using natural sea water, which was previously collected from the coastal waters off Hillary's Beach, Perth, Western Australia.

The seawater was first charcoal filtered and autoclaved prior to the addition of sterile nutrients. Initial algal cell cultures were grown in 250 mL Erlenmeyer flasks, under continuous cool-white fluorescent illumination at incident intensity of around 200  $\mu$ mol photons m⁻² s⁻¹ (PAR).

## Initiation and progress of microalgal growth on the surface of PHEMA hydrogels

PHEMA hydrogel disks were initially placed at the centre of a sterile and transparent 6-well tissue culture plate with an internal diameter of 3.5 cm and a depth of approximately 1.5 cm (Cellstar[®]). Specified amounts of algal culture solutions were slowly added on top of larger (Ø: 17 mm; 0.4 mL) and smaller (Ø: 8 mm; 50 µL) disks (refer to Figure S1 in File S1), which were selected according to the near-maximum capacity of the fluid that would stay at the surface of each disk without falling from the sides. Chlorophyll content and quantum yield measurements were both used to validate the uniformity of the initial cell concentrations by inoculating from the algal culture flasks with dark-adapted quantum yields of  $\sim$ 0.65  $\pm$  0.05, which have the initial total-chlorophyll contents as  $\sim 1.5 \text{ mg L}^{-1}$  D. salina,  $\sim 0.7 \text{ mg L}^{-1}$  Nannochloropsis sp., and  $\sim$ 0.4 mg/L *B. braunii* cells (refer to the File S1 for the calculations of chlorophyll contents). Initial guantum yields of the microalgal cultures were measured after 20 min of dark-adaptation at room temperature, using a portable fluorometer AquaPen-C (Photon Systems Instruments, Czech Republic). After the first introduction of the cells, the disks were kept on the bench for 2 days without any additional processing under an illumination of natural cool white fluorescent light with an intensity of  $50 \pm 5 \mu$ mol photon  $m^{-2} s^{-1}$  and at a temperature of  $25 \pm 2^{\circ}$  C (Figure S1, File S1). This phase is followed by the addition of sterile algae-specific growth media (AF-6⁴⁸ medium for *B. braunii*; F medium⁴⁹ for *D. salina* and F/2 medium for Nannochloropsis sp.) by slowly dripping from the side of the culture-well (4 mL for larger disks, 500 µL for smaller disks). Then the disks were measured for their dark-adapted photosynthetic activity every 3 days. At the end of the growth experiments (with a total duration of 15 days), culture solutions were discarded from the containers followed by the addition of ~3 mL ethanol (70% v/v) by vigorously spraying on the surface of all disks. 3 mL of sterile deionized water were then added on top of the ethanol solution, in such a way that the entire disks were completely immersed within this mixture. These disks, inside the ethanol-water mixture, were kept on the bench for 2 days, followed by removal of the ethanol-water solution and washing with sterile deionized water at least three times. In order to assure that the surfaces of the disks were then free of cells, photosynthetic activities of the remaining disks were measured. Recycled disks were kept ready within sterile deionized water until their further usage for algal immobilization.

Cell growth of all cultures was carried out on the surface of hydrogel disks under batch conditions at around  $25 \pm 2^{\circ}$  C and under artificial diurnal illumination (12 h light / 12 h dark cycle). The light periods of the cycle were provided by natural cool white fluorescent light at a light intensity of  $50 \pm 5 \mu$ mol photon m⁻² s⁻¹.

#### **Algal growth measurements**

#### Minimum fluorescence yield

Algal growth was examined by evaluating the photosynthetic activity of the cells through measuring the minimum fluorescence yields (Fo) of the biofilms after their dark-adaptation for 20 min before each measurement. Fo values were recorded using a Handy-PEA chlorophyll fluorimeter (Hansatech Instruments, UK) that contains high-intensity LED arrays delivering red light at a peak wavelength of 650 nm, while the infrared region of any light source can be blocked by its NIR short-pass filters.⁵⁰ The dark-adaptation process allows the re-oxidation of the photosystem-II reaction centre of algal cells, which leads to calculation of the minimum fluorescence yield under the lack of any photochemical or non-photochemical quenching of the fluorescence yield.^{50–52} Released fluorescence values from the biofilms were later recorded by the integrated software of the fluorimeter, PEA Plus V1.10. All experiments were conducted in triplicate and the standard deviation of each value is given in the form of error bars within the related figure.

#### Confocal laser scanning microscopy

The viability and thickness of the biofilm were observed under confocal laser scanning microscopy (CLSM), Nikon C2+ multispectral laser scanning confocal microscope, which is equipped with 405 nm, 458 nm, 488 nm, 514 nm, 561 nm and 647 nm lasers. The fresh sample of algal biofilm was used for this observation. The surface of each disk was cut into thinner layers so that the light and the laser can easily penetrate the sample. The sample was placed upside down on a 35 mm diameter glass bottom dish. The lugol solution was added to Dunaliella salina culture to stop the movement of algal cells prior to microscopic imaging. No solution addition was necessary for the non-motile species of Botryococcus braunii and Nannochloropsis sp. A 20× objective was used to capture three-dimensional biofilm structure and thickness, while a Mito Tracker Deep Red laser (640.0 nm) was used to observe the algal cells. Owing to their chlorophyll contents, the algal cells autofluorophore in red.^{53,54} The images were recorded and processed by a software package (Nikon Imaging Software (NIS)-Elements) that converted the images into their three-dimensional forms with two constant dimensions (L:632  $\mu$ m  $\times$  W:632  $\mu$ m) and varying biofilm depths (shown as 'D' in Fig. 5). This observation was made in two replicates.

#### Scanning electron microscopy

Surface analysis of hydrogels, with and without attached algal cells, were investigated by scanning electron microscopy (SEM, NEON 40EsB) analysis at an accelerating voltage of 3 kV on samples coated with platinum (layer thickness ~3 nm). The hydrogel samples were freeze-dried for a day prior to the SEM analysis. The hydrated hydrogels were previously kept overnight at  $-40^{\circ}$ C inside a freezer, then the frozen samples were transferred into a vacuum chamber connected to a condenser and cooled to  $-55^{\circ}$ C. For SEM analysis of the microalgal cultures, given in Fig. 2, 200 µL of liquid cultures were initially placed on the surface of the SEM pin stubs with carbon adhesive tabs, and allowed to dry under the laminar hood before being coated with a layer of platinum for SEM analysis. Size measurements, such as the pore diameters and the sizes of microalgal cells, were calculated with the aid of ImageJ 1.50i software.

#### **RESULTS AND DISCUSSION**

## Immobilization efficiencies of PHEMA hydrogel disks with different porosities

The first challenge of the present work was to test the immobilization capabilities of two different PHEMA hydrogel disks with different porosities. Opaque E25 PHEMA and transparent E60 PHEMA



**Figure 1.** SEM images of as prepared (a, b) E25 PHEMA; (c, d) E60 PHEMA hydrogels; and (e, f) gelatin-modified E20 PHEMA at low and high magnifications. Differences in the magnifications of the different hydrogels are due to presenting the best magnification to show the detailed surface structure.

were the hydrogel specimens used in this study, where the numbers represent the percentage of HEMA in the polymerization solutions (see Table S1 in File S1) and the capital letter 'E' symbolizes the crosslinking agent EDMA. Porosities of PHEMA hydrogels are known to be affected by variations in the ratios of HEMA to water concentrations present in the polymerization mixtures.^{20,27,34,39,42} Figure 1 shows SEM images of as-prepared E25 and E60 PHEMA specimens before the introduction of any algal cells. SEM images indicated that the porosity of the disks increased as the percentage of HEMA was decreased (or percentage of water increased), making the E25 sample more porous than E60 (Fig. 1). On the other hand, the E60 PHEMA specimen's surface was composed of flakes rather than porous holes (Fig. 1(c,d)). The reason to continue testing the E60 sample for algal growth was its more transparent nature compared with E25 PHEMA, which facilitated the photosynthesis process by allowing the transfer of light throughout the entire disk. The diameter of the pores at the surface of E25 PHEMA had a range between 0.6 and 2.5 µm, with an average diameter of around 1.6  $\mu$ m (Fig. 1(a,b)). It should be noted that these pore sizes are calculated based on the SEM imaging of the freeze-dried samples, which might be slightly higher in their actual moist conditions.⁵⁵ The porous structure of E25 PHEMA disk makes it a good candidate for serving as a supporting matrix of microalgal cells, mainly for the Nannochloropsis sp. and D. salina cells due to their smaller sizes than B. braunii cells (Fig. 2). According to the SEM images of those cells, Nannochloropsis sp. and D. salina

cells have spheroidal shapes with average diameters of around 2.8  $\mu$ m (Fig. 2(a,b)) and 3.5  $\mu$ m (Fig. 2(c,d)), respectively. On the other hand, B. braunii cells form large colonies of around 30 µm that are made of pyriform-shaped individual cells with an average length of around 9.5 µm (Fig. 2(e,f)). One of the advantages of porous surface structures is the possibility to allow replication of the cells on their surfaces by facilitating the diffusion of nutrients towards the cells, while transferring the cellular waste/by-products from the cells through their environment.^{1,9} It is worth noting that porous PHEMA hydrogels produced in the presence of large amounts of water, as reported in this paper, are termed 'phase separation' hydrogels.²⁴ These materials are well known for their applications as medical implants and tissue engineering scaffolds in which the presence of interconnected pores and the non-toxic nature are essential to facilitate the growth, proliferation and migration of animal cells.^{24,25,39,56} Such hydrogels have also been studied for sustained delivery of therapeutic drugs^{34,38} and gas transportation and storage.⁵⁷ The feature of open pore channels and its correlation with the transportation characteristics of drugs and gases have been extensively studied.58-61

#### Immobilization of microalgal strains on the gelatin-modified PHEMA hydrogel and its comparison with the unmodified PHEMA hydrogel disks

The next challenge was to explore the attachment of the microalgal cells on gelatin-modified E20 PHEMA disks. The porosity of



Figure 2. SEM images of air-dried (a, b) Nannochloropsis sp.; (c, d) D. salina; (e, f) B. braunii cell cultures at low and high magnifications.

the gelatin-modified E20 PHEMA hydrogel disks was also explored under SEM, showing a highly porous structure forming a hollow network – hollow sizes mostly ranged between 10 and 45  $\mu$ m, with an average diameter of around 25  $\mu$ m) (Fig. 1(e)), which has various internal porous passages with smaller pore sizes at diameters ranging from 0.5–4.5  $\mu$ m (Fig. 1(f)). The greater pores observed in gelatin modified E20 PHEMA are largely due to the higher amounts of water, in comparison with E25 and E60, used in the polymerization process.^{27,34,40}.

The bioactivity of the hydrogel disks was explored by monitoring the growth of microalgal cells on the surface of their solid supports at different time intervals. Due to the impracticalities of several cellular growth analyses, such as the difficulties observed during cell counting of the colony forming B. braunii cells or motile D. salina cells, along with the harshness of the chlorophyll extraction from the surface of the disks; an in situ analysis was conducted by measuring the photosynthetic activity of the cells as an indication of the cellular growth without harming either the cells or the disks during progression of the growth experiment. Minimum fluorescence measurements in the dark-adapted state ( $F_{0}$ ) is known to have a significant positive correlation with the growth of microalgal cells, due to the observed linear relationship between F_o and Chl *a*, allowing us to use F_o results as a non-invasive proxy tool for estimating algal biomass.^{51,62,63} According to this information, we measured dark-adapted (20 min) minimum fluorescence values using a Handy-PEA chlorophyll fluorimeter (Hansatech Instruments, UK) as an indication of the bioactivity of the tested hydrogels. Figure 3 shows the variations observed for the photosynthetic activities of three different microalgal cells at different time intervals. The comparative growth tests between E25 and E60 PHEMA specimens indicated the clear success of the more porous E25 PHEMA sample for being a better support for all of the tested microalgal species (Fig. 3(a-c)). Figure 3(d-f) showed that the gelatin-modified E20 PHEMA hydrogels provided the best support for Nannochloropsis sp. and D. salina cells (Fig. 3(d,e));

whereas unmodified PHEMA disks were more attractive for the cells of B. braunii (Fig. 3(f)). When gelatin-modified E20 PHEMA was compared with E25 PHEMA specimens at similar dimensions (Ø: 8 mm), Nannochloropsis sp. cells had around 20 times higher photosynthetic activity on the surface of the gelatin-modified hydrogel (Fig. 3(d)), while this increase was only around 5-fold for D. salina cells (Fig. 3(e)), where both differences were highest on the 6th day of the growth experiments. It should be noted that larger E25 PHEMA hydrogel (Ø: 17 mm) had better biofilm activities than its smaller counterpart (Ø: 8 mm) due to the presence of greater area for the cells to form biofilms. Growth of microalgal cells was also observed visually, where the colour changes on the surface of each disk can be seen on the real-time images taken at the beginning, 6th day, and by the last day (15th day) of the growth experiments (Fig. 4). The increasing green colour on the surfaces is due to the increased concentration of algal cells on the hydrogel mats. Larger E25 and E60 PHEMA disks had their greenest colour by the 6th day due to their coverage with Nannochloropsis sp. (Fig. 4N(b,e)) or D. salina cells (Fig. 4D(b,e)), whereas their gelatin-modified E20 PHEMA equivalents kept their green colour until the 15th day of the experiment with a slight increase on their growth during the second-half of the experiment (Figs 3(d,e) and 4N(i),D(i)). It should be noted that the overall duration of the growth experiments for Nannochloropsis sp. and D. salina cell cultures also lasted for 15 days in order to compare them with B. braunii cells with slower growth. Due to the characteristic slow-growth rates of *B. braunii* cells, 50,64 more pronounced delay was observed to reach the maximum cellular activity, as can be seen in Figs 3(c,f) and 4(B). For the case of E25 and E60 PHEMA disks with B. braunii cells (Fig. 4B(a-f)), less area is covered with green colour, which is mostly localized on specific places rather than being spread throughout the hydrogel surface. This might be mainly due to the colony-forming nature of the cells (Fig. 2(e,f) in addition to showing lower photosynthetic activities than Nannochloropsis sp. or D. salina cells (Fig. 3). B. braunii cells



**Figure 3.** Photosynthetic activities of (a, d) *Nannochloropsis* sp.; (b, e) *D. salina*; (c, f) *B. braunii* cells on the surface of E25 PHEMA (white columns), E60 PHEMA (black columns) and gelatin modified E20 PHEMA (grey columns) hydrogel disks, based on the minimum fluorescence measurements at different time intervals. Note that the upper part (a – c) shows the results obtained for the larger disks of E25 and E60 PHEMA having diameters (Ø) of 17 mm; whereas the lower parts (d – f) are for the smaller disks of gelatin-modified E20 PHEMA and E25 PHEMA hydrogel that were initially cast identical (Ø: 8 mm).

grown on the surface of gelatin-modified E20 PHEMA showed the lowest cellular activities, as also observed with the least colour change during the time course of the growth experiment (Fig. 4B(g-i)).

Surface activity of each disk was also monitored by CLSM using a Nikon C2+ confocal microscope (Fig. 5), along with the red autoflorescence assets of the algal cells due to their chlorophyll content.^{53,54} The red fluorescence of algal cultures, Fig. 5, indicates the viability of the cells on hydrogel surfaces, which establishes the nonlethal effect of PHEMA hydrogels on them. It should be noted that the samples were imaged on the 7th day after the initiation of algal growth, representing the approximate half-time of the growth experiment. Gelatin-modified E20 PHEMA had the most concentrated Nannochloropsis sp. (Fig. 5(c)) and D. salina (Fig. 5(f)) cells on their surfaces based on the coverage area and the biofilm thickness (D) on each mat. E25 PHEMA hydrogel hosted more B. braunii cells on their surfaces (Fig. 5(g)), although relatively large biofilm thicknesses of around  $40 \pm 2 \,\mu$ m were observed on the surfaces of all hydrogel mats covered with B. braunii cells (Fig. 5(q-i)). Accumulation of thicker *B. braunii* biofilms is due the larger sizes of each individual cell ( $\sim 10 \,\mu$ m) that also form large colonies (refer to Fig. 2(e,f)), in comparison with the non-colony forming Nannochloropsis (Fig. 2(a,b)) and D. salina (Fig. 2(c,d)) cells with smaller cell diameters of around  $3-3.5 \mu m$ .

Combination of the aforementioned results revealed the success of PHEMA disks for the cellular attachment of *N. chloropsis* and *D. salina* cells in descending order as the gelatin-modified E20 > E25 PHEMA > E60 PHEMA, whereas this order is different for *B. braunii* cells as E25 PHEMA > E60 PHEMA > gelatin-modified E20 PHEMA. As the two most successful mats, gelatin-modified E20 PHEMA and E25 PHEMA specimens have macroporous structures in common (Fig. 1), revealing their physical availability to entrap

various microorganisms. SEM images of both E25 PHEMA (Fig. 6) and gelatin-modified E20 PHEMA (Fig. 7) were also investigated after immobilization of the cultures, displaying the entrapment of cells within the porous matrices with the exception of the larger B. braunii colonies (Fig. 2(e,f)) that are mostly attached on the surfaces of E25 PHEMA hydrogel mats rather than being embedded within its pores, which are relatively smaller than the sizes of the colonies (Fig. 6(e,f)). For the case of the gelatin-modified E20 PHEMA hydrogels, spherical morphologies of both Nannochloropsis sp. and D. salina microalgal cells can be distinguished from their immobilizing hydrogel surfaces by the larger sizes of the algal cells (varying between  $\sim$ 2.5 and  $\sim$ 4 µm; Fig. 2) than the more distorted spherical droplets of PHEMA polymers (average size  $\sim 1.5 \,\mu\text{m}$ ; Fig. 1), which is in agreement with the reported observation of freeze-dried E20 PHEMA-only hydrogels by Paterson et al.⁵⁵ Additionally, Nannochloropsis sp. and D. salina microalgal cells appear to have smoother surfaces under SEM imaging, which is a typical observation for various algal cells.⁶⁵

Structural properties of the solid surfaces such as their porosities, sizes of the pores, and surface geometries; morphology of the cells; hydrophobicity and surface charges of both the cells and their solid supports are some of the important parameters that contribute to the efficiency of cellular attachment onto the supporting solid matrix.⁶⁶ As two of the unmodified PHEMA hydrogels, significant differences were observed for the bioactivities of macroporous E25 PHEMA and nonporous E60 PHEMA specimens, which showed the importance of the porous surface structures for a proper algal immobilization process. According to the current literature, various porous surfaces had been tested for the immobilization of microalgae, such as electrospun nanofibers of chitosan⁹; polycarbohydrate with grooves⁶⁷; controlled-pore glass⁶⁸; cotton cloth⁶⁹; and cellulose nitrate filter paper⁵⁰ revealing the successful



**Figure 4.** Progress of the algal growth on the surfaces of E25, E60 and gelatin-modified E20 PHEMA hydrogel disks (1st-row) at the beginning, (2nd-row) after 6 days, and (3rd-row) after 15 days from the start of the growth experiments. 'N' letter represents *Nannochloropsis* sp.; whereas 'D' and 'B' letters represent *D. salina* and *B. braunii* cells, respectively. Please refer to the online version for the actual colours of these images.



**Figure 5.** Confocal laser scanning microscopic images of (a–c) *Nannochloropsis* sp.; (d–f) *D. salina*; (g–i) *B. braunii* cells on E25 (first row), E60 (middle row) and gelatin-modified PHEMA hydrogel disks (last row). 'D' represents the depth/thickness of the biofilm on a three-dimensional image with other dimensions constant at 632  $\mu$ m width (W) × 632  $\mu$ m length (L). Note that the observed red autofluorescence is due to the presence of active chlorophyll pigments in microalgal cells.^{53,54} Please refer to the online version of this article for colourful images.



Figure 6. SEM images of immobilized (a, b) Nannochloropsis sp.; (c, d) D. salina; (e, f) B. braunii cells on the surface of E25 PHEMA hydrogel mats at low and high magnifications (samples are taken on the 7th day of the experiment).



Figure 7. SEM images of immobilized (a, b) *Nannochloropsis* sp.; (c, d) *D. salina*; (e, f) *B. braunii* cells on the surface of gelatin-modified PHEMA hydrogel mats at low and high magnifications (samples are taken on the 7th day of the experiment).

entrapment capabilities of porous structures with sufficient pore sizes that can allow adhesion of the targeted-cells.

Compatible surface hydrophobicity of the solid supports and the cells is an important parameter for defining the mechanism of cellular adhesion to the solid surfaces. The cell wall of an individual *B. braunii* cell is known to have an internal fibrillary layer made of mucilaginous polysaccharides and an external trilaminar sheath,^{70,71} while several individual cells adhere by being embedded within an extracellular matrix composed of oils and various cellular excretes.⁷⁰ The hydrophobic nature of the terpenoid substrates present in the extracellular matrix of *B. braunii* microalgal colonies – particularly botryococcene and associated carotenoid hydrocarbons for the B-race *B. braunii* cells^{70,72-75} – might be the main reason for the decreased affinity of those cells on the surfaces of the gelatin-modified E20 PHEMA hydrogels with enhanced hydrophilic properties due to gelatin.

Hydrophilic surface proteins⁷⁶ and plasma membrane proteins with extracellular hydrophilic moieties⁷⁷ are some of the main mechanisms for the adhesion of D. salina cells on solid surfaces. It has also been stated that the salt concentration of the culture media is an important parameter for defining the hydrophilicity of the cell membrane of Dunaliella cells, as solutions with sodium chloride concentrations lower than 3 mol L⁻¹ (i.e. natural sea water, and the F medium used within the current study⁴⁹) results in domination of the cell membrane by polar groups, indicating increased hydrophilic properties.⁷⁸ Dunaliella salina cells are also known to accumulate extracellular polymeric substances (EPS) on their surfaces with hydrophilic nature (mainly composed of various proteins, polymers, phospholipids, and nucleic acids) that are principally used as a carbon and energy storage material during starvation conditions.⁷⁹ These EPSs are heterogeneous mixture of polyelectrolytes that include some groups such as primary amine, aliphatic alkyl, halide-group, aromatic compounds and polysaccharides;^{79,80} which are compatible with the surfaces of both unmodified PHEMA and the more hydrophilic gelatin-modified PHEMA. The unique motility assets of D. salina cells⁸¹ also increase their cellular migration and replication on the surface of the attachment matrix.

Nannochloropsis sp. cells possess high oil contents,⁸² like the oil-rich B. braunii cells, without forming any colonies. Previous studies revealed that Nannochloropsis cell walls have a bilayer structure made of a cellulosic inner layer surrounded by an outer hydrophobic algaenan layer.⁸³ Despite this hydrophobic algaenan outer layer, presence of negative surface charges might have played a more effective role on the attachment of Nannochloropsis sp. cells that clearly showed a higher affinity with the modified PHEMA surfaces with hydrophilic gelatin molecules. The presence of hydrophilic functional groups, such as -OH (hydroxyl), -NH₂ (amine), and -COOH (carboxyl), can generate surface charges depending on the pH of the environment.9,84,85 Most of the algal cells have negative zeta potentials, as those aforementioned surface groups create negative surface charges by their deprotonation at higher pH conditions above their isoelectric points,^{84,86} which is the case for the salt-water media used for Nannochloropsis sp. and D. salina cells having a pH value around 8.0.49 The isoelectric point of 4.7-5.2 range for gelatin (type B)87,88 shows the presence of negative surface charges on gelatin molecules under basic physiological conditions, revealing lower electrostatic attraction to the negative surface charges of microalgae. This might show that the amino groups present on the Nannochloropsis sp. and D. salina surfaces would be one of the main sources of electrostatic interactions with the negative groups of gelatin. When the repulsive energy barrier between two surfaces is low enough, the absorption of negatively charged surfaces onto the surfaces showing zeta potentials of the same sign is still possible, which is more pronounced for the low zeta potentials that are associated with low repulsive energies.⁸⁹ It should be noted that the saltwater species (i.e. Nannochloropsis sp. and D. salina) showed lower absolute zeta potential values than the microalgal species living in freshwater media (i.e. B. braunii),84 which was explained by the high ionic strength of the saltwater medium that decreases the thickness of the electrical double layer formed at the solid-water interfaces by compressing it around the cells.^{84,90,91} Lower absolute zeta-potential values of salty water species (i.e. around -18.5 mV for Nannochloropsis⁹²) compared with the freshwater B. braunii cells (around -30 mV^{84,93}), overcomes the

repulsive energy barrier to show electrostatic attraction between PHEMA hydrogels (around  $-10 \text{ mV}^{57}$ ) or type-B gelatin molecules (around  $-12.5 \text{ mV}^{94,95}$ ) that also have negative zeta potentials at physiological conditions.^{57,66,96,97} A comprehensive understanding of the position of attachment-sites, comprising their detailed surface morphology and chemical composition, are still needed to fully endorse the interactions between the cells and their immobilization matrices.

Material recycling experiments also revealed that cleaning the used hydrogel disks with an ethanol/water mixture at the end of each cycle was sufficient to re-immobilize new cells on the surface of the recycled disks while yielding similar bioactivities even after three consecutive cycles (data not shown), providing that the disks were kept in a solvent between the experiments.

#### **CONCLUSIONS**

We have established the use of macroporous PHEMA hydrogel-disks as a water-insoluble and non-toxic support for microalgal growth. Both unmodified PHEMA and gelatin-modified PHEMA were proven to be highly durable polymer supports, with the disks still retaining their integrity after several years if kept moist within an aqueous solution. Holding the advantage of recycling the disks can contribute to cost reduction of the overall process. PHEMA hydrogels also have the benefit of adjustable porosity, as it can be simply customized by regulating the chemical composition of the polymerization mixture to meet the specific needs of the application. Attachment efficiencies of PHEMA hydrogels can be simply altered by binding various natural molecules on their surfaces, as also revealed here with the gelatin-modified PHEMA. Algae immobilized PHEMA systems will be further developed by integrating them with waste treatment processes and the generation of various microalgal bioproducts including biodiesel, photopigments and fatty acids.

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#### **Supporting Information**

Supporting information may be found in the online version of this article.

#### REFERENCES

- 1 Mallick N, Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *BioMetals* **15**:377–390 (2002).
- 2 Liu Y, Rafailovich MH, Malal R, Cohn D and Chidambaram D, Engineering of bio-hybrid materials by electrospinning polymer-microbe fibers. *Proc Natl Acad Sci U S A* **106**:14201–14206 (2009).
- 3 Eroglu E, Smith SM and Raston CL, Application of various immobilization techniques for algal bioprocesses, in *Biomass and Biofuels from Microalgae*, ed. by Moheimani NR, McHenry MP, de Boer K and Bahri PA. Springer International Publishing, Switzerland, pp. 19–44 (2015).
- 4 de la Noue J and de Pauw N, The potential of microalgal biotechnology: a review of production and uses of microalgae. *Biotechnol Adv* 6:725–770 (1988).

- 5 Lebeau T and Robert J-M, Biotechnology of immobilized micro algae: a culture technique for the future, in *Algal Cultures, Analogues of Blooms and Applications*, ed. by Rao S. Science Publishers, Enfield, pp. 801–837 (2006).
- 6 De-Bashan LE and Bashan Y, Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresour Technol* 101:1611–1627 (2010).
- 7 Eroglu E and Raston CL, Nanomaterial processing strategies in functional hybrid materials for wastewater treatment using algal biomass. *J Chem Technol Biotechnol* **92**:1862–1867 (2017).
- 8 Zeng X, Danquah MK, Halim R, Yang S, Chen XD and Lu Y, Comparative physicochemical analysis of suspended and immobilized cultivation of Chlorella sp. J Chem Technol Biotechnol 88:247–254 (2013).
- 9 Eroglu E, Agarwal V, Bradshaw M, Chen X, Smith SM, Raston CL et al., Nitrate removal from liquid effluents using microalgae immobilized on chitosan nanofiber mats. Green Chem 14:2682–2685 (2012).
- 10 Aksu Z, Biosorption of heavy metals by microalgae in batch and continuous systems, in *Wastewater Treatment with Algae*, ed. by Wong YS and Tam NFY. Springer-Verlag, Berlin Heidelberg, Springer, pp. 37–53 (1998).
- 11 Wahid MH, Eroglu E, Chen X, Smith SM and Raston CL, Functional multi-layer graphene–algae hybrid material formed using vortex fluidics. *Green Chem* **15**:650–655 (2013).
- 12 Aksu Z, Gönen F and Demircan Z, Biosorption of chromium (VI) ions by Mowital[®] B30H resin immobilized activated sludge in a packed bed: comparison with granular activated carbon. *Process Biochem* 38:175–186 (2002).
- 13 Spolaore P, Joannis-Cassan C, Duran E and Isambert A, Commercial applications of microalgae. *J Biosci Bioeng* **101**:87–96 (2006).
- 14 Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C et al., Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Res* 1:20–43 (2008).
- 15 Chisti Y, Biodiesel from microalgae. Biotechnol Adv 25:294-306 (2007).
- 16 Borowitzka L, Borowitzka M and Moulton T, The mass culture of Dunaliella salina for fine chemicals: from laboratory to pilot plant. Hydrobiologia 116:115–121 (1984).
- 17 García-González M, Moreno J, Manzano JC, Florencio FJ and Guerrero MG, Production of *Dunaliella salina* biomass rich in 9-cis- $\beta$ -carotene and lutein in a closed tubular photobioreactor. *J Biotechnol* **115**:81–90 (2005).
- 18 Mäki-Arvela P, Hachemi I and Murzin DY, Comparative study of the extraction methods for recovery of carotenoids from algae: extraction kinetics and effect of different extraction parameters. *J Chem Technol Biotechnol* **89**:1607–1626 (2014).
- 19 Chauton MS, Reitan KI, Norsker NH, Tveterås R and Kleivdal HT, A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities. *Aquaculture* 436:95–103 (2015).
- 20 Peppas NA, Moynihan HJ and Lucht LM, The structure of highly crosslinked poly (2-hydroxyethyl methacrylate) hydrogels. *J Biomed Mater Res* **19**:397–411 (1985).
- 21 Peppas NA and Khare AR, Preparation, structure and diffusional behavior of hydrogels in controlled release. Adv Drug Deliv Rev 11:1–35 (1993).
- 22 Sun Y-M and Lee H-L, Sorption/desorption properties of water vapour in poly (2-hydroxyethyl methacrylate): 1. Experimental and preliminary analysis. *Polymer* **37**:3915–3919 (1996).
- 23 Dragusin D-M, Van Vlierberghe S, Dubruel P, Dierick M, Van Hoorebeke L, Declercq HA *et al.*, Novel gelatin–PHEMA porous scaffolds for tissue engineering applications. *Soft Matter* 8:9589–9602 (2012).
- 24 Chirila TV, Hicks CR, Dalton PD, Vijayasekaran S, Lou X, Hong Y *et al.*, Artificial cornea. *Prog Polym Sci* **23**:447–473 (1998).
- 25 Hicks CR, Morrison D, Lou X, Crawford GJ, Gadjatsy A and Constable IJ, Orbital implants: potential new directions. *Expert Rev Med Devices* 3:805–815 (2006).
- 26 Flynn L, Dalton PD and Shoichet MS, Fiber templating of poly (2-hydroxyethyl methacrylate) for neural tissue engineering. *Biomaterials* **24**:4265–4272 (2003).
- 27 Li C, Zheng Y-F and Lou X, Calcification capacity of porous pHEMA–TiO2 composite hydrogels. *J Mater Sci Mater Med* **20**:2215–2222 (2009).
- 28 Montheard J-P, Chatzopoulos M and Chappard D, 2-Hydroxyethyl methacrylate (HEMA): chemical properties and applications in biomedical fields. J Macromol Sci Polymer Rev 32:1–34 (1992).

- 29 Vacanti FX, PHEMA as a fibrous capsule-resistant breast prosthesis. Plast Reconstr Surg **113**:949–952 (2004).
- 30 Imai Y and Masuhara E, Long-term in vivo studies of poly (2-hydroxyethyl methacrylate). *J Biomed Mater Res A* **16**:609–617 (1982).
- 31 Voldřrich Z, Tománek Z, Vacík J and Kopečcek J, Long-term experience with poly (glycol monomethacrylate) gel in plastic operations of the nose. J Biomed Mater Res A 9:675–685 (1975).
- 32 Hsiue G-H, Guu J-A and Cheng C-C, Poly (2-hydroxyethyl methacrylate) film as a drug delivery system for pilocarpine. *Biomaterials* 22:1763–1769 (2001).
- 33 Dziubla T, Torjman M, Joseph J, Murphy-Tatum M and Lowman A, Evaluation of porous networks of poly (2-hydroxyethyl methacrylate) as interfacial drug delivery devices. *Biomaterials* 22:2893 – 2899 (2001).
- 34 Lou X, Munro S and Wang S, Drug release characteristics of phase separation pHEMA sponge materials. *Biomaterials* **25**:5071–5080 (2004).
- 35 Arica MY, Alaeddinoğlu NG and Hasirci V, Immobilization of glucoamylase onto activated pHEMA/EGDMA microspheres: properties and application to a packed-bed reactor. *Enzyme Microb Technol* 22:152–157 (1998).
- 36 Bayramoğlu G, Kacar Y, Denizli A and Arıca MY, Covalent immobilization of lipase onto hydrophobic group incorporated poly (2-hydroxyethyl methacrylate) based hydrophilic membrane matrix. J Food Eng 52:367–374 (2002).
- 37 Arica MY and Hasirci V, Immobilization of glucose oxidase: a comparison of entrapment and covalent bonding. *J Chem Technol Biotechnol* 58:287–292 (1993).
- 38 Lou X, Wang S and Tan S, Mathematics-aided quantitative analysis of diffusion characteristics of pHEMA sponge hydrogels. Asia-Pac J Chem Eng 2:609–617 (2007).
- 39 Chirila TV, Chen YC, Griffin BJ and Constable IJ, Hydrophilic sponges based on 2-hydroxyethyl methacrylate. I. Effect of monomer mixture composition on the pore size. *Polym Int* **32**:221–232 (1993).
- 40 Lou X, Vijayasekaran S, Sugiharti R and Robertson T, Morphological and topographic effects on calcification tendency of pHEMA hydrogels. *Biomaterials* 26:5808–5817 (2005).
- 41 Lou X, Dalton P and Chirila T, Hydrophilic sponges based on 2-hydroxyethyl methacrylate part VII: modulation of sponge characteristics by changes in reactivity and hydrophilicity of crosslinking agents. *J Mater Sci Mater Med* **11**:319–325 (2000).
- 42 Lou X and van Coppenhagen C, Mechanical characteristics of poly (2-hydroxyethyl methacrylate) hydrogels crosslinked with various difunctional compounds. *Polym Int* **50**:319–325 (2001).
- 43 Takahashi K, Okita K, Nakagawa M and Yamanaka S, Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc* 2:3081–3089 (2007).
- 44 Paguirigan A and Beebe D, Gelatin based microfluidic devices for cell culture. Lab Chip 6:407–413 (2006).
- 45 Iturriaga R and Sullivan C, Long-term preservation of microalgal cells and their optical properties. *Oceanography* **3**:2 (2015).
- 46 Zhang K, Zhang H, Hu X, Bao S and Huang H, Synthesis and release studies of microalgal oil-containing microcapsules prepared by complex coacervation. *Colloids Surf B Biointerfaces* 89:61–66 (2012).
- 47 Carbonetto S, Gruver MM and Turner DC, Nerve fiber growth in culture on fibronectin, collagen, and glycosaminoglycan substrates. *J Neurosci* 3:2324–2335 (1983).
- 48 Watanabe M, Kawachi M, Hiroki M and Kasai F, NIES-collection, List of strains: Microalgae and Protozoa. Microbial Culture Collection. National Institute for Environmental Studies, Tsukuba (2000).
- 49 Guillard RRL, Culture of phytoplankton for feeding marine invertebrates, in *Culture of Marine Invertebrate Animals*, ed. by Smith WL and Chanley MH. Springer, Boston, pp. 29–60 (1975).
- 50 Wijihastuti RS, Moheimani NR, Bahri PA, Cosgrove JJ and Watanabe MM, Growth and photosynthetic activity of *Botryococcus braunii* biofilms. *J Appl Phycol* **29**:1123–1134 (2017).
- 51 Honeywill C, Paterson D and Hagerthey S, Determination of microphytobenthic biomass using pulse-amplitude modulated minimum fluorescence. *Eur J Phycol* **37**:485–492 (2002).
- 52 Schreiber U, Schliwa U and Bilger W, Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res* **10**:51–62 (1986).
- 53 MacIsaac E and Stockner JG, Enumeration of phototrophic picoplankton by autofluorescence microscopy, in *Handbook of Methods in*

Aquatic Microbial Ecology, ed. by Kemp PF, Sherr BF, Sherr EB and Cole JJ. Lewis Publishers, Boca Raton, FL, pp. 187–197 (1993).

- 54 Schulze K, López DA, Tillich UM and Frohme M, A simple viability analysis for unicellular cyanobacteria using a new autofluorescence assay, automated microscopy, and ImageJ. *BMC Biotechnol* **11**:118 (2011).
- 55 Paterson SM, Casadio YS, Brown DH, Shaw JA, Chirila TV and Baker MV, Laser scanning confocal microscopy versus scanning electron microscopy for characterization of polymer morphology: sample preparation drastically distorts morphologies of poly (2-hydroxyethyl methacrylate)-based hydrogels. J Appl Polym Sci 127:4296–4304 (2013).
- 56 Li C, Liu S, Zheng Y and Lou X, Collagen modified porous pHEMA-TiO₂ composite hydrogels for tissue engineering. *J Control Release* 172:e143-e143 (2013).
- 57 Ding A, Yang L, Fan S and Lou X, Reversible methane storage in porous hydrogel supported clathrates. *Chem Eng Sci* **96**:124–130 (2013).
- 58 Wang S, Mahali SM, McGuiness A and Lou X, Mathematical models for estimating effective diffusion parameters of spherical drug delivery devices. *Theor Chem Acc* **125**:659–669 (2010).
- 59 Wang S and Lou X, Numerical methods for the estimation of effective diffusion coefficients of 2D controlled drug delivery systems. *Optim Eng* **11**:611–626 (2010).
- 60 Wang S and Lou X, An optimization approach to the estimation of effective drug diffusivity: from a planar disc into a finite external volume. *J Ind Manag Optim* **5**:127–140 (2009).
- 61 Shi B-H, Fan S-S and Lou X, Application of the shrinking-core model to the kinetics of repeated formation of methane hydrates in a system of mixed dry-water and porous hydrogel particulates. *Chem Eng Sci* **109**:315–325 (2014).
- 62 Serôdio J, Marques da Silva J and Catarino F, Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using in vivo chlorophyll a fluorescence. J Phycol **33**:542–553 (1997).
- 63 Jesus B, Perkins R, Mendes C, Brotas V and Paterson D, Chlorophyll fluorescence as a proxy for microphytobenthic biomass: alternatives to the current methodology. *Mar Biol* **150**:17–28 (2006).
- 64 Vazquez-Duhalt R and Arredondo-Vega BO, Haloadaptation of the green alga *Botryococcus braunii* (race A). *Phytochemistry* **30**:2919–2925 (1991).
- 65 Wahid MH, Eroglu E, Chen X, Smith SM and Raston CL, Entrapment of *Chlorella vulgaris* cells within graphene oxide layers. *RSC Adv* **3**:8180–8183 (2013).
- 66 Harkes G, Feijen J and Dankert J, Adhesion of *Escherichia coli* on to a series of poly (methacrylates) differing in charge and hydrophobicity. *Biomaterials* **12**:853–860 (1991).
- 67 Cui Y, Yuan W and Cao J, Effects of surface texturing on microalgal cell attachment to solid carriers. *Int J Agri Biol Eng* **6**:44–54 (2013).
- 68 Elmahadi H and Greenway GM, Speciation and preconcentration of trace elements with immobilized algae for atomic absorption spectrophotometric detection. *J Anal At Spectrom* **9**:547–551 (1994).
- 69 Prasad R, Shabnam N and Pardha-Saradhi P, Immobilization on cotton cloth pieces is ideal for storage and conservation of microalgae. *Algal Research* **20**:172–179 (2016).
- 70 Banerjee A, Sharma R, Chisti Y and Banerjee U, Botryococcus braunii: a renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol 22:245–279 (2002).
- 71 Largeau C, Casadevall E, Berkaloff C and Dhamelincourt P, Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. *Phytochemistry* **19**:1043–1051 (1980).
- 72 Eroglu E and Melis A, Extracellular terpenoid hydrocarbon extraction and quantitation from the green microalgae *Botryococcus braunii* var. Showa. *Bioresour Technol* **101**:2359–2366 (2010).
- 73 Metzger P and Largeau C, *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl Microbiol Biotechnol* **66**:486–496 (2005).
- 74 Moheimani NR, Cord-Ruwisch R, Raes E and Borowitzka MA, Non-destructive oil extraction from *Botryococcus braunii* (Chlorophyta). *J Appl Phycol* **25**:1653–1661 (2013).

- 75 Wijffels RH, Barbosa MJ and Eppink MH, Microalgae for the production of bulk chemicals and biofuels. *Biofuels Bioprod Biorefin* **4**:287–295 (2010).
- 76 Fisher M, Gokhman I, Pick U and Zamir A, A structurally novel transferrin-like protein accumulates in the plasma membrane of the unicellular green alga Dunaliella salina grown in high salinities. *J Biol Chem* 272:1565–1570 (1997).
- 77 Katz A, Waridel P, Shevchenko A and Pick U, Salt-induced changes in the plasma membrane proteome of the halotolerant alga *Dunaliella salina* as revealed by blue native gel electrophoresis and nano-LC-MS/MS analysis. *Mol Cell Proteomics* 6:1459–1472 (2007).
- 78 Curtain CC and Snook H, Method for harvesting algae. US Patent No 4554390 (1985).
- 79 Mishra A, Kavita K and Jha B, Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina*. *Carbohydr Polym* **83**:852–857 (2011).
- 80 Mishra A and Jha B, Isolation and characterization of extracellular polymeric substances from micro-algae *Dunaliella salina* under salt stress. *Bioresour Technol* **100**:3382–3386 (2009).
- 81 Oren A, A hundred years of *Dunaliella* research: 1905–2005. *Saline Sys* 1:2 (2005).
- 82 Vadiveloo A, Moheimani NR, Cosgrove JJ, Bahri PA and Parlevliet D, Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp.(Eustigmatophyceae). *Algal Res* 8:121–127 (2015).
- 83 Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, Goodenough U *et al.*, Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. *Eukaryot Cell* **13**:1450–1464 (2014).
- 84 Ozkan A and Berberoglu H, Physico-chemical surface properties of microalgae. Colloids Surf B Biointerfaces 112:287–293 (2013).
- 85 Hadjoudja S, Deluchat V and Baudu M, Cell surface characterisation of *Microcystis aeruginosa* and *Chlorella vulgaris*. J Colloid Interface Sci 342:293–299 (2010).
- 86 Henderson R, Parsons SA and Jefferson B, The impact of algal properties and pre-oxidation on solid – liquid separation of algae. *Water Res* 42:1827–1845 (2008).
- 87 Gaihre B, Khil MS, Lee DR and Kim HY, Gelatin-coated magnetic iron oxide nanoparticles as carrier system: drug loading and in vitro drug release study. Int J Pharm 365:180–189 (2009).
- 88 Kommareddy S and Amiji M, Poly (ethylene glycol) modified thiolated gelatin nanoparticles for glutathione-responsive intracellular DNA delivery. *Nanomed Nanotechnol Biol Med* 3:32–42 (2007).
- 89 McKetta JJ, Unit Operations Handbook: Volume 2. CRC Press, New York, (1992).
- 90 Wang B, Wu D, Chu KH, Ye L, Yip HY, Cai Z et al., Removal of harmful alga, Chattonella marina, by recyclable natural magnetic sphalerite. J Hazard Mater 324:498–506 (2017).
- 91 van Oss CJ, Interfacial Forces in Aqueous Media. CRC Press, Boca Raton, (2006).
- 92 Hu Y-R, Wang F, Wang S-K, Liu C-Z and Guo C, Efficient harvesting of marine microalgae *Nannochloropsis maritima* using magnetic nanoparticles. *Bioresour Technol* **138**:387–390 (2013).
- 93 Hena S, Fatihah N, Tabassum S, Lalung J and Jing S, Magnetophoretic harvesting of freshwater microalgae using polypyrrole/Fe3O4 nanocomposite and its reusability. J Appl Phycol 28:1597–1609 (2016).
- 94 Azarmi S, Huang Y, Chen H, McQuarrie S, Abrams D, Roa W et al., Optimization of a two-step desolvation method for preparing gelatin nanoparticles and cell uptake studies in 143B osteosarcoma cancer cells. J Pharm Pharm Sci. 9:124–132 (2006).
- 95 Masuelli MA and Sansone MG, *Hydrodynamic Properties of Gelatin-Studies from Intrinsic Viscosity Measurements*. In Products and Applications of Biopolymers, ed. by Verbeek C, INTECH Open Access Publisher, London, pp. 85–116 (2012).
- 96 Moro DG, John JVS, Shannon KF and Ponder BC, Method of formation of shape-retentive aggregates of gel particles and their uses. US Patent 7811605 (2010).
- 97 Van Vlierberghe S, Vanderleyden E, Dubruel P, De Vos F and Schacht E, Affinity study of novel gelatin cell carriers for fibronectin. *Macromol Biosci* **9**:1105–1115 (2009).