Effect of spacer length on the antimicrobial activity of the bound peptide, chrysopsin-1

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Statement of Purpose: Each year, greater than 2 million fracture fixation devices and 600,000 joint prostheses are implanted in the US but infection rates associated with such devices are 5-14 % and 1-2% respectively, ultimately costing $250 million dollars in extended hospital stays and revision surgeries [1][2]. Currently, they are treated with systemic antibiotics, debridement, and implant removal; but the high local antibiotic concentrations needed to kill colonized bacteria are only achieved over a short time and can be cytotoxic to surrounding cells [3]. Thus, finding novel ways to prevent infection is needed. As an alternative, antimicrobial peptides (AMPs) are short, cationic molecules associated with the innate immunity of many species have broad spectrum antimicrobial activity. AMPs use fundamentally different mechanisms to kill bacteria than conventional antibiotics, reducing the threat of bacterial resistance. Recently, functionalizing surfaces with AMPs has emerged as a technology to combat the spread of infection, and our objective is to improve biomedical devices using a therapy based on surface-tethered AMPs. However, immobilization of AMPs has been shown to reduce activity [4]. Previously, we have covalently linked the AMP chrysophsin-1 (CHY1) with a cysteine modification (C-CHY1) to silicon dioxide (SiO_2) using a poly(ethylene glycol) (PEG) spacer molecule to allow flexibility and maintain activity[5]. Determining the effect of spacer length on antimicrobial activity will help determine the mechanism by which the peptide acts, thus allowing for more rational design of antibacterial surfaces as well as improve patient outcomes. Previous studies show increased resistance to physical stress and bactericidal activity compared with physically-bound chrysophsin-1 (CHY1) and unmodified surfaces. Thus far, an efficacy of 82+/-11% against our gram negative model bacteria, *Escherichia coli HB101 (EC)* and 41+/-9% against our gram positive model, *Staphylococcus aureus ATCC43866 (SA)*.

Methods: A quartz crystal microbalance with dissipation monitoring (QCM-D) (Biolin Scientific, Sweeden) was used to monitor the attachment of both the PEG spacer molecules and chrysophsin-1 peptides to SiO_2-coated quartz crystal surfaces. Both peptides, CHY1 and C-CHY1 were studied. For the covalently linked system,, the QCM-D steps were: (1) crystals were functionalized using 3-(aminopropyl)trimethoxysilane (APTMS), (2) one of three PEG spacer lengths, molecular weight (MW) 866 (Thermo Scientific Rockford, IL), 2000 or 7500 (JenKem Technology USA Inc. Allen, TX), were flowed through, (3) 10uM C-CHY1 was flowed, (4) phosphate-buffered saline rinse, (5) either EC or SA bacteria were flowed. For the physically-adsorbed system, APTMS was not used and CHY1 was flowed over the bare SiO_2. Steps (4) and (5) remained the same. Additional control experiments included only APTMS functionalization and APTMS plus PEG addition. The crystals were stained using a LIVE/DEAD BacLight Bacterial Viability Kit (Life Technologies Corp., NY) to determine the killing percent.

Results: Table 1 summarizes the major killing percentages as a function of spacer length for the different situations studied with the QCM-D. For C-CHY1 versus EC, the highest killing achieved was 82+/- 11% with a MW 866 PEG. The killing percent did not increase with PEG length;32 +/-17% and 54%+/-22% killing for MW2000 and MW 7500 was determined, respectively. CHY-1 only acheived 38% +/-17%. For C-CHY1 versus SA, killing was found to be 42+/-9% and 25+/-15% for PEG MW866 and MW 2000 PEG spacers, respectively. CHY1 produced a higher killing in this case of 57 +/-12%. for the physically adsorbed chrysophsin-1. In solution, 10μM for both peptides achieved 99.9% killing against both microbes.

E. coli HB101				*S. aureus (ATCC43866)*		
	Killing %	**Std. Dev.**			**Killing %**	**Std. Dev.**
Physically Adsorbed	37.8	17.0		**Physically Adsorbed**	56.6	11.8
PEG866	82.0	10.6		**PEG866**	41.6	9.3
PEG2000	32.4	17.3		**PEG2000**	25.2	15.0
PEG7500	53.7	22.3		**PEG7500**	-	-
APTMS only	40.0	26.1		**APTMS only**	25.9	15.5

Table 1. Killing Percent of *E.Coli HB101*and *S. Aureus*

Conclusions: Contrary to other studies [4], we found that for EC, the highest killing percent was achieved with the shortest PEG. However, for SA, the highest killing percent was achieved with the physically adsorbed peptide. This suggests that for CHY1, its mechanisms differ depending on the type of microbe it faces. Despite the lack of gram positive killing by the tethered peptide, it could have a bacteriostatic effect [6] which has not been studied. This study shows that it is possible to covalently tether AMPs to surfaces while retaining some antimicrobial activity. Future studies will involve similar chemistries to attach these peptides to other substrates specific to the biomedical implant industry, such as titanium [7].

References:

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