Published in final edited form as:
Laryngoscope. 2011 November ; 121(11): 2418–2428. doi:10.1002/lary.22226.

Biosimulation of Acute Phonotrauma: an Extended Model

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Abstract

Objectives/ Hypothesis—Personalized, pre-emptive and predictive medicine is a central goal of contemporary medical care. The central aim of the present study is to investigate the utility of mechanistic computational modeling of inflammation and healing in order to address personalized therapy for patients with acute phonotrauma.

Study Design—Computer simulation.

Methods—Previously reported agent-based models (ABMs) of acute phonotrauma were extended with additional inflammatory mediators as well as extracellular matrix components. The models were calibrated with empirical data for a panel of biomarkers – interleukin (IL)-1β, IL-6, IL-8, IL-10, tumor necrosis factor-α and matrix metalloproteinase-8, from individual subjects following experimentally induced phonotrauma and a randomly assigned voice treatment namely

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The authors have no other funding, financial relationships, or conflicts of interest to disclose.

voice rest, resonant voice exercise and spontaneous speech. The models' prediction accuracy for biomarker levels was tested for a 24-hr follow-up time point.

Results—The extended ABMs reproduced and predicted trajectories of biomarkers seen in experimental data. The simulation results also agreed qualitatively with various known aspects of inflammation and healing. Model prediction accuracy was generally better following individual-based calibration as compared to population-based calibration. Simulation results also suggested that the special form of vocal fold oscillation in resonant voice may accelerate acute vocal fold healing.

Conclusions—The calibration of inflammation/healing ABMs with subject-specific data appears to optimize the models' prediction accuracy for individual subjects. This translational application of biosimulation might be used to predict individual healing trajectories, the potential effects of different treatment options, and most importantly, provide new understanding of health and healing in the larynx and possibly in other organs and tissues as well.

Level of Evidence—N/A

Keywords

Acute phonotrauma; computer simulation; danger signals; vocal folds; inflammation; wound healing; cytokines

INTRODUCTION

Behavioral voice therapy is usually preferred as the first-line approach for patients with phonotrauma[1]. Unfortunately, as for many other disease processes, treatment outcomes for this approach appear to be highly individualized[2]. A possible factor is that phonotraumatic lesions display wide individual variability in their presentation and in their response to therapy (for review, see[2]). The clinical decision-making process of voice management professionals is therefore highly complex, and has become increasingly daunting in an era emphasizing personalized and preventive medicine.

Systems biology is a fast-advancing field that has significant potential to help us address this challenge by combining experimental and computational techniques to enhance our ability to make longer-term predictions of complex biological phenomena such as inflammation and healing. The more that genomics, proteomics, and related fields reveal about biological processes, the more apparent it becomes that the current understanding of health and disease is not ideally situated to address such complexity. Systems biology places biology on a rational, multi-dimensional foundation to make the task of understanding biological complexity more tractable. The current study, based on one such approach, builds on a series of computational modeling studies focused on the inflammatory and healing responses to mechanical damage of vocal folds[2–4].

Preliminary individual-specific agent-based models (ABMs) of acute phonotrauma[3] were previously reported. The first-generation models were calibrated using biomarker data from the laryngeal secretions of individuals subjected to experimentally induced acute phonotrauma and subsequent behavioral treatment assignments (voice rest, resonant voice exercises and spontaneous speech)[5]. In this previous work, the predicted outputs of biomarker levels from these models showed good correspondence with empirical data. However, the early models are limited in terms of (1) their representation of vocal fold extracellular matrix (ECM) and its biochemical roles in mediating inflammation and healing, (2) their representation of inflammatory mediators and growth factors and responses to biomechanical stress and (3) insufficient calibration to individual biomarker data. The

present study was designed to address these gaps to improve the model's biological representation and prediction accuracy to vocal fold biosimulation.

MATERIALS AND METHODS

Experimental protocol for acute phonotrauma using different treatment modalities

The study was approved by the Institutional Review Board at the University of Pittsburgh. The experimental protocol used to induce and treat acute phonotrauma, which provided the necessary empirical data for the previous and current modeling studies, was described previously[3]. In brief, a total of nine subjects participated in the study, six females (21–46 years) and three males (21–29 years). Subjects were randomly assigned to one of three voice treatment conditions (voice rest, resonant voice exercises or spontaneous speech) following a vocal loading task. One of the female subjects (Subject 3) also participated in a within-subjects design, which involved exposure to all three "treatment" conditions. For the procedures, first, secretions were suctioned from the vocal fold surfaces bilaterally following local anesthetization[5]. Then, all subjects were exposed to a vocal loading task, which aimed to induce acute phonotrauma. The vocal loading protocol entailed repeating cycles of 15 minutes of loud phonation (~75 – 90 dB @ 25 cm microphone-to-mouth distance) followed by 5 minutes of silence, for a total of 3 cycles over 60 minutes. Following a second sampling of laryngeal secretions, subjects were randomly assigned to one of the three aforementioned treatment groups for 4 hrs in the clinic, under the careful supervision of a voice trainer, who was blinded to the experimental hypotheses. The resonant voice exercise protocol involved cycles of 4 minutes of exercise followed by 16 minutes of rest, whereas the spontaneous speech treatment involved cycles of 16 minutes of conversational speech followed by 4 minutes of silence during the 4-hr treatment. In the voice rest condition, subjects did not phonate at all during the 4-hr period. After the 4-hr in-house treatment, subjects underwent laryngeal secretion sampling a third time and were discharged to home with instructions to continue to follow their corresponding treatment condition, with slight modifications for the resonant voice and spontaneous speech groups. The next morning, all subjects were required to observe complete voice rest until their arrival at the clinic the following morning. At that time, they underwent a fourth and final sampling of laryngeal secretions.

Assessment of inflammatory analytes in laryngeal secretion

A total of 4 secretion specimens were collected from each subject at 4 different times per treatment condition: at baseline, immediately after vocal loading, immediately following the 4-hr in-clinic treatment and 24 hr post-baseline. Standard enzyme-linked immunosorbent assays (ELISAs) were performed for interleukin (IL)-1β, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, and matrix metalloproteinase (MMP)-8 utilizing the manufacturer's recommended protocol (R&D Systems, Minneapolis, MN). All samples were run in duplicates on the same kit to avoid inter-kit variability.

Inspection of the laryngeal secretion data was carried out in order to identify the cleanest data for the development of ABM, as described previously[3]. To do so, the secretion data were sorted into three main categories for each subject and inflammatory marker: (1) data showing high baseline concentrations of pro-inflammatory markers ("pre-inflamed" data); (2) data showing normal baseline concentrations of markers but paradoxically decreasing post loading ("non-responsive" data); and (3) data showing normal baseline concentrations of markers and increase after loading ("responsive" data). As a result of this process, a core data set was identified using data from three subjects (Subjects 1 – 3), for whose mediator data were considered "responsive" and "not pre-inflamed" according to the schematic used. In addition, data from two subjects (Subject 8 and Subject 9) were considered invalid altogether due to thick secretions that compromised interpretation of ELISA results.

Components and rules of the vocal fold ABM

Biological components—The previous vocal fold phonotrauma ABM[3] was composed of (1) platelets, (2) cells (neutrophils, macrophages and fibroblasts), (3) a growth factor (TGF-β1) and three cytokines (IL-1β, TNF-α, IL-10) involved in inflammation and wound healing, (4) a matrix substance (collagen type I) and (5) a tissue damage function analogous to alarm/danger signals[6]. In the present study, six new elements were added into the model: two cytokines (IL-6 and IL-8), a collagenase (MMP-8), a growth factor (bFGF), and two extracellular matrix substances (elastin and HA).

The additional cytokines, collagenase and growth factor represent the essential signaling molecules that regulate distinct aspects of cell behavior, as well as in ECM production and remodeling, during different phases of inflammation and healing. This model extension would provide a more complete simulation of the interplay among cells, inflammatory mediators, growth factors, and ECM substances in the progression of vocal fold healing following acute injury. Also, the panel of ECM substances was extended in order to better simulate the ECM environment in the vocal folds. Whereas the published ABM used collagen type I as the sole ECM representative for the virtual vocal folds, the present model also incorporated elastin and hyaluronan (HA). These substances are known to have important biomechanical roles in the regulation of vocal fold vibratory properties[7,8]. Moreover, a growing literature suggests that the idea that these ECM components also have biochemical roles in regulating the wound healing process. Studies to date have shown that aberrant scarring/ fibrosis is at least partly due to the response of fibroblasts to both inflammatory mediators and ECM components in the wound area[9]. In particular, fragments of some ECM substances are known to constitute alarm/ danger signals (also known as damage-associated molecular pattern molecules [DAMP]) that influence cellular responses in the wound environment[6,10–12]. Thus, in the updated ABM, fragments of the ECM substances ensuing from injury or inflammation were incorporated as indicators of “tissue damage”.

Detailed literature on inflammation and healing was reviewed to identify rules for the further development of the ABMs in the present context[12–19]. Further, relevant literature on the vocal folds[20–22] was used to specify the model to the setting of vocal fold injury. The cell source and biological functions of the existing and augmented (in italics) models are summarized in Table I. For each simulation, the user could define the initial levels of biomarkers (IL-1β, TNF-α, IL-6, IL-8, IL-10 and MMP-8), add a phonotraumatic event, and then a 4-hr treatment event (voice rest, resonant voice exercises or spontaneous speech). The changes in temporal concentration of platelets, cells, mediators, tissue damage, and extracellular matrices were plotted and refolded into the model at each time step.

Treatment components—The three voice treatments studied herein can be thought of on a two-dimensional continuum involving vibratory stress (none — voice rest; intermediate — spontaneous speech and resonant voice) and impact stress (none — voice rest; low — resonant voice; or intermediate — spontaneous speech)[23,24]. In the current ABM, phonation-induced impact stress was constructed as “damaging stress”. This assumption was gleaned from the literature in voice science and exercise physiology. First, impact stress between the vocal folds during phonation has been suggested to be destructive to vocal fold mucosal tissue and is regarded as a major causative factor of phonotraumatic lesions[25–27]. Second, intense muscle loading from exercise has been found to induce an immediate “para-inflammatory” response both systematically and locally, without necessary overt tissue injury. Systemic/ local pro-inflammatory mediator levels have been found to increase after the onset of exercise and return to baseline quickly after exercise[28]. The algorithm of impact stress from the 4-hr treatment event was constructed in a similar way, in which levels of the

pro-inflammatory mediators IL-1β and TNF-α increased in different amounts, depending on the magnitude of impact stress generated from a particular treatment (Table II).

On the other hand, vibratory stress was constructed as stress arising from tissue stretching and viewed as "healing stress", which was linked to the functions of anti-inflammatory responses in the model, based on the literature in voice science and exercise physiology[29,30]. First, the effects of cyclic equibiaxial tensile strain (CTS) on rabbit vocal fold fibroblast cultures have been evaluated, in the presence or absence of IL-1β-induced inflammation[29]. The relevant in vitro study concluded that comparatively low magnitude and high frequency mobilization (6% CTS & 0.5 Hz) might assist in healing for acute vocal fold inflammation by attenuating the expression of various pro-inflammatory mediators. Second, researchers in exercise physiology have looked for an "exercise factor" that mediates the beneficial health effects of exercise. Of the various cytokines involved in the response to exercise, IL-6 was identified as the "exercise factor" at least in muscle[30]. Essentially, the level of IL-6 in circulation was zero during rest but had a rapid increase (about 100-fold) in response to exercise and decreased rapidly in the post-exercise period[30]. Also, exercise-induced IL-6 was shown to have inhibitory effects on the expression of pro-inflammatory mediator IL-1β and TNF-α, while having stimulatory effects on the expression of anti-inflammatory mediators IL-10 and possibly TGF-β1 in exercised tissue[31–33]. Given these data, IL-6 was chosen as the "exercise mediator" in the current ABM. The algorithm for the vibratory stress was constructed in which IL-6 levels increased in different amounts, depending on the magnitude of simulated vibratory stress generated from a particular treatment (Table II). Based on the exercise literature described above, IL-6 was included in the inhibitory term for the rules governing the secretion of the pro-inflammatory mediators IL-1β and TNF-α, as well as in the stimulatory term for the rules governing the secretion of the anti-inflammatory mediator IL-10.

Parameter estimation: iterative model calibration and validation process

The earlier ABMs were calibrated with empirical data of biomarker levels in laryngeal secretions from a specific group of individuals. Subsequently, the calibrated models were used to simulate not only for that specific group of subjects but also for another group of subjects whose biomarker data had *not* been used for model calibration[3]. The prediction accuracy of the ABMs for the biomarker levels was satisfactory for the former group (80% of cases) but less than satisfactory for the latter group (50% of cases). In the present study, the extended ABMs were calibrated for all individuals. Two variations of calibration methods, population-based and individual-based approaches (Figure 1), were employed in this study. For the population-based approach, the models were calibrated and verified for three subjects (Subjects 1 – 3) since, as already noted, their data were considered both responsive to the phonotrauma insult and not pre-inflamed (both conditions being necessary for a proper, generic baseline of inflammation). Thus, three ABMs were specified to represent each of three voice treatments (Figure 1). The calibrated models were subsequently applied to predict the inflammatory response for Subjects 1 – 3 as well as for the remaining subjects (Subjects 4 – 7), none of whose data were used for model calibration. Regarding the individual-based calibration approach, models were calibrated and verified for each of the seven subjects independently (Figure 1). Each personalized model was evaluated for its ability to predict the subsequent course for that particular individual, not for other individuals.

Nevertheless, in both population- and individual-based calibration methods, a "*Leave-One-Out-Cross-Validation*" was used to evaluate the predictive power of computational models of biological systems. During the calibration process, the patterns of simulation-generated data curves for each biomarker was compared with those of the empirical data at the first two time points (immediately after phonotrauma and following a 4-hr treatment), excluding

the last time point (24-hr post baseline) for validation purposes. The model's parameters were calibrated iteratively until the model eventually yields a satisfactory match between simulation data and empirical data. For the validation process, the ABM was evaluated statistically for its accuracy in predicting biomarker levels at the 24-hr time point. Due to the inherent stochasticity of the ABM framework, each model was run 100 times for up to three simulated days in order to generate a representative data pool for subsequent statistical analysis. The ABM-predicted levels of each biomarker were compared with the corresponding, experimentally-determined biomarker levels at 24 hr for each subject. A 95% confidence interval was computed for each biomarker, i.e., 6 confidence intervals in total (one for each of the inflammatory biomarkers assessed: IL-1β, IL-6, IL-8, IL-10, TNF-α and MMP-8) at the 24-hr time point from the simulation runs. If the empirical result for a given marker fell within the 95% confidence interval from the simulation runs, the mode was considered capable of predicting the experimentally-determined levels of inflammatory biomarkers. Unadjusted α levels were used because Type II (β) error as much as from Type I (α) error were opted to protect at this early stage of inquiry in predicting biological responses following phonotrauma.

RESULTS

Predicted trajectories of biomarkers

The predicted biomarker trajectories for Subjects 1 (voice rest), 2 (resonant voice) and 3 (spontaneous speech), under both population- and individual-based calibration methods, are shown in Figures 2 – 7. The trajectories of the remaining subjects under the other two treatment conditions, are attached in *Additional Files 1–12*. For Subjects 1 – 3, predicted 24-hr biomarker values were within the 95% confidence intervals in 73% of cases under both calibration methods (22/30: 18 biomarker measurements from 3 between-group subjects, each with one of the 3 different treatments; 18 biomarker measurements from one within-group subject (Subject 3) who received each of the treatments (at widely-spaced time intervals); minus 6 biomarker measurements to account for double-counting the "spontaneous speech" treatment for Subject 3 in both between- and within-group measurements; hence, 30 different mediator measurements in total; Table III). For Subjects 4 – 7, the prediction accuracy was 38% (9/24) under the population-based calibration approach (Table III). When the models were personalized with the data from Subjects 4 – 7, the prediction accuracy rose to 63% (15/24).

Further *post hoc* analyses were carried out to compare the prediction accuracy for "valid" and "non-responsive and/ or pre-inflamed" biomarkers at the 24-hr time point in the between-subject group (Table IV). For "valid" biomarkers, the prediction accuracy was 82% (14/17) under both calibration approaches. For "non-responsive and/ or pre-inflamed" biomarkers, the prediction accuracy was 36% (9/25) under the population-based calibration method and rose to 60% (15/25) under the individual-based calibration approach.

Based on the analysis of the simulated trajectories of Subjects 1 – 3 (Figures 2 – 7), the biomarker trajectories were distinctive across treatment assignment. For the spontaneous speech condition (Figures 6 – 7), the ABM predicted that the inflammatory response would be escalated, i.e., involving massive secretion of both pro- and anti-inflammatory mediators following the 4-hr treatment. Specifically, the concentrations of pro-inflammatory mediators (IL-1β, TNF-α, and IL-8) and collagenase (MMP-8) reached their peaks at Day 1 post-injury and returned to baseline concentrations at approximately Day 2 – 3 post-injury. One anti-inflammatory mediator, IL-10, was also predicted to be secreted in great quantities by wound macrophages. The concentration of IL-6 was increased after the 4-hr spontaneous speech treatment and remained elevated up to Day 1 post-injury.

In contrast, under conditions of voice rest (Figures 2 – 3) and resonant voice exercise (Figures 4 – 5), concentrations of pro-inflammatory mediators (IL-1β and IL-8) and collagenase (MMP-8) dropped rapidly after the 4-hr treatment and then remained low through the end of simulation, i.e., Day 3. IL-1β was particularly down-regulated following resonant voice exercise. The anti-inflammatory mediator IL-10 was predicted to be secreted rapidly after the 4-hr treatment and remained at a high level at Day 1 post-injury. The concentration of "exercise factor" IL-6 was predicted to be strongly secreted following the 4-hr treatment and drop rapidly to a minimal level at Day 1 post-injury. The degree of drop in IL-6 was lower following resonant voice compared to the voice rest condition.

Predicted trajectories of cells and ECM synthesis

In addition to studying biomarker trajectories, computer simulation of cells and ECM synthesis is equally important to provide insights into vocal fold healing, because *in vivo* measurements of vocal fold cell counts and ECM concentrations in human subjects are not yet available and may be difficult –if not impossible due to human subjects considerations – to obtain. The ABM predicted massive platelet infiltration and remarkable resident fibroblast activation during the first 12-hr period post-injury, without the intervention of resonant voice or spontaneous speech (Figure 8a). Inflammatory cells (neutrophils and macrophages) were predicted to arrive at relatively later time points, between 12 hr and Day 1 post injury. Fibroblasts were the dominant cell type throughout the simulation period. HA was predicted to be secreted in large quantities by activated fibroblasts during the acute phase of healing (Figure 8b). Minimal collagen type I and elastin secretion was predicted following the injury.

DISCUSSION

The present report is part of a series of systems biology-driven studies aimed at developing a computational platform with which to aid researchers and clinicians in 1) investigating the complex processes of inflammation and healing involved in the pathogenesis of vocal fold lesions, and 2) testing the effects of behavioral and ultimately pharmaceutical treatments on stressed/ traumatized vocal folds[2–4]. In the present study, the panel of mediators and ECM substances was extended over the initial scope encompassed in the previous ABM[3]. Also, two different calibration methods were employed in the process of fitting the ABMs to inflammation biomarker data. The ABMs generally exhibited poorer prediction accuracy for Subjects 4 – 7 as compared to Subjects 1 – 3 following the population-based calibration approach. These findings suggested that the parameters of the models are indeed patient-specific (in this case to Subjects 1 – 3) and did not produce accurate population-general predictions. When the parameters of the models were calibrated with Subjects 4 – 7 under the individual-based calibration method, the prediction accuracy of the biomarker levels in these subjects was improved. This finding is not entirely unexpected, given the known individual variability in the inflammatory and healing responses following vocal fold injury[2].

In the current study, the extended ABMs showed better prediction accuracy for the "valid" biomarkers as compared to the "pre-inflamed and/ or non-responsive" markers, corroborating the previous report[3] in general. Current findings suggest unknown factors due to prior injury (perhaps accounting for the "pre-inflamed" phenotype described above for some subjects), unaccounted for in the simulations, and may need to be modeled in order to account for the discrepancy between experimental data and model predictions in the setting of pre-inflamed / chronically inflamed vocal folds. The current phonotrauma ABMs primarily relied on data and assumptions derived from experimental and clinical studies in larynges presumed to have no prior history of injury or stress. Thus, the current phonotrauma ABM may not be applicable for the simulation of pre-inflamed conditions that

existed prior to a discrete phonotraumatic event. This basic phenomenon, which is known as inflammatory conditioning[34], has been studied by other inflammatory models. Both empirical and simulation results concomitantly suggested that prior exposure to an inflammatory stimulus could alter a secondary response to the same or similar stimulus[35].

In addition to the need for extending the vocal fold ABM's with additional variables that may account for other conditions that affect inflammation, the improved predictions accuracy of ABM's calibrated based on single-subject data (i.e., tuning a given individual's inflammation-related parameters) may be associated with genetic variation and epigenetic factors known to influence the evolving inflammatory state. Genetic variability in inflammation and wound healing is typically mediated via single-nucleotide gene polymorphisms (SNPs) of various inflammatory mediators[36,37]. SNPs are variations in short deoxyribonucleic acid (DNA) sequences. In various inflammatory diseases, SNPs in the cytokine genes dictate the ultimate production of that cytokine in response to a defined stimulus[38]. Essentially, such genetic variation is captured in the process of tuning model parameters to the inflammation biomarker data of an individual, as was done herein and in the previous study[3]. Epigenetic factors, such as age, gender and ethnicity, may also affect individual's course and outcome of inflammation[39]. Future model development may need to include not only inflammatory history but also genetic and epigenetic factors in order to simulate various inflammatory and healing behaviors in reality.

In contrast to the situation for mediator prediction, the trajectories of ECM generated from the ABMs were not quantitatively calibrated with any empirical data from specific human subjects in this study. Surprisingly, the predictions for the ECM were consistent with findings in the general healing literature. First, the accumulation of HA during the earliest phase of inflammation is considered one of the cardinal signs of acute inflammation, namely edema (swelling)[10,11]. In a previous report, vocal fold edema was observed visually in a subject immediately following 1 hr of loud phonation (74 – 121 dB 30 cm)[40], suggesting a similar mechanism of rapid HA accumulation and edema in the vocal folds as in other tissues. In contrast, the ABM predicted minimal accumulation of collagen and elastin compared to HA following acute phonotrauma. This prediction suggested that microscopic tissue injury from a single event of phonotrauma, – at least phonotrauma of the magnitude studied – is unlikely to lead to substantial loss of structural proteins (collagen and elastin), in which fibroblasts would need to secrete elevated levels of collagen and elastin to repair the loss. In fact, abnormal collagen and elastin accumulation has rarely been reported in acute phonotrauma, but has been commonly reported in chronic benign vocal fold lesions (presumably from repeated phonotrauma) and in vocal fold scar (surgical trauma)[41].

In addition, the phonotrauma ABM had the capability of testing three voice treatment regimes *in silico*. A novel debate that has emerged from the empirical data set is whether voice rest or resonant voice exercise is a better intervention for patients with acute vocal fold injury[42] . Traditional wisdom suggests that voice rest is the ideal approach. However, the current phonotrauma ABM suggested that biomechanical signals generated from resonant voice exercises might optimally assist stressed tissues to restore their homeostasis by regulating an array of pro-inflammatory and anti-inflammatory mediators. The induction of IL-6 was enhanced immediately following the resonant voice treatment (i.e., the time point following a 4-hr treatment). Exercise-induced IL-6 has been suggested to stimulate the production of IL-10 and inhibit the synthesis of TNF-α and IL-1β in exercised tissues[31–33]. Both empirical and simulation data in our series showed that during the post-treatment period (i.e., the 24-hr post baseline time point), although IL-10 was on an upward trajectory in both voice rest and resonant voice exercise, the trajectory was sharper following resonant voice exercise. In other words, although an anti-inflammatory process may be already programmed physiologically (as indicated in the voice rest condition), vibratory stress from

resonant voice enhanced the pre-existing anti-inflammatory process by augmenting the anti-inflammatory effects of IL-10 through the induction of IL-6. Also, marked decrease of pro-inflammatory mediators (TNF-α and IL-1β) were noted following resonant voice exercises at the 24-hr post-baseline time point. These observations suggest that TNF-α/ IL-1β-induced destruction events might be optimally attenuated following resonant voice exercise, at least for some individuals and some conditions of resonant voice exercise.

CONCLUSION

This study shows that individual-specific ABMs had reasonable ability to predict 24-hr biological outcomes of acute phonotrauma. The models in this study coupled with animal models reported elsewhere also show good correspondence with a theoretical framework for inflammation, which postulates different biological results depending on the level of initial biomarker profile and associated treatment. Results from this study added to those from existing studies on vocal fold inflammation and inflammation in other tissues indicate that ABM may represent a powerful computational technique in biological simulation, which provides a real framework to assemble and fit many pieces of the inflammation puzzle together and produces detailed route maps of biological networks. A proposal is that ABM simulation results can be used to chart individualized healing trajectories and help clinicians determine when, where, and how to intervene. The success of this research program will reduce animal testing, have better targeted clinical trials and help to advance the prerogative of personalized medicine.

Acknowledgments

The authors would like to acknowledge the following clinicians and investigators: Dr. Clark Rosen, Dr. Kim Steinhauer, Dr. Ryan Branski and Dr. Elizabeth U. Grillo, for their contributions to this work. We would also like to thank Dr. John Durrant and Dr. Susan Shaiman for providing valuable advice to improve this project, conducted as part of the doctoral dissertation project of Dr. Li.

Source of Funding

The study was funded by the National Institutes of Health grants R01-DC-008290 and P50-GM-53789.

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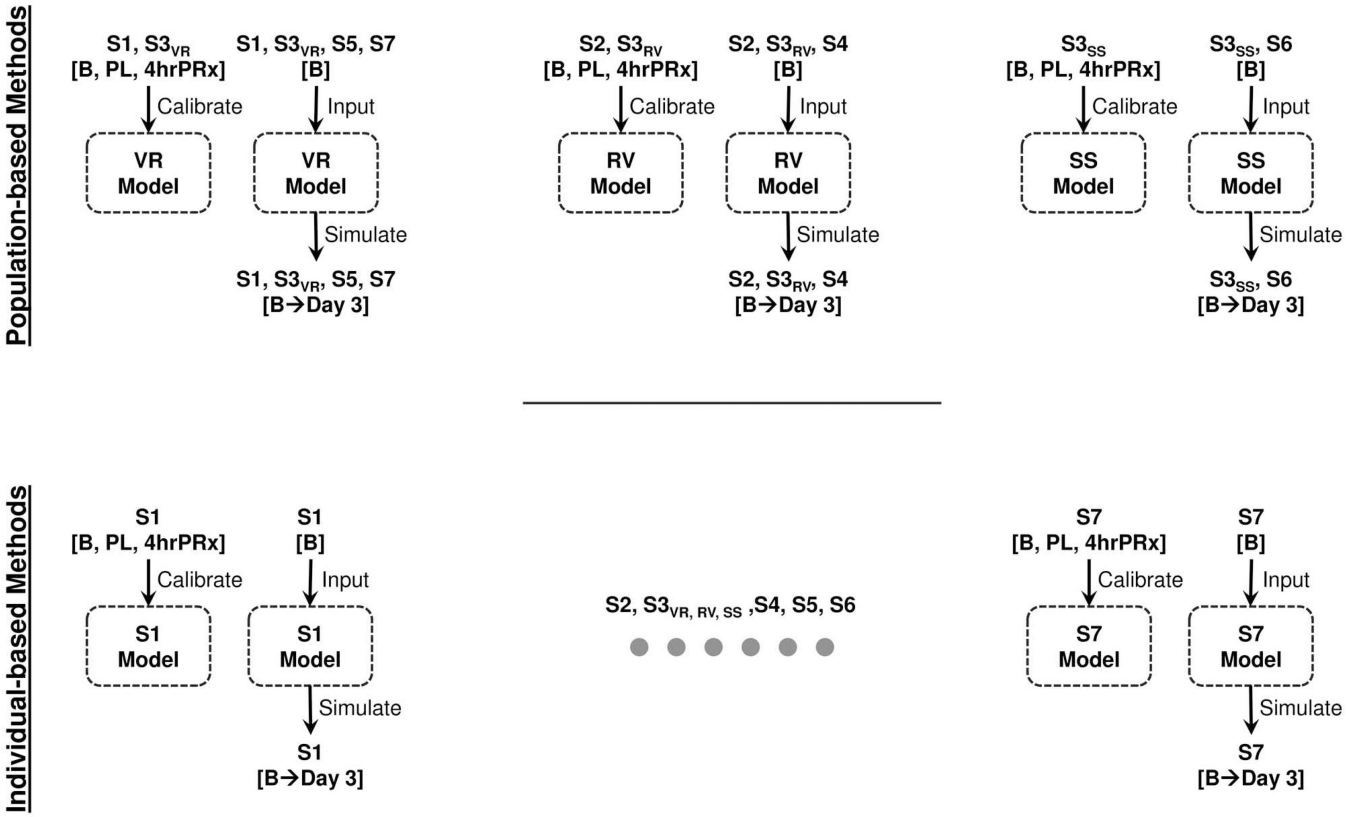

Figure 1. Population-based versus individual-based calibration approaches

For population-based calibration, empirical data for the first three time-points (baseline, post-loading, 4-hr post treatment onset) from Subjects 1 – 3 were used to calibrate the ABMs and to generate three ABMs represent each of treatment assignments. Subsequently, these ABMs were used to predict all seven subjects by inputting individual-specific biomarker profile and their corresponding treatment assignments. For individual-based calibration, each model was personalized to each subject's empirical data. Each subject's empirical data for the first three time-points (baseline, post-loading, 4-hr post treatment onset) were used to calibrate the ABMs and seven personalized models were generated. Subsequently, individual-specific biomarker profile and the corresponding treatment assignment were input to each personalized model for simulation. S: Subject; B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. VR: voice rest; RV: resonant voice; SS: spontaneous speech.

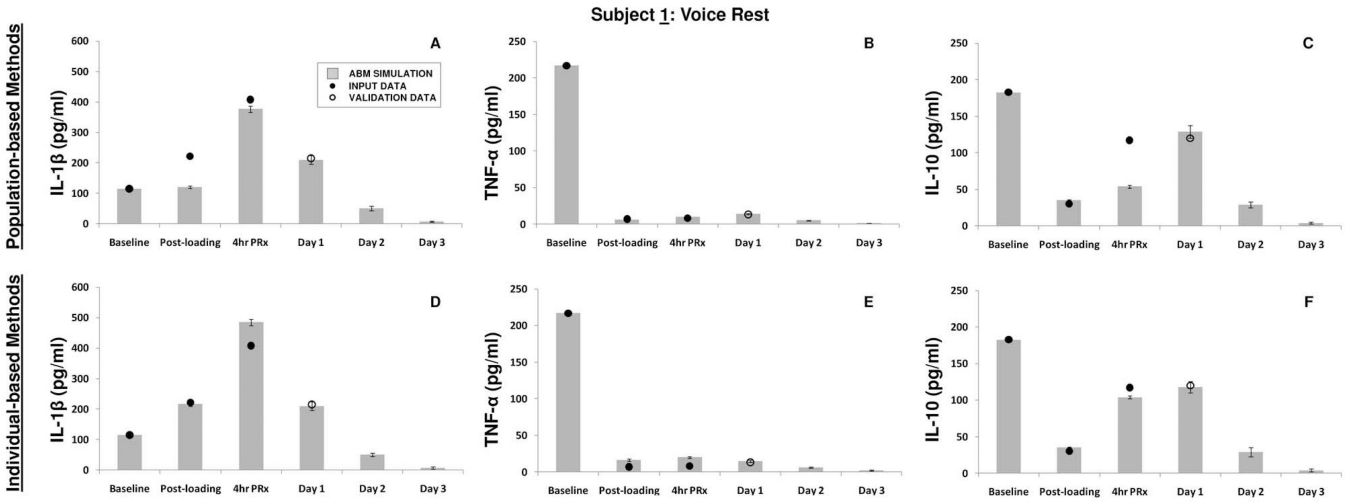

Figure 2. Empirical and model-predicted IL-1β, TNF-α and IL-10 levels in Subject 1 under voice rest condition

Panels A, B, and C display empirical and predicted trajectories of IL-1β, TNF-α and IL-10 following population-based calibration. Panels D, E, and F show empirical and predicted trajectories of the same biomarkers following individual-based calibration. Biomarker concentrations are in pg/ml. The grey bars represent the mean of the simulated data, and the error bars represent 95% confidence intervals in the simulated data. The dark circles represent the input data for the first three time-points (baseline, post-loading, 4-hr post treatment onset), obtained from human laryngeal secretion data. The empty circles represent the validation data at the 24-hr time point from the human laryngeal secretion data. B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. Note that human validation data for Days 2–5 have not yet been generated.

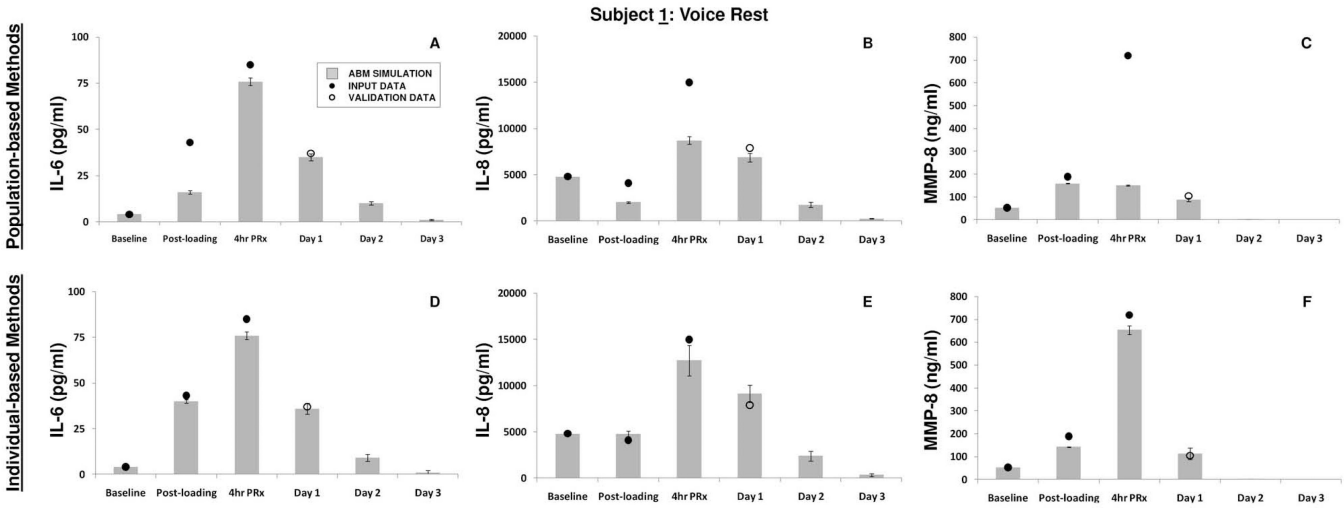

Figure 3. Empirical and model-predicted IL-6, IL-8 and MMP-8 levels in Subject 1 under voice rest condition

Panels A, B, and C display empirical and predicted trajectories of IL-6, IL-8, and MMP-8 following population-based calibration. Panels D, E, and F show empirical and predicted trajectories of the same biomarkers following individual-based calibration. Biomarker concentrations are in pg/ml except MMP-8 in ng/ml. The grey bars represent the mean of the simulated data, and the error bars represent 95% confidence intervals in the simulated data. The dark circles represent the input data for the first three time-points (baseline, post-loading, 4-hr post treatment onset), obtained from human laryngeal secretion data. The empty circles represent the validation data at the 24-hr time point from the human laryngeal secretion data. B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. Note that human validation data for Days 2–5 have not yet been generated.

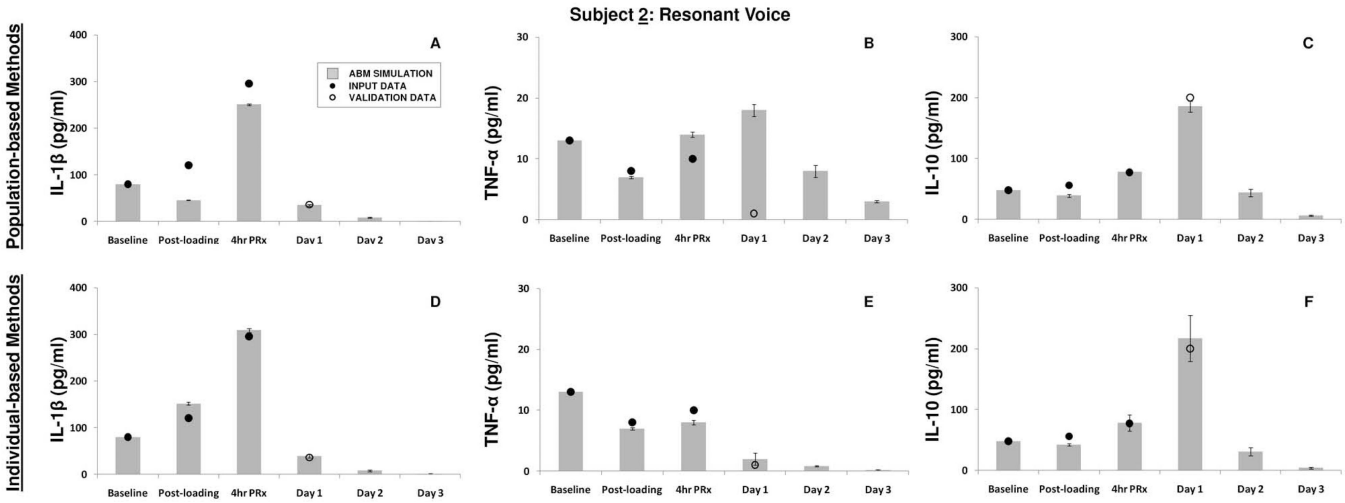

Figure 4. Empirical and model-predicted IL-1β, TNF-α and IL-10 levels in Subject 2 under 4-hr resonant voice condition

Panels A, B, and C display empirical and predicted trajectories of IL-1β, TNF-α, and IL-10 following population-based calibration. Panels D, E, and F show empirical and predicted trajectories of the same biomarkers following individual-based calibration. Biomarker concentrations are in pg/ml. The grey bars represent the mean of the simulated data, and the error bars represent 95% confidence intervals in the simulated data. The dark circles represent the input data for the first three time-points (baseline, post-loading, 4-hr post treatment onset), obtained from human laryngeal secretion data. The empty circles represent the validation data at the 24-hr time point from the human laryngeal secretion data. B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. Note that human validation data for Days 2–5 have not yet been generated.

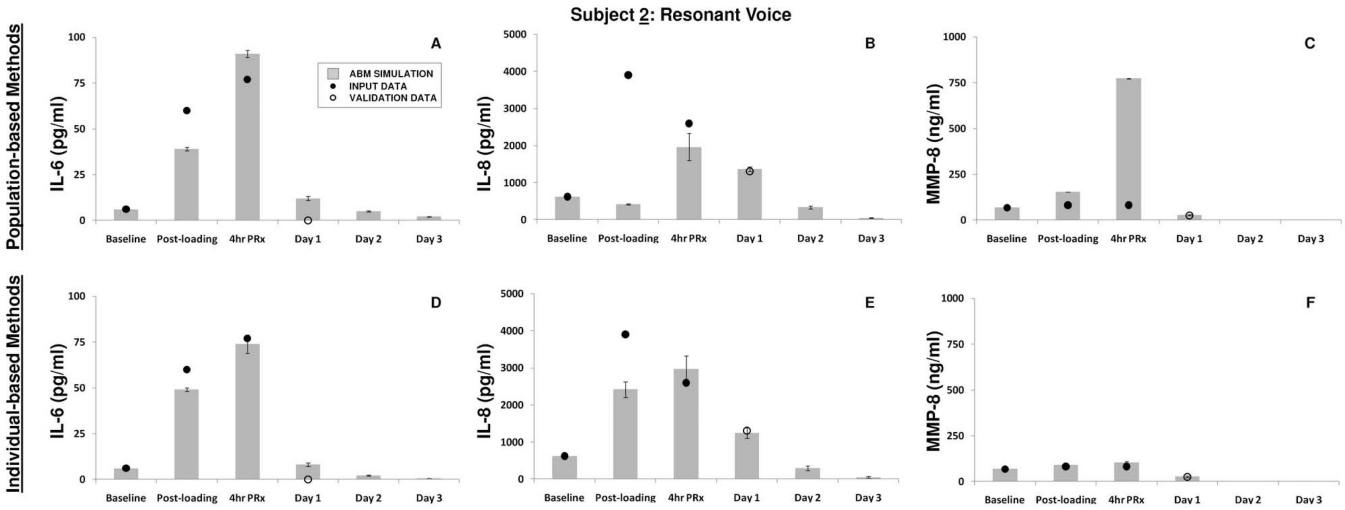

Figure 5. Empirical and model-predicted IL-6, IL-8 and MMP-8 levels in Subject 2 under resonant voice condition

Panels A, B and C display empirical and predicted trajectories of IL-6, IL-8, and MMP-8 following population-based calibration. Panels D, E, and F show empirical and predicted trajectories of the same biomarkers following individual-based calibration. Biomarker concentrations are in pg/ml except MMP-8 in ng/ml. The grey bars represent the mean of the simulated data, and the error bars represent 95% confidence intervals in the simulated data. The dark circles represent the input data for the first three time-points (baseline, post-loading, 4-hr post treatment onset), obtained from human laryngeal secretion data. The empty circles represent the validation data at the 24-hr time point from the human laryngeal secretion data. B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. Note that human validation data for Days 2–5 have not yet been generated.

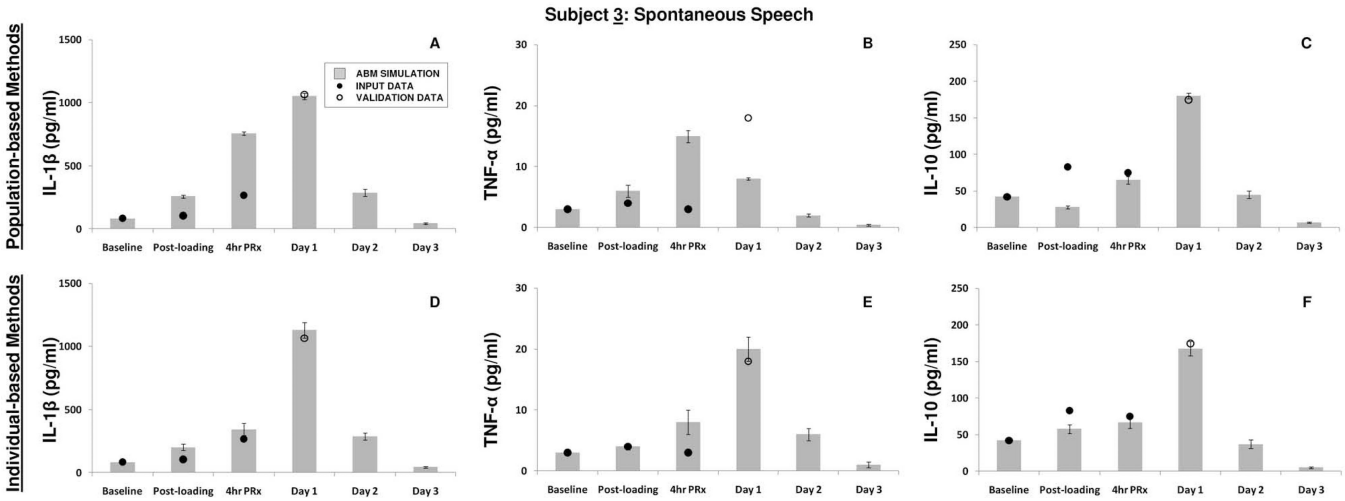

Figure 6. Empirical and model-predicted IL-1β, TNF-α and IL-10 levels in Subject 3 under spontaneous speech condition

Panels A, B, and C display empirical and predicted trajectories of IL-1β, TNF-α and IL-10 following population-based calibration. Panels D, E, and F show empirical and predicted trajectories of the same biomarkers following individual-based calibration. Biomarker concentrations are in pg/ml. The grey bars represent the mean of the simulated data, and the error bars represent 95% confidence intervals in the simulated data. The dark circles represent the input data for the first three time-points (baseline, post-loading, 4-hr post treatment onset), obtained from human laryngeal secretion data. The empty circles represent the validation data at the 24-hr time point from the human laryngeal secretion data. B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. Note that human validation data for Days 2–5 have not yet been generated.

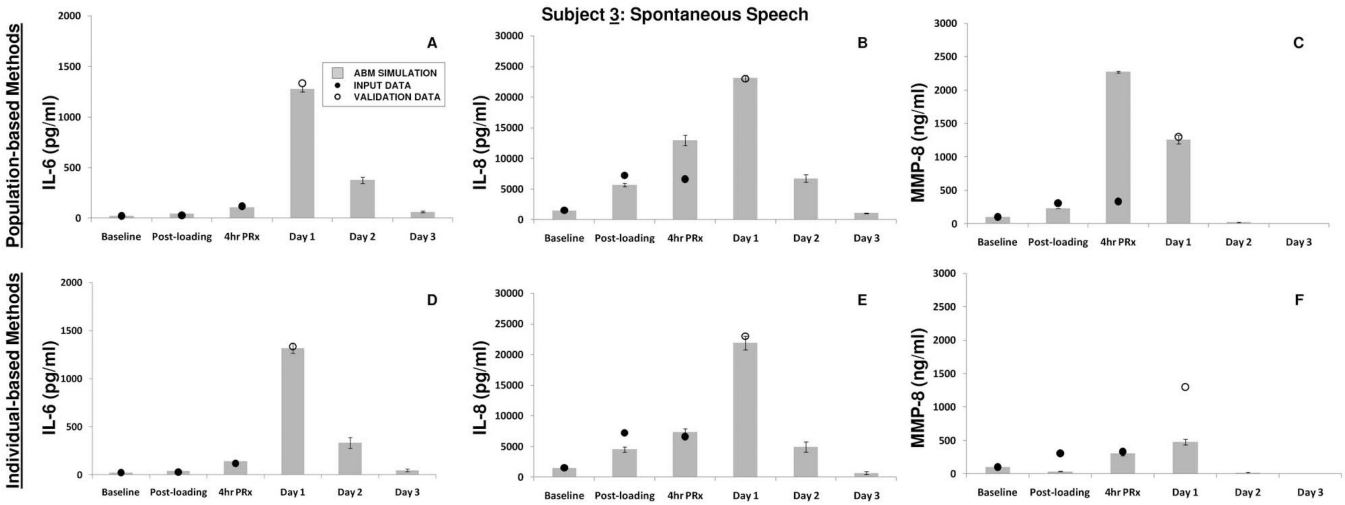

Figure 7. Empirical and model-predicted IL-6, IL-8 and MMP-8 levels in Subject 1 under spontaneous speech condition

Panels A, B, and C display empirical and predicted trajectories of IL-6, IL-8, and MMP-8 following population-based calibration. Panels D, E, and F show empirical and predicted trajectories of the same biomarkers following individual-based calibration. Biomarker concentrations are in pg/ml except MMP-8 in ng/ml. The grey bars represent the mean of the simulated data, and the error bars represent 95% confidence intervals in the simulated data. The dark circles represent the input data for the first three time-points (baseline, post-loading, 4-hr post treatment onset), obtained from human laryngeal secretion data. The empty circles represent the validation data at the 24-hr time point from the human laryngeal secretion data. B: baseline; PL: post vocal loading; 4hrPRx: following a 4-hr treatment. Note that human validation data for Days 2–5 have not yet been generated.

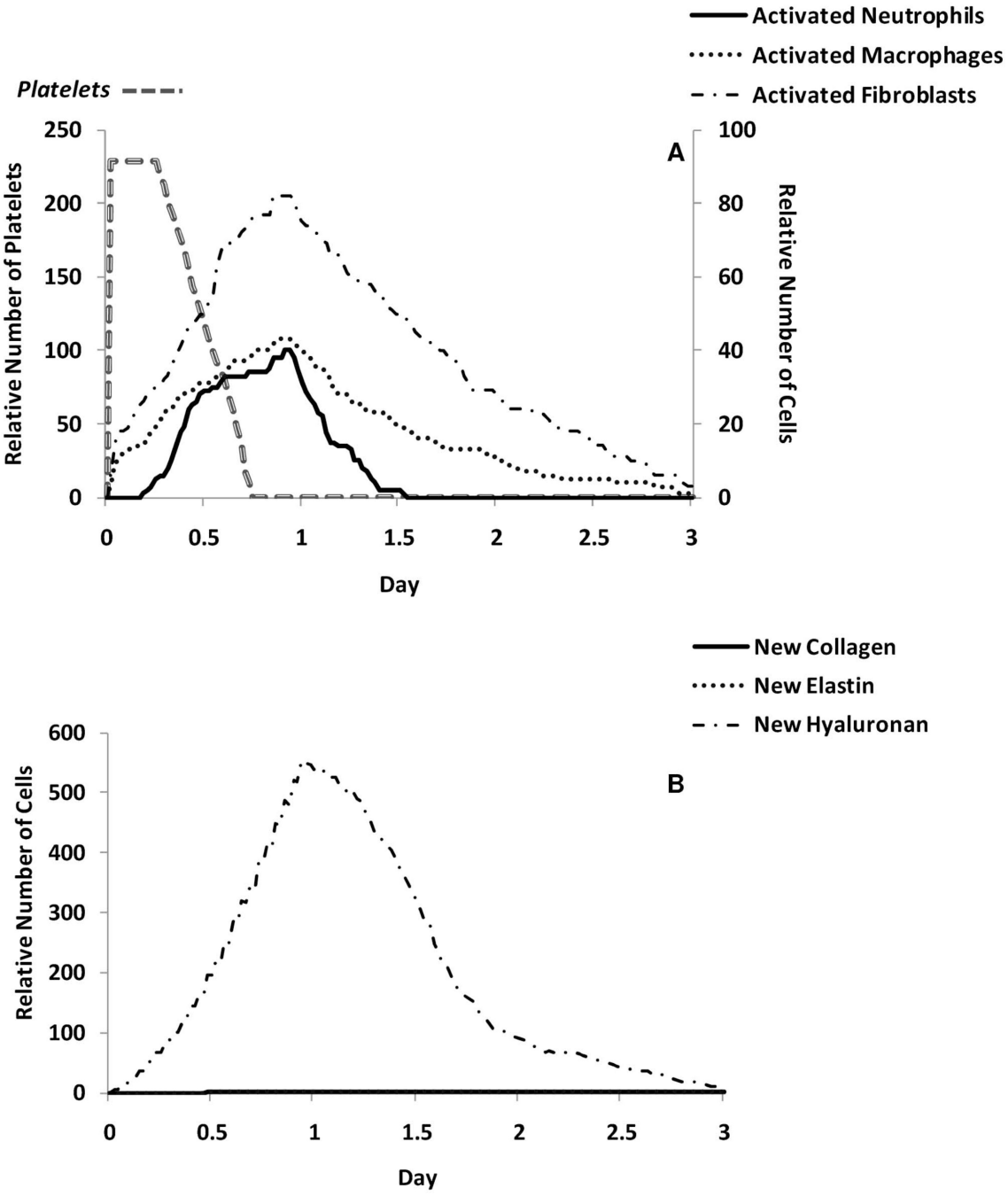

Figure 8. Representative ABM predictions of cell counts and extracellular matrix (ECM) synthesis in human acute phonotrauma up to 3 simulated days following injury

Panel A is predicted cell trajectories for platelets, activated neutrophils, activated macrophages and activated fibroblasts. Panel B is predicted ECM trajectories for new collagen, new elastin and new hyaluronan. No tissue mobilization treatments (resonant voice or spontaneous speech) were applied in these predictions. The predictions are up to 3 simulated days following injury and are in relative units.

Table I
Summary of the components involved in the ABM

The items in *italics* represent the extension of the existing ABM.

Substances	Cell Sources	Biological Functions in Wound Healing used in ABM
TGF-β1	Platelets Macrophages Fibroblasts	Chemotactic to neutrophils, macrophages and fibroblasts
		Inhibit expression of TNF-α in neutrophils, macrophages and fibroblasts
		Inhibit expression of MMP-8 in neutrophils
		Inhibit expression of IL-1β in macrophages (minimal effect)
		Activate resting fibroblasts
		Mitogenic to fibroblasts (proliferation)
		Stimulate collagen synthesis in fibroblasts
		Stimulate elastin synthesis in fibroblasts
		Stimulate hyaluronan synthesis in fibroblasts
bFGF	*Macrophages Fibroblasts*	*Chemotactic to neutrophils and macrophages*
		Mitogenic to fibroblasts (proliferation)
		Stimulate fibroblast migration
		Inhibit collagen synthesis in fibroblasts
		Inhibit elastin synthesis in fibroblasts
		Stimulate hyaluronan synthesis in fibroblasts
TNF-α	Neutrophils Macrophages Fibroblasts	Chemotactic to neutrophils and macrophages
		Activate neutrophils and macrophages
		Stimulate expression of MMP-8 in neutrophils
		Stimulate expressions of TNF-α, IL-1β, *IL-6 and IL-8* in macrophages
		Stimulate expression of TGF-β in macrophages and fibroblasts
		Mitogenic to fibroblasts (proliferation)
		Stimulate expression of IL-6 in fibroblasts
		Inhibit elastin synthesis in fibroblasts
		Stimulate hyaluronan synthesis in fibroblasts
		Induce tissue damage
IL-1β	Platelets Macrophages	Chemotactic to neutrophils and macrophages
		Activate macrophages
		Stimulate expressions of TNF-α, IL-1β, *IL-6 and IL-8* in macrophages
		Mitogenic to fibroblasts (proliferation)
		Inhibit collagen synthesis in fibroblasts
		Inhibit elastin synthesis in fibroblasts
		Stimulate hyaluronan synthesis in fibroblasts
IL-6	*Macrophages Fibroblasts*	*Chemotactic to neutrophils*
		Stimulate collagen synthesis in fibroblasts
IL-8	*Macrophages Fibroblasts*	*Chemotactic to neutrophils*
		Inhibit collagen synthesis in fibroblasts
IL-10	Macrophages	Inhibit expression of TNF-α in neutrophils, macrophages and fibroblasts
		Inhibit expression of IL-1β in macrophages
		Inhibit expressions of IL-6 and IL-8 in macrophages and fibroblasts

Substances	Cell Sources	Biological Functions in Wound Healing used in ABM
		Stimulate expression of TGF-β in macrophages and fibroblasts
		Stimulate expression of IL-10 in macrophages
		Inhibit activated neutrophil survival
		Inhibit activation of neutrophils and macrophages
MMP-8	*Platelets Neutrophils*	*Degrades collagen to produce collagen fragments*
Collagen (type I)	*Fibroblasts*	Collagen repairs tissue damage
		Collagen fragments are chemotactic to neutrophils and macrophages
Elastin	*Fibroblasts*	Elastin repairs tissue damage
		Elastin fragments are chemotactic to macrophages
Hyaluronan (HA)	*Fibroblasts*	*HA repairs tissue damage*
		HA inhibits expression of TNF-α and IL-8 in fibroblasts
		HA inhibits collagen synthesis in fibroblasts
		HA fragments stimulate expressions of TNF-α, IL-1β and IL-8 in macrophage
		HA fragments are mitogenic to fibroblasts (proliferation)
		HA fragments stimulate collagen synthesis in fibroblasts

Table II

Magnitude of ABM-simulated phonatory stress (range 0 – 10 in arbitrary units)

Phonation Type	Magnitude of Simulated Vibratory Stress (in arbitrary unit)	Magnitude of Simulated Impact Stress (in arbitrary unit)
Voice Rest	0	0
Resonant Voice	10	5
Spontaneous Speech	10	10

Table III

Comparison of Model Prediction Accuracy of 24-hr Biomarker Values Between Two Variations of Calibration Methods

Group 1 was subjects 1–3 whose data were used for both calibration methods. Group 2 was subjects 4–7 whose data were not used under population-based calibration but only under individual-based calibration.

Subjects	Population-based Calibration	Individual-based Calibration
Group 1	73% (22/30)	73% (22/30)
Group 2	38% (9/24)	63% (15/24)
All	57% (31/54)	69% (37/54)

Table IV

Comparison of Model Prediction Accuracy of "Valid" Biomarkers Versus "Non-responsive and/or Pre-inflamed" Biomarkers

Data were from the between-subject group at the 24-hr time point under two variations of calibration methods.

Markers	Population-based Calibration	Individual-based Calibration
Valid	82% (14/17)	82% (14/17)
Non-responsive and/ or Pre-inflamed	36% (9/25)	60% (15/25)