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UNDERSTANDING PLATELET THROMBOGENICITY CASCADE OF THE BIOCOMPATIBLE CHITOSAN-DERIVATES IN VON WILLEBRAND DISEASE

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A	**PROJECT DETAILS**
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B	ABSTRACT OF RESEARCH

UNDERSTANDING PLATELET THROMBOGENICITY CASCADE OF THE BIOCOMPATIBLE CHITOSAN-DERIVATIVES IN VON WILLEBRAND DISEASE

Introduction: Chitosan extracted from the shells of arthropods have becoming one of the most promising local hemostatic agents because it is of particular interest as it functions independently on platelets and normal clotting mechanisms. **Objectives:** This work verified the underlying mechanisms of chitosan-induced platelet thrombogenicity cascades and comprises experimental tests such as degradation ability; coagulation analysis and the investigations of hemostatic mediators: von Willebrand Factor (vWF), Factor 8 (FVIII), Thromboxane A_2 (TXA_2), $P2Y_{12}$, glycoprotein IIbIIIa (GpIIbIIIa), Transforming Growth Factor- Beta 1 (TGF-β1) and Platelet Derived Growth Factor-AB (PDGF-AB) in normal donors and von Willebrand disease (vWD) patients *in vitro*. **Materials and Methods:** Comparative studies have been conducted to measure the hemostatic capacity of biodegradable: 7% N,O-Carboxymethylchitosan (NO-CMC) (with 0.45 mL collagen), 8% NO-CMC, Oligo-chitosan (O-C) and O-C 53. Lyostypt, the topical hemostatic agent was used as a positive control. This study was conducted using scanning electron microscope, enzyme-linked immunosorbent assay, westergren, coagulation analyzer, western blotting and flow cytometry techniques. Fourteen vWD and normal subjects were recruited in this study with provided informed written consent. **Results and Discussions:** O-C type of chitosans are able to enzymatically degrade, possess better porosity and the scaffold pores are sufficient to allow nutrients and cells to enter and by encouraging platelet activities to accelerate hemostasis and wound healing process. O-Cs exert a combined effect on thrombogenesis by causing platelets to adhere, activate, aggregate and forms insoluble fibrin network to strengthen platelet plug formation by elevating the studied mediators.O-C was capable to induce the expression levels of vWF, FVIII and TXA_2 receptor signals. This signaling pathway assists the platelet aggregation. Also, GpIIbIIIa and $P2Y_{12}$ analysis showed that O-C group of chitosan are capable of activating platelets by providing a good surface for blood hemostatic mediators and signals to facilitate thrombin generation. O-C-activated platelets lead to the release of growth factors, mainly TGF-β1 and PDGF-AB. Therefore, this exhibited that greater expression level of O-C group of chitosan assists in mediating wound healing process. **Conclusion:** Tested chitosan-stimulated-mediators potentially initiate the platelet actions and expedite the hemostasis processes *in vitro*. Based on the outcome of this research, the O-C and O-C 53 stimulated hemostasis process and worked better and equal to the commercially available lyostypt in normal donors or subjects and vWD patients *in vitro*.

PEMAHAMAN MEKANISMA SEL PLATELET UNTUK MENGANALISA KEBERKESANAN KITOSAN TERHADAP PENYAKIT VON WILLEBRAND

Pengenalan: Biobahan kitosan diperolehi daripada cengkerang hidupan laut seperti udang dan ketam, mempunyai potensi yang hebat bagi kegunaan klinikal kerana ia dapat bertindak balas dengan sel-sel platelet secara bebas bagi membantu proses hemostasis. **Objektif Kajian:** Penyelidikan ini mengesahkan kebolehan biobahan kitosan untuk merangsangkan mekanisma platelet terhadap penderma darah normal dan pesakit von Willebrand disease (*vWD*) *in vitro*. Ujian eksperimen penting seperti menguji kebolehan degradasi; analisis koagulasi dan analisis pengantara hemostatik: *von Willebrand Factor (vWF)*, Faktor 8 (FVIII), *Thromboxane A_2 (TXA_2), $P2Y_{12}$, glycoprotein IIbIIIa (GpIIbIIIa), Transforming Growth Factor- Beta 1 (TGF-β1)* dan *Platelet Derived Growth Factor-AB (PDGF-AB)* telah dijalankan. **Bahan dan Kaedah kajian**: Kajian perbandingan telah dijalankan dengan menggunakan dua jenis kitosan yang terdiri daripada *7% N,O-Carboxymethylchitosan (NO-CMC)* (dengan 0.45 mL kolagen), *8% NO-CMC, Oligo-chitosan (O-C)* dan *O-C 53. Lyostypt* yang memainkan peranan sebagai agen hemostatik topikal telah digunakan sebagai kawalan positif. Kajian ini dijalankan dengan menggunakan teknik-teknik ujikaji seperti *enzyme-linked immunosorbent assay, westergren, coagulation analyzer, western blotting, flow cytometry* dan *scanning electron microscopy.* Seramai 14 orang orang pesakit *vWD* dan individu biasa telah direkrutkan dengan keizinan bertulis yang disediakan. **Hasil Kajian dan Perbincangan:** Hasil kajian ini menunjukkan bahawa kitosan jenis O-C mempunyai ciri-ciri biodegradasi serta memiliki keliangan *(scaffold)* yang lebih baik. Liang *scaffold* ini membolehkan nutrien dan sel-sel menembus keluar dengan menggalakkan aktiviti platelet untuk mempercepatkan proses hemostasis dan proses penyembuhan luka. O-C memberi implikasi positif dengan menyebabkan platelet melekat, mengaktifkan, menggumpal serta membentuk rangkaian fibrin larut untuk mengukuhkan pembentukan platelet plug dengan merangsangkan mekanisma pengantara hemostatik yang telah dikaji. O-C mampu merangsangkan reseptor *vWF*, *FVIII*, *TXA_2*, *GpIIbIIIa*, *$P2Y_{12}$*, *TGF-β1* dan *PDGF*-AB bagi mempercepatkan aktiviti platelet. Dalam pada masa yang sama, analysis juga menunjukkan yang O-C mengaktifkan aktiviti platelets dengan menyediakan permukaan yang baik untuk mediator hemostatik darah bagi memudahkan generasi thrombin dan penyembuhan luka. **Kesimpulan:** Berdasarkan hasil kajian ini, O-C dan O-C 53 mampu berrfungsi lebih baik dan sama dengan lyostypt bagi merangsang proses hemostasis dengan mengaktifkan reseptor *vWF*, *FVIII*, *TXA_2*, *GpIIbIIIa*, *$P2Y_{12}$*, *TGF-β1* dan *PDGF*-AB di kalangan invidual normal dan pesakit vWD *in*

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