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Review

Gel fiberglass membranes for affinity chromatography columns and their application to cancer detection

Igor Zusman

Laboratory of Teratology and Experimental Oncology, Koret School of Veterinary Medicine, Faculty of Agriculture, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Box 12, 76100 Rehovot, Israel

Abstract

The development of mechanical supports for biochemically active compounds serving as immunochemical sensors has been the goal of many studies. A new compound in the form of gel fiberglass (GFG) membranes was recently developed as an example of such supports. These membranes prepared from glass fibers covered with oxysilanes to create a matrix 'gel fiberglass' (R. Zusman, USA Patent #08/112,087, 1993) were used to prepare affinity chromatography columns for the isolation of soluble p53 protein from the serum of cancer patients. A thin layer of protein, trapped in gel glass during its preparation, was deposited on a lattice of glass fibers. Derivatization of the support eliminated nonspecific adsorption of proteins. Under such conditions, external agents percolating through a membrane may contact a maximum number of protein molecules trapped in the gel glass. The membranes are very stable, and can be stored in dry conditions for several months at room temperature. Affinity chromatography columns were prepared from the GFG membranes and used to isolate various proteins, including tumor-associated antigens (TAA). The capacity of the columns was calculated as the amount of protein (mg ml^{-1}) isolated from TAA-containing solution, and amounted to up to 9 mg ml^{-1} of serum in colon cancer patients. The cytoplasmic p53 protein was one of the main components of TAA isolated in our experiments. Its concentration was determined by HPLC. This protein was isolated from the serum of cancer patients in the highest concentration yet reported, up to 5 mg ml^{-1}. The described method allows an easy and highly effective isolation of TAA and can be used for important goals including cancer diagnosis. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Gel fiberglass membranes; p53 protein

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1. Introduction

Immunochemical methods are widely used for diagnostic purposes; however the results are not always unequivocal [1–3], and research for additional approaches continues. Many studies during the last two decades have aimed at developing mechanical supports for biochemically active compounds which

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can act as immunochemical sensors [4–6]. Various glass beads have been used for electroelution of proteins from SDS–polyacrylamide gels [7]. Different fractions of human serum IgG have been isolated by affinity chromatography on jacalin–sepharose [8]. A cellulose and acryl composite matrix has been utilized to immobilize protein A and purify immunoglobulins from the serum [9]. Specific antibodies were isolated from the blood by attachment to glass coated with poly-*N*(2-hydroxyethyl)acrylamide [10]. Glycyrrhizin-affinity chromatography columns were used to isolate lipoxygenase from soybean [11] and casein kinase, from mouse liver [12]. Concanavalin A immobilized on chlorotriazine-bead cellulose was used for affinity purification of glycoenzymes and glycoproteins [13]. Cellulose-based chelating supports were used for affinity chromatography of human growth hormones [14]. Small particles of dialdehyde cellulose were used to prepare immunoadsorbents for affinity isolation of proteins [15]. A synthetic peptide derivative alanyl leucyl morpholide is suggested as a specific ligand for affinity chromatography of subtilisin-like serine proteases [16]. PbCS 242 310 polypeptides corresponding to the C-terminal region of *Plasmodium berghei* CS protein were purified by a combination of affinity chromatography and methionine chemistry [17].

A new effective affinity chromatography membrane was developed using sol–gel glass as a support system [18]. In this method, the isolation of large amounts of affinity proteins can be achieved without nonspecific proteins. Several attempts have been made to isolate proteins [18,19] and to immobilize enzymes [20,21] using sol–gel glass as a support system.

More recently, gel fiberglass (GFG) was used to prepare a new class of affinity supports for the isolation of proteins [22,23]. Rabbit anti-colon-tumor IgG entrapped in GFG columns was shown to enable the isolation of tumor-associated antigens (TAA) from sera of colon cancer patients [24]. The main component of TAA isolated in our experiments was p53 protein in its cytoplasm or soluble form [22,24]. Data concerning two forms of p53 protein, nuclear and cytoplasmic, were published elsewhere [25–27]. In our studies, the isolation of the cytoplasmic p53 from the serum was performed by affinity reaction of serum antigens with polyclonal anti-p53 IgG [22–24]. Herein, we have reviewed our previous publications devoted to the possibility of isolating human p53 antigen using GFG columns with entrapped polyclonal anti-p53 IgG.

2. Preparation of gel fiberglass membranes and study of their biochemical characteristics

A variety of methods have been described for the analytical separation of proteins. Many of these, including the affinity chromatography columns, are based on the chemical binding of different active reagents to a solid support [1,5,7,10]. Sol–gel glass is one of the new supports enabling the direct trapping of different substances [28,29] that can be prepared at room temperature [30,31]. Such support was obtained together with chemical [32] and biochemical reagents using polymerization of the support and its derivatization [12,33]. The immobilization of some enzymes [20,21] and the successful isolation of sheep IgG [19] using sol–gel glass columns have been previously described. In spite of the fact that the sol–gel glass is a porous material, part of the chemical and biochemical compounds remained inside the gel–glass mass, inaccessible to any external reagent. This decreases the amount of trapped materials available for performing analytical reactions, especially in biochemical and immunochemical studies. In order to have access to all of the reagent molecules, one has to crush the prepared glass into a powder. Glass lattice (SiO_2) resulting from the reactions of hydrolysis and polycondensation of tetramethoxysilane (TMOS) is insoluble in water. The alkaline solutions react with SiO_2 and, it in turn, dissolves silicate salt Na_2SiO_3. This reaction occurs very rapidly with the silica powder, which makes such columns unstable under the effects of alkaline buffers, which are used for extraction and purification of tissue proteins.

A new approach has been developed to avoid the above-mentioned defects. In this method, small particles of newly formed gel–glass are formed on the surface of a lattice of glass fibers [34]. This allows the external reagents to percolate through GFG columns to get in contact with the maximal number of molecules of compounds trapped in the glass and avoids the necessity for grinding the glass into powder. The time required for preparation of

GFG membranes decreases to a few hours instead of two weeks in the previous method [18]. This support has shown to have high stability under storage for 10 months in dry conditions at room temperature.

Biotechnological characteristics of the GFG support such as stability, effectiveness, capacity and specificity are essential for an affinity chromatography matrix and have been described previously [23]. The effectiveness of the method was evaluated in relation to the concentration of different detergents that were used to prevent the leaching of gel glass. Different chemical compounds were used to derivatise sol–gel glass and to eliminate nonspecific protein absorption. The capacity of the GFG columns was studied in relation to the chemical composition and pH of elution buffers and to concentrations of protein entrapped in the GFG supports. Both normal and tumor proteins have been used to evaluate the isolation-capacity range of these supports [22,23].

The preparation of a GFG membrane has been described in detail previously [22–24]. Fiberglass were initially washed in distilled water and treated for 5 min in acid solution as described [34]. Five milligrams of such fiberglass were mixed with 45 μg of TMOS, 50 μl of polyethylenglycol 6000 in the form of 20% solution in distilled water, 20 μl of 0.1 *M* glycine, 0.2 mg of polyvinylpyrrolidone (PVP) and 0.1 mg of cetyldimethylethylammonium bromide. The mixture was shaken for 15 min and cooled on ice. One milligram of protein dissolved in 10 μl of glycine was added to the mixture. After additional shaking of 15–20 min, the prepared gel-mass was kept for 4–5 h at 37°C to complete polymerization. 3-Aminopropyltriethoxysilane dissolved in distilled water (1:20) was added (2% of membrane weight). After 16–18 h of drying at room temperature, the GFG membranes were ready for use.

Affinity chromatography columns prepared with GFG have been used to isolate different types of proteins [22–24]. Different IgG (10–25 mg) were entrapped in GFG membranes (0.5×10 mm) during their preparation (one membrane contained 1 mg of protein). These membranes were arranged in pre-column barrels with the porous nylon membranes. The columns were used to isolate specific polyclonal IgG from rabbit sera [22], heat shock proteins from rat serum [35] or TAA from the human serum of cancer patients [24,36].

The principal procedure for isolation of the soluble p53 antigen is shown in Table 1. One milliliter of a primary antigenic extract, or serum, with protein concentrations of 16 to 26 mg/ml was dissolved 1:3 in 0.01 *M* Tris with 0.15 *M* NaCl, pH 7.5, and percolated three times through GFG columns with entrapped IgG [23,24,36]. The rate of percolation was 0.1–0.2 ml/min. The columns were washed with the same buffer until proteins disappeared in the rinsing water. Elution of antigens was performed with 0.1 *M* glycine buffer, pH 2.5. The pH of the eluted protein solution was immediately adjusted to 7.5. The proteins eluted from the GFG columns were dialyzed against distilled water and concentrated to 1 ml. Immunochemical methods as 12% SDS–PAGE, ELISA and Western immunoblotting were used to determine the profile of isolated proteins and their affinity to rabbit anti-tumor serum and commercial monoclonal antibodies (PAb) [37].

Gel fiberglass has a highly dispersed surface. Under a scanning electron microscope, it appears as small particles located on the surface of a lattice of

Table 1
The scheme of isolation of the soluble p53 protein from serum (After Refs. [24,36,38])

No. of procedure	Description	Resulting product
1	Isolation of TAA from tumors, immunization of rabbits	Antitumor IgG
2	Trapping of anti-tumor IgG in the GFG columns and percolation the human serum through the columns	Serum TAA
3	Isolation of p53 from the serum–TAA and immunization of rabbits	Anti-p53 IgG
4	Trapping of anti-p53 IgG in the GFG columns and percolation the human serum through the columns	Serum p53 antigen

glass fibers [23]. Utilization of the columns can be accompanied by leaching of entrapped proteins without the destruction of the gel glass. Three detergents (cetyldimethylethylammonium bromide, cetylpyridinium bromide and cetrimide) were studied to prevent this destruction. The highest amount of protein eluted after percolation of the serum through GFG columns was obtained from the column containing cetyldimethylethylammonium bromide [23]. The life time of GFG prepared without any detergent was short and such columns cannot be utilized. The usable lifetime of the column prepared with the best surfactant was the same as the storage lifetime of the column and it continues for a few months.

Nonspecific adsorption developed in nonderivatized glass causes the elution of nonspecific proteins. Between 0.5% and 1.0% of the nonspecific protein of initial protein weight was found in the first washing water. This adsorption can be eliminated by derivatization of sol–gel glass with a previously used 3-aminopropyltriethoxysilane moiety [19]. To completely block the nonspecific centers, we also used PVP as an additional hydrophobic moiety. The addition of PVP proved to be the most effective for the elimination of nonspecific protein adsorption and for obtaining the highest amount of specific protein eluted [23]. GFG prepared without derivatization binds many different nonspecific proteins.

The capacity of GFG columns was determined as the amount of eluted proteins. This parameter depends on the amount of TMOS in the matrix: the capacity of GFG columns was higher when the amount of TMOS was 0.45 to 0.5 mg/10 mg of GFG [23]. The capacity of the GFG columns also depends on the amount of IgG entrapped in a column. A column with 10 mg of entrapped anti-tumorous IgG isolated about 7 mg of proteins from TAA-containing solutions. Ten to 13 mg of proteins were isolated from columns with 20 and 30 mg of entrapped IgG, respectively [38]. The capacity of the GFG columns ranged from 29.3% to 46.0% of the amount of entrapped proteins and from 36.0% to 50.0% of the amount of percolated proteins [23,38]. Up to 9 mg of TAA were isolated from the sera of colon cancer patients using GFG columns entrapped with 20 mg of anti-colon cancer IgG isolated from rabbit antisera (Table 2) [38].

3. Clinical application of the method to cancer detection

Detection and isolation of TAA from cancer tissues and sera are used in oncological diagnostic practice [39–41]. These methods, however, are restricted by isolation of TAA in extremely small amounts, perhaps due to the fact that the authors seeked the so-called ‘nuclear proteins’. We have described a method for the isolation of large amounts of the low-molecular-mass soluble TAA located in the cellular cytoplasm. The serum levels of these TAA were shown to increase in parallels to the progress of tumorigenesis (Table 2). In patients with recurrent cancer, this parameter was similar to the serum level of tumor marker such as carcinoembryonic antigen (CEA), a fact that is in accordance with the observations in the literature about correla-

Table 2
Serum levels of total tumor-associated antigens (TAA) and carcinoembryonic antigen (CEA) (mean±S.D.). (After Refs. [24,36])

Groups of patients	n	TAA		CEA (ng/ml serum)
		mg/ml serum	μg/mg total protein	
Healthy people	26	1.95±0.86	28.2±2.12	
Gastrointestinal disorders	76	2.66±1.46	46.8±4.24	
Polyps in colon	38	4.66±2.03	69.5±.29.6	
Primary colon cancer	98	8.12±4.89	110.6±24.0[a,b]	
Recurrent cancer with metastases	50	9.2±5.8[a]	94.8±23.8[a]	75.7±67.7

[a] Significantly different from the healthy people ($P<0.05$).
[b] Significantly different from people with polyposis ($P<0.05$).

tion between serum concentration of tissue polypeptides-specific-antigen and CEA in colon cancer patients [42].

SDS–PAGE analysis of isolated TAA, ELISA and Western immunoblotting performed with rabbit anti-tumor IgG and commercial anti-human p53 PAb DO1 revealed that antigens isolated from human cancer serum have affinity for antibodies entrapped in the columns and that 64-kDa and 53-kDa proteins are the main components of these TAA (Fig. 1). Both of these proteins were significantly different in their amino acid content [25]. Moreover, Western immunoblotting showed that only 53-kDa protein has a strong affinity reaction with the commercial anti-human p53 PAb (Fig. 2). The 64-kDa protein belongs to a group of stress-related proteins [35,43,44]. There was no difference between its concentration in cancer and in noncancer patients ($r=-0.27$). Therefore it cannot be considered to be related to cancer development, and should be evaluated as accompanying noncancer disorder [24,36].

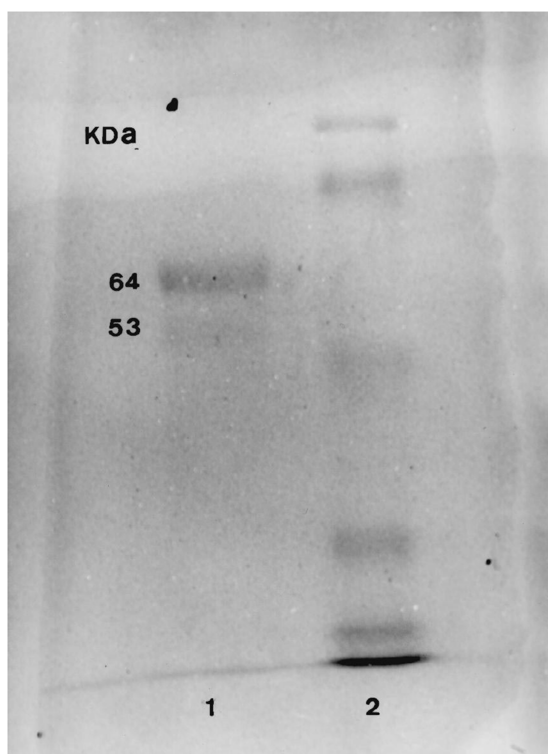

Fig. 1. (1) 12% SDS–PAGE profiles of tumor-associated antigens isolated by the GFG columns from the human serum obtained from a colon cancer patient. (2) Low molecular mass standard. Note that 64- and 53-kDa proteins are the only proteins eluted in significant amounts.

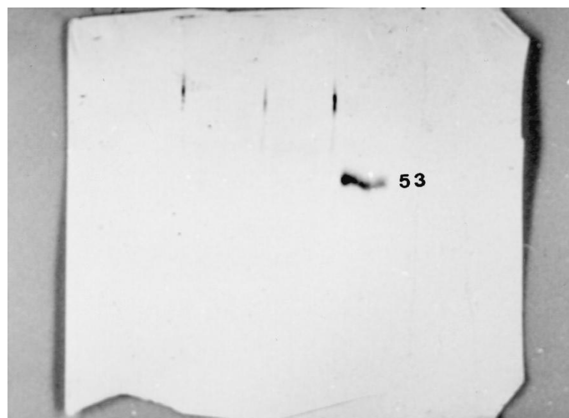

Fig. 2. Western immunoblotting of TAA presented in Fig. 1. A 10-μg protein sample was loaded on a 12% SDS–PAGE gel electrophoresis, and transferred to a polyvinylidene difluoride membrane. The commercial anti-human p53 PAb DO1 was applied to a well at a dilution of 1:10^{6} and binding detected by means of the avidin–biotin complex. Note that p53 TAA isolated from the serum exhibited a strong affinity reaction with the commercial anti-p53 PAb.

p53 protein is considered an important marker for cancer detection mainly by histochemical methods [45]. In the last years, serological methods have been shown to be successful for this purpose [46–49]. Using ELISA and commercial recombinant p53 antigen, the authors observed p53 antibodies in form of the nuclear p53 protein in the sera of cancer patients. In colon cancer, a strong correlation has been shown between the blood-level of p53 antibodies and colorectal cancer prognosis [50,51]. It is known that the p53 gene and its protein play an important role in the regulation of the cell cycle, and that alterations in the p53 gene are considered one of the stages in the initiation of cancer [52]. The role of the p53 protein in cancer development has been reviewed recently [40,41,53–56].

The p53 protein, as has been mentioned above, can be identified in cytoplasmic, soluble, and nuclear nonstable forms [25–27]. Immunochemical determination of the nuclear p53 protein in sera by commonly-used methods cannot be considered of clinical significance: the methods used in diagnostic laboratories have given low positive results in most of the cancer types [57,58]. Although, for example, in one group of lung cancer patients, the plasma level

of the nuclear p53 protein has a tendency to increase with progress in the carcinoma stage [59], this trend was not statistically significant in colon cancer [60] or the other group of lung cancer patients [61].

Cytoplasmic expression of the p53 protein, on the contrary, has been shown to be related to the early development of malignancy in chemically induced colon cancer in rats [62] and to colorectal [26,27] and breast tumors in humans [63]. These data were obtained in histochemical studies.

Our modification of affinity chromatography columns with GFG membranes allowed us to isolate the cytoplasmic form of p53 protein from the serum of cancer patients, and to use the obtained results for cancer detection [24,36,64,65]. The latter was possible due to the determination of p53 concentration using high-performance liquid chromatography (HPLC) (Fig. 3). The reversed-phase HPLC carried out on a Vydac RP-18 column (Hitachi-Merck, Germany) and a mobile phase consisting of 0.1% (v/v) trifluoroacetic acid (TFA)–2-propanol solution (90:10) were used to separate the proteins isolated by GFG columns and to determine their concentration [36,38]. Identification of proteins was performed according to the manufacturer's protocol and by previous isolation on HPLC of the commercial anti-human p53 PAb. The final concentration of proteins was evaluated in relation to the amount of TAA isolated by GFG columns.

We have shown that colon cancer is accompanied by a sharp increase in the level of the cytoplasmic form of the p53 protein in the serum of patients. We found that the serum level of this protein ranged between 0.24 to 0.94 mg/ml in patients with noncancer diseases (gastric, colon, liver or renal disorders), reached 1.0 and 2.0 mg/ml in patients with polyps and in high risk groups, respectively, increased to over 2.0 mg/ml in primary colon cancer patients and up to 5.0 mg/ml in cancer patients with metastases (Table 3).

Data in the literature showed that the Duke's colon cancer stages are weakly associated with serum levels of p53 antibody [66]. The determination of the serum p53 antigen revealed moderate correlation between the serum level of p53 protein and the progress in colon cancer: correlation and regression coefficients were 0.48 and 0.88 respectively, $P<$ 0.01–0.001 [24,36]. The method also allows the detection of patients with polyposis or those of high

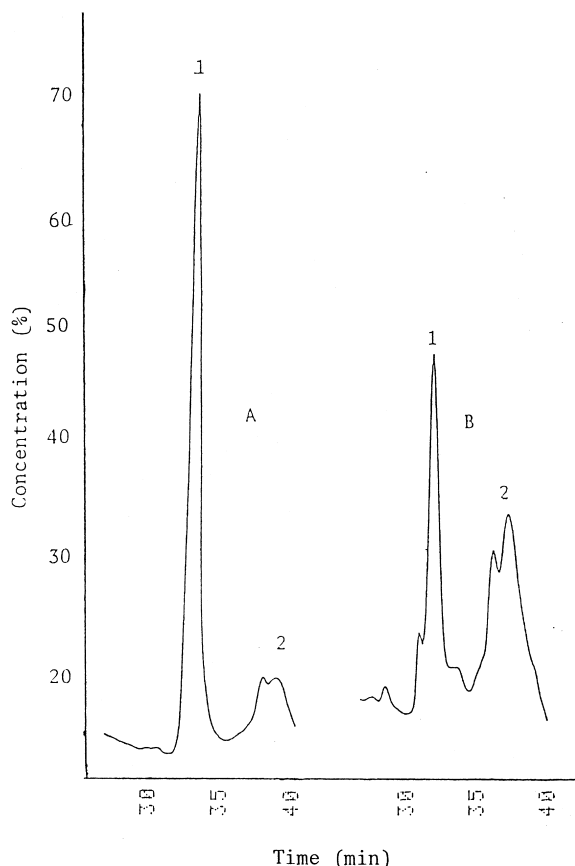

Fig. 3. HPLC profiles of TAA isolated with GFG columns from the serum of a patient with a noncancer disorder (A) and a patient with colon cancer (B). The HPLC instrument was equilibrated with water 0.1% TFA–2-propanol solution (90:10). Proteins were detected with an increasing gradient of 2-propanol in water 0.1% TFA (10–50%) for 30 min and 0.5 ml of each portion was collected each minute. Detection of proteins was performed at 220 nm and was expressed as rate (mV). The final concentration of proteins was evaluated in relation to the amount of TAA isolated by GFG columns. (A) A noncancer disorder: main protein was eluted at 32 min as 64 kDa; (B) colon cancer: two proteins were eluted at 32 and 37 min as 64 kDa and the soluble p53 protein. Note that high concentration of p53 protein was found only in a cancer patient serum. (After Refs. [24,36,38]).

risk groups who exhibit a real tendency to develop colon cancer.

Our method has proved to be more effective than the commercial immunochemical methods: sensitivity and specificity in our method amounted to 92% and 96% respectively (Table 4), as compared to 25.5% or 32% of colon cancer patients detected with commercial methods [50,51,66]. The average accuracy of our method reached 88%. The relatively low

Table 3
The amount of main antigens isolated from 1 ml of the serum obtained from colon cancer and noncancer patients (mean±S.E.) (After Refs. [24,36])

Groups of patients	n	Antigens	
		64 kDa (mg/ml)	53 kDa (mg/ml)
Healthy people	26	1.23±0.21	0.22±0.15
Disorders of digestive organs	76	2.01±0.36	0.43±0.14
High risk group	32	2.35±0.61	1.72±0.67[a]
Polyps in the colon	38	4.55±0.68[a,b]	1.32±0.29[a]
Colon adenomas	28	3.37±1.32[a]	1.82±0.82[a,b]
Colon adenocarcinoma	70	2.98±0.86[a]	3.55±1.46[a,b,c]
Recurrent colon cancer with metastases	50	3.96±0.72[a,b]	5.62±1.06[a,b,c]

[a] Significantly different from healthy people ($P<0.05$).
[b] Significantly different from non cancer groups ($P<0.05$–0.01).
[c] Significantly different from the group with polyps ($P<0.05$–0.01).

sensitivity among high risk groups or patients with colon polyps should be considered as an additional evidence of the effectiveness of the method in revealing suspected cancer cases.

Most of our studies were performed by isolation of p53 protein in the form of soluble antigen. Recently, we have shown the possibility of isolating this protein from the serum of colon cancer patients as p53 antibodies [67]. An HPLC technique was used to measure serum levels of both forms of p53 protein after their partial isolation on GFG-affinity chromatography columns. Tumor-associated proteins (TAP) in the form of either antigens or antibodies comprised about 4% of the total protein isolated from the serum of colon cancer patients. The absolute amount of each of the two types of TAP was also similar: 14.69±2.88 and 18.29±3.85 mg/ml, respectively. The amount of p53 antibodies seems higher than those of p53 antigen, but the difference was not significant: 9.35±3.48 and 6.19±3.87 mg/ml, respectively ($P>0.05$). A high correlation was found between the total amount of TAP and the amount of p53 antigen ($r=0.69$), the total amount of IgG and the amount of p53 antibodies ($r=0.46$), and between both forms of p53 protein ($r=0.46$). A high regression coefficient reflecting the interdependency between the parameters compared was found by comparing the total amount of TAA and the amount of p53 antigen ($b=2.42$). Correlation between Duke's stage in colon cancer and the serum levels of p53 protein was weak: 0.33 and −0.32 for p53 antigen and its antibodies, respectively. Serum determination of p53 antibodies has no advantage over the determination of p53 antigen. Both forms of p53 protein can be isolated with extremely high accuracy for the pathological diagnosis of cancer (87–93%). The specificity of the method was proved using commercial anti-human p53 PAb DO1: the GFG columns with this antibody were able to isolate the same proteins which were isolated by GFG columns with anti-p53 IgG. Moreover, the isolation of p53 protein was performed with higher effectiveness using the GFG columns with entrapped anti-p53 IgG than by using the columns with commercial DO1 PAb [67].

Immunohistochemical studies have shown that

Table 4
Oncological parameters (%) which can be achieved for colon cancer diagnosis by the determination of the p53 serum level. (After Refs. [24,36])

Groups of patients	n	Sensitivity[a]	Specificity[a]	False negative[b]	Cancer suspect[c]
Healthy people	26	–	96	–	4
Disorders of digestive organs	76	–	92	–	8
High risk group	32	–	82	–	18
Polyps in colon	38	–	67	–	33
Primary colon cancer	98	92	–	8	–
Recurrent colon cancer with metastases	50	92	–	8	–

[a] This parameter was evaluated independently of the clinical diagnosis with subsequent comparison with results of pathological studies.
[b] False negative results were found in the patients with Duke's stage D.
[c] This parameter was evaluated as predictive diagnostic method.

some noncancer disorders are accompanied by the accumulation of p53 protein in the cytoplasm of cells [64–69]. We have found an accumulation of this type of p53 protein in the blood of noncancer patients [64,65]. A repeated assay yielding a high concentration of this protein in noncancer patients with chronic diseases should be performed in order to detect the transformation of such diseases into cancer. A high serum level of p53 protein from a single determination is not necessarily indicative; however, a consistent high level over time strongly implies the transformation of a disease into a state of cancer.

p53 protein was found in various noncancer tissues [70,71], in serous effusions [72], in the serum of noncancer patients [24,36] and even in healthy people [60], but it has not been always determined in cancer tissues [73]. These findings suggest that p53 protein cannot be considered as a strictly specific cancer marker. Recent reviews have shown that none of the commercial cancer markers are specific, because they were detected not only in tumor tissues but also in normal tissues (see reviews, [40,41,74,75]). Our findings of high serum levels of the cytoplasmic p53 protein in patients with noncancer diseases are due to the participation of the p53 gene and its protein in cell-cycle control apoptosis accompanying any disease, since a repeated test at a later date shows lowered levels.

The determination of p53 gene mutations and of the overexpression of the nuclear p53 protein was not statistically associated with the prognosis of some recurrent malignancies such as renal [76] or ovarian [77] cancer. The clinical significance of these markers remains to be studied, and further research will be necessary to determine whether they could serve in the evaluation of the malignant potential of benign tissues [78].

Our method can be useful in detecting cancer development either as a primary illness, including cancer developing from noncancer diseases, or as recurrent disorders. It is possible to conduct follow-up examinations of patients with chronic diseases (colitis, liver or renal disorders, polyposis) and to detect transformation of these diseases into cancer. This transformation, which has been shown in diseases such as ulcerative colitis [79–81], gastritis [82,83] and liver cirrhosis [84], can be detected by a repeated increase in the serum level of the p53 protein. Conversely, the determination of the serum level of this protein in former cancer patients makes this method suitable for the follow-up on such patients in order to detect the recurrence of cancer as early as possible.

4. Conclusions

Nonderivatized glass exhibited marked nonspecific protein adsorption: almost all of the proteins that percolated through the GFG column were retained, and could not be eluted from this glass column. In our study with sol–gel columns [19], it has been shown that nonspecific protein adsorption was eliminated by derivatization of sol–gel glass with a hydrophobic moiety such as γ-aminopropyltriethoxysilane. In experiments with GFG columns, we found that a combination of this oxysilane and PVP was more suitable for derivatization of gel glass than derivatized sol–gel glass alone, which, prepared without protein, did not bind measurable amounts of protein [19]. As a result of the preliminary treatment [34], the H^+ and OH^- groups appeared on the large surface of the glass fibers. The activated glass fibers yielded better adhesion to sol–gel glass. The capacity of the GFG columns was much higher than that of the other types of matrix for affinity chromatography described in the literature [15,85,86].

Affinity GFG columns have high sensitivity and are highly effective for the isolation of TAA. Detection and isolation of TAA from cancer tissue and sera of cancer patients is used in oncological research and practice [39–41]. All commonly used methods, however, permit to isolate TAA in extremely small amounts. We have described a method for the isolation of high yields of TAA, sufficient for further use and analyses by methods such as GFG affinity chromatography columns and HPLC [24,36].

p53 protein has been isolated from the serum of colon cancer patients as a major fraction of TAA [22,24]. Two approaches have been described in the literature for the purification of p53 protein: under native conditions by immunoaffinity chromatography or in denaturated form with subsequent refolding [87–89]. All of these procedures are complicated and do not yield high resolution.

The method developed in our laboratory [22–24] is highly effective, and enables the isolation of the

soluble p53 protein from the serum in spite of its very low concentration. This was impossible to achieve with the other commonly utilized methods [88,90]. For example, 6.88 μg of the soluble p53 protein per 1 mg total protein was isolated with GFG columns from the serum of colon cancer patients [24], whereas only 0.6 μg of the nuclear p53 protein per 1 mg total protein was isolated from the colon cancer cell lyzates by the commonly used method [91]. We have shown that the source of IgG entrapped in GFG columns does not affect their capacity and the amount of proteins isolated [39].

Gel fiberglass can be regarded as a new generation of supports for preparative and analytical chemistry and immunochemistry. Such membranes do not require the processes of activation, attachment and space determination for preparation of affinity chromatography columns. Different compounds can be immobilized in large amounts within these membranes during their preparation. One example of the many possible applications is the isolation and purification of proteins from blood [92]. It has been found that the GFG columns have a high capacity, and can be used for isolation of p53 protein in high yields: 36% to 50% of the amount of TAA isolated [24,36]. The specificity of the affinity GFG columns was highly effective for the isolation of TAP in the form of either antigens or antibodies [67]. We believe that the GFG membranes have great potential for isolating macromolecules, utilizing various ligands, and can be widely used for diagnostic purposes. We hope that the finding opens an easy and highly effective method for the isolation of antigens from different organs, both animal and human, which can be used for therapy and generation of specific antibodies.

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