In vitro adsorption of a broad spectrum of inflammatory mediators with CytoSorb® hemoadsorbent polymer beads

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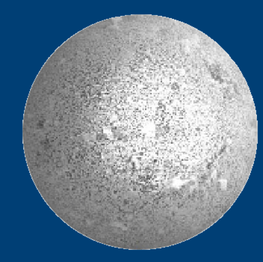
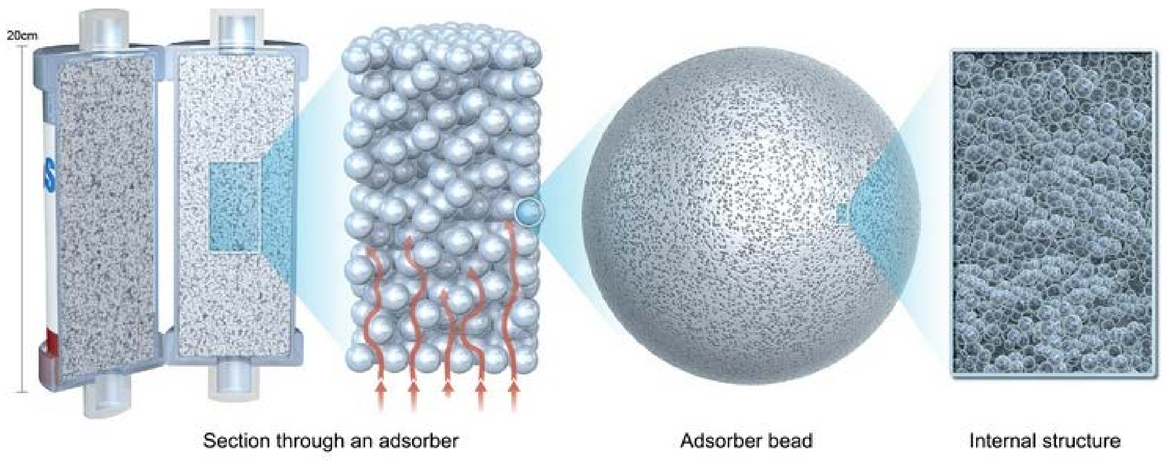


INTRODUCTION

Sepsis is a life-threatening class of severe systemic inflammation in response to invading pathogens or direct tissue insults (trauma, burns, etc.). Pathogen-associated molecular pattern molecules (PAMPs), such as bacterial exotoxins, trigger an immune response in the host to fight the infection leading to the production of high levels of cytokines that damage tissues causing the extracellular release of damage-associated molecular pattern (DAMPs) molecules into the bloodstream. Excessive, persistent circulating levels of cytokines and DAMPS contribute to organ injury and identify those patients who have the highest risk of multiple organ dysfunction syndrome (MODS) and death in community acquired pneumonia and sepsis. Experimental studies[1] and clinical reports[2-5] have demonstrated the benefits of cytokine reduction with extracorporeal blood filtration with polymer beads yet do not fully explain the mechanism. This study set out to investigate the capability of the polymer beads to remove a broad selection of inflammatory PAMPs and DAMPs, in addition to cytokines, in an *in vitro,* single compartment blood recirculation model. It is hypothesized that such removal may contribute to the control of the SIRS response in critically-ill patients.

TECHNOLOGY

Each polymer bead is roughly the size of a grain of salt and contains millions of pores and channels that capture and absorb different blood contaminants based on pore size and surface adsorption. Substances that are larger than the pores, such as blood cells, cannot get into the pores and go around the beads. Very small substances, such as electrolytes and other blood chemistries, also are not captured. Appropriately sized molecules, in the 5-55 kDa range, get trapped in the vast network of pores and channels in every bead and are permanently eliminated from the blood.

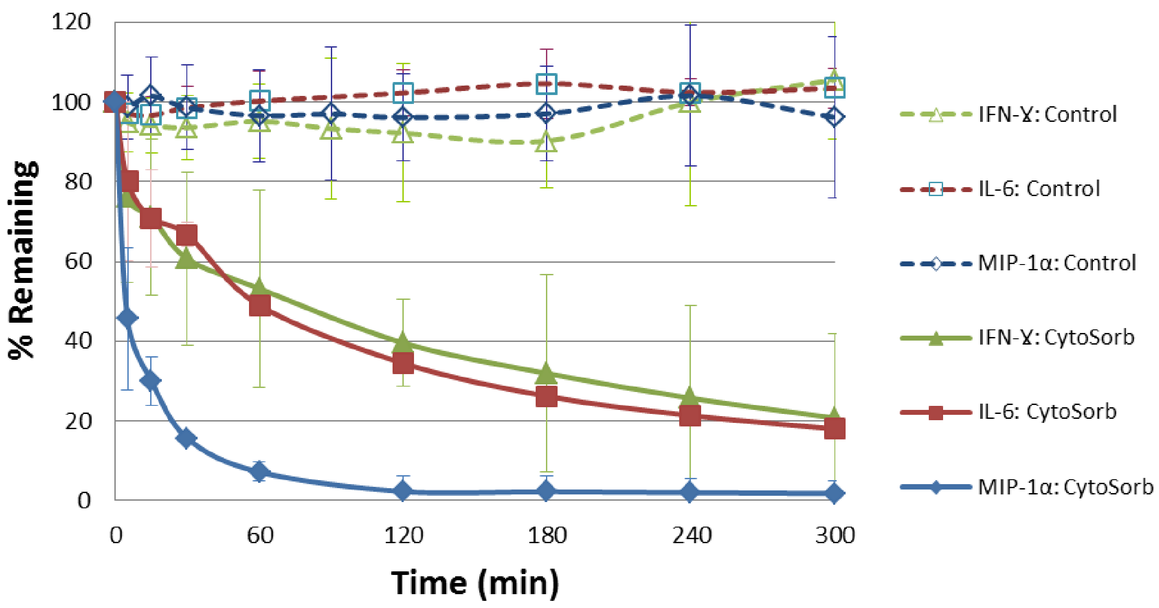


METHODS

Purified proteins S100 A8 (50 ng/mL), C5a (25 ng/mL), procalcitonin (16 ng/mL), HMGB-1 (100 ng/mL), MIP1-α (400 pg/ml), IL-6 (3,000 pg/mL), IFN-ɣ (400 pg/mL), TNF-α (100 pg/mL), *Strep* pyrogenic exotoxin B SpeB (100 ng/ml), *Staph* enterotoxin TSST-1 (2.0 µg/mL) and aflatoxin B1 were added at clinically relevant concentrations to 265 ml 3.8% citrated whole bovine blood and recirculated through a 20 mL CytoSorb® (CS) device or control (no bead) device at a flow rate of 140 mL/min for five hours. Fetal bovine serum was used for the *Staph* α-toxin (α-hemolysin; 1.5 µg/mL) due to the prevalence of anti-hemolysin antibodies in blood obtained from adult donors. Each analyte was normalized to the T0 sample collected from the reservoir prior to initiation of the recirculation. Plasma was collected following centrifugation of blood for 10 min at 1500 g at 4°C and stored at -20°C until analysis. Proteins were analyzed by ELISA (R&D Systems duosets n=3; bacterial proteins: Toxin Technologies) according to recommendations.

CYTOKINES

In Vitro Adsorption of Cytokines from Blood with CytoSorb® or Control Device

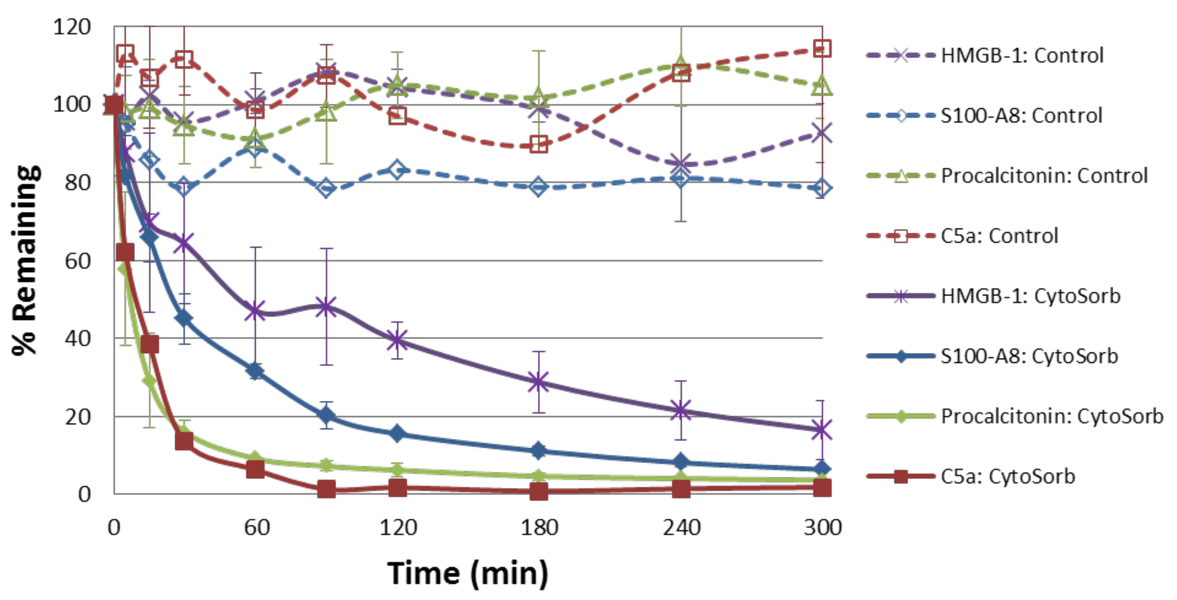


RESULTS

Hemoperfusion of whole blood through porous polymer bead devices for five hours significantly reduced the levels of a broad spectrum of PAMPS, DAMPS and cytokines whereas the levels of the inflammatory proteins were altered by <20% during hemoperfusion through a control device.

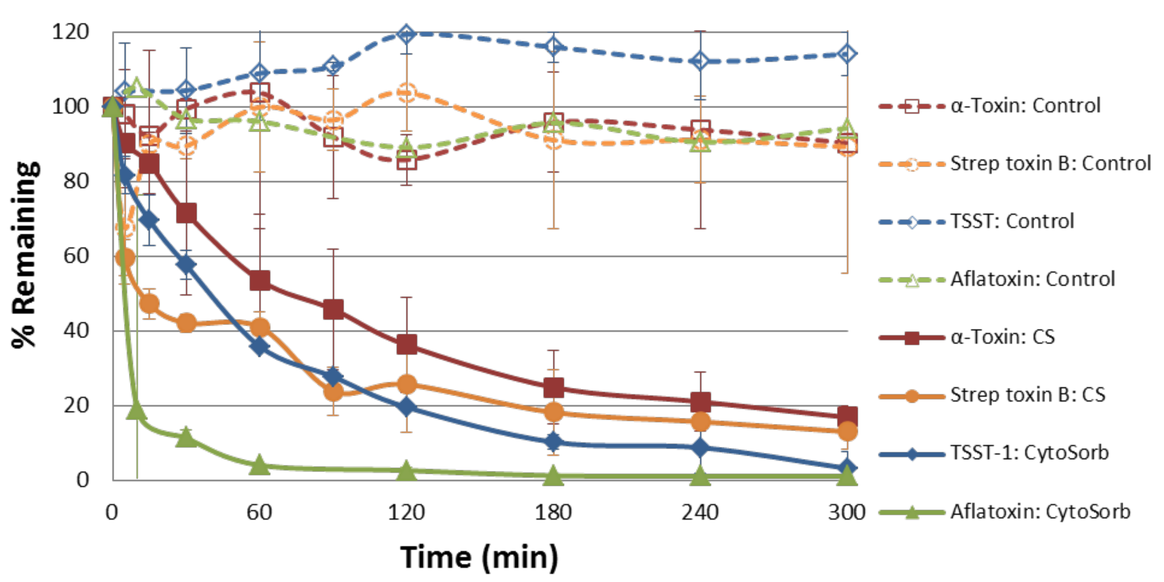
DAMPS

In Vitro Adsorption of DAMPS from Blood with CytoSorb® or Control Device



PAMPS

In Vitro Adsorption of PAMPS from Blood with CytoSorb® or Control Device



Staph α-toxin forms pores on cells causing cell lysis and tissue destruction; SpeB helps evade the immune response by degrading host factor required for phagocytic activity; TSST-1 is a superantigen that activates T cells causing massive cytokine release; and aflatoxin B1 causes severe acute hepatotoxicity.

CONCLUSIONS

This study demonstrates that CytoSorb® hemoadsorbent polymer beads are capable of reducing a broad range of toxic DAMPS and PAMPS from blood providing a means, in addition to cytokine reduction, of reducing the uncontrolled inflammatory cascade that contributes to a maladaptive SIRS response, organ injury, multiple organ dysfunction syndrome (MODS) and death in patients with a broad range of life-threatening inflammatory conditions such as sepsis, trauma, lung injury, pancreatitis, acute respiratory distress syndrome and many others. The specific removal of bacterial and fungal toxins highlights an under appreciated potential mechanism in the treatment of serious infections such as MRSA, aspergillosis, and other toxin producing infections. Further study to elucidate the potential clinical impact is warranted.

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