Inflammation and cell toxicity, impact of chitosan acetylation pattern

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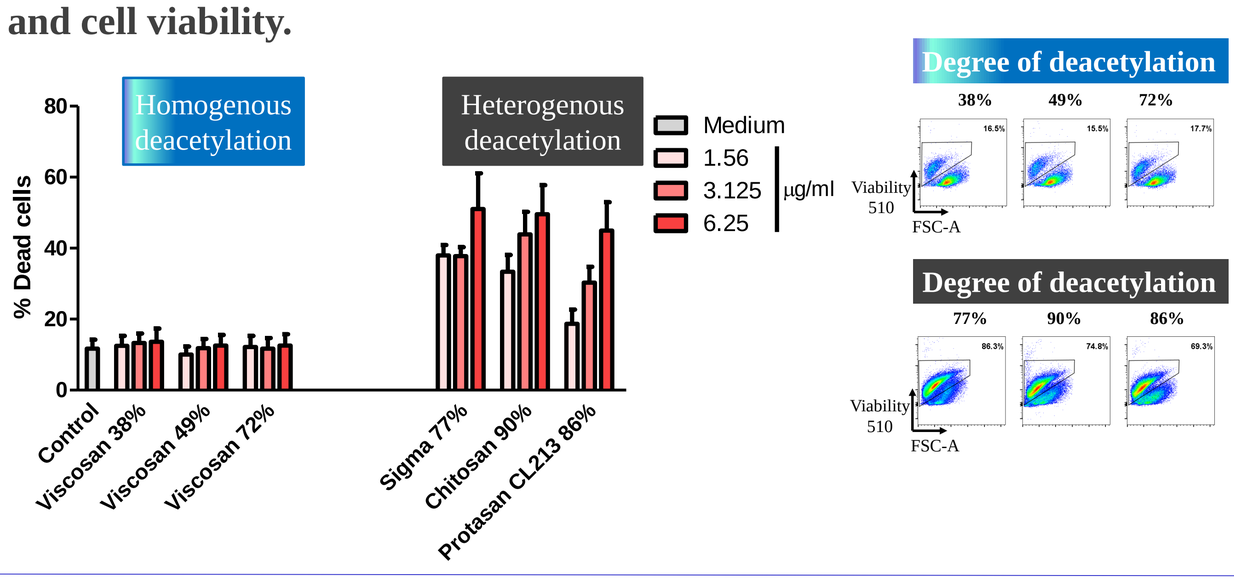
Background

It is well known that there are physical and biological differences between homogenously and heterogeneously deacetylated chitosan, e.g their solubility[1,2] and biodegradation rate[3]. However, their role in inflammatory reactions is still debatable and the scientific literature is contradictive and often inconclusive. Is inflammation induced by chitosan or impurities therein and how correlates the magnitude of an inflammatory response to the degree of acetylation, the molecular weight and the physical form of a chitosan preparation? Many studies have been performed to bring clarity in this matter but most often the outcome of these investigations have been inconclusive and overshadowed by uncertainties related to impurities or poor characterization. Can inflammation and cell toxicity be controlled or even eliminated by selection of the right chitosan[4]? In this study we have used well characterized chitosan/Viscosan[5] preparations having different types of acetylation pattern and studied their impact on inflammatory markers and cell viability.

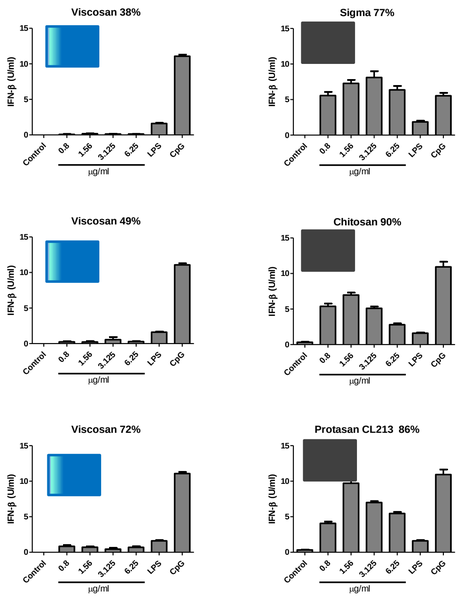
Cell viability

BMDCs were stimulated with medium, Viscosan or chitosan, Protasan CL213 at a range of concentrations (1.56, 3.125, 6.25 µg/ml) for 24h. Cell death was measured by Viability 510



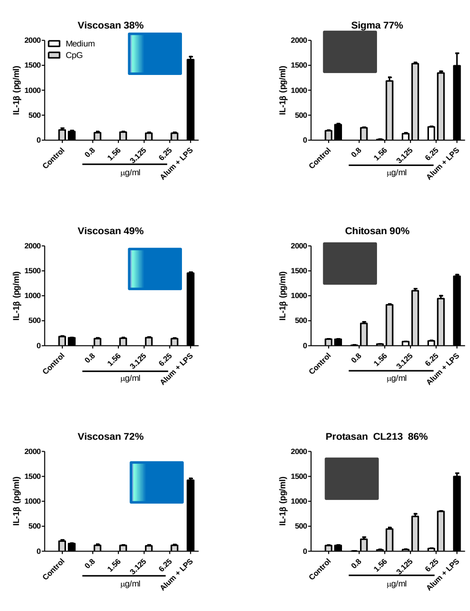
Inflammatory markers

IFN-β secretion by BMDCs



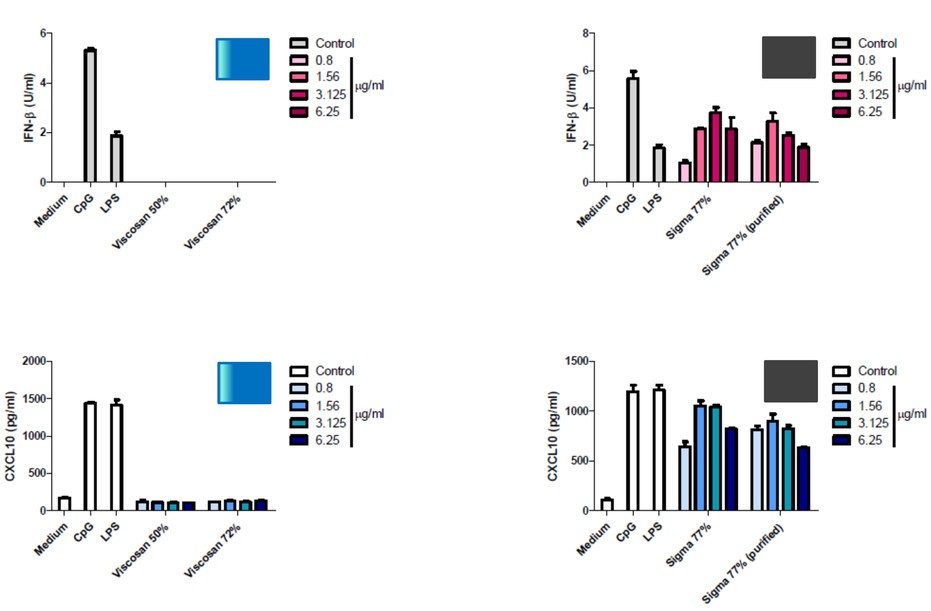
BMDCs were stimulated with medium, CpG (4 µg/ml), LPS (10 ng/ml) or chitosan's (0.8, 1.56, 3.125, 6.25 µg/ml) for 24h. Levels of IFN-B were measured by ELISA.

Inflammasome activation



BMDCs were stimulated with medium, CpG (4 µg/ml), LPS (10 ng/ml) or chitosan's (0.8, 1.56, 3.125, 6.25 µg/ml) +/- CpG for 24h. Levels of IL-1B were measured by ELISA.

Induction of type I interferon secretion



BMDCs were stimulated with medium, CpG (4 µg/ml), LPS (10 ng/ml), or chitosan's (0.8, 1.56, 3.125, 6.25 µg/ml) for 24h. Levels of IFN-B and CXCL-10 were measured by ELISA.

Chitosans

Type of* material	DD % ^{1}H-NMR	Viscosity (1% HOAc)	~ Mw (D)	Endotoxin EU/g
Viscosan	38	100 mPas	250 000	n.a. (<20)
Viscosan	49	490 mPas	600 000	n.a. (<20)
Viscosan	50	570 mPas	650 000	n.a. (<20)
Viscosan	72	120 mPas	260 000	n.a. (<20)
Viscosan	77	1380 mPas	<1 000 000	n.a. (<20)

Homogeneously Deacetylated Chitosans

** Viscosan is a homogenously deacetylated chitosan*

Type of material	DD % ^{1}H-NMR	Viscosity (1% HOAc)	~ Mw (D)	Endotoxin EU/g
Chitosan	91	1400 mPas	<1 000 000	n.a
Sigma (LMW) STBF8219V	77	146 mPas	300 000	<100
* (LMW) STBF8219V	77	80 mPas	200 000	<25
Protasan CL213	86	-	296 000	n.a

Heterogeneously Deacetylated Chitosans

Conclusions

- **Chitosan polymers with a heterogenous deacetylation pattern are more toxic to cells *in vitro* compared to those with a homogenous pattern of deacetylation which induce minimal toxicity**
- **Chitosan polymers with a heterogenous pattern of deacetylation induce an inflammatory response as measured by type I interferon secretion and inflammasome activation compared to those with a random pattern of deacetylation that appear to lack the ability to induce an immune response**
- **The results obtained in this study indicate that both the deacetylation pattern and the degree of deacetylation is of great importance for the immunological responses, whereby chitosans with a homogenous distribution and low degree of deacetylation are non-inflammatory and non-toxic in nature compared to highly deacetylated, heterogenous chitosans.**

References

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