Peptide self-assembly and its effect on hydrogel properties, platelet and complement activation

Aditi Saini and Larry D. Unsworth
Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, Canada
National Research Council (Canada)-National Institute for Nanotechnology, Edmonton, Alberta, Canada

Statement of Purpose: Ionic self-assembling peptides have immense potential for many cardiovascular nanomedicine applications. Particularly, $RADA_4$ (Type I), $[COCH_3]$-RADARADARADARADA-$[CONH_2]$, has been shown to form antiparellel β – sheets, which further self-assemble into nanofibers that then develop into a viscoelastic 3D matrix in aqueous solution. Specifically, amino acids of various physicochemical properties are added to the self-assembling peptide domain to determine their effect on nanofiber properties, platelet and complement activation, for understanding the host response to biomaterials. Peptide nanofibers will be characterized so as to determine their charge and hydration state. However, the effect that nanofiber-water interactions have upon the fate determining molecular-level interactions between these nanofibers and plasma proteins are largely unknown. This information is of critical importance as it is well understood that plasma protein-surface interactions can lead to the initiation of many deleterious host responses, including platelet activation. Thus, the goal of this study is to understand how peptide physico-chemical properties ultimately affect peptide self-association, vicinal water structure platelet and complement activation. Specifically, the C-terminus of $RADA_4$ peptides will be modified to include five repeats of lysine ($RADA_4$-K_5) and serine ($RADA_4$-S_5), so as to investigate the effect of polar amino acids on peptide self-assembly, vicinal water structure, plasma protein interactions and platelet activation. The ultimate goal of this research study is to correlate material properties to platelet and complement activation, which is of fundamental importance to the development of peptide based materials for a plethora of nanomedicine applications.

Methods: $RADA_4$ of 95% purity was obtained from Alberta Peptide Institute (Edmonton, Canada) and used without further purification. $RADA_4$-S_5 and $RADA_4$-K_5, with respective purities of 88% and 95%, were obtained from SynBioSci (Livermore, CA). Circular Dichroism (CD, Jasco J-810) spectra (180-260 nm) was collected on samples prepared by diluting stock peptide solution in water to a working concentration of 0.5% (w/v) of $RADA_4$, and analyzed at room temperature. Differential Scanning Calorimetry (DSC, TA DSC/TGA Thermal Analyzer) was conducted on samples prepared by diluting stock peptide solution in PBS (pH 7.4) to a working concentration of 0.5% (w/v) of $RADA_4$. Platelet and complement activation studies were conducted according to well established protocols described by Kainthan *et.al.* (2007). Briefly, nanofibers were incubated in platelet rich plasma at 37°C, and aliquots of the mixture removed at 10, 30 and 100 min. Platelet activation marker expression (3 donors) of CD62P and the pan-platelet marker CD42 was assessed using a double staining method. To assess complement activation, the cleavage of complement component C3 was investigated by measuring the formation of its activation peptides, C3a and C3a des arg, using a commercial C3a enzyme immunoassay kit. Nanofibers were incubated in plasma (3 donors) at 37°C for 30 min. The morphology of platelets was prepared by the platelet-rich plasma method, and was assessed by modified Kunicki morphology scoring (Kunicki, 1975). One hundred platelets in total were counted (3 donors) and morphology score was calculated as described by Levin *et.al* (2008).

Results: CD studies confirmed the presence of β-sheet structure and hence, nanofiber formation in 100% $(RADA)_4$-I and $(RADA)_4S_5$ but not in $(RADA)_4K_5$. Moreover, hydration state analysis revealed a dose dependant correlation of lysine modified peptides with non-frozen water fraction, which increased with increasing amounts of $RADA_4$-K_5 present. As the lysine residues are positively charged at a pH of 7.4, it is thought that the increase in non-frozen waters is due to a stronger interaction between the positively charged nanofibers and the vicinal water, suggesting that charge localization along the nanofiber does affect the vicinal water structure. Platelet activation studies reveal that $RADA_4$ and $RADA_4$-S_5 have similar activity as those shown for negative controls. However, $RADA_4$-K_5 samples showed a significant activation of the platelets for all 3 donors and exhibited a dose dependency (2 of 3 donors) in response to the presence of CD62 as well as PAC-1. Nevertheless, in complement activation studies, $(RADA)_4$-S_5 activated more of C3a when compared with the rest. Serine resides are expected to be complement activating as free hydroxyl groups are thought to induce complement activation (Law, 1983). Despite the donor to donor variability, the overall trend in C3a expression among the three donors remained similar. Also, when compared with the negative controls, all the hydrogel systems showed a higher C3a activation. Morphology score of 385 and above was achieved for all the systems, implying a high platelet survival upon the treatment with hydrogels.

Conclusions: Although work is on-going, it is apparent that peptide chemistry is pivotal in directing the self-assembly process, nanofiber hydration state, platelet and complement activation.

Acknowledgements: We would like to acknowledge Dr. D. Devine for helpful discussion and advice on biocompatibility studies. We also acknowledge the technical assistance of Dr. K. Serrano in biocompatibility assays. The study was funded by NSERC and the travel award by Canadian Blood Services.

References:

Kainthan RK. Biomat. 2007;28:4581-4590.
Kunicki TJ. Transf. 1975;15:414-421
Law SKA. Ann N Y Acad Sci. 1983;421:246-58
Levin E. Transf. 2008;48:2331-2337