A Novel Drug Delivery System into Fish?

In Vitro and *In Vivo* Evaluation of Fish Vaccine Nano-Carriers

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Aims:

- To optimise a nanomaterial-based vaccine delivery system into fish against Tenacibaculosis
- To address the efficacy and efficiency of vaccine delivery system using different nano-carriers
- To prepare for the immune response assessment in cells and fish (*in vivo)* after vaccine delivery

Introduction:

Tenacibaculosis is a threatening bacterial infection caused by *Tenacibaculum maritimum,* which affects a significant number of marine fish in recent years. They are associated with macroscopic skin lesion, eroded mouth, necrosis, septicaemia and ulcerative condition in particular. To build up the immune response against this pathogen, bacterial outer membrane protein is used as vaccine component and this research will focus on how to modify and deliver the vaccine into model fish, Seabass. Halloysite, nano-clay is selected to be an optimal carrier system due to its large surface area, tubular shape and good biocompatibility.

Grafting Process:

Halloysite (**Clay**)
↓
Halloysite + APTES (**Clay APTES**)
↓
Halloysite + APTES + Glutaraldehyde
↓
Halloysite + APTES + Glutaraldehyde + Chitosan (**Clay Chitosan**)
Halloysite + APTES + Glutaraldehyde + Peptide (**Clay Peptide**)

Methods:

1. Characterisation of Materials:
- Fourier Transform Infrared Spectroscopy (FTIR), Zetasizer, Field Emission Scanning Electron Microscope (FESEM)

2. *In Vitro* Experiments:
- Measure the sustained delivery kinetic of Bovine Serum Albumin (BSA- standard protein) at pH 5 and pH 7
- Green Fluorescence Protein (GFP) nanoparticle delivery and Resazurin Test

3. *In Vivo* Preparation:
- Fish Immersion into vaccine solution

Results & Discussion:

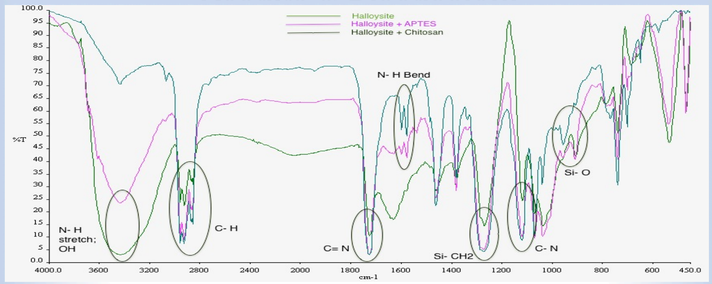
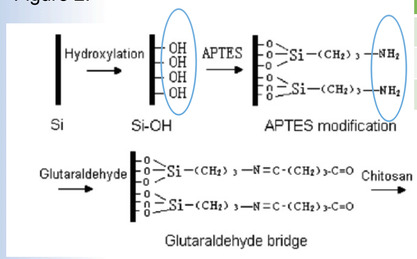


Figure 1:
FTIR: As compared to **clay**, **clay APTES** and **clay chitosan** have more distinct peaks at around 2850- 3000cm^{-1} (C-H bond) and 1600cm^{-1} (N-H bond)with two sharp bands. Besides, **clay** also has strong, broad peaks at approximately 3400 cm^{-1} with the contribution of O-H bond.

- However, there are some unnecessary peaks present in the graph due to KBr pellet contamination

Figure 2:



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Nanoparticles	Zeta Potential (mV)
Clay	-21.5
Clay APTES	+26.3
Clay Chitosan	+32.5

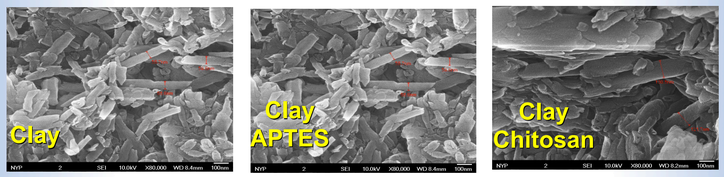
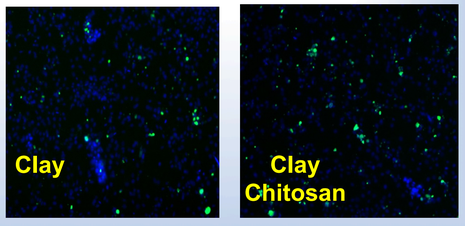


Figure 3:
FESEM: **Clay** has an average diameter of 57.6cm whereas 64.1cm in **clay APTES**. **Clay chitosan** has the greatest average diameter of 126.3cm.

Figure 4:
GFP Delivery: **Clay chitosan** has more green fluorescence than clay itself. This shows that **clay chitosan** exhibits better efficiency in delivering drug into cells than **clay**.



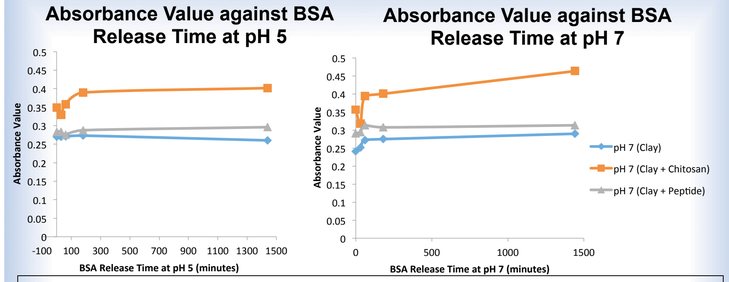


Figure 5: **Clay Chitosan** shows a higher amount of BSA release in relative to **clay** and **clay peptide**. The BSA release in **clay chitosan** is also higher in pH 5 than pH 7. It was believed that the drug was retained longer in acidic condition and showed sustained behaviour in biological activity.

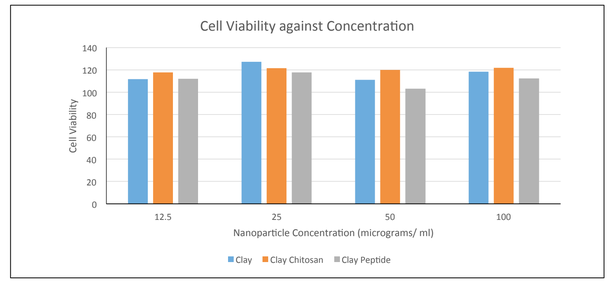


Figure 6:
Resazurin Test: It was shown that neither of the nanoparticles showed toxic effects to the BF2 cells. In spite of different concentrations of nanoparticles administered to the cells, they were still alive. **Clay chitosan** shows the most stable cell viability across different nanoparticle concentrations.

- During *in vivo* experiment, fish were immersed in seven different solutions and some of them experienced stress and were dead after staying overnight
- RT- PCR and the rest of *in vivo* experiments are currently being done by other colleagues

Conclusion:

- C**lay chitosan** is an optimal nano-carrier to be used in delivering BSA protein into fish as compared to **clay** and **clay peptide**
- It is possibly a better drug delivery system due to its good biocompatibility, no toxicity, yet higher, sustained and controlled release of drug (efficiency and efficacy shown)
- Future works include assessment of immune response and fish cells using RT- PCR (mainly *in vivo* part)

References:
- Bermudez R., Failde L.D., Losada L.P. *et al.* (2013) "*Tenacibaculum Maritimum* Infection: Pathology and Immunohistochemistry in Experimentally Challenged Turbot" *Microbial Pathogenesis* **65**: 82-88.

If you are interested, please contact me at s.s.winnie-hii@newcastle.ac.uk for further details. Thank you.