ASEE 2014 Zone I Conference, April 3-5, 2014, University of Bridgeport, Bridgeport, CT, USA.

Graphene Biosensor for Early Detection of Cancer Utilizing Antigen Concentrations in Saliva

Andrew Martin
Graduate Student
Dept. of Biomedical Engineering
University of Bridgeport
Bridgeport, CT USA
anmartin@my.bridgeport.edu

Prof. Prabir Patra
Associate Prof. of Biomedical Engineering
University of Bridgeport
Bridgeport, CT USA
ppatra@bridgeport.edu

***Abstract*— In this paper we present an idea for a graphene biosensor to detect cancer antigens in human bodily fluids. By electro spinning a solution of poly-vinyl alcohol, graphene and silk onto a printed circuit board then seeding it with cancer antibodies we plan to create a sensing platform that will detect the presence of cancer antigens in bodily fluids such as saliva. It will utilize antibody/antigen binding behavior to act as a resistor in a circuit providing impedance data that will allow us to detect and quantify cancer antigens in a saliva sample and thereby detect the presence of cancer in the human body.**

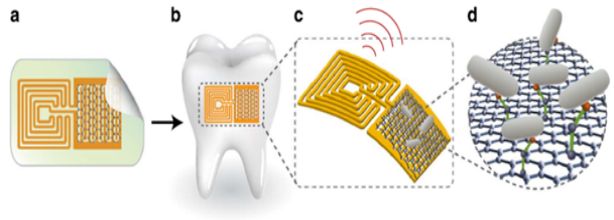
Keywords—Biosensor, Graphene, Silk, Electro spinning, Antigen, Antibody, Resistance, Lab-on-a-chip

I. INTRODUCTION

Cancer is one of the leading causes of death in the world today [29; 30; 31; 44; 53; 54; 55; 56; 62; 63]. It is the second most common cause of death in the United States. It is responsible for 1 out of every 4 deaths annually and was responsible for approximately 580,350 deaths, or more than 1,600 deaths per day, in 2013[1]. Early detection and monitoring of the advancement or remission of cancer is of the utmost importance in oncological medicine today and pprevious research has shown early detection of cancer increases the odds of patient survival[17; 46]. For high risk patients, such as those with a genetic pre-disposition to cancer and/or those who have already been diagnosed and treated for the disease, periodic monitoring for early detection of recurring cancer has been shown to be vital to long term survival rates in these types of patients[14; 52]. Currently, these patients must undergo continuous examinations and occasional biopsies by their health care professional to monitor the regression or progression of the disease. These procedures are expensive, time-consuming and invasive. We propose a new biosensor that uses a micrometer size circuit incorporating a polymer, graphene and silk bio-sensing platform seeded with cancer antibodies that creates a device for the detection of cancer antigens in human bodily fluids such as saliva. Previous research has shown the emerging importance of saliva as a diagnostic tool [26; 35; 37; 45]. Our device will allow quick, inexpensive, sensitive and accurate detection of cancer antigens in bodily fluids and hopefully provide unprecedented early detection of cancer in the human body**.**

The biosensor technology we propose to use as a starting point for our device already exists and was designed and built by Mannoor and company to detect bacteria in the saliva *(fig 1)* [42]. Their device consists of a micro scale resonant circuit overlaid on a graphene sheet foundation and attached to an inductive coil antenna. Peptide linker molecules, attached to the graphene attract and bond with bacteria in the saliva and the accumulation of the bacteria on the surface of the circuit act as a resistor and change its resistance. This change in resistance is detected wirelessly through the inductive coil antenna.

Figure 1



***(a)** Graphene film with resonant circuit and inductive coil antenna is printed onto bioresorbable silk substrate, **(b)** Transfer of the sensor onto tooth surface, **(c)** Magnified view of sensor and inductive coil antenna, **(d)** Binding of bacteria on graphene surface.* [42]

We have developed an idea for using this successful research by Mannoor and expanding on it. We plan to create a biosensor that utilizes antigen concentrations in a bodily fluid such as saliva to detect the presence of cancer in the human body by creating a sensor that uses natural silk and a synthetic polymer as a matrix. With graphene as a conductor, and cancer antigens as a resistor, we plan to create a sensing platform that can detect and quantify concentrations of cancer antigens in the saliva and hopefully diagnose the presence of cancer. We hypothesize that by analyzing resistance data provided by our sensor we will be able to determine the concentration of cancer antigens in the sample and ultimately the presence of cancer in the body.

Although we used Mannoor and company's biosensor as our inspiration, our concept is unique and novel and differs from theirs in several key aspects. Where Mannoor used sheet graphene and bacteria linker molecules overlayed onto a gold resonant circuit to detect bacteria, we plan to electro spin a polymer, silk, graphene solution onto a printed circuit board then seed it with cancer antibodies and use it to detect cancer antigens. The function will be similar but the construction will be very different. We will be creating a "fabric" of nanoscale polymer fibers impregnated with graphene and antibodies and applying it over a small circuit. The operational concept of our biosensor is that the circuit on the printed circuit board (PCB) will conduct an applied electric current through the sensor. The graphene will disperse electricity through the silk/polymer matrix and the antibodies will act as a resistor. When a bodily fluid sample such as saliva is placed onto the PCB, cancer antigens, if present, will be attracted to the immobilized antibodies attached to the silk/polymer surface and will spontaneously self-assemble and accumulate on the sensor surface. Since graphene is conductive and will deflect part of the electrical current path in the circuit through the silk, the binding of the antigens with the antibodies attached to the silk should affect the electrical properties of the circuit, namely its resistance. Our hypothesis is that any permanent deviation in the impedance of the circuit from a predetermined baseline after application of a saliva sample will be due to the binding of antigens to the antibodies on the silk/polymer matrix. This change can then be measured, quantified and analyzed to estimate the concentration of antigen in the sample and then in the body.

Our device has the potential of high sensitivity, high selectibility and significantly decreased response time in detecting cancer antigens in the body. With this sensor we hope be able to detect cancer at its earliest possible stages, long before tumor formation. This unprecedented early cancer detection should lead to quicker intervention with medical treatment and will hopefully result in more positive outcomes in cancer patients.

II. Research Methods

A. Literature Review

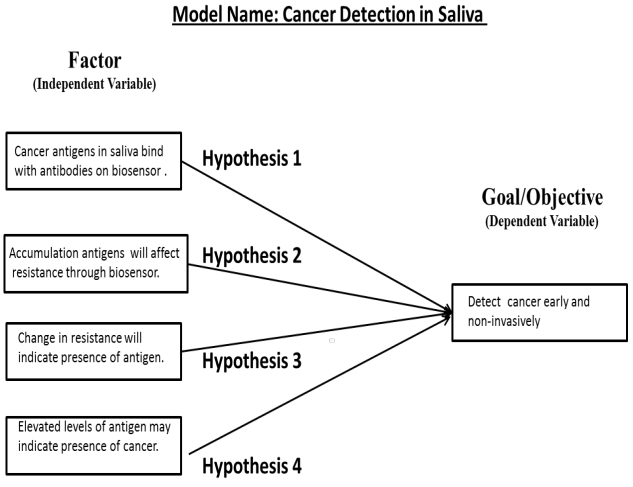
This paper utilizes the literature review centric research approach according to LePine & Wilcox in which a researcher reviews "existing theories and research", with the focus on combining the most important "previously established studies and concepts" and which "synthesizes recent advances and ideas into fresh new theory"[27]. I have done extensive research searching the scientific literature for pre-existing research on this subject and to check for feasibility of concept. I have searched several databases and checked hundreds of journal papers to date and I have found no previous literature utilizing this type of biosensor specifically to detect cancer antigens in the human saliva. I have also reviewed many articles on all the different technologies that are involved in the construction of this biosensor. I researched the literature for cancer antibody/antigen behavior [5; 7; 23], graphene as a biosensor platform [11; 42], gold resistor circuits and impedance [10; 42], and antibody/antigen binding effects on impedance [66]. The goal of my literature research was to determine if these technologies could be combined into one bio sensor to detect cancer. I found records of successful research on all technologies of biomedical engineering necessary to build this device and I found no reasons why these technologies cannot be combined to create our biosensor. Although our physical research to build a working proto-type has just begun, when complete, we are confident it will perform as expected and confirm the feasibility of the concept.

B. Scientific Causal Model

The model consists of a cause and effect tree diagram describing how our device will interact with the saliva at various stages in the function of the biosensor to detect the presence of cancer antigens (*fig 2)*. Recent research has shown the growing importance of saliva as general diagnostic tool[47] and particularly in the detection and diagnosis of cancer[4; 35; 49; 68]. The goal in our model (dependent variable) is to detect cancer early and non-invasively in the human body. "Detecting antigens in saliva" and "detecting cancer" are two very different things and we feel we need to exercise caution here. There has been plenty of research linking antigens in the body and the presence of cancer[3; 5; 6; 15; 40; 41] but the exact relationship between antigen levels in the saliva and the presence of cancer in the body still remains unclear and has led to misdiagnosis in the past[16; 58; 61]. Our ultimate goal is to detect cancer in the body at the earliest possible stage of the disease progression and our hypothesis is that future research will show a direct correlation between the concentration of cancer antigens in the saliva and the presence of cancer in the body.

The model also contains four factor boxes (independent variables). Each box is a hypothesis about a biosensor function in the presence or absence of cancer antigens in the saliva.

Figure 2



The hypothesis in factor #1 is that cancer antigens, if present in the patient saliva, will spontaneously bind with the antibodies attached to the silk/PVA platform incorporated in the biosensor. Antibody/antigen binding behavior has been

well studied and there is ample scientific research literature available on the subject. Prostate Specific Antigen (PSA) binding behavior is routinely used to predict the presence of prostate cancer[36]. Binding behavior of monoclonal antibody 19-9 has been used to identify pancreatic and gastrointestinal cancers [18, 26, 61]. Human lung cancer antigen binding has been used to detect lung cancer [28]. Research has been done with antigen binding of HER-2 for the diagnosis and treatment of breast cancer [33, 34]. And, mouse monoclonal antibodies have been used in binding assays to target cancer cell lines [35]. We feel confident that this resource of existing research will successfully guide us when the time comes for us to incorporate antibody/antigen binding behavior into our device.

The hypothesis in factor #2 is that bound cancer antibody/antigen structures will affect the internal resistance of the graphene circuit. Previous research has been done on the effects of antibody-antigen binding and impedance[64; 67]. Manoor and other researchers have successfully used bacteria as a resistor in their experimentation [19; 42; 51; 66]. The change in impedance in the circuit due to this binding activity will allow us to obtain quantifiable data to analyze how binding is affected by antigen concentration. Due to some biomarkers being gram-positive and some being gram-negative this change in impedance will be realized as a net increase or net decrease in resistance respectively. The change in resistance will then be detectable as a variation from the baseline resistance point of the circuit.

The hypothesis in factor #3 is that antibody/antigen binding and its effect on resistance in the biosensor will translate to a correlation with antigen concentrations in the saliva. The degree of variation from the base resistance point will have some correlation to antigen concentration in the saliva but the exact correlation is yet unknown and will required experimentation to quantify. We theorize that it will depend greatly on the specific antigen being targeted due to individual antigen binding behaviors. Manoor and company found there to be a linear relationship with their biosensor when describing the effect of bacteria *S.aureus* being bound on a graphene RLC circuit [42]. Other research has shown this to be a linear relationship also when dealing with similar research to ours involving antigens and resistance[66]. We expect to see a similar linear relationship between antigen concentration in saliva and circuit impedance also when we build our biosensor. A linear relationship is desirable because it will allow us to easily predict the results of future assays and estimate levels of antigens in saliva and their probability of being indicative of cancer in the body.

The hypothesis in factor #4 is that elevated levels of cancer antigens in the saliva will be indicative of cancer in the body. There is a great deal of previous research indicating a correlation between elevated cancer antigens in the body and the presence of cancer [20; 61] but as stated earlier we need to proceed with caution here because the exact link to antigen levels and cancer is not yet well understood and has led to misdiagnosis in the past[16; 36]. Over-expression of prostate stem cell antigen (PSCA) has been linked to pancreatic cancer[3]. Cancer testis antigen (CTA) has been linked to hepatocellular carcinoma [21] and antigens c-erbB-2 and HER2 have been seen in elevated levels in breast cancer patients [15; 34]. Several antigen biomarkers have been used to detect prostate cancer [61]. It is safe to say there is an abundance of literature showing that there are elevated levels of various cancer antigens present in patients with active cancers [20; 28; 32]. We are unsure at this point as to which cancer antigen we will use as a biomarker going forward in our research. Our selection of which antigen to pursue will be based on additional research findings to determine which one is the best candidate for reliable detectability in the saliva combined with proven correlation with a specific cancer. Since our biosensor should be adaptable to just about any antigen, our first choice of target antigen is simply to prove feasibility of our concept. Future research will more than likely be done to modify our device to detect many different antigens, maybe even multiple antigens on one device creating a "lab-on-a-chip" device that will be able to screen for multiple cancers with one salivary test. Our basic assumption going forward in this research is that we will find correlations between elevated cancer antigens and specific types of cancer for use in our research.

C. Experimental Research

The goal of our experimental research is build a working prototype of the biosensor described above. It is a device that will utilize the effect antibody/antigen binding has on the impedance of a circuit to determine antigen concentration in the saliva or other bodily fluid. Our method will consist of electro-spinning a solution that contains poly-vinyl alcohol, silk, graphene onto a printed circuit board, creating a "fabric" that covers the circuit. Then we will seed that fabric with antibodies to attract a specific antigen. Our experimental research method consists of several steps to design, build and test this device but first, it is important to list the components we used and the reasons why we chose them.

1) Components

We chose **poly-vinyl alcohol (PVA)** for our electro spinning polymer for several reasons. It is water soluble and has been widely used in previous electro spinning research including electro spinning involving graphene [18; 22; 39; 48; 60] and has been used in similar research to ours involving the anchoring of antibodies[12; 65]. It has also been successfully used in electro-spinning research by UB's own Ashish Aphale and Prof. Prabir Patra utilizing a electro-spinning machine of their own design and construction[2]. Its familiarity, desired properties, ease of accessibility and the use of our own custom spinning machine made this the obvious choice for our synthetic polymer base.

The **silk** we chose to use in our research is Sericin Bombyx mori purchased from Sigma-Aldrich Inc, Allentown PA. It is available in a processed powdered form, having been already boiled and degummed. This option saved us valuable lab time in preparing the silk for use in solution. Bombyx mori silk is a widely used protein polymer for biomedical applications. It has remarkable mechanical properties, is bio-compatible and has been previously used to immobilize growth factors [50]. It has been used in similar research to ours as a substrate to immobilize enzymes [9] and has been previously used in

electro spinning applications [18; 69]. It is also readily available and is relatively inexpensive.

Graphene is used for this device due to its unique physical properties. It is biocompatible, incredibly strong and highly conductive due to its nanoscale structure. Single atom thick graphene possesses amazing electrical, mechanical and analyte sensing properties which were essential in our biosensor design. It has already been successfully used in many micro and nanoscale electronic devices [8; 13; 38]. It was used by Manoor and company in their device[42] and has been used in other similar biosensor devices [10]. Graphene is highly conductive[59] and has been used in previous research similar to ours to bind antibodies and antigens[25]. It has also been shown to provide excellent intersurface adhesion to different substrates due to the high van der Waal forces of its nanoscale structure [33]. This adhesion property may prove important in the evolution of this sensor into an "*in-vivo*" device. All of these characteristics make graphene a perfect choice for a conductive platform for our biosensor.

We have not decided on which **cancer antibody** to use in our research but, as stated earlier, previous research has shown there are several cancer antigens that can be targeted in human saliva that present in elevated levels in cancer patients[26; 35; 43; 57; 68]. Other biomarkers are present in other bodily fluids also. HER2 is present in most patients with breast cancer[15]. Prostate Specific Antigen (PSA) is a biomarker for prostate cancer[16]. Antigen 19-9 presents in patients with pancreatic and gastrointestinal cancers[24]. We feel it is safe to assume that previously published cancer antibody/antigen binding behavior research data will be able to be adapted and applied in our future research involving this biosensor.

2) Experimental Methods

We devised several steps to build and test this device. First, we needed to create our polyvinyl alcohol solution for electro-spinning. We did this by creating a 6% v/v solution of PVA and buffered H2O. This ratio has been used in previous research here at UB for electro spinning purposes and based on previous results we felt this concentration will work well for our purposes. We separated several 1ml samples from this solution to be used to create different concentrations of final graphene and silk solutions. We took small quantity of this solution and applied it to a glass slide and set it aside to dry. This was to be used later as a control when testing different solutions for their conductive properties.

Next we added graphene to two of the 1ml 6%PVA/H2O samples we previously made. We created two solutions of 2.5% w/v and 5.0% w/v. Again, we took small samples from these solutions and applied them on glass slides and set them aside to dry for later conductivity testing.

We then created the silk solution. To be useful in our research the silk must be prepared for electro spinning. The fibroin proteins in natural silk must be dissolved in solution for use in regenerative silk protein fiber experimentation. This is accomplished by dissolving the degummed silk in a lithium bromide (LiBr) solution. We followed the preparation instructions provided by Chen, et al in their paper on silk solutions[9] and created a 9.3M LiBr/buffered water solution to be used in our experiments. This is also the concentration used by Mannoor in preparing their silk[42]. Powdered Bombyx mori silk was then added in a 5% w/v ratio to the LiBr/H2O solution. The finished solution was then thoroughly mixed on a magnetic mixer for at least 30 minutes to ensure the dissolving of the silk fibers. Again, we created glass slides from this solution to be used later as a control in our graphene conductivity testing. We then added this solution to our previous 6%PVA/graphene solutions (both 2.5% and 5.0%) in a 1:1 ratio. We once again took samples from these solutions and created glass slides. These were our primary samples for graphene/PVA/silk conductivity testing.

Special Note: It was at this point we noticed a problem with our samples. The idea was to smear samples of each solution onto glass slides, let them air dry and then test each one for conductivity. After plating numerous slides we found that some did not air dry when left out at room temperature, even after one week. We tried heating some of these slides to dry them. When exposed to 105 ° C heat for approx. 30 minutes the samples appeared to dry but to our surprise, shortly after removal from the heat, the samples would return to a gel state. After some additional experimentation we found it to be the lithium bromide and water solution that would not precipitate. According to the instructions by Chen, et al, that we followed to create the 9.3M LiBr/H2O solution, it should air dry at room temperature (20ºC and 50% humidity) in 2–3 days[9]. Ours did not. At the time of this writing we are still unclear as to why this is occurring and are working to figure it out.

We then tested each slide for conductivity. We did this by attaching electrodes to either end of the sample. We then connected a constant 10V DC source to the electrodes, passing a current through the samples *(fig 3)*. We then measured the voltage potential in the sample on each slide. The conductivity results showed that the PVA/graphene/silk solution is indeed conductive *(table 1)*. It is clear that the samples that contained the graphene were much more conductive than the control slides. Although two of the control slides had dried into a solid and the test samples did not, we don't feel this has affected our data. Control slide 3 remained a gel and in comparing that with test slides 1 and 2 it is clear that test slides with the graphene are much more conductive than control slide.

Table 1

Control 1	Control 2	Control 3	Test 1	Test 2
Plain glass slide	6% PVA (SOLID)	6% PVA 5% Silk (GEL)	6% PVA 5% Silk 2.5% Graphene (GEL)	6% PVA 5% Silk 5.0% Graphene (GEL)
3.2mV	6.3mV	1.2V	3.6V	4.7V

Figure 3

This concludes the extent of our experimental progress so far. We believe we have produced a conductive polymer/graphene solution that can be electro spun and then seeded with antibodies. Our future ongoing research will involve further conductivity testing on our control sample slides including developing methods to quantify resistance though the samples. We will be electro spinning our PVA/silk/graphene solution onto a simple printed circuit board. We will also be selecting a cancer antibody and seeding it into the electro spun polymer/graphene fabric. Finally we will apply saliva samples containing the targeted antigen to our newly constructed biosensor and then test them for changes in resistance. Hopefully we will see a correlation between antigen accumulation and resistance.

III. Sensitivity and Selectibility

Previous research by Mannoor with their bacteria biosensor which is similar to ours has shown that micro-scale graphene circuits are able to detect minute changes in resistance due to accumulated biomarkers on its surface [42]. The results of experiments by Mannoor and company show sensitivity and selectibility of their sensor on targeted bacteria and its effects on circuit impedance and are relevant to our research.

For sensitivity testing they prepared their graphene/micro circuits with bonding peptides to attract *S.aureus* bacteria. They soaked the sensor in a 1 µL solution containing approximately 100 *S.aureus* bacteria cells for 400 seconds. They then thoroughly rinsed the circuit with DI water to remove any bacteria not bonded and loosely attached to the surface. They noted a dramatic change in resistance over time even after rinsing, indicating permanent bacteria binding with peptides on the biosensor surface.

Mannoor conducted additional assays to determine the lower limit of detectability with the goal of detecting a single bacterium. Biosensors were prepared with binding peptide linkers for *E.coli.* bacteria. A single *E.coli* bacterium was then introduced to the sensor surface. Their results showed a consistent and permanent change in resistance over time due to the binding of the single bacterium to a peptide linker on the sensor. Assays like this were also done with various concentrations of *S.aureus* in solution.

Two additional assays were prepared to determine selectivity. They prepared an assay where a non-functionalized sensor with incomplete peptide bonding linkers was exposed to *S.aureus* in solution. They also used a fully functionalized sensor exposed to solution with non-target erythrocytes. In both assays, there was a temporary shift in resistance but once the sensor is rinsed with DI water, resistance goes back to close to control levels indicating permanent binding of non-target biomarkers did not take place.

If we can expect results similar to that obtained by Manoor our biosensor should be able to detect minute increases in antigen concentrations in the saliva and be highly selective against unwanted substances affecting resistance.

IV. Conclusion

Our device could revolutionize early cancer detection. The proven technology involved along with high expected sensitivity will result in a biosensor that is cheap and accurate. "Off the shelf" technology will allow economy and scalability of manufacture and should result in a very low per unit cost. If our device proves to have comparable sensitivity seen by Mannoor and company we may be able to detect antigen concentrations as small as 100 antigens per µL of saliva. This kind of sensitivity could provide early cancer detection at levels never seen before. We should be able to detect rises in antigen concentrations in the body almost in real time and certainly well before any tumor growth. Our device was originally conceived for use in high risk patients who need monitoring over an extended period of time but, this device may be able to be used as part of a routine check-up where a saliva sample is tested for cancers in the doctor's office. Other possibilities may be an in-vivo device where a small biosensor patch is placed on the patients tongue for 5-10 minutes then checked for cancer antigen concentrations. This idea may also be adaptable to a "lab-on-a-chip" concept where a single patch has the ability to detect multiple antigens in a single saliva sample. One patch could detect many different types of cancer. This could lead to a single comprehensive test for the screening of all cancers in the body. We envision a single, in-office device to screen for all cancers at all levels in the human body. That may be possible in the future with the evolution of this cancer antigen detection device.

V. Acknowledgements

I would like to acknowledge and thank my good friends Dleer Sabir and Faris Sahhaf, both engineering graduate students at University of Bridgeport, for developing the original idea for this paper with me in 2012.. I would also like to acknowledge Prof. Christian Bach, University of Bridgeport, School of Biomedical Engineering for his assistance in writing this paper and for teaching me how to do proper research. Special thanks to Akinwumi Akinkunmi, my present lab and research partner and Prof. Prabir Patra, Associate Dean, University of Bridgeport, School of Biomedical Engineering, who is my advisor on this research.

REFERENCES

[1] American Cancer Society Cancer Facts and Figures (2013). from http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-036845.pdf

[2] Aphale, e. a. (2012). A new collector design technique for alignment of polyvinyl alcohol nanofibers. *ASEE-NE.ORG, sp_3122.pdf*.

[3] Argani, P., Rosty, C., Reiter, R. E., Wilentz, R. E., Murugesan, S. R., Leach, S. D., . . . Jaffee, E. M. (2001). Discovery of New Markers of Cancer through Serial Analysis of Gene Expression Prostate Stem Cell Antigen Is Overexpressed in Pancreatic Adenocarcinoma. *Cancer research, 61*(11), 4320-4324.

[4] Bigler, L. R., Streckfus, C. F., Copeland, L., Burns, R., Dai, X., Kuhn, M., . . . Bigler, S. A. (2002). The potential use of saliva to detect recurrence of disease in women with breast carcinoma. *Journal of oral pathology & medicine, 31*(7), 421-431.

[5] Bostrom, J., Yu, S.-F., Kan, D., Appleton, B. A., Lee, C. V., Billeci, K., . . . Wiesmann, C. (2009). Variants of the antibody herceptin that interact with HER2 and VEGF at the antigen binding site. *Science, 323*(5921), 1610-1614.

[6] Brockhaus, M., Wysocka, M., Magnani, J. L., Steplewski, Z., Koprowski, H., & Ginsburg, V. (1985). Normal Salivary Mucin Contains the Gastrointestinal Cancer‐Associated Antigen Detected by Monoclonal Antibody 19–9 in the Serum Mucin of Patients1. *Vox sanguinis, 48*(1), 34-38.

[7] Carter, P., Presta, L., Gorman, C. M., Ridgway, J., Henner, D., Wong, W., . . . Shepard, H. M. (1992). Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proceedings of the National Academy of Sciences, 89*(10), 4285-4289.

[8] Chen, C., Rosenblatt, S., Bolotin, K. I., Kalb, W., Kim, P., Kymissis, I., . . . Hone, J. (2009). Performance of monolayer graphene nanomechanical resonators with electrical readout. *Nature nanotechnology, 4*(12), 861-867.

[9] Chen, X., Knight, D. P., Shao, Z., & Vollrath, F. (2001). Regenerated< i> Bombyx</i> silk solutions studied with rheometry and FTIR. *Polymer, 42*(25), 09969-09974.

[10] Chien, J., Cheng, Y., Wang, P., Yang, C., & Chen, P. (2006). A radio frequency biosensor with gold nanoparticle probes. *Biosensors & bioelectronics*.

[11] Choi, B. G., Park, H., Park, T. J., Yang, M. H., Kim, J. S., Jang, S.-Y., . . . Hong, W. H. (2010). Solution chemistry of self-assembled graphene nanohybrids for high-performance flexible biosensors. *ACS nano, 4*(5), 2910-2918.

[12] Choi, J. S., Lee, S. J., Christ, G. J., Atala, A., & Yoo, J. J. (2008). The influence of electrospun aligned poly (ε-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. *Biomaterials, 29*(19), 2899-2906.

[13] Choi, W., Lahiri, I., Seelaboyina, R., & Kang, Y. S. (2010). Synthesis of graphene and its applications: a review. *Critical Reviews in Solid State and Materials Sciences, 35*(1), 52-71.

[14] Cote, R. J., Rosen, P., Lesser, M., Old, L., & Osborne, M. (1991). Prediction of early relapse in patients with operable breast cancer by detection of occult bone marrow micrometastases. *Journal of Clinical Oncology, 9*(10), 1749-1756.

[15] Dowsett, M., Cooke, T., Ellis, I., Gullick, W. J., Gusterson, B., Mallon, E., & Walker, R. (2000). Assessment of HER2 status in breast cancer: why, when and how? *Eur J Cancer, 36*(2), 170-176. doi: http://dx.doi.org/10.1016/S0959-8049(99)00264-6

[16] Elgamal, A.-A. A., Ectors, N. L., Sunardhi-Widyaputra, S., Van Poppel, H. P., Van Damme, B. J., & Baert, L. V. (1996). Detection of prostate specific antigen in pancreas and salivary glands: a potential impact on prostate cancer overestimation. *The Journal of urology, 156*(2), 464-468.

[17] Etzioni, R., Urban, N., Ramsey, S., McIntosh, M., Schwartz, S., Reid, B., . . . Hartwell, L. (2003). The case for early detection. *Nature Reviews Cancer, 3*(4), 243-252.

[18] Gandhi, M., Yang, H., Shor, L., & Ko, F. (2009). Post-spinning modification of electrospun nanofiber nanocomposite from< i> Bombyx mori</i> silk and carbon nanotubes. *Polymer, 50*(8), 1918-1924.

[19] Geng, P., Zhang, X., Meng, W., Wang, Q., Zhang, W., Jin, L., . . . Wu, Z. (2008). Self-assembled monolayers-based immunosensor for detection of< i> Escherichia coli</i> using electrochemical impedance spectroscopy. *Electrochimica Acta, 53*(14), 4663-4668.

[20] Gohring, J. T., Dale, P. S., & Fan, X. (2010). *Detection of HER2 breast cancer biomarker using the optofluidic ring resonator biosensor*.

[21] Grizzi, F., Franceschini, B., Hamrick, C., Frezza, E. E., Cobos, E., & Chiriva-Internati, M. (2007). Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. *J Transl Med, 5*(3).

[22] Hai-Yan, K., & Ji-Huan, H. (2012). SUPERTHIN COMBINED PVA-GRAPHENE FILM. *Thermal Science, 16*(5).

[23] Hansson, G., Karlsson, K., Larson, G., McKibbin, J., Blaszczyk, M., Herlyn, M., . . . Koprowski, H. (1983). Mouse monoclonal antibodies against human cancer cell lines with specificities for blood group and related antigens. Characterization by antibody binding to glycosphingolipids in a chromatogram binding assay. *Journal of Biological Chemistry, 258*(7), 4091-4097.

[24] Hansson, G., & Zopf, D. (1985). Biosynthesis of the cancer-associated sialyl-Lea antigen. *Journal of Biological Chemistry, 260*(16), 9388-9392.

[25] Haque, A.-M. J., Park, H., Sung, D., Jon, S., Choi, S.-Y., & Kim, K. (2012). An electrochemically reduced graphene oxide-based electrochemical immunosensing platform for ultrasensitive antigen detection. *Analytical chemistry, 84*(4), 1871-1878.

[26] Hu, S., Arellano, M., Boontheung, P., Wang, J., Zhou, H., Jiang, J., . . . Wong, D. T. (2008). Salivary proteomics for oral cancer biomarker discovery. *Clinical cancer research, 14*(19), 6246-6252.

[27] INSIGHT, D. N. T. (2010). EDITORS'COMMENTS: DEVELOPING NOVEL THEORETICAL INSIGHT FROM REVIEWS OF EXISTING THEORY AND RESEARCH. *Academy of Management Review, 35*(4), 506-509.

[28] Itzkowitz, S. H., Bloom, E. J., Kokal, W. A., Modin, G., Hakomori, S. I., & Kim, Y. S. (1990). Sialosyl‐Tn. A novel mucin antigen associated with prognosis in colorectal cancer patients. *Cancer, 66*(9), 1960-1966.

[29] Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA Cancer J Clin, 61*(2), 69-90.

[30] Jemal, A., Siegel, R., Xu, J., & Ward, E. (2010). Cancer statistics, 2010. *CA Cancer J Clin, 60*(5), 277-300.

[31] Jemal, A., Ward, E., Hao, Y., & Thun, M. (2005). Trends in the leading causes of death in the United States, 1970-2002. *JAMA: the journal of the American Medical Association, 294*(10), 1255-1259.

[32] Kannagi, R., Fukushi, Y., Tachikawa, T., Noda, A., Shin, S., Shigeta, K., . . . Hakomori, S.-i. (1986). Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing fucosyl or sialyl-fucosyl type 2 chain polylactosamine. *Cancer research, 46*(5), 2619-2626.

[33] Koenig, S. P., Boddeti, N. G., Dunn, M. L., & Bunch, J. S. (2011). Ultrastrong adhesion of graphene membranes. *Nature nanotechnology, 6*(9), 543-546.

[34] Leitzel, K., Teramoto, Y., Konrad, K., Chinchilli, V. M., Volas, G., Grossberg, H., . . . Lipton, A. (1995). Elevated serum c-erbB-2 antigen levels and decreased response to hormone therapy of breast cancer. *Journal of Clinical Oncology, 13*(5), 1129-1135.

[35] Li, Y., John, M. A. S., Zhou, X., Kim, Y., Sinha, U., Jordan, R. C., . . . Park, N.-H. (2004). Salivary transcriptome diagnostics for oral cancer detection. *Clinical cancer research, 10*(24), 8442-8450.

[36] Lilja, H. (2003). Biology of prostate-specific antigen. *Urology, 62*(5), 27-33.

[37] Liu, J., & Duan, Y. (2012). Saliva: A potential media for disease diagnostics and monitoring. *Oral Oncology, 48*(7), 569-577. doi: http://dx.doi.org/10.1016/j.oraloncology.2012.01.021

[38] Liu, Y., Yu, D., Zeng, C., Miao, Z., & Dai, L. (2010). Biocompatible graphene oxide-based glucose biosensors. *Langmuir, 26*(9), 6158-6160.

[39] Lu, B., Li, T., Zhao, H., Li, X., Gao, C., Zhang, S., & Xie, E. (2012). Graphene-based composite materials beneficial to wound healing. *Nanoscale, 4*(9), 2978-2982.

[40] Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H., & Ginsburg, V. (1982). A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *Journal of Biological Chemistry, 257*(23), 14365-14369.

[41] Magnani, J. L., Steplewski, Z., Koprowski, H., & Ginsburg, V. (1983). Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody 19-9 in the sera of patients as a mucin. *Cancer research, 43*(11), 5489-5492.

[42] Mannoor, M. S., et al. (2012). Graphene-based wrireless bacteria detection on tooth enamel. *nature communications*, 1-8. doi: 10.1038

[43] Matthews, A. M., Kaur, H., Dodd, M., D'Souza, J., Liloglou, T., Shaw, R. J., & Risk, J. M. (2013). Saliva collection methods for DNA biomarker analysis in oral cancer patients. *British Journal of Oral and Maxillofacial Surgery, 51*(5), 394-398. doi: http://dx.doi.org/10.1016/j.bjoms.2012.09.017

[44] Mokdad, A. H., Marks, J. S., Stroup, D. F., & Gerberding, J. L. (2004). Actual causes of death in the United States, 2000. *JAMA: the journal of the American Medical Association, 291*(10), 1238-1245.

[45] Nagler, R. M. (2009). Saliva as a tool for oral cancer diagnosis and prognosis. *Oral Oncology, 45*(12), 1006-1010. doi: http://dx.doi.org/10.1016/j.oraloncology.2009.07.005

[46] Parker, S. L., Tong, T., Bolden, S., & Wingo, P. A. (1996). Cancer statistics, 1996. *CA Cancer J Clin, 46*(1), 5-27.

[47] Pfaffe, T., Cooper-White, J., Beyerlein, P., Kostner, K., & Punyadeera, C. (2011). Diagnostic potential of saliva: current state and future applications. *Clinical chemistry, 57*(5), 675-687.

[48] Qi, Y., Tai, Z., Sun, D., Chen, J., Ma, H., Yan, X., . . . Xue, Q. (2013). Fabrication and characterization of poly (vinyl alcohol)/graphene oxide nanofibrous biocomposite scaffolds. *Journal of Applied Polymer Science, 127*(3), 1885-1894.

[49] Righini, C. A., De Fraipont, F., Timsit, J.-F., Faure, C., Brambilla, E., Reyt, E., & Favrot, M.-C. (2007). Tumor-specific methylation in saliva: a promising biomarker for early detection of head and neck cancer recurrence. *Clinical cancer research, 13*(4), 1179-1185.

[50] Rockwood, D. N., Preda, R. C., Yücel, T., Wang, X., Lovett, M. L., & Kaplan, D. L. (2011). Materials fabrication from Bombyx mori silk fibroin. *Nature protocols, 6*(10), 1612-1631.

[51] Ruan, C., Yang, L., & Li, Y. (2002). Immunobiosensor Chips for Detection of Escherichia c oli O157: H7 Using Electrochemical Impedance Spectroscopy. *Analytical chemistry, 74*(18), 4814-4820.

[52] Schiessel, R., Wunderlich, M., & Herbst, F. (1986). Local recurrence of colorectal cancer: effect of early detection and aggressive surgery. *British journal of surgery, 73*(5), 342-344.

[53] Shopland, D. R., Eyre, H. J., & Peachacek, T. F. (1991). Smoking-attributable cancer mortality in 1991: is lung cancer now the leading cause of death among smokers in the United States? *Journal of the National Cancer Institute, 83*(16), 1142-1148.

[54] Siegel, R., Naishadham, D., & Jemal, A. (2012). Cancer statistics, 2012. *CA Cancer J Clin, 62*(1), 10-29.

[55] Siegel, R., Naishadham, D., & Jemal, A. (2013). Cancer statistics, 2013. *CA Cancer J Clin, 63*(1), 11-30. doi: 10.3322/caac.21166

[56] Siegel, R., Ward, E., Brawley, O., & Jemal, A. (2011). Cancer statistics, 2011. *CA Cancer J Clin, 61*(4), 212-236.

[57] Streckfus, C., Bigler, L., Tucci, M., & Thigpen, J. T. (2000). A preliminary study of CA15-3, c-erbB-2, epidermal growth factor receptor, cathepsin-D, and p53 in saliva among women with breast carcinoma. *Cancer investigation, 18*(2), 101-109.

[58] Tan, D. S., Yap, T. A., Hutka, M., Roxburgh, P., Ang, J., Banerjee, S., . . . Kaye, S. B. (2013). Implications of BRCA1 and BRCA2 mutations for the efficacy of paclitaxel monotherapy in advanced ovarian cancer. *Eur J Cancer, 49*(6), 1246-1253. doi: 10.1016/j.ejca.2012.11.016

[59] Tan, Y.-W., Zhang, Y., Bolotin, K., Zhao, Y., Adam, S., Hwang, E., . . . Kim, P. (2007). Measurement of scattering rate and minimum conductivity in graphene. *Physical Review Letters, 99*(24), 246803.

[60] Tan, Y., Song, Y., & Zheng, Q. (2012). Hydrogen bonding-driven rheological modulation of chemically reduced graphene oxide/poly (vinyl alcohol) suspensions and its application in electrospinning. *Nanoscale, 4*(22), 6997-7005.

[61] Troyer, D., Mubiru, J., Leach, R., & Naylor, S. (2004). Promise and challenge: markers of prostate cancer detection, diagnosis and prognosis. *Disease markers, 20*(2), 117-128.

[62] Tufte, E. Cancer Survival Rates: Tables, Graphics and PP. from http://www.edwardtufte.com/bboard/q-and-a-fetch-msg?msg_id=0000Jr

[63] Twombly, R. (2005). Cancer surpasses heart disease as leading cause of death for all but the very elderly. *Journal of the National Cancer Institute, 97*(5), 330-331.

[64] Wang, M., Wang, L., Wang, G., Ji, X., Bai, Y., Li, T., . . . Li, J. (2004). Application of impedance spectroscopy for monitoring colloid Au-enhanced antibody immobilization and antibody–antigen reactions. *Biosensors and Bioelectronics, 19*(6), 575-582.

[65] Yoshimoto, H., Shin, Y., Terai, H., & Vacanti, J. (2003). A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. *Biomaterials, 24*(12), 2077-2082.

[66] Yu, X., Lv, R., Ma, Z., Liu, Z., Hao, Y., Li, Q., & Xu, D. (2006). An impedance array biosensor for detection of multiple antibody-antigen interactions. *Analyst, 131*(6), 745-750. doi: 10.1039/B517148B

[67] Zayats, M., Raitman, O. A., Chegel, V. I., Kharitonov, A. B., & Willner, I. (2002). Probing antigen-antibody binding processes by impedance measurements on ion-sensitive field-effect transistor devices and complementary surface plasmon resonance analyses: development of cholera toxin sensors. *Analytical chemistry, 74*(18), 4763-4773.

[68] Zhang, L., Farrell, J. J., Zhou, H., Elashoff, D., Akin, D., Park, N. H., . . . Wong, D. T. (2010). Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. *Gastroenterology, 138*(3), 949-957. e947.

[69] Zuo, B., Dai, L., & Wu, Z. (2006). Analysis of structure and properties of biodegradable regenerated silk fibroin fibers. *Journal of materials science, 41*(11), 3357-3361.