Modification of Living Diatom, *Thalassiosira weissflogii* by Calcium Precursor as a Sacrificial Template for Development of Next Generation of Structural Biomaterials

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Keywords : Biomedical Engineering, Material Science

ABSTRACT

Diatoms are single-cell organisms, which can be easily found in freshwater and marine environments. It is an exceptional templates due to its highly-ordered pores, large surface area, species-specific architecture and flexibility for surface modifications. Comprised largely of amorphous silica, this diatom structure is characterised by an intricate species-specific architecture of arrays of highly-ordered pores, ribs and has many functions that can be utilised for biomaterials platforms. Existing application have focused on surface modification of the diatom as a drug delivery microparticle, however, to fully utilize their application, alteration of the chemical composition of the intricate diatom morphology is essential[2,3]. The hypothesis of this study is that calcium can be up taken by the living diatom using the same mechanism as employed for silica uptake without altering the topography of its unique three-dimensional structure. The specific objective of this study is to optimize the incorporation of calcium hydroxide into the diatom during the frustule synthesis and monitor the effects on the diatom architectural with surface morphological analysis. Additionally, the amount of calcium incorporated was investigated using bulk analysis.

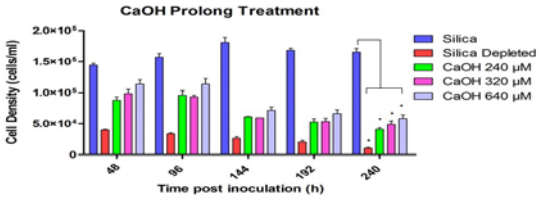
EXPERIMENTAL METHODS

Axenic *Thalassiosira weissflogii* cultures were grown in artificial seawater. Cultures were silica depleted for a minimum of 48 hours before inoculation with the calcium precursor and were inoculated at 5 x 10^4 cells/mL in a final volume of 200 mL in a polystyrene tissue culture flask. Sodium metasilicate (Na_2SO_3) or calcium hydroxide (CaOH) was added to cultures and grown at a 14 hour/10 hour, light/dark cycle at a light intensity of 3000 lux and temperature range of 16–22ºC. Multiple additional cultures received further additions of Na_2SO_3 or CaOH at 48, 96, 144 and 192 hours. Cultures were collected at 240 hours post-inoculation and cleaned of organic matter. Cell density was monitored during growth using a haemocytometer. The silica and calcium formation was studied using PDMPO and Calcium Green staining. The morphology of the frustule was investigated by SEM, TEM, AFM and the amounts of calcium were quantitatively determined by ICP-MS. To obtain further structural information, FTIR and XPS were used.

RESULTS AND DISCUSSION

Fluorescence microscopy and XPS analysis confirmed that Ca was incorporated into the frustule of CaOH-modified *T.weissflogii* (Figure 1, 2). This amount was dependent on the dosing regimen of Ca that the culture received. No adverse effect was observed on the topography of the modified diatom.

a



b

Sample ID	Calcium (%)	Silica (%)
Unmodified	2.28 ± 0.01	15.67 ± 0.17
CaOH - 240uM	27.57 ± 0.11	1.83 ± 0.10
CaOH - 320uM	21.74 ± 0.11	1.83 ± 0.14
CaOH - 640uM	25.27 ± 0.08	1.23 ± 0.09

Figure 1. *a) Growth profile of T.weissflogii for unmodified and CaOH-modified diatom. Two way ANOVA revealed an effect of time and treatment, statistical significant with * $p < 0.05$($n = 3$). b) Measurement of calcium and silica in the diatom frustule using ICP-MS. An increase ratio of calcium to silica is observed when increasing the treatment dose. Data presented as the mean ± standard deviation.*

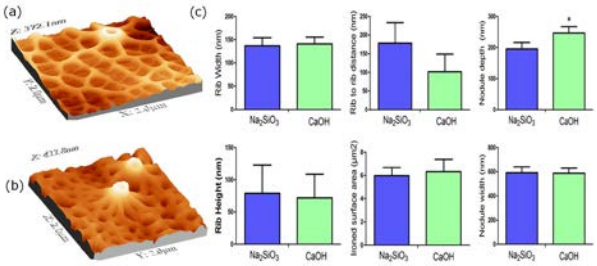


Figure 2. *AFM Image of (a) Unmodified diatom and (b) CaOH modified diatom of the nodule and rib structure (c) Architectural parameter relating to the rib structure on the valve surface are altered in CaOH modified T.weissflogii. T-test reveals significant difference for Na_2SiO_3 vs CaOH * $p<0.05$. (n=4 diatoms per treatment)*

CONCLUSION

The modification of the diatom frustule with calcium has been archieved and the characteristic architecture of gross features was unaltered.

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ACKNOWLEDGMENT

Majlis Amanah Rakyat (Grant no: R0115969) for providing financial support to this project. MB is an SFI SIRG Co-fund fellow, Grant No. 11/SIRG/B2135