

Inkjet-Printed Graphene Sensors for the Bedside Detection of Tear Film pH

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Purpose: To determine whether an inexpensive, graphene thin-film electronic pH sensor could be used to measure tear film pH.

Methods: The pH-sensitive electrolyte-gated graphene field-effect transistors (EG-GFETs) were fabricated by patterning graphene ink and ultraviolet-cured dielectric onto 125 μm -thick polyimide substrate using a nanomaterials inkjet printer. A flow-cell was used to exchange test solutions and record current flow through the EG-GFET. Laboratory reference pH test solutions were used to calibrate the sensor. Contrived tears with lipids were pH buffered using HCL (1 M) or NaOH (1 M) to produce tear solutions ranging in pH from 2.0 to 9.5. A laboratory-reference pH meter was used to verify the pH of each solution. Dirac curves that demonstrate pH-dependent changes in current flow through the EG-GFET were measured for each test solution, using dual sourcemeters.

Results: Graphene EG-GFET devices were highly sensitive to changes in artificial tear-film pH. The Dirac voltage was defined as the gate voltage at which minimum source drain current was measured. The relationship between Dirac voltage and tear film pH was highly linear with a slope of 17.2 mV per pH unit over the range of solutions tested, from pH 2.0 to pH 9.5 ($r^2 = 0.977$).

Conclusions: Graphene field-effect transistors accurately measure tear film pH and may be useful in the emergency management of ocular adnexal exposure to acids or bases.

Translational Relevance: Thin-film graphene sensors are low cost and can rapidly map tear-film pH at multiple sites on the ocular surface and within the conjunctival fornices, avoiding subjective, colorimetric test-paper methods.

Introduction

Ocular surface burns represent 7% to 18% of the eye injuries seen in the emergency department and chemical exposure accounts for 84% of these.¹ Chemical burns, particularly those involving the cornea, are considered true ophthalmologic emergencies that require prompt assessment, pH measurement, pH neutralization, and continued monitoring of ocular and adnexal surface pH to minimize morbidity.^{2–7} Acute tissue damage occurs when cellular proteins are denatured or coagulated and lipid cell membranes are saponified, leading to cell death. Vascular injury may lead to ischemic damage and cicatrization of the ocular surface and adnexae. Furthermore, limbal stem cell loss may be associated with recurrent epithelial defects,

corneal melting, scarring, and eventual conjunctival and neovascular invasion into the corneal stroma.

The severity of ocular injury due to chemical exposure is directly correlated with the solution pH, volume, duration of contact, and penetration depth through tissue. Critical to the acute diagnosis and management of these cases is the accurate measurement of ocular surface pH over multiple locations on the eye surface, repeated over the entire course of acute management to assure rapid and complete pH neutralization. Copious irrigation with buffered saline solution is the mainstay of treatment. This is continued until pH measurements of the cornea, bulbar, forniceal, and palpebral conjunctiva are neutral. Thus the ability to accurately measure pH, rapidly, from multiple tiny surface sites is critical. At the present time, pH test paper is commonly used. While these thin strips can

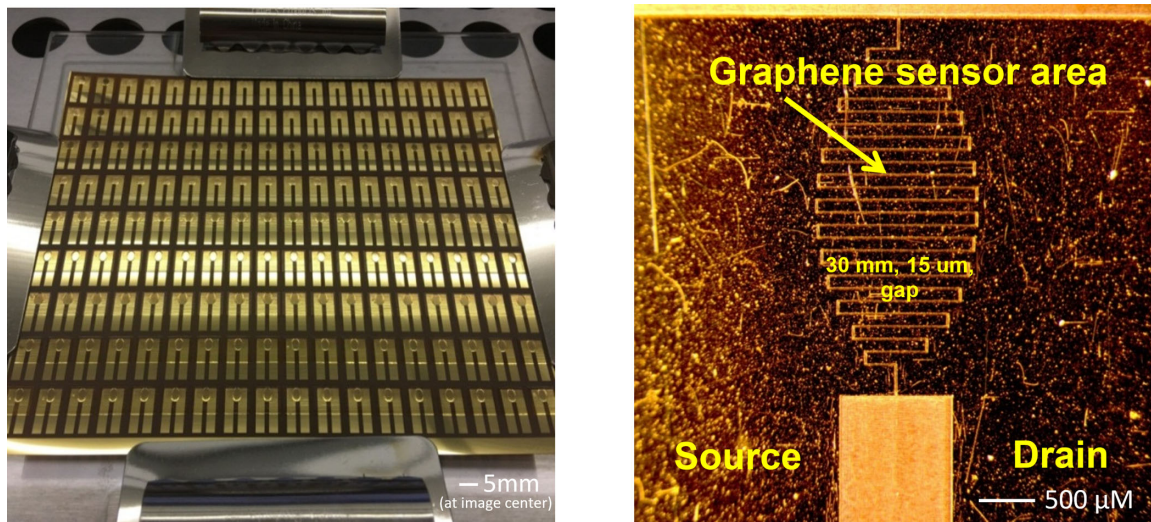


Figure 1. Array of as-printed EG-GFET sensors (*left image*); individual sensor (*right image*).

access the tight spaces of the forniceal conjunctiva, they are colorimetric, and measurements may be erroneous because they are subject to qualitative interpretation and cannot be used by colorblind providers.

Electronic devices that measure pH are commonplace; however, most are not designed to be applied to the ocular surface. If, however, an electronic pH sensor was designed to measure ocular surface pH, clinicians could map pH across the ocular surface and adnexae in patients with chemical exposure on presentation and repeatedly during the course of surface irrigation to assure proper treatment.

The goal of the present work was to develop a disposable thin film pH sensor, similar in geometry to a standard pH test strip that can be applied to multiple locations on the ocular surface for the diagnosis and management of acute chemical ocular surface injury. The device is quantitative, inexpensive, disposable, accurate and provides an electronic readout with the ability to monitor trends in pH change. Critical to the development of this device is a process for applying a two-dimensional (2-D) pH-sensitive graphene thin film semiconductor onto a micromachined flexible circuit board.

Methods

Device Fabrication

Graphene field-effect transistors were fabricated upon 125-μm thick polyimide substrate (Kapton-HN; 3M Corp, St. Paul, MN, USA). A 100-nm thick gold conductive film was sputtered onto the polyimide

film substrate using a titanium adhesion layer (Astral Technologies, White Bear Lake, MN, USA). Electrical circuits were then patterned into the gold film using a direct-wright 342 nm wavelength femtosecond laser (FSL) micromachining system using approximately 4 watts of output power. The laser was used to create a nominally 10-μm gap in the gold film of 30 mm length, interleaved, underlying the sensing region (Fig. 1). Devices were then singulated from the parent polyimide sheet to precise dimensions using the FSL. The pH-sensing portion of the device is an oval of approximately 3 mm in its longest dimension; however, this can be modified to virtually any size or geometry. Ethyl cellulose-capped graphene micro-platelet suspension ink (Sigma Aldrich, St. Louis, MO, USA) was inkjet-printed onto the sensor area using a nanomaterials inkjet printer (Dimatix, DMP-2831; Fujifilm, Tokyo, Japan). Before printing, the polyimide/gold substrate was cleaned using methanol and ethanol wipes. Further cleaning, surface etching, and surface energy modification were performed using an argon-filled plasma chamber at 50 watts for five minutes (Plasma Etch, Carson City, NV, USA). This treatment was used to promote graphene inkjet droplet adsorption to the surface of the gold/polyimide circuit. After printing, the graphene film was then thermally cured at 350 degrees Centigrade for 30 minutes to render it conductive. This curing process removes the ethyl cellulose capping agent that is used to stabilize the graphene ink by preventing Van der Waals force-driven graphene platelet agglomeration when in solution. The devices were electrically insulated using two layers of inkjet-printed ultraviolet-cured dielectric polymer (Sun Chemical, Parsippany, NJ, USA).

After thermal curing, devices were passivated through an overnight incubation with a standard pH = 2.00 solution and thoroughly rinsed with de-ionized water. The passivation process neutralizes negative charges by covalently binding hydrogen ions to oxygen impurities at the graphene edges.⁸

Device Testing

Contrived tear solution with lipids was used to simulate natural tears during pH testing (Ursa BioScience, Bel Air, MD, USA). Hydrochloric acid (1 M) and sodium hydroxide (1 M) solutions were used to buffer the various tear solutions to the desired pH values for testing. Thin film EG-GFET devices were used to test tear solutions of pH 3.91, 6.01, 7.45, 8.50, and 9.54. A calibrated laboratory reference pH meter with an accuracy of 0.001 pH units was used to measure and verify the pH reference solutions, as well as the pH of the tear solutions we tested.

Electrolyte gated, graphene field effect transistor sensors were tested using two sourcemeter units (Keithley 2450, Cleveland, OH, USA). One sourcemeter was used to provide a clamped voltage bias of +0.3V across the source and drain terminals of the device and measure the current, I_{SD} . The other was used as a potentiostat between the gate and source electrodes, feedback-controlled via the Ag/AgCl reference electrode. The gate voltage, V_G , was swept between ± 0.3 V and the current across the source and drain terminals, I_{SD} was recorded. When I_{SD} is graphed as a function of V_G , the resulting curves are called Dirac curves, and the point of minimum trans-conductance where semiconductor holes are equal to electrons (charge equivalence) is termed the Dirac point. Shifts in the Dirac point reflect the field-effect of analytes on sensor conductance and provide an electronic measurement of solution pH.

The devices were placed in a fixture that allowed solution exchange. Initially, five Dirac curves were run in the standard pH = 2.00 solution to establish an accurate baseline measurement and verify the stability of the device. The pH = 2.00 solution was then exchanged with the tear solutions of different pH values (pH = 3.91, 6.01, 8.50, and 9.54) in random sequence, and Dirac curves were recorded two minutes after each exchange. After each Dirac curve was recorded, pH = 2.00 reference solution was then reapplied to the sensor, and the Dirac curve was recorded to monitor for any changes in response baseline. This was done to validate that each of the measured Dirac shifts was, in fact due to the analyte pH and not due to the irreversible binding of hydrogen ions to oxygen impurities in the graphene.

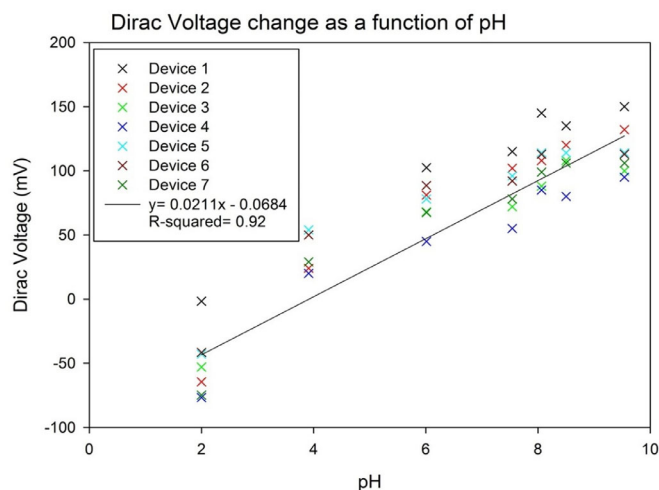


Figure 2. Dirac voltages recorded for the different tear solutions tested for each sensor plotted as a function of increasing pH. As the pH value increased, changes in the device's Dirac point also increased. The coefficient of determination $R^2 = 0.92$.

The Dirac point was then plotted as a function of pH unit for each sensor tested using SigmaPlot software and a single linear regression analysis was generated by SigmaStat software (Systat Software, Inc., San Jose, CA, USA).

Raman Spectroscopy

Raman microscopy (Renishaw, InVia) was used to collect Raman signals from the device. The Raman spectra were recorded using 532 nm laser, 600 l/cm, CCD detector and exposure time of 10 seconds.

Atomic Force Microscopy (AFM)

AFM was performed to characterize the sensor surface (Bruker, FastScanBio). An icon head and scanasyst air probe with spring constant 0.4N/m, resonant frequency 70 kHz and tip radius 2 to 12 nm was used. The experiment was conducted in air. The device was immobilized onto the AFM stage by vacuum. We used peak force quantitative nanomechanical property mapping to obtain surface topographical details using following parameters: peak force amplitude –100 nm, peak force frequency 2 kHz and peak force setpoint 1 nN.

Results

The EG-GFET sensors demonstrated a Dirac Voltage shift that was linearly related to pH (Fig. 2). As the pH was increased, the Dirac Voltage shifted in the positive direction, consistent with a p-doping effect

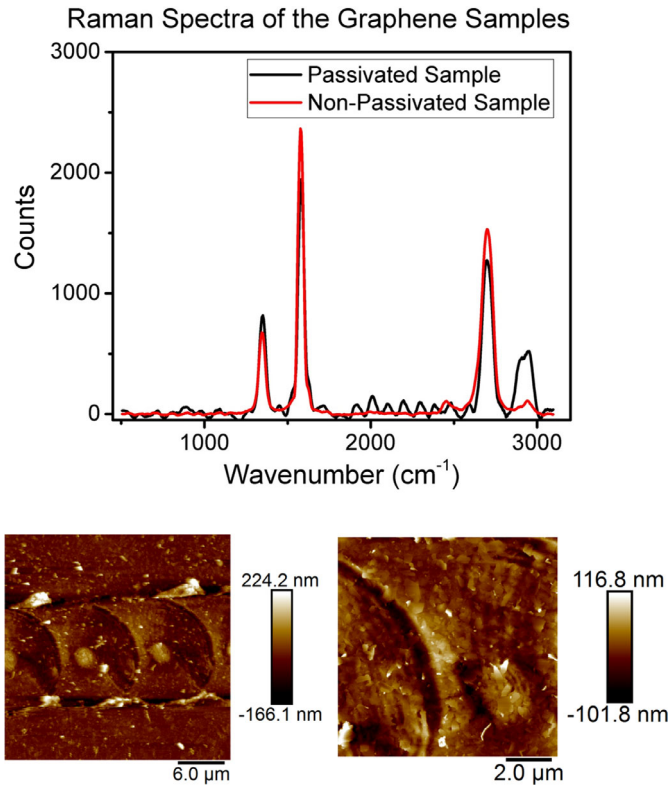


Figure 3. Raman spectrogram of inkjet-printed graphene (*upper image*). Atomic force imaging of graphene within sensor gap (*lower image*). Bottom left represents a 30 μm × 30 μm scan, and bottom right represents 10 μm × 10 μm scan.

in the graphene, as expected. Conversely, as pH was decreased, EG-GFET sensors demonstrated a negative Dirac Voltage shift, consistent with n-doping. This was shown to be reproducible over eight devices tested. The Dirac points recorded for device 1 were −55.0 mV at pH = 2.00, +24.0 mV at pH = 4.00, +81.0 mV at pH = 6.00, +102.0 mV at pH = 7.40, +107.0 mV at pH = 8.00, +120.0 mV pH = 8.50, and +132.0 mV at pH = 9.00. The coefficients of determination for the linear function demonstrated for the sensors tested were 0.9340, 0.8584, 0.7799, 0.9551, 0.8988, 0.9553, 0.9631, and 0.8259. The mean Dirac Voltage shift per pH unit, across all sensors tested was 26.15 mV/pH unit [range: 22.6 to 29.9 mV/pH]. The pH was reported using the linear Equation 1.

$$pH = \frac{(V_{Dirac} - 0.0684)}{0.0211} \quad (1)$$

As shown in the Figure 3, we monitored peaks at ~1580 cm⁻¹ and ~2690 cm⁻¹ wavenumbers that correspond to characteristic primary in-plane vibrational mode G and a second-order tone 2D bands of graphene in both the samples. The first-order disorder peak: D (1350 cm⁻¹) is present in both samples, but

the combination scattering peak D + G at 2940 cm⁻¹ is prominent only for pH2 passivated graphene samples. The first order D peak is not visible in pristine graphene because of the crystal symmetries. In our samples we see significant amount of excitation of charge carrier and inelastic scattering by phonon.

A 30 μm × 30 μm AFM scan displays the continuity of laser bombardment during the sample devise preparation and further magnification reveals more details of surface morphology (as shown in the bottom panel of Fig. 3).

Discussion

Graphene is a one-atom-thick sheet of pure carbon atoms arranged into a 2-D hexagonal sp²-hybridized lattice. Because the carbon atoms are bound to each other in only two dimensions, a cloud of unbound electrons called the Dirac fermion forms above and below the graphene sheet. This electron cloud acts as a massless charge carrier, rendering graphene highly semiconductive with a negative surface charge. Electric field changes induced within the Dirac fermion by the proximity of externally-applied charged molecules alter the conductivity of graphene as measured by electrodes placed across the graphene layer. In solution, an electric double layer forms in which hydrated positively charged cations, attracted to the negative surface charge, arrange themselves uniformly to form the outer Helmholtz plane over the graphene surface, and create an insulating water dielectric layer of a few nanometers (nm) thickness, called the inner Helmholtz plane. This allows non-Faradaic electric fields to interact with the Dirac fermion to modulate the number of holes or electrons in the graphene plane, thus altering the conductance within the graphene sheet, resulting in and electrolyte-gated graphene field-effect transistor (EG-GFET).^{9–12} Changes in solution pH, above the liquid dielectric layer, alter the Dirac fermion according to the concentration of H⁺ and OH⁻ ions, allowing the EG-GFET to report pH as a change in the conductance of the graphene layer. EG-GFETs have also been extensively studied as high performance gas, humidity, chemical, and biological sensors.^{8,13–22}

The pH sensing mechanism of EG-GFET devices is related to the redox state of oxygen-containing functional moieties, such as hydroxyl, carboxyl and aldehyde groups present along the edges of the graphene platelets that we applied via inkjet printing. Hydroxyl and hydronium ions in the test solution protonate or deprotonate these oxygen-containing functional moieties, thus altering the electric field

within the electrical double layer and the Dirac fermion at the graphene surface. This alters the density of holes and electrons in the graphene semiconductor, changes the doping state and modulates the EG-GFET source-drain conductance, thus providing a rapid electronic readout of solution pH. We observed highly reproducible pH-induced shifts in the Dirac Voltage when tear solutions of varying pH were tested.

Fu et al., have demonstrated that chemical vapor deposited graphene films, which primarily form what is known as pristine graphene without carbon edge defects, demonstrate very weak Dirac voltage shifts when the pH of the buffer is changed. They measured a value of 6 ± 1 mV/pH, which was further reduced to ~ 0 mV/pH when the surface was passivated with a hydrophobic organic layer.²³ Furthermore, the article discussed the addition of a thin oxide layer to the graphene strongly increased the pH-induced Dirac voltage shift to 17 ± 2 mV/pH. This suggests that pristine graphene, devoid of oxygen-containing functional groups, cannot sense the proton concentration or pH of a solution.

Our results strongly suggest that the range of pH-induced gate shifts we observed in our experiments are due to oxygen-containing functional groups with the graphene. The Dirac voltage shift/pH values we recorded fall below the thermodynamically allowed maximal shift, the so called Nernst value (60 mV/pH at room temperature). The positive Dirac voltage shift to increasing pH is expected for a partially oxidized graphene surface.^{15–17} In this model the terminal OH groups on the surface can be neutral in the form of OH, protonated to OH^{+2} or deprotonated to O^{-} . At a large pH value, the equilibrium is shifted toward a deprotonated surface that is negatively charged, shifting the Dirac Voltage in the positive direction.

Because of the thin-film nature of these devices and their capacity to be miniaturized, they serve as effective pH sensors in patients with chemical exposure and would mitigate the subjective aspects of current pH test-strip colorimetric methods. Because these devices are only 125 μm thick, they can easily be applied to the ocular surface or inserted deep into the conjunctival fornices where repeated, rapid measurement of tear-film pH is critically important during treatment to neutralize acid or alkali pH abnormalities introduced by chemical splash injuries.

Although the above devices have worked well in our laboratory, this study has some limitations. For this study, we used a commercially-available tear film model; however, we have not tested these devices in a prospective human clinical trial. Before those studies, we should test our devices using human tears collected via capillary tubing, ideally in patients with

chemical exposure. Further we limited the pH range tested between 2.0 and 9.5. In other studies we have performed, these devices have demonstrated excellent linearity between pH 2 and 14. However, with the kind of repeated testing we used for each device in this study, we did note delamination of the graphene at pH levels above 10. In clinical use, when exposed to pH above 10, sensors should be discarded after each ocular pH map measurement set. In this study, we used a linear model to analytically describe the sensor response to pH, this was done primarily to illustrate the relationship between Dirac voltage (sensor output) and pH. In an actual clinical application, a more precise method for reading out pH would be the use of a look-up table to compensate for device non-linearity. Future work will miniaturize the sensor control system to use an embedded microcontroller with touchscreen display and potentiostat integrated circuits to eliminate the benchtop reference potentiostats used in this study.

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References

1. Xiang H, Stallones L, Chen G, Smith GA. Work-related eye injuries treated in hospital emergency departments in the US. *Am J Ind Med*. 2005;48(1):57–62.
2. Tuft S, Shortt A. Surgical rehabilitation following severe ocular burns. *Eye (Lond)*. 2009;23(10):1966–1971.
3. Merle H, Gerard M, Schrage N. Ocular burns. *J Fr Ophthalmol*. 2008;31(7):723–734.
4. Hodge C, Lawless M. Ocular emergencies. *Aust Fam Physician*. 2008;37(7):506–509.
5. Spector J, Fernandez W. Chemical, thermal, and biological ocular exposures. *Emerg Med Clin North Am*. 2008;26(1):125–136, vii.
6. Pfister D, Pfister R. Acid injuries of the eye. *Fundamentals of Cornea and External Disease*. Cornea. 2005;2:1277–1284.
7. Pfister R, Pfister D. Alkali injuries of the eye. *Fundamentals of Cornea and External Disease*. Cornea. 2005;2:1285–1293.

8. Sun Sang K, Yi J, Lee WW, et al. Reversible and irreversible responses of defect-engineered graphene-based electrolyte-gated pH sensors. *ACS Appl Mater Interfaces*. 2016;8(1):834–839.
9. Chen Z, Lin Y, Rooks M, Avouris Ph. Graphene nano-ribbon electronics. *Physica E*. 2007;40:228–232.
10. Wang X, Li X, Zhang L, et al. N-doping of graphene through electrothermal reactions with ammonia. *Science*. 2009;324:768–771.
11. Zhang Y, Tang TT, Girit C, et al. Clinical analysis of occult macular dystrophy [Chinese]. *Nature*. 2009;459:820–823.
12. Ang P, Chen W, Wee A, Loh K. Solution-gated epitaxial graphene as pH sensor. *J Am Chem Soc*. 2008;130:14392–14393.
13. Das Sarma S, Adam S, Hwang E, Rossi E. Electronic transport in two-dimensional graphene. *Rev Mod Phys*. 2011;83.2:407.
14. Fu W, Nef C, Knopfmacher O, et al. Graphene transistors are insensitive to pH changes in solution. *Nano Letters*. 2011;11(9):3597–3600.
15. Bousse L, De Rooij N, Bergveld P. Operation of chemically sensitive field-effect sensors as a function of the insulator-electrolyte interface. *IEEE Trans Electron Devices*. 1983;30:1263–1270.
16. Bergveld P. Thirty years of ISFETOLOGY: what happened in the past 30 years and what may happen in the next 30 years. *Sensors and Actuators B*. 2003;88:1–20.
17. Chen S, Bommer J, Van der Wiel W, Carlen E, Van den Berg A. Top-down fabrication of sub-30 nm monocrystalline silicon nanowires using conventional microfabrication. *ACS Nano*. 2009;3(11):3485–3492.
18. Ang P, Chen W, Wee A, Loh K. Solution-gated epitaxial graphene as pH sensor. *Am Chem Soc*. 2008;130:14392–14393.
19. Ristein J, Zhang W, Speck F, Ostler M, Ley L, Seyller T. Characteristics of solution gated field effect transistors on the basis of epitaxial graphene on silicon carbide. *Phys D: Appl Phys*. 2010;43(34):345303.
20. Cheng Z, Li Q, Li Z, Zhou Q, Fang Y. Suspended graphene sensors with improved signal and reduced noise. *Nano Letters*. 2010;10:1864–1868.
21. Ohno Y, Maehashi K, Yamashiro Y, Matsumoto K. Electrolyte-Gated Graphene Field-Effect Transistors for Detecting pH and Protein Adsorption. *Nano Letters*. 2009;9:3318–3322.
22. Heller I, Chatoor S, Männik J, et al. Influence of electrolyte composition on liquid-gated carbon nanotube and graphene transistors. *J Am Chem Soc*. 2010;132:17149–17156.
23. Wangyang Fu, Nef C, Knopfmacher O, et al. Graphene transistors are insensitive to pH changes in solution. *Nano Lett*. 2011;11(9):3597–3600.