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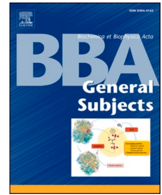

Affinity purification of fibrinogen using an Affimer column

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ABSTRACT

Background: Fibrinogen is an abundant plasma protein with an essential role in blood coagulation and haemostasis thus receiving significant research interest. However, protein purification is time consuming and commercial preparations often have protein contaminants. The aim of this study was to develop a new method to purify high quality and functional fibrinogen.
Methods: Fibrinogen-specific Affimer protein, isolated using phage display systems, was immobilised to SulfoLink resin column and employed for fibrinogen purification from plasma samples. Fibrinogen was eluted using a high pH solution. Commercial human fibrinogen was also further purified using the Affimer column. Fibrinogen purity was determined by SDS-PAGE and mass spectrometry, while functionality was assessed using turbidimetric analysis.
Results: Affimer-purified fibrinogen from human plasma showed purity at least comparable to commercially available preparations and was able to form physiological fibrin networks. Further purification of commercially available fibrinogen using the Affimercolumn eliminated multiple contaminant proteins, a significant number of which are key elements of the coagulation cascade, including plasminogen and factor XIII.
Conclusions: The Affimercolumn represents a proof of concept novel, rapid method for isolating functional fibrinogen from plasma and for further purification of commercially available fibrinogen preparations.
General significance: Our methodology provides an efficient way of purifying functional fibrinogen with superior purity without the need of expensive pieces of equipment or the use of harsh conditions.

1. Introduction

Fibrinogen is one of the most abundant plasma proteins, circulating at 2–4 mg/mL under normal physiological conditions, and has a key role in coagulation and maintenance of normal haemostasis. Moreover, qualitative and quantitative changes in the protein play an important role in pathological thrombotic and bleeding disorders, making fibrinogen a focus of translational and clinical research studies [1,2]. Fibrinogen is a soluble glycoprotein of ~340 kDa, composed of two sets of three polypeptide chains Aα, Bβ, and γ [3]. Purified fibrinogen from human plasma can be obtained from commercial sources; however, often investigators are required to purify fibrinogen from individuals with thrombotic or bleeding tendencies to study protein characteristics [4,5] or to further eliminate impurities found in commercial preparations that can compromise experiments and complicate the interpretation of results [6,7].

Different techniques have been described for the purification of fibrinogen from human plasma, which are often labour intensive and require multiple steps such as a combination of Cohn ethanol fractionation, virus inactivation, glycine and sodium chloride precipitation, and lysine-Sepharose affinity chromatography [8]. Other methods include cryo-, ammonium sulfate or β-alanine precipitation, chromatography methods using ristocetin agarose, protamine-agarose or n-alkylagaroses [6,9–15]. Affinity of *Staphylococcus aureus* clumping factor A [7] and synthetic peptides have also been used for fibrinogen purification [16–18] as well as different monoclonal antibodies [19,20]. Although all the methodologies yield purified protein, there are drawbacks that should not be underestimated. First, some methods are labour intensive

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and therefore can be difficult to undertake when purification is required for large numbers of samples. Second, the need for specialist equipment and high cost reagents with some methods, e.g. the use of monoclonal antibodies, which limits widespread use. Third, some methods use harsh conditions that may alter protein functionality, making interpretation of subsequent experiments problematic. Finally, some of the necessary reagents for high purity protein isolation, such as IF-1 antibody are no longer available, limiting options when high quality protein is required.

The aim of this study was to develop an easy method for purifying high quality fibrinogen that retains its biological activity. Our methodology provides an efficient way to purify fibrinogen from a complex mixture of plasma proteins using an affinity column packed with resin covalently coupled to an Affimer protein. Affimers are small proteins of approximately 13 kDa, composed of a scaffold containing two variable region loops [21]. Affimer proteins are easily produced recombinantly in large quantities, and are thermally/chemically stable to withstand conditions required during washing and protein elution steps as part of protein purification protocols [22]. High affinity fibrinogen-binding Affimers were raised using our phage display system and one Affimer was chosen for immobilisation to a column for fibrinogen purification from plasma samples. Our methodology also enables us to further purify commercially available fibrinogen in order to obtain a 'cleaner' protein for more accurate results in sensitive experiments.

Data presented in this study represent a proof of concept of a novel methodology that employs Affimer protein for fibrinogen purification from plasma samples and also provides an alternative method for increasing the purity of commercially available fibrinogen preparations.

2. Materials and methods

2.1. Screening for fibrinogen binding Affimer proteins

Fibrinogen-binding Affimer proteins were isolated from the Affimer phage library as previously described [21]. Affimer proteins of interest were produced to contain a C-terminal cysteine tag in addition to their polyhistidine tag. Affimer His tag was used for column purification of Affimer proteins using Ni-NTA sepharose (IBA, Loughborough, UK) as previously described [22].

2.2. Surface Plasmon Resonance (SPR) binding assay

In order to characterise Affimer-fibrinogen interactions, SPR experiments were conducted. Human fibrinogen (ERL) in 0.1 M sodium acetate buffer, pH 5.6 at 5 μg/mL was immobilised to 2000 RUs by amine-coupling using an NHS/EDC-activated CM5 chip followed by deactivation with ethanolamine/HCl using a Biacore 3000 (GE Healthcare, Buckinghamshire, UK). A reference surface was also prepared by activating and deactivating the dextran without the addition of protein. Binding assays were performed as previously described [21]. Briefly, a dilution series of the Affimer (50–800 nM) in running buffer (100 mM NaCl, 50 mM Tris, 2 mM $CaCl_2$, 0.1% (*v*/v) Tween-20, pH 7.4) were injected for 120 s at 50 μl/min in three replicate experiments. Affinity and rate constants for association and dissociation were analysed using a 1:1 Langmuir binding model with BIAevaluation 3.1 software.

2.3. Immobilisation of Affimer to SulfoLink resin

The Affimer column was prepared by adding sulfhydryl-containing Affimer (15 mg in 3 mL of coupling buffer; 50 mM Tris, 5 mM EDTA-Na; pH 8.5) in a SulfoLink resin column pre-equilibrated with coupling buffer (containing 2 mL of 6% crosslinked beaded agarose) and mixing by rotation for 30 min at ambient temperature. The Affimer column was then incubated for a further 30 min without mixing according to manufacturer's instructions (Thermoscientific, Loughborough, UK). The flow-through unbound Affimer (1 mg) was collected in order to determine the amount of Affimer bound to the column. L-Cysteine – HCl (15 mg in 2 mL of coupling buffer) was added to the column and mixed by rotating for 20 min followed by 30 min incubation without mixing at ambient temperature to block nonspecific binding sites. The Affimer column was finally washed and equilibrated with 100 mM NaCl, 50 mM Tris, pH 7.4.

2.4. Fibrinogen purification using the Affimer column

Commercial human fibrinogen (2 mg) from Calbiochem (Feltham, UK) or human fibrinogen depleted of plasminogen, von Willebrand factor (vWF) and fibronectin (2 mg) from Enzyme Research Laboratories (ERL, Swansea, UK) or 4 mL of diluted (1 in 5) human normal pooled plasma (1 mL plasma+4 mL buffer) in 100 mM NaCl, 50 mM Tris, pH 7.4 was passed through a 0.22 μm filter and loaded into the Affimer column. Human plasma sample was loaded onto the column in two batches of 2 mL. After one-hour incubation at room temperature with rocking, the column was centrifuged at 1000 ×*g* for 1 min according to the manufacturer's instructions and flow-through containing unbound plasma proteins was collected. The column was washed four times with 100 mM NaCl, 50 mM Tris, pH 7.4 and fibrinogen was eluted with 0.1 M sodium carbonate (elution buffer) in 2 mL tubes containing 100 μL of 1 M citrate buffer, pH 3.0. The eluted fibrinogen samples were pooled and dialysed overnight into 100 mM NaCl, 50 mM Tris, pH 7.4 using Pur-A-Lyzer™ dialysis tubes with molecular weight cut-off of 6–8 kDa (Merck, Hertfordshire, UK) and then concentrated using a centrifugal concentrator with molecular weight cut-off of 100 kDa (Sartorius, Goettingen, Germany). The Affimer column was washed three times with 100 mM NaCl, 50 mM Tris, pH 7.4 and then stored in 100 mM NaCl, 50 mM Tris, pH 7.4 containing sodium azide at a final concentration of 0.05%.

2.5. Determination of fibrinogen purity

Protein purity was determined using SDS-PAGE analysis and mass spectrometry.

2.5.1. SDS-PAGE

Samples of the initial plasma loaded onto the column, unbound plasma proteins and eluted fibrinogen were prepared for SDS-PAGE by adding NuPAGE LDS sample buffer and NuPAGE sample reducing agent (Thermo Fisher Scientific, Loughborough, UK). Samples were heated for 5 min at 95 °C and loaded onto 4–12% Bis-Tris gels (Thermo Fisher Scientific, Loughborough, UK). The gels were stained with GelCode Blue gel stain (Thermo Fisher Scientific, Loughborough, UK).

After SDS-PAGE, the proteins were transferred to PVDF membranes (Thermo Fisher Scientific Loughborough, UK) for 60 min at 100 Volts in transfer buffer (25 mM Tris, 384 mM glycine, 20% (*v*/v) methanol, pH 8.3). The membranes were blocked overnight with 4% (*w*/*v*) milk powder in Tris-Buffered Saline with Tween (TBS-T) (50 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.4). Rabbit anti-human fibrinogen (1:5000), goat anti-rabbit HRP (1:10000) antibodies (Dako, Agilent Technologies, Stockport, UK) were used to detect fibrinogen and mouse anti-his antibody (1:200) (Roche, Welwyn Garden City, UK), rabbit anti-mouse-HRP (1:1000) (Dako, Agilent Technologies, Stockport, UK) to detect Affimer protein. Supersignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Loughborough, UK) was used to develop the membranes.

2.5.2. Mass spectrometry

To identify all the proteins that were present in the purified samples and the commercially available preparations, mass spectrometry analysis was carried out at the Mass Spectrometry Facility in the University of Leeds as previously described [23].

2.6. Functionality of purified fibrinogen

2.6.1. Clot formation and lysis

Affimer column purified fibrinogen at 0.5 mg/mL (final concentration) was tested for its ability to clot and to lyse, by using a validated turbidimetric assay as described [21,24,25]. An activation mix

containing 0.05 U/mL thrombin (Merck Millipore, Watford, UK) and 2.5 mM $CaCl_2$ and a lysis mix containing 39 ng/mL tissue plasminogen activator (tPA; TC Technoclone, Surrey, UK) and 3 μg/mL plasminogen (ERL, Swansea, UK) (all final concentrations) were added to fibrinogen to initiate clot formation and subsequent lysis. Commercially available fibrinogen from Calbiochem and ERL were also included as control fibrinogen. Samples were added to a 96-well plate in duplicate and measurements of the optical density at 340 nm were taken every 12 s on a Multiskan Go plate reader (Thermo Scientific, Loughborough, UK).

2.6.2. Clottability

To find the percentage of fibrinogen that remained clottable after elution from the Affimer column, the OD of an aliquot of the purified fibrinogen was measured using Nanodrop (Wilmington, USA) prior to addition of 5 U/mL thrombin (Merck, Hertfordshire, UK) and 5 mM $CaCl_2$ (final concentrations). The sample was then incubated for 1 h at 37 °C, centrifuged at 13,000 rpm for 10 min and finally protein concentration of non-clottable fibrinogen was determined by measuring the OD in the supernatant. The value of the final OD (non-clottable) divided by the value of the initial OD of fibrinogen determines the percentage of non-clottable fibrinogen hence the remaining percentage corresponds to the percentage of clottable fibrinogen.

3. Results

3.1. Isolation of fibrinogen-binding Affimers

A number of fibrinogen-binding Affimers were isolated using the phage display system and one Affimer was selected for immobilisation to the affinity column for fibrinogen purification. SPR experiments were used to determine the kinetics of the interaction of this Affimer with fibrinogen. It was demonstrated that the Affimer had a binding affinity with K_D value of 99 ± 30.8 nM, association rate with k_a value of 8.4 ± 6.42 × 10^4 M^{-1} s^{-1} and dissociation rate with kd value of 0.007 ± 0.002 s-1 (Supplemental Fig. 1). The particular Affimer was selected due to its ability to pull down fibrinogen from plasma in pull down assays (Supplemental Fig. 1). Fibrinogen-binding Affimers can interfere with protein function, as we have previously shown [21]. Therefore, we undertook turbidimetric analysis to investigate whether the Affimer chosen for fibrinogen purification altered protein function in case of Affimer protein contamination during fibrinogen purification. Commercially available fibrinogen from ERL was assessed in the presence of the selected Affimer (containing a C-terminal cysteine), at 5:1 Affimer:fibrinogen molar ratio. There was a small reduction in maximum clot absorbance without an effect on clot lysis time (Supplemental Fig. 2).

3.2. Purification of fibrinogen from normal pooled plasma and assessment of functionality

The Affimer column was used to purify fibrinogen from normal pooled plasma. The purity of the eluted (purified) fibrinogen was analysed using SDS-PAGE under reducing conditions as shown in Fig. 1A. The initial pooled plasma (before loading onto the column) is shown in lane 1, non-bound plasma proteins (flow-through) in lane 2 and purified fibrinogen in lane 3. Commercially available fibrinogen from Calbiochem (lane 4) and ERL (lane 5) were also loaded onto the gel to compare the purity of the purified fibrinogen. The distinct protein bands of fibrinogen as the three subunits Aα (68 kDa), Bβ (54 kDa) and γ (48 kDa) are indicated in Fig. 1A. Western blot using anti-fibrinogen antibody was used to confirm the protein identity, detecting the three fibrinogen chains in all fibrinogen samples (Supplemental Fig. 3A).

A clottability assay was used to determine the functionality of purified fibrinogen, which revealed that 93% was clottable. A turbidimetric assay showed that purified fibrinogen had lower maximum absorbance and increased lag time compared with the commercially available fibrinogen from ERL, but did not differ from fibrinogen from Calbiochem (Fig. 1B). The eluted fibrinogen exhibited purity that was comparable to two of the commercially available fibrinogen preparations, containing the three expected bands of human fibrinogen, in addition to some extra bands that were common in all fibrinogen samples (lanes 3–5) (Fig. 1A). To determine whether the differences in maximum absorbance of the three fibrinogen preparations reflected protein purity, rather than a difference in clotting ability, we next analysed the composition of the fibrinogen samples using mass spectrometry.

3.3. Identification of proteins present in fibrinogen samples

Fibrinogen purified from human plasma using the Affimer column, and fibrinogen samples commercially available from Calbiochem and ERL were analysed by mass spectrometry in order to identify all proteins present in these samples. The list of proteins that were potential matches to extra proteins found in fibrinogen samples are shown in Table 1

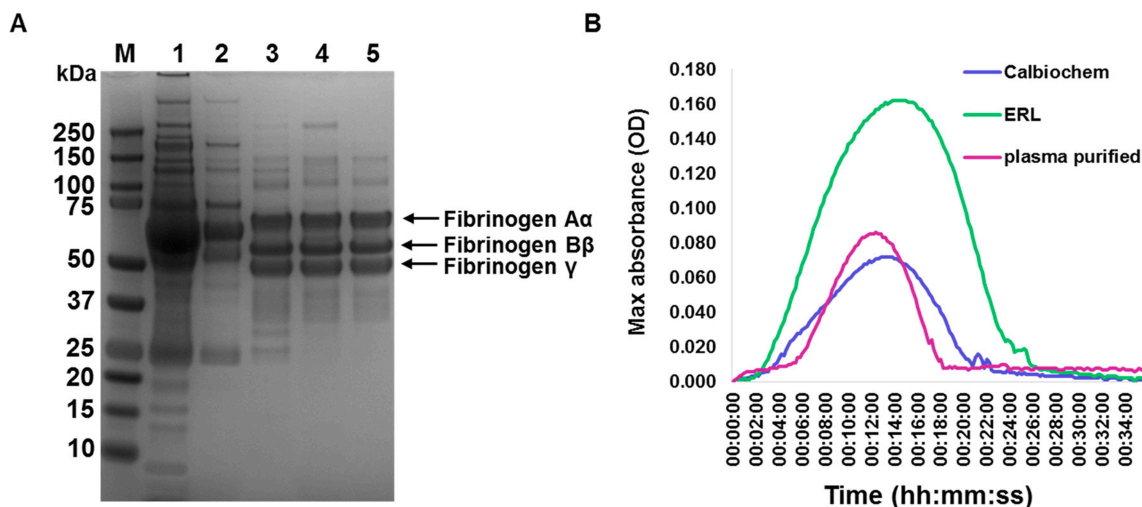

Fig. 1. (A) Purification of fibrinogen from normal pooled plasma. SDS-PAGE analysis of initial normal pooled plasma sample (before column) (lane 1), the unbound plasma proteins (flow-through) (lane 2), purified fibrinogen eluted from the Affimer column (lane 3), commercially available fibrinogen from Calbiochem (lane 4) and ERL (lane 5). Lanes 3–5 each contain 7 μg of fibrinogen sample. (B) Fibrin clot formation and lysis. A turbidimetric assay was used to investigate the ability of purified fibrinogen to form clots, and to lyse. Fibrinogen purified using the Affimer column was compared to commercially available fibrinogen from Calbiochem and ERL.

Table 1
Proteins identified in fibrinogen samples using mass spectrometry. The samples analysed and presented in the table (from left to right) are as follows: fibrinogen purified from human plasma using the Affimer column, fibrinogen commercially available from Calbiochem, fibrinogen commercially available from ERL (which is sold as plasminogen, vWF and fibronectin depleted), Calbiochem fibrinogen further purified using the Affimer column, ERL fibrinogen further purified using the Affimer column.

	Affimer purified fibrinogen from plasma	Commercially available Fibrinogen (Calbiochem)	Commercially available Fibrinogen (plasminogen, vWF, fibronectin depleted) (ERL)	Calbiochem fibrinogen further purified using Affimer column	ERL fibrinogen further purified using Affimer column
Fibrinogen alpha chain	✓	✓	✓	✓	✓
Fibrinogen beta chain	✓	✓	✓	✓	✓
Fibrinogen gamma chain	✓	✓	✓	✓	✓
Prothrombin	✓				
Serum albumin	✓	✓	✓	✓	✓
Complement C3	✓	✓	✓	✓	✓
Immunoglobulin gamma-1 heavy chain	✓		✓		
Alpha-2-macroglobulin	✓	✓	✓	✓	✓
Complement C4-A	✓	✓	✓		
Alpha-1-antitrypsin	✓	✓	✓	✓	✓
Immunoglobulin heavy constant mu	✓	✓	✓		✓
von Willebrand factor		✓	✓		
Coagulation factor XIII B chain		✓	✓	✓	
Plasminogen		✓	✓	✓	✓
Coagulation factor XIII A chain		✓	✓	✓	✓
C4b-binding protein alpha chain		✓	✓		✓
Immunoglobulin lambda constant 3		✓	✓		
Immunoglobulin heavy constant alpha 1	✓	✓	✓	✓	✓
Alpha-2-antiplasmin		✓	✓	✓	✓
Fibronectin	✓	✓	✓	✓	
Complement C5		✓	✓		
Kininogen-1		✓	✓		
Protein AMBP	✓	✓	✓		
Complement factor H		✓	✓		
Carboxypeptidase N subunit 2		✓	✓		
Angiotensinogen		✓	✓	✓	
Glutathione peroxidase 3		✓	✓		
Immunoglobulin heavy constant alpha 2	✓				
Apolipoprotein A-I	✓	✓	✓		✓
Clusterin	✓				✓
Fibulin-1		✓	✓	✓	✓
Vitronectin	✓				
Immunoglobulin kappa constant	✓			✓	✓
Immunoglobulin kappa light chain	✓		✓		
Immunoglobulin heavy constant gamma 3			✓		
Serotransferrin	✓				
EGF-containing fibulin-like extracellular matrix protein 1		✓	✓		✓
Transthyretin	✓				

(*continued on next page*)

Table 1 (*continued*)

	Affimer purified fibrinogen from plasma	Commercially available Fibrinogen (Calbiochem)	Commercially available Fibrinogen (plasminogen, vWF, fibronectin depleted) (ERL)	Calbiochem fibrinogen further purified using Affimer column	ERL fibrinogen further purified using Affimer column
Fibulin-1	✓				
Galectin-3-binding protein		✓	✓		
Complement C1q subcomponent subunit B		✓	✓		
Apolipoprotein A-II	✓				
Serum paraoxonase/ arylesterase 1	✓				
Complement C1q subcomponent subunit C		✓	✓	✓	
Vitamin K-dependent protein C	✓				
Extracellular matrix protein 1		✓	✓		
Vitamin K-dependent protein S	✓				
Haptoglogin-related protein		✓	✓		
Antithrombin-III		✓			
Apolipoprotein E					✓
Complement C4-B		✓			
Alpha-1-antichymotrypsin		✓			
Basement membrane-specific heparin proteoglycan core protein		✓			
Immunoglobulin heavy constant gamma 1	✓	✓			
Inter-alpha-trypsin inhibitor heavy chain H2	✓				
Total proteins	29	38	36	17	19

(column 1). Only the proteins with at least 5 peptides detected were considered as real matches and are presented in the table. Reassuringly, the three chains of fibrinogen (alpha, beta and gamma chains) were identified in all samples. Other proteins also found to be present in all samples were serum albumin, complement C3, alpha-2-macroglobulin and immunoglobulin heavy constant alpha 1. Interestingly, commercial fibrinogen from Calbiochem and ERL contained a large number of additional proteins (38 and 36 proteins in total respectively) while our Affimer-purified fibrinogen contained 29 proteins in total. Some proteins were identified in the commercial samples that are involved in coagulation and fibrinolysis, and these were not present in the Affimer-purified fibrinogen, e.g. plasminogen, coagulation factor XIII A chain, complement C5 or vWf (Table 1).

3.4. *Further purification of commercially available fibrinogen*

Commercially available fibrinogen from ERL was found to contain plasminogen, vWF and fibronectin, despite being depleted of these proteins by the manufacturer (Table 1). Therefore, we decided to use our Affimer column to further purify commercially available fibrinogen preparations in order to eliminate more contaminant proteins. Remarkably, fibronectin and vWF were removed from ERL fibrinogen sample after purification using the Affimer column. Additionally, other proteins such as alpha-2-antiplasmin were also eliminated after fibrinogen purification using the Affimer column (Table 1). Further purification of Calbiochem fibrinogen using the Affimer column reduced the total number of proteins from 38 to 17, and in ERL fibrinogen from 36 to 19.

The fibrinogen samples were analysed by SDS-PAGE and protein bands present in each sample are shown in Fig. 2. The three main protein bands at molecular weights of 68, 54 and 48 kDa correspond to the fibrinogen Aα, Bβ and γ chains respectively. No Affimer bands were evident in the gel, and this was confirmed with a Western blot using an anti-his antibody, indicating that there was no loss of Affimer protein from the affinity column during the protocol (Supplemental Fig. 3B).

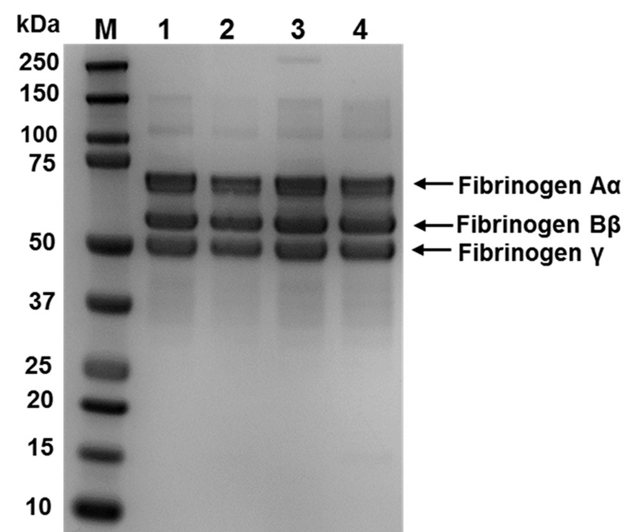

Fig. 2. Purification of commercially available fibrinogen. SDS-PAGE analysis of fibrinogen samples as they were purchased and after further purification using the Affimer column. Fibrinogen from ERL (lane 1), ERL fibrinogen further purified using the Affimer column (lane 2), fibrinogen from Calbiochem (lane 3) and Calbiochem fibrinogen further purified using the Affimer column (lane 4). 7 μg of each protein sample was loaded in each lane.

4. Discussion

An affinity column capable of isolating fibrinogen from human pooled plasma was successfully prepared using Affimer protein. A fibrinogen binding Affimer was immobilised to resin via a covalent linkage using the protein's C-terminal cysteine. In addition to purification of fibrinogen from a complex mixture of plasma proteins, the Affimer column was also successful at further purifying commercially available fibrinogen preparations to remove contaminant proteins.

We and others have used the monoclonal antibody IF-1 for the affinity purification of fibrinogen [19], however, this antibody is no longer produced. Therefore, there was a need to develop new methodology for the purification of fibrinogen from human pooled plasma or individual patient plasma samples. Recovery of purified fibrinogen using the Affimer column was relatively good approaching 70%, in 2 h around 1.2 mg of fibrinogen was purified from 1 mL of plasma and 1.3 mg of fibrinogen was purified from 2.5 mg commercial fibrinogen that was loaded onto the Affimer column (Table 2).

Our work demonstrates that Affimer-purified fibrinogen shows high integrity with no suggestion of protein degradation on SDS-PAGE. Moreover, our Affimer-purified fibrinogen is functional as demonstrated in clottability and turbidimetric assays. However, fibrin network formation and lysis with Affimer-purified fibrinogen can differ when compared with fibrin clots made from commercial fibrinogen. This may be due to changes in protein function but more likely due to the presence of additional protein contaminants in commercial fibrinogen samples. In particular, unlike Affimer-purified fibrinogen, both Calbiochem and ERL fibrinogen contained key coagulation proteins, such as plasminogen and FXIII, which may have affected clot formation and/or lysis.

There are clear advantages to this new methodology. First, it is the relative ease by which fibrinogen purification is undertaken and without the need for specialist equipment. Second, it is a low-cost technique particularly as the column can be reused a number of times without loss of binding capacity (Table 2). Third, the purified fibrinogen is relatively pure with fewer protein contaminants in Affimer-purified fibrinogen compared with two commercially available preparations, which are regularly used in research studies.

There are a number of limitations of our methodology, including the small scale fibrinogen purification, which can be problematic when large amounts of protein are required. However, this could easily be resolved by preparing a bigger column with a larger volume of agarose beads that are modified with the same iodoacetyl chemistry. This would allow more Affimer protein to be immobilised and thus bind more fibrinogen from loaded samples at any one time. Moreover, despite the higher purity compared with commercial fibrinogen preparations, protein contaminants are still evident, although these do not seem to affect protein clottability. Finally, we have shown that the column can be regenerated and reused but we are yet to evaluate shelf-life and effectiveness of the column in the long-term. In summary, our work represents a proof of concept for a new methodology employing Affimer technology for fibrinogen purification. Further work is required to identify fibrinogen-binding Affimer proteins that have more favourable fibrinogen-binding characteristics, to improve binding, purity and elution efficiency. An ideal Affimer would exhibit relatively strong but reversible binding to fibrinogen, fast association rates with predictable dissociation characteristics to allow protein elution without the need for harsh conditions (e.g. extremes of pH) that risk fibrinogen protein functionality. We continue to work on isolating additional fibrinogen-binding Affimers that may exhibit the above characteristics, allowing the purification of functional fibrinogen protein with superior purity.

Table 2
Binding capacity of the column and fibrinogen recovery. The amount of fibrinogen loaded and bound into two Affimer columns in various repeats showed a high binding capacity and efficiency of each column. The yield of fibrinogen determined as the amount of the eluted fibrinogen out of the bound fibrinogen also highlighted the ability of the column to be used multiple times while keeping its efficiency. Experiments of purification of fibrinogen were performed many times, representative data are shown at the table for Column#1 and Column#2. ND = not determined

Fibrinogen sample	Loaded fibrinogen	Bound fibrinogen (binding capacity)	Eluted fibrinogen (recovery)
Column #1			
Calbiochem	1.85 mg	1.72 mg (93%)	1.37 mg (79.6%)
Plasma	2 mg	ND	1.4 mg (58%)
Plasma	2 mg	ND	1.15 mg (70%)
Column #2			
Plasma	2 mg	ND	0.7 mg (41%)
ERL	2.5 mg	1.95 mg (78%)	1.2 mg (61.5%)
Calbiochem	2.5 mg	1.9 mg (76%)	1.32 mg (69.5%)
Calbiochem	2.2 mg	1.7 mg (77%)	0.9 mg (53%)

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Conflict-of-interest disclosure

M.J.M. and D.C.T. are co-inventors of the Adhiron/Affimer technology and own personal shares in Avacta Life Sciences. The Adhiron/Affimer patent (patent application number PCT/GB2014/050435) is owned by the University of Leeds and licensed to Avacta Ltd. N.P. and R. A.A. received research funding from Avacta Life Sciences. The remaining authors declare no competing financial interests.

CRediT authorship contribution statement

Nikoletta Pechlivani: Methodology, Validation, Investigation, Writing – Original Draft, Writing – Review and Editing, Visualisation. **Katherine J. Kearney:** Conceptualisation, Methodology, Investigation, Writing – Review and Editing, Visualisation. **Christian Tiede:** Resources. **Ramsah Cheah:** Investigation. **Fladia Phoenix:** Investigation. **Sreenivasan Ponnambalam:** Writing - Review and editing. **James R. Ault:** Investigation. **Michael J. McPherson:** Resources. **Darren C. Tomlinson:** Resources. **Ramzi A. Ajjan:** Conceptualisation, Writing – Review and Editing, Supervision, Project Administration, Funding Acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbagen.2022.130115.

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