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A true theranostic approach to medicine: Towards tandem sensor detection and removal of endotoxin in blood ☆

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ABSTRACT

Sepsis is one of the leading causes of death around the world. The condition occurs when a local infection overcomes the host natural defense mechanism and suddenly spreads into the circulatory system, triggering a vigorous, self-injurious inflammatory host response. The pathogenesis of sepsis is relatively well known, one of the most potent immuno-activator being bacterial lipopolysaccharide (LPS) – also known as 'endotoxin'. Tests exist to detect endotoxin in bodily fluids, but are expensive, not necessarily user-friendly and require reporter molecules. In addition, the situation for safe and effective anti-endotoxin therapy is problematical. At the present time, endotoxin removal through cartridge hemoperfusion is one of the better alternatives to combat sepsis. The capability to both measure endotoxemia levels and offer an adapted response treatment in a timely manner is crucial for better management and improved prognosis, but is currently unavailable. In this context, we describe herein preliminary research towards the development of an alternative LPS biosensor and an innovative LPS neutralization cartridge to be eventually combined in an all-integrated configuration for the theranostic, personalized treatment of blood endotoxemia/sepsis. LPS detection is performed in a real-time and label-free manner in full human blood plasma, using ultra-high frequency acoustic wave sensing in combination with ultrathin, oligoethylene glycol-based mixed surface chemistry imposed on piezoelectric quartz discs. Biosensing platforms are functionalized with polymyxin B (PMB), a cyclic peptide antibiotic with high affinity for LPS. Analogous surface modification is used on glass beads for the therapeutic cartridge component of the combined strategy. Incubation of LPS-spiked whole blood with PMB-bead chemistry resulted in a significant decrease in the production of pro-inflammatory TNF-α cytokine. LPS neutralization is discussed in relation to the perturbation of its supramolecular chemistry in solution.

1. Introduction

Many diseases are highly heterogeneous in terms of their expression (Nie et al., 2007). This fact results in the consequence that therapies are only effective for certain sub-populations of afflicted patients. Moreover, there are often severe limitations with respect to our ability to produce treatment options that are viable in more than one stage of disease development (Xie et al., 2010). This is a very common issue as it relates, for example, to the treatment of cancer (Kelkar and Reineke, 2011). Recognition of these problems has led, in recent times, to a more 'personalized' form of medicine, where therapy is targeted on an individual patient basis. One development that has emerged from this type of treatment is the protocol involving *combined* therapy with diagnosis (Liu et al., 2010). This rapidly growing area of medicine is now referred to as *theranostics*, a term originally coined by Funkhouser (2002). The key point in this approach is that therapeutic and diagnostic capabilities are employed via a single platform to treat a specific disease in a pseudo-simultaneous fashion (Janib et al., 2010). A broader definition of the strategy is the use of an appropriate diagnostic measure to personalize a separate therapeutic intervention (Pene et al., 2009).

A very recent example of the technology is the emergence of nanoparticles as a vehicle for theranostic treatment. To operate as a theranostic nanoparticle, the entity must be multifunctional and carry a therapeutic agent in addition to a contrast probe for imaging purposes (Haglund et al., 2009). Many types of nanoparticle have been formulated to act in this fashion, including vesicles, micelles, drug conjugates and complexes, dendrimers, microbubbles, carbon nanotubes, and core–shell nanoparticles/quantum

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dots (Crawley et al., 2014). Aside from attached drugs that target particular tissues, other treatment methods comprise radiation therapy, photothermal ablation and photodynamic therapy. Imaging modalities using contrast agents attached to the nanoparticle generally involve the procurement of a set of *static* images in order to provide a snapshot of a patient's medical condition (Nahrendorf et al., 2009). Techniques typically employed for visualization include magnetic resonance imaging, (single photon emission) computed tomography, ultrasound and fluorescence imaging. Despite the promise offered by theranostic-nanoparticle treatment, there are few such systems in clinical trials at the present time (Miele et al., 2009; Malam et al., 2009). The reality is that there are a number of problems to be addressed before the technology becomes widespread in use, including production costs, formulation stability and innate particle toxicity (Medina et al., 2007; Tinkle, 2010). The latter has actually spawned a new field of research termed 'nanotoxicology', where the interaction of nanoparticles with cellular components and tissue is the subject of detailed study (Durán et al., 2014).

In contrast to the static detection nanosystem mentioned above, we describe 'macro-theranostics', which involves an instrument capable of both *real-time* diagnostics and therapy. In particular, we introduce 'theranostic circuit devices' (TCDs) that consist of an in-line detection system (biosensor) employed in tandem with a therapeutic cartridge component to clean and remove harmful species from blood circulated extracorporeally (Fig. 1). The concept is well established in terms of membrane-based dialysis for patients who suffer from renal failure – with the exception that measurements of creatinine and other offending molecule concentrations are in this case not performed in conjunction with therapy. Herein, we are concerned with endotoxemia, a condition associated with sepsis and caused by an elevated blood concentration of LPS (Fig. 2), also known as endotoxin, which is the major constituent of the outer membrane of Gram-negative bacteria (Ronco et al., 2010). The role of bacterial LPS in the pathogenesis of human disease has been comprehensibly reviewed elsewhere (Opal, 2007; Munford, 2008). Concisely, LPS binds to toll-like receptor 4 (TLR-4) on the surface of immunocompetent cells and triggers a cascade of signal transduction events, which culminate in the synthesis of pro- and anti-inflammatory species including TNF-α, IL-1, IL-6 and IL-10, to mention a few. The toxic component of LPS has been unequivocally shown to be the epitope region known as 'lipid A' (Gutsmann et al., 2007) (Fig. 2), which is generally conserved among most pathogenic Gram-negative bacteria and can trigger the altered expression of thousands of genes. Although a litany of anti-endotoxin therapies attempting to block the toxic effects of LPS have failed clinical trials, studies by Ronco et al. (2010) have suggested that endotoxin sorption via hemofiltration may have clinical benefit and reduce mortality in a cohort of patients with severe sepsis/septic shock following emergency abdominal surgery. From a mechanistic point of view, the effective treatment of endotoxemia is certainly also complicated by the fact that LPS is an amphipathic molecule that can exhibit complex supramolecular chemistry of polymorphic aggregates, micelles and vesicles (Petsch and Anspach, 2000) on which its bioactivity appears to depend (Mueller et al., 2004; Seydel et al., 2000; Gutsmann et al., 2007).

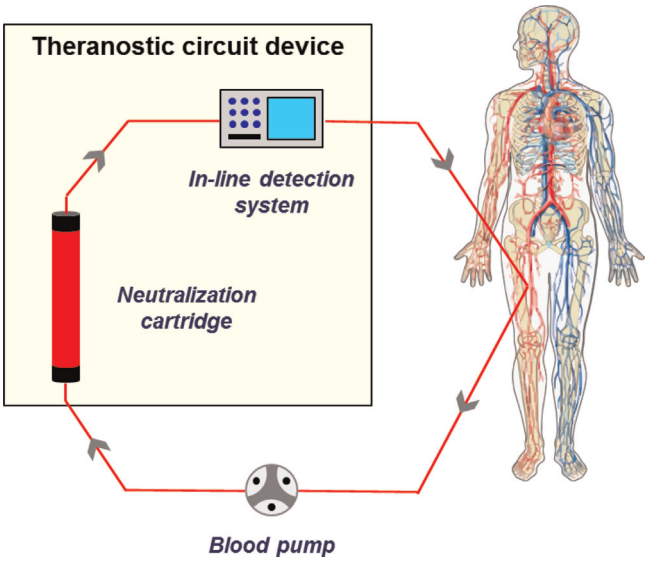

Fig. 1. Schematic representation of a generic 'theranostic circuit device' (TCD), which involves a therapeutic cartridge component to neutralize harmful species from blood circulated extracorporeally, and an in-line detection system capable of real-time and label-free measurements.

The oldest such hemofiltration device still in use clinically is the 'Toraymyxin' cartridge developed by Toray Industries Inc. (Shoji, 2003; Ronco et al., 2010). Briefly, blood flows into the cartridge through a central pipe and is then passed through sidewalls radially into a knitted fabric composed of polystyrene fibers, before exiting through an outlet at the top of the cartridge (Shoji, 2003). The surface of the polymer is modified with polymyxin B (PMB – Fig. 2), a cyclic peptide antibiotic known to neutralize endotoxin (Garidel and Brandenburg, 2009; Shoji, 2003). PMB is polycationic stemming from its five primary amino functional groups and, as such, will interact on an ionic basis with the negative lipid A portion of LPS from many different strains of Gram-negative bacteria (Garidel and Brandenburg, 2009; Shoji, 2003). The endotoxin removal capacity of the cartridge has been shown to be intimately linked to the number of free amino groups presented by surface-bound PMB residues (Shoji, 2003). Although purportedly successful for the removal of endotoxin from blood, the configuration does not function currently in tandem with in-line sensing. Assays related to the level of such removal are usually performed in a static batch process, using such tests as the '*Limulus* amebocyte lysate' (LAL) assay (Hurley, 1995) or the more recent 'endotoxin activity assay' (EAA) (Romaschin et al., 1998). However, these are expensive, relatively fastidious to implement and require reporter molecules. Finally, there are also health-related concerns and questions connected to the possibility of PMB leaching from the cartridge (Mitzner et al., 1993; Petsch and Anspach, 2000; Mohorčič et al., 2010). In fact, PMB is associated with neurotoxicity and nephrotoxicity (Petsch and Anspach, 2000), which contraindicates its systemic use (Mitzner et al., 1993; Garidel and Brandenburg, 2009).

In this context, we describe herein research towards both a new, alternative biosensing interface for LPS detection (Das et al., 2014) to be operated in concert with an innovative LPS neutralization cartridge based on glass beads rather than polymer fibers. Both diagnostic and therapeutic platforms adopt the same proprietary surface chemistry, which relies on the covalent attachment of PMB to silica substrates through organosiloxane mixed adlayer coatings constructed from a binary mixture of mono- and oligoethylene glycol (MEG and OEG) trichlorosilane surface modifiers (Thompson et al., 2013; Sheikh et al., 2010) (Fig. 2). Whereas surface modification is performed on amorphous glass beads for the cartridge component, the biosensing aspect of the work requires the employment of monolithic quartz discs with piezoelectric properties as detection is based on the electromagnetic piezoelectric acoustic sensor (EMPAS) (Thompson et al., 2003). This highly sensitive, built in-house flow-through device – that provides real-time and label-free measurement capability – was recently employed for the serological detection of anti-HIV antibodies (Sheikh et al., 2011). The EMPAS work presented herein is preliminary in nature but these initial experiments conducted in