ASSESSING THE CYTOTOXICITY OF MULTIMODAL CARBON BEADS

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Introduction

This study investigated the cytotoxic potential of phenolic resin derived activated carbon (AC) with multimodal controlled pore structure (MAST Carbon International Ltd) developed for potential applications as medical devices [1,2]. According to the international standard which addresses medical device biocompatibility (ISO 10993), cytotoxicity testing is a key element [3]. Preliminary studies on these uncoated AC beads showed that they exhibited good haemocompatibility [4]. AC beads were assessed for their *in vitro* cytotoxicity using two separate tests to measure cell viability and cell membrane integrity. Results indicate that although AC beads were not cytotoxic, it was necessary to adapt standard methodologies to account for the large adsorption potential of these materials.

Experimental

Carbon bead analysis. Synthetic, medical grade pyrolysed carbon beads were produced with defined porous structure from a number of synthetic organic co-polymers such as phenolic resin, by MAST Carbon International Ltd using patented carbon technology [5]. The AC beads (MAST 1 &2) contained micro-, meso- and macropores. Carbon bead porosity was characterized by scanning electron microscopy (SEM) and mercury porosimetry.

Cytotoxicity Assessment. Carbon cytotoxicity for human cells was testing using two standard colorimetric assays, the MTS and LDH cytotoxicity assays. The MTS assay determines viable cell number following incubation with carbon leachate. The MTS tetrazolium compound is bioreduced by metabolizing cells into a coloured formazan product that is proportional to the number of living cells in culture. The LDH assay measures cell membrane integrity following incubation with carbon leachate. Lactase dehydrogenase (LDH) is a stable cytosolic enzyme that is released upon cell lysis and can be used to quantify cell death. Materials consisted of: MAST carbon beads (1&2), a commercially available cytotoxic carbon control and a positive control cytotoxic PVC polymer (0.57% Dibutyltin maleate). 0.1g of each material was incubated for 24hrs at 37°C with 2ml of cell culture media (DMEM) containing 10% fetal bovine serum. Material extracts were used neat (100%) or diluted from 50% in DMEM and added to V79 fibroblast type hamster lung cells ($1x10^4$/well) in a 96 well culture plate and incubated for 24hrs at 37°C, 5% CO_2. The MTS and LDH assay were then conducted following manufacturers' (Promega) instructions. The MTS assay results are expressed as percentage cell activity compared to the no extract control (media only). The LDH assay results were expressed as percentage extract cell lysis compared to total cell lysis for each extract.

Results and Discussion

The MAST carbon bead size and macroporous (>50 nm) surface were visible under SEM (Fig. 1), and the mesoporosity was visible under higher magnification (Fig. 2).

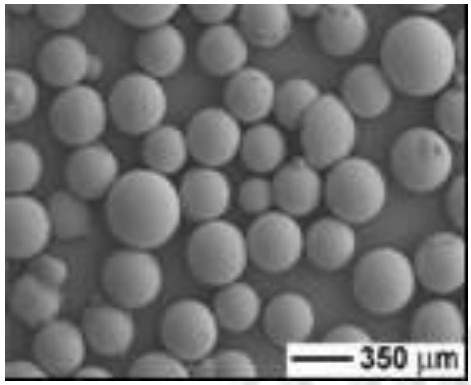

Fig.1 SEM image of MAST 1 beads

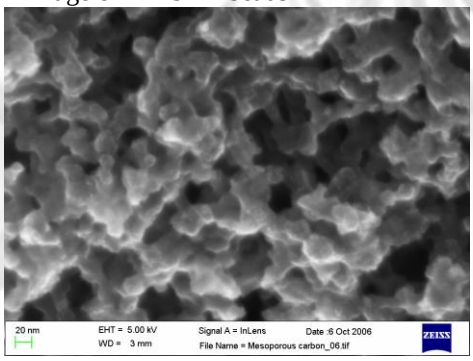

Fig 2. High magnification SEM image of mesoporous domains of MAST 1 (EVO series SEM, Carl Zeiss SMT Ltd).

Mercury porosimetry revealed the multimodal structure of the MAST 1&2 carbons, with micropore structure and large meso-macropores (40-80nm) (results not shown).

Validation studies of the MTS and LDH assay (Fig 3&4) after cell incubation with extracts for 8 and 24 hrs, indicate that although MAST 2 displays no cytotoxicity after 8 hrs at either % extract, at 24hrs the 100% extract appears cytotoxic (MTS-reduced % normal cell activity, LDH-increase % cell lysis). However, when the MAST 2 extract is diluted to 50% at 24hrs, it is no longer cytotoxic, although this dilution effect was not observed for the positive control cytotoxic polymer. Due to the high porosity and surface area of MAST 2, the results suggest that the observed cytotoxicity of the 100% extract at 24hrs may be caused by adsorption of nutrients from the culture medium which consequently interfered with cell metabolic activity (MTS) and membrane integrity (LDH). Subsequent carbon bead cytotoxicity

assessments were made using 50% extracts with a cytotoxic carbon control.

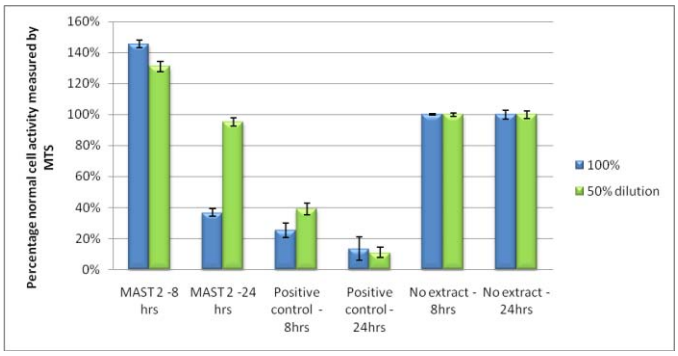

Fig. 3. The effect of material extract on cell metabolism after 8 & 24hrs, measured using the MTS assay (mean n=3, +/- sem).

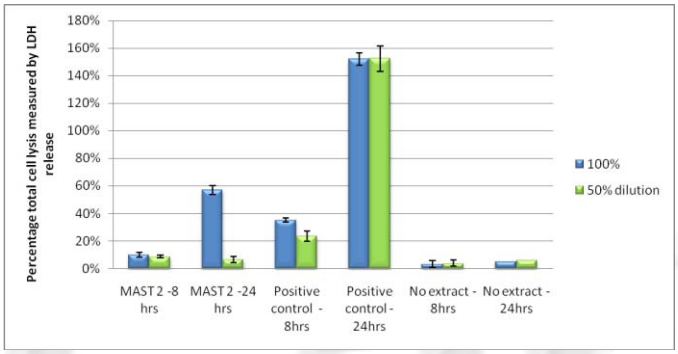

Fig. 4. The effect of material extract cytotoxicity after 8 & 24hrs incubation, expressed as percent cell lysis measured by LDH release (mean n=3, +/-sem).

MAST 1&2 and cytotoxic carbon cytotoxicity assessment using 50% extracts incubated with cells for 24 hrs and measured by the MTS and LDH assay are displayed in Fig 5&6.

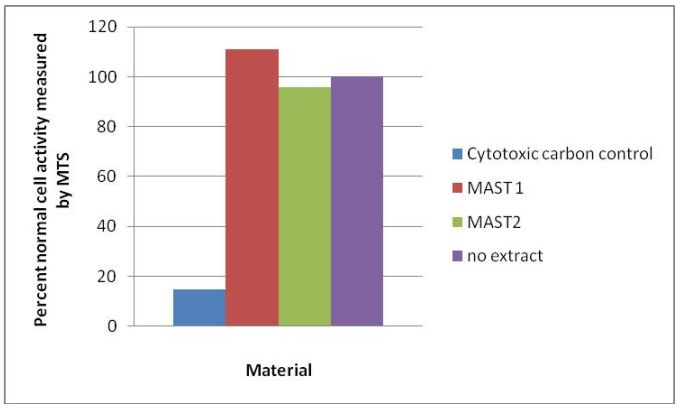

Fig 5. The effect of 50% carbon extract on cell metabolism measured using the MTS assay.

Results indicate that 50% extracts of MAST 1&2 carbons, are not cytotoxic when measured by the MTS and LDH assay, as levels compare to the no extract control. Whereas the 50% extract of the cytotoxic carbon control display cytotoxicity for both assays (MTS-reduced % cell activity, LDH- high % cell lysis).

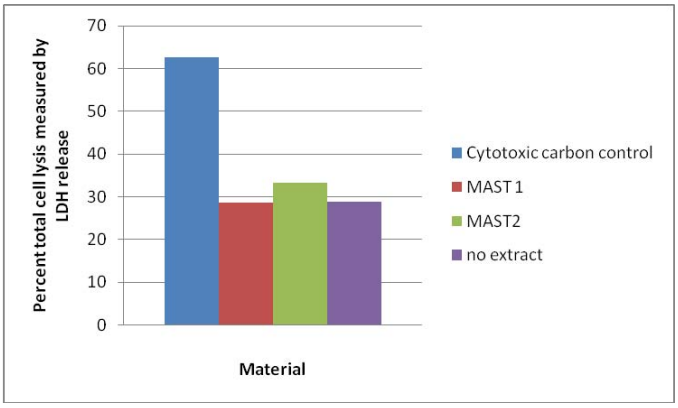

Fig 6. The cytotoxic effect of 50% carbon extract observed by measurement of LDH release following cell lysis.

Conclusions

The MTS and LDH cell cytotoxicity assays can be used to measure carbon material cytotoxicity with adaption of the standard protocol. Assessment of 50% extracts overcome the nutrient adsorption potential of the carbon and provide a means of determining material cytotoxicity.

The MAST 1&2 carbon show no cell cytotoxic effect, and a device incorporating these beads holds potential for use as an extracorporeal treatment for conditions such as sepsis and renal failure where current treatment strategies are sub-optimal.

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References

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