A comprehensive review on staphylococcal protein A (SpA): Its production and applications

Garshasb Rigi[1,2]
Samira Ghaedmohammadi[3]
Gholamreza Ahmadian[4*]

[1]Department of Genetics, Faculty of Basic Science, Shahrekord University, Shahrekord, 881 863 4141, Iran

[2]Department of Industrial Biotechnology, Research Institute of Biotechnology, Shahrekord University, Shahrekord, Iran

[3]Department of Cellular and Molecular Biology, Estahban Higher Education Center, Estahban, Iran

[4]Associate Professor, Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

Abstract

The *Staphylococcus aureus* protein A (SpA) can be obtained through the culture of wild-type *S. aureus* and also as a recombinant protein in safe bacterial hosts. Several methods have been used to purify SpA among which ion-exchange chromatography, affinity chromatography, gel filtration, and per aqueous liquid chromatography (PALC) are common. SpA has a wide range of biochemical, biotechnological, and medical applications and is most commonly used in test methods such as immunoprecipitation, enzyme-linked immunosorbent assay, and Western blotting. SpA has also been widely utilized in pharmaceutical applications to bind to immune complexes and serum immunoglobulins. SpA also directly binds to the B-cells preventing initiation of infectious diseases as well as having a role in the development of various autoimmune diseases. This review considers different applications of SpA in biotechnology and its novel clinical application for effective treatment of autoimmune diseases. It also discusses various strategies for expression and purification of the SpA including types of column chromatography that are commonly used in protein purification and developing SpA surface display technologies. Finally, this review highlights the potential and novel applications of SpA immobilization, SpA typing, protein engineering for further development of immunological and biochemical research, and also application of SpA as a diagnostic biosensor. Volume 66, Number 3, Pages 454–464, 2019

Keywords: *IgG purification, pharmaceutical application, protein A, recombinant expression, surface display*

Abbreviations: *β-Ga, β-galactosidase; CALB, candida antarctica lipase B; CPR, cytochrome P450 reductase; ECL, electrochemiluminescent; EDC, ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; EFSA, European food safety authority; ELISA, enzyme-linked immunosorbent assay; EU, endotoxin units; Fab, fragment antigen-binding; FC, fragment crystallizable region; FDA, food and drug administration; His-tag, polyhistidine affinity tag; Ig, immunoglobulin; ITP, idiopathic thrombocytopenic purpura; kDa, kilodalton; LAL, limulus amebocyte lysate; LPA, low molecular weight SpA; MALDI-TOF mass spectrometry, matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry; MLST, multi locus sequence typing; MRSA, methicillin-resistant S. aureus; NHS, N hydroxysulfosuccinimide; PALC, per aqueous liquid chromatography; PCR, polymerase chain reaction; PFGE, purpose include pulsed-field gel electrophoresis; Psi, porous silicon; QPS, qualified presumption of safety; RA, rheumatoid arthritis; repPCR, repetitive sequence-based PCR; RSM, response surface methodology; RIA, radioimmunoassay; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SELEX, systematic evolution of ligands by exponential enrichment; SpA, staphylococcal protein A; UV-Vis, ultraviolet-visible; VL, light chain variable domain.*

*Address for correspondence: Gholamreza Ahmadian, Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
Tel: (+9821) 0912-4187608, Fax: (+9821) 44580-395;
e-mail: ahmadian@nigeb.ac.ir.

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1. Introduction

Staphylococcus aureus protein A (SpA) is a 40–60 kDa surface protein that comprises of 4 or 5 homologous immunoglobulin (Ig)-binding domains (E, D, A, B, and C) of 56–61 residues followed by a polymorphic variable repeat region, Xr, and a conserved region, Xc, which includes a cell-wall attachment sequence [1]. Among these domains, the interaction between domain D of SpA and the Fab 2A2 from a human IgM antibody is noteworthy [2]. SpA domain D can interact to the Fab heavy chain but not bind to VL domain of antibody (Fig. 1A) [2]. Variable heavy chain of Fab and the residues of SpA domain D and Fab 2A2 involved in the interaction were presented in Figs. 1B and 1C, respectively [2]. Alignment of the amino acid sequences of the five SpA domains (Fig. 1D) showed the binding of SpA domain D, Fab, and Fcγ region of antibody (Figs. 1E and 1F) [2]. This protein is covalently anchored in the staphylococcal cell wall via its carboxyl terminal end by a transpeptidase enzyme called sortase A [3]. SpA is also widely utilized for antibody and Fc-tagged protein immunoprecipitation and for immobilization of antibodies for affinity chromatography and biosensing [4]. SpA is one of the most well-known and interesting cell wall proteins in Gram-positive bacteria because it can interact, with varying specificity, with the Fc region of a wide range of mammalian IgG and enables SpA to be used in a variety of radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) procedures. Immobilized SpA can interact with IgG molecule without interrupting its antigen-binding ability, which has great developments in immunological applications [5].

SpA binds to IgG in two different ways: each of the five domains can bind to both the fragment crystallizable region (Fc region) and Fab (part of Ig that identifies antigen). The binding site to Fc is in the open region at a distance between CH2 and CH3 regions of IgG subgroups, and this property has been extensively used for labeling and purification of antibodies. The binding site within the Fab is less understood, but includes a location in a variable region of the heavy Ig chain [6].

2. Production of Protein A

SpA can be obtained from large-scale culture of wild-type *S. aureus* followed by lysis of cell suspensions or in the supernatant from cultures of mutant or secretory strains such as *S. aureus* A676. In both cases, purification is usually performed employing affinity chromatography with fixed IgG. About 7% (weight) of the cell wall of Cowan I strain of *S. aureus*, which is a suitable strain for production, is composed of SpA, which is equivalent of 1.7% of the total bacterial protein content. Nevertheless, handling large volumes of *S. aureus* culture is not recommended because of its pathogenicity and so molecular methods such as expression of SpA in safe bacterial hosts such as *Escherichia coli* are commonly used for large-scale production [2]. This helps to avoid the need for the higher safety levels required for work with wild-type strains of *S aureus* lowering the otherwise high costs. Optimization of the production conditions is an important component in SpA expression and production to improve the yields. For this purpose in our laboratory, expression and secretion of full-length and truncated recombinant forms of SpA containing just five IgG-binding domains in *E. coli* using the SpA native signal sequence were optimized using response surface methodology (RSM). The application of RSM resulted in a five-fold increase in the secretion of SpA and a minimum of 10-fold higher expression for the truncated protein compared with that of the full-length recombinant form. The function of these two forms of protein A was then studied using ELISA, which revealed a higher activity for the truncated form in IgG-binding, compared with the full-length protein [7–9]. Schematic illustration of plasmid construct is shown in Supplementary Data 1.

In our previous works, the full-length and truncated forms of the SpA were secreted, expressed, and purified using a novel, cost-effective, and powerful separation technique of per aqueous liquid chromatography (PALC), for the first time. The combination of the silica column and a gradient elution of water: acetonitrile, with ammonium acetate as an additive was used. In this method, the purified SpA fraction derived from high water/low acetonitrile elution solvent is the correctly folded form of SpA, which retains the desired bioactivity. Purification of both forms of SpA was confirmed by SDS-PAGE, silver staining, Western blotting, and mass spectrometry analysis. The purification of SpA was also confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis. The purified proteins retained their function and activity, as confirmed by ELISA and other functional assays [10, 11]. Using the ProtScale Tool and the methods proposed by Parker et al and Kyte-Doolittle on (https://web.expasy.org/protscale/) to give a general view on the protein hydrophobicity indicated that the protein is hydrophobic based on the sharp peaks and positive values in the hydropathy plot. Difficulties in purification of SpA due to the partially hydrophobic nature of the protein remain a major challenge and several methods have been employed to purify SpA. The methods used include ion-exchange chromatography [12], affinity chromatography [13], and gel filtration. The ion-exchange and gel filtration chromatography methods cannot entirely eliminate contaminants and so may not be suitable preparations of SpA for therapeutic applications. The reagents used in the affinity chromatography technique are expensive and have lower binding capacity. In our studies, the recombinant SpA produced was free of the polyhistidine affinity tag (His-tag), which affects the expression levels [14]. Additionally, a His-tag might affect the function of the recombinant protein and when the recombinant protein is to be used as drug the tag should be removed [15]. Removal of the tag will require additional steps using proteases that imposes additional cost on production. In addition, a common problem in using polyhistidine affinity tags for protein purification is nonspecific binding of untagged proteins on the nickel-based columns used in affinity chromatography. Although histidine occurs relatively infrequently (2% of all protein residues are histidine), some cellular proteins contain two or more adjacent histidine residues,

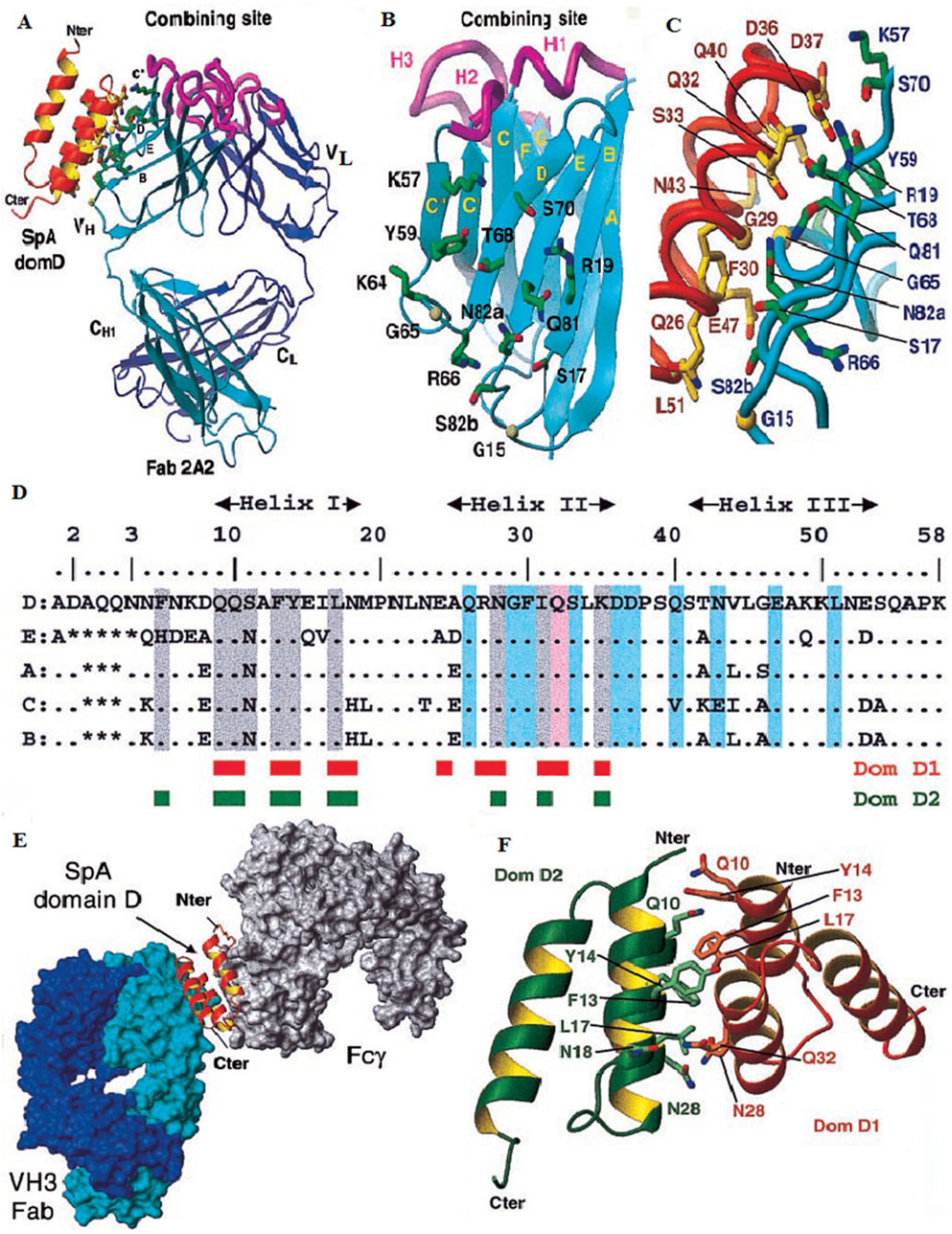

FIG. 1

Interaction between SpA domain D and Fab 2A2 from a human IgM. (A) Side view showing SpA domain D (red) interacted to the Fab heavy chain (cyan). The VL domain, which is not bind to these molecules, is presented in dark blue. (B) Ribbon structure of the VH region of Fab showing the regions of the residues that bind to domain D. (C) Details of the residues of SpA domain D interact with the Fab 2A2. (D) Alignment of the amino acid sequences of the five SpA domains. Domain D residues shared in interaction with Fab 2A2 are highlighted in cyan. (E) Binding of a VH3 Fab (cyan) and a Fcγ (gray) by the D domain of SpA (red). (F) Interaction between domain D monomers, dom-D1 (red) and dom-D2 (green). (adapted from https://www.pnas.org/content/97/10/5399) [2].

which may interfere with affinity purification. Another point to be considered when working on biomimetic therapeutic proteins is that for United States Food and Drug Administration (US FDA) approval, the recombinant protein needs to be similar to the native sequences with no added amino acids [16]. The comparison of the SpA purification and production methods are presented in Table 1.

2.1. Intra- and extracellular expression of recombinant SpA

Over recent years, various strategies have been employed for heterologous expression of SpA. The commercially available SpA is expressed as an intracellular form and is then purified after cell lysis using Ni-beads that bind to the histidine-tag [17]. Protein expression as a secreted protein can be beneficial and have a number of advantages over intracellular production [18]. Abrahmsén et al. [19] expressed a secreted form of the recombinant SpA using the SpA promoter in *E. coli*. They reported the rate of SpA expression in different fractions as follows: intracellular fraction: 2%, periplasmic fraction: 89% and extracellular fraction: 9%. In another study by von Roman et al [20], the recombinant SpA was produced using the pelB signal peptide to secrete the protein into the periplasmic space of *E. coli*. In these studies, the proportion of SpA in the periplasmic fraction was higher than that in the extracellular fraction, which necessitated introduction of an extra step for the extraction of periplasmic proteins. The increased costs of extracting periplasmic proteins and subsequent removal of contaminating bacterial endotoxin is a major concern when using *E. coli* expression system. Studies of recombinant SpA expression in our own laboratory gave a distribution of 37% in the intracellular fraction, 9% in the periplasmic fraction, and 54% in the extracellular fraction [8, 9]. Therefore, our expression system offers attractive advantages for industrial production of SpA. In addition, our approach has the advantage of not requiring cell lysis for preparation, which would otherwise release some endotoxin and other cellular impurities into the medium, which would have to be removed in the downstream processes. This is particularly advantageous when the recombinant protein is to be used as a drug. The US FDA has set the following maximum permissible endotoxin levels for drugs distributed in the United States: Drug (injectable, intrathecal) = 0.2 EU/kg body weight, drug (injectable, non-intrathecal) = 5 EU/kg body weight, and sterile water = 0.25–0.5 EU/mL (depends on intended use) [21]. We have measured the endotoxin level after different stages of purification and found that the level is sufficiently below acceptable levels that it can be ignored [22]. The level of endotoxin in our protein preparations assessed using the Limulus amebocyte lysate endotoxin assay was 0.008 EU/mL for the truncated form of the SpA and 0.021 EU/mL for full-length SpA. Production of SpA in *Pichia pastoris* has also been successfully described [23]. However, expression of the recombinant SpA using the *E. coli* expression system still has several advantages, including lower costs, easier handling, and the higher level of expression.

3. Applications of SpA

3.1. Applications of SpA from the general perspective

SpA is a reactive antibody-binding agent that binds to Fc region of all human antibodies (except IgG3) and certain antibodies of animals including pig, dog, rabbit, goat, and mouse [24]. SpA is the main component of a wide range of biochemical, biotechnological, and medical applications. One of the most widely used application of SpA is in the affinity chromatography in which SpA is bound to various matrices such as silica, agarose, cellulose, or cross-linked dextrose to produce hybrid chromatography resins for purification of antibodies, proteins, peptides, and so on. SpA-based chromatography is the most efficient method for capturing Fc-containing proteins and purification of therapeutic IgG on an industrial scale. SpA-based fusion systems for hybrid purification is another application of SpA in the last decade.

Uhlen et al. (1986) prepared a protein fusion consisting of IgG-binding domains of SpA and active β-galactosidase and revealed that this protein can bind to IgG-sepharose. Nevertheless, the proteolytic destruction of the fused protein reduced the efficiency of this system [1]. In 1986, Uhlen et al. also designed double-vector systems to overcome this problem. Both vectors had been designed such that the product produced by hybrid chromatography with IgG could be purified [19]. SpA may bind to various polymeric supports such as agarose and is used in safe immunoprecipitation deposition to refine antibodies from biological solutions. SpA is most commonly used in test methods such as aggregation, ELISA, and Western blot. It has also been widely used in pharmaceutical applications to bind to immune complexes and serum Igs [25]. Proteins binding to Ig have been used as reporters in large numbers of indirect primary binding assays, especially in veterinary diagnosis due to their applicability to many species [26, 27]. Lawman et al. (1986) [28] used radioactive-labeled SpA to track bovine antibody, especially IgG2 subclass raised against *Brucella abortus*. In addition, enzyme-bound SpA has been used in tracking anti-brucella antibodies in buffalo, polar bear, cattle, and humans. SpA has also been used in coagglutination testing for human antibody against *Brucella sp.* [28].

The *Caulobacter crescentus* can be used to display heterologous peptides on its cell surface for research applications. In this work one of the binding domains to IgG was connected to the protein G (GB1) of the bacterial surface protein RsaA so that the whole cell could act as an immune-reactive particle [29]. SpA-based chromatography is commonly used as the best choice for the IgG binding and purification of the Fc-containing proteins [30]. However, researchers have tried to develop various SpA-based methods for antibody purification with more efficiency and reduced cost of. McCaw et al. (2014), applied a novel methacrylate-based SpA matrices called Amsphere, for IgG purification at the industrial scale and compared it with a commercially available protein-A resins of MabSelect and they confirmed that Amsphere showed the similar binding capacity and reduced the host cell proteins contamination compared

TABLE 1 *Production and purification methods of SpA: strengths and weaknesses*

Methods	*Strengths or weaknesses*	*References*
SpA production methods		
SpA obtained from culture of wild-type *S. aureus*	Handling the large volumes of *S. aureus* culture is not recommended because of its pathogenicity.	[2, 33–36]
Expression of SpA in safe bacterial hosts such as *Escherichia coli*	This avoids the need for the higher safety levels required for work with wild-type strains of *S. aureus* reducing otherwise high costs.	[7–9]
Intracellular expression of recombinant SpA in *E. coli*	Requiring cell lysis. Increased costs of extracting intracellular proteins and subsequent removal of contaminating bacterial endotoxin is a major concern when using *E. coli* expression system.	[40–54]
Extracellular expression of recombinant SpA	Not requiring cell lysis for preparation, which would otherwise release some endotoxin and other cellular impurities into the medium, which would have to be removed in the downstream processes. Cost effective.	[8, 9]
Production of SpA in *Pichia pastoris*	Expression of the recombinant SpA using the *E. coli* expression system has several advantages, including lower costs, easier handling, and the higher level of expression in comparison of its production in *Pichia pastoris*.	[23]
SpA purification methods		
Per aqueous liquid chromatography (PALC)	Cost effective and powerful separation technique. The purified SpA fraction derived from high water/low acetonitrile solvent elution is the correctly folded form of SpA which retains the desired bioactivity.	[10, 11]
Ion-exchange chromatography	Cannot entirely eliminate contaminants and so may not be suitable preparation of SpA for therapeutic applications.	[12, 37]
Affinity chromatography	The reagents used in the affinity chromatography technique are and have lower binding capacity.	[13, 37]
Gel filtration	Expensive	[38]
Polyhistidine affinity tag (His-tag)	Affects expression levels. His-tag will frequently affect the function of the recombinant protein. Removal of the tag will require additional steps using proteases that imposes additional cost on production. Nonspecific binding of untagged proteins on the nickel base column used in affinity chromatography due to time dependence of His-tag removing process. Some cellular proteins contain two or more adjacent histidine residues which may interfere with affinity purification. Biomimetic therapeutic proteins, for US FDA approval, needs to be similar to the native sequences with no added amino acids.	[14–16, 39–44]

with MabSelect [31]. Murphy et al. [32], suggested these matrices are suitable for the economical antibody purification because of using the low volume of resin and buffers along with reducing the time it takes to carry out the process.

3.2. Pharmaceutical application of SpA

A primary therapeutic application of SpA is its recent use as a medication against autoimmune diseases. SpA directly binds to monocytes and subsets of the B-cells that have a role in the development of various autoimmune diseases, and the resulting interaction has been reported to lead to better control of the disease [33]. Two of these autoimmune diseases are mentioned below.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a highly serious autoimmune disease, in which the patients' immune system mistakenly produces antibodies that attack the joint surface, causing inflammation and pain [33].

Idiopathic thrombocytopenic purpura

Idiopathic thrombocytopenic purpura (ITP) is a rare disorder in which blood does not clot. This happens when blood platelets are not recognized by the immune system and are identified as foreign, and are subsequently targeted [34].

Use of SpA had a significant effect on treatment of ITP and RA patients. Preclinical studies have shown that a small dose of this medication has equal or greater effect on these two diseases, with fewer side-effects than currently available medicinal products on the market to treat autoimmune diseases [34]. Two advantages of the application of SpA are that it is used at a significantly lower dose than other medications, and due to its long-lasting effect, frequent use of that is not necessary.

SpA has also been the drug target for treatment of infection caused by methicillin-resistant *S. aureus* (MRSA) in some studies. In one study an anti-*S. aureus* surface SpA monoclonal antibody has been used as an antibody-based immunotherapeutic for MRSA infections [35].

3.3. Immobilization of SpA on solid supports

One of the most common techniques for antibody purification uses SpA immobilized on various solid supports [36]. Given that SpA is the protein of choice for antibody purification with high purity and efficiency, which is an important factor to reduce time and cost; therefore, it can be used in industrial application of antibody-based anticancer drugs [37]. However, changes in the methods of immobilization of SpA for improvement of antibody extraction is still ongoing. SpA can also be immobilized covalently on a polymeric support using enzymatic methods. One of the efficient methods of enzymatic attachment of proteins on the surface is by the use of the *S. aureus* transpeptidase called sortase. Recently, SpA was attached covalently on the surface of graphene oxide nanoparticles using sortase-mediated immobilization with some modifications and improvements and the immobilized SpA was able to efficiently purify IgG from the serum [38]. The explanation of this method will be provided in the next subtitle, 3.3.1. Khodaei et.al. [39] have also investigated the immobilization of SpA on the surface of chitosan using two different methods. The first involved direct coupling of SpA on chitosan by using glutaraldehyde and the second used the functionalization of amino groups of chitosan with tris(2-aminoethyle)amine to produce amino-double branched moieties followed by activation with glutaraldehyde. The idea of using double-branched linkers for surface modification is driven by the expected improvement in the loading capacity of chitosan for the protein immobilization and purification of IgG [39].

Site-specific immobilization of SpA on nanoparticles for one-step and high efficiency purification of IgG

Immobilization of proteins on a solid support is an efficient way to make these processes more cost effective as the immobilized proteins can be easily recovered and used in the continuous rounds of the reaction [40]. There are various immobilization methods such as: adsorption [41], covalent bonding [42], entrapment [43], polymerization [44], and encapsulation [45]. In these above methods, covalent anchored proteins on a solid support is usually suggested when leaching of protein from the support is the main concern [46]. From other points of view, protein immobilization can be categorized into random and oriented/site-directed immobilization [47]. In random immobilization, proteins are bonded through side chain amino groups to the surface of carrier from different sites, thus exposing various orientation of the immobilized protein to the surrounding environment [48]. Therefore, the protein native function can be affected by this unpredictable orientation.

Site-specific immobilization of proteins on the surface of nanoparticles allows the proteins to retain their function as this kind of coupling is oriented and uniformly done through specific motifs or amino acids. Different approaches have been developed for the oriented immobilization of proteins which are exemplified below.

In one of the approaches, a specific sequence namely capture sequence is fused to the structure of target protein, which will then recognize a motif on a solid support. Oriented immobilization by avidin is an example of this category. Chemoenzymatic approach is one of the most efficient strategy that takes advantage of affinity and selectivity of the enzymes for their specific substrate. *S. aureus* sortase is a well-characterized highly specific transpeptidase, which naturally and covalently anchor a variety of surface proteins to the cell wall [49]. Sortase recognizes LPxTG motif and cleaves amide bond between threonine and glycine, followed by the nucleophilic attack of a multiglycine, which results in covalent attachment. Recently our laboratory developed a site-specific immobilization of protein A on amine linker and pentaglycine functionalized silica and graphene oxide nanoparticles by using a cost-effective sortase-mediated immobilization strategy [38]. In this approach, silica and graphene oxide nanoparticles were also functionalized using a pentaglycine peptide as a nucleophilic amine on the support. This approach allowed a one-step

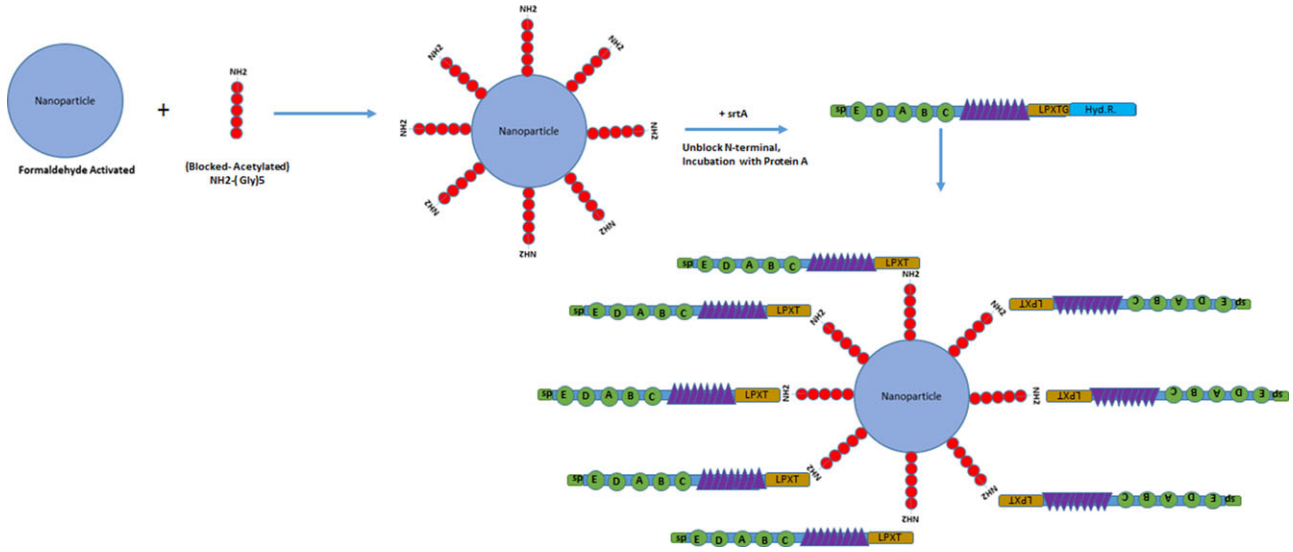

FIG. 2 *Graphic presentation of protein A linked to the surface of nanoparticles after Sortase-mediated site-specific covalent immobilization. The protein A molecules are cleaved between the threonine (T) and the glycine (G) residues within the LPXTG sortase recognition motif, followed by ligation of the C-terminal threonine to an N-terminal pentaglycine.*

purification and immobilization of the proteins and to retain their functional properties using two different linkers. Immobilization of SpA in this approach showed a high efficiency in purification of IgG from human serum, which is affected by the type of carrier used. The presence of a sec-dependent signal peptide at the N-terminal part and a sortase recognition motif, LPxTG, at the C-terminal end of SpA, makes it ideal substrate and model for the sortase-mediated immobilization on different carriers (Fig. 2).

3.4. Surface display of SpA on bacterial and eukaryotic cells for immunoprecipitation of IgG-antigen complex

Surface display of protein A has an application in immunoprecipitation of IgG-antigen complex like pansorbin from EMD Millipore Company or formalin fixed *S. aureus* from Sigma–Aldrich. Given that *S. aureus* is a human pathogen, working with this organism requires safety level 3. In a measure to stop using this organism, our laboratory has developed various novel surface display methods in the safe microorganism including *Bacillus subtilis*, *E. coli*, and *Lactobacillus* strains. To avoid the potential problem caused by this organism, we displayed *S. aureus* protein A on the surface of spores, and vegetative form of *B. subtilis* and also *E. coli*. Subsequently, related content is provided by referencing.

Binding proteins to the outer surface of viruses [50], microbial [51], mammalian, and insect cells is a suitable solution in surface display of heterologous proteins, which has many applications in industry, medicine, and biotechnology. For this purpose, the heterologous protein should pass the cytoplasmic membrane after binding to one of the host cell surface proteins in the form of a fusion protein. This requirement is a significant limitation of this approach but this and several other limitations can be overcome using bacterial spores as a biotechnological tool. Some advantage of spore-based display systems that spores are formed through a natural sporulation process in the mother cell and also some chaperones are formed during this process that help the proper folding of proteins [52]. Other advantages are the simple and economic production of large amounts of spores, safety, high stability, and resistance of spores, which are in part due to the presence of the spore coat [53].

Bacillus is a classic genus of Gram-positive bacteria that form spores when placed in a harsh environment. In Europe *Bacillus subtilis* has been granted qualified presumption of safety status by the European Food Safety Authority (EFSA, 2008) [54]. Among the various *Bacillus* species, *B. subtilis* has additional advantages because of the detailed knowledge of its spore structure [55] and the availability and ease of advanced genetic tools [56] and genomic data that facilitate the construction of recombinant spores [57]. Spore formation is a cell response to environmental stress and various mechanisms of bacterial sporulation include the formation of exospores, myxospores, and akinetes. In this review, only endospores will be discussed (hereafter referred to as spores).

The spore is one of the most durable biodynamic structures. They are metabolically dormant and partially dehydrated because most of the water has been replaced with Ca^{2+}-dipicolinic acid. The genome of the organism is placed in the central core and surrounded by the inner spore membrane, germ cell wall, cortex, outer spore membrane and coat, respectively from the inside to the out [58]. The spore coat is itself composed of three layers: a lamellar inner coat, a more coarsely layered outer coat and crust [58].

The spore coat has many different functions such as: protection from the environmental stresses like extreme heat or

UV irradiation, regulation of germination in suitable conditions, coat proteins show many diversity between different species, which may have an important role in defining the ecological niches of spore-forming bacteria [58].

The bacterial spore coat is a proteinaceous structure with approximately 1 mm in diameter that surrounds spores and is composed of about 70 different proteins [59]. In the situation of nutrient depletion or some other environmental threat, the spore-producing bacteria attempt to divide asymmetrically resulting in a large mother cell and a smaller cell called the forespore. These cells have the same genetic material but a different fate. The forespore is then engulfed by the mother cell enclosing it as the double membrane organelle within the cytosol of the mother cell. Finally, the mother cell lyses and the forespore is released into the environment. Coat proteins are synthesized in the mother cell during the process of sporulation and these localize onto the surface of the forespore [60, 61]. Five proteins are essential for proper coat formation; the morphogenetic proteins (SpoIVA, SpoVM, SpoVID, SafA, and CotE) and three proteins are crucial for crust morphogenesis (CotX, CotY, and CotZ) [52]. So far four spore coat proteins which are located in the outermost layer of the coat have been used for surface display of enzymes or antigens in spore-based vaccines. These are CotB, CotC, CotG, and CotX [62]. Recently, a crust protein CotZ was used as a new anchor protein useful in spore surface display [63].

The cell-based display system and also phage- and spore-based systems rely on genetic engineering of the host to make a recombinant organism as a live biotechnological tool to display peptides or proteins [64]. The main drawback of genetic approach of spore surface display as a carrier to display heterologous protein is that this it requires the release of a live recombinant organisms, raising concerns over the use and clearance of genetically modified microorganisms [65]. To overcome this problem, a non-recombinant approach of spore-based display system has been proposed. In the first study, mammalian NADPH cytochrome P450 reductase was overexpressed in sporulating *B. subtilis* cells, adsorbed on the spore surface, and released into the culture medium after sporulation by autolysis of the mother cell [66]. In a separate study, a collections of the purified antigens TTFC of *Clostridium tetani*, PA of *Bacillus anthracis*, Cpa of *Clostridium perfringens*, and glutathione S transferase of *Shistosomas japonic*) were adsorbed onto the *B. subtilis* spores and shown to be able to induce specific and protective immune responses in mucosally immunized mice [67]. It was suggested that the combination of electrostatic and hydrophobic interactions between spores and heterologous proteins will drive the adsorption, which is dependent on negatively charged and hydrophobic surface of the spore and not dependent on specific spore coat components [67]. Hydrophobic and electrostatic interactions were suggested as the main forces involved also in the interaction between the *E. coli* phytase and spores of *B. polyfermenticus* [68]. Spore surface display, a powerful technique that can effectively display the bioactive molecules on the surface of spores, has been recognized as a suitable alternative for biotechnological processes in recent years.

In 2012, Sirec et al. [68] used β-galactosidase of the thermoacidophilic bacterium *Alicyclobacillus acidocaldarius* [69], as a model to study enzyme adsorption on *B. subtilis* spores. Their results indicated that spores bind β-Gal without affecting its properties but, instead, stabilizing it at acidic pH and high temperatures [68]. In 2014, Gashtasbi et al. [70] immobilized the alpha-amylase enzyme on the spore surface by the covalent method using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N hydroxysulfosuccinimide. In contrast to that study in which just one domain of SpA was displayed, in 2015, Ghaedmohammadi et al. [53] displayed both full-length and truncated forms of SpA containing 5 IgG-binding domains on the spore surface. In the latter study, SpA was displayed on the surface of *B. subtilis* spores by two nongenetically approaches including spontaneous adsorption of SpA onto the spore surface, and covalent linkage of this protein to the spore surface proteins. The covalent immobilization method using glutaraldehyde as an efficient and low-cost crosslinker had been developed for the first time to display SpA on the spore surface. The data showed that spore-bound SpA can be recycled and utilized several times because it retained its IgG-binding activity, even after several consecutive binding and washing steps. They also suggested that the covalent approach is more efficient than adsorption regarding protein attachment to the spore surface [53, 71].

3.5. Clinical application of SpA in *S. aureus* typing (SpA typing)

SpA gene typing has been used to study relatedness of *S. aureus* isolates [72]. One methods used for this purpose include pulsed-field gel electrophoresis (PFGE), multi locus sequence typing (MLST), repetitive sequence-based PCR, and various restriction fragment length polymorphism-based methods. SpA typing has in general higher discriminative power than MLST and lower discriminative power than PFGE [73]. The SpA typing method is based on sequencing of the polymorphic X region of the SpA. X-region has variable number of 24-bp repeats and is highly polymorphic [74]. Deletion and duplication of repeats, and point mutations are happen in this region. SpA typing is the result of the sequence and order of specific repeats [75].

3.6. Directed evolution of SpA

As described above, SpA contains five homologous IgG-Fc binding domains, namely, E, D, A, B, and C, and a cell-wall anchoring region, XM, respectively from N terminus [51]. As in all IgG purification steps, an extra cleaning-in-place step by treatment with sodium hydroxide is required to clean up and reuse the column. Therefore, tolerance to high alkaline condition is an essential feature for these kind of matrices [76]. Although SpA is relatively resistant to alkaline conditions, this resistance is not enough to tolerate highly alkaline conditions of more than 0.1 M NaOH [77]. Therefore, attempts have

TABLE 2 *The summary of the SpA applications*

SpA applications	*References*
Pharmaceutical application of SpA	
Use as a medication against autoimmune diseases such as: *rheumatoid arthritis (RA)* and *idiopathic thrombocytopenic purpura (ITP)*	[33, 34]
Use as the target drug for treatment of infection caused by methicillin-resistant *S. aureus* (MRSA)	[35]
Immobilization of SpA on solid supports	
Use for antibody purification	[31, 32, 36, 37]
Site-specific immobilization of SpA on nanoparticles for one-step and high efficiency purification of IgG	[38]
SpA immobilization on the surface of chitosan for purification of IgG	[39]
Surface display of SpA on bacterial and eukaryotic cells for immunoprecipitation of IgG–antigen complex	
Displaying both full length and truncated forms of SpA containing 5 IgG-binding domains on the surface of *Bacillus subtilis* spores by two nongenetically approaches	[53, 71]
Clinical application of SpA in *S. aureus* typing (SpA Typing)	
SpA gene typing has been used to study relatedness of *S. aureus* isolates	[72–75]
Application of SpA in diagnostic biosensors	
Rapid and low-cost detection technique for diagnosis of pathogen *S. aureus* in different environments is critical for infectious diseases control	[80–82]
Using SpA-targeting aptamers conjugated to a porous silicon (PSi) nanostructure to specifically quantify SpA in a range of 2–50 μM in less than 2 H	[80]
Using carboxyl graphene as the carrier of IgG to develop electrochemiluminescent (ECL) biosensor to detect *S. aureus* because of specific reaction between SpA and IgG molecules	[83]

been made to improve the alkaline stability of the IgG-binding domains of the SpA using genetic engineering methods. The C domain has been shown to be fairly resistant to alkaline conditions compared with the other domains, probably due to the presence of Thr-23 instead of the Asn-23 in other domains [78]. Two particular mutations in the C domain of interest are A1V and G29A [27, 77]. The engineered immobilized SpA introduced that enable milder elution pH for use in affinity chromatography using point mutations to the SpA amino acids. A use of these compounds is shown with a pH-sensitive molecule prone to association in acidic situations [79]. A mutated and low-molecular-weight SpA including the domains, E, D, A, B, and 13 amino acids of the C-domain with a thermal stability was immobilized on silk fibroin to prospect its engineering application.

3.7. Novel application of SpA in diagnostic biosensors

S. aureus is a human pathogen and it causes several diseases such as bacteremia, endocarditis, and sepsis. It also excretes several endotoxins and causes food-borne diseases. Therefore, developing a reliable, rapid, and low-cost detection technique for diagnosis of this bacteria in different environments like water, air, food, and body fluids is critical for infectious diseases control. Various approaches have been developed for the identification and diagnosis of *S. aureus*. The traditional gold standard includes the characteristics of the bacterial colony using selective agar. Although these bacteriological methods are simple and accurate, they are time consuming and take several days to yield results. Molecular methods such as PCR is also available, which allows more rapid identification ability and more accuracy than the old method, but it is a rather expensive process with more equipment and materials. However, sample preparation and results analysis require expert staff [80].

More recently SpA is used for rapid, accurate, and low-cost detection of *S. aureus* using techniques such as real-time PCR, ELISA, aptamer-based, and nanobiotechnology-based methods. Protein A is an ideal molecular marker for the detection of

S. aureus because its gene is highly conserved and just have one mutation in 70 months [80].

Aptamers are single-stranded DNA or RNA oligonucleotides, which can specifically bind their target due to their three-dimensional structures. Recently, a variety of aptamers as a capture probes have been developed for stable and reliable biosensing of *spa* gene. The SELEX technology (Systematic Evolution of Ligands by Exponential enrichment) is widely applied as an *in vitro* selection and amplification method to generate target-specific aptamers [80]. Strehlitz and coworkers [81] have developed a modified variant of SELEX technology named FlugMag-SELEX in which magnetic beads are used for target immobilization and fluorescent labels in aptamer quantification. Urmann et al. [80], used SpA-targeting aptamers conjugated to a porous silicon (PSi) nanostructure. This biosensor could specifically quantify SpA in a range of 2–50 μM in less than 2 H [80].

Many scientists have used gold nanoparticles colorimetric method for detection of the conserved region sequence of *spa* gene in the environment. A color change can easily be detected by just a portable UV–visible spectrophotometer and is very useful for rapid detection of pathogens in the environment [82].

In one study, Yue et al. [83] used carboxyl graphene as the carrier of IgG to develop electrochemiluminescent biosensor to detect *S. aureus* because of specific reaction between SpA and IgG molecules.

The important applications of SpA are summarized in Table 2.

4. Conclusions

SpA has multiple applications spanning from industry to medicine with signs of its use in controlling serious disease. Production of high quality and high yield materials free of contaminants to permit therapeutic use is essential. Genetic engineering approaches using various host organisms are being tested and used but there is room for improvement by targeting the molecule to be secreted to enhance purification and reduced contamination. Meanwhile, genetic engineering looks to promise improvements in characteristics that aid in both function and to assist in fundamental aspects such as reuse of columns and so on.

Considering the unique role of SpA in biotechnology, according to the provided content, its production and purification in the large scale is recommended. Another part that needs to be considered is that the native form of SpA signal peptide and its derivatives) have been used for efficient secretion of several recombinant proteins through sec pathway [19, 51]. In our laboratory, nSpA signal peptide was previously used to secret a few recombinant proteins including two mentioned forms of and organophosphorus hydrolase in *E. coli* [7–10, 84]. We also designed a modified and codon optimized SpA signal peptide (mSpA) to drive the secretion of the CALB into the periplasm of *E. coli* [85].

Recently, the novel application of SpA as a diagnostic biosensor has been reported. Finally, as using SpA as a drug for treatment of RA and other autoimmune diseases has recently been started, the further evaluation of SpA effects on immune system and its animal and clinical studies should be highly recommended.

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