Heparin–cellulose–charcoal composites for drug detoxification prepared using room temperature ionic liquids

Tae-Joon Park,[a] Sang-Hyun Lee,[a] Trevor J. Simmons,[b] Jeffrey G. Martin,[bc] Shaker A. Mousa,[d] Elisaveta A. Snezhkova,[e] Veronika V. Sarnatskaya,[e] Vladimir G. Nikolaev*[e] and Robert J. Linhardt*[abc]

Received (in Cambridge, UK) 10th June 2008, Accepted 16th July 2008
First published as an Advance Article on the web 2nd September 2008
DOI: 10.1039/b809791g

We report novel heparin–cellulose–charcoal composites prepared using room temperature ionic liquids (RTILs) to enhance the biocompatibility and blood compatibility of activated charcoal beads while decreasing the size of their active pores.

Activated charcoal is useful for treating individuals in danger from oral drug overdose of depressants such as alcohol, barbiturates, and benzodiazepines, or stimulants such as ecstasy, cocaine, and amphetamines.[1,2] However, uncoated activated charcoal generally results in thromboresistance when used in direct hemoperfusion[3] to such an extent that modified cellulose–charcoal composites have been used for drug detoxification,[4–7] since the first literature on cellulose coating of charcoal was shown in 1975.[8] Unfortunately, the use of cellulose–charcoal composites in hemoperfusion still requires such additional measures as whole blood citratization and the addition of human serum albumin due to the lack of blood compatibility with cellulose–charcoal composites. To address this problem, we prepared novel biocompatible and blood compatible heparin–cellulose activated charcoal bead composites using room temperature ionic liquids (RTILs). This coating decreases the active pore size of the activated charcoal, thus diminishing its rate of protein adsorption, without decreasing the effective removal of free-diluted and protein-bound small drug molecules. These composites are useful for the rapid and safe removal of small, hydrophobic protein-bound drug molecules from the digestive system or from the blood of overdose patients in an extracorporeal circuit. A model system for blood detoxification, containing biocompatible and blood compatible charcoal composites, is examined using the hydrophobic small molecule, phenytoin, and the large protein molecule, bovine serum albumin (BSA).

[a] *Department of Chemical and Biological Engineering, Biotechnology 4005, Rensselaer Polytechnic Institute 110, 8th Street, Troy, NY 12180, USA*
[b] *Department of Chemistry and Chemical Biology, Biotechnology 4005, Rensselaer Polytechnic Institute 110, 8th Street, Troy, NY 12180, USA*
[c] *Department of Biology, Biotechnology 4005, Rensselaer Polytechnic Institute 110, 8th Street, Troy, NY 12180, USA. E-mail: linhar@rpi.edu; Fax: (+1) 518-276-3405*
[d] *Pharmaceutical Research Institute, Albany College of Pharmacy, Albany, New York, 12208, USA*
[e] *Department of Physico-Chemical Mechanisms of Adsorptive Detoxification, R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, 45, Vasilkovskaya Str., 03022 Kiev, Ukraine. E-mail: aos@onconet.kiev.ua*

Cellulose (20 mg, M_w = 5 800 000) was added to 1 g of the RTIL, 1-butyl-3-methylimidazolium chloride ([bmIm][Cl]).[9] This mixture was then heated at 70 °C for 30 min to fully dissolve the cellulose (2% (w/w) cellulose in [bmIm][Cl]). Imidazolium heparin was prepared from pharmaceutical grade heparin as previously described.[10,11] Imidazolium heparin (10 mg) was added to 1 g of 1-ethyl-3-methylimidazolium benzoate ([emIm][ba]), mixed by vortexing and heated at 35 °C for about 20 min, affording a clear solution (1% (w/w) heparin in [emIm][ba]). The 2% cellulose solution (20 mg in 1 g of [bmIm][Cl]) was combined with an equal volume of 1% heparin in [emIm][ba] and mixed by vortexing for 2 min, resulting in a final concentration of 1% (w/w) cellulose and 0.5% (w/w) heparin in [bmIm][Cl] + [emIm][ba]. Uncoated activated charcoal beads (100 mg, prepared from resin pyrolysis) were added to the heparin–cellulose solution and this mixture was then heated at 50 °C for 2 min and mixed by vortexing for 2 min to fully coat the charcoal. The resulting suspension was placed in syringes and introduced drop-wise into excess ethanol. The resulting heparin–cellulose coated charcoal beads were washed with ethanol using a rotary shaker (50 rpm) for 24 h to completely remove the RTILs. Neither cellulose nor heparin are ethanol soluble, thus, the ethanol selectively removes the RTILs from the coated charcoal beads. After removing the ethanol, the charcoal composite was washed with a 16% NaCl solution using a shaker (50 rpm) for 24 h to convert the imidazolium heparin to sodium heparin and to remove all leachable heparin from the heparin–cellulose coated charcoal beads. Finally, the coated charcoal beads were washed with double distilled water using a rotary shaker (50 rpm) for another 3 h to remove residual sodium chloride. The heparin–cellulose coated charcoal bead composite was recovered from the water and dried in a desiccator.

The weight of 500 uncoated charcoal beads (bulk density, γ = 0.20 g mL^{-1}) was determined to be 14.6 mg, and 500 heparin–cellulose charcoal beads (γ = 0.23 g mL^{-1}) weighed 21.9 mg. Thus, 5 mg charcoal preparation contained 5 mg uncoated charcoal beads, and 7.5 mg heparin–cellulose–charcoal composites contained 2.5 mg heparin–cellulose on 5 mg of uncoated charcoal beads (50% of the weight of the uncoated charcoal). Charcoal beads can typically be coated with polymers at ~10 wt%.[12] Recently, 30 wt% coatings of poly(4-vinylpyridine) on activated charcoal have been reported.[13] Thus, it was unexpected that highly viscous RTILs could afford cellulose–heparin coatings of 50 wt% on charcoal

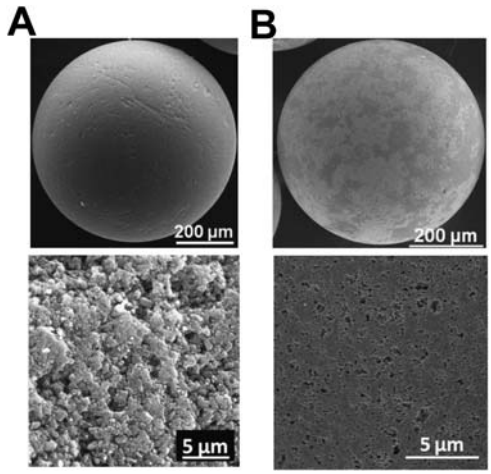

Fig. 1 FESEM images: (A) uncoated charcoal (B) heparin–cellulose–charcoal composite.

that preserved the original adsorption characteristics of uncoated charcoal.

Field emission scanning electron microscopy (FESEM) was used for the surface characterization of both uncoated charcoal beads and heparin–cellulose–charcoal composites. The FESEM image of uncoated charcoal beads shows a rough, highly porous structure with large, multimicron-sized pores both capable of allowing the penetration of large proteins and small drug molecules (Fig. 1A). The FESEM image of heparin–cellulose–charcoal composites (Fig. 1B) shows the smooth, uniformly coated surface with a large number of small, nano-sized pores, potentially capable of inhibiting the adsorption of proteins while permitting small drug molecules to adsorb to the underlying charcoal bead.

Activated partial thromboplastin time (APTT), used in evaluating the blood compatibility of heparinized polymer surfaces,[10,14] was carried out to measure anticoagulant activity of heparin–cellulose–charcoal composites in human plasma (Fig. 2). Plasma did not clot over the course of 1 h, when exposed to 7.5 mg (2.5 mg heparin–cellulose on 5 mg uncoated charcoal) of heparin–cellulose–charcoal composite, giving no measurable APTT. To obtain measurable APTT for heparin–cellulose–charcoal composite, 3 mg (1 mg heparin–cellulose on 2 mg uncoated charcoal) of sample were used to afford an APTT value of 79.9 ± 2.5 s. This compares favorably to an APTT value of 50.4 ± 0.9 s for 2 mg of uncoated charcoal bead. Thus, heparin–cellulose–charcoal composites clearly offer excellent blood compatibility characteristics, which should prove useful in the application of these heparin–cellulose–charcoal composites in extracorporeal blood detoxification.

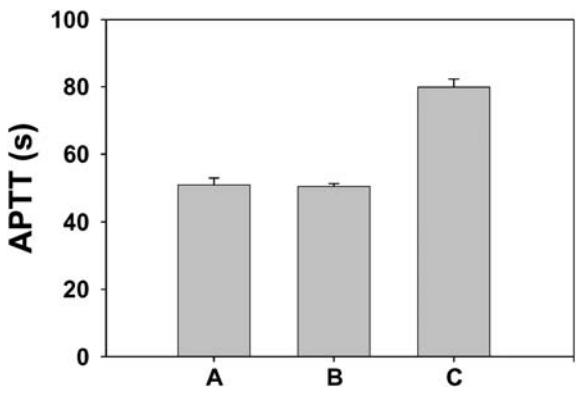

Fig. 2 APTT studies on 2 mg of charcoal preparation; (A) control (with no added charcoal), (B) uncoated charcoal, (C) heparin–cellulose–charcoal composite.

Phenytoin (5,5-diphenylhydantoin sodium, M_w 274.3) and BSA (M_w 66 430) adsorption, on both charcoal samples, were next examined (Fig. 3). Heparin–cellulose–charcoal composites showed reduced BSA adsorption compared to uncoated charcoal beads, while retaining the same ability to adsorb phenytoin. Since both uncoated charcoal beads and heparin–cellulose–charcoal composites removed 100% of the phenytoin within a period of 1 h, the capacity of phenytoin adsorbed onto the heparin–cellulose–charcoal composite was similar to uncoated

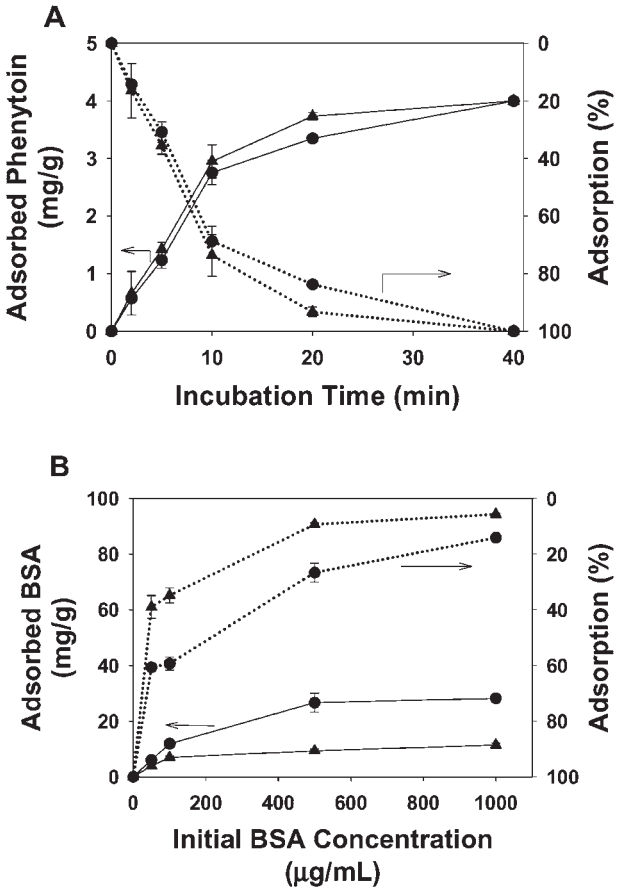

Fig. 3 The phenytoin and BSA adsorption on heparin–cellulose–charcoal composites (▲), and uncoated charcoal beads (●); the concentration of non-adsorbed (soluble) BSA was measured using the Pierce BCA Protein Assay and the concentration of non-adsorbed (soluble) phenytoin was measured by UV absorbance at 230 nm. The plots with solid lines correspond to the *y*-axis on the left and those with dotted lines to the *y*-axis on the right: (A) each 5 mg charcoal preparation was shaken (70 rpm) in 1 ml phenytoin stock solution (20 μg mL^{-1} at pH 6.3) at room temperature. (B) Each 5 mg charcoal preparation was shaken (70 rpm) at room temperature in different concentrations of 1 ml BSA stock solution (5, 50, 100, 500, and 1000 μg mL^{-1} at pH 6.3) until equilibrium is reached (24 h).

charcoal beads (Fig. 3A). The heparin–cellulose–charcoal composites resulted in a 9% decrease in BSA from 500 μg BSA in 1 mL stock solution after 24 h, while the uncoated charcoal composites resulted in a 27% decrease in BSA.

Biocompatible and blood compatible heparin–cellulose–charcoal composites have successfully been prepared using RTILs. This report introduces a new approach for coating activated charcoal to reduce its surface porosity to large molecules. FESEM clearly shows a decrease in the surface pore size in the coated charcoal preparations, which results in decreased protein adsorption while maintaining the bulk material's ability to adsorb small drug molecules. These biocompatible and blood compatible charcoal composites might be useful for direct hemoperfusion to remove free-diluted and protein-bound toxins of small size, or as potential oral agents in the cases when strict preservation of large molecules, proteins, is necessary. This successful development of novel heparin–cellulose–charcoal composites still requires *in vivo* evaluation, which we currently plan to perform in rabbits.

Notes and references

1 T. Nakamura, Y. Oida, K. Matsumoto, N. Kawasaki and S. Tanada, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, 2002, **37**, 905–912.

2 M. Melillo, G. J. Phillips, J. G. Davies, A. W. Lloyd, S. R. Tennison, O. P. Kozynchenko and S. V. Mikhalovsky, *Carbon*, 2004, **42**, 565–571.

3 V. V. Sarnatskaya, W. E. Lindup, P. Walther, V. N. Maslenny, L. A. Yushko, A. S. Sidorenko, A. V. Nikolaev and V. G. Nikolaev, *Artif. Cells, Blood Substitutes, Immobilization Biotechnol.*, 2002, **30**, 113–126.

4 L. K. Mathur, J. M. Jaffe, J. L. Colaizzi and R. W. Moriarty, *Am. J. Hosp. Pharm.*, 1976, **33**, 717–719.

5 P. Crome, G. Hampel, B. Widdop and R. Goulding, *Postgrad. Med. J.*, 1980, **56**, 763–766.

6 R. G. Peterson and L. N. Peterson, *Pediatr. Clin. North Am.*, 1986, **33**, 675–689.

7 J. F. Winchester and C. D. Ronco, *Adv. Ren. Replace. Ther.*, 2002, **9**, 19–25.

8 E. Denti, M. P. Luboz and V. Tessore, *J. Biomed. Mater. Res.*, 1975, **9**, 143–150.

9 R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *J. Am. Chem. Soc.*, 2002, **124**, 4974–4975.

10 S. Murugesan, S. Mousa, A. Vijayaraghavan, P. M. Ajayan and R. J. Linhardt, *J. Biomed. Mater. Res., Part B*, 2006, **79**, 298–304.

11 G. Viswanathan, S. Murugesan, V. Pushparaj, O. Nalamasu, P. M. Ajayan and R. J. Linhardt, *Biomacromolecules*, 2006, **7**, 415–418.

12 S. Elkheshen, H. Zia, T. E. Needham, A. Badawy and L. A. Luzzi, *J. Microencapsulation*, 1992, **9**, 41–51.

13 D. Gang, R. K. Kadari and B. Deng, *J. Environ. Eng.*, 2007, **133**, 834–838.

14 S. Murugesan, T.-J. Park, H. Yang, S. Mousa and R. J. Linhardt, *Langmuir*, 2006, **22**, 3461–3463.