Adsorbents in blood purification: From lab search to clinical therapy

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Various types of porous resins adsorbents based on polystyrene, polyvinyl alcohol, cellulose as matrixes and DNA, amino acids with other biological active molecules as ligands were extensively studied at Nankai University. Molecular recognition between the ligand and pathogenic molecule was investigated. Several commercialized products are now widely used in hospitals all over China. Whole blood hemoperfusion are used to treat patients suffering from autoimmune diseases, uremia acute intoxication and hyperbilirubinemia. Clinical performances of hundreds and thousands of patients, treated by whole blood sorption therapy are safe, efficient (90%) and cost-effective.

resin adsorbent, whole blood purification, autoimmune disease, detoxification, hemoperfusion

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In the early 1980s, a research group headed by Professor Yu was established at Nankai University and aimed to synthesize various types of adsorbents for the therapy of severe diseases, which conventional medical treatments show little or no efficiency. Bioactive ligands such as DNA and amino acids were carefully designed and linked to porous resins of polystyrene, polyvinyl alcohol, and cellulose. The adsorbents thus made were used to selectively remove pathogenic molecules in blood of patients suffering from auto-immune diseases, liver, kidney failure, hyperlipidemia, endotoxemia and acute intoxication by hemoperfusion. In order to reduce the burden of health-care agencies and enabling a large number of patients to be treated in the hospitals, the Nankai group used whole blood perfusion instead of plasmapheresis in hemoperfusion. Therefore, biocompatible adsorbents were carefully designed and tailor made for the safety of the treatment.

The present paper mainly focused on the development, commercialization and clinical performance of various kinds of adsorbents in sorption perfusion.

1 Polystyrene based adsorbent

1.1 Carbonized resin immobilized DNA

In 1983 carbonized resin beads was made by the Nankai group from a styrene copolymer by pyrolysis at high temperature. DNA was then coated on the resin with a colloidal solution to form an immuno-adsorbent. Whole blood from systemic lupus erythematosus (SLE) patients was perfused through a cartridge containing the immuno-adsorbent.

Clinical reports of 23 patients showed that the pathogenic anti-DNA antibody was removed at an average of 56%, immuno-complexes were sharply reduced and symptoms were relieved. After perfusion no significant changes in hemoglobin, leukocyte count, total protein and electrolytes were observed. Platelet depletion was in the permissible range. Whole blood hemoperfusion with the immuno-adsorbent was effective (90%) for treatment of SLE and the commercialized cartridges are now used in hospitals all over China [1–9].

1.2 Large pore-size porous resin

Various macroporous resins were tailor-made by suspension

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polymerization of styrene and divinyl benzene with various amounts of pore-forming materials at Nankai University. Resins with different pore diameters and surface areas were studied for the adsorption capacity of unconjugated bilirubin (Table 1).

From the data shown above, we can clearly see that NK-110 resin having a pore size of 160 Å and surface area of 500 m²/g showed the highest adsorption capacity for bilirubin. This is because the large size of the unconjugated bilirubin molecule could diffuse freely through the large pores of the resin. This does not mean that the larger the pore size, the higher adsorption capacity, because as the pore size increases the surface area decreases, resulting in a decrease of adsorption capacity. Therefore a balance between the two parameters should be optimized in order to obtain the highest adsorption capacity. From the above findings, a slightly modified resin was commercialized and successfully used in clinics for the treatment of hyperbilirubinemia patients in whole blood hemoperfusion [10].

1.3 High surface area resin

High surface area of 1076 m²/g porous resin was made from polystyrene by the Nankai group [11]. The bead type porous resin has a high adsorption capacity for small molecules such as drugs and chemicals (Table 2).

The physiological and blood compatible properties of the adsorbent were approved by China Food and Drug Administration (SFDA). The resin is now produced on an industrial scale and used clinically for detoxification of acute intoxicated patients in hospitals all over China.

2 Cellulose based adsorbent

Cellulose is a natural biomaterial that is biodegradable and

has good biocompatibility properties. Various adsorbents based on cellulose as matrixes were extensively studied (Table 3). An adsorbent for the removal of bacterial endotoxins was developed by immobilizing lysine covalently onto cellulose beads [12]. *In vivo* studies showed that the mean blood endotoxin concentration in rabbits was reduced significantly from 5.56±0.54 to 0.41±0.26 EU/mL after perfusion without significant side effects. It has a high potential of clinical application for treatment of endotoxemia patients [13].

Another approach showed cellulose beads linked with phenylalanine ligand can remove rheumatoid factors [14]. *In vivo* results show that the adsorbent holds promise as a highly effective and safe adsorbent in clinical therapy for rheumatoid arthritis patients by hemoperfusion [15]. It is well known that dendrimers have a large number of terminal functional groups. A synthetic approach involved arching of PAMAM onto cellulose beads by reaction with epichlorohydrin was conducted [16]. The adsorbent showed a high amount of ligands linked and an enhanced adsorption percentage for rheumatoid factors [17].

In order to remove the anti-DNA antibody from the blood of patients with systematic lupus erythematosus (SLE), DNA immuno-adsorbent was prepared by coupling calf thymus DNA to epichlorohydrin-activated cellulose beads. *In vitro* adsorption tests showed that the DNA immuno-adsorbent could remove 40%–70% of anti-DNA antibody from the plasma (incubation of 1.0 mL of adsorbent with 3.0 mL of plasma) [18]. In the circulation tests, 30 mL of plasma was circulated through a column containing 3 mL of adsorbent. The maximum decrease of anti-DNA level was 80% after 60 min. The high adsorption capacity and rate suggest the immuno-adsorbent may be used for clinical treatment [19].

Immuno-adsorbents for removing pathogenic antibodies of myasthenia gravis were prepared by coupling tryptophan onto cellulose beads. *In vivo* hemoperfusion experimental

Table 1 Various surface area and pore size of resins versus their adsorption capacity for bilirubin

	Resin	Specific surface area (m ² /g)	Average pore size (Å)	Adsorption capacity (%)
Average pore diameter larger than 120 Å	NK-X-5110	500	160	87.5
	X-5	483	152	79.3
	A-6	330	125	41.0
	A-7	200	250	28.0
	D-14	38	500	22.0
Average pore diameter lower than 120 Å	Amberlite XAD-4	750	50	16.7
	D-6	466	73	29.0
	58-9	388	43	13.8
	Amberlite XAD-2	300	90	12.0

Table 2 Physical properties and adsorption capacities of the resin

Resin	Density (g/mL)	Specific surface area (m ² /g)	Pore diameter (Å)	Porosity (%)	Pore volume (mL/g)	Particle size (mm)	Adsorption capacity (%)			
							Chlorpromazine	Barbital	Methaqualone	Valium
NK-107	0.37	1086	90	64	1.27	0.6–1.2	88.7	90.6	94.8	82.9

Table 3 Cellulose based adsorbent

Ligands	Pathogenic molecules	Adsorption efficacy (%)
Lysine	Endotoxin	80
Phenylalanine	Rheumatoid factors	60
Calf thymus DNA	Anti-DNA antibody	70
Tryptophan	Acetylcholine receptor antibodies	25
Phosphate	Low density lipoprotein	3.12 ^{a)}
Taurine	Low density lipoprotein	57
Cholesterol and sulfonic	Low density lipoprotein	88

a) Adsorption capacity (mg/mL).

results showed that the removal of acetylcholine receptor antibodies was 25% [20,21].

Hyperlipidemia is one of high risk for the development of atherosclerosis. In order to remove high levels of low density lipoprotein (LDL), especially in familial hypercholesterolemia patients, cellulose based LDL adsorbent with phosphate functional groups was prepared. The adsorption capability was 3.12 mg/mL, which proved the phosphate adsorbent had high adsorption capability and excellent adsorption selectivity. The autoclave and storage test showed the adsorbent was stable [22]. Cellulose based LDL adsorbent with taurine functional group and PAMAM spacer also showed high adsorption capacity for LDL [23].

In order to enhance the adsorption capacity of the developed adsorbent, a new amphiphilic adsorbent for the removal of LDL was prepared by introducing cholesterol and sulfonic groups onto cellulose beads [24,25]. *In vitro* studies showed that the amphiphilic adsorbent had a high adsorption capacity for LDL without significantly adsorbing high-density lipoprotein. The adsorption capacity of the adsorbent for the removal of LDL, total cholesterol (TC) and TG was 1.916, 2.132, 1.349 mg/mL, respectively. After 2 h hemoperfusion on hyperlipidemia rabbits, the LDL levels from 3.619 ± 0.354 decreased to 0.724 ± 0.07 mmol/L, demonstrating the adsorbent could effectively remove LDL [26].

3 Polyvinyl alcohol based adsorbent

Polyvinyl alcohol has been widely used in biomedical applications and has good blood compatibility. Macroporous polyvinyl alcohol microspheres were prepared and L-phenylalanine was immobilized as the ligand. The adsorbent could remove high levels of rheumatoid factors (RFs) from the blood of rheumatoid arthritis patients at a capacity of 750 IU/mL [27].

A series of bilirubin adsorbents were generated by linking of trimethylamine, triethylamine and 1,6-hexanediamine on macroporous polyvinyl alcohol beads. The adsorption capacity of bilirubin reached 80% [28,29].

Recently adsorbents for sepsis were prepared by incorporating hexane diamine onto macroporous polyvinyl alcohol microspheres. The adsorbents had high adsorption effi-

ciencies for TNF- α (72%), IL-6 (91%) and endotoxin (96%).

4 Polymethylmethacrylate (PMMA) based adsorbent

Various adsorbents have been investigated for removing endotoxin from protein solutions by PMMA based adsorbents coupling with dimethylamine ligand. Experimental results indicated that the adsorbent having a hydroxyl group at the beta-site of the ligand had an enhanced adsorption capacity for endotoxin. The computer simulation showed that the hydroxyl group at the beta-site could form hydrogen bonds with endotoxin, as a result an octatomic ring was formed. The spacer of the adsorbent and the long alkyl chain of endotoxin were located at the same side of the octatomic ring. In this situation, electrostatic interaction, hydrogen bond, cooperative effect of octatomic ring and hydrophobic intermolecular interaction effected simultaneously. The combination of endotoxin with adsorbent was tight and adsorption capacity was effectively increased [30].

5 Adsorbents with other matrixes and ligands

Agar or agrose was covalent linked on heat aggregated human IgG as ligand for the removal of rheumatoid factor. The adsorbent capacity for IgMRF, IgGRF, IgARF was 3400, 2240, and 2400 IU/g respectively [31].

Chitosan based adsorbent was also studied for the removal of LDL adsorption with a capacity of 2.72 mg/mL [32].

6 Molecular recognition mechanism between ligand and toxic molecules

In general, there are four types of interacting forces involved between the ligand and the toxic molecules i.e. electrostatic interaction, hydrophobic interaction, hydrogen bonding and Van der Waals' force. The author found that electrostatic force plays an important role and enhance the adsorption capacity drastically in the case where the toxic molecule to be removed has a high polarity [32,33]. Hydrophobic interaction is crucially important in the case where the ligand and toxic molecule are non polar [34]. Antibody-antigen interaction contributes to the specificity between the molecular recognition. Computer modeling was also used to study the reaction mechanism [31–35]. These findings attribute to the designing of more efficient adsorbent.

7 Spacer effect

The spacers have a significant effect on the adsorption

property of resin adsorbents. It can reduce the steric hindrance between the ligand and the large toxic molecules, resulting in an increase of adsorption capacity of the adsorbent. Different spacers have an obvious effect on the adsorption properties of adsorbents. When IgG was coupled by *N,N'*-carbonyldiimidazole (CDI), the immuno-adsorbent had an adsorption efficiency of 30%–35% for IgA *in vitro* experiments [36]. When it was coupled by glycidyl 1,4-butanediol, the adsorption efficiency was 60% which was much higher than that of CDI coupling method [37]. And when it was coupled by epoxy chloropropane and glutaraldehyde, the adsorbent efficiency reached 74% [38].

The length of a spacer (number of carbon atoms) influences significantly the adsorption capacity of the resin. As shown in Figure 1 the adsorption capacity increases as the number of carbon atoms increased (about 12%) [17]. Spacer, not only has a profound effect on the performance of adsorbents, recently it was also shown that it could increase the adhesion of biological cells to the matrix.

8 Scale-up production and clinical performance

In China, at present there are 5–6 companies engaged in the production of adsorbents on an industrial scale of which Jianfan Biological Science and Technology Company in Zhuhai is the largest. They used the technology and patents provided by the Nankai group, several products were produced for the treatment of SLE, hyperbilirubinemia, uremia and various acute intoxication patients by whole blood hemoperfusion. Statistic studies provided by Jianfan Biological Science and Technology Company, shown that more than 200000 cartridges were produced yearly and used in 2300 hospitals in China. The productive value was over 200 million RMB (~3.2 million USD). Furthermore, new adsorbents were developed for the treatment of sepsis and multi-failure organs. Going back to the 1980s, when the adsorbents were produced and clinical trials needed to be performed, but the medical doctors at that time did not recognize the techniques of sorption perfusion and refused to carry out clinical trials. So the first trial was carried on an

Figure 1 The effect of spacer length on the adsorption property of resin adsorbents.

acute intoxicated patient who was in deep coma with heart beads permanently stopped and on a young woman suffering from severe SLE (coma grade II) complicated with renal failure. Life saving and recovery of patients encouraged doctors to continue further clinical trials. Other difficulties arose from the company that was to maintain the quality of the adsorbents in scale up production which the Nankai group helped and gave instructions. Marketing is crucially important to recoup millions of capital outlay. Various measures and issues were conducted such as organizing teaching courses, training classes for doctors and product exhibitions were displayed to advertise and teach the new sorption technology. A new method for the treatment of severe diseases was achieved after more than 10 years of persisting effort in scaling up production and clinical trials. Clinical performances have proved that it is beneficial in enhancing the life quality of patients. Up to date, whole blood sorption therapy was performed on hundreds and thousands of patients suffering autoimmune diseases, various cases of acute intoxication such as suicidal, drug overdose, accidental contact of organo-phosphorous insecticides, snake poison or miss taking puffer fish (tetrodotoxin) were successfully treated. Symptoms were relieved, lives were saved, no significant side effects have occurred and an efficiency of 90% was achieved. More important was that whole blood perfusion with locally produced adsorbents are cost-effective. A cartridge of a few hundred USD is available, so a large number of patients are able to afford it, which drastically reduces the high medical burden of health-care agencies.

9 Concluding remarks and perspectives

Thirty years of research and development in adsorbents for whole blood purification is well established in China for the treatment of autoimmune diseases, hyperbilirubinemia, uremia and various acute intoxication patients. Clinical performances have proved that it is safe, efficient and cost effective. In the future, clinical performance of large pore size, tailor made resin adsorbents would have a high efficiency for the removal of cytokines in sepsis. Investigation in the removal of pathogenic virus by adsorbents with specific ligands has a high impact in blood purification.

For the outstanding achievement, the Nankai group was rewarded the National prize of Scientific and Technological Progress, the Ho Leung Ho Lee prize, the Chinese Invention prize and the Special Contribution Prize of the 9th World Biomaterial Congress. At this point, we are grateful to our national and local governmental departments who have financially supported the Nankai research group for so many years. Sincerely thanks goes to the local and international colleagues for their strong scientific supports and assistance.

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