The Effect of Human Cryopreserved Viable Amniotic Membrane on The Biofilm Formation by *P.aeruginosa*

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Statement of Purpose: Biofilms, a population of bacteria grown within self-produced extracellular polymeric substances, are often resistant to antibiotic treatment and remain as one of the key challenges in treating chronic wounds. A recent clinical study showed that human cryopreserved viable amniotic membrane (hCVAM) promoted closure of chronic diabetic foot ulcers and reduced wound-related infections [1]. The intrinsic antimicrobial activity of hCVAM against a spectrum of wound-associated pathogens under planktonic (floating) culture condition was also demonstrated in an *in vitro* study [2]. The goal of this study is to evaluate the effect of hCVAM on the formation of biofilms by *P. aeruginosa*, a pathogen commonly associated with chronic wounds.

Methods: In this study, the formation of biofilms on polystyrene surface and dermal tissue surface were evaluated. In order to avoid the physical disturbance of hCVAM on the biofilm formation, we prepared conditioned medium from hCVAM. The *P. aeruginosa* was inoculated to conditioned medium or assay medium (a control) in wells of a polystyrene plate. The plates were incubated for 48 h, and after that, gentamicin was added to the wells at 20x MIC (minimal inhibitory concentration) for 24 h to eliminate the planktonic bacteria. The biofilms in the wells of the polystyrene plates were washed and stained with crystal violet. In addition, we adapted a wound-relevant porcine dermal biofilm model [3] to confirm the effect of hCVAM on the biofilm formation. Pieces of porcine dermis were soaked in the hCVAM conditioned medium or assay medium for 6 h. The *P. aeruginosa* was inoculated onto the dermal tissues lying on agar plates. After incubation for 72 h and the removal of planktonic bacteria, the *P. aeruginosa* in the biofilm was extracted and quantified by serial dilution and CFU counting.

Results: We demonstrated that the formation of biofilm was significantly reduced when *P. aeruginosa* was inoculated in hCVAM conditioned medium compared to the control assay medium. We observed the inhibition of biofilm formation on dermal tissue in the presence of hCVAM-derived conditioned medium.

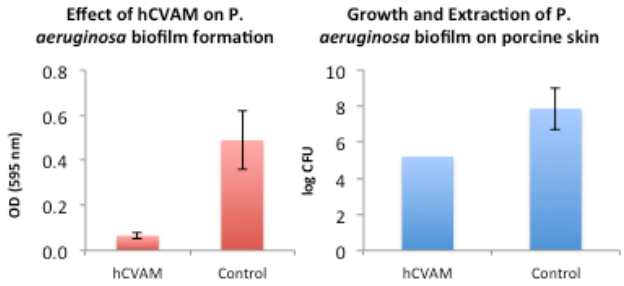

Figure 1. Growth of P. aeruginosa after treatment with hCVAM

Conclusions: Altogether, our results demonstrate that hCVAM has antimicrobial activity inhibiting the biofilm formation by *P. aeruginosa*.

References:

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