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Model First and Ask Questions Later: Confessions of a Reformed Experimentalist

This paper is an invited perspective written in association with the awarding of the 2018 American Society of Mechanical Engineers Van C. Mow Medal. Inspired by Professor Mow's collaboration with Professor Michael Lai and the role mathematical modeling played in their work on cartilage biomechanics, this article uses our group's work on myocardial infarct healing as an example of the potential value of models in modern experimental biomechanics. Focusing more on the thought process and lessons learned from our studies on infarct mechanics than on the details of the science, this article argues that the complexity of current research questions and the wealth of information already available about almost any cell, tissue, or organ should change how we approach problems and design experiments. In particular, this paper proposes that constructing a mathematical or computational model is now in many cases a critical prerequisite to designing scientifically useful, informative experiments. [DOI: 10.1115/1.4043432]

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Introduction

I was offered the opportunity to write this article as the winner of the 2018 Van C. Mow medal. Receiving the medal is a tremendous honor, because it is conferred by the professional society I have always considered my primary academic and scientific home. It is also special for me on a personal level, because Van Mow was the department chair who hired me for my first faculty job, and I had the chance to work closely with him in building a new Department of Biomedical Engineering at Columbia University. In thinking about a topic that would be fitting for this occasion, my thoughts kept returning to Van's partnership with Mike Lai, and the role that mathematical modeling played in their efforts to understand the biomechanics of cartilage [1–3]. I realized how dramatically my own view of modeling has changed over my career: my Ph.D. thesis was 90% experimental, with a small modeling chapter thrown in at the end more or less because my committee made me do it; 20 years later, I am not sure we should ever do another experiment in the lab without making a model first.

This evolution in thinking about the interplay between modeling and experiments was driven by repeated attempts to test an apparently straightforward hypothesis about heart mechanics and wound healing. However, our own experience is just one example

of broader trends in cardiovascular biomechanics and in biomedical science that are increasing the importance of modeling. We are no longer solving the “easy” problems, if there ever were any. Any cardiovascular device or drug we design today will be deployed against a background of the current standard of care, which will likely include multiple drugs and possibly other devices or interventions. Data on gene expression and other aspects of cardiovascular biology are so readily available that most of the challenges lie in interpreting the data rather than acquiring it, a situation reflected in the rise of both biomedical data sciences and personalized medicine. In this article, I use our attempts to answer a specific question about myocardial infarct healing as an illustrative example to argue that the complexity of the problems we are addressing should change how we make our initial approach. Or perhaps it would be more accurate to say that I am arguing that none of us is making an initial approach. We are all starting in the middle of the story, and to make progress we must build on prior work. I have come to believe that models offer the most useful summary of that prior work, because they facilitate the development of specific, quantitative hypotheses to guide informative experiments.

The Question: What Determines Collagen Alignment Following Myocardial Infarction?

One of my primary research interests is scar formation after myocardial infarction (MI). Following MI, damaged muscle is

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replaced by scar tissue, and over the years we have discovered that the alignment of the collagen fibers in that scar varies in different animal models [4,5] and infarct locations [6]. This is potentially important because we have also shown that scar anisotropy is an important determinant of heart function [7–9], and therefore might be a target for therapeutic manipulation [10,11]. However, it is difficult (and risky) to manipulate something we do not understand, so from the beginning of our work we have sought to answer the seemingly simple question of what determines collagen alignment in postinfarction scar.

The Hypothesis

Our first clue emerged from a series of descriptive experiments where we simply tracked the evolution of regional mechanics following myocardial infarction in pigs and then measured collagen structure in the postinfarction scar at the end of the study [4,12]. In a normal heart, the fibers on the epicardial (outer) surface run diagonally at an angle of about 60 deg clockwise from the circumferential (hoop) direction. This angle rotates gradually with depth into the wall, until at the inner surface the fibers are oriented roughly 60 deg counterclockwise (Fig. 1). In postinfarction scar, we found that this transmural gradient was shallower, with most collagen fibers oriented near the circumferential direction. At the same time, we learned that while normal myocardium shortens to a similar extent in both the circumferential and longitudinal directions during each heartbeat, these infarcts stretched primarily in the circumferential direction throughout healing. These results

Fig. 1 Muscle and collagen fiber orientation and mechanics in healing pig infarcts: (a) schematic showing the circumferential (0 deg) and longitudinal directions (90 deg) used to define all orientation measurements in this article. Muscle fiber orientation normally varies with depth between the outer surface (epicardium) and inner surface (endocardium). (b) In this experiment, the orientation of undamaged muscle fibers varied from -55 deg to $+60$ deg (gray bars), while the mean orientation of collagen fibers 3 weeks after infarction ranged from -45 deg to $+30$ deg (red bars, data re-analyzed and replotted from Ref. [4]; epi = epicardium, endo = endocardium). (c) Tracking midwall strains using implanted radiopaque markers in this same animal model showed that healing infarcts stretched primarily in the circumferential direction (black bars) with little stretch in the longitudinal direction (gray bars), suggesting a role for mechanics in influencing scar collagen structure (based on data reported in Ref. [12], control = immediately prior to infarction, acute = 15 min after coronary ligation).

suggested the hypothesis that would prove so difficult to test: that local mechanics during healing are the primary determinant of collagen fiber orientation in postinfarction scar.

The Test, Version 1: Do Hundreds of Experiments

Experimentally testing the hypothesis that local mechanics are the primary determinant of collagen fiber orientation in postinfarction scar requires a method for altering mechanics. But it also requires a list of alternative hypotheses, so that the experimental design can both test the primary hypothesis and exclude the alternatives. At the time, the most obvious alternative hypothesis was contact guidance—that pre-existing extracellular matrix (ECM) surrounding myocytes in the normal heart would survive the initial injury and provide a template to guide fibroblast alignment and collagen deposition during healing. Thus, our first experiment [13] contained an untreated group, a group where we altered mechanics by making a cut to physically disconnect the infarct from adjacent muscle in the circumferential direction (slit group), and a group where we altered the pre-existing matrix template by removing a block of infarcted tissue, rotating it 90 deg, and suturing it back into position (plug group).

The results proved impossible to interpret definitively. The slit gradually scarred closed over the course of the experiment, producing a time-varying mechanical stimulus and relatively modest alterations in collagen structure. It proved technically difficult to fully reconnect the rotated plug to surrounding tissue, so the plug group ended up with altered mechanics as well as an altered ECM template. And in both cases the surgical procedures likely altered cell migration and chemokine gradients, factors that were difficult to measure and even more difficult to control in vivo. We concluded that deformation, pre-existing ECM, and cell migration all played a role—an unsatisfying statement we could have made before performing the experiment [13].

As I transitioned through a postdoctoral fellowship and into my first faculty position, I did what my experimental training had taught me to do: I took a step back and developed an in vitro system where I could better control potential determinants of fibroblast and collagen alignment [14]. Together with Kevin Costa, a colleague at Columbia, we showed that mechanical boundary conditions alone are sufficient to drive fibroblast alignment and collagen reorganization in fibroblast-populated collagen gels [15–17], and that mechanics can overcome contact guidance when those cues are intentionally placed into conflict with one another [18]. Yet in many ways our work in collagen gels brought us no closer to testing our original hypothesis. While we had shown that mechanics could dominate other alignment signals in some situations, refining our experiments to test all of the cues that might plausibly drive fibroblast alignment in a healing infarct—alone and in combination, across ranges that reasonably reflect the in vivo setting—would have taken many more years.

The Test, Version 2: Do Dozens of Experiments, and Use a Model to Interpret Them

Around this time, I moved to the University of Virginia, where the Biomedical Engineering department houses an outstanding systems biology group [19]. Andrew Rouillard, a graduate student in the lab, realized something during a systems biology class that completely changed how we thought about understanding scar formation. The problem was not that we did not have enough data on how fibroblasts behave, it was that we did not know how to predict behavior in settings (like in vivo wound healing) where cells were integrating multiple different signals at once. Starting from a class project, Andrew built a data-driven, agent-based model (ABM) of scar formation in the heart [20]. Cellular alignment responses to each individual signal (stretch, contact guidance, chemokine gradients, etc.) were governed by experimental dose-response curves based on published in vitro studies, with a

Fig. 2 ABM predictions of collagen alignment in multiple animal models. (a) schematic illustrating two rat cryoinfarct locations and shapes for which data and predictions are presented here. (b) Cryoinfarcts at the equator healed with circumferentially aligned collagen, regardless of the infarct shape, as illustrated in this histologic section stained with picrosirius red and imaged under circularly polarized light to highlight collagen fibers. (c) By contrast, cryoinfarcts at the apex healed with randomly oriented collagen. (d) The model predicted preferred alignment in the circumferential direction for rat cryoinfarcts located near the equator (red dotted line) but nearly random orientation for cryoinfarcts located at the apex (blue solid line); both predictions matched experimental measurements 3 weeks following infarction (red circles and blue triangles mean $\pm$ SD; model results replotted from [6], data replotted from [20]). (e) Simulations of multiple transmural layers in healing pig infarcts with the same ABM predicted shifts in the transmural distribution of fiber angles that closely matched our previously reported data (see Fig. 1).

simple first-pass guess about how to model the integration of multiple cues. Remarkably, this model reproduced measured in vivo collagen fiber alignment across multiple animal models (Fig. 2) and even explained the shifts in transmural fiber angle distribution we had observed in our initial pig experiments nearly two decades earlier [6,20].

The fact that the model fit so much data from different experiments provided some confidence that we were capturing some of the key features of scar formation. Parameter sensitivity studies then allowed us to develop a better understanding of how the model behaves, and why. In the model, mechanics are the primary determinant of cell and collagen alignment, but not because we weighted mechanics more heavily when integrating alignment cues. Instead, strains in a healing infarct are large enough to induce near-maximal alignment based on in vitro stretch-alignment curves, while the levels of other signals such as chemokine gradients are quite low relative to measured response thresholds. Understanding how a model behaves might be interesting in its own right, but for me the key point is that the model represents a quantitative, specific hypothesis (or set of hypotheses) consistent with available data about what factors guide scar formation and how they interact.

The Test, Version 3: Use a Model to Design One Really Good Experiment

If a model is a quantitative hypothesis, it should also be a powerful tool for designing experiments to test that hypothesis. Armed with our model, we returned to the idea of perturbing regional mechanics. We modeled simple, surgically feasible experiments: sewing Dacron patches over infarcts to remove stretch in the circumferential direction, the longitudinal direction, or both directions. Circumferential reinforcement provided the most interesting potential test of the role of stretch, since the model predicted that collagen in the midwall of the heart would gradually align perpendicular to the pre-existing matrix (Fig. 3). Importantly, the time course of that realignment is determined primarily by the relative weighting of stretch versus contact guidance in the model, so mapping this time course experimentally would provide not only a test of the hypothesis that mechanics are the dominant signal, but also a test of the degree to which contact guidance plays a secondary role. When we performed these experiments [21], collagen aligned longitudinally as predicted, confirming the dominant role of stretch in determining collagen orientation (Fig. 3). The collagen aligned more quickly and more

Fig. 3 Predicted time course of midwall collagen alignment with and without circumferential surgical reinforcement in the rat. ((a) and (b)) The ABM predicted that the mean angle of collagen fibers would remain near 0 deg in the untreated animals (baseline), while surgical reinforcement (patch) will cause collagen accumulation in the direction of the predominant longitudinal strain, causing the mean angle to switch to longitudinal (± 90 deg) by 3 weeks. The experimental data showed the predicted reorientation of collagen, but it occurred more quickly than predicted by the model, suggesting a less important role for contact guidance in vivo than assumed in the model. (Note: error bars not shown because mean vector length presented in panels C and D quantifies the variation between subjects.) **((c) and (d))** The ABM predicted that mean vector length (a measure of collagen alignment ranging from 0 to 1) would decrease to near zero (random) in untreated rat infarcts (baseline), while surgical reinforcement (patch) would cause a drop and then rise in alignment strength as longitudinal deposition initially dilutes and then overwhelms the pre-existing collagen. The experimental data showed higher alignment than predicted, again reflecting a more dominant role for mechanics versus other factors than assumed in the model.

strongly than the model predicted, suggesting that contact guidance exerts less influence in vivo than we expected. These experiments also confirmed that we are approaching one of our primary goals—prospectively predicting responses to perturbations well enough to begin using the model to design novel therapeutic interventions.

Lessons Learned: Why Not Model First?

By far the most important lesson I learned from this odyssey is that we should be much more aggressive about making models when we first approach a problem. A model is a quantitative, concise summary of (some of) what is already known, and that is never a bad place to start when designing a new experiment. The act of constructing the model helps drive the initial literature review and in my experience tends to force that review to be more comprehensive than it might be otherwise. Similarly, the act of formulating equations rather than worded hypothesis statements

requires attention to time constants, concentrations, and other factors that are critical in designing good experiments.

Once we engage with a problem, we enter a cycle of iterating between model and experiment. Many others have written eloquently about this cycle [22–26] and the influence of simulation on science more generally [27]. For example, in his cardiovascular solid mechanics textbook [28], Jay Humphrey first discusses the general scientific framework of making an observation, developing a hypothesis, testing that hypothesis experimentally, and refining the hypothesis and iterating. He then specializes this framework for the problem of formulating, testing, and refining constitutive models. Most of us learn and adopt a similar framework during our training, but in my experience the divide that often arises between experimentalists and modelers can prevent needed crosstalk. Modelers readily accept that they need experimental data to test and refine their models, but may be less likely to view themselves as playing a critical role in designing new experiments. Experimentalists are certainly comfortable with

formulating and testing hypotheses, but often less comfortable with using a model as part of that process.

For students entering the field today, does that mean that the demands of training have doubled? Becoming good at any one technique in any one field is demanding enough; it would be daunting to think that everyone must become an expert in both experimental and computational aspects of his or her discipline. I believe the answer lies in collaboration. Just as many of us work closely with physicians when seeking to improve medical therapies, collaboration allows experimentalists to incorporate modeling into their work, and modelers to integrate experimentation. At its best, the conversation between collaborators continually challenges each partner and improves the quality of all aspects of the work. In fact, the turning point in our own work on scar healing came from engaging with systems biology colleagues who challenged us to think differently about both the benefits of modeling and the types of modeling approaches we employ.

Fig. 4 Proposed explanation for the failure of MMP inhibitors to alter collagen content in healing scar (simulations based on model first reported in Ref. [10]): (a) a simple single-equation model with time-varying MMP concentrations derived from literature (gray bars) provides a good match to measured collagen area fractions in healing rat infarcts (red bars, mean $\pm$ SD). Simulated 75% inhibition of MMPs (black bars) takes several weeks to produce an effect size that would be detectable given the variability in the measurements. (b) Reported MMP activity (green dashed line) peaks within a few days after infarction in rodents and then falls, while collagen gradually accumulates (blue solid line); the product of these curves (gray dotted line) reflects the expected rate of collagen degradation, which rises much more slowly than would have been expected from the MMP curve alone.

Another factor that may make incorporating models into experiments seem less daunting is that even models as simple as a single equation can improve experimental design. For example, in the early 2000s there was a burst of studies that tested the hypothesis that inhibiting matrix metalloproteinases (MMPs) would enhance collagen accumulation in healing infarct scars. This hypothesis is so straightforward that it seems nearly destined to be true, and so simple that nobody would have thought to model it. Collagen accumulation is a balance between deposition and degradation, so blocking degradation will obviously increase accumulation. Yet in multiple published studies using both pharmacologic inhibitors and genetic knockouts, scar collagen content did not change. We showed recently that modeling collagen turnover with a single differential equation provides a plausible explanation for this surprising result [10]. The equation

$$\frac{dc}{dt} = k_{\text{dep}} - k_{\text{deg}} * c$$

where c is the collagen concentration, k_{dep} is a rate constant for collagen deposition, and k_{deg} is a rate constant for collagen degradation, is a straightforward and simple representation of collagen turnover. Yet prescribing literature-derived time-varying values for k_{deg} based on reported fibroblast numbers and levels of collagen gene expression and for k_{deg} based on reported MMP activity provides an excellent fit to measured collagen concentrations or area fractions (Fig. 4). More importantly, this equation highlights an uncontroversial but key concept: that the rate of degradation of collagen by MMPs is proportional both to activity of MMPs (the enzyme) and to the concentration of collagen (the substrate). Because collagen concentrations are very low in the first week or two following infarction, degradation rates are also low, and there is little impact from simulated MMP inhibition until much later time points (Fig. 4). Yet many of the published studies waited only 1–2 weeks to assess the impact of their interventions. This model certainly leaves out many of the complexities of MMP regulation and processing of collagen, but making this model first would have allowed the subsequent studies to reach more definitive conclusions by including later time points and/or including additional measurements to assess each of the terms affecting collagen degradation.

Other lessons from our work on scar formation are not specific to modeling. The most exciting developments in our research arose from encouraging students to take risks, from enrolling in courses outside their comfort zone to pursuing science far outside our lab's comfort zone (with good support from other committee members). Another lesson learned over the years is to be as flexible as possible about methods—we have used a much wider range of modeling and experimental approaches than I would have ever thought possible—while remaining stubborn about our objectives.

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