WBC 2020 virtual
11TH WORLD BIOMATERIALS CONGRESS
11 - 15 December 2020

Biocompatibility of pharmacotherapeutic nanogels for the treatment of arthrosic articulations

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INTRODUCTION

- Osteoarthritis (OA) is a degenerative disease of the cartilage, affecting over 300 million people worldwide left without curative treatment[1,2]
- Synergic antagonism of ETA and BKB1 receptors by two peptides can reduce nociception and regenerate the cartilage in an OA rat model[3]
- However, the avascular nature of the cartilage leads to the poor control of drug delivery in it
- Nanogels show interesting properties for osteoarticular diseases such as[4,5]:
  - targeted delivery of IPA
  - high-water content
  - a biopolymeric matrix mimicking the ECM

AIM

To develop biocompatible and biodegradable biopolymeric nanogels (NGs) for the local administration of therapeutic peptides to increase their effectiveness for the long-term treatment of OA

METHOD

1. **Chitosan/Hyaluronic acid NGs synthesis by ionic gelation** from a negatively charged hyaluronic acid (HA) solution and a chitosan (CS) solution, positively charged into an acidic solution of :
   - citric acid 10% (w/v) → NGs CS-CA10
   - acetic acid 2% (v/v) → NGs CS-AA2
2. *In vitro* toxicity assessment of the two formulae by **MTS/LDH assays** on human primary osteoblasts (OB), chondrocytes (CD) and synoviocytes (SYN) :
   - dose-response : up to 400 ug/mL of NGs
   - time-response : 24h, 48h and 72h
3. *In vivo* toxicity assessment of the two formulae on *Danio rerio (*zebrafish) **embryos** regarding **survival**, **hatching** and **malformations** rates for 4 days.

RESULTS

1. Blank NGs synthesis by ionic gelation

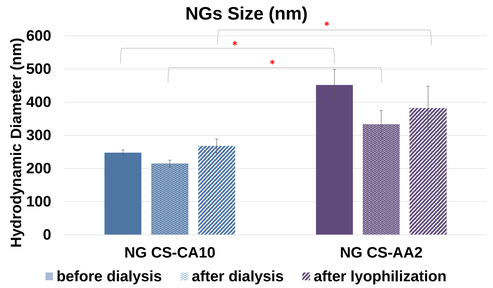

*Figure 1: NGs size (nm) before and after dialysis and after lyophilization and reconstitution; n = 3 batches for NG CS-CA10 and n= 4 batches for NG CS-AA2; * p < 0,05, Student T-test*

	NG CS-CA10				NG CS-AA2			
	Size (nm)	Pdi	ZP (mv)	pH	Size (nm)	Pdi	ZP (mv)	pH
before dialysis	248 ± 8	0,18 ± 0,02	28 ± 3	1,91 ± 0,01	452 ± 47	0,28 ± 0,03	55 ± 7	3,31 ± 0,02
after dialysis	215 ± 10	0,24 ± 0,03	27 ± 3	4,16 ± 0,23	333 ± 42	0,32 ± 0,02	37 ± 6	5,83 ± 0,16
after lyoph.	268 ± 21	0,26 ± 0,02	27 ± 8	3,86 ± 0,41	382 ± 66	0,32 ± 0,02	40 ± 10	5,36 ± 0,58

Table 1: NGs size (nm) and PolyDispersity Index (PdI) obtained by dynamic light scattering DLS, Zeta Potentiel (ZP) obtained by electrophoretic light scattering ELS, Nanobrook Omni, Brookhaven, n = 3 batches for NG CS-CA10 and n=4 batches for NG CS-AA2

2. *In vitro* toxicity studies

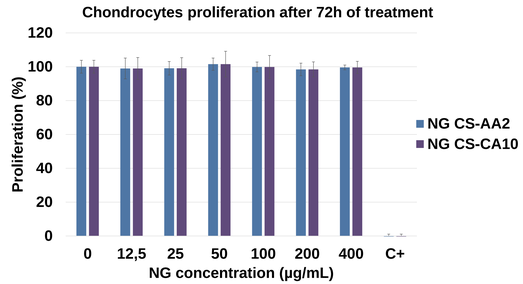

Figure 2: Relative proliferation of chondrocytes 72h post-treatment. Proliferation was assed by an MTS assay kit and proliferation percentage was calculated as follow : % proliferation = ($Abs_{treated\ group}$/$Abs_{control\ group}$)x100. n = 3 independent experiments for each dose and formula in quadruplets

MTS results at 24 and 48 hours were similar and there was no significant difference in proliferation rate for both formulae at all doses compared to the control group. LDH assays confirmed these results showing no sign of significant membrane leakage when treated with the NGs.

3. *In vivo* toxicity studies

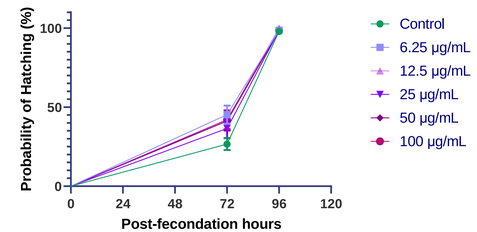

Figure 3: Hatching probability of zebrafish (Danio rerio) embryos treated with NG CS-AA2. Hatching probability was obtained from Kaplan-Meier curves with logrank test, Hatching curves were significatively different from control (P value < 0,05). n = 2 independent experiments per dose and per formula, including 20 embryos per dose and per test.

Hatching probability of zebrafish embryos treated with NG CS-CA10 did not show significant difference with the control group (Kaplan-Meier curves, Logrank test; p > 0,05).

Survival probability of zebrafish embryos treated either with NG CS-AA2 or CS-CA10 was not significatively different from untreated animals (Kaplan-Meier curves, Logrank test; p-value > 0,05), similarly to the malformation rate at 96 pfh (one-way ANOVA test, Dunnett's comparison, p > 0,05).

CONCLUSIONS

- Toxicity of our preliminary results has successfully been avoided by optimizing the synthesis and purification process of the NGs.
- Both formulae demonstrated good biocompatibility towards chondrocytes, synoviocytes and osteoblasts cells, and *Danio rerio* zebrafish embryos.
- NGs seem to enhance the hatching step of embryos for both NG CS-CA10 and CS-AA2, but without inducing any over-mortality and over-malformation.
- NGs CS-CA10 seems to be the most promising formula, allowing good NGs size control and safe use at the same time.
- NG CS-AA2 could also be used since the pH is less acidic than for NG CS-CA10, and is also safe for its use in osteo-articular applications.

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ACKNOWLEDGEMENTS

We would like to thank Fonds de Recherche Nature et Technologies (FRQNT) for their financial support

Fonds de recherche Nature et technologies Québec

We would also like to thank Dylan Bergozza and Noémie Duchanaud for their implication in the project

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