

TOWARDS A NOVEL CARBON DEVICE FOR THE TREATMENT OF SEPSIS

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1. Introduction

Sepsis is a systemic inflammatory response to infection in which the balance of pro- and anti-inflammatory mediators, which normally isolate and eliminate infection, is disrupted [1]. Gram negative sepsis is initiated by bacterial endotoxin release which activates macrophages and circulating monocytes to release TNF and IL-1 β followed by IL-6 and other inflammatory cytokines [2]. As the disease progresses, an unregulated inflammatory response results in, tissue injury, haematological dysfunction and organ dysfunction. Severe sepsis, involving organ hypoperfusion may be further complicated by hypotension that is unresponsive to adequate fluid replacement, resulting in septic shock and finally death [3].

Despite improvements in anti-microbial and supportive therapies, sepsis remains a significant cause of morbidity and mortality in ICUs worldwide [4]. The complexity of processes mediating the progression of sepsis suggests that an extracorporeal device combining blood filtration with adsorption of a wide range of toxins, and inflammatory mediators offers the most comprehensive treatment strategy. However, no such device exists at present. A novel, uncoated, polymer pyrolysed synthetic carbon device is proposed which combines the superior adsorption properties of uncoated activated carbons with the capacity to manipulate porous structure for controlled adsorption of target plasma proteins and polypeptides [5]. Preliminary haemocompatibility and adsorptive capacity was assessed using a carbon matrix prototype.

2. Experimental

2.1 Material synthesis

Novel phenol-formaldehyde-aniline based pyrolysed carbons were synthesised using monolithic polymer technology patented to Mast Carbon Ltd. Carbon wells of 19 mm diameter (figure 1) were manufactured for preliminary *in vitro* testing of carbon biocompatibility and adsorption/filtration capacity. Carbon well A exhibits a mainly microporous structure, Carbon wells B-D have an increasing ratio of mesoporous : microporous structure as displayed in figure 2..



Fig 1. Carbon well (19mm diameter).

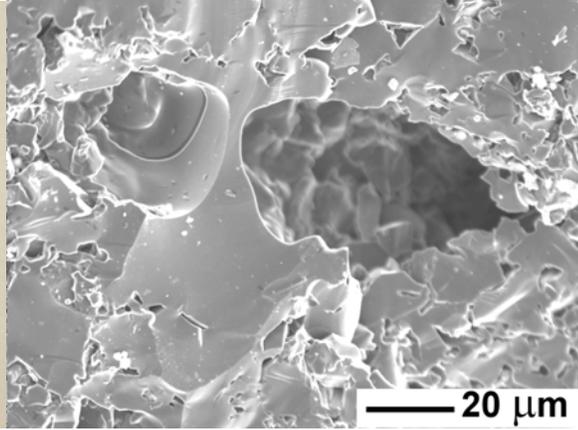


Fig 2. SEM of internal pore structure (mag x 1000).

2.2 Cytokine spiked-plasma adsorption assay.

Fresh frozen human plasma (NBS) was defrosted and spiked with the recombinant human cytokines; TNF, IL6 & IL8 (BD Biosciences) at a concentration of 1000, 2000 and 500pg/ml respectively. The levels are comparable to those measured in the plasma of sepsis patients [6]. Spiked plasma (1.8ml) was added to the surface of the carbon wells and tissue culture (tc) plastic controls, prior to incubation overnight at 37°C. Filtrate samples were collected from the base of the carbon wells and tc controls and stored at -20°C. Samples were diluted 1:8 (TNF,IL8) and 1:20 (IL6) in assay diluent, prior to the analysis of cytokine concentration using ELISA's (BD Biosciences).

3. Results and Discussion

Figure 3, indicates that all three cytokines (TNF,IL6 & IL8) when present in human plasma are removed to some extent by filtration/adsorption when allowed to flow through the carbon wells A-D. The largely microporous structure of Carbon well A, suggests why it is more successful in removing the smallest cytokine IL8 (8 kDa) from plasma. Whereas TNF (17kDa) forms a biologically active trimer and IL6 (21-28 kDa) complexes with proteins such as Alpha 2M when in plasma, thus both these cytokines exhibit a larger molecular size. As the results if figure 4 indicate, the more mesoporous carbon wells C&D display greater % removal from plasma of these two cytokines.

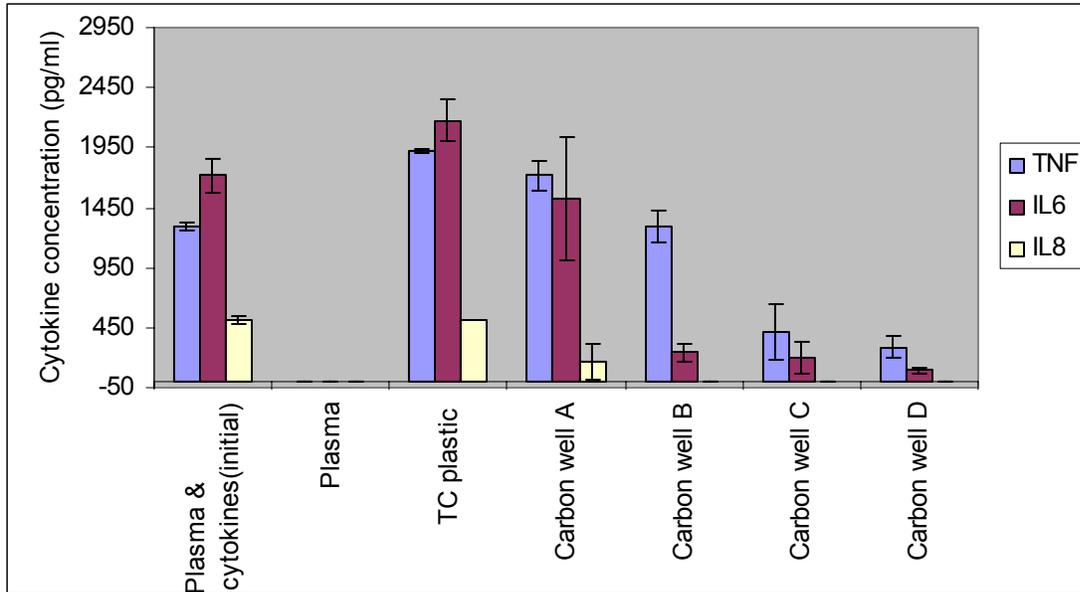


Fig 3. Carbon well adsorption/filtration of the cytokines TNF, IL6 & IL8 in human plasma. Plasma spiked with cytokines was collected after filtration through the carbon wells A-D, and assayed for cytokine concentration by ELISA. (n=3, mean +/- SEM).

Cytokine	Initial conc in plasma (pg/ml)	% removed from plasma by Carbon well A	% removed from plasma by Carbon well B	% removed from plasma by Carbon well C	% removed from plasma by Carbon well D
TNF	1926.25	10.93	32.86	78.20	85.01
IL6	2177.24	30.20	88.85	90.86	95.82
IL8	520.71	69.30	100	100	100

Fig 4. Carbon well adsorption/filtration of the cytokines TNF, IL6 & IL8 in human plasma. Data displays the % of each cytokine removed from the plasma by each carbon well A-D (n=3).

3. Conclusion

Current haemoperfusion adsorbents suffer from a number of inherent problems. They have low affinities for the major pro-inflammatory cytokines and the amberlite-type adsorbents have a very broad pore size distribution, rendering them non-selective on the basis of molecular size. Finally, all the adsorbents are poorly biocompatible (7) and coating the carbons to avoid direct blood contact effectively inhibits the adsorption of molecules with larger molecular weights.

The results obtained in our preliminary studies using the uncoated carbon well prototypes, conclude that by manipulation of the porous composition of the carbon matrix to create a differing mesoporous/microporous structure for our proposed device. We will be able to tailor the device for maximal adsorption/filtration of pro-inflammatory cytokines or bacterial toxins, which supports the further development of this device for the treatment of sepsis.

References

- [1] Shulman R. Current drug treatment of sepsis. *Hospital Pharmacist* 2002;9:97-101.
- [2] Matot I, Sprung CL. Definition of sepsis. *Intensive Care Med* 2001;27:S3-S9.
- [3] Paterson RL, Webster NR. Sepsis and the systemic inflammatory response syndrome. *J.R.Coll.Surg.Edinb* 2000;45:178-182.
- [4] Stegmayr BG. Is there a future for adsorption techniques in sepsis? *Blood Purif* 2000;18:149-155.
- [5] Tennison SR. Phenolic-resin-derived activated carbons. *Applied Catalysis A:General* 1998;173:289-311.
- [6] Heering P, Morgera S, Schmitz FJ, Schmitz G, Willers R,, Schultheiss HP, Strauer BE, Gabensee B. Cytokine removal and cardiovascular hemodynamics in septic patients with CVVT. *Int Care Med* 1997;23:228-296
- [7] Hughes RD, Williams R. *Blood Purif* 1993;11(3):163-169