Endothelial Colony Forming Cell Rolling and Adhesion Supported by Peptide Combinations

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Statement of Purpose: The aim of this study was to investigate the ability of newly designed peptides to support rolling and adhesion of circulating endothelial colony forming cells (ECFCs) under shear flow which has the potential to advance rapid cardiovascular device endothelialization. Despite substantial progress in the development of biocompatible surface technologies, endothelialization remains the single truly effective long-term means of preventing this undesirable narrowing of the vessel lumen. ECFCs are a highly proliferative EC cell source, thus promising for use in rapid endothelialization.

The mechanisms that regulate recruitment and retention of circulating ECFCs at a site of injury are still not well understood. Generally, recruitment of circulating cells occurs through a dynamic adhesion process consisting of cell tethering, rolling, and firm adhesion. Currently relatively little work has been done to study the cell surface receptor-mediated, especially integrin-mediated, ECFC dynamic adhesion process for reendothelialization. [1,2]

This study investigated the ability of novel peptides and peptides combinations containing $\alpha_4\beta_1$ or $\alpha_5\beta_1$ integrin-binding sequences to support dynamic adhesion of ECFCs under shear flow. Both single peptides and peptide combinations were coupled to poly(ethylene glycol) hydrogels, and the induction of tether events and velocity fluctuations during the ECFC rolling process were assessed, in addition to firm adhesion.

Methods: Peptides, synthesized with a peptide synthesizer, were first conjugated to acryloyl-PEG-succinimidyl valerate and then grafted onto poly(ethylene glycol) diacrylate (PEGDA, 6 kDa) hydrogels through photocrosslinking. A pre-developed parallel plate flow chamber system [1] was used to mimic physiological fluid shear. The interaction of ECFCs with the peptides and their combinations under different shear rates was recorded using a high-speed camera and assessed by an optical cell tracking analysis system [3]. Tested peptides are in Fig.1.

Integrin	Peptide	Abbreviation
	RGDSG	RGDS
$\alpha_5\beta_1$	PHSRNSGSGSGSGSGRGDSG	P_RGDS
	CRRETAWAC	CRRETAWAC
	REDVG	REDV
$\alpha_4\beta_1$	PHSRNSGSGSGSGSGREDVG	P_REDV
	KSSPHSRNSGSGSGSGSGREDVG	KSSP_REDV

Figure 1. Peptides being tested.

PEGDA hydrogels without grafted peptides were used as a negative control; by providing a "blank slate" surface with minimal protein adsorption, specific interactions with the grafted peptides can be evaluated [1]. The velocity pattern, including new metrics of "tether percentage" and "velocity fluctuation," and number of captured ECFCs were quantified for at least 3 separately prepared hydrogels.

Results: Individually $\alpha_5\beta_1$ integrin-binding peptides, which support ECFC static adhesion but slow ECFC rolling only minimally, supported a low level of ECFC capture, with the highest rates found for CRRETAWAC (Fig. 2). Importantly, this study found that combining these peptides with REDV-containing peptides, which we have previously shown to significantly slow ECFC rolling over other peptides [1], resulted in an over 3x increase in the percentage of ECFCs captured. In addition, when peptides lengths were adjusted to be similar, the peptide combinations synergistic ability to capture ECFCs was increased. Peptide combinations with similar lengths, including CRRETAWAC + REDV, P_RGDS + KSSP_REDV, and P_RGDS + P_REDV, had higher tether percentages, velocity fluctuation and ECFC capture.

To understand the factors contributing to peptide cell capture capabilities, new parameters for quantifying the velocity patterns during rolling process were established: tether percentage and velocity fluctuation. Tether percentage reflects how well the peptides maintain ECFCs at a low cell rolling velocity; velocity fluctuation measures how well the peptides induce velocity changes. It was found that having high values for both tether percentage and velocity fluctuation indicates that peptides will also display a higher cell capture percentage. Unlike cell capture, both tether percentage and velocity fluctuation can be rapidly assessed, providing valuable new metrics for evaluating biomaterial capture molecules.

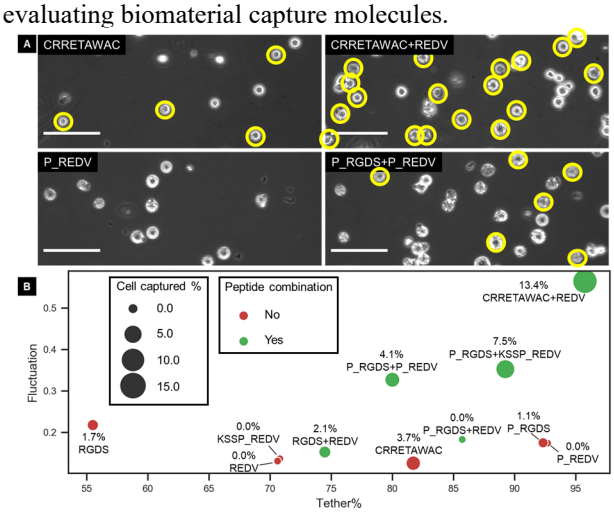

Figure 2. Peptides with high percentages of capture also had high tether percentage and velocity fluctuation. (A) Representative images of ECFC capture (yellow) on peptide-grafted hydrogels. No capture was seen on P_REDV-grafted. (B)Percentage of ECFCs captured with different peptide-grafted hydrogels plotted with its corresponding tether percentage and velocity fluctuation at shear rate 20 s^{-1}. Total concentration of grafted peptide in each condition was 0.7 μmol/mL.

Conclusions: In this work, we identified novel peptides and peptide combinations to capture ECFCs under shear conditions and evaluated their performance by monitoring the velocity patterns of the cell rolling process, including tether percentage and velocity fluctuation, together with final cell adhesion. Overall, this work developed a new methodology for evaluating capture molecules and identified new peptide candidates for intravascular biomaterial design and prevention of stenosis.

References:[1] Seeto et al. Acta biomaterialia, 2013, 9(9): 8279-8289.
[2] Angelos et al. Biophysical Journal, 2010, 99(11): 3545-54.
[3] Seeto et al. Review of Scientific Instruments, 2016, 87(3): 033705.