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Quantitation of basic fibroblast growth factor by immunoassay using BIAcore™ 2000

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Abstract

A sensitive, accurate, and efficient immunoassay using a BIAcore™ 2000 biosensor instrument for the quantitation of basic fibroblast growth factor (bFGF) in HEPES-buffered saline containing 100 μg/ml heparin (HHBS) has been developed and validated. In this method, anti-bFGF monoclonal antibody 48.1 (MAb 48.1) was selected as a binding ligand and immobilized to the matrix surface of Sensor Chip CM5 by amine coupling. A high immobilization level of MAb 48.1 (12643 ± 816 RU, mean $\pm$ S.D., $n = 5$) was achieved with high reproducibility (i.e. coefficient of variation (CV) was 6.5%). This immobilized MAb 48.1 sensor surface was used to detect and quantify bFGF. This assay has a range of reliable BIAcore™ response from 5.65 to 1440 ng/ml bFGF in HHBS, which was well fitted with a sigmoidal model. The immobilized MAb 48.1 was found to be stable for at least 150 regeneration cycles and for at least 9 days at room temperature. Intra- and interassay CVs ranged from 0.9 to 5.9% and from 2.7 to 8.5%, respectively. Matrices such as serum, bovine serum albumin (BSA), and two pharmaceutical excipients (Pluronic® F127 surfactant and sodium carboxymethylcellulose) did not interfere with bFGF analysis over the sensor surface. Therefore, this validated assay has good precision, accuracy and specificity, and has been found useful in quantifying bFGF in several research and development studies.

Keywords: Basic fibroblast growth factor; BIAcore™ immunoassay; BIAcore™ 2000 biosensor; Antibody immobilization; bFGF quantitation

1. Introduction

Basic fibroblast growth factor (bFGF; FGF-2), an important angiogenic factor, is characterized by potent mitogenic activity for a variety of mesoderm- and neuroectoderm-derived cells such as fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, osteoblasts, and melanocytes. Proposed clinical uses include wound healing, collateral blood vessel formation in coronary artery disease, neuroprotection in stroke, and osteogenesis in bone fracture [1]. FIBLAST® trafermin, a proprietary form of recombinant human bFGF with 154 amino acid residues, was developed at

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Scios Inc. [2]. This product is in various phases of development for neurological and vascular conditions. One important issue during development is accurate and efficient quantitation of bFGF in different complex matrices such as serum, cell culture medium, or samples containing pharmaceutical excipients. Because concentrations of bFGF in samples of interest may be very low (e.g. ng/ml) and significant interference from the matrix can occur, quantitation of the growth factor using analytical techniques such as HPLC and colorimetric assays are often problematic. Currently enzyme immunoassay [3–5] and direct radiometric assay using labeled agent [4,6] are the most commonly used methods to quantify bFGF. These methods are usually time consuming and labor-intensive. The methods can also be difficult to validate. Therefore, the purpose of this study was to facilitate analysis of bFGF in complex media by developing a novel biosensor-based immunoassay that is efficient, sensitive, accurate, and reproducible. This biosensor immunoassay represents a biomolecular interaction analysis (BIA) and a BIAcore™ 2000 biosensor instrument was used to perform the assays.

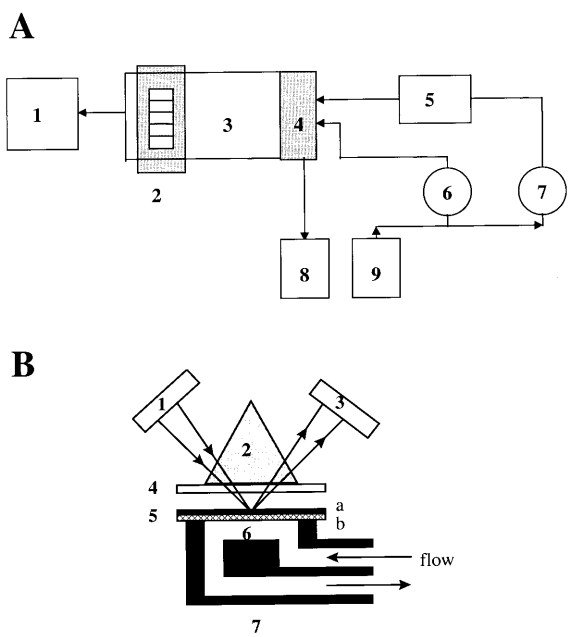

Fig. 1. (A) Basic components of BIAcore™ 2000 instrument: (1) optical detection system; (2) sensor chip; (3) integrated microfluidic cartridge (IFC); (4) connector block; (5) autosampler; (6) eluent pump; (7) autosampler pump; (8) waste bottle; (9) buffer bottle. (B) The structure of the optical system and flow cell: (1) light source; (2) prism; (3) detector array; (4) opto interface; (5) sensor chip (a, gold film; b, matrix coupled to ligand); (6) flow cell; (7) microfluidic cartridge.

2. Materials and methods

2.1. Instrumentation and principles of BIA technology

The BIAcore™ 2000 biosensor instrument is manufactured by Pharmacia Biosensor AB (Uppsala, Sweden). It mainly consists of a sensor chip, a surface plasmon resonance (SPR) detector unit, and a liquid handling system that has two precision pumps and an integrated microfluidics cartridge (IFC). The autosampler and the liquid handling system together control delivery of sample plugs into a buffer stream that passes continuously across the sensor chip surface (Fig. 1A). The entire system is computer-controlled, including data collection and analysis, resulting in a fully automated analytical system.

The sensor chip (signal transducer) is a glass slide with a thin layer (50 nm) of gold deposited on one side. The gold film is in turn covered with a covalently bound matrix on which biomolecules can be immobilized. The most widely used chip is the Sensor Chip CM5 (Pharmacia Biosensor AB, Uppsala, Sweden), with a surface matrix of carboxymethyl dextran (100 nm), which can be coupled to a macromolecule using the Amine Coupling Kit (Pharmacia Biosensor AB, Uppsala, Sweden). The coupled macromolecule, or immobilized ligand, can be used to interact with an analyte in solution. One wall of a sensor chip has four flow cells where interactions are monitored. The solution being studied is pumped over the matrix-covered side of the flow cell. The other side is illuminated by near-infrared and plane-polarized incident light which passes through the glass to strike the gold film (Fig. 1B). The evanescent wave, one component of the incident light momentum, penetrates the liquid/gold interface and interacts with free oscillating electrons or

plasmons in the metal surface, causing a decrease in the incident light intensity. This phenomenon, SPR, occurs only at a precisely defined angle of the incident light. This SPR angle is dependent on the refractive index of the medium close to the gold film surface. The interactions between the analyte in buffer solution and the immobilized ligand on the chip cause a change in refractive index in close proximity to the gold film surface that translates into a change in the resonance angle. The resonance angle (or SPR signal) is measured in resonance units (RU). A sensorgram is a plot of the SPR signal as a function of time and displays the progress of the interaction at the sensor surface. Continuous monitoring of the SPR signal allows quantitation of interaction changes between ligand and analyte. BIA technology has been used to determine the amount of analyte in a sample [7–9] as well as the specificity and kinetics of interactions [10–12].

Concentration measurement with BIA relies on a specific interaction between the analyte and a chosen immobilized ligand. Antibodies are most commonly used as the immobilized ligand, which can specifically interact with analytes (e.g. peptides or proteins) in solution. A typical biosensor-based immunoassay for concentration measurement would involve: (1) immobilization of antibody onto the sensor chip surface using the amine coupling kit or other methods; (2) injection of analyte onto the immobilized antibody sensor surface to generate the response caused by binding of analyte to antibody on the sensor surface; and (3) injection of regeneration solution to remove non-covalently bound analyte for the next analysis cycle. A typical sensor surface can be used repeatedly for 50–100 cycles or more, depending on the stability of the immobilized antibody. For antigen–antibody interactions, the surface can usually be regenerated by injection of 10–100 mM HCl [13,14].

The important parameters for validation of a biosensor-based immunoassay should include [13]: (1) sensitivity, which depends on type and amount of immobilized antibody and injection time; (2) stability of the immobilized antibody; (3) reproducibility of the sensor surface; (4) precision and accuracy of analysis; and (5) specificity.

2.2. Reagents

HEPES-buffered saline (HBS, containing 10 mM HEPES, 150 mM NaCl, 0.05% Tween 20, and 3 mM EDTA, pH 7.4) and the amine coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-diethylaminopropyl)-carbodiimide (EDC) and ethanolamine hydrochloride (pH 8.5, 1 M) were from Pharmacia Biosensor AB. Recombinant human bFGF is a lyophilized product (lot no. E0004A) from Scios Inc. (Sunnyvale, CA). Heparin (lot no. 95H06055), bovine serum albumin (BSA, lot no. 24H01751) and anti-bFGF monoclonal antibody (MAb FB-8, lot no. 28H4813) were purchased from Sigma Chemical Co. (St Louis, MO). Sodium carboxymethylcellulose (lot no. FP1012340) was obtained from Aqualon Division, Hercules (Wilmington, DE). Pluronic® F127 surfactant (lot no. 549926) was supplied by BASF Co. (Mount Olive, NJ). Anti-bFGF monoclonal antibodies 4.2, 11.1 and 48.1 (MAb 4.2, MAb 11.1 and MAb 48.1) were prepared against peptide fragments 10–27, 22–27 and 94–155, respectively of bFGF at Scios Inc. (Sunnyvale, CA). Rat serum was obtained from Sprague–Dawley rats at Scios Inc. All other reagents were of analytical grade or purer and purchased from commercial suppliers.

2.3. Selection of MAb for bFGF quantitation using BIAcore™ 2000

To select a proper MAb for bFGF quantitation, bFGF was immobilized onto a flow cell of a CM5 sensor chip using the amine coupling kit according to the procedure described by the manufacturer [13,14]. During immobilization, HBS was used as a mobile phase at a flow rate of 10 μl/min. The carboxymethyl dextran matrix of the sensor chip surface was first activated with an injection of 70 μl of the EDC/NHS reagent mixture. Then, 70 μl of bFGF at 100 μg/ml in 10 mM NaOAc (pH 5.7) was injected and allowed to covalently couple to the sensor surface. Finally, the unreacted sites were blocked by injection of 70 μl of 1 M ethanolamine (pH 8.5). A BIAcore™ response of $\sim$6000 RU was achieved after immobilization.

The second step was to determine the binding affinity for bFGF of all the anti-bFGF MAbs (e.g. FB-8, 4.2, 11.1 and 48.1). This was done by injecting 10 μl of each antibody at 10 μg/ml in HBS buffer onto the immobilized bFGF flow cell. The responses after 110, 140 and 170 s, starting from the time point of injection, were recorded and averaged. The flow cell was regenerated by an injection of 10 μl of 15 mM HCl plus 0.5 M NaCl and then switched back to HBS.

2.4. BIAcore™ assay for bFGF quantitation

Monoclonal antibody 48.1 was selected for bFGF analysis and immobilized onto the sensor surface. The procedure was similar to that used for bFGF immobilization, which was described in Section 2.3, except that 140 μl of EDC/NHS mixture and 140 μl of MAb 48.1 at 50 μg/ml in 10 mM NaOAc buffer (pH 5.7) were used to immobilize MAb 48.1.

The bFGF samples were assayed over the immobilized MAb 48.1 sensor surface at room temperature. The mobile phase was HBS at a flow rate of 10 μl/min. During analysis, 10 μl of bFGF sample was injected and passed over the sensor surface. The amount of bFGF bound onto the surface was determined by recording the responses at 110, 140 and 170 s starting from the time of injection. Regeneration of the sensor surface was achieved by injecting 10 μl of 15 mM HCl plus 0.5 M NaCl, followed by a return to HBS. Total assay time was typically $\sim$12.5 min.

2.5. Validation of BIAcore™ assay for bFGF quantitation

2.5.1. Construction of bFGF standard curve

A series of eight twofold dilutions from 1440 to 5.65 ng/ml of bFGF in HBS containing 100 μg/ml heparin (HHBS) were prepared and assayed. Heparin (100 μg/ml) was added to HBS to stabilize bFGF in samples during the assay because heparin was shown to enhance the stability of bFGF [15]. The relationship between the BIAcore™ response and bFGF concentration was described by a four-parameter sigmoidal model:

$$R = R_{max} + (R_{min} - R_{max})/[1 + (C/EC_{50})^r] \quad (1)$$

where R is BIAcore™ response, R_{max} is the estimated maximum of the function, R_{min} is the estimated minimum of the function, C is bFGF concentration, EC_{50} is the estimated midpoint of the regression line, and r is the slope of the apparent linear region of the curve.

2.5.2. Quantitation limit

The quantitation limit was evaluated by repeatedly assaying the lowest level standard of 5.65 ng/ml bFGF in HHBS and analyzing the coefficient of variation (CV).

2.5.3. Reproducibility of the immobilized MAb 48.1 sensor surfaces

MAb 48.1 was immobilized onto five different sensor chip surfaces under the same immobilization conditions described in Section 2.4. The binding capacity of the sensor surfaces was determined by assaying the standards of 5.65–1440 ng/ml bFGF in HHBS.

2.5.4. Stability of the immobilized MAb 48.1 sensor surface

The effect of the number of analysis cycles on the stability of the immobilized MAb 48.1 surface was examined by consecutively running the standard samples from 5.65 to 1440 ng/ml of bFGF in HHBS over a freshly prepared sensor surface. The stability as a function of time was evaluated by running the standard samples after the sensor chip was stored at room temperature for 1, 3, 4, 7 and 9 days.

2.5.5. Precision and accuracy of the assay

To establish intraassay precision, three samples of bFGF were prepared in HHBS at concentrations of 20.0, 100 and 1000 ng/ml and assayed six times each. For interassay precision assessment, the samples were assayed on 6 different days. Estimates of precision were expressed as a CV relative to the overall mean observed concentration for all analytical runs at each concentration level. Estimates of accuracy were expressed as the percentage of the overall mean observed concentrations versus the corresponding actual concentration.

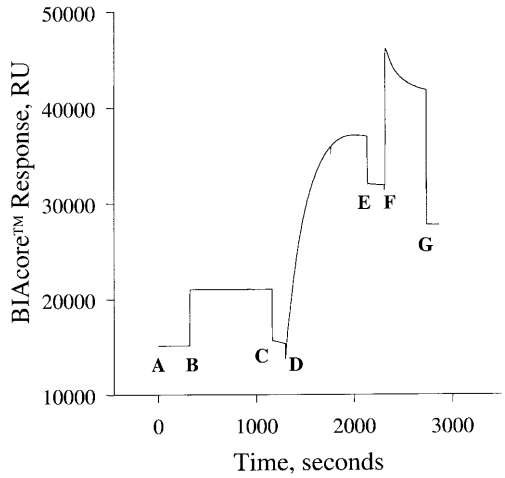

Fig. 2. Sensorgram showing the immobiliaztion of MAb 48.1 on the sensor surface using the amine coupling method. Immobilization conditions: HBS as a mobile phase at a flow rate of 10 μl/min; EDC/NHS volume: 140 μl; 50 μg/ml MAb 48.1 volume: 140 μl; ethanolamine volume: 70 μl. See text for definitions of events A–G.

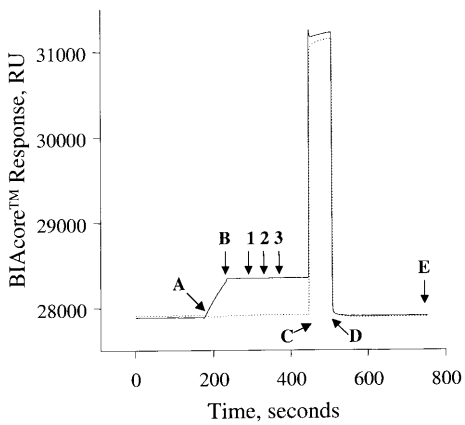

Fig. 3. Sensorgram showing bFGF analysis on the immobilized MAb 48.1 sensor surface. Injected samples: 10 μl HHBS (- - -) and 10 μl bFGF at 1440 ng/ml in HHBS (—). Analysis conditions: HBS as a mobile phase at a flow rate of 10 μl/min; 10 μl of 15 mM HCl plus 0.5 M NaCl as a regeneration buffer. See text for definitions of events A–E and 1, 2 and 3.

2.5.6. Specificity of the assay

The specificity of the assay was tested by adding 100 ng/ml of bFGF to HHBS containing 0.1% of rat serum, BSA, Sodium carboxymethylcellulose, or Pluronic® F127 surfactant and assaying the prepared samples to test the effect on binding of bFGF to the immobilized MAb 48.1 sensor surface.

3. Results and discussion

3.1. Analysis of BIAcore™ sensorgrams

Fig. 2 shows a sensorgram from a typical immobilization sequence using the amine coupling method to immobilize MAb 48.1 onto the sensor chip CM5 surface. The lettered points refer to different stages in the immobilization procedure. Point A is the baseline for the unmodified sensor chip surface with continuous HBS flow. Starting from point B to point C is an injection of 140 μl of EDC/NHS to activate the carboxylated dextran matrix. The increase in BIAcore™ response is caused by the change in bulk refractive index. After the surface is activated, 140 μl of MAb 48.1 is injected and the antibody is covalently coupled to the surface matrix through amide bonding, which corresponds to the time interval from D to E. From point F to G, 70 μl of ethanolamine hydrochloride is injected to deactivate the unreacted NHS-esters of the matrix and remove any remaining electrostatically bound MAb 48.1. Therefore, the increase in BIAcore™ response from point A to point G represents the amount of the immobilized MAb 48.1 on the sensor chip. For concentration measurements using the BIAcore™ instrument, a higher level of immobilized ligand will give a broader dynamic range and better sensitivity of the assay. In this study, a very high level (mostly around 12 000 RU) of immobilized MAb 48.1 was achieved under the conditions described in Section 2.4.

Fig. 3 shows the sensorgram of bFGF analysis over the immobilized MAb 48.1 sensor surface. A 10-μl bFGF sample is injected starting from point A and the injection is finished at point B, so the total injection time (or contact time of bFGF on the surface) is 1 min since the flow rate is 10 μl/min. After point B, the sensor surface bound with bFGF is exposed to the mobile phase again and the loosely bound bFGF is washed away by the mobile phase. The BIAcore™ responses at points 1, 2 and 3 relative to the baseline at point

Table 1
Determination of binding affinity of anti-bFGF monoclonal antibodies on the immobilized bFGF sensor surface (mean ± S.E., $n = 3$)

Anti-bFGF antibodies	BIAcore™ response (RU)
MAb 4.2	43.5 ± 1.6
MAb FB-8	574.6 ± 59.0
MAb 11.1	729.1 ± 67.9
MAb 48.1	1235.1 ± 142.6

A represent the amount of bound bFGF on the sensor surface. From point C to D, 10 μl of the regeneration solution (15 mM HCl plus 0.5 M NaCl) is injected to remove the bound bFGF for the next cycle of analysis. The difference in BIAcore™ response between point A and E shows the efficiency of regeneration. Insufficient regeneration can affect the binding capacity of the immobilized ligand. In this study, 15 mM HCl plus 0.5 M NaCl is a good regeneration solution, which can efficiently remove the bound bFGF without affecting the stability of the immobilized MAb 48.1, as shown in Section 3.5.

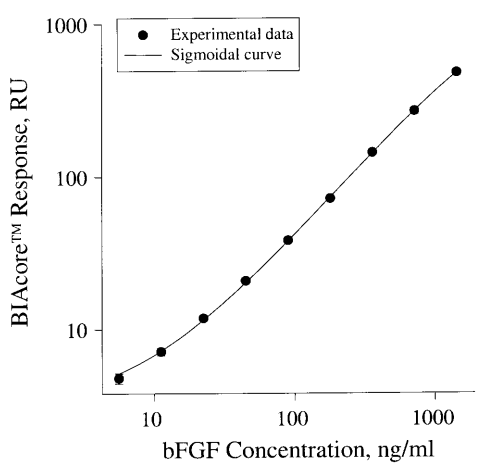

Fig. 4. The standard curve of bFGF qunatitated by BIAcore™ immunoassay in HHBS buffer. The fitted curve was based on the four-parameter sigmoidal model (average ± S.E., $n = 3$).

Table 2
Results of the bFGF standard curve fitted with a four-parameter sigmoidal model

bFGF (ng/ml)	BIAcore™ response (RU)		
	Experimental[a]	Predicted[b]	Residue[c]
5.65	4.8 ± 0.2	5.1	−0.3
11.3	7.1 ± 0.2	7.2	−0.1
22.5	11.8 ± 0.2	11.5	0.3
45	20.7 ± 0.3	20.2	0.6
90	38.1 ± 0.4	37.9	0.2
180	71.7 ± 0.7	73.3	−1.6
360	143.4 ± 2.5	142.1	1.4
720	267.6 ± 6.1	268.0	−0.5
1440	476.7 ± 10.7	476.6	0.1

[a] Mean ± S.E., $n = 3$.
[b] Based on Eq. (1), the estimated parameters are: $R_{max} = 1849.2$ RU, $R_{min} = 3.1$ RU, $EC_{50} = 3999.75$ ng/ml, $r = 1.0419$.
[c] Residue is the value of the experimental response minus predicted response.

3.2. Selection of MAb for bFGF quantitation using BIAcore™ 2000

The sensitivity, specificity, and dynamic range of the BIAcore™ immunoassay are largely determined by the immobilized antibody. An antibody with a higher affinity for analyte will give a higher response. To select the antibody with the highest binding affinity for bFGF, a set amount of MAb 4.2, FB-8, 11.1 or 48.1 was injected onto the immobilized bFGF sensor surface. The results are presented in Table 1. Monoclonal antibody 48.1, with the greatest BIAcore™ response, has the highest binding affinity for bFGF, followed by MAb 11.1, FB-8 and MAb 4.2. Therefore, MAb 48.1 was chosen as the immobilized ligand for bFGF quantitation.

3.3. Curve fitting for bFGF standard curve

A series of eight 2-fold dilutions from 1440 to 5.65 ng/ml of bFGF in HHBS was prepared and assayed; the resulting standard curve is shown as a log–log plot in Fig. 4. A four-parameter sigmoidal model was used to fit the standard curve. As shown in Table 2, at each bFGF level, the value predicted by the model is close to the exper-

Table 3
Evaluation of the limit of quantitation

No.	BIAcore™ response (RU) 5.65 ng/mL bFGF	Observed bFGF[a] (ng/ml)
1	4.9	6.8
2	4.1	5.0
3	3.5	3.6
4	4.0	4.7
5	4.3	5.4
6	4.4	5.6
Mean	4.2	5.2
S.D.	0.5	1.0
CV (%)	11.9	19.2

[a] Calculated from Eq. (2) based on the estimated parameters of a standard curve prepared on the day of analysis.

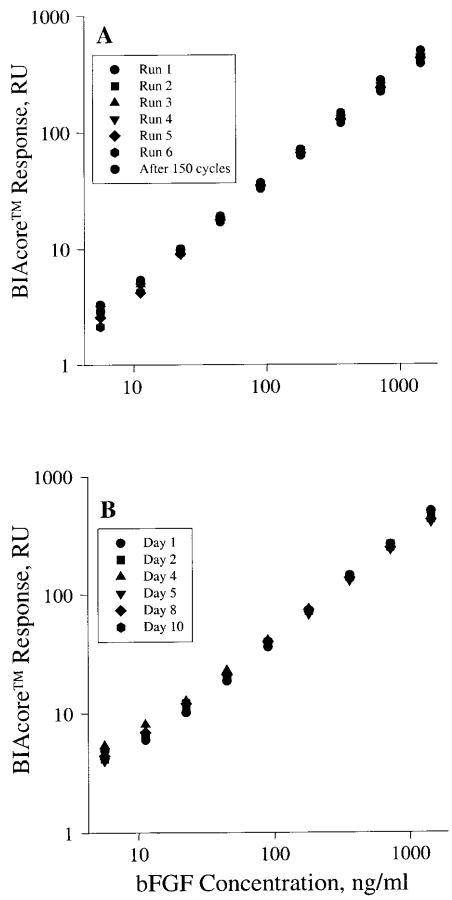

Fig. 5. The stability of the immobilized MAb 48.1 sensor surface as a function of the number of analysis cycles (A) and the storage time at room temperature (B).

imental data, which indicates the model fits well to the bFGF standard curve over the concentration range. Based upon Eq. (1), the observed concentration can be calculated from the following inverse equation:

$$C = EC_{50} \times [(R - R_{min})/(R_{max} - R)]^{1/r} \quad (2)$$

3.4. Determination of the limit of quantitation

To determine whether the lowest concentration on the standard curve can be measured with acceptable accuracy and variability, bFGF solution at 5.65 ng/ml was assayed six times. The results are presented in Table 3. The CV values are 11.9 and 19.2% for the BIAcore™ response and observed concentration, respectively. The mean value of observed concentration is 92% of the actual concentration. Therefore, the concentration of 5.65 ng/ml, as a lower limit of quantitation of this assay, can be measured with good accuracy and precision according to the criteria for validation of analytical methods [16].

3.5. Stability of immobilized MAb 48.1 surface

One important parameter for the validation of a biosensor-based immunoassay is the stability of the immobilized ligand. The immobilized ligand is repeatedly exposed to regeneration solutions, which are usually highly acidic or basic, or contain highly concentrated salts [13]. It is imperative to determine how many times the immobilized ligand can be used and regenerated without significant loss of assay sensitivity or accuracy. As shown in Fig. 5A, the consecutive assays of six standard curves generated almost superimposible curves, which suggests that the binding capacity of the immobilized MAb 48.1 does not change over the initial 60 cycles (54 samples plus 6 blanks not shown). After the sensor surface was used for 150 cycles, the responses of the standard curve still did not exhibit any appreciable change (Fig. 5A).

The stability of the immobilized MAb 48.1 as a function of time is shown in Fig. 5B. Compared with the standard curve run over the freshly prepared MAb 48.1 surface, no significant loss of binding capacity was observed after the chip had

Table 4
Reproducibility of the immobilized MAb 48.1 sensor surfaces and their binding capacity for bFGF

No.	1	2	3	4	5	mean	S.D.	CV, %
MAb level (RU)[a]	11770.2	12141.5	13921.4	12771.3	12612.7	12643.4	816.2	6.5
bFGF (ng/mL)	bFGF Binding Response (RU)							
5.65	4.4	4.4	5.0	3.7	4.9	4.5	0.5	11.8
11.3	6.8	6.6	7.5	5.9	7.6	6.9	0.7	10.3
22.5	11.9	11.8	12.1	9.8	10.1	11.1	1.1	9.6
45	21.7	20.8	21.2	18.8	18.7	20.2	1.4	7.0
90	41.2	38.9	39.0	35.3	36.1	38.1	2.4	6.2
180	77.6	62.8	73.1	68.6	71.9	70.8	5.5	7.8
360	145.5	126.5	147.3	140.2	143.7	140.6	8.3	5.9
720	281.8	252.8	277.7	270.3	262.9	269.1	11.6	4.3
1440	490.1	430.2	496.1	497.3	501.4	483.0	29.8	6.2

[a] The BIAcore™ response after immobilization of MAb 48.1 onto the sensor chip.

been kept in the BIAcore™ instrument at room temperature for 9 days. This result confirms that the immobilized MAb 48.1 is very stable and can be repeatedly used for more than 1 week without loss of binding capacity.

3.6. Reproducibility of immobilized MAb 48.1 surface

Because the amount of immobilized ligand directly affects the binding capacity of the sensor surface, the consistency of immobilization on different occasions determines the reproducibility of the assay between chips. Table 4 lists the results of immobilization of MAb 48.1 onto different sensor chips in five individual experiments. As shown, the mean immobilization response has a CV value of 6.5%, which indicates the immobilization procedure has good reproducibility. The responses at each level on the standard curve of bFGF over the corresponding sensor surface also show good precision, i.e. all the levels have CV values of below 15%. Therefore, these results confirm that by using the immobilization conditions described in Section 2.4, the responses from both the antibody immobilization and the bFGF analysis are highly reproducible.

3.7. Precision and accuracy of the assay

The CV values for six injections at each concentration level are all below 6% (Table 5). Each mean value calculated from Eq. (2) is above 85% of the actual value. These results confirm that the assay has good intraassay precision and accuracy. For interassay reproducibility assessment, the samples were assayed on six different days. The

Table 5
Determination of the accuracy and precision of intraassay and interassay

	bFGF Concentration (ng/ml)		
	20.0	100	1000
Intraassay (n = 6)			
Observed mean[a] (ng/ml)	17.7	90.1	959.2
S.D. (ng/ml)	1.0	5.4	8.7
CV (%)	5.7	5.9	0.9
Accuracy (%)	88.6	90.1	95.9
Interassay (n = 6)			
Observed mean[b] (ng/ml)	18.3	93.4	975.3
S.D. (ng/ml)	1.6	2.5	44.0
CV (%)	8.5	2.7	4.5
Accuracy (%)	91.6	93.4	97.5

[a] From six replicates.
[b] From six independent assays, each in triplicate.

Table 6
The effect of different matrices on binding of bFGF to the immobilized MAb 48.1 sensor surface (mean ± S.E., $n = 3$)

Matrix	BIAcore™ response (RU)		
	Blank	100 ng/ml bFGF	Net value[a]
HHBS	0.8 ± 0.1	38.7 ± 0.6	37.9 ± 0.6
0.1% Pluronic® F127 surfactant/HHBS	1.8 ± 0.6	39.5 ± 0.4	37.7 ± 0.4
0.1% Sodium carboxymethylcellulose/HHBS	1.7 ± 1.0	40.2 ± 1.5	38.5 ± 1.5
0.1% BSA/HHBS	2.7 ± 0.1	43.4 ± 0.9	40.7 ± 0.9
0.1% Rat serum/HHBS	4.0 ± 0.6	42.4 ± 0.2	38.4 ± 0.2

[a] Net value = Response (100 ng/ml bFGF) − Response (blank).

CV values at each level are all below 9% (Table 5). All mean accuracy values are above 90%. Therefore, this assay also has good interassay precision and accuracy.

3.8. Specificity of the assay

Samples in our development studies generally represent serum, cell culture medium, or other matrices containing polypeptides such as albumin or pharmaceutical excipients such as surfactants and suspending agents. These matrices usually complicate the analysis of bFGF using traditional analytical methods such as HPLC. To check whether these matrices affect the BIAcore™ responses of bFGF, 0.1% of each matrix was added to HHBS and then each resulting mixture was spiked with 100 ng/ml of bFGF. The BIAcore™ responses of the mixtures are listed in Table 6. Compared with HHBS alone, addition of these matrices to HHBS indeed gave small BIAcore™ responses in the absence of bFGF, among which BSA and rat serum had the more obvious effect. The results indicate that these matrices themselves have a weak binding affinity for the sensor surface. When 100 ng/ml of bFGF was added to each mixture, the BIAcore™ responses mainly were from bFGF because the net responses of the samples were very close to each other. This result indicates that addition of these matrices did not affect the binding of bFGF to the immobilized MAb 48.1. Therefore, these results have shown this assay has good specificity for bFGF and no significant interference from several types of matrices. These results further suggest this assay would be very useful in quantitating bFGF in samples from several kinds of studies such as bioassays, protein stability tests, and excipient compatibility evaluations.

4. Conclusions

In conclusion, a sensitive, accurate and efficient novel immunoassay for the quantitation of bFGF was successfully developed and validated using the BIAcore™ 2000 biosensor instrument. Over the stable immobilized MAb 48.1 sensor surface, this assay has a range of reliable response in HHBS of 5.65–1440 ng/ml bFGF. The validation results show this assay has good precision, accuracy, and specificity. The method described herein may allow for efficient quantitation of bFGF in several research and development studies.

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