Role of peptide valency on PEG-b-PPS micelles for integrin receptor blocking and anti-angiogenesis

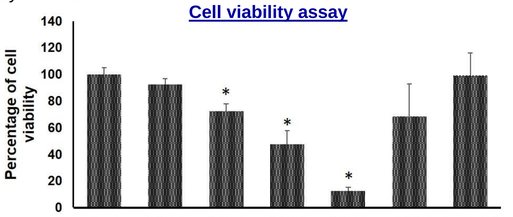
Neha Phani Bhushan[1], Trevor Stack[2], Benjamin Mathew[3], Evan A Scott[2], Mathew T Mathew[1], Divya Bijukumar[1]*

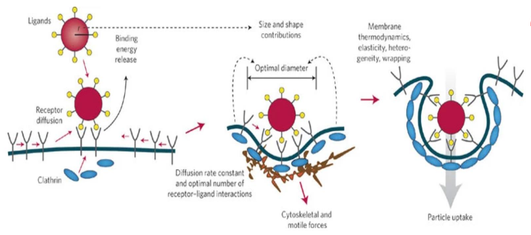
[1]Department of Biomedical Science, University of Illinois College of Medicine at Rockford, Rockford, IL [2]Department of Biomedical Sciences, Northwestern University, Evanston, IL

[3]Department of Ophthalmology and Visual Sciences, University of Illinois Chicago, Chicago, IL; Email: drbiju2@uic.edu

INTRODUCTION

- Anti-integrin therapy was recently established as a promising strategy for angiogenesis-related disease, including Diabetic Retinopathy (DR), Retinopathy of Prematurity (ROP), Age-related macular degeneration (AMD) [1],[2].
- It was previously reported that PEG-b-PPS micelles bound anti-integrin peptide Serine-Aspartic acid-Valine (SDV) demonstrated enhanced anti-angiogenesis at lower peptide concentration [3].
- However, optimization of the surface density of peptides is imperative to improve integrin clustering and subsequent cell signaling.
- The abnormal growth of blood vessels in the eye can be destroyed by cell death or apoptosis.
- Hence for triggering apoptosis, an optimum valency of peptides must be presented on the surface of the micelles to bind to the αvβ3 integrin and block it.





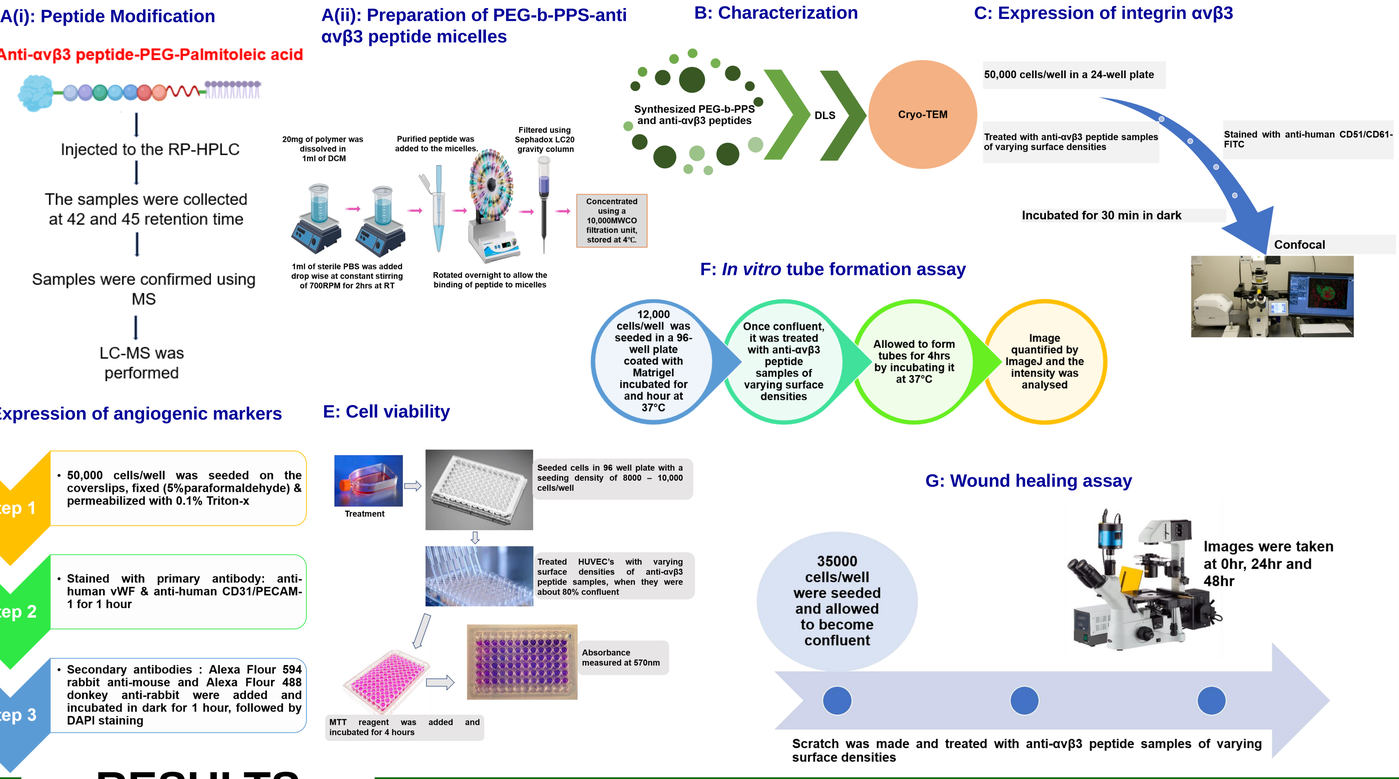
HYPOTHESIS

We **hypothesize** that incorporating an optimum density of anti-integrin peptides on micelles will significantly increase the efficiency of anti-angiogenesis.

OBJECTIVES

The **objective** of this study is to synthesize and characterize PEG-b-PPS micelles with a different surface density of anti-integrin peptides and study the angiogenic ability using primary human umbilical vein endothelial cells (HUVEC).

MATERIALS & METHODS



RESULTS

Liquid Chromatography-Mass Spectrometry (LC-MS)

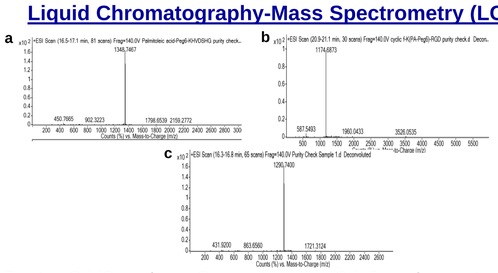


Fig 1: LC-MS analysis. Graph showing LC-MS analysis with the purity of the peptide samples. The peak obtained on the chromatogram co-relates to the molecular weight of the peptide. **(a)** SDV peptide has a mol wt of 1350g/mol, **(b)** SGV- 1291 g/mol, **(c)** cRGD-1174 g/mol

Particle size and Zeta potential

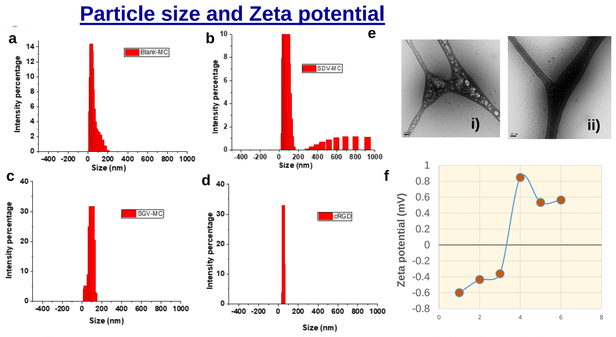


Fig 2: The DLS spectrum represents the average particle size of the peptides. **(a)** Blank micelles **(b)** SDV **(c)** SGV and **(d)** cRGD bound micelles. About 77% of the blank micelles, 90% of the SDV peptide, 89% of the SGV peptide and 96% of cRGD were found to be in the range of 20-50nm. **(e)** Cryo-TEM of (i) blank and (ii) Peptide loaded MCs. **(f)** The zeta potential of MCs lie between -30 to +30mV suggesting that the peptide is stable and the surface potential increases as peptide density increases.

Cell viability assay

Fig 3 : **Cell viability analysis using MTT assay**. Graph showing cell viability of HUVEC cells after 24 h incubation with micelles of different peptide densities, blank micelles compared cRGD bound micelles and control endothelial cells. p<0.05 is considered as significant

Expression of angiogenic markers

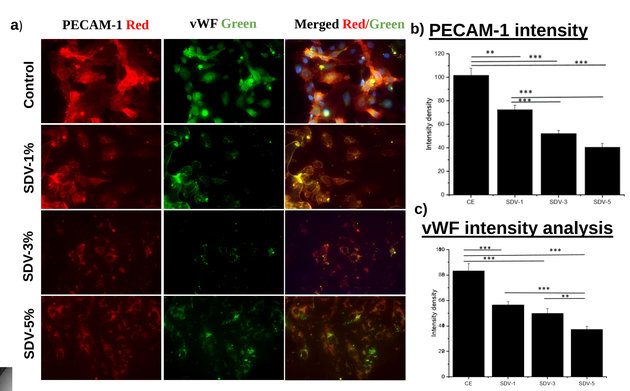


Fig 4:**a)** Immunostaining with angiogenic markers Platelet Endothelial Cell Adhesion molecule and von Wilebrand factor (vWF-red) showing the decrease in intensity from control to SDV-5%. Quantitative expression of **b)** PECAM-1 and **c)** vWF

Invitro tube formation assay

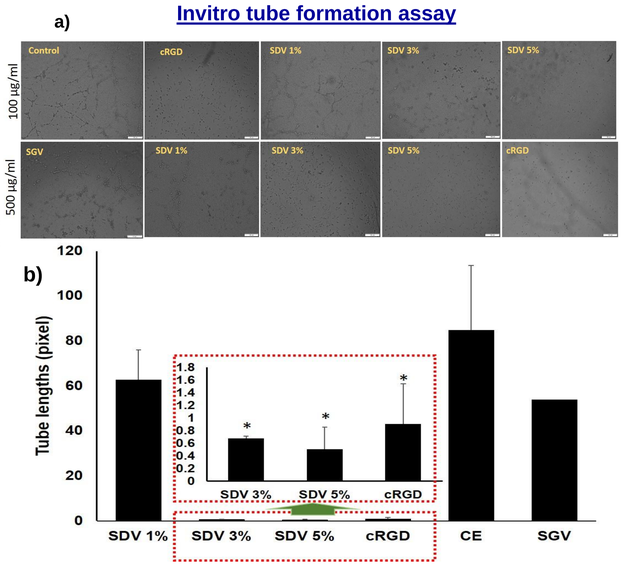


Fig 5: **a)**The in vitro angiogenesis was inhibited in the HUVEC cells by the PEG-b-PPS- anti αvβ3 peptides in a dose dependent manner and **b)** the tube lengths were analyzed using anti-angiogenesis analyzer plugin Image J. p<0.05 is considered as significant

Expression of integrin αvβ3

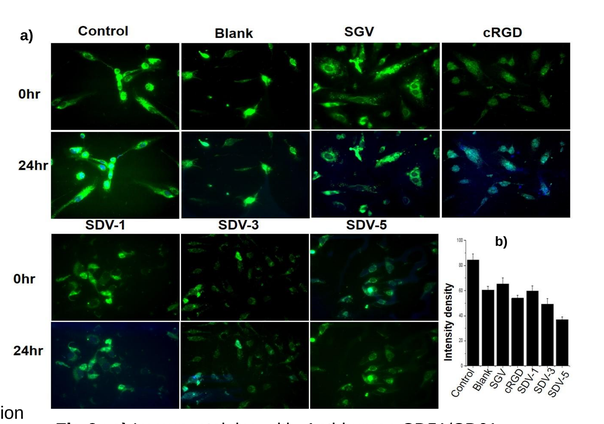


Fig 6 : **a)** Immunostaining with Anti-human CD51/CD61 (Integrin αvβ3) showing the inhibition of integrin αvβ3 by SDV peptides with varying surface densities and comparing it scrambled peptide and cRGD and **b)** quantitative expression of Integrin αvβ3

Wound-healing assay

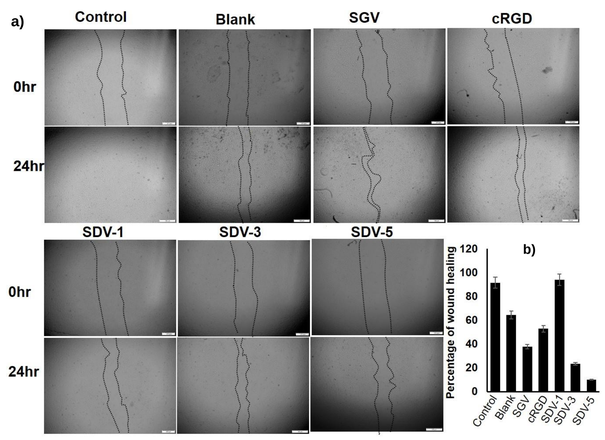


Fig 7: **a)**The inhibitory effect of the PEG-b-PPS anti αvβ3 peptides on migration of HUVEC's after 24hrs of treatment and **b)** the %of wound healing analyzed using ImageJ software. SDV-5% showed maximum inhibition than SDV-3% and SDV-1%.

DISCUSSION

- LC-MS determined the purity of the peptide samples and the DLS confirmed the particle size of the peptides to be between 20-50nm.
- The expression of angiogenic markers vWF which is responsible for the formation of blood vessels and PECAM-1 which is responsible for vascular integrity confirmed that the HUVEC's used in our experiment have angiogenic properties . A significant increase in the inhibition of vWF and PECAM-1 was also observed when the surface density of the peptides was increased from 1% to 5%.
- Similarly with the increase in the peptide density from SDV-1% to SDV-5%, the increase in the inhibition of integrin αvβ3 was observed. This led to the decrease in the intensity of the anti-human CD51/CD61 stained cells.
- At 500µg/ml concentration, SDV-1, 3, and 5 induced significantly higher toxicity (27.71±5.43%, 52.54±10.48%, 87.27±2.70%, respectively) compared to control. However, cRGD-MC showed approximately 40% toxicity at both concentrations studied, whereas random peptide SGV-MC did not show significant toxicity at any concentrations studied.
- SDV-5 showed inhibition of tube formation when compared to SDV-3 and SDV-1 suggesting that better anti-angiogenic effect can be expected from SDV-5%.
- Similarly, the migration of the HUVEC's in the wound healing assay saw maximum inhibition by SDV-5% than SDV-3 and SDV-1.
- However, one major limitation of this study is that the efficiency of the peptide to inhibit angiogenesis is analyzed *in vitro* but, the effect of the peptide *in vivo* to inhibit angiogenesis is yet to be studied.

FUTURE DIRECTION

- To develop an efficient PEG-b-PPS –anti αvβ3 peptide for sustained release and to bring about an effective anti-angiogenic response for patients suffering from diseases like diabetic retinopathy (DR).
- Perform in vivo studies using diabetic retinopathy animal model to confirm the sustained release kinetics, tissue distribution and anti-angiogenic efficiency.

CONCLUSIONS

- From this study, it can be concluded that peptide density on the micellar surface is crucial in enhancing integrin clustering for anti-angiogenesis.
- The study provided a detailed understanding of the development of a novel ligand targeted therapeutic strategy, which can be utilized for various disease conditions.

REFERENCES

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ACKNOWLEDGMENTS

- MBT program