Submission Summary

Conference Name
International Conference on Nanoscience and Nanotechnology

Paper ID
143

Paper Title
Functional analysis of the role of extracellular vesicles in pathogenesis of diabetic foot ulcers

Abstract
Extracellular vesicles (EV, 20-300nm) are recognized as key mediators of host-pathogen interactions in infectious diseases such as diabetic foot ulcers (DFU). EV cargo has been associated with functions such as antimicrobial resistance and immune modulation that enhance microbial virulence. A functional analysis of microbial EVs with host immune cells can help us better understand microbial pathogenesis and aid in development of efficacious wound management strategies. Towards this, we have characterized and studied the interaction between membrane vesicles produced by bacterial pathogens on host cells. DFU pathogens (Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA)) were cultured and their interactions with neutrophils, primary host molecules of innate immunity, was studied. DFU isolates showed distinct phenotypes in terms of motility and pigment production. Size and shape of outer membrane vesicles of PA and cytoplasmic membrane vesicles of SA, isolated with density gradient ultracentrifugation were confirmed using Zetasizer Nano system and scanning electron microscopy. EVs shared an average diameter of ~200nm with intact circular morphology. Neutrophils were treated with (a) whole cells (MoI 1:10) and (b) cell free supernatants of PA and SA (enriched with EVs) in axenic and co-culture conditions followed by visualization of neutrophil extracellular traps (NET) with Sytox® Green dye. Altered levels of NET production was observed with a significant reduction in co-culture conditions. Growing rise of antimicrobial resistance necessitates the need to gain a mechanistic and functional understanding of key molecules that contribute to microbial pathogenesis, that can aid in development of efficacious therapeutic strategies and improve public health.

Created on
1/15/2024, 2:50:13 PM

Last Modified
1/15/2024, 2:54:47 PM

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