Supporting Information

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Branched Poly(ethylene imine)s as Anti-algal and Anti-cyanobacterial Agents with Selective Flocculation Behavior to Cyanobacteria over Algae

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Supporting Information. Polymer characteristics (**Supplementary Table S1**), additional characteristics of studied phototrophic microorganisms (**Supplementary Table S2**), summary of IC_{50} values for growth-inhibitory effect (**Supplementary Table S3**), cidal, aggregation and flocculation activities of B-PEIs (**Supplementary Figure S1-5**) (PDF).

TABLES

Supplementary Table S1. Structural characteristics of B-PEIs used in this study.

B-PEIs[a)]	**MW**[b)]	$\bar{M}_n$[c)] (GPC)	$\bar{M}_w$[d)] (GPC)	$\bar{M}_w / \bar{M}_n$	pK_{a1}[e)]	pK_{a2}[e)]
B-PEI$_{0.5}$	600	470	2,100	4.5	9.4	6.2
B-PEI$_{1.1}$	1,800	1,100	1,400	1.3	9.6	6.2
B-PEI$_{12}$	10,000	12,000	19,000	1.6	9.0	5.8

[a)] See the text for denotation; [b)] MW and volume reported by a supplier; [c)] The number average molecular weight; Gibney *et. al.* (2012)[1]; [d)] The weight average molecular weight; Gibney *et. al.* (2012)[1]; [e)] Gibney *et. al.* (2012)[1]

Supplementary Table S2. Growth-inhibitory effect of low MW PPI-DEN and B-PEIs on freshwater green algae *Chlamydomonas reinhardtii* (*CR*) and *Desmodesmus quadricauda* (*DQ*) and cyanobacteria *Synechococcus elongatus* (*SE*) and *Microcystis aeruginosa* (*MA*) based on monitoring optical density (λ = 680 nm). IC_{50} values are expressed for comparison in μg mL^{-1} (**A**) and in μM (**B**).

A

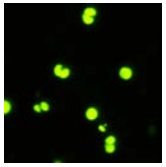
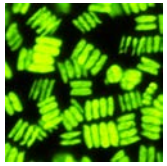
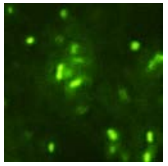
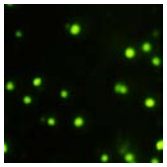
POLYMER	GREEN ALGAE						CYANOBACTERIA				
	CR			***DQ***			***SE***			***MA***	
	IC_{50}[a] (μg mL^{-1})			**IC_{50}** (μg mL^{-1})			**IC_{50}** (μg mL^{-1})			**IC_{50}** (μg mL^{-1})	
	24 h[b]	48 h	72 h	48 h	72 h	96 h	24 h	48 h	72 h	72 h	96 h
PPI-DEN	**0.22** (0.09-0.55)	**0.22** (0.09-0.74)	**0.24^{aA}** (0.21-0.36)	**3.04** (1.27-7.30)	**2.72^{aB}** (1.63-4.55)	**2.21** (1.46-3.34)	**0.23** (0.10-0.49)	**0.21** (0.12-0.36)	**0.21^{aA}** (0.14-0.33)	**0.28^{aA}** (0.10-0.49)	**0.33** (0.23-0.50)
B-PEI$_{0.5}$	**0.35** (0.23-0.56)	**0.18*** (0.08-0.38)	**0.14*^{abA}** (0.13-0.14)	**3.39** (1.94-5.90)	**2.59^{aB}** (1.42-4.74)	**2.43** (0.80-7.38)	**0.35** (0.11-1.17)	**0.33** (0.19-0.56)	**0.36^{bC}** (0.30-0.45)	**0.22^{aAC}** (0.10-0.47)	**0.26** (0.16-0.43)
B-PEI$_{1.1}$	**0.32** (0.16-0.65)	**0.21** (0.17-0.27)	**0.21^{bA}** (0.14-0.32)	**4.06** (2.91-5.65)	**3.36^{aB}** (1.92-5.87)	**2.71** (1.09-6.75)	**0.23** (0.19-0.29)	**0.24** (0.19-0.29)	**0.27^{abA}** (0.21-0.34)	**0.27^{aA}** (0.17-0.43)	**0.26** (0.20-0.34)
B-PEI$_{12}$	**0.74** (0.35-1.53)	**0.56** (0.45-0.70)	**0.58^{cA}** (0.48-0.69)	**~[c]2.5-40** (40-44 %)	**2.5-40^{aB}** (40-47 %)	**~2.5-40** (40-47 %)	**0.39** (0.19-1.13)	**0.35** (0.27-0.50)	**0.36^{bA}** (0.27-0.55)	**0.24^{aA}** (0.15-0.39)	**0.19** (0.09-0.40)

B

PPI-DEN	**0.70** (0.28-1.74)	**0.68** (0.28-2.34)	**0.74^{aA}** (0.67-1.14)	**9.60** (4.01-23.06)	**8.59^{aB}** (5.15-14.37)	**6.98** (4.61-10.55)	**0.73** (0.32-1.55)	**0.66** (0.38-1.14)	**0.66^{aA}** (0.44-1.04)	**0.88^{aA}** (0.32-1.55)	**1.04** (0.73-1.58)
B-PEI$_{0.5}$	**0.74** (0.49-1.19)	**0.38*** (0.17-0.81)	**0.30*^{bA}** (0.28-0.30)	**7.21** (4.13-12.55)	**5.51^{abB}** (3.02-10.09)	**5.17** (1.70-15.70)	**0.74** (0.23-2.49)	**0.70** (0.40-1.19)	**0.77^{aC}** (0.64-0.96)	**0.47^{bAC}** (0.21-1.00)	**0.455** (0.34-0.91)
B-PEI$_{1.1}$	**0.29** (0.15-0.59)	**0.19** (0.15-0.25)	**0.19^{cA}** (0.13-0.29)	**3.69** (1.62-3.14)	**3.05^{bB}** (1.75-5.34)	**2.46** (0.99-5.14)	**0.21** (0.17-0.26)	**0.22** (0.17-0.26)	**0.25^{bA}** (0.19-0.31)	**0.25^{cA}** (0.15-0.24)	**0.24** (0.18-0.31)
B-PEI$_{12}$	**0.06** (0.03-0.13)	**0.05** (0.04-0.06)	**0.05^{dA}** (0.04-0.06)	**~[c]0.2-3.3** (40-44 %)	**~0.2-3.3^{bB}** (40-47 %)	**~0.2-3.3** (40-47 %)	**0.03** (0.02-0.09)	**0.03** (0.02-0.04)	**0.03^{cC}** (0.02-0.05)	**0.02^{dA}** (0.01-0.03)	**0.02** (0.01-0.03)

[a] The concentration of polymer causing 50% inhibition of the algal or cyanobacterial growth. The IC_{50} value was determined for each independent experiment and the geometric mean with 95% confidence limits (CI) was calculated (n≥3); [b] Exposure time; [c] A clear concentration-dependent effect at the concentrations of 0.3-2.5 μg mL^{-1} (0.03-0.2 μM) was followed by a plateau value of growth inhibition of about 40-47% observed along the concentration range from 5 to 40 μg mL^{-1} (0.4-3 μM). Asterisk denotes the significant difference from the IC_{50} value after 24 (48) h ($P \leq 0.05$; one-way ANOVA with Dunnett's method or unpaired two-tailed t-test). Values with different lowercase letter superscripts differ significantly ($P \leq 0.05$; one-way ANOVA with Tukey's method) among the polymers within the same microorganism tested. Values with different italic uppercase letter superscripts differ significantly ($P \leq 0.05$; one-way ANOVA with Tukey's method) among different microorganisms tested within the same polymer. B-PEI, branched polyethylenimine; PPI-DEN, poly(propylenimine)-dendrimer.

Supplementary Table S3. Characteristics of studied freshwater phototrophic microorganisms

Genus	Species	Photo	Cell shape[2]	Length/ width (μm)[a)]	Volume[b)] (μm³)	Dt[c)] (h)	Characteristics & impact Negative	Cell envelope (cell wall & membrane)
Green algae – eukaryotic cell type	***CR***		Sphere	6-12	113-904	19	• **ubiquitous algae** • common species of extensive **water blooms** • **model organisms** for antialgal tests and for biofouling	• **multilayers** composed of high-molecular-weight **glycoproteins** rich in hydroxyproline, arabinose and galactose (40-100 nm thick)[3] • **plasma membrane** - mainly phospholipids PG and PE, β-carotene, betaine lipid DGTS[4]
	DQ		Prolate spheroid	10-18/ 3.5-7	64-462	26		• **multilayers** composed of **hemicellulosis** and **sporopollenin**[3a,5] • **plasma membrane** - mainly phospholipids PG, PE and PC, β-carotene[4]
Cyanobacteria – prokaryotic cell type	***SE***		Rod-shaped ⇒ Sphere model[2]	0.6-1.6	0.1-2	14	• **common cyanobacteria** in freshwater sources • a **model freshwater cyanobacterium** for anticyanobacterial tests	• **external surface layers** (slime or sheath) • **outer membrane** (neutral or low negative charge) - phospholipids, lipopolysaccharide (**LPS**), glycolipids, carotenoids and porins[6] • **peptidoglycan** (multilayers - thicker and with a higher degree of cross-linking between PG chains than those in other Gram-negative bacteria)[6] • **plasma membrane** - mainly glycolipids MGDG, DGDG and SQDG, and phospholipid PG[4b,7]
	MA		Sphere	3.5-6	22-113	27	• produces cyano**toxins** microcystins • presence of gas vesicles • causes **harmful water blooms** of health, economic and ecological importance	

[a)] Protist Information Server, URL: http://protist.i.hosei.ac.jp/; [b)] Calculated based on geometric models for phytoplankton[2]; [c)] Doubling time (Dt) under the conditions of this study. DGDG, digalactosyldiacylglycerol; DGTS, Diacylglyceryltrimethylhomoserine; MGDG, monogalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

FIGURES

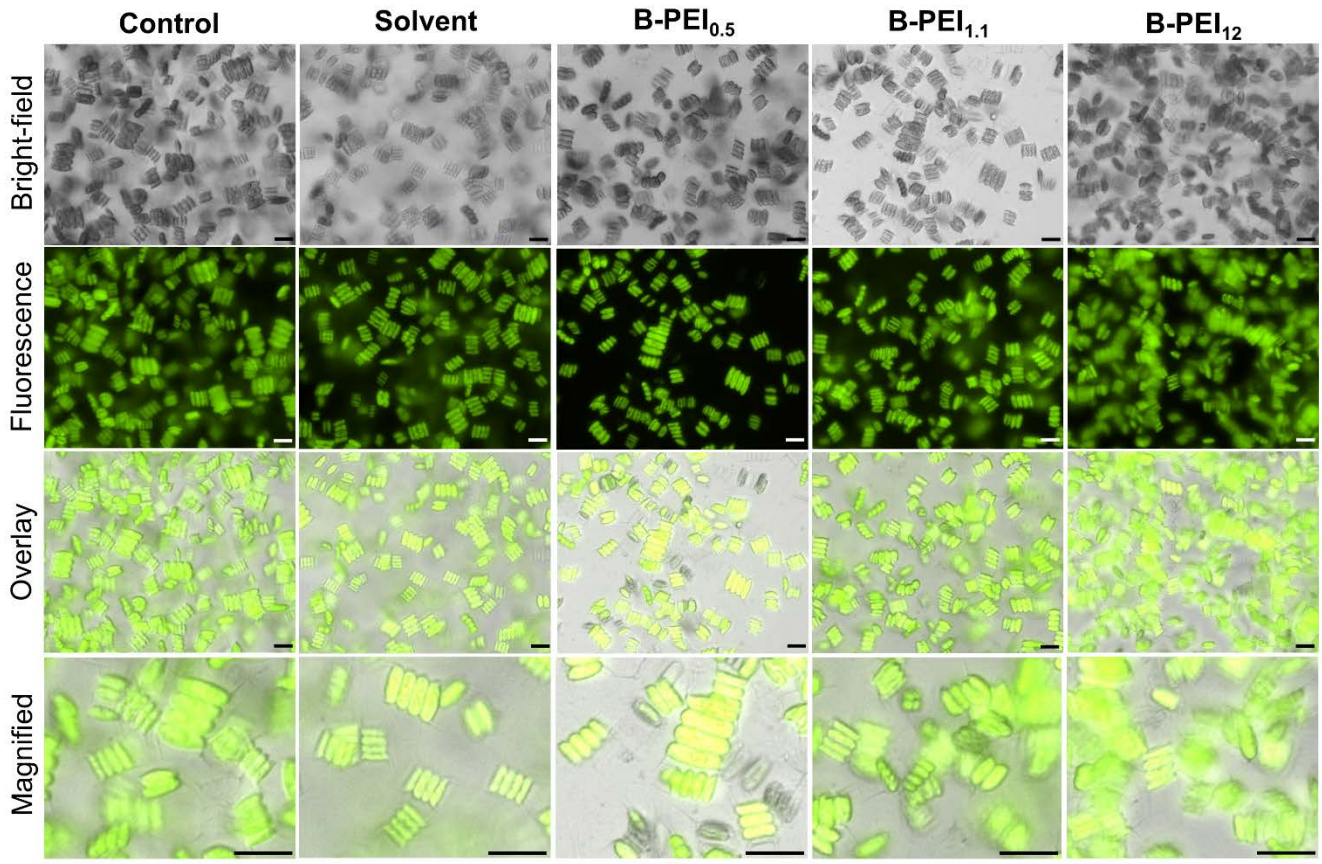


Figure S1. **Freshwater green algae *DQ* after treatment with B-PEIs.** Representative bright-field, fluorescence, overlay and magnified images of non-treated cells (naïve and solvent controls) and cells treated with B-$PEI_{0.5}$, B-$PEI_{1.1}$ and B-PEI_{12} at the concentration of 20 μg mL^{-1} for 72 h. *DQ*, *Desmodesmus quadricauda*. Scale bar = 20 μm.

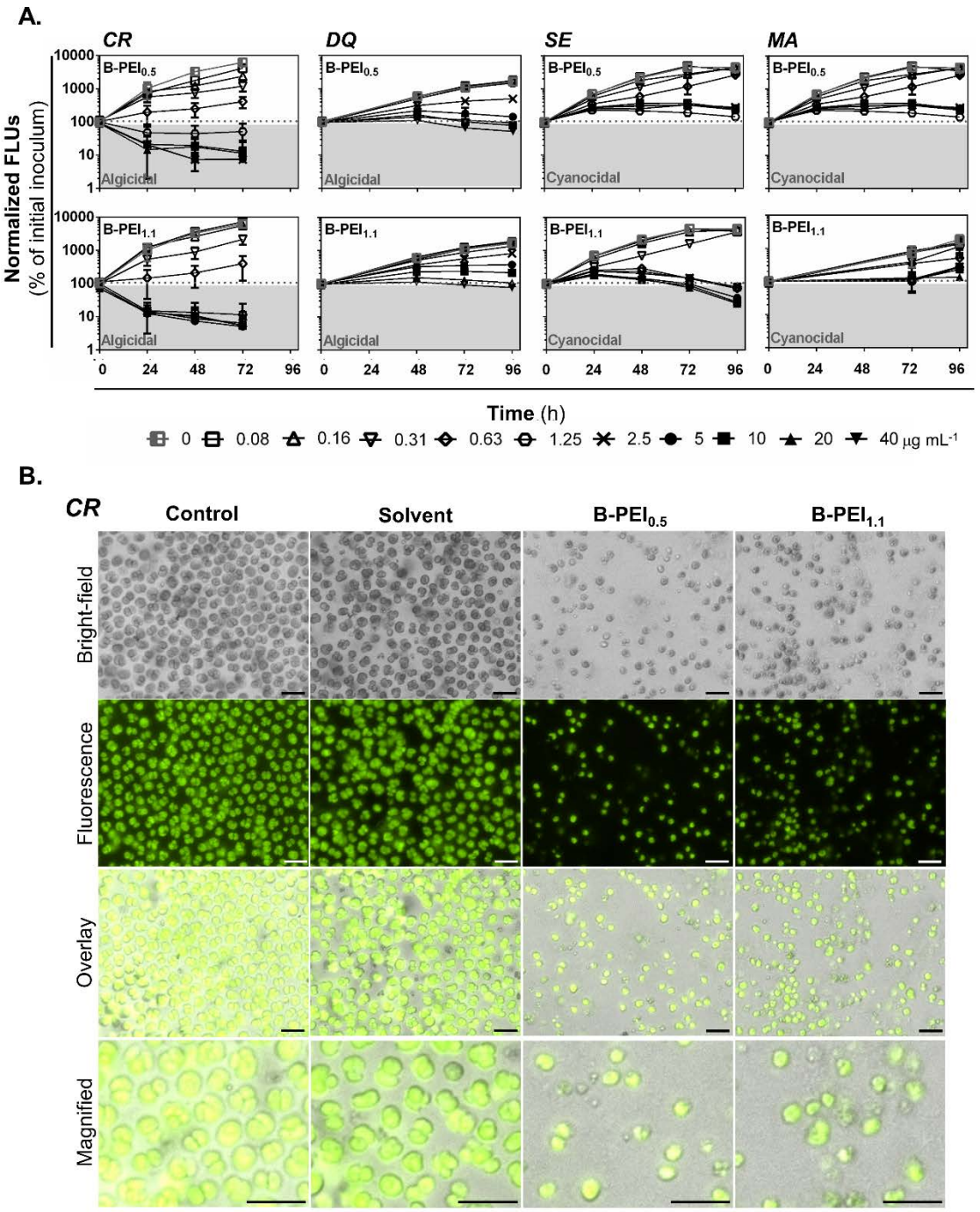
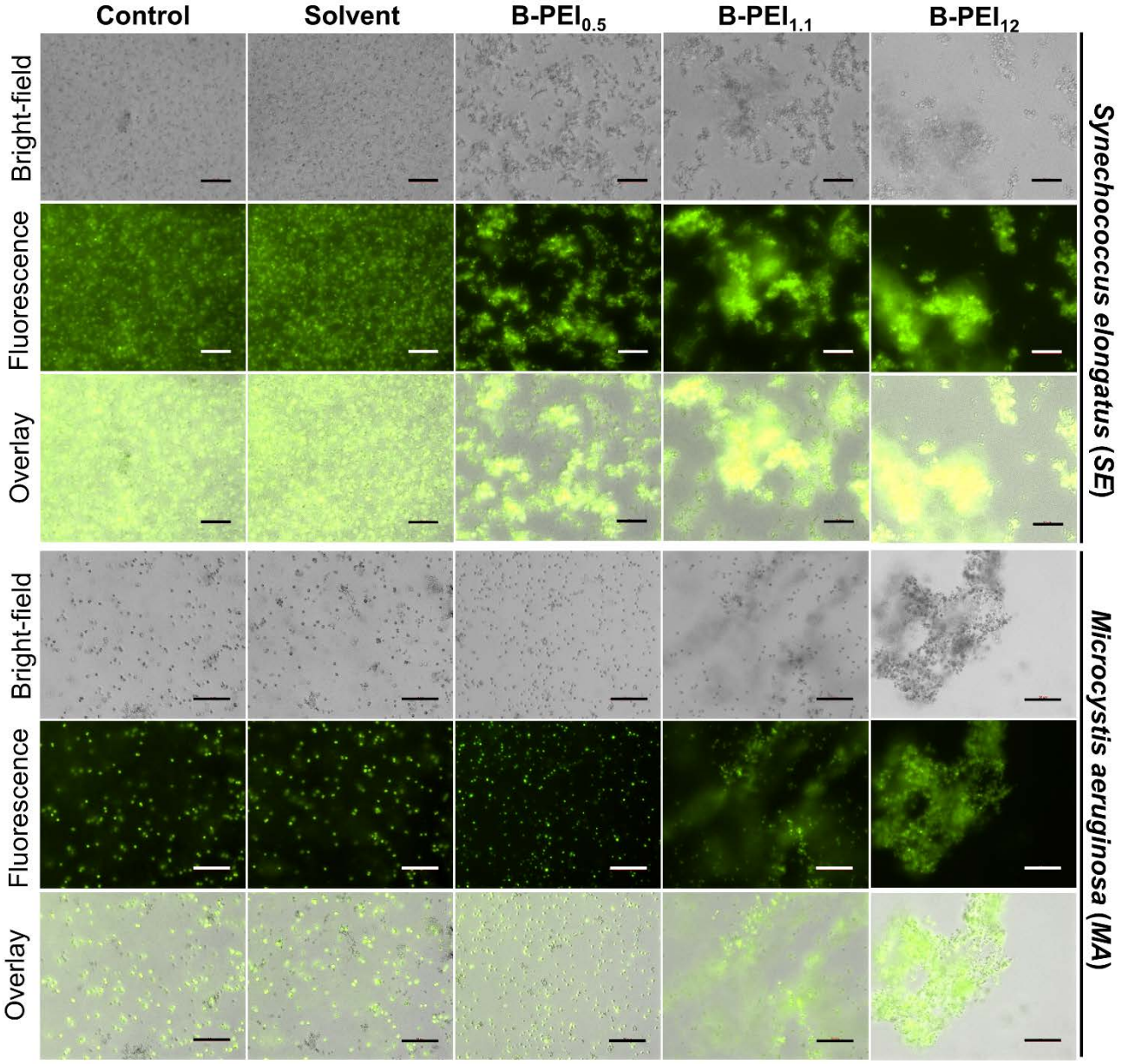
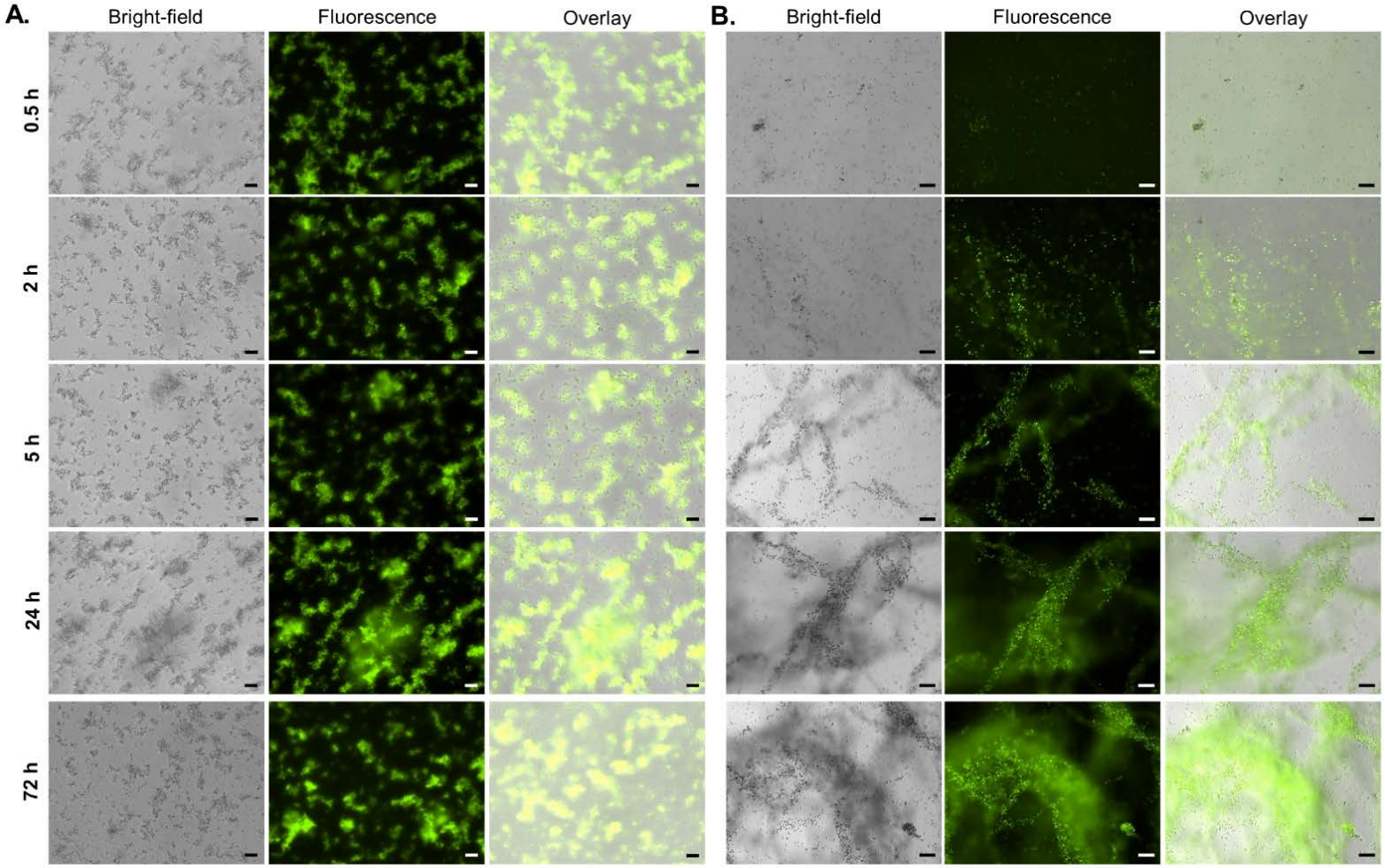


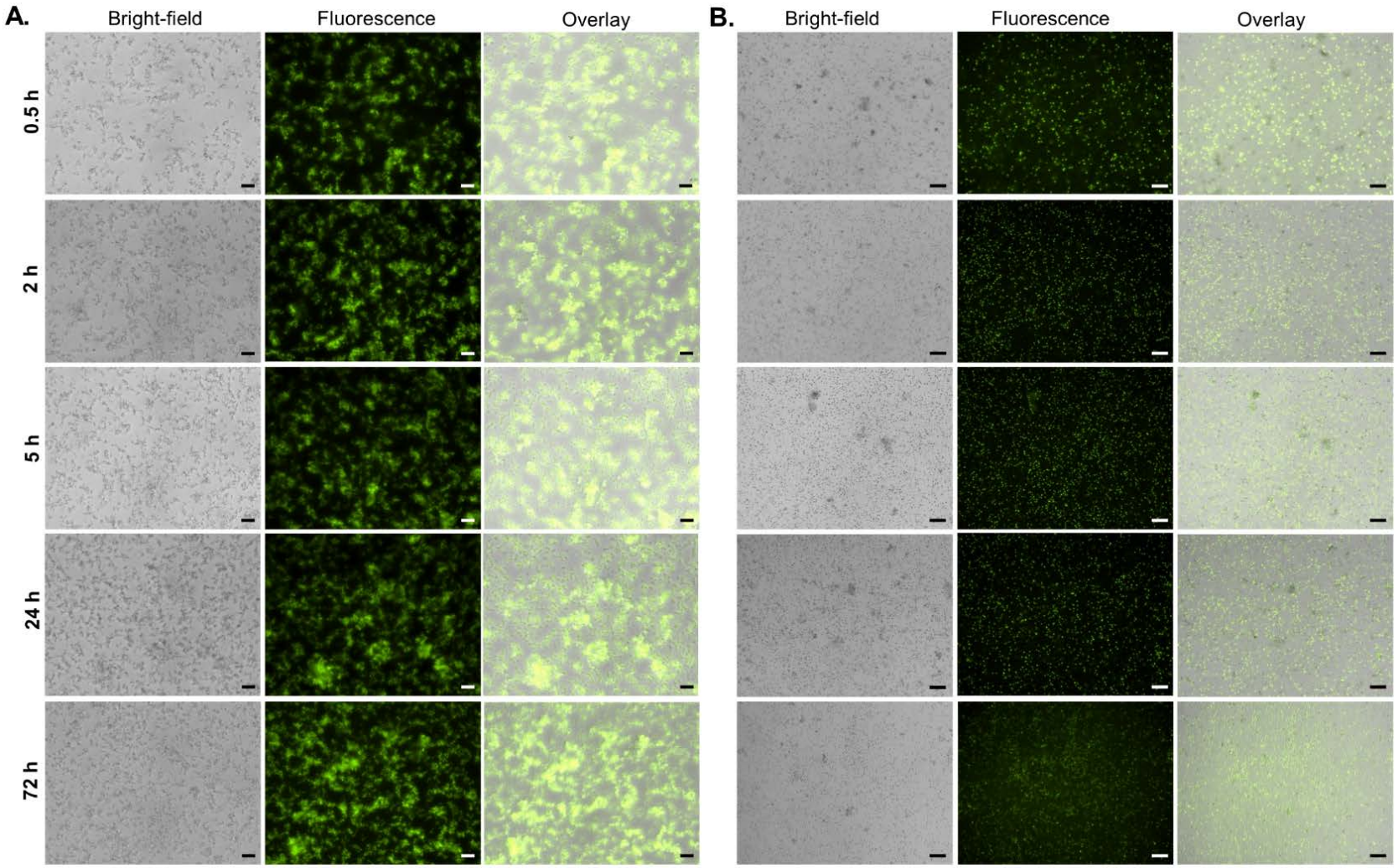
Figure S2. **Cidal activity of B-PEIs to algae *CR*, but not to algae *DQ* and cyanobacteria *SE* and *MA*. (A)** B-PEI$_{0.5}$ and B-PEI$_{1.1}$ against *Chlamydomonas reinhardtii* (*CR*) and *Desmodesmus quadricauda* (*DQ*) and cyanobacteria *Synechococcus elongatus* (*SE*) and *Microcystis aeruginosa* (*MA*) as assessed by autofluorescence (λ_{ex} = 485 nm/λ_{em} = 675 nm) at different time points (24-96 h). B-PEIs concentrations: 0-40 μg mL^{-1}. Data are expressed as a percentage of fluorescence units (FLUs) for initial inoculum and presented as means (SD; n = 3-5). **(B)** *CR* cells after treatment with B-PEI$_{0.5}$ or B-PEI$_{1.1}$. Representative bright field, fluorescence and overlaid images of non-treated *CR* cells (naïve and solvent controls) and *CR* cells treated with B-PEI$_{12}$ at the concentration of 20 μg mL^{-1} for 72 h. Scale bar = 20 μm.



Supplementary Figure S3. **Cyanobacteria *SE* and *MA* after treatment with B-PEIs.** Representative bright-field, fluorescence and overlaid images of non-treated cells (naïve and solvent controls) and cells treated with B-$PEI_{0.5}$, B-$PEI_{1.1}$ and B-PEI_{12} at the concentration of 20 μg mL^{-1} for 72 h. B-PEI, branched polyethylenimine; *MA*, *Microcystis aeruginosa; SE*, *Synechococcus elongatus*. Scale bar = 20 μm (*SE*)/ 50 μm (*MA*).



Supplementary Figure S4. **Time-dependent aggregation and flocculation activities of B-PEI$_{1.1}$ inducing clustering of both cyanobacterial cell types.** Representative bright-field, fluorescence and overlay images of cyanobacteria *Synechococcus elongatus* (*SE*: **A**) or *Microcystis aeruginosa* (*MA*: **B**) treated with B-PEI$_{1.1}$ at the concentration of 20 μg mL^{-1} for 0.5, 2, 5, 24, and 72 h. The aggregated cells (flocs) were still autofluorescent, suggesting the presence of live cells with intact photosynthetic pigments in the aggregates. Scale bar = 20 μm (*SE*)/ 50 μm (*MA*).



Supplementary Figure S5. **Time-dependent aggregation and flocculation activities of B-PEI$_{0.5}$ inducing clustering of cyanobacterial *SE* cells, but not MA cells.** Representative bright-field, fluorescence and overlaid images of cyanobacteria *Synechococcus elongatus* (*SE*: **A**) or *Microcystis aeruginosa* (*MA*: **B**) treated with B-PEI$_{0.5}$ at the concentration of 20 μg mL^{-1} for 0.5, 2, 5, 24 and 72 h. The aggregated cells (flocs) of *SE* were still autofluorescent, suggesting the presence of live cells with intact photosynthetic pigments in the aggregates. Scale bar = 20 μm (*SE*)/ 50 μm (*MA*).

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