Abstract of Exhibit
Taiwan International Science Fair

CATEGORY: **Chemistry**

TITLE: **Isolation, characterization of β-chitin from squid pens and calcium carbonate crystallization on the chitin scaffold**

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Introduction and Purpose

Chitin, a polysaccharide common in biocomposites, has an interwoven organic framework that can act as a scaffold for mineralization in natural systems. Acidic amino acids, namely aspartic (Asp) and glutamic (Glu) acids, are the primary active molecules at biomineralisation interphases in mollusks and play an important role in controlling the polymorph and morphology of the associated mineral.

In this study, chitin was extracted from squid pens and used as a scaffold for crystallization. The chitin scaffold was functionalized with glutamic acid and aspartic acid separately to create an artificial microenvironment for the study of biomineralization of calcium carbonate. The objective was to investigate the usefulness of the extracted chitin to serve as a scaffold for calcium carbonate crystallization, especially the fidelity of the polymorph nucleated to the amino acids and proteins used.

Procedures

To investigate the above-mentioned propositions, chitin was extracted from pens of the common squid, demineralized with acid, deproteinated with alkali, then soaked in solutions of the amino acids overnight. Saturated calcium carbonate was made by Kitano's method and used for crystallization by slow evaporation of the saturated calcium carbonate solution. Proteins from the bone of cuttlefish, *Sepia latimanu*s, a mollusk, was included in one experiment. X-ray diffractometer (XRD) and Scanning Electron Microscopy (SEM) images were used to investigate the polymorph and morphology of the crystals that resulted.

Data and Discussion

The chitin extracted was pure, as was confirmed by the comparison of the Fourier Transform Infrared Spectroscopy (FTIR)-spectra with that reported in the literature. XRD was employed to identify the resulting crystal polymorph from chitin funtionalised with Glu and Asp. The presence of the diffraction peak at $2\theta \approx 29^{o}$ for calcium carbonate grown over chitin in absence of amino acids, indicated that the polymorph formed was calcite. Mineralization of $CaCO_3$ over Glu functionalised chitin nucleated pure calcite as can be seen from the diffraction peaks. Interestingly, no diffraction peak was observed with $CaCO_3$ grown over chitin functionalized with Asp,

suggestive of inhibition of the nucleation of calcite. From the experiment with proteins from cuttlefish, the presence of the proteins over the chitin-Glu assembly highly influences the morphology of the crystals nucleated, suggesting that the proteins play an important role in the biomineralisation of mollusks.

Conclusion

Our experimental results proved that the chitin extraction from squid pens gives very high yields of the biopolymer, indicating that squid pens can be a rich chitin source. Chitin is a low-cost biopolymer with potential applications in the fields of medicine and materials science. The isolated chitin has proved to be an effective scaffold for mineral growth. Glu over chitin is shown to induce nucleation of calcite whereas Asp functionalized-chitin probably favours formation of amorphous phase. This is interesting in that, the acidic amino acids being structurally similar could exercise such high controls on the polymorph selectivity. Also, we attempted to explain the biomineralisation of mollusks by using a chitin scaffold and proteins isolated from a mollusk, *Sepia latimanus*. It was inferred that mollusk proteins did significantly influence the morphology of the crystals nucleated thus suggestive of playing an important role in biomineralisation. Further experiments are underway to ascertain all our present assumptions