FACULTY OF BIOSCIENCE ENGINEERING

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POLYMER MEDIATED DELIVERY OF dsRNA IN LEPIDOPTERA FOR RNAi-BASED CONTROL OF PEST INSECTS

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Introduction

RNA interference (RNAi) for insect pest management has gained significant interest, however several limitations have been encountered for the Lepidoptera order. These include cellular uptake from the gut environment and dsRNA stability in the digestive system, due to enzymatic degradation and a high pH (>pH 9). This study investigates the potential for nanoparticles to improve the delivery of double-stranded RNA (dsRNA) by preventing its nucleolytic degradation and increasing the cellular uptake.

Materials and methods

Optimal ratio determination

Determined by gel retardation. The polymer:dsRNA (polyplex) charge ratio (N/P ratio), was calculated as the molar ratio of the polymer amino charges (N) to the phosphate (P) groups of dsRNA.

Protection assay

Gut juice from the lepidopteran, army beetworm, (*Spodoptera exigua)* was collected in pH 11 PBS and the polyplex was incubated in it for different time points.

Cellular uptake

The polyplex and the naked dsRNA were incubated in a continuous cell line of Spruce budworm (*Choristoneura fumerana*) midgut cells (CF203) and confocal images were obtained after 4 hours of incubation.

Results and discussion

Complexation was successful at different N/P ratios. At N/P ratio 2/1, no bands are visible on the agarose gel therefore all the dsRNA is complexed with the polymer, G23 (Fig 1A). For the *ex vivo* assays, 2:1 N/P ratio was used. After incubation, the polyplexes were decomplexed and the naked dsRNA was run on 1.5% agarose gel to observe the stability. Results show a lasting stability from 1 to 30 hours (Fig 1B).

Confocal microscopy results showed no significant difference in the uptake between naked dsRNA and polyplex after 4 hours of incubation (Fig 2.). Furthermore, the polyplex is mostly uptaken as a complex. However, naked dsRNA can also be observed inside the cells. Regarding the location, the polyplex appears to be concentrated in late endosome/lysosomes (Fig 3.)

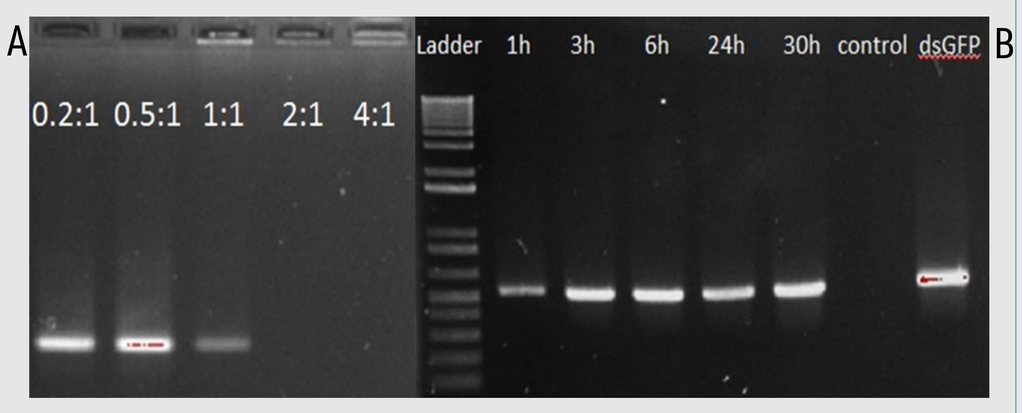

Figure 1. Characterization of activity of G23 and ability to protect dsRNA against nucleolytic degradation. **(A)** Complexation of the polymer G23 with the dsRNA at different polymer:dsRNA charge ratios (0.2:1, 0.5:1, 1:1, 2:1 and 4:1) **(B)** dsRNA polyplexes (2:1 N/P ratio) were incubated in collected gut juice for different time periods (1h-30h) to investigate the stability of the dsRNA when complexed with the polymer,G23. The control represents naked dsRNA incubated in the gut juice.

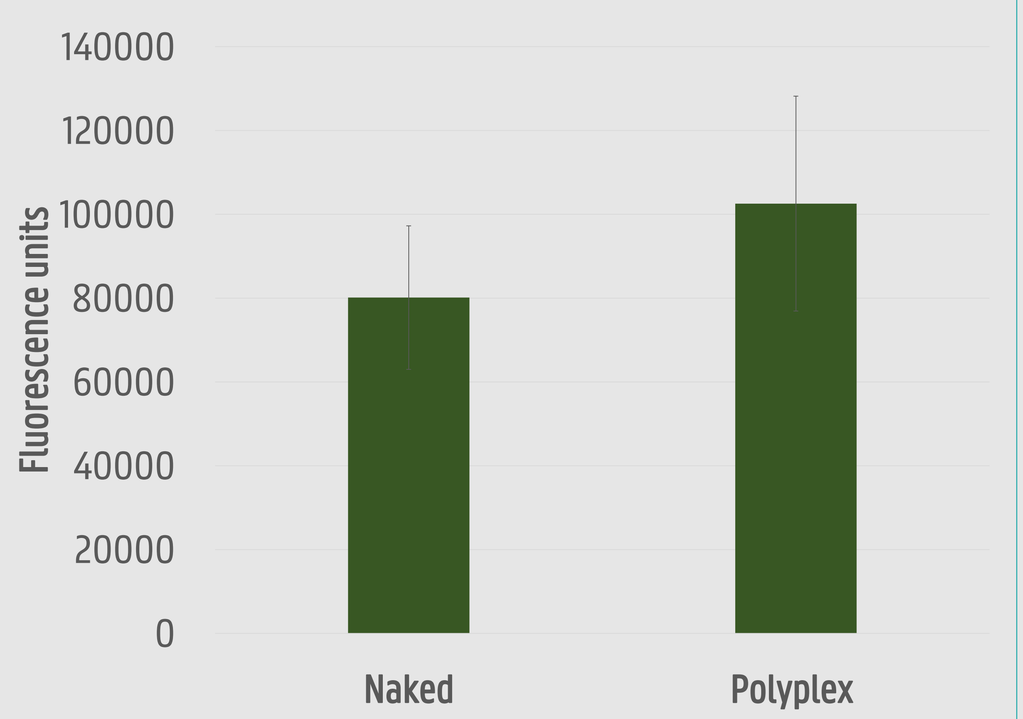

Figure 2. Corrected Total Cell Fluorescence (CTCR) between treatments (p=0.514). Independent samples t-test was used to compare means between the two groups.

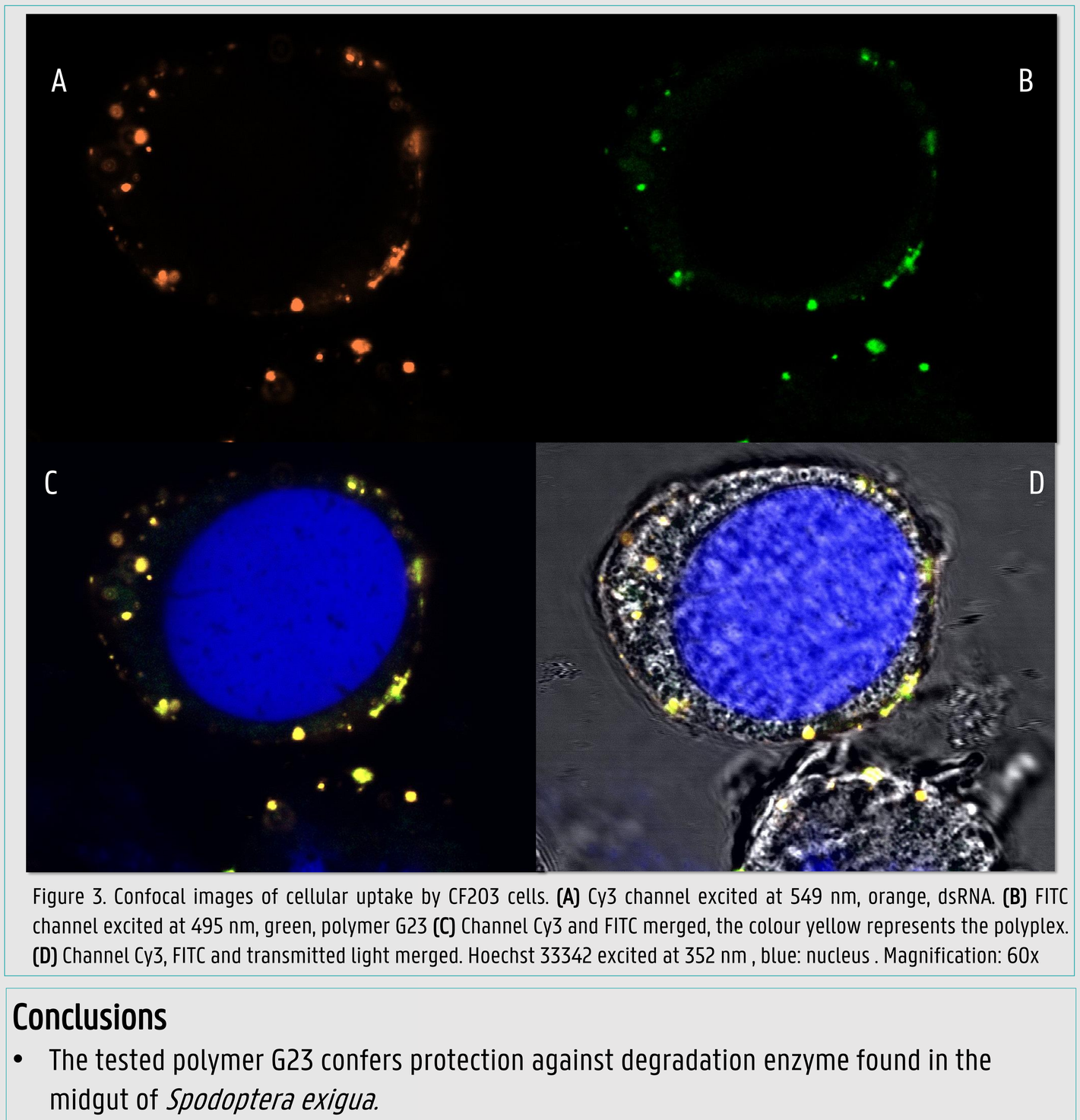

Figure 3. Confocal images of cellular uptake by CF203 cells. **(A)** Cy3 channel excited at 549 nm, orange, dsRNA. **(B)** FITC channel excited at 495 nm, green, polymer G23 **(C)** Channel Cy3 and FITC merged, the colour yellow represents the polyplex. **(D)** Channel Cy3, FITC and transmitted light merged. Hoechst 33342 excited at 352 nm , blue: nucleus . Magnification: 60x

Conclusions

- The tested polymer G23 confers protection against degradation enzyme found in the midgut of *Spodoptera exigua.*
- More research is necessary to quantify the amounts of dsRNA taken up inside the target cells and also on the localization intracellularly.

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