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Simulation of perforated rectangular cantilever immunosensor for estimation of bacterial pathogens

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ABSTRACT

Micro fabricated and multilayered perforated cantilever beam immunosensor was modeled using CoventorWare for the estimation of bacterial antigens of *Bacillus Anthrax, Pseudomonas aeruginosa, Coryne Bacterium Diptheria and Treponema pallidum*. A rectangular cantilever beam with perforations was simulated with dimensions as length-200 µm, width-10 µm and thickness-0.5 µm. Each perforation is rectangular with length-10 µm, width-5 µm and thickness-0.5 µm. The theoretical and FEM simulations were carried out with five immunoglobulin antibodies, IgA, IgD, IgE, IgG and IgM for the estimation of bacterial antigens. The effect of perforation in cantilever beam and molecular size of antibody and antigen on the performance of the sensor has been studied. The cantilever beam without perforation showed a deflection of 1.8 e + 02 µm whereas the cantilever beam with perforation showed additional deflection of 1.9 e – 02 µm. With IgG, the difference between analytical and simulation values is positive and low especially with low molecular weight antigens *Pseudomonas aeruginosa and Treponema pallidum.* The low molecular weight IgG influences the antigen-antibody interaction more favourably. The simulated perforated rectangular cantilever beam with IgG antibody is a more promising model for the fabrication of a sensor for the estimation of highly motile *Pseudomonas aeruginosa and Treponema pallidum.*

Keywords: Modeling; Perforated Rectangular Cantilever Beam; Immunosensor; Immunoglobulin Antibodies; Estimation of Bacterial Pathogen Antigen

1. INTRODUCTION

Identification and quantification of bacterial pathogens are essential for the potential treatment of the diseases. However, it is difficult to treat for gram negative and positive bacteria due to inherent and acquired resistance to antimicrobial agents. Endospore-forming gram-positive bacteria produce a unique resting cell called an endospore. *Bacillus Anthrax* spore formers cause anthrax in domestic animals, which may be transmitted to humans. *Pseudomonas aeruginosa* pathogen is generated from gram-negative bacteria. It is the quintessential opportunistic pathogen of humans. It is a leading cause of hospital-acquired infections (nosocomial infections). It is difficult to eradicate due to its resistance to most antimicrobial agents. There is probably no tissue that cannot become infected by Pseudomonas if the host defenses are weakened. It is usually involved in soft tissue infections, urinary tract infections and pneumonia. The best known and most widely studied species is *Coryne Bacterium Diptheria*, gram-positive bacteria, the causal agent of Diptheria. The genus *corynebacterium* consists of a diverse group of bacteria including animal and plant pathogens, as well as saprophytes. Some coryne bacteria are part of the normal flora of humans, finding a suitable niche in virtually every anatomic site. One of the major pathogens of humans from spirochetes a phylogenetically distinct group of bacteria, is *Treponema pallidum*, the agent of syphilis, a sexually transmitted disease.

Infectious diseases are generally detected with immunohistochemistry (IHC), flow cytometry, immuno-electron microscopy, ELISA, western blotting, polymerase chain reaction (PCR) etc. These systems use an antibody-based method to detect a specific protein. One of the main drawbacks with IHC staining is difficulties in overcoming specific or non-specific background. Optimisation of fixation methods and times, pretreatment with blocking agents, incubating antibodies with high salt, and optimising post-antibody wash buffers and wash times influence the quality immunostaining. Flow cytometry is less effective for detecting extremely rare cell populations. Immuno-electron microscopy can be technically challenging, expensive and require rigorous optimisation. ELISA test does not identify the amount of antigen present in

the sample and is expensive and time consuming. Western blot test is technically demanding, expensive, subject to interpretation, presence or absence of bands, intensity of those bands. Major disadvantages of the PCR protocol include length of time needed (2-3 days). PCR tests also require more sophisticated equipment and greater expertise and hence expensive. Urine and blood tests takes a long time to analyze. The test should be carried out in the lab only. Errors may occur due to the carelessness of the technician.

Immunosensors are a type of biosensor, which uses antibodies or antigen as the biospecific sensing element, and are based on the ability of an antibody to form complexes with the corresponding antigen. The reaction between the antibody-antigen reactions is highly selective, and is analogous to a lock and key fit. The piezoelectric sensing method is thought to be one of the most sensitive analytical instruments developed to date, being capable of detecting antigens in the picogram range. This transduction method is relatively easy to use, cost effective, and offers direct label-free analysis and overcomes all the dis-advantages of the existing system. In addition, it is able to provide the option of several immunoassay formats for increased sensitivity and specificity.

Development of a simple and multifunctional transducer to detect two or more species by one cantilever at one time is a challenge. The cantilever deflection and output voltage can be influenced by various forces acting on the probe molecule such as molecular size of the probe and target molecules, DNA hybridization of probe molecule [1], nature of grafting of probe molecules on the surface whether it is ordered or is disordered [2], grafting density [3] and design and thickness of the cantilever beam. Therefore it is relevant to develop simulation methods and design rules that will enable the design of microcantilever-based biosensing systems. In this paper the effect of molecular size of both antibody and antigen on the deflection of microcantilever upon adsorption of probe molecules and binding of target molecules has been studied by modeling perforated rectangular cantilever with immunoglobulin for the detection of bacterial pathogens of *Bacillus Anthrax, Pseudomonas aeruginosa, Coryne Bacterium Diptheria and Treponema pallidum.*

2. MODELING AND ANALYSIS

2.1. Modeling of Perforated Rectangular Cantilever Beam

The design of the perforated rectangular cantilever beam was simulated using commercial CoventorWare-DESIGNER software. The first step involved in the modeling of the perforated rectangular cantilever beam starts with the Process Editor, where the properties of the materials for cantilever beam are applied. The antibodies IgG, IgM, IgD, IgA and IgE are added to the process editor with their properties like Young's modulus, density, weight etc.

Crystalline silicon (Young modulus 155.8 Gpa, Poisson coefficient 0.21 and density 2330 Kg/m^3) was used as the substrate with thickness, 5 μm in the present simulation. Boron phosphor silicate glass (BPSG) material was deposited for a thickness, 35 μm (selected from the Materials Database) as a temporary support for the cantilever beam using Stack Material option. For construction of the cantilever beam, certain region in the layer has been etched in the BPSG using the options Delete and Straight Cut. Deposition and selective etching of the silicon material was then carried out using Planar Fill option and Straight Cut options respectively. A layer of gold was deposited to give good attachment of the antibodies. Deposition and selective etching of the gold on the perforated cantilever was then carried out using Planar Fill option and Straight Cut options respectively. The sequence of modeling of the cantilever beam using the Process Editor is given in **Figure 1**. Once the cantilever beam was constructed the antibodies were coated in the beam surface.

To improve the sensitivity of the immunosensor to detect nanogram material, a simple way was to reduce the size of the beam. The final simulated perforated rectangular cantilever beam sensor has dimensions length-300 μm, width-100 μm and total thickness-40.5 μm. The thickness of the cantilever beam is 0.5 μm (**Figure 2**). Once the whole structure was built in the 2D the structure was exported to the MEMMECH analysis, where the boundaries were fixed and meshing was done. Finite Element Method was used for the analysis. The model of the mesh of cantilever beam is given in **Figure 2**. After meshing, the pressure was applied at the top surface of the cantilever equivalent 1 mole (molecular weight in gram) of antigen for the sensing. The pressure/stress equivalent 1 mole (molecular weight in gram) of antigen was calculated using the equation,

$$\text{Pressure (kg/cm}^2) = \frac{(\text{Weight of antigen} \times 1.66090210\,e^{-27})}{((L \times B) \times (e^{-04})^2)}$$

where L is length of the cantilever, and B is width of the cantilever. The pressure in Kg/cm^2 was converted into pressure in Mpa.

2.2. Detection of Antigens

Four different diseases viz, anthrax, nosocomial infections, diphtheria and syphilis and the corresponding bacteria were considered for the present study. In the present modeling static deflection mode was considered

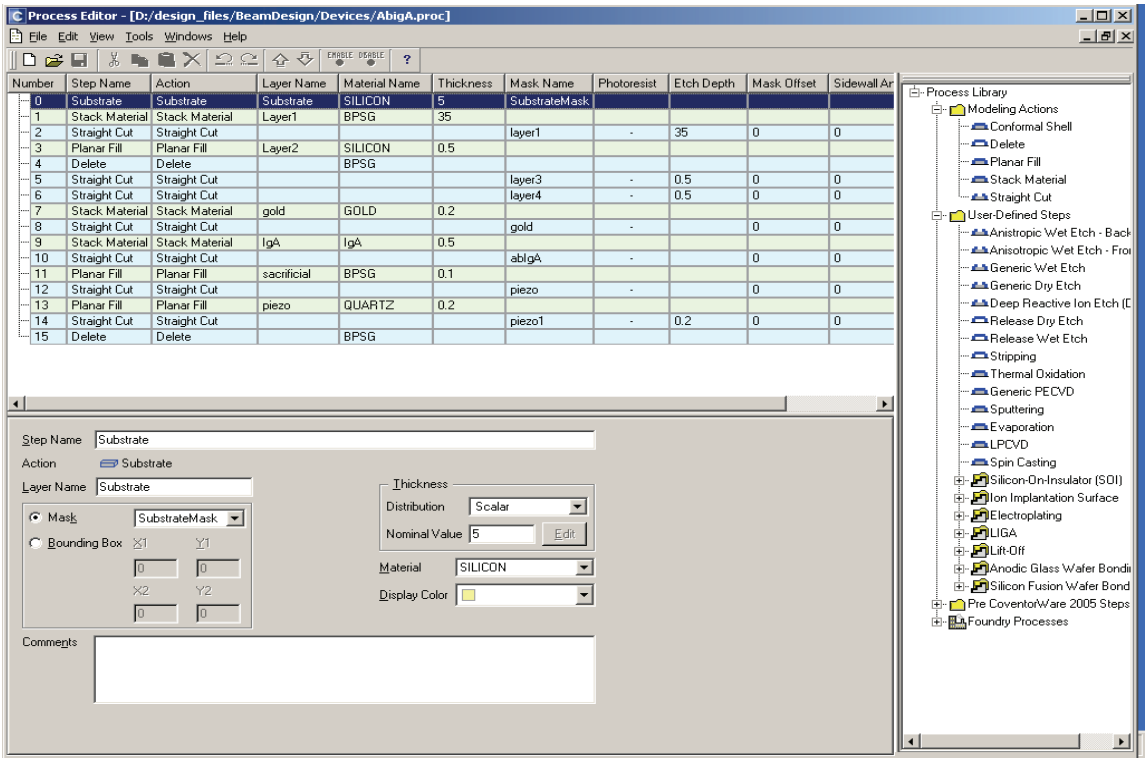

Figure 1. Sequence of modeling of cantilever using process editor of the coventorware.

for the detection. For static deflection, one can apply Stoney's formula, $R = \frac{Et^2}{6\Delta\sigma(1-\nu)'}$ where R is the cantilever's radius of curvature, ν is Poisson's ratio, E is the substrate's Young modulus, t is the thickness of the cantilever, and $\Delta\sigma$ is the differential surface stress. In the present modeling, uniaxial and one dimensional approximation was followed in which Poisson coefficient was not taken in to account. However, in order to get accurate results, simulation was carried out with a non zero value of Poisson coefficient. When analytes bind to only one side of the cantilever's surface, the cantilever bends up or down depending on the side to which the analytes bind. The deformation of the cantilever, which arises from variations in surface stress ($\Delta\sigma$), is measured from changes in the resistance of the piezoresistive material. The equivalent pressure corresponding to 1 mole of antigen was applied to the beam and the displacement, voltage and stress was noted. The deformation induces the current in the quartz piezo-patch. The output voltage at the upper surface of the piezo-patch is measured. The deformation of the beam depends on the applied pressure as well as on the geometry of the device, molecular size of the antibody and antigen-antibody interaction. According to the deflection of the beam and out put voltage, the intensity of the disease could be judged.

3. RESULTS AND DISCUSSION

The antibody and antigen reaction is an important protective mechanism in human being against invading foreign substances. The antibody and antigen reaction, together with phagocytosis, constitute the immune response (humoral immune response). Invading foreign substances are antigens while the antibodies (immunoglobulins), are specific proteins generated (or previously and present in blood, lymph or mucosal secretions) to react with a specific antigen. Gamma globulins are produced by B lymphocytes when antigens enter the body. Immunoglobulins (Ig) have been selected as substrate antibodies for the construction of cantilever. Immunoglobulins (Ig) have a basic four-chain monomeric structure consisting of two identical heavy chains and two identical light chains with interchain disulfide bonds. There are five heavy chain classes (M, D, G, E and A), four G subclasses (G1-4), and two A subclasses (A1,2). There are two light chain isotypes, K (kappa) and L (lamda).

The biological characteristics of human immunoglobulins are given in **Table 1**. IgA molecules have an average molecular weight of 250,000 daltons. IgA is found in body fluids such as tears, saliva, mucosa, and other bodily secretions. It provides a first line of defense against invading pathogens and allergens. IgD appears to act in

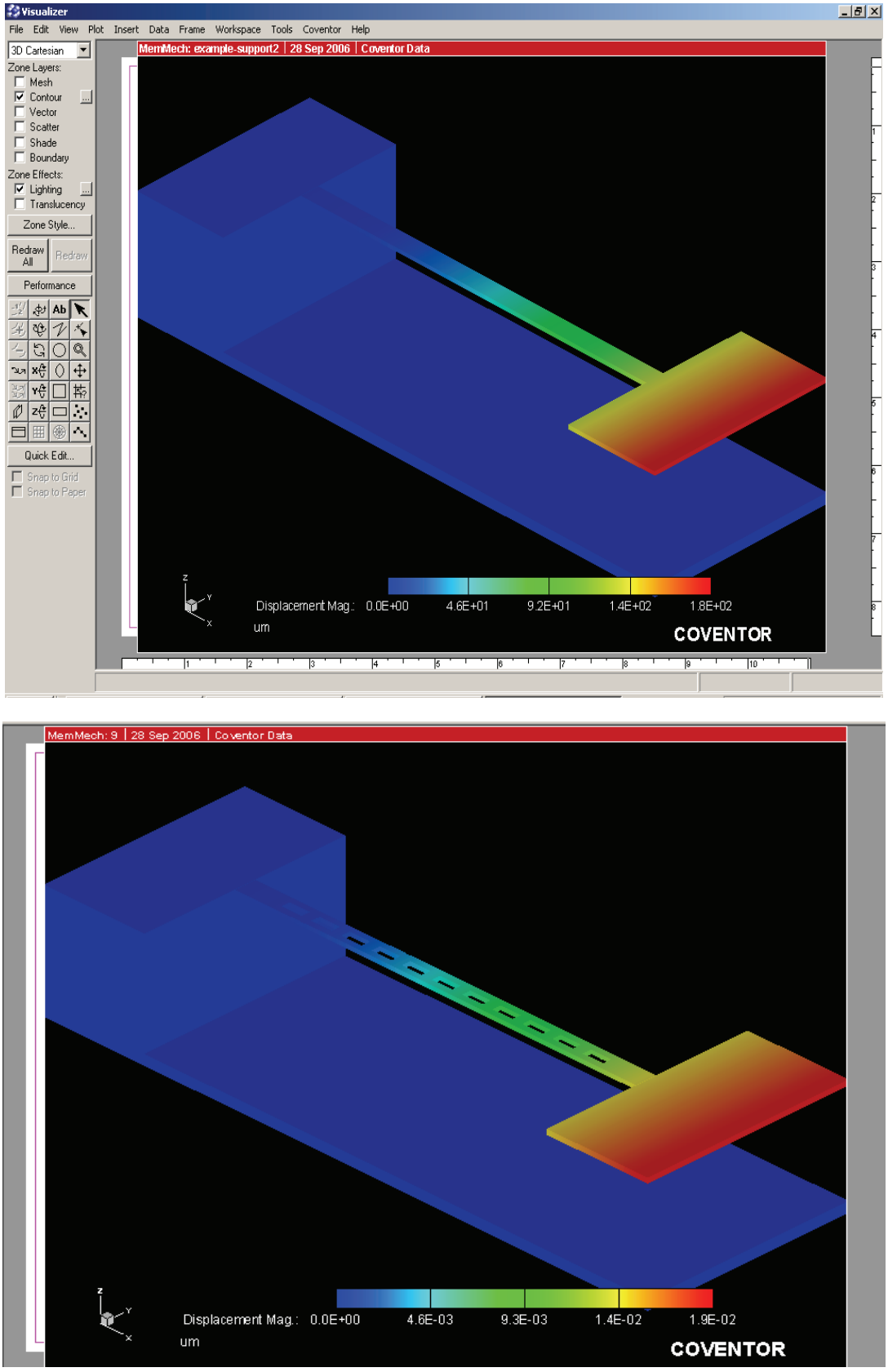

Figure 2. Displacement profile of simulated plain and perforated rectangular cantilever beams under applied pressure.

conjunction with B and T cells to help them in location of antigens. IgG is the most common type of antibody. It is the most common immunoglobulin against microbes. It acts by coating the microbe to hasten its removal by other immune system cells. It gives lifetime or long-standing immunity against infectious diseases. It is highly mobile, passing out of the blood stream and between cells, going from organs to the skin where it neutralizes surface bacteria and other invading microorganisms. IgE is responsible for allergic reactions. IgE acts by attaching to cells in the skin called mast cells and basophil cells. In the presence of environmental antigens, IgE releases histamines from the mast cells. The histamines cause the nasal inflammation. IgM is the largest among the immunoglobulins. IgM usually form clusters that are in the shape of a star. It is effective against larger microorganisms. Because of its large size (it combines 5 Y-shaped units), it remains in the bloodstream where it provides an early and diffuse protection against invading antigens. IgG is produced by the plasma cells. IgD and IgE are susceptible to denaturation by the treatment with heat and reducing agents under conditions that have little effect on other immunoglobulins.

The present selected bacteria, *Bacillus Anthrax* (1), *Pseudomonas aeruginosa* (2), *Coryne Bacterium Diptheria* (3), *and Treponema pallidum* (4), have different molecular weight and physical characteristics (**Table 2**). All immunoglobulins are in a Y-shape with differences in the upper branch of the Y. These structural differences of in each of the immunoglobulins enable the individual immunoglobulins to recognize an antigen. An antigen has on its surface a combining site that the immunoglobulin recognizes from the combining sites on the arms of its Y-shaped structure. In response to the antigen that has called it forth, the immunoglobulin wraps its two combining sites like a "lock" around the "key" of the antigen combining sites. The mode of action of the present immunoglobulin varies with different types of antigens. With its two-armed Y-shaped structure, the immunoglobulin can interact two antigens at the same time with each arm. Adsorption of biomolecules on a surface of a microcantilever generates surface stresses that cause the cantilever to deflect [4-6]. In the present studies, adsorption of various antigens on the perforated rectangular cantilever influence displacement, voltage and stress.

To improve the sensor performance and to enhance the signal transduction to detect nanogram level antigen, a novel sensor platform with perforated rectangular cantilever beam was modeled. By modeling and simulation, the high surface stress region on the cantilever was identified. Optimization of cantilever width, thickness and perforation was carried out with the software packages. Specific perforated cantilever designs were targeted to optimize stress localization at the base of the cantilever. The cantilever beam without perforation showed a deflection of 1.8e + 02µm whereas the cantilever beam with perforation showed additional deflection of 1.9e – 02µm (**Figure 2**). Therefore the cantilever beam with perforation was modeled. The layout of perforated can- tilever beam is given in **Figure 3**.

Table 1. Biological characteristic of human immunoglobulins.

Antigen species	Shape	Gram staining	Motility	Intra/Extracellular	Molecular Weight (Dalton)
Bacillus Anthrax Toxin (1)	Rods	Gram-positive	Nonmotile	Extracellular	86,000
Pseudomonas Aeruginosa (2)	Rods	Gram-negative	Motile	Extracellular	33,000
Coryne Bacterium Diptheria (3)	Small, slender, pleomorphic rods	Gram-positive (unevenly)	Nonmotile	Extracellular	60,000
Treponema Pallidum (4)	Long, slender, flexible, spiral- or corkscrew-shaped rods	Gram-negative, but stains poorly	Highly motile	Extracellular	34,000

Table 2. Characteristics of antigen species.

Characteristics	IgA	IgD	IgE	IgG	IgM
Molecular weight (Dalton)	250000 (150000-350000)	180000	190000	150000	900000
Carbohydrate (Approx %)	7	12	12	3	12
Sedimentation coefficient ($S_{20,w}$)	7 (9-15)	7	8	7	19
Biological survival (plasma T ½ days)	6	3	2	3	5
Serum concentration (mg per 100ml)	250	3	0.01	1100	100

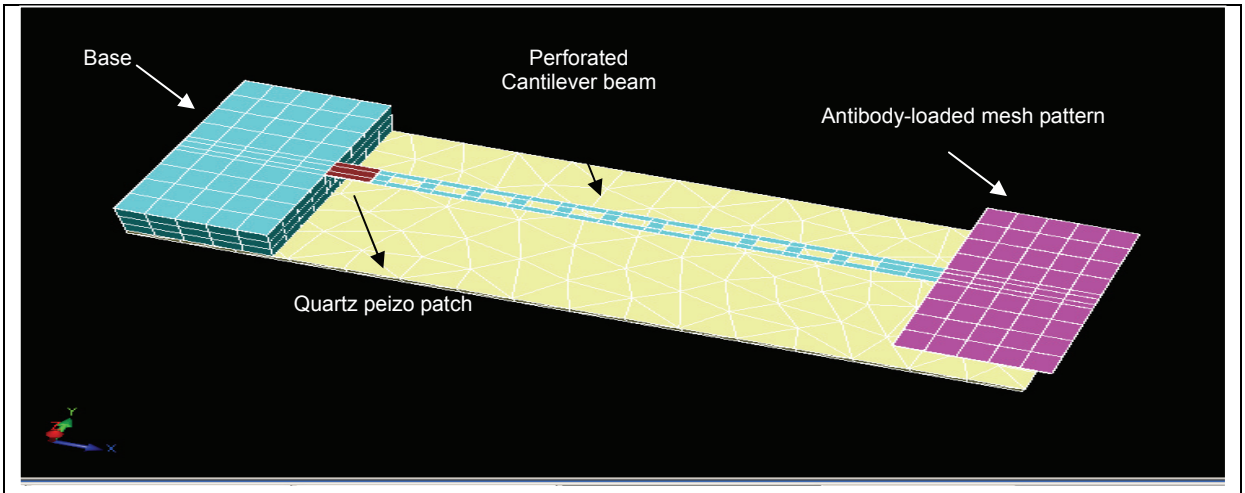

Figure 3. Layout of perforated cantilever beam.

The data on the interaction of antigen with IgA, IgD, IgE, IgG and IgM are given in **Table 3**. The analytical and simulation data reveal the variation of displacement, voltage and stress with the interaction of 1 mole of antigen with the antibody. With all toxins, the displacement, voltage and stress increases with the increase of molecular weight of the antigen. The variation of cantilever response with molecular weight of antigen is given in **Figure 4**. The difference between analytical and simulation values is attributed to the uniaxial and one dimensional approximation. Moreover the point of interaction of antigen and antibody in the length of the cantilever is also an influential factor. Only the part from the clamping edge to the force applying point produces a stress. Ramos *et al*. [7,8] analyzed the effect of bacterial adsorption onto cantilevers and claimed that the cantilevers' response depends on the stiffness of the sample as well as on its mass. The authors also reported that a stiffer cantilever is relatively insensitive to the mechanical properties of bioparticles but that its mass sensitivity is limited. A pliant cantilever usually has better mass sensitivity, but its response may be complex [9].

The force applying point is again influenced by the molecular weight of immobilised-antibody and antigen and concentration of binding sites. The variation of cantilever response with molecular weight of the all immobilised-antibodies is given in **Figure 5**. With a particular antigen the output voltage during simulation decreases with increase of molecular weight of the all immobilised-antibodies except with IgM (**Table 4, Figure 5**). However, stress decreases only up to the molecular weight of 180000. Afterwards the stress increases with increase of molecular weight of the immobilised-antibody (**Figure 5**). In the case of displacement, the displacement decreases only up to the molecular weight of 190000. Afterwards the displacement increases with increase of molecular weight of the immobilised-antibody (**Figure 5**). IgM having high molecular weight due to polymeric nature, the relationship does not hold good. With all other antibodies, the low molecular size influences the antigen-antibody interaction more effectively. Antibody, IgG having low molecular weight yields higher output voltage with *Bacillus Anthrax Toxin* which has higher molecular weight in comparison with all other antigens. Antibody, IgG yields lower output voltage with *Pseudomonas aeruginosa* which has lower molecular weight. While comparing the output voltage of all the antibodies, IgG yields higher out put voltage. IgG having low molecular weight may allow lesser strain on the cantilever beam in comparison with the high molecular weight immunoglobulin. Immunoglobulin having low molecular weight may favour molecular level changes in terms of physical and morphological nature of adsorbed molecules.

It has been reported that the physical and morphological nature of the probe molecules influence the deflection. Hagan and Chakraborty have investigated the effect of the structure of the adsorbed layer of probe molecules on the cantilever on the transport of targets on to the layer and subsequent organization of complementtary regions leading to nucleation and completion of hybridization [1]. The nature of grafting of probe molecules on the surface whether it is ordered or is disordered influence the deflection [2]. Disordered distributions, which are most likely in practice, lead to much larger

cantilever deflections. Molecular level self-assembly controls the response of the microdevice. The grafting density also influences the deflection [3]. At larger grafting density hybridization of probe molecule does not occur, but rather, the target chain can bridge across more than one probe molecule.

Table 3. Response of immunoglobulins to 1 mole of antigen.

Antigen	Analytical			Simulation			Difference between simulation and analytical		
	Displacement (nm)	Output voltage (V)($x10^{11}$)	Stress (Mpa) ($x10^{8}$)	Displacement (nm)	Output voltage (V)($x10^{11}$)	Stress (Mpa) ($x10^{8}$)	Displacement	Output voltage	Stress
IgA immunoglobulin									
I	0.318	13.30	5.7	0.063	5.06	5.8	-80.2	-61.5	1.8
II	0.150	5.49	2.3	0.026	2.09	2.5	-82.7	-61..9	8.7
III	0.265	9.97	4.2	0.047	3.80	4.5	-82.3	-61..9	7.1
IV	0.153	5.65	2.4	0.027	2.15	2.7	-82.4	-61..9	12.5
IgD immunoglobulin									
I	0.308	12.61	5.7	0.170	15.88	4.6	-44.8	25.9	-19.3
II	0.142	5.20	2.3	0.071	6.55	1.9	-50.0	26.0	-17.4
III	0.234	9.46	4.2	0.130	11.91	3.4	-44.4	25.9	-19.1
IV	0.145	5.36	2.4	0.073	6.75	2.0	-49.7	25.9	-16.7
IgE immunoglobulin									
I	0.310	12.94	5.7	0.051	5.83	5.8	-83.5	-55.2	1.8
II	0.146	5.34	2.3	0.021	2.40	2.4	-85.6	-55.1	4.3
III	0.240	9.71	4.2	0.038	4.37	4.4	-84.2	-55.0	4.8
IV	0.149	5.50	2.4	0.022	2.46	2.7	-85.2	-51.3	12.5
IgG immunoglobulin									
I	0.295	14.01	5.7	0.220	22.1	6.0	-25.4	57.7	17.1
II	0.092	5.64	2.3	0.093	9.11	2.5	1.1	61.5	8.7
III	0.221	10.25	4.2	0.170	16.56	4.5	-23.1	61.6	7.1
IV	0.093	5.81	2.4	0.096	9.39	2.7	3.2	61.6	12.5
IgM immunoglobulin									
I	0.029	12.30	5.7	0.077	6.93	5.8	165.5	-43.7	1.8
II	0.021	5.07	2.3	0.032	2.86	2.5	52.3	-43.6	8.7
III	0.030	9.22	4.2	0.057	5.20	4.7	90.0	-43.6	11.9
IV	0.021	5.23	2.4	0.033	2.95	2.8	57.1	-43.6	16.7

I: *Bacillus Anthrax Toxin*, II: *Pseudomonas Aeruginosa*, III: *Coryne Bacterium Diptheria*, IV: *Treponema Pallidum* Difference between simulation and analytical (%) = $100(\Delta z_{sim}-\Delta z_{anal})/-\Delta z_{anal}$

Table 4. Variation of output voltage with molecular weight of antibody.

Antigen	Output voltage with simulation (V) ($x10^{11}$)				
	IgA	**IgD**	**IgE**	**IgG**	**IgM**
Bacillus Anthrax Toxin (I)	5.06	15.88	5.83	22.1	6.93
Pseudomonas Aeruginosa (II)	2.09	6.55	2.40	9.11	2.86
Coryne Bacterium Diptheria (III)	3.80	11.91	4.37	16.56	5.20
Treponema Pallidum (IV)	2.15	6.75	2.46	9.39	2.95

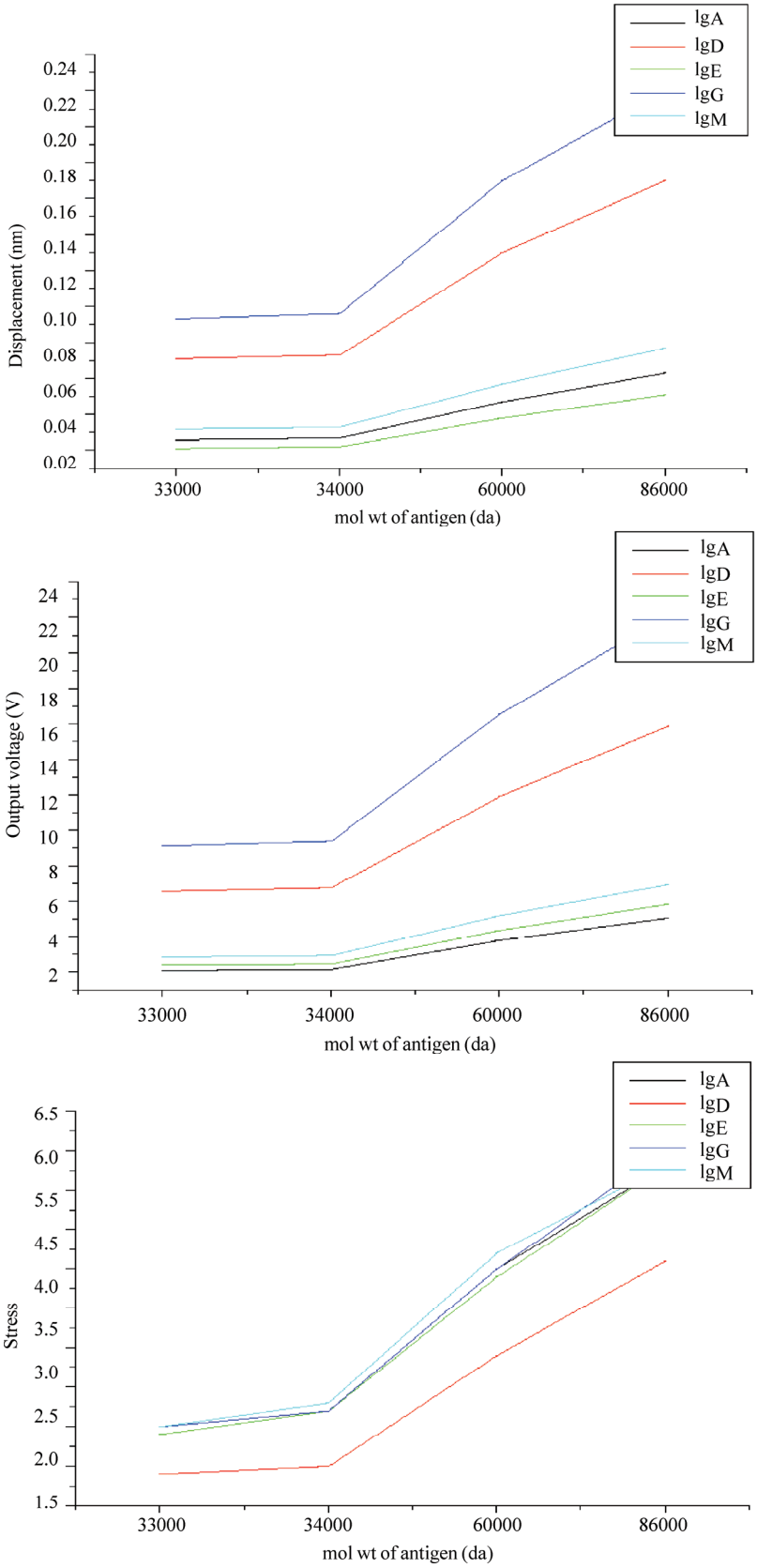

Figure 4. Variation of cantilever response with molecular weight of antigens.

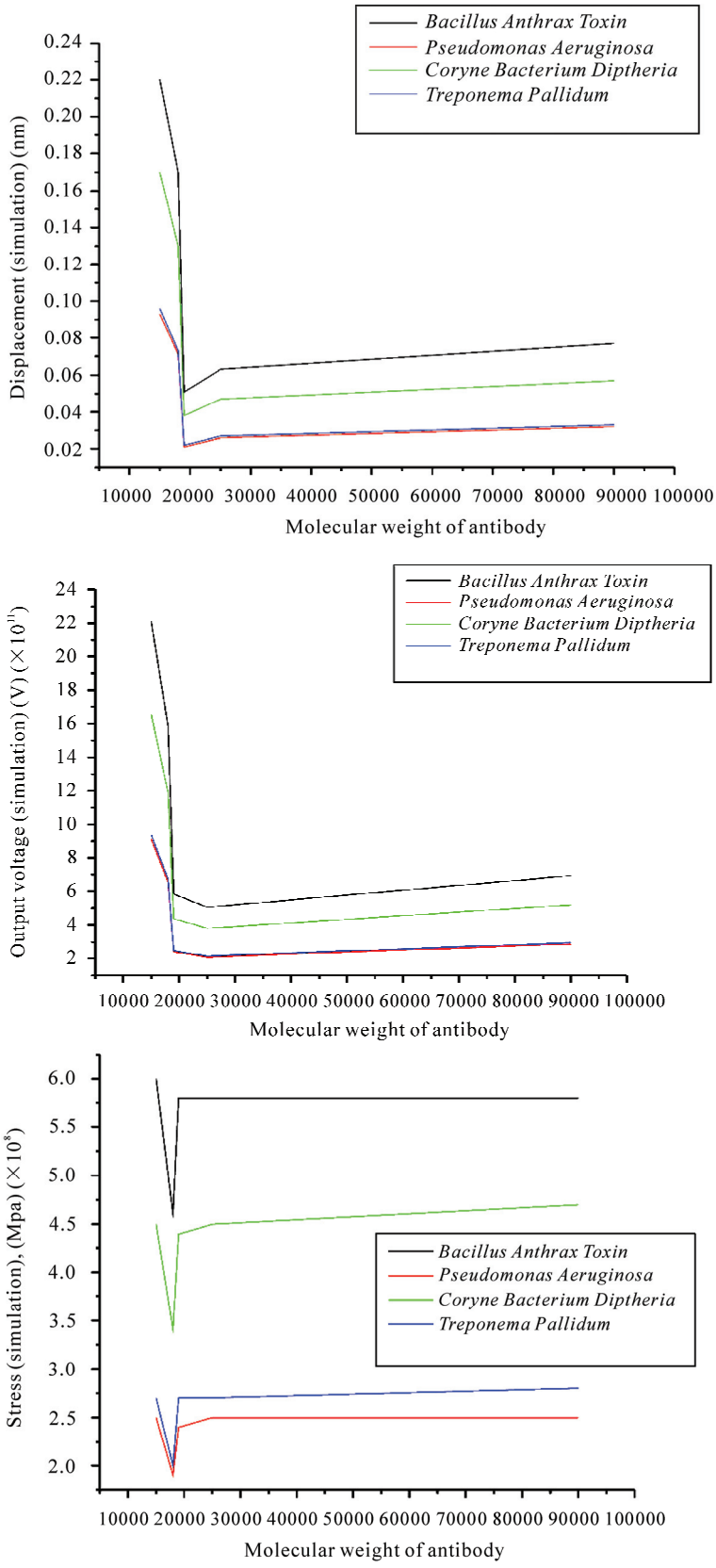

Figure 5. Variation of cantilever response with molecular weight of immobilized-antibody.

With IgG, the difference between analytical and simulation values is positive especially with low molecular weight and motile antigens *Pseudomonas aeruginosa and Treponema pallidum.* The signal transduction is influenced by the motility of the target molecules. It has been reported by Schnell and Turner [10] that if signal transduction occurs on time scales that are slow compared to the motility of the molecules and organelles that constitute the crowding elements, the effects of crowding are qualitatively the same as in a homogeneous 3-dimensional medium. In contrast, if signal transduction occurs on a time scale that is much faster than the time over which the crowding elements move, the effects of varying the extent of crowding are very different when reactions occur both in 2 and 3-dimensional space. For fast signaling, crowding agents attenuate signaling and never enhances signaling. In contrast, slow signaling cascades can be both enhanced and attenuated by crowding agents [11]. Therefore for the signal transduction in the case of low molecular weight and motile antigens *Pseudomonas aeruginosa and* Treponema *pallidum,* IgG is an appropriate probe molecule.

4. CONCLUSION

The theoretical and FEM simulations were carried out for plain and perforated rectangular cantilever with different immunoglobulin antibodies for the detection of bacterial antigens. The theoretical and computational approaches have identified important variables that affect design of the cantilever beam which are essential for the design of the prototype device. The cantilever beam with perforation showed an improved response. With IgG, the difference between analytical and simulation values is positive and low especially with low molecular weight antigens *Pseudomonas aeruginosa and Treponema pallidum.* The studies reveal that simulated perforated rectangular cantilever beam sensor (length-300 µm, width-100 µm and thickness-40.5 µm) with IgG antibody is a more promising system for the fabrication for the detection of highly motile *Pseudomonas aeruginosa and Treponema pallidum.* Monoclonal IgG antibody could be a more suitable probe for the detection of *Pseudomonas aeruginosa and Treponema pallidum* antigens.

5. ACKNOWLEDGEMENT

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