Collaboration with NUS MD6 iHealthTech in developing anti-IL11 antibody (X203) Layer-by-Layer (LbL) Nanoparticles to achieve Prolonged Sustained Released Effects for Therapeutic Applications.

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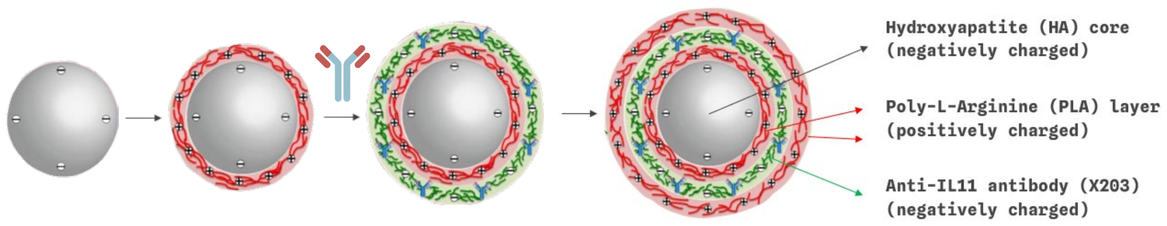
Abstract

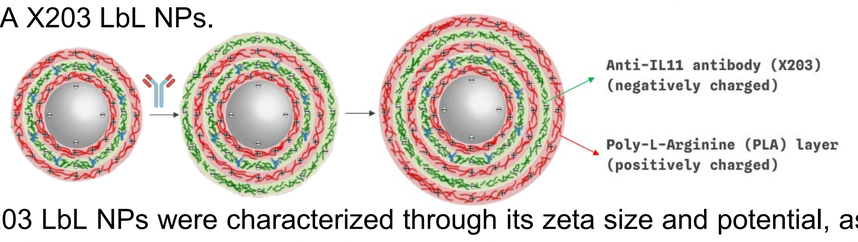
This project aims to design and fabricate anti-IL-11 antibody (X203) layer-by-layer (LbL) nanoparticles (NPs) to achieve prolonged sustained released effects for therapeutic applications. Anti-IL-11 antibodies (X203) were incorporated into the layer-by-layer nanoparticles (NP) system utilized as nanocarriers. Fabricated single-layered X203 LbL NPs had characterization values of 441.80 ± 20.90 in hydrodynamic size, a polydispersity index (PDI) of 0.30 ± 0.16, and a surface charge of 27.00 ± 1.15mV. While fabricated double-layered X203 LbL NPs had characterization values of 439.17 ± 8.49 in hydrodynamic size, a polydispersity index (PDI) of 0.39 ± 0.15, and a surface charge of 10.45 ± 2.68mV. The successfully fabricated X203 LbL NPs were then used to conduct release studies to determine their release activity, providing a controlled release effect of up to 14 days for single-layered X203 LbL NPs, and up to 70 days for the double-layered X203 LbL NPs. Thus, the fabricated LbL nanoparticle systems meet the project's objective of achieving a prolonged sustained release effect.

Design/Methods

Hydroxyapatite (HA) is used as the core structure as it is negatively charged due to the presence of hydroxide ions. HA particles were used for the LbL NPs as it is the most stable calcium phosphate derivative which makes it a good carrier for the release of substance. Poly-l-arginine (PLA) is used as a layer due to its unique properties such as its high transfection efficiency and cellular uptake. In addition, it is biocompatible, representing a cationic biopolymer composition of physiologically active L-arginine amino acid. The presence of the guanidinium group is responsible for the net positive charge and hydrophobic character. Antibody X203 is negatively charged to ensure solubility and successful coating between the positively charged PLA layers. Hence, it is prepared in NaCl (0.1M, pH 9.5), causing it to have a net negative charge. This is due to an antibody's isoelectric point (pI) of 8 – 9, where an antibody will carry a net negative charge when the pH is above the pI [1].



The negatively charged structural core HA is coated with the positively charged polymer PLA, followed by the negatively charged anti-IL-11 antibody (X203), and a final coating of positively charged PLA layer to encapsulate the X203. Layers are coated through electrostatic interactions to form the HA X203 LbL NPs.



Subsequently, the HA X203 LbL NPs were characterized through its zeta size and potential, as well as its encapsulation efficiency measurements. Its release studies were also conducted to determine the amount of X203 release per timepoint to ensure prolonged sustained release.

Project Task & Milestones

ID	Task/Activities Name	Apr				May				June				July				Aug				Sept			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Riffaie Fathan Bin Rahim (2104384I)																								
	Fabrication of nanocarriers with 1 layer of X203 loaded																								
1	**Design and Fabrication of X203 Nanocarriers**																								
	a) Determine the appropriate numbers of chemicals and reagents																								
	b) Determine appropriate experimental procedures																								
	c) Design and fabricate the X203 Nanocarriers with characterization testing (Zeta Size and Zeta Potential)																								
2	**Fluorescent Tagging of X203**																								
	a) Using a Lightning-Link kit to fluorescent tag the X203 antibody																								
	b) Dividing tagged X203 into 10 parts of equal amounts for storage																								
3	**Conduct release studies of the HA PLA X203 PLA LbL NP**																								
	a) Replace the release buffer with new buffer																								
	b) Measure the collected release buffer with the TECAN machine																								
	c) Process the results and update the cumulative release graph with the results																								
4	**Report Experimental Content**																								
	a) Report the experimental methodology, results, discussions, and future works																								
5	**Optimize the Single-layer X203 loaded nanocarriers to promote higher sustained released effects**																								
	a) Calculate and change the required amounts of chemicals, reagents and experimental procedures used in fabricating and designing of X203 loaded nanoca																								
	b) Measure characterization and release studies of newly formulated X203 loaded nanocarriers																								
	c) Report the experimental methodology, results, discussions, and future works																								
	Nuruddini Hidni Binte Khalid (2101391G)																								
	Fabrication of nanocarriers with 2 layers of X203 loaded																								
1	**Design and Fabrication of X203 nanocarriers**																								
	a) Determine the appropriate numbers of chemicals and reagents																								
	b) Determine appropriate experimental procedures																								
	c) Design and fabricate the X203 Nanocarriers with characterization testing (Zeta Size and Zeta Potential)																								
2	**Fluorescence tagging of X203**																								
	a) Using a Lightning-Link kit to fluorescent tag the X203 antibody																								
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	a) Replace the release buffer with new buffer																								
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4	**Report Experimental Content**																								
	a) Report the experimental methodology, results, discussions, and future works																								
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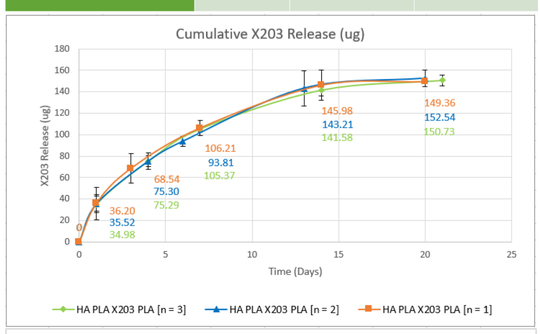
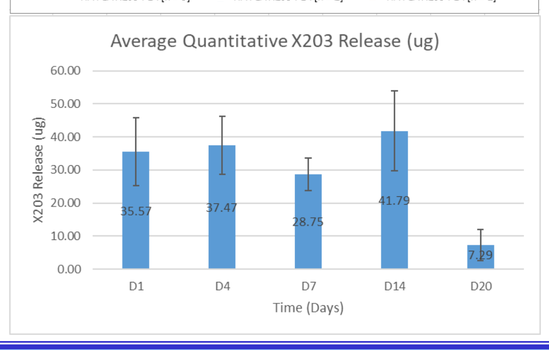
	c) Report the experimental methodology, results, discussions, and future works																								

Results & Data Analysis

Single Layer X203

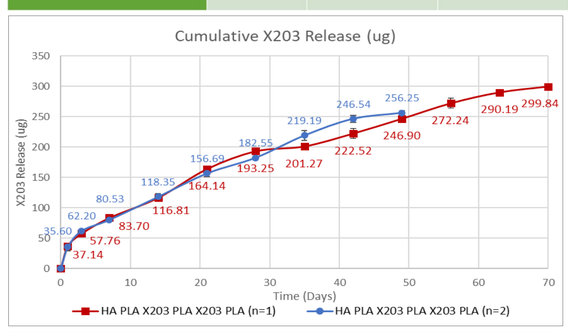
Particle configurations	Zeta size (dnm)	PDI	Zeta potential (mV)
HA| PLA	174.70 ± 3.84	0.24 ± 0.01	31.83 ± 2.20
HA| PLA| X203	363.77 ± 23.02	0.29 ± 0.01	-13.37 ± 1.21
HA| PLA| X203| PLA	441.80 ± 20.90	0.30 ± 0.16	27.00 ± 1.15

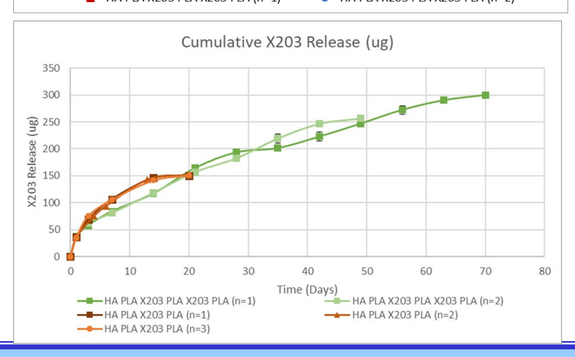




Double Layer X203

Particle configurations	Zeta size (dnm)	PDI	Zeta potential (mV)
HA|PLA	204.40 ± 2.27	0.23 ± 0.01	34.03 ± 0.97
HA|PLA|X203	313.90 ± 2.85	0.19 ± 0.10	-14.03 ± 0.90
HA|PLA|X203|PLA	331.57 ± 0.06	0.31 ± 0.02	22.93 ± 3.18
HA|PLA|X203|PLA|X203	492.07 ± 6.81	0.36 ± 0.04	-4.38 ± 0.50
HA|PLA|X203|PLA|X203|PLA	439.17 ± 8.49	0.39 ± 0.15	10.45 ± 2.68





Discussion/Evaluation

The fabricated HA X203 LbL NPs can be characterized as a nanocarrier as it meets the requirement of below 500nm in size which is small enough to be taken up in cells. In addition, it shows the successive coating of layers and encapsulation from the alternating charges of zeta potential and the increase in hydrodynamic size. Moreover, the particle's final surface charge was positive, giving them the ability to attach efficiently electrostatically onto cellular membranes (negatively charged) and improve cell uptake when dosed to cells. HA is negatively charged due to the presence of hydroxide ions, a PLA layer is used to coat the negatively charged HA as PLA is positively charged due to the presence of guanidino group. To coat X203 electrostatically, it is important to disperse it in the ideal pH-adjusted medium to ensure maximum charge. To do so, its isoelectric point (pI) must be considered, which is essentially the pH at which the antibody has no net electrical charge. When the pH of the dispersant is below pI, the antibody will carry a net positive charge, with the reverse being true. As human antibodies have a pI range of around 8 – 9 [2], the X203 solution is prepared in NaCl (0.1M, pH 9.5), causing it to carry a maximum net negative charge. As a result, the layers were successfully coated through electrostatic interactions hence the alternating charges. Therefore, the HA PLA X203 PLA LbL NPs were successfully fabricated with the desired characterization specifications.

In addition, according to the release studies conducted, the fabricated and characterized triplicates of HA PLA X203 PLA LbL NPs were functioning as intended, as they provide a prolonged sustained release of X203 of up to 14 days for single-layered X203 LbL NPs, and up to 70 days for the double-layered X203 LbL NPs. The prolonged sustained X203 release was made possible with the LbL system. The X203 layer was sandwiched between PLA layers. For the X203 release to happen, the outermost PLA layer must first defoliate. The PLA layers not only act as a platform for slow X203 release, they also help in protecting the X203 from degradation during fabrication and application.

In comparison to double-layered X203 LbL NPs, the release profile of the single-layered X203 LbL NPs shows a higher release rate compared to the double-layered X203 LbL NP. This might be due to the smaller charge of the outermost X203 layer of the double-layer X203 NP (-4.38 ± 0.50mV) as compared to the X203 layer of the single-layer X203 NP (-13.37 ± 1.21mV). The smaller charge of the double-layer X203 NP shows that lesser X203 was coated on the second X203 layer. Hence, the single-layer X203 LbL NPs tend to release more as it has much more X203 coated on than the outer layer of the double-layered X203 LbL NPs.

Conclusions

In conclusion, the project objectives have been successfully met by designing and developing nanocarriers (LbL NPs), using a negatively charged HA as the structural core and positively charged PLA as polymer layers to incorporate anti-IL-11 antibody (X203) with the expected characterization specifications to provide a prolonged sustained release effect. Nanocarriers developed were of hydrodynamic size less than 500nm, with the X203 successfully encapsulated between PLA layers through electrostatic interactions. All in all, the nanocarriers developed were successful as they were able to provide a prolonged sustained release effect of X203 of over 14 days for single-layered X203 LbL NPs, and up to 70 days for double-layered X203 LbL NPs through conducted release studies.

However, these nanocarrier systems can still be improved upon, such as exploring other molecular weight of PLA. In addition, as size of particle and encapsulation is highly depended on parameters of the microfluidic device (IGNITE machine) (flow rate, flow ratio, and concentration), further optimization of microfluidic device setting is also recommended.

References

[1] Schneider, Z. (2017, June 28). Importance of isoelectric point (pi) of antibodies. *The Antibody Society*. [Online]. Available: https://www.antibodysociety.org/new-articles/importance-isoelectric-point-pi-antibodies/

[2] Morga, M., Batys, P., Kosior, D., Bonarek, P., & Adamczyk, Z. (2022). Poly-L-Arginine Molecule Properties in Simple Electrolytes: Molecular Dynamic Modeling and Experiments. *International journal of environmental research and public health*, 19(6), 3588. [Online]. Available: https://doi.org/10.3390/ijerph19063588

Acknowledgement:

The author wishes to thank the collaboration partner National University of Singapore, Institute of Health and Technology for their generosity and kind assistance: Prof. Subbu Venkatraman, Principal Investigator; Dr. Yang Fei Tan, Research Fellow; Mr. Muhammad Aminuddin Bin Anuar, Research Engineer. The author would also like to extend their gratitude towards MP supervisor, Dr. Raja Rangaswamy, Senior Lecturer, for his guidance and support.

MP ID: 1M23323

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