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The efficient removal of anthrax toxin from human blood using monoclonal antibodies immobilized on a disposable polymeric cryogel

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Introduction

- Anthrax toxin is produced by *Bacillus anthracis*, the causative agent of anthrax, and is responsible for the major symptoms of the disease. The toxin consists of a single receptor-binding moiety, protective antigen (PA), and two enzymatic moieties, edema factor (EF) and lethal factor (LF). After release from the bacteria as nontoxic monomers, these three proteins diffuse to the surface of a mammalian cell and assemble into toxic, cell-bound complexes (Fig. 1).

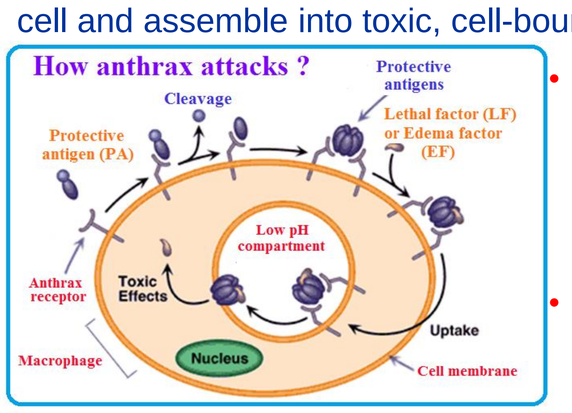


Figure 1. The different steps of anthrax toxins entry.

- A number of therapeutic human protective antigen (PA) specific monoclonal antibodies have been developed or are in process of being developed, for the treatment of individuals who have developed anthrax.
- While experiments to date have focused on delivering these antibodies by injection there is also interest in assessing their efficacy as part of a haemoperfusion system.

Objectives

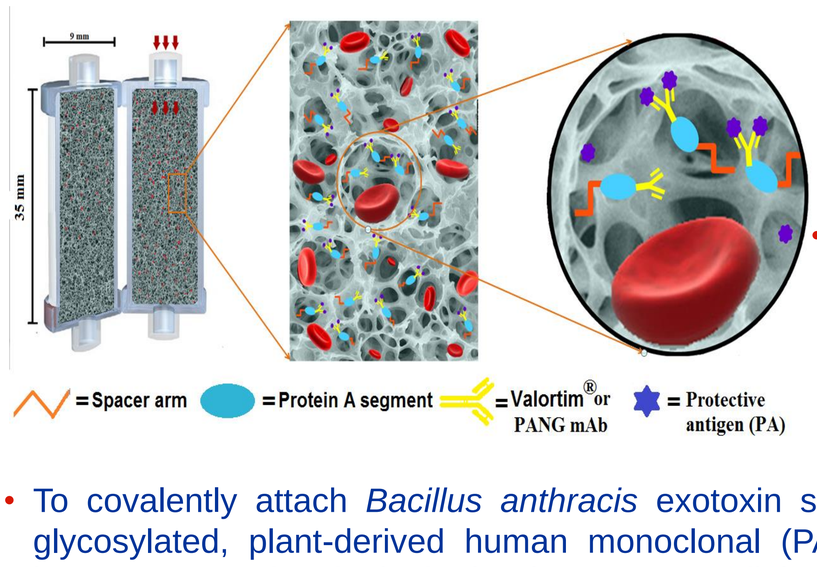


Figure 2. Vertical cross section of antibody bound adsorbent column and cartoon illustration showing antibody-antigen interactions within porous structure

- To develop monolithic cryogel materials with surface functionalities suitable for bioligand binding (protein A and monoclonal antibodies).
- To covalently attach *Bacillus anthracis* exotoxin specific antibodies, a non-glycosylated, plant-derived human monoclonal (PANG) and a glycosylated human monoclonal (Valortim®), on cross-linked macroporous polymer columns, synthesized by a cryogelation method, for the removal of anthrax protective antigen from infected human blood (Fig. 2).

Methods

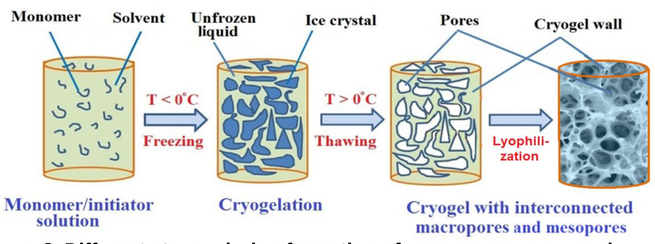


Figure 3. Different stages during formation of macroporous cryogel.

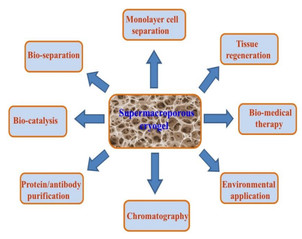


Figure 4. Diverse applications of macroporous cryogels.

- Macroporous monolithic materials produced from polymers by cryogelation technique (Fig. 3) have previously been used for a number of biomedical applications (Fig. 4) [1-4].
- Cryogels are efficient carriers for the immobilisation of biomolecules because of their unique macroporous structure, mechanical stability and wide surface chemical functionalities.

Antibody immobilization

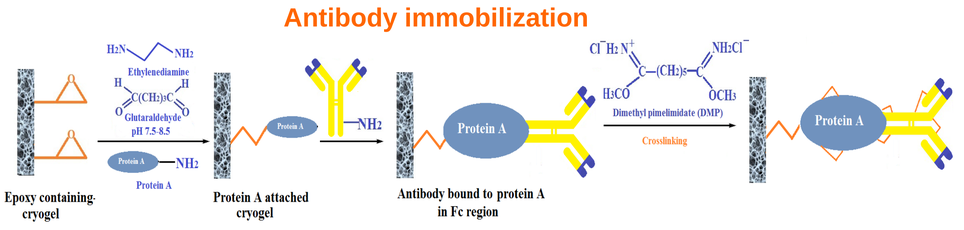


Figure 5. Schematic representations showing antibody binding approach on cryogel through Protein A.

Characterizations and results

Swelling Properties

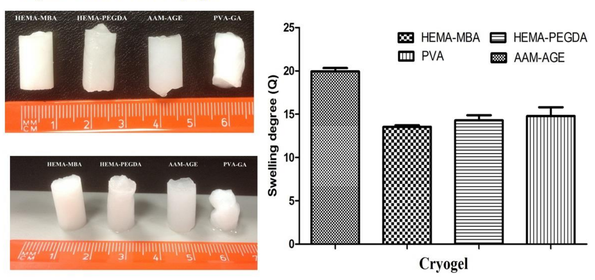
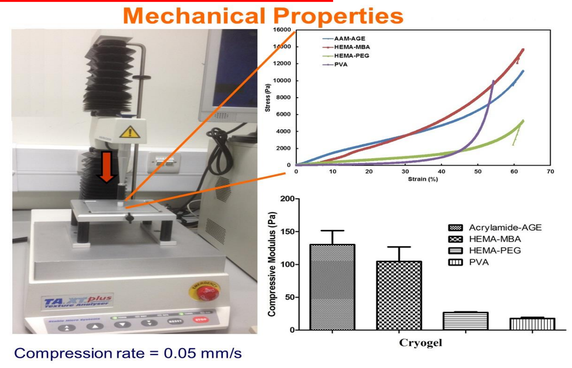


Figure 6. Macroscopic images of different cryogels and swelling ratios of equilibrated swollen cryogels.

Mechanical Properties



Compression rate = 0.05 mm/s

Figure 7 Compression testing and compression modulus of cryogels.

Pore size distribution

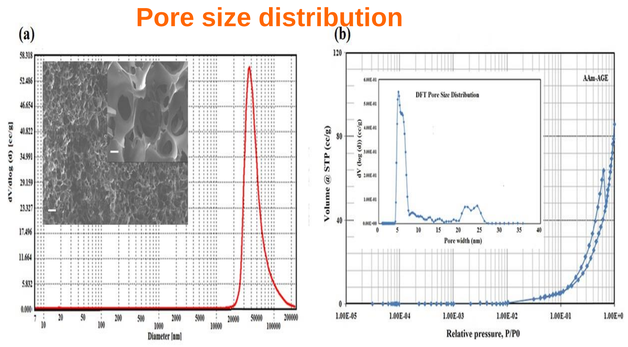


Figure 8. Macroporous pore size distribution by (a) mercury porosimetry with inset image representing porous structure and (b) N_2 adsorption isotherm with inset image representing mesopore size distribution.

Cryogel cytotoxicity and cell viability

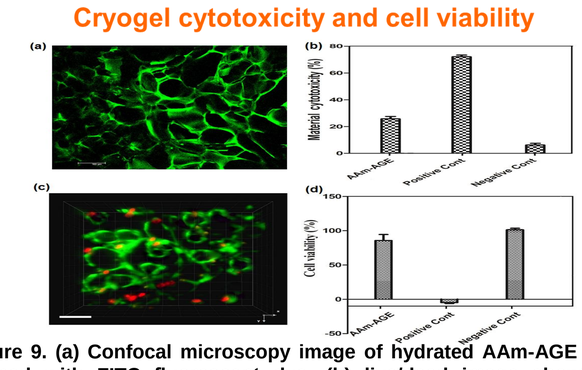


Figure 9. (a) Confocal microscopy image of hydrated AAm-AGE cryogels stained with FITC fluorescent dye, (b) live/dead image showing high viability of V79 hamster lung fibroblast at 1 week culture, (c) the cytotoxicity of cryogel extracts determined by LDH assay, and (d) cell viability by MTS assay after 24 h incubation with cryogel extracts (Scale bar = 100 µm).

Antibody binding capacity

Cryogel	Protein-A mg/g of adsorbent/cryogel	PANG mg/g of adsorbent/cryogel	Valortim® mg/g of adsorbent/cryogel
Aam-AGE-MBA	96.4 ± 10.4	**108.0 ± 19.3**	**117.0 ± 13.4**
HEMA-MBA	47.3 ± 13.4	58.7 ± 11.2	72.3 ± 11.0
HEMA-PEGDA	38.3 ± 8.6	46.3 ± 9.3	49.8 ± 20.3
PVA-GA	84.2 ± 15.5	92.3 ± 21.4	95.2 ± 7.4

Table 1. Binding capacities of cryogel towards protein-A and antibodies.

- The antibody binding capacity of the AAm-AGE-MBA-protein A cryogel column was significantly higher ($p < 0.05$) for Valortim than for PANG (Table.1).

Antibody-antigen interactions

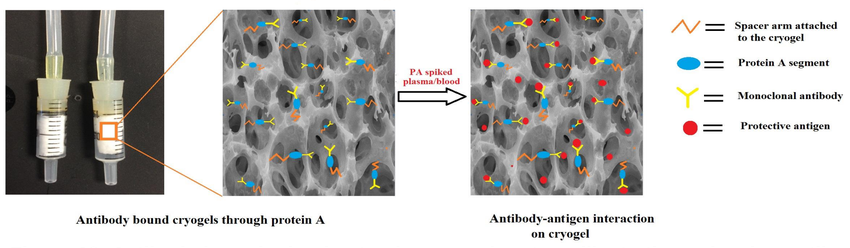


Figure 10. Antibody bound adsorbent columns and cartoon illustrations showing antibody-antigen interactions within porous structure.

Lab scale *ex vivo* experimental set up

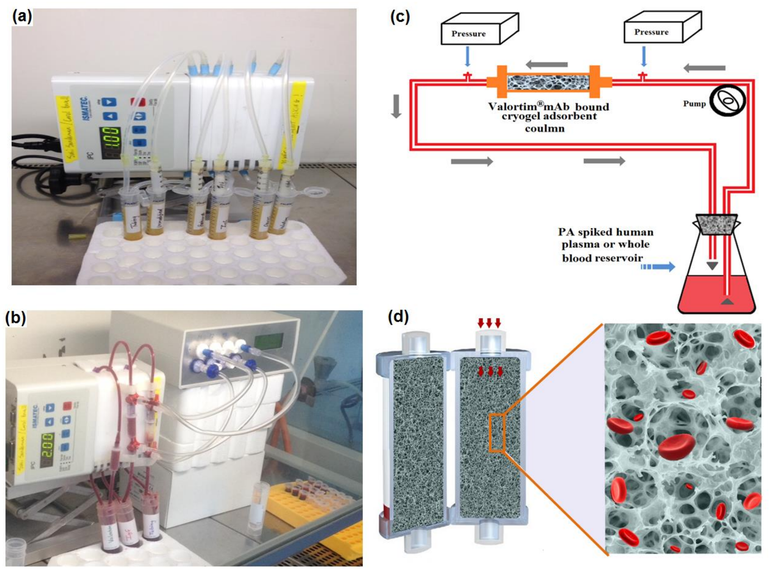


Figure 11. Lab scale *ex vivo* experimental set up for circulation of PA spiked (a) human plasma, (b) whole blood (from healthy donor) through cryogel matrix for the removal of PA, (c) Schematic representation of the *ex vivo* circuit utilized for the blood perfusion study, and (d) cross section of cryogel adsorption device illustrating interconnected porous morphology and flowing blood cells.

- An *ex vivo* PA removal studies were carried out using lab scale experimental flow set up with PA spiked fresh frozen human plasma (Fig. 11a) and freshly withdrawn human whole blood (Fig. 11b).
- For each recirculation experiment, syringe like plastic casing packed with cryogel and connected in line with a peristaltic pump (Fig. 11c).
- Removal of PA by Valortim bound AAm-AGE cryogel from human whole blood showed similar results as like plasma.

Anthrax protective antigen removal

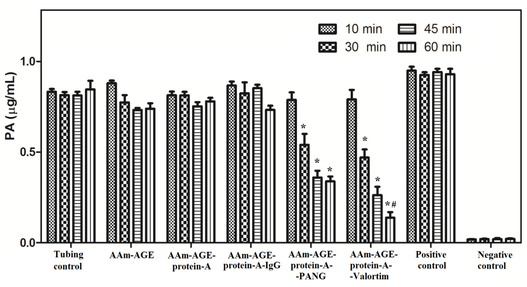


Figure 12. The concentration of PA remained in the plasma at each time points by competitive ELISA method.

- The PA adsorption from plasma by Valortim® bound cryogel column was considerably higher than the PANG bound cryogel column, over 60 minutes of recirculation (Fig. 12).
- The AAm-AGE-Valortim cryogel column removed 60% (1 to 0.40µg/mL) and 72% (1 to 0.28µg/mL) of PA at 45 and 60 minutes recirculation, respectively from whole blood (Fig. 13).

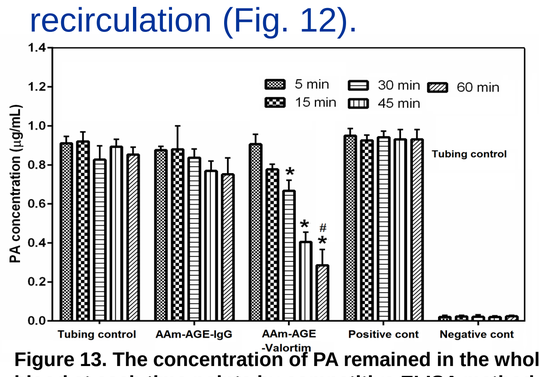


Figure 13. The concentration of PA remained in the whole blood at each time points by competitive ELISA method.

Conclusions

- We managed to chemically bind protein A with an epoxy group on the surface of cryogel and utilize protein A to link anthrax specific antibodies PANG and Valortim® for efficient removal of PA from spiked human plasma and whole blood.
- The efficacy of these PANG and Valortim® bound cryogels in adsorbing anthrax toxin PA from spiked blood extracorporeally suggest that this approach could be useful in developing therapeutically relevant agents to combat possible future risk of bioterrorism involving anthrax toxins.

Acknowledgments

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