

Autoimmune Diseases and Immunoabsorption Therapy

Otoimmün Hastalıklar ve İmmunoabsorpsiyon Tedavisi

Review Article / Derleme

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ABSTRACT

The human immune system is structured to recognize, respond to, and destroy a wide variety of potentially harmful microorganisms. One major component of this defense system depends on its ability to recognize foreign antigens versus healthy cells or tissues. When the immune system begins to destroy vital cells and organs within the body, the resulting reaction can be the basis for certain autoimmune diseases. Immunoabsorption, an extracorporeal technique for the removal of autoantibodies from patient plasma, offers some advantages over plasmapheresis. Immunoabsorption appeared relatively rapid and safe for the extensive removal of pathogenic antibodies and immune-complexes. During the mid 1970's the first application of therapeutic intervention with immunoabsorbents was reported. In this review, some selected applications on immunoabsorption therapies mainly for removal of anti-dsDNA antibodies from systemic lupus erythematosus (SLE) and rheumatoid factors from rheumatoid arthritis patients plasmas are briefly discussed.

Key words

Autoimmune diseases, immunoabsorption, affinity adsorption, affinity therapy

ÖZET

İnsan bağışıklık sistemi, zararlı olabilecek bir çok mikroorganizmayı tanıma, karşılık verme ve yok etmeye yönelik düzenlenmiştir. Bu savunma sisteminin temel bileşeni, sağlıklı hücre ve dokulardan yabancı antijenleri ayırt edebilme özelliğine dayanmaktadır. Bağışıklık sisteminin vücut içerisindeki önemli hücre ve dokuları yok etmeye başlamasıyla oluşan reaksiyon bir otoimmün hastalığa sebep olabilmektedir. Hasta plazmasından otoantibadilerin uzaklaştırılması için uygulanan bir ekstrakorporal teknik olan immunoabsorpsiyon, plazmafereze göre önemli avantajlar sunmaktadır. İmmunoabsorpsiyon, patojenik antibadilerin ve immunkomplekslerin uzun süreli uzaklaştırılmasında daha hızlı ve güvenilirdir. İmmunoabsorpsiyonla ilk tedavi amaçlı müdahale 1970'lerin ortalarında uygulanmıştır. Bu derlemede, sistemik lupus eritematos'lu ve romatoid artrit'li plazmalarından anti-dsDNA antibadilerin ve romatoid faktörlerinin uzaklaştırılması için uygulanan immunoabsorpsiyon tedavilerine ait seçilen bazı makaleler kısaca tartışılmıştır.

Anahtar Kelimeler

Otoimmün hastalıklar, immunoabsorpsiyon, afinite adsorpsiyonu, afinite tedavisi

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INTRODUCTION

Autoimmunity and Autoimmune Diseases

The human immune system is organized to recognize, respond to, and destroy a wide variety of potentially harmful microorganisms. One major component of this defense system depends on its ability to recognize foreign antigens versus healthy cells or tissues. When the immune system begins to destroy vital cells and organs within the body, the resulting reaction can be the basis for certain autoimmune diseases [1]. Autoimmune diseases are a heterogeneous group of disorders in which recognition of self-antigens by lymphocytes is centrally involved in pathologic organ damage. Autoimmune diseases can be classified according to how the damage is induced; humoral-mediated autoimmune diseases (e.g., myasthenia gravis, autoimmune hemolytic anemia and immune thrombocytopenic purpura) and cell-mediated autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, and insulin-dependent diabetes mellitus) [2]. Another classification is based on the number of organs afflicted. Autoimmune diseases can be divided into either organ-specific disorders and systemic disorders (Table 1).

Immunoabsorption

Immunoabsorption (i.e., extracorporeal therapy) was first introduced as a treatment method for pathogenic diseases that caused an increase in the body's production of toxic substances or interfered with processes that interrupted an end-organ's systemic filtration ability [3]. Especially, immunoabsorption with affinity adsorbents has become increasingly utilized a therapeutic modality to remove pathogenic antibodies from plasma of patients. During the mid 1970's the first ex vivo application of immunoabsorption came from Terman and his coworkers [4]. They removed DNA antibodies from the plasma of positively immunized rabbits by circulating their blood through an extracorporeal shunt containing DNA attached charcoal. They also reported removal of antibodies from the blood of dog previously injected with antibodies using albumin/collodion/charcoal adsorbent system [5]. The first clinical trial of immunoabsorption was also achieved by Terman et al in 1979, where they

used DNA attached charcoal to treat a female patient suffering from systemic lupus erythematosus (SLE) [6]. One of the early advancements related to immunoabsorption came from studies of Ray and his coworkers, who have attempted extracorporeal adsorption of pathogenic antibodies and immune complexes in various diseases including cancer [7]. They used protein A containing *Staphylococcus aurei* of Cowan I strain for IgG removal from the patients with chronic lymphocytic leukemia and autoimmune hemolytic anemia. Protein A attached agarose based adsorbents have been also used successfully to treat patients with hemophilia complicated to factors VII and IX, respectively [8,9]. Hoshimoto et al used a dextran sulphate column for immunoabsorption in SLE patients with nephritis and observed antibody and immune complex reduction as well as clinical improvement under a drug therapy restricted to corticosteroids [10]. Palmer et al found therapeutic protein A immunoabsorption to be effective in glomerulonephritis when combined with corticosteroids and cyclophosphamide [11].

Protein A has high affinity for IgG, antigen-bound IgG and IgM complexes such as rheumatoid factors and circulating immune complexes. The protein A immunoabsorption column used in the treatment of several rheumatoid arthritis has failed to respond to the traditional treatment with diseases modifying anti-rheumatic drugs [12]. Staphylococcal protein A is immunogenic and any leakage from the column is capable of inducing immunological stimulation, together with hypertension [13]. The most common adverse effects of immunoabsorption treatment with protein A was joint pain, followed by fatigue, joint swelling, abdominal pain, headache, nausea and dizziness; the total of these complaints were experienced by 25% of patients. Other side effects included infectious complications due to central line placement for venous access and decreases in hemoglobin and hematocrit in a population already affected with anemia from chronic disease [14-16].

Several groups have investigated the feasibility of immunoabsorption columns for the selective removal of anti-dsDNA antibodies from SLE, plasma patient [17-23]. These studies have been performed with different adsorbents bearing dextran sulphate,

Table 1. Classification of autoimmune diseases [3].

Disease	Target organ/tissue
<i>Systemic</i>	
Systemic lupus erythematosus	Skin, joints, kidneys, brain, heart, lungs
Rheumatoid arthritis	Joints, lungs, skin, pericard
Sjogren's syndrome	Exocrine glands (particularly paratoid and lacrimal)
Diffuse systemic sclerosis	Skin, joints, kidneys, gastrointestinal tract, lungs
Goodpasture's syndrome	Kidneys, lung
<i>Organ specific</i>	
Myasthenia gravis	Muscles
Polymyositis	Muscles
Graves' disease	Thyroid
Hoshimoto's disease	Thyroid
Pernicious anemia	Stomach
Addison's disease	Adrenal
Insulin-dependent diabetes	Pancreas
Primary biliary cirrhosis	Liver
Autoimmune hemolytic anemia	Red blood cells
Idiopathic thrombocytopenic purpura	Platelets
Pempigus vulgaris	Skin, mucosal membranes

phenylalanine, tryptophan, protein A and antibody as ligand molecule. In principle, adsorption is based on physicochemical forces (Van der Waals forces, electrostatic interactions, hydrophobic interactions or hydrogen bonds).

Phenylalanine, tryptophan and dextran sulphate bind their targets by physicochemical forces, while protein A and antibodies recognize via biological forces (antigen-antibody/Fc binding, Table 2).

Dextran sulphate, which has negatively charged characteristics, has a high affinity for anti-DNA

antibodies. tryptophan has both of hydrophilic and hydrophobic groups in its molecular structure. therefore, pathogenic antibodies are adsorbed on the adsorbent by ionic and hydrophobic interactions [24].

IMMUNOADSORPTION in SLE

Systemic lupus erythematosus (SLE) is a multi-system, auto-immune, connective-tissue disorder with a broad range of clinical presentations [25]. SLE affects predominantly women, with an incident of 1 in 700 among women between the ages of 20 and 60 about 1 in 250 black women)

Table 2. Immunoabsorbents: mode of action [23].

Ligand	Binding force	Principle
Anti-IgG, anti-LDL	Antigen-antibody	Biological interaction
Protein A	Fc	Biological interaction
Phenylalanine, tryptophan	Hydrophobic	Physicochemical interaction
Dextran sulphate	Ionic	Physicochemical interaction

and a female: male ratio of 10:1. Ethnic groups, such as those with African or Asian ancestry, are at greatest risk of developing disorder, which can be more severe than in white patients [26]. The principle clinical manifestations are skin rashes, arthritis and glomerulo-nephritis, but hemolytic anemia, thrombocytopenia, and central nervous system involvement are also common.

Genetic susceptibility to SLE is inherited as a complex trait and studies have proposed that several genes could be important [26]. Sunlight is the most obvious environmental factor that can exacerbate the disease (Table 3). Other factors have been considered and occupational exposure of crystalline silica was postulated as a risk for development of SLE. Epstein-Barr virus has also been identified as a possible factor in the development of lupus. Oral contraceptive use was associated with a slightly increased risk of disease.

Many different autoantibodies are found in patients with SLE. The most frequent are antinuclear, particularly anti-dsDNA antibodies, and others include antibodies against ribonucleoproteins and histones. Anti-dsDNA antibodies were first described in the sera of patients with SLE more than 40 years ago [27]. Since then, anti-dsDNA antibodies have emerged as a central focus in the investigation of the pathogenesis of SLE and of autoimmunity in general. Antibodies against DNA serve as markers of diagnostic and prognostic significance in SLE, and there is compelling evidence for an association between anti-dsDNA antibodies and tissue damage [28]. Since many of the clinical manifestations of this disease can be attributed to immune complex deposition, the concept has arisen that antibodies against DNA mediate tissue damage by the formation of DNA-anti-dsDNA antibody

immune complexes which localize throughout the body, most prominently in the kidneys [29]. However, this model, while consistent with many clinical and serologic findings, has been difficult to verify. For example, although there is suggestive evidence for DNA-anti-dsDNA antibody complexes in patient sera, such complexes have not been shown either consistently or conclusively [30]. The level of anti-dsDNA antibodies correlates well with the disease activity and organ involvements, such as nephritis and cerarthritis.

Today, intravenous pulse cyclophosphamide (IVCP) treatment is the standard treatment for severe SLE with major organ involvement [31]. IVCP can cause leucopenia and is contraindicated in some situations such as pregnancy. Among other therapeutic effects, IVCP treatment significantly reduces autoantibodies, but this effect takes time [3]. In such cases the removal of anti-dsDNA antibodies from plasma may lead to a clinical improvement. Analysis of plasmapheresis has shown that plasma exchange acts by removing autoantibodies [32]. But, risks of plasmapheresis therapy stem from the nonspecific elimination of all plasma components according to their plasma content. Necessary substitutions may induce allergic reactions and plasma replacement can convey infective diseases. The hierarchy of the specificity of different techniques for the therapeutic apheresis technology is given in Figure 1. Because of the disadvantages of plasmapheresis, efforts were made to develop a more specific extracorporeal technique to remove the autoantibodies from plasma. Therefore, immunoadsorption treatments that directly remove anti-dsDNA antibodies have been used for patients with SLE with life threatening disease.

Immunoadsorption with affinity adsorbents has become increasingly utilized a therapeutic modality to remove autoantibodies containing from plasma of patients. Nicolaev et al. applied DNA-attached activated carbon containing hemoperfusion column to the treatment of psoriasis patients [33]. Ventura et al. utilized poly(ethylene vinyl alcohol) hollow fiber carrying L-histidine for the removal of anti-dsDNA antibodies in in-vitro system [34]. Zhu et al. used DNA-attached non-woven poly(ethylene terephthalate) fabric fibers for treatment of SLE [35]. Yu and He prepared DNA-attached hydroxyethyl crosslinked

Table 3. Factors associated with development of SLE [3].

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- Sunlight
 - Occupational exposure silica, mercury and pesticides
 - Epstein-Barr virus
 - Abnormalities of apoptosis
 - Drugs
 - Abnormal signal transduction tool like receptors
 - Cytokine patterns
 - Genes
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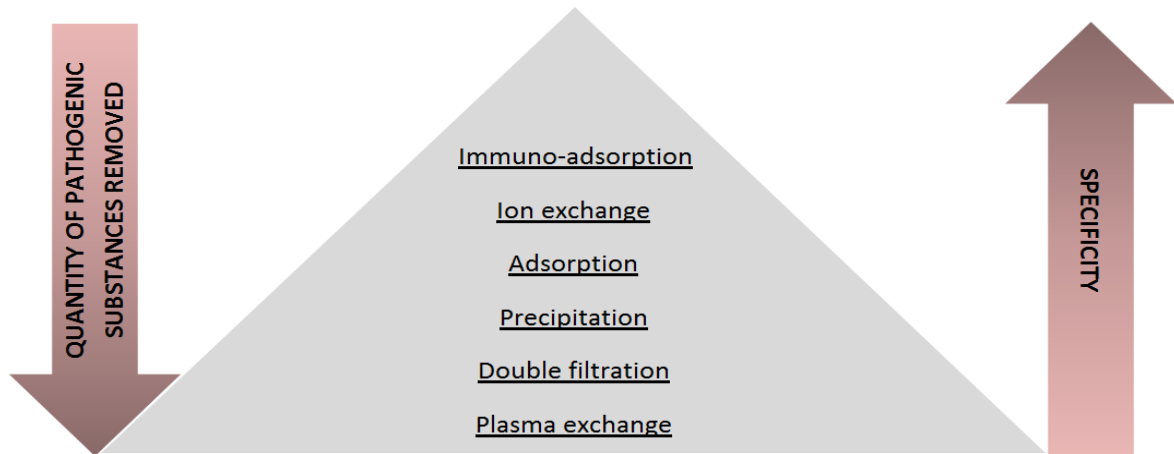


Figure 1. Hierarchy of specificity of different techniques for therapeutic apheresis.

chitosan particles as immunoabsorbents for specific removal of anti-dsDNA antibodies in SLE serum [36]. Kato and Ikada reported anti-dsDNA antibody adsorption using DNA-carrying poly(ethylene terephthalate) microfibers [37]. Hershko and Naparstek used the Prosorba silica based system which utilizes the non-selective binding of the *Staphylococcus aureus* protein A to the Fc portion of the immunoglobulins in order to remove them from the blood. This therapeutic modality has been approved by the Food and Drug Administration for the treatment of refractory rheumatoid arthritis and for resistant idiopathic thrombocytopenic purpura [38]. Aotsuka et al used polyanionic ligands bearing repeating negative charges such as dextran sulphate, polyacrylic acid and monoionic sulphonic acid for binding anti-dsDNA antibodies [39]. They found that the cellulose gels coupled polyanionic ligands adsorbed anti-dsDNA antibodies effectively from SLE patient plasma. Three different high affinity columns have been successfully used to treat SLE patients by Biesenbach et al. [40]. Their ligands are sheep IgG directed against human Ig, staphylococcal protein A and the synthetic peptide GAM146. Their data showed that all the columns are adequately effective in controlling key parameters of SLE. Rech et al used a combination of immunoabsorption with a monoclonal antibody to CD20 to deplete the circulating B cell pool [41]. Immunoabsorption was carried out with a synthetic peptide (GAM146) attached on Sepharose CL4B. They suggested that anti-CD20 monoclonal antibody with extracorporeal immunoabsorption might be a promising approach to treatment in severe SLE. Yan et al studied the preparation and clinical trial

of DNA immunoabsorbent for hemoperfusion in SLE therapy [42]. They reported that whole-blood hemoperfusion with spherical carbonaceous adsorbent bearing DNA is an effective treatment for patients with SLE nephritis and central nervous system symptoms. The removal of pathogenic substances was as high as 97.8% during 30 min hemoperfusion.

Membranes for anti-dsDNA antibody removal from SLE patient plasma

The traditional hemoperfusion column is effective and widely used for diverse applications. However, it has several disadvantages, such as the compressibility of the particles, the fouling, and particularly slow flow-rate through the column (5-20 ml/min) [43]. The smaller the particles are, the greater the cost of pressure drop will be. One significant trend has been to replace the particle packing by porous membranes that allow operation at low pressure [44]. Especially, when dealing with blood, contact with the membrane in a stacked-system is desirable because of high convective transport rates without cell damage [45]. The desirable properties of affinity membranes are high porosity, large internal surface area, high chemical, biological and mechanical stabilities, hydrophilicity, low non-specific adsorption of blood proteins and the presence of functional groups for derivatization [46]. Due to these advantages, affinity membranes provide higher efficiency.

Jia et al established a protein A tangential flow affinity membrane chromatography cartridge for

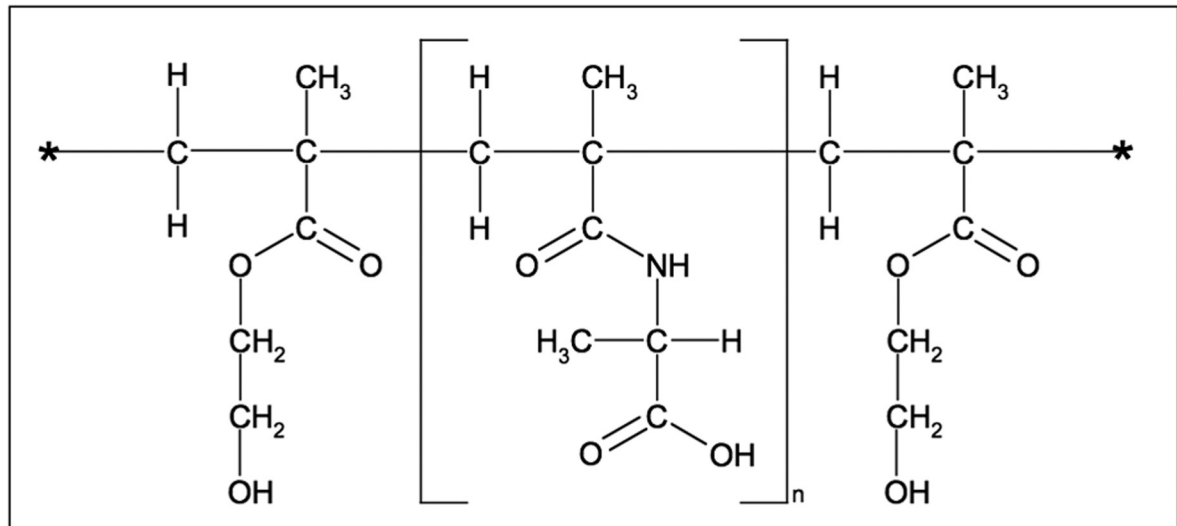


Figure 2. The molecular formula of PHEMAAL.

therapeutic extracorporeal immunoadsorption [47]. Their results showed that the pressure drop increased with the increasing flow rate of water, plasma and blood, demonstrating reliable strength of membrane at high flow rate. Their experiments in vitro and in vivo confirmed that protein A tangential flow affinity membrane cartridge mainly adsorbed immunoglobulin G and a little of other plasma proteins, and that blood cell damage was negligible. Pitiot et al showed the capability of the immobilized L-histidine-poly(ethylene vinyl alcohol) hollow fiber membrane cartridge for the removal of specific pathogenic autoantibodies using a patient's plasma with SLE and primary antiphospholipid syndrome [48,49]. They optimized biochemical and hydrodynamic conditions so performed in vitro preferentially removal of autoantibodies.

An interesting approach for the preparation of blood compatible affinity membrane for application in immunoadsorption devices was reported by Denizli et al [50]. They prepared DNA bearing poly(hydroxyethyl methacrylate) (PHEMA) based affinity membrane for selective removal of anti-dsDNA antibodies from SLE patient plasma in in-vitro. In order to increase blood-compatibility of membrane, the aminoacid based comonomer N-methacryloyl-L-alanine (MAAL) was included in the polymerization medium.

Molecular formula of PHEMAAL membrane is given in Figure 2. The PHEMAAL membrane was produced by a photo-polymerization technique

and then characterized by scanning electron microscope (SEM).

The SEM images given in Figure 3 show the surface structure and the cross-section of the PHEMAAL membrane. As seen from the SEM images, the membranes have large interconnected pores; the pore sizes are around in the range of 5-10 μm . The membrane surface seems rough and heterogeneous. The large pores reduce diffusional resistance and increase mass transfer rate of anti-dsDNA antibodies. This also provides higher DNA binding and enhances higher antibody adsorption amount.

They reported a negligible anti-dsDNA-antibody adsorption amount onto the PHEMAAL membrane, about 78 IU/g. The maximum anti-dsDNA-antibody adsorption from SLE plasma is found to be 68×10^3 IU/g onto the PHEMAAL-DNA membrane. The affinity constant of DNA-anti-dsDNA antibody complex is $4.9 \times 10^9 \text{ M}^{-1}$. Anti-dsDNA-antibody concentration decreased significantly from 875 IU/ml to 144 IU/ml with the time (Figure 4).

There are many studies are available for the extracorporeal removal of autoantibodies by various columns. A wide range of equilibrium adsorption times have been reported in the literature. Suzuki used dextran sulfate cellulose columns for removal of anti-dsDNA antibodies from the circulating blood of SLE patients [17].

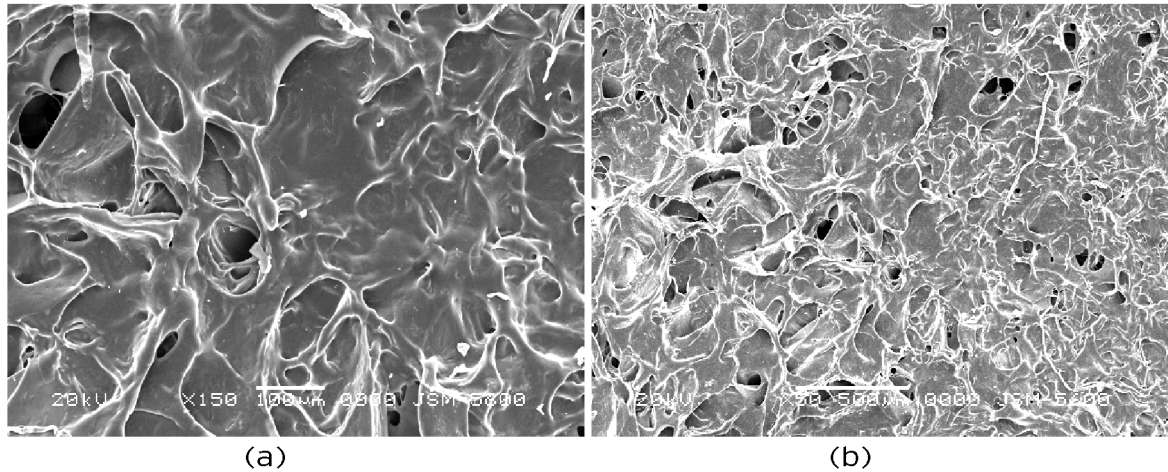


Figure 3. The SEM images of PHEMAAL membranes; (a) Surface and (b) Cross-sectional area.

The apheresis had an immediate effect on the elimination of anti-dsDNA antibodies, the titer of anti-dsDNA antibodies was decreased from 100 IU/ml to 15 IU/ml. Schneider et al studied immunoabsorption plasma perfusion in SLE patients using phenylalanine and tryptophan carrying commercial IM-P columns and reported 2 h treatment time [20]. They observed that anti-dsDNA antibody level decreased from 610 IU/ml to 80 IU/ml. Anti-dsDNA antibodies of the IgG class were reduced by 33.9% in this study. Sugimoto et al used adsorption columns with phenylalanine ligand (Immisorba PH-35) for eliminating pathogenic molecules such as anti-DNA antibody and immune complexes from the serum of patients with systemic autoimmune diseases and they reported that mean serum anti-dsDNA antibody decreased from 84 IU/ml to 5.8 U/ml [21]. Stummvoll et al used IgG Therasorb Columns for the removal of anti-dsDNA antibody in active SLE [31]. Pretreatment anti-dsDNA serum levels decreased from 391 IU/ml to 146 IU/ml. Zhu et al considered 2 h as a treatment time in their anti-dsDNA antibody adsorption kinetic studies in SLE patient, in which they used poly(ethylene terephthalate) microfibers as adsorbent [35].

Uzun et al reported that the SLE patient plasma was incubated with the PHEMAAL-DNA membrane for 2 h. Both the equilibrium adsorption time (60 min) and decreasing amount (from 875 IU/ml to 144 IU/ml) observed with the PHEMAAL-DNA membrane seem to be very promising [50]. Müller et al studied immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy on a column contained polyclonal anti human

immunoglobulin antibodies produced in sheep and each session lasted a mean of 5.6 ± 2.1 h [51]. Jansen et al considered 4-5 h as an effective treatment time in their coagulation inhibitors removal studies from patients with haemophilia A, in which they used Ig-Therasorb column contained sepharose coupled with polyclonal sheep antibodies [52]. Some patients with acquired hemophilia have been treated using commercial Immunosorba[®] column around 5 h effective treatment time [53] for the removal of IgG and inhibitors. Yu and He investigated the adsorption of anti-dsDNA antibodies in SLE serum on DNA attached hydroxyethyl crosslinked chitosan particles and reported that equilibrium adsorption time is 2 h [36]. Braun et al treated a

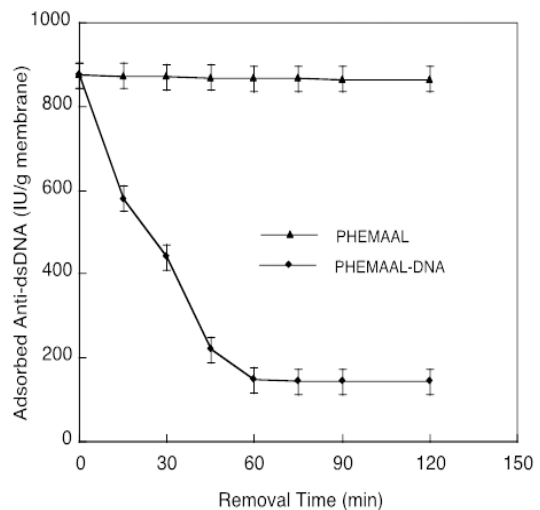


Figure 4. Effect of time on the anti-dsDNA antibody removal: DNA loading: 7.8 mg/g; anti-dsDNA-antibody concentration: 875 IU/ml; T: 25°C [50].

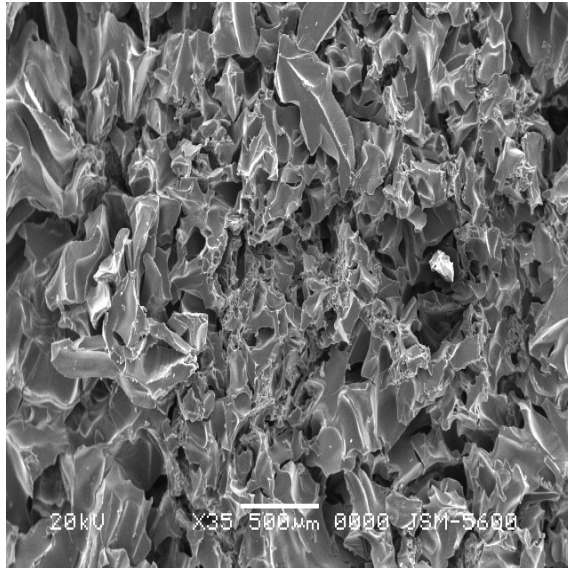
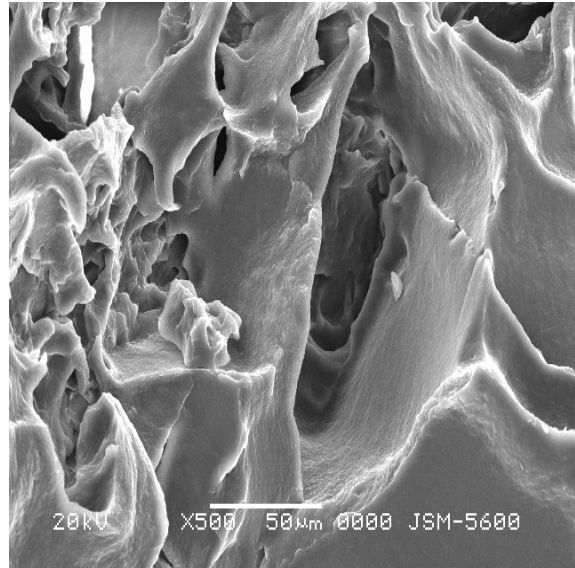


Figure 5. SEM images of the PHEMA cryogel.



patient of therapy-resistant SLE with commercial Protein A-Sepharose column (Immunosorba), which is an SPA-based immunoabsorption system, approved by the FDA for the treatment of SLE [54].

They reported that anti-dsDNA antibody level declined from 800 IU/ml to 30 IU/ml after the immunoabsorption. Kong et al prepared DNA carrying cellulose based immunoabsorbent for the specific removal of anti-DNA antibodies from the blood of patients with SLE [55]. They reported that anti-dsDNA antibody level decreased from 3500 IU/ml to 400 IU/ml in 60 min. Kong et al also removed anti-DNA antibodies from a patient's plasma with SLE using DNA attached Sepharose 4FF [56]. Takahashi et al showed that dextran sulphate carrying cellulose is a potent adsorbent of anti-dsDNA antibody [57].

They noted that anti-dsDNA antibody level decreased from 67 IU/ml to 38.5 IU/ml. The anti-DNA antibody in the plasma (2248 IU/ml) was almost completely removed by the Sepharose-DNA adsorbents. It should be also noted that the immunoabsorption therapy takes between 2-5 h with including commercial columns ProSORBA® and EXCORIM® [58,59].

Cryogels for anti-dsDNA antibody removal from SLE patient plasma

Cryogels are a very good alternative to remove harmful substances from human plasma with

many advantages including large pores, short diffusion path, low pressure drop and very short residence time [60-63].

Recently, Ozgur et al selected PHEMA which is one of the most widely used hydrophilic polymers in biomedical applications, by considering possible applications of these adsorbents in direct hemoperfusion extracorporeal therapy, in which blood compatibility is one of the main concerns [64-66]. The SEM images of the PHEMA cryogel is shown in Figure 5. The PHEMA cryogel have non-porous walls and large continuous interconnected pores (10-200 µm in diameter) that provide channels for the mobile phase to flow. The pressure drop needed to drive the liquid through any system should be as low as possible [67].

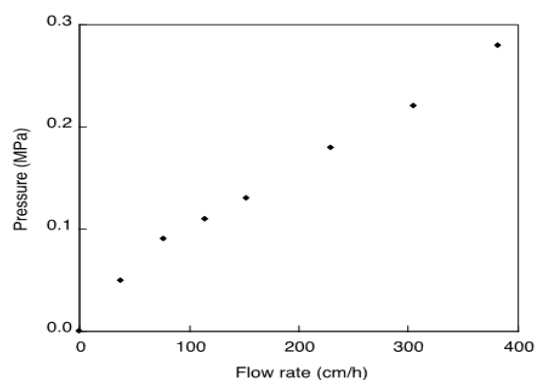


Figure 6. Pressure drop at different flow rates.

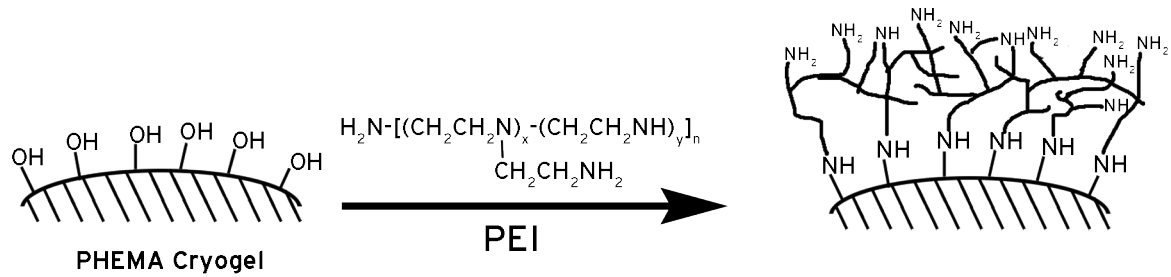


Figure 7. PEI attached PHEMA cryogel.

Pressure drop studies through the PHEMA cryogel were performed in water as equilibration medium, and at linear flow rates from 38 to 382 cm/h (Figure 6). The water was passed through the column for 1 min at different flow-rates. Due to the presence of large and interconnected macropores, the PHEMA cryogel column has very low flow resistance. PHEMA cryogel column had low back pressure indicating the porous structure with large size of the interconnected macropores.

In order to have high adsorption capacity for DNA, PEI was attached on the PHEMA cryogel (Figure 7). It was demonstrated that the attachment of PEI chains onto the adsorbent to form tentacle-type supports could sufficiently increase the adsorption capacity of biomolecules [68].

Ozgur et al recently reported the use of selective anti-dsDNA antibody adsorption using DNA-attached PHEMA cryogel [69]. Traditional columns have a major limitation: incapability of processing whole blood. Blood cells are trapped between the particles of the column resulting in increased flow resistance and complete blockage of the flow.

Expanded-bed chromatographic set-up overcomes the problem of handling viscous solutions. However, the high shear stresses occurring in expanded bed chromatographic set-up could be detrimental for the integrity of blood cells. It is attractive to have a chromatographic adsorbent with pores large enough to accommodate blood cells without being blocked (Figure 8). The supermacroporosity of cryogels makes them appropriate candidates as the basis for such materials [70-72]. Owing to supermacroporosity and interconnected pore-structure, such a chromatographic matrix has a very low flow resistance [73-76].

The most important properties required of immunoadsorbents are high capacity and specificity of adsorbent. Ozgur et al noted that negligible amount of anti-dsDNA antibody adsorbed on the PHEMA cryogel, which was about 90 IU/g.

DNA attachment significantly increased the anti-dsDNA antibody adsorption amount of the PHEMA-PEI-DNA up to 70×10^3 IU/g. With

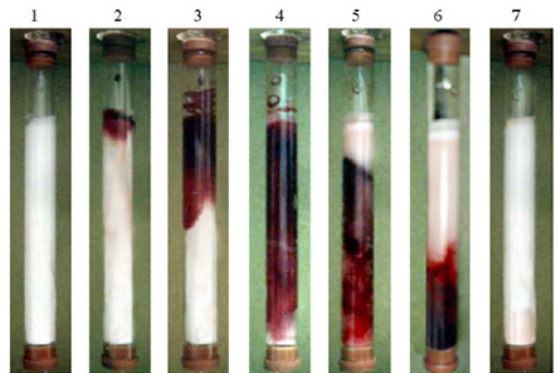


Figure 8. Whole blood is passed through a PHEMA cryogel column showing the convective flow of blood.

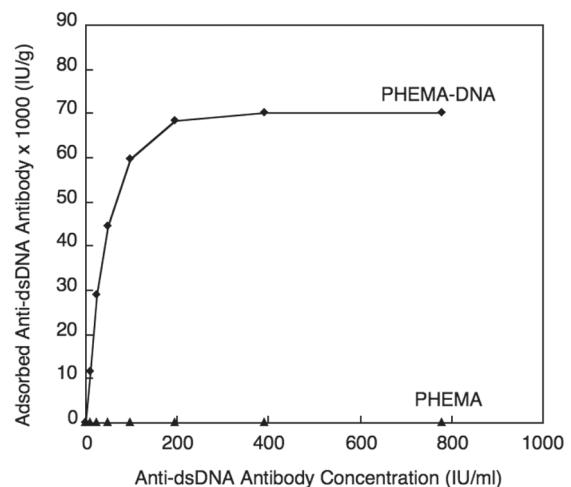


Figure 9. Effect of anti-dsDNA antibody concentration on adsorption capacity; DNA loading: 53.4 mg/g; Flow-rate: 0.5 ml/min; T: 25°C [69].

increasing anti-dsDNA antibody concentration, the amount of anti-dsDNA antibody adsorbed amount per unit mass increased below about 195 IU/ml, then increased less rapidly (Figure 9). It reached saturation level when the anti-dsDNA antibody concentration is greater than 200 IU/ml.

Magnetic particles for anti-dsDNA antibody removal from SLE patient plasma

Conventional immunoadsorption therapies used to remove pathogenic antibodies perform poorly due to low accessibility, insufficient adsorption capacities, low efficiency and economic limitations [77-79]. Denizli and his coworkers used DNA-attached mPHEMA particles (80-120 μm in diameter) as a specific affinity adsorbent for removal of anti-dsDNA antibodies from SLE patient plasma in magnetically stabilized fluidized bed (MSFB) for the first time in literature [80,81]. mPHEMA particles are hydrophilic and cross-linked structures [82]. The surface morphology and internal structure of non-magnetic PHEMA and mPHEMA particles are examined by the SEM pictures in Figure 10. mPHEMA particles have a rough surface containing pores due to the abrasion of magnetite crystals during the

polymerization (Figure 10A). However, the surface of the non-magnetic PHEMA particles contained no pores (Figure 10B). The SEM pictures in Figure 10C and 10D were taken with broken particles to observe the internal structure of both non-magnetic and mPHEMA particles. The presence of pores within the particle interior was clearly seen in these photographs. They concluded that the mPHEMA particles have a macroporous bulk structure and rough surface.

MSFB combines the best characteristics of both packed bed and fluidized bed. These include the efficient mass transfer properties, low pressure drop, good fluid-solid contact, elimination of clogging [83]. Especially, when dealing with blood contact with the magnetic adsorbent in a MSFB is desirable because of high convective transport rates without hemolysis. Denizli et al used DNA as the affinity ligand for specific binding of anti-dsDNA antibody [82]. They reported that the maximum anti-dsDNA antibody adsorption capacity was 97.8 mg/g in 2 h. The SLE patient plasma was passed through the column containing particles for 2 h. The removal rates obtained with the mPHEMA/DNA particles us seem to be very promising.

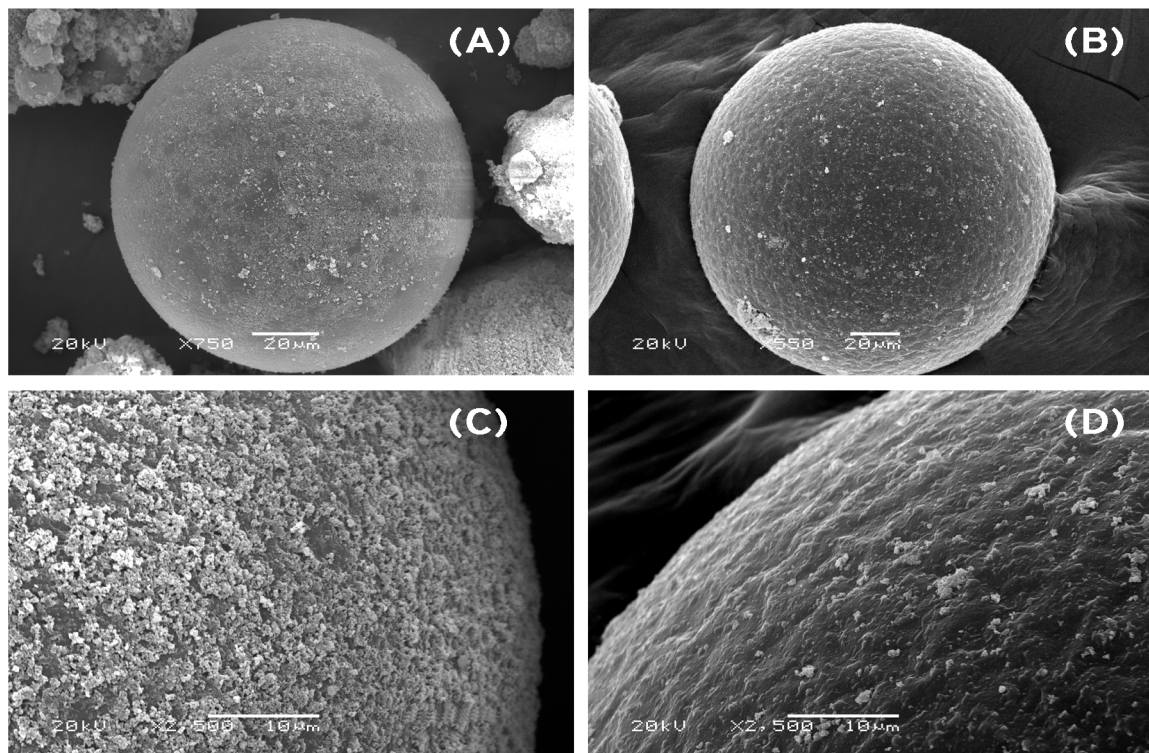


Figure 10. SEM pictures of polymeric particles: (A) surface of mPHEMA; (B) surface of non-magnetic PHEMA; (C) cross-section of mPHEMA; (D) cross-section of non-magnetic PHEMA.

Table 4. Adsorption capacities for anti-dsDNA antibody of various adsorbents.

Support	Ligand	q (IU/g)	Reference
Cellulose particles	Dextran sulphate	2667	[17]
Poly(vinyl alcohol) gel	Phenylalanine	3533	[20]
	Tryptophan		
Poly(vinyl alcohol) gel	Phenylalanine	521	[21]
Non-woven PET fabric	DNA	3600	[35]
Poly(ethylene terephthalate)	DNA	49800	[37]
Cellulose particles	Dextran sulphate	417	[39]
	DNA	438	
PHEMAAL membrane	DNA	68000	[50]
Cellulose particles	DNA	40400	[55]
Sepharose 4FF	Calf thymus DNA	35980	[56]
PHEMA cryogel	Herring testes DNA	70000	[69]
Cellulose particles	Dextran sulphate	1089	[84]
Poly(vinyl alcohol) gel	Phenylalanine	2158	[85]
Cellulose cotton	Calf thymus DNA	245	[86]

The most important properties required adsorbents are high capacity and specificity of binding. For the removal of anti-DNA antibodies from plasma by various columns, a wide range of adsorption capacity have been reported in the literature. Some studies including commercially available adsorbents are given in Table 4. Differences of anti-dsDNA antibody adsorption capacities are due to the properties of each adsorbent such as macromolecule structure, reactive functional groups, ligand loading amount, porosity, pore size, pore size distribution and accessible surface area.

IMMUNOADSORPTION in RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic, progressive, debilitating autoimmune disease that is characterized by chronic polyarthritis and destruction of multiple joints. RA affects approximately 0.5-1.0% of the population worldwide [87]. Standardized prevalence rate of RA in Turkey is 0.4% which is similar to other mediterranean countries. Over time, RA results in significant disability due to persistent inflammation, involvement of periarticular tissue,

and joint deformity. Studying patients with RA, the researchers found IgM antibodies (i.e., rheumatoid factor) in serum samples [88]. Rheumatoid factors are the characteristic autoantibodies of RA, which bind to the Fc regions of IgG molecules [89]. Rheumatoid factors are detected in 60-70% of the patients affected by rheumatoid arthritis. Continual production of rheumatoid factors at a high level contributes to disease progression [90]. As no cure exists, the therapeutic goal is to control the underlying inflammatory process and maintain or improve function. Because it compromises or prevents patients from engaging in desired physical activities, rheumatoid arthritis impacts most aspects of daily living [91]. RA generally has been regarded as a "non-fatal" disease in most patients; however, studies of life expectancy have revealed that survival among patients with RA is approximately 3 to 18 years shorter than that of the general population [92,93]. Treatment of RA incorporates the use of first-line drugs (aspirin and corticosteroids for pain and inflammation) and second line drugs (methotrexate and hydroxychloroquine to prevent joint destruction and promote remission). The continued use of methotrexate in the treatment of RA has been

implicated in a variety of undesirable side effects including gastrointestinal toxicity, stomatitis, hematological toxicity, pulmonary toxicity [94].

Plasma exchange has been applied to remove autoantibodies in the treatment of severe forms of various autoimmune diseases [95]. In patients with RA, plasma exchange had shown no significant clinical benefits [96]. Plasma exchange is a non-selective method and the requirement for plasma substitutes such as albumin is very high. Moreover, the dangers of hepatitis or immune reaction accompany this therapy while using plasma products. It would be most desirable to selectively remove any pathogenic substances. For this purpose, adsorption was suggested as an alternative to plasma exchange for removing pathogenic substances from the plasma of patients with autoimmune diseases refractory to conventional treatments [95]. Immunoabsorption has also been used for other conditions such as treatment of cancer, paraneoplastic syndroms and Guillain-Barré syndrome. Immunoabsorption is more selective than plasma exchange, as it does not remove plasma proteins such as albumin and clotting factors. In addition, adsorption does not require administration of plasma substitutes and therefore does not expose patients to the potential side effects of these compounds [97].

Recently, Yilmaz et al studied and assessed the poly(2-hydroxyethyl methacrylate-N-methacryloyl-(L)-histidine-methylester) (PHEMAH) particles for its capability to remove the auto-antibodies in human plasma with rheumatoid arthritis [98]. In the first part, N-methacryloyl-(L)-histidine-methylester (MAH) was synthesized by methacryloyl chloride and L-histidine methylester as a comonomer and/or pseudo-specific ligand for IgM-antibody molecules. The PHEMAH particles are mostly in the size range of 80-120 μm . Surface area of the PHEMAH particles is 18.3 m^2/g . Mercury porosimetry data showed that the average pore diameter of the PHEMAH particles was 250 nm. This indicated that the PHEMAH particles contained mainly mesopores. IgG is a compact sphere with approximate diameter of 8-14 nm (Fab' and Fc fragment) [99]. Therefore, this pore diameter range of PHEMAH particles is possibly available for diffusion of the IgG molecules.

Ligand loading is an important design parameter in the chromatographic operations [100]. In order to determine the effect of the amount of MAH content on the adsorption of IgM-antibody, the amount of MAH incorporated into the particles was changed in the range of 2.4-35.4 $\mu\text{mol}/\text{g}$. Figure 11 shows the effect of MAH incorporation on IgM-antibody adsorption. The IgM-antibody adsorption capacity increases rapidly at low MAH incorporation (< 11.8 $\mu\text{mol}/\text{g}$). Then, there is a decrease in the adsorption amount after 11.8 $\mu\text{mol}/\text{g}$ MAH incorporation value. The maximum adsorption amounts that corresponding this MAH incorporation was 69.2 mg/g. But lower IgM-antibody adsorption capacities were observed at higher MAH incorporation due to steric hindrances.

Alkan et al reported IgM-antibody removal from human plasma with supermacroporous poly(hydroxyethyl methacrylate) [PHEMA] cryogel carrying protein A [101]. The PHEMA cryogel was prepared by bulk polymerization which proceeds in an aqueous solution of monomer frozen inside a plastic syringe (cryo-polymerization). After thawing, the PHEMA cryogel contains a continuous matrix having interconnected macro-pores of 10-200 μm size. Pore volume in the PHEMA cryogel was 71.6%. Protein A molecules were covalently attached onto the PHEMA cryogel via cyanogen bromide (CNBr) activation.

The PHEMA cryogel was contacted with blood in in-vitro system for determination of blood-

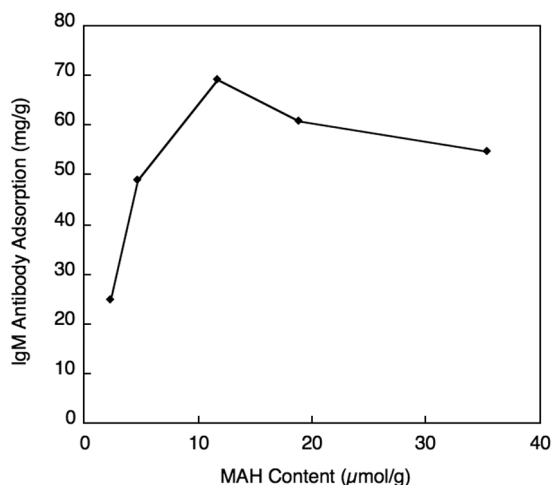


Figure 11. Effect of MAH content on IgM-antibody adsorption: IgM-antibody concentration: 2.85 mg/ml; Flow-rate: 0.5 ml/min; T: 25°C [97].

compatibility. The supermacroporous structure of the PHEMA cryogel makes it possible to process blood cells without blocking the cryogel column. IgM-antibody adsorption capacity decreased significantly with the increase of the plasma flow-rate. The maximum IgM-antibody adsorption amount was 42.7 mg/g. IgM-antibody molecules could be repeatedly adsorbed and eluted without noticeable loss in the IgM-antibody adsorption amount.

Some selected applications

Selective immunoabsorption has been experimentally implemented in myasthenia gravis patients using a peptide originating from the human α -subunit of the acetylcholine receptor [102]. Myasthenia gravis is an autoimmune disease in which the neuromuscular transmission disorder is caused by inflammatory and degenerative changes at the neuromuscular junction resulting in an autoimmune reaction against acetylcholine receptors [103]. A study encompassing 22 randomly selected patients showed significant reduction of blocking antibodies with concomitant clinical improvement in more than half of these patients. Patients with myasthenia gravis were treated with Ig-Adsopak immunoabsorption columns [104]. The amounts of IgG, IgM and IgA removed were respectively 55.7%, 42.8% and 43.7% of the initial level. Benny et al used the immunoabsorption columns containing agarose-protein A adsorbent [105]. They reported that the patients with myasthenia gravis respond to treatment with plasma immunoabsorption. Ronspeck et al described a new generation adsorbents for autoantibody elimination based on synthetic peptides and their use in immunoabsorption therapy [106]. The adsorber Coraffin is the first adsorber for specific removal of β_1 -adrenergic autoantibodies in idiopathic thrombocytopenia cardiomyopathy. Coraffin has been developed on the basis of peptide epitopes binding to β_1 -adrenergic autoantibodies.

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