Modeling the dual oxygen- and pH-stimulated response of hemoglobin-loaded polyampholyte hydrogel for oxygen-pH coupled biosensor platform

Goh, Kek Boon; Li, Hua; Lam, Khin Yong

2019

Goh, K. B., Li, H., & Lam, K. Y. (2019). Modeling the dual oxygen- and pH-stimulated response of hemoglobin-loaded polyampholyte hydrogel for oxygen-pH coupled biosensor platform. *Sensors and Actuators B: Chemical*, 286, 421–428. doi:10.1016/j.snb.2019.01.138

<https://hdl.handle.net/10356/144963>

<https://doi.org/10.1016/j.snb.2019.01.138>

© 2019 Elsevier B.V. All rights reserved. This paper was published in *Sensors and Actuators B: Chemical* and is made available with permission of Elsevier B.V.

Downloaded on 26 Apr 2023 23:08:56 SGT

Modeling the dual oxygen- and pH-stimulated response of hemoglobin-loaded polyampholyte hydrogel for oxygen-pH coupled biosensor platform

K.B. Goh, Hua Li* lihua@ntu.edu.sg, K.Y. Lam

*School of Mechanical and Aerospace Engineering, Nanyang Technological University,
Nanyang Avenue, Singapore 639798, Republic of Singapore*

*Corresponding author, Tel.: + 65 6790 4953; Fax: + 65 6792 4062

Highlight

- The hemoglobin-rich polyampholyte hydrogel is studied as a function of O₂-pH level.
- This is achieved by a multiphysics model, which couples mechano-electrochemical effects together.
- The hydrogel has wide linear pH (4-6 and 8-10) and oxygen (1-30 mmHg) ranges.
- The hydrogel can detect and capture bioactive solutes in biological fluids.

Abstract: This paper presents the dual oxygen- and pH-stimulated responsive performances of hemoglobin-loaded polyampholyte hydrogel, where a multiphysics model is formulated for the

numerical characterization of the hydrogel, incorporating electrical interactions of the fixed-mobile charge groups and chemical reactions of the hemoglobin-oxygen complexes. A developed constitutive relation is integrated into the model to capture bioactivity of the immobilized hemoglobin as a function of ambient oxygen coupled with environmental pH. After examination with published experimental observations, it is concluded that the multiphysics model can accurately characterize both neonatal hemoglobin oxygen saturation and pH-driven volume transition behaviors of hemoglobin-loaded hydrogel. The results show that the swelling deformation of polyampholyte hydrogel changes in a bowl-shaped like pattern with increase of environmental pH value, whereas the rate of oxygen loaded into the hydrogel enlarges linearly with increase of physiological oxygen level from 1 to 30 mmHg. Consequently, these findings demonstrate that hemoglobin-loaded polyampholyte hydrogel could provide an innovative avenue for sensing and storing both bioactive oxygen and hydrogen solutes in biological fluids, pointing to a novel material platform for developing oxygen-pH stimuli coupled responsive biosensor, as well as oxygen- and/or pH-driven bio-actuators.

Keywords: Dual oxygen-pH-sensitive hydrogel; Hemoglobin-loaded polyampholyte hydrogel; Sensing and storing O_2 and H^+ ; Multiphysics model

1. Introduction

As well known, the oxygen O_2 and pH are two critical biomarkers in the human system, which facilitate: (i) the detection of diseases, such as breast and liver cancers [1] and (ii) the navigation of gas transport in hemoglobin-rich blood [2]. As a result, it is important for an advanced biosensor to detect and measure these two important biomarkers in biological fluids, simultaneously. In addition, in terms of the hydrogel-based biofuel cell [3], particularly the enzyme mediates pH-driven one [4], their performances are usually limited by the oxygen O_2

concentration in the microenvironment [5], due to the low solubility of the physiological gas in the water-loaded polymeric system. Therefore, it is necessary to incorporate the hemoglobin into the polyampholyte hydrogel for developing and characterizing the oxygen-pH stimuli coupled biosensing or oxygen-rich biofuel material platform [6], in which the hemoglobin is inherently associated with the attractive oxygen O_2 sensing and capturing properties [7], while the acidic-basic groups in the hydrogel undergo ionization/deionization in response to environmental pH. In other words, incorporation of both the hemoglobin and fixed charge groups onto the polymeric network chains of the hydrogel give rise to its dual oxygen- and pH-reactive behaviors.

Unfortunately, literature searches unveiled that the responsive behaviors of hemoglobin-loaded polyampholyte hydrogel remains poorly understood. To the best of our knowledge, no efforts were conducted experimentally and theoretically to investigate the performance of hemoglobin-loaded polyampholyte hydrogel as a function of ambient oxygen O_2 coupled with environmental pH. In general, free hemoglobin consists of two components, the organic and inorganic parts [8-10], where the inorganic heme iron complex in the sensing domain of hemoglobin binds with oxygen O_2 molecules [11-13], forming oxygen-heme iron complexes [14]. Interestingly, the binding of oxygen O_2 with the heme iron complex leads to a tense-to-relax structural change in the hemoglobin, altering oxygen O_2 affinity of the free hemoglobin [15-18]. In addition, formation of the free oxygen-hemoglobin complex is also influenced by environmental pH, where the affinity of hemoglobin towards oxygen O_2 is decreased, when acidity of the environmental solution increases. However, the fundamental mechanism of the hemoglobin operating in the polyampholyte hydrogel still remains unclear, particularly when subjected to the oxygen-pH coupled stimuli. Therefore, it is worthwhile and necessary to elucidate the responsive behaviors of the hemoglobin-loaded hydrogel, especially via theoretical

modelling for systematically tailoring such materials as the biosensing and biofuel cell platforms [19, 20].

The aim of this paper is to obtain a greater insight into the dual oxygen- and pH-responsive behaviors of the hemoglobin-loaded polyampholyte hydrogel in salt solution via a multiphysics model. It includes the Poisson-Nernst-Planck equation couples with a nonlinear mechanical equation, which accounts for the conversion of electrochemical energy into a mechanical one. In addition, two correlations are integrated into the multiphysics model to capture: (i) the biochemical interactions amongst the hemoglobin, dissolved oxygen O_2 molecules and the hydrogen H^+ ions; and (ii) the electrical activity between the fixed and the mobile charges in the hydrogel. The predictive capability of the multiphysics model is then examined by comparing the present numerical results with the experimental data published in open literature, in which a good agreement is achieved. This paper is organized as follows. After the introduction presented in the first section, the multiphysics model is developed in the second section, followed by the section for validation and results discussions. Finally, several conclusions are drawn in the last section.

2. Formulation of the multiphysics model

In order to investigate the responsive behaviors of hemoglobin-loaded polyampholyte hydrogel, a multiphysics model is developed to characterize: (a) the movement (diffusion and migration) of ions and oxygen O_2 between the microenvironment of polyampholyte hydrogel and the environmental solution; (b) the biochemical binding/unbinding reaction between the mobile solutes (oxygen and ions) and the immobilized functional groups (hemoglobin and charge group); (c) the electrical interactions between the mobile (both co- and counter-) ions in the

microenvironment and the immobilized charge groups (both anionic and cationic) in the hydrogel; and (d) the oxygen- and pH-responsive actuation behaviors of the hydrogels.

The present multiphysics model is formulated based on the following assumptions:

- (i) The ambient oxygen O_2 dissolves in environmental solution, such that the concentration of oxygen O_2 in the environmental solution is a function of ambient oxygen O_2 level, namely $O_2 = \alpha pO_2$, where α is the solubility of oxygen O_2 in the water and pO_2 is the ambient oxygen O_2 level (mmHg).
- (ii) The oxygen O_2 reversibly binds to the immobilized hemoglobin in the hydrogel, and thus the oxygen O_2 concentration in the hydrogel is summation of mobile (dissolved in microenvironment) and fixed (oxygen-hemoglobin complex) oxygen O_2 concentrations.
- (iii) The net charge density of the hemoglobin-loaded polyampholyte hydrogel is contributed by both the fixed (acidic and basic groups) and mobile (co and counter ions) charges, where the ionizable group density on the immobilized hemoglobin is neglected.
- (iv) The anti-polyelectrolyte behavior of polyampholyte hydrogel is accounted into the model via the Debye-Huckel theory, in which a correction term is incorporated into the mechanical equation, accounting for the electrostatic interactions between the acidic and basic groups.

2.1 Hemoglobin reaction mechanism

In this work, the hemoglobin-loaded hydrogel is subjected to variation of ambient oxygen O_2 levels, where the oxygen-hemoglobin interactions is given as

where Hm is the heme group located in the immobilized hemoglobin Hb, O_2 is the dissolved oxygen in the hydrogel, O_2Hm is the immobilized oxygenated heme site, and the association constant K_{O_2} is characterized by the ratio of its forward k_{OxyHb} rate to the backward k_{Oxy} one.

Furthermore, the hydrogel is also immersed in a solution with varying pH values, influencing the ionization of both the reduced Hm and oxygenated O_2Hm heme sites, where the equilibrium position of these phenomena are described by

where HmH^+ and O_2HmH^+ are both the protonated reduced and oxygenated heme sites, respectively. k_{F1} and k_{F2} are the forward rates, and k_{B1} and k_{B2} are the backward rates (where subscript 1 refers to the reduced heme sites and subscript 2 denotes the oxygenated heme sites).

Therefore, the total hemoglobin concentration [Hb] in the hydrogel is thus written as

$$Hb = Hm + O_2Hm + [HmH^+] + [O_2HmH^+] \quad (4)$$

where the immobilized hemoglobin involves in two important chemical reactions: (i) the oxygenation/deoxygenation of heme group and (ii) both the protonization of reduced and oxygenated heme groups.

The saturation of hemoglobin S with the oxygen O_2 is given as

$$S = \frac{HbO_2}{Hb} \quad (5)$$

where the oxygenated hemoglobin concentration HbO_2 can be described as follows

$$\begin{aligned} HbO_2 &= O_2Hm + [O_2HmH^+] \\ &= K_{O_2} O_2 Hm + K_2 [H^+] O_2Hm \\ &= K_{O_2} O_2 Hm (1 + K_2 [H^+]) \end{aligned} \quad (6)$$

in which K_{O_2} and K_2 are defined by the ratio of its forward and backward rates and can be given

as $K_{O_2} = O_2Hm / O_2 Hm$ and $K_2 = [O_2HmH^+] / [H^+] O_2Hm$,

and the total hemoglobin concentration Hb can be expressed as

$$\begin{aligned} Hb &= Hm + HmH^+ + HbO_2 \\ &= Hm + K_1 [H^+] Hm + K_{O_2} O_2 Hm (1 + K_2 [H^+]) \\ &= Hm (1 + K_1 [H^+] + K_{O_2} O_2 (1 + K_2 [H^+])) \end{aligned} \quad (7)$$

while K_1 is expressed by the ratio of $[HmH^+] / [H^+] Hm$

The rate of oxygenation of hydrogel can be characterized mathematically by

$$r_{O_2} = k_{OxyHb} (1 - S_{O_2} Hb) + k_{Oxy} S_{O_2} Hb \quad (8)$$

where the first term on the right characterizes the increase of concentration of the fixed oxygen O_2 in the form of oxygen-hemoglobin complex and the second term on the right describes the increase of dissociation of mobile oxygen O_2 from the fixed hemoglobin, which both enrich the microenvironment with oxygen O_2 . There is no consumption of oxygen O_2 in the system, explaining the absent of an oxygen O_2 sink term in Eq. (8).

2.2 Formulation of the multiphysics model

In formulating the multiphysics model for the hemoglobin-loaded polyampholyte hydrogel, the reaction-diffusion equation describing the oxygenation of hemoglobin-loaded hydrogel consists of three components: (i) diffusion of mobile oxygen O_2 from environmental solution to microenvironment of the hydrogel, (ii) binding/unbinding of oxygen O_2 with the hemoglobin immobilized in the hydrogel, and (iii) diffusion of mobile oxygen O_2 from the hydrogel to the environmental solution.

For the development of the model, at least six diffusive species is considered in the system, namely, oxygen (O_2), hydrogen ion (H^+), hydroxide ion (OH^-), a cation and an anion solutes. The mass conservation law is employed to described the mass transport between hydrogel-solution [21]

$$\nabla \cdot \mathbf{N}_k + r_k = 0 \quad k = O_2, H^+, OH^-, \text{anion, cation} \quad (9)$$

where $\mathbf{N}_k$ indicates molar flux (mM/s) and r_k refers to rate of chemical reaction for the k th species. For a charged hydrogel system, under the influence of a concentration gradient coupled with an electrical field, the mass transport between the hydrogel and environmental solution is described mathematically by a Nernst-Planck-like equation [22]

$$\nabla \cdot \mathbf{C}^{-1} D_k \left[\nabla C_k + \frac{z_k F}{RT} C_k \nabla \Phi \right] + r_k = 0 \quad k = O_2, H^+, OH^-, \text{anion, cation} \quad (10)$$

in which D_k , C_k , z_k and r_k refer to diffusion coefficient (m^2/s), mobile solutes concentration (mM), valence number, and rate of chemical reaction. R , T , F , and Φ denote the universal gas constant (8.314 J/mol K), absolute temperature (K), Faraday constant (96,487 C/mol), and electrical potential of the hydrogel. $\mathbf{C}^{-1}$ is inverse of the right Cauchy-Green tensor which arises due to fact that the system is developed in the reference configuration.

In order to capture impacts of electrical potential strength on mobile ions concentrations in the hydrogel, Eq 11, or so-called Poisson equation, is incorporated into the model which gives the relationship between the electrical potential of the polyampholyte hydrogel and the net charge density of the system [22, 23]

$$\nabla \cdot (\epsilon_r \epsilon_0 \nabla \Phi) = - \frac{F}{Cv} \left(\sum_k z_k C_k - C_A + C_B \right) \quad (11)$$

where ϵ_r is relative dielectric constant of environmental solution and ϵ_0 is permittivity of vacuum, and C is number of moles of water molecule and v is volume of a mole of fluid molecules. C_A and C_B are concentrations of the fixed acidic and basic charge groups, respectively [24], where based on Langmuir theory it can be written as [25]

$$C_A = \frac{C_A^0}{H} \frac{K_A}{K_A + C_{H^+}} \text{ and } C_B = \frac{C_B^0}{H} \frac{C_{H^+}}{K_B + C_{H^+}} \quad (12)$$

where K_A , and K_B , are the dissociation constant for fixed acidic and basic [26] groups, respectively. H is the local hydration of the hydrogel system which is defined as ratio of the volume of fluid V_f to the volume of solid V_o inside the hydrogel. Eq 12 defines the ionized fixed charge group density a function of initial concentration of fixed charge group, its ionization strength, hydration state and hydrogen ion concentrations in the hydrogel.

In order to characterize the responsive actuation behaviors of the hydrogel, the osmotic swelling pressure acting on the hydrogel-solution interface is assumedly contributed by: (i) the elastic polymeric network chains, (ii) the ionic concentration difference between the microenvironment and the environmental solution, (iii) the interactions between the polymer and the solution, and (iv) the electrical interactions between the fixed charge groups and the mobile ions, which accounts for the anti-polyelectrolyte behaviors [27]. At swelling equilibrium, the

hydrostatic pressure p over the hydrogel-solution interface is defined as the sum of the osmotic pressure, given as [22]

$$p = RT \sum_k \left(\frac{C_k}{H} - \frac{\kappa^3}{24\pi} \sigma \right) - RT \sum_k \left(\bar{C}_k - \frac{\bar{\kappa}^3}{24\pi} \bar{\sigma} \right) - N_k k_B T \left[\frac{1}{1 + C\nu} \left(\frac{1}{3} \text{tr } \mathbf{C}_e - \frac{1}{1 + C\nu} \right) \right] - \frac{RT}{\nu} \left(\ln \frac{C\nu}{1 + C\nu} + \frac{1}{1 + C\nu} + \frac{\chi}{1 + C\nu} \right) \quad (13)$$

where κ is inverse screening length, N_k is the number of polymer chains per volume of the dry polymer ($2.43 \times 10^{25} \text{ m}^{-3}$), $k_B T$ is the absolute temperature in unit of energy and χ polymer-solvent interaction parameter which measures the dis-affinity between solvent-hydrogel. The first term on the right-hand side arises due to the imbalance of mobile species concentration between hydrogel-environmental solutions which accounts for the anti-polyelectrolyte behaviors; the second term is due to contractive force by the polymeric network chains to prevent dissolution of the hydrogel during hydration and the last term is due to the mixing between the polymeric network chains and solvent [28].

The inverse screening length κ can be written as [27]

$$\kappa = \sqrt{\frac{F^2}{\epsilon_r \epsilon_0 RT} \sum C_k z_k^2 + C_f z_f^2} \quad (14)$$

and σ is given as [29]

$$\sigma_k \kappa a = 1 - \frac{3}{2} \kappa a + \frac{9}{5} \kappa a^2 \quad (15)$$

where κ and a refer to inverse screening length and ion radius

It is well-established that, at swelling equilibrium condition, the osmotic force-induced swelling of the hydrogel progresses, until it is balanced by the contractive forces expressed by

the polymeric network chains to achieve an equilibrium hydration state, where the swelling deformation λ_s of the free surface between hydrogel-solution interface can be written as [22]

$$\lambda_s = \frac{N_k k_B T}{p} = \frac{L_{gel}}{L_{gel}^0} \quad (16)$$

where $N_k k_B T$ is ground shear modulus (*i.e.* 0.1 MPa), p is hydrostatic pressure, and L_{gel} and L_{gel}^0 are final and initial lengths of the hydrogel, respectively.

In this paper, only one-dimensional steady-state simulations of the multiphysics model are performed for the responsive characteristics of a cylindrical hemoglobin polyampholyte hydrogel in response to the environmental stimuli. The swelling deformation of the hydrogel is the radial direction and restricted in the axial direction, such that Neumann boundary conditions are taken at the center of the hydrogel $\frac{\partial C_k}{\partial X} = 0$, $\frac{\partial \Phi}{\partial X} = 0$ ($= H^+, OH^-, \dots, N$) and the Dirichlet boundary conditions are assumed at the environmental solution edges $C_k = \bar{C}_k$, $\Phi = 0$.

3. Result and discussions

3.1 Validation of the multiphysics model

In order to validate the multiphysics model, the examination between the present numerical results and the published experimental data is performed for: (i) the oxygen O_2 association with hemoglobin at different ambient levels and (ii) the pH-actuated swelling hydration deformation of the hemoglobin-loaded hydrogel.

Fig. 1(a) visualizes the saturation of neonatal hemoglobin with the oxygen O_2 by the present numerical results and the published experimental data [30], responding to the changes of ambient oxygen O_2 level. In the experimental work conducted by Shiao and Ou (2007), the association of

oxygen O_2 with the neonatal hemoglobin is measured from the ambient oxygen O_2 level 30 to 100 mmHg at environmental pH=7.4, whereas the inputs required by the model to characterize numerically the oxygen saturation of the hemoglobin are provided in Table. 1. As observed in Fig. 2(a), the results show that the saturation of hemoglobin with the oxygen O_2 increases in a sigmoid-like pattern with the enlargement of the ambient oxygen O_2 level. Thereby, the present numerical results are in a good agreement with the published experimental data, with a difference of $\sim 1.3\%$, and thus the multiphysics model is able to capture well the oxygen-responsive behaviors of the hemoglobin.

In addition, Fig. 1(b) probes the pH-induced responsive swelling characteristics of the hemoglobin-loaded hydrogel via both the present numerical results and published experimental data [31], where the material parameters required by the model are given as $L = 250 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $K_A = 10^{-5}$, $C_A^0 = 180 \text{ mM}$, with concentration of immobilized hemoglobin 0.01 mM [31], while the remaining inputs are provided in Table. 1. For the present simulation, the hydrogel is immersed in a 10 mM salt solution, where the environmental pH is taken from 1 to 13 and ambient oxygen O_2 level 160 mmHg, with temperature 37 °C. The pH-induced volume transition behaviors of the hemoglobin-loaded hydrogel are divided into four stages: (i) the hydrogel is at a collapse state with the increment of the environmental pH lesser than 3, as majority of the fixed charge group still remains unionized, (ii) the hydrogel undergoes a collapse-to-swelling transition behavior from environmental pH 3 to 7, due to increase of the concentration of ionized fixed charge group, (iii) the hydrogel remains at the swelling state from environmental pH 7 to 9, as the fixed charge is still dominantly ionized, and (iv) the hydrogel collapses with the further increase of the environmental pH, due to suppression of the osmotic swelling by the largely concentration of counter-ions in the environment solution.

After comparison with the experimental data, it is concluded that the multiphysics model can accurately characterize: (i) the oxygen O_2 saturation of hemoglobin as function of ambient oxygen O_2 level and (ii) the pH-induced volume transition behaviors of the hemoglobin-loaded hydrogel, and thus the model is validated.

3.2 Dual oxygen- and pH-sensitive behaviors of polyampholyte pNIPAM hydrogel

In general, the oxygen O_2 is stored in the hemoglobin by forming the oxygen-hemoglobin complex (oxyhemoglobin), where typically the concentration of oxyhemoglobin in a free hemoglobin solution enlarges in the sigmoid-like fashion with increase of ambient oxygen O_2 level. However, the effect of ambient oxygen O_2 coupled with the environmental pH on the responsive behaviors of the hydrogel remains unclear in the current literature. In order to address the paucity of information, the multiphysics model is necessarily performed, where the material parameters required by the model for the pNIPAM-based hydrogel are obtained from referenced experimental and numerical investigations, given as $L = 250 \mu\text{m}$, $L_{gel}^0 = 50 \mu\text{m}$, $K_A = 10^{-6}$, $K_B = 10^{-8}$, $C_A^0 = 0.1 \text{ M}$ and $C_B^0 = 0.1 \text{ M}$ with concentration of immobilized hemoglobin 1 mM [32-35], while the remaining inputs are provided in Table. 1. For the present simulation, unless specified otherwise, the hydrogel is immersed in the salt solution of $\bar{C}_{NaCl} = 100 \text{ mM}$ at temperature 37°C , where the environmental pH is taken from 1 to 13 and ambient oxygen O_2 from 0.1 to 1000 mmHg.

Fig. 2 investigates the loading influence of the functional group on the dual oxygen- and pH-responsive behaviors of the hemoglobin-loaded polyampholyte hydrogel. Fig. 2(a) visualizes the impact of the hemoglobin concentration on the oxygen-driven swelling deformation of polyampholyte hydrogel at environmental pH=7.4 (blood plasma pH), in which the hydrogel demonstrates the swelling response for the oxygen O_2 , ranging from 1 to 100 mmHg, even for

the hydrogels loaded with different hemoglobin concentrations. This suggests the independence between the hydrogel linear swelling deformation response and the hemoglobin concentration in the hydrogel. Fig. 2(b) examines the effect of fixed charge group density on the pH-actuated swelling deformation of the hydrogel at the oxygen O_2 concentration of 160 mmHg (atmospheric level), where the increase of fixed charge group concentration causes the enlargement of the swelling deformation of the hydrogel. However, its pH-driven swelling deformation pattern remains unchanged for hydrogel incorporated with different fixed charge group concentrations. Therefore, Fig. 2 unveils that the linear swelling deformation response of the present hydrogel is independent of the functional component loading, including the fixed charge group density and the immobilized hemoglobin concentration.

Fig. 3(a) visualizes the pH-driven morphology of hemoglobin-loaded polyampholyte hydrogel mechanical behaviors at different ambient oxygen O_2 levels, where the hydrogel undergoes a bowl-shape like swelling deformation pattern with the increase of environmental pH. As observed in Fig. 3(a), the hydrogel experiences swelling, to collapse, and then re-swelling, when the environmental pH is changed from acidic, to neutral, and then to basic one. On the one hand, the swelling-to-collapse volumetric swelling behavior is caused by the increase of the electrical attraction between the acidic-basic charged groups on the polymeric network chains [36], resulting in the hydrogel to shrink when the environmental pH is approaching neutral conditions. On the other hand, the hydrogel experiences collapse-to-swelling volumetric transition behaviors when the net charges in the polymeric system increases, enlarging in the electrochemical potential between the hydrogel-solution interface which then drives the ingestion of environmental solution into the hydrogel. Furthermore, when the ambient oxygen O_2 level becomes higher than 100 mmHg, the immobilized hemoglobin reaches a

saturation state with the oxygen O_2 , which then results in the independence between the ambient oxygen O_2 and the pH-induced swelling behavior of the hydrogel in the salt solution. Therefore, as seen in Fig. 3(a), this material could be employed as a simple and affordable biosensor for simultaneously sensing the oxygen O_2 and pH in biological fluids, where the swelling deformation response of the hydrogel can be quantified, and then correlated to the concentration of physiological oxygen O_2 and pH [37].

Fig. 3(b) examines the oxygen-actuated morphology of hemoglobin-loaded polyampholyte hydrogel mechanical behaviors at different environmental pH values, where it can be seen that the oxygen-responsive swelling deformation behavior of the hydrogel is categorized into three stages: (i) the swelling deformation remains unchanged with the increment of the ambient oxygen O_2 level smaller than 0.1 mmHg, (ii) the hydrogel starts to swell significantly, when the ambient oxygen O_2 level is larger than 1mmHg, and (iii) the swelling of the hydrogel remains almost unchanged with the further increase of the ambient oxygen O_2 level above 100 mmHg, due to the saturation of the immobilized hemoglobin with the oxygen O_2 . Interestingly, the oxygen-actuated swelling deformation of the hydrogel coincides with each other, when the hydrogel is subjected environmental pH 2 and 12. As seen in Fig. 3(a), the pH-driven swelling deformation pattern of the hydrogel appears to be a mirror image at the environmental pH 7, such that the hydrogel demonstrates a similar swelling actuation behavior at the environmental pH 2 and 12 (which are 5 pH units away from the left and right of the environmental pH 7). As such, Fig. 3(b) shows that the hydration-induced swelling deformation of the hydrogel is highly responsive at the physiological oxygen O_2 levels (*i.e* from 40 to 95 mmHg), and thus showing potential as a physiologically sound oxygen biosensor.

Fig. 4(a) is plotted to address an important issue how the oxygen O_2 affinity of the hemoglobin-loaded hydrogel changes with the environmental pH at different ambient oxygen O_2 levels. At the lower ambient oxygen O_2 level, the oxygen-sensitive behavior of the hydrogel is highly responsive to the changes of environmental pH, where the oxygen O_2 affinity of the immobilized hemoglobin is enhanced in a sigmoid-like pattern, when the environmental solution becomes more alkaline [38]. However, at the higher ambient oxygen O_2 level, the majority of heme-active sites in the immobilized hemoglobin are associated with the oxygen O_2 , making the

immobilized hemoglobin be relatively insensitive towards the changes of environmental pH [39]. In other words, the sensitivity of the hydrogel-based biosensor works well within the physiological range of both the pH and oxygen O_2 levels, and thus it is biocompatible with the human system [40].

Fig. 4(b) investigates the oxygen O_2 concentration in the hemoglobin-loaded polyampholyte hydrogel as a function of environmental pH at different ambient oxygen O_2 levels. Similarly, at the lower ambient oxygen O_2 level, the oxygen O_2 concentration enlarges in a sigmoid-like pattern with the increase of environmental pH, reflecting the pH-sensitive oxygen O_2 affinity of the hydrogel, as illustrated in Fig. 4(a). However, at the higher ambient oxygen O_2 level, the oxygen O_2 in the microenvironment is relatively abundant, which then results in the saturation of hemoglobin with the oxygen O_2 to remain unchanged due to environmental pH increase. This makes the oxygen O_2 concentration in the hydrogel be highly dependent on its hydration state, where the increase of hydration in the polymeric system dilutes the microenvironment, consequently decreasing the oxygen O_2 concentration in the hydrogel, as seen in Figs. 3(b) and 4(b). Therefore, to the best of our knowledge, the present hemoglobin-loaded hydrogel is one of few examples of biomaterials, which are capable to sense, capture, and also store oxygen O_2 , exhibiting potential as the oxygen-responsive biosensor or oxygen-rich biofuel cell platform. In terms of the biofuel cell system, the hemoglobin-loaded hydrogel can supply adequate amount of oxygen O_2 that enhances the oxygen-induced chemical reaction, such as glucose- [41, 42], ethanol- [43], lactate- [44], or glycerol- [45] based generation of electrical power in the biofuel cells, making it a suitable material for biofuel cell platform development.

Fig. 5(a) illustrates the effects of fixed hemoglobin density on the oxygenation rate of the hemoglobin-loaded polyampholyte hydrogel at environmental pH=7.4 with variation of ambient

oxygen O₂ levels. The increase of the fixed hemoglobin density from 1.0 to 3.0 mM enlarges the linear slope of the oxygen O₂ uptake rate response from 0.52 to 1.26 mM s⁻¹/ mmHg (enhances by 142.3%), which improves the loading of oxygen O₂ into the hydrogel. In addition, Fig. 5(a) also shows that the hydrogel undergoes three stages of oxygenation rate, revealing that: (i) the hydrogel acts only as the oxygen O₂ carrier with the increment of the ambient oxygen O₂ level smaller than 1 mmHg, since the oxygenation rate of the hydrogel is insensitive towards the changes of this lower oxygen O₂ levels (ii) the hydrogel behaves as both oxygen O₂ carrier and sensor, as the rate of oxygenation of the hydrogel increases linearly with the further increase of the ambient oxygen O₂ level, until it reaches 100 mmHg, and (iii) the hydrogel again, behaving only as an oxygen carrier O₂ with the subsequent increase of the ambient oxygen O₂ level above 100 mmHg, as the oxygenation rate of the hydrogel becomes unresponsive towards the changes of these larger ambient oxygen O₂ levels.

Fig. 5(b) describes the impacts of fixed hemoglobin density on the oxygen O₂ concentration in the hemoglobin-loaded polyampholyte hydrogel with variation of ambient oxygen O₂ levels. Fig. 5(b) unveils that the oxygen O₂ concentration in the hydrogel increases in a sigmoid-like shape behavior with the increasing ambient oxygen O₂ level. To achieve the equilibrium state, the oxygen O₂ is constantly dissociated from the hemoglobin, due to the higher oxygen O₂ level in the fixed oxyhemoglobin than its microenvironment, consequently increasing the oxygen O₂ concentration in the hydrogel [46]. Indeed, the fixed hemoglobin also re-associates with the oxygen O₂ to replenish the oxygen O₂ loss from its active sites. However, the net oxygen-hemoglobin interaction causes the oxygen O₂ to diffuse down its concentration gradient from the hemoglobin to the microenvironment, enriching the hydrogel with oxygen O₂, and thus shows potential as an oxygen O₂ supplier-carrier.

4. Conclusions

In this work, a multiphysics model is developed to investigate dual oxygen- and pH-stimulated response of hemoglobin-loaded polyampholyte hydrogel, accounting for the multi-physical interactions between immobile functional components and environmental solution. The model includes a Poisson-Nernst-Planck (PNP) equation for the transport of mobile species between the microenvironment and the environmental solution, which is coupled with a nonlinear mechanical equation to account for the conversion of biochemical cues into its mechanical counterpart. For model validation, a comparison between present numerical result and published experimental observation is conducted, in which a good agreement is achieved. As discussed above, the numerical result unveils that the hydrogel has a wide linear pH from 4 to 6 and 8 to 10 which covers both acidic and basic pH values, whereas the hydrogel swells linearly from ambient oxygen level of 1 to 30 mmHg. Thereby, the model allows systematic investigation of the hydrogel where the dual oxygen- and pH-stimulated responses of hemoglobin-loaded polyampholyte hydrogel can be elucidated. In future work, the multiphysics model can be utilized to get a greater insight into the fundamental mechanism of the hydrogel to systematically design and optimize such materials.

Acknowledgement

The authors gratefully acknowledge the financial support from Nanyang Technological University through the project (No: M4081151.050) and NTU Research Scholarships

K. B. GOH received the B.Eng. degree with first class honours in manufacturing engineering from University of Malaya, Malaysia, in 2013. He is currently pursuing the Ph.D. degree in mechanical engineering with Nanyang Technological University, Singapore. His research interests include multiphysics modelling of soft materials and design of hydrogel-based biosensors and bioelectronics.

Hua Li received his B.Sc and M.Eng degrees in Engineering Mechanics from Wuhan University of Technology, P.R.C., in 1982 and 1987, respectively. He obtained his Ph.D degree in Mechanical Engineering from the National University of Singapore in 1999. From 2000 to 2001, Dr. Li was a Postdoctoral Associate at the Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign. At the end of 2005, he was a Visiting Scientist on invitation at the Department of Chemical and Biomolecular Engineering in Johns Hopkins University. From 2001 to 2006, he was a Research Scientist in the A*STAR Institute of High Performance Computing. Dr Li joined Nanyang Technological University (NTU) as an Assistant Professor in June 2006 and he was promoted to Associate Professor in March 2013. He is currently in the School of Mechanical & Aerospace Engineering at NTU.

His research interests include the multiphysics modelling of soft matters (smart hydrogel in BioMEMS and biological cell in microscale fields); development of highly efficient numerical computational methodology (meshless & multiscale algorithms); simulation of sustainable energy (building energy efficiency and fuel cell system); and structural dynamics (high-speed rotating shell and composite materials structure). He has so far authored/co-authored 3 monograph books and more than 140 refereed journal papers in these areas.

Dr. Li has served as the members of editorial boards for 3 international journals. He also received the awards from IBM and Singapore Maritime Institute.

K. Y. Lam was born in Singapore. He received the B.Sc. (Eng.) degree (Hons.) in mechanical engineering from Imperial College, University of London, U.K., in 1980, and the master's and Ph.D. degrees from the Massachusetts Institute of Technology, Cambridge, MA, USA, in 1982 and 1985, respectively.

He joined the Department of Mechanical Engineering, National University of Singapore, Singapore, in 1985. In 1998, he founded the Institute of High Performance Computing, Singapore. From 2003 to 2006, he was the Founding Executive Director of the Agency for Science, Technology, and Research, Graduate Academy, Singapore. He is currently the Vice President (Research) and a Professor with the School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore.

Prof. K. Y. Lam attended the Advanced Management Program at the Harvard Business School, Boston, MA, USA, in 2000. In 2016, he was awarded the Honorary Professorship Diploma from Lomonosov Moscow State University, Russia.

References

- [1] Chen M, Chen C, Shen Z, Zhang X, Chen Y, Lin F, et al. Extracellular pH is a biomarker enabling detection of breast cancer and liver cancer using CEST MRI. *Oncotarget* 2017;8:45759.
- [2] Antonini E, Brunori M. (1970) Hemoglobin and myoglobin in their reactions with ligands. *Front Biol* 1954;21:279-82.
- [3] Falk M, Andoralov V, Silow M, Toscano MD, Shleev S. Miniature biofuel cell as a potential power source for glucose-sensing contact lenses. *Analytical chemistry* 2013;85:6342-8.
- [4] Giroud F, Gondran C, Gorgy K, Pellissier A, Lenouvel F, Cinquin P, et al. A quinhydrone biofuel cell based on an enzyme-induced pH gradient. *Journal of Power Sources* 2011;196:1329-32.
- [5] Li H, Luo R. Modeling and characterization of glucose-sensitive hydrogel: Effect of Young's modulus. *Biosensors and Bioelectronics* 2009;24:3630-6.
- [6] Patton JN, Palmer AF. Engineering temperature-sensitive hydrogel nanoparticles entrapping hemoglobin as a novel type of oxygen carrier. *Biomacromolecules* 2005;6:2204-12.
- [7] Yu C, Qian D, Huang X, Han F, Bao N, Gu H. Construction of biconcave hemoglobin-based microcapsules and electrochemical evaluation for its ability of oxygen carry. *Sensors and Actuators B: Chemical* 2018;256:217-25.
- [8] Gilles-Gonzalez MA, Gonzalez G, Perutz MF, Kiger L, Marden MC, Poyart C. Heme-based sensors, exemplified by the kinase FixL, are a new class of heme protein with distinctive ligand binding and autoxidation. *Biochemistry* 1994;33:8067-73.
- [9] Gilles-Gonzalez M-A, Gonzalez G. Heme-based sensors: defining characteristics, recent developments, and regulatory hypotheses. *Journal of inorganic biochemistry* 2005;99:1-22.
- [10] Martínková M, Kitanishi K, Shimizu T. Heme-based globin-coupled oxygen sensors: linking oxygen binding to functional regulation of diguanylate cyclase, histidine kinase, and methyl-accepting chemotaxis. *Journal of Biological Chemistry* 2013;288:27702-11.
- [11] Benabbas A, Sun Y, Poulos TL, Champion PM. Ultrafast CO kinetics in heme proteins: adiabatic ligand binding and heavy atom tunneling. *Journal of the American Chemical Society* 2017.
- [12] Bouzahir-Sima L, Motterlini R, Gross J, Vos MH, Liebl U. Unusual Dynamics of Ligand Binding to the Heme Domain of the Bacterial CO Sensor Protein RcoM-2. *The Journal of Physical Chemistry B* 2016;120:10686-94.
- [13] Key J, Šrajer V, Pahl R, Moffat K. Time-resolved crystallographic studies of the heme domain of the oxygen sensor FixL: structural dynamics of ligand rebinding and their relation to signal transduction. *Biochemistry* 2007;46:4706-15.
- [14] Kruglik SG, Jasaitis A, Hola K, Yamashita T, Liebl U, Martin J-L, et al. Subpicosecond oxygen trapping in the heme pocket of the oxygen sensor FixL observed by time-resolved resonance Raman spectroscopy. *Proceedings of the National Academy of Sciences* 2007;104:7408-13.
- [15] Mihailescu M-R, Russu IM. A signature of the T $\rightarrow$ R transition in human hemoglobin. *Proceedings of the National Academy of Sciences* 2001;98:3773-7.
- [16] Perutz MF, Wilkinson A, Paoli M, Dodson G. The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annual review of biophysics and biomolecular structure* 1998;27:1-34.

- [17] Safo MK, Ahmed MH, Ghatge MS, Boyiri T. Hemoglobin–ligand binding: Understanding Hb function and allostery on atomic level. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 2011;1814:797-809.
- [18] Vitagliano L, Vergara A, Bonomi G, Merlino A, Verde C, Prisco Gd, et al. Spectroscopic and crystallographic characterization of a tetrameric hemoglobin oxidation reveals structural features of the functional intermediate relaxed/tense state. *Journal of the American Chemical Society* 2008;130:10527-35.
- [19] Devineau S, Inoue K-i, Kusaka R, Urashima S-h, Nihonyanagi S, Baigl D, et al. Change of the isoelectric point of hemoglobin at the air/water interface probed by the orientational flip-flop of water molecules. *Physical Chemistry Chemical Physics* 2017;19:10292-300.
- [20] Devineau S, Zargarian L, Renault JP, Pin S. Structure and function of adsorbed hemoglobin on silica nanoparticles: relationship between the adsorption process and the oxygen binding properties. *Langmuir* 2017;33:3241-52.
- [21] Lai F, Li H. Transient modeling for kinetic swelling/deswelling of the ionic-strength-sensitive hydrogel. *The European Physical Journal E* 2010;31:269-74.
- [22] Goh KB, Li H, Lam KY. Development of a multiphysics model to characterize the responsive behavior of urea-sensitive hydrogel as biosensor. *Biosensors and Bioelectronics* 2017;91:673-9.
- [23] Li H, Luo R, Birgersson E, Lam KY. A chemo-electro-mechanical model for simulation of responsive deformation of glucose-sensitive hydrogels with the effect of enzyme catalysis. *Journal of the Mechanics and Physics of Solids* 2009;57:369-82.
- [24] Kono H, Oeda I, Nakamura T. The preparation, swelling characteristics, and albumin adsorption and release behaviors of a novel chitosan-based polyampholyte hydrogel. *Reactive and Functional Polymers* 2013;73:97-107.
- [25] Grimshaw P, Nussbaum J, Grodzinsky A, Yarmush M. Kinetics of electrically and chemically induced swelling in polyelectrolyte gels. *The Journal of Chemical Physics* 1990;93:4462-72.
- [26] Beretta S, Chirico G, Arosio D, Baldini G. Role of ionic strength on hemoglobin interparticle interactions and subunit dissociation from light scattering. *Macromolecules* 1997;30:7849-55.
- [27] Baker JP, Blanch HW, Prausnitz JM. Swelling properties of acrylamide-based ampholytic hydrogels: comparison of experiment with theory. *Polymer* 1995;36:1061-9.
- [28] Goh K, Li H, Lam K. Modeling the Urea-Actuated Osmotic Pressure Response of Urease-Loaded Hydrogel for Osmotic Urea Biosensor. *Sensors and Actuators B: Chemical* 2018;268:465-74.
- [29] McQuarrie D. *Statistical mechanics*. 2000. Sausalito, Calif: University Science Books 2004;12:641.
- [30] Shiao S-YPK, Ou C-N. Validation of oxygen saturation monitoring in neonates. *American Journal of Critical Care* 2007;16:168-78.
- [31] Bajpai S. Hemoglobin-Crosslinked Polyacrylamid Hydrogels: Swelling Behaviour Analysis. *Asian Journal of Chemistry* 1998;10:964.
- [32] Feicht SE, Khair AS. A mathematical model for electrical impedance spectroscopy of zwitterionic hydrogels. *Soft matter* 2016;12:7028-37.
- [33] Ogawa K, Kokufuta E. Enzymatic formation of pH gradients within polyelectrolyte gels with immobilized urease. *Langmuir* 2002;18:5661-7.

- [34] Ogawa Y, Ogawa K, Kokufuta E. Swelling– shrinking behavior of a polyampholyte gel composed of positively charged networks with immobilized polyanions. *Langmuir* 2004;20:2546-52.
- [35] Ogawa Y, Ogawa K, Wang B, Kokufuta E. A biochemo-mechanical system consisting of polyampholyte gels with coimmobilized glucose oxidase and urease. *Langmuir* 2001;17:2670-4.
- [36] Goh KB, Li H, Lam KY. Modeling the impact of pH-and oxygen-coupled stimuli on osmotic pressure and electrical potential responses of hemoglobin-loaded polyampholyte hydrogel. *ACS Applied Bio Materials* 2018.
- [37] Zhang Y, Angelidaki I. A simple and rapid method for monitoring dissolved oxygen in water with a submersible microbial fuel cell (SBMFC). *Biosensors and Bioelectronics* 2012;38:189-94.
- [38] Nagatomo S, Okumura M, Saito K, Ogura T, Kitagawa T, Nagai M. Interrelationship among Fe–His Bond Strengths, Oxygen Affinities, and Intersubunit Hydrogen Bonding Changes upon Ligand Binding in the β Subunit of Human Hemoglobin: The Alkaline Bohr Effect. *Biochemistry* 2017;56:1261-73.
- [39] Patton JN, Palmer AF. Physical Properties of Hemoglobin– Poly (acrylamide) Hydrogel-Based Oxygen Carriers: Effect of Reaction pH. *Langmuir* 2006;22:2212-21.
- [40] Krushinitskaya O, Tønnessen TI, Jakobsen H, Johannessen E. The assessment of potentially interfering metabolites and dietary components in blood using an osmotic glucose sensor based on the concanavalin A–dextran affinity assay. *Biosensors and Bioelectronics* 2011;28:195-203.
- [41] Boussema F, Gross AJ, Hmida F, Ayed B, Majdoub H, Cosnier S, et al. Dawson-type polyoxometalate nanoclusters confined in a carbon nanotube matrix as efficient redox mediators for enzymatic glucose biofuel cell anodes and glucose biosensors. *Biosensors and Bioelectronics* 2018.
- [42] Cadet M, Gounel S, Stines-Chaumeil C, Brilland X, Rouhana J, Louerat F, et al. An enzymatic glucose/O₂ biofuel cell operating in human blood. *Biosensors and Bioelectronics* 2016;83:60-7.
- [43] Gouranlou F, Ghourchian H. Enhancement of ethanol–oxygen biofuel cell output using a CNT based nano-composite as bioanode. *Biosensors and Bioelectronics* 2016;78:337-43.
- [44] Reid RC, Minter SD, Gale BK. Contact lens biofuel cell tested in a synthetic tear solution. *Biosensors and Bioelectronics* 2015;68:142-8.
- [45] Ratautas D, Tetianec L, Marcinkevičienė L, Meškys R, Kulys J. Bioanode with alcohol dehydrogenase undergoing a direct electron transfer on functionalized gold nanoparticles for an application in biofuel cells for glycerol conversion. *Biosensors and Bioelectronics* 2017;98:215-21.
- [46] Longeville S, Stingaciu L-R. Hemoglobin diffusion and the dynamics of oxygen capture by red blood cells. *Scientific Reports* 2017;7:10448.
- [47] Plawsky JL. Transport phenomena fundamentals: CRC Press; 2014.
- [48] Bae YH, Okano T, Kim SW. Temperature dependence of swelling of crosslinked poly (N, N alkyl substituted acrylamides) in water. *Journal of Polymer Science Part B: Polymer Physics* 1990;28:923-36.
- [49] Gibson QH. The reaction of oxygen with hemoglobin and the kinetic basis of the effect of salt on binding of oxygen. *Journal of Biological Chemistry* 1970;245:3285-8.
- [50] Antonini E, Brunori M. Hemoglobin and Myoglobin: Elsevier; 1971.

ACCEPTED MANUSCRIPT

Fig. 1. The comparison between the present numerical result and the published experimental observations [30, 31]: (a) the oxygen saturation with neonatal hemoglobin as a function of ambient oxygen levels; (b) the pH-induced swelling deformation of the hemoglobin-loaded hydrogel.

Fig. 2. (a) The effect of hemoglobin concentration on the oxygen-induced swelling deformation of the hemoglobin-loaded polyampholyte hydrogel (the horizontal axis is plotted in the log scale). (b) The influence of fixed acidic and basic groups densities on the pH-driven responsive behaviors of the hemoglobin-loaded polyampholyte hydrogel.

Fig. 3. The responsive behaviors of the hemoglobin-loaded polyampholyte hydrogel for: (a) the pH-driven swelling deformation of the hydrogel at variation of ambient oxygen levels.; (b) the oxygen-induced swelling behaviors of the hydrogel at different environmental pH values where it appears that the swelling performance of the hydrogels coincide with each other at environmental pH 2 and 12, and the inset of Fig. 3(b) visualizes the swelling behaviors at environmental pH 12 (the horizontal axis is plotted in the log scale).

Fig. 4. (a) The pH-induced oxygen saturation with immobilized hemoglobin in the polyampholyte hydrogel at different ambient oxygen levels and (b) The pH-driven oxygen loading in the polyampholyte hydrogel at several ambient oxygen levels.

Fig. 5. (a) The rate of oxygen-loaded in the polyampholyte hydrogel as function of ambient oxygen level at different hemoglobin concentrations. (b) The oxygen-loaded (fixed and mobile forms) in the hydrogel with varying of immobilized hemoglobin concentrations, responding to the changes of ambient oxygen level (the horizontal axis is plotted in the log scale).

ACCEPTED MANUSCRIPT

Table. 1. Input data for the numerical simulation of the multiphysics model.

Parameter	Value
Diffusion coefficients D_{H^+} [47]	$9.3 \times 10^{-9} \text{ m}^2/\text{s}$
Diffusion coefficients D_{OH^-} [47]	$5.2 \times 10^{-9} \text{ m}^2/\text{s}$
Flory interaction parameter χ for pNIPAM [48]	0.906
Permittivity $\epsilon_r \epsilon_0$ (water)	$7.1 \times 10^{-10} \text{ A s/V.m}$
Forward rate k_{OxyHb} ; Backward rate k_{Oxy} [49]	$3.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$; 50 s^{-1}
Association constant: K_1 ; K_2 [50]	$\frac{1}{10^{-7.58}}$; $\frac{1}{10^{-7.72}}$
Ion radius a [27] Solubility of oxygen O_2 α	$3 \text{ \AA}$ $1.34 \mu\text{M/mmHg}$