Towards Mechanistically Explanatory Biomimetic Analogs for Fracture Healing

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Abstract

Impaired bone healing is a medical challenge that entails significant patient morbidity and cost to society. There are now two competing theories about the mechanism of bone healing. One theory has cartilage cells fill the fractures site and turn into bone cells. The other theory has the cartilage cells die and be replaced by invading bone cells. Both theories are supported by wet-lab studies. Deciding on the correct mechanism or combining the mechanisms into singular theory will necessitate several costly wet-lab experiments. We present a method by which repeated cycles of virtual (in silico) experimentation together with the occasional necessary wet lab experiments are used to advance mechanistic insight into the true mechanism of bone healing. Biomimetic analogs are developed using a variety of models of computation to simulate biologic phenomena. Target Attributes are defined together with similarity measures and are used in an Iterative Refinement protocol for implementing and validating/falsifying mechanistic hypotheses.

Keywords: Computational biology, bone healing, in silico, mechanisms, requirements, software engineering, virtual experimentation

1 Introduction

The biomedical problem on which we focus is impaired bone healing. Long-term, we seek improved therapeutic interventions that result from actionable explanatory modeling of bone healing. Currently we lack modeling systems that can adequately explain the complexity and multifactorial attributes of biological healing. We argue that experimenting on different yet equally plausible concretized adaptive bone healing mechanisms that have been instantiated in software can be a fruitful new means to improve explanatory insight into this challenging, multiscale problem. Doing so is expected to enable discovering novel intervention strategies that otherwise may not be apparent. The envisioned approach is not yet operational. Making it so has proven challenging because requirements specification draws simultaneously on state-of-the-art best practices in software engineering, in vitro biomedical research, and translational science. We characterize improving mechanistic insight into impaired bone healing phenomena as the pursuit of (software) mechanistic models that 1) better explain impaired bone-healing phenomena and 2) enable scientifically useful virtual experiments. A brief summary of the biomedical problem helps explain why we will need models designed to satisfy a variety of use cases, and provides an essential contextual foundation for our approach to requirements specification.

1.1 Synopsis of Impaired Bone Healing

Of the patients who sustain fractures each year in the United States, approximately 800,000 have impaired bone healing [8]. Management of impaired bone healing (delayed unions and non-unions) entails considerable cost, risk, and patient discomfort [2,16]. Autologous bone transplantation are the most common clinical treatment to enhance bone healing [1], making bone the second most commonly transplanted tissue. However, the procedure faces a number of significant problems: limited donor tissue availability, the requirement for additional surgeries, and donor site pain. Bone graft substitutes and bone tissue engineering represent alternatives, yet despite recent progress, failure rate remains in the range of 15-35% [10,21]. Given the prevalence of these failures new therapeutic strategies for improved bone healing are necessary.

1.2 Synopsis of Cell and Molecular Level Events

During fracture repair the majority of bones heal through the process of endochondral ossification, in which bone forms secondarily to cartilage [20]. In the axial skeleton this is also the mechanism by which long bones form. Mechanistically, endochondral bone repair is *presumed* to parallel developmental cellular and molecular sequences. During long bone development, mesenchymal cells condense and undergo chondrogenic differentiation to form a cartilaginous model of the future bone [18]. Chondrocytes within the bone anlagen, organize into functionally distinct domains, begin secreting the molecules that form the extracellular matrix, and undergo maturation into hypertrophic chondrocytes. Current models hold that these hypertrophic chondrocyte undergo programed cell death (apoptosis), and that blood vessels

invade the matix to degrade the cartilage and form bone from invading osteoprogenitors.

Bahney *et al*. experimented recently with using cartilage rather than bone grafts for treatment of tibia bone defects in a mouse model [3]. Genetic labeling of the donor and host tissue enabled identification of the source of generated bone. In contrast to the current models, the new bone formed from the transplanted chondrocytes. If verified, we must conclude that, at least in mice, there is a second mechanism of bone formation, one in which chondrocytes transform directly into osteoblasts. Improving explanatory insight into a potentially new mechanism of bone formation is expected to identify opportunities for new therapeutic interventions.

1.3 Toward Virtual Experiments

It is now understood that there can be multiple, equally plausible, mechanistic explanations for generating any particular complex phenomenon such as bone healing [15]. However, it should be obvious that it is infeasible to rely exclusively on using costly wet-lab experiments to explore possible mechanisms space and shrink it to a manageable set of more plausible mechanisms that merit challenge. As has been argued elsewhere [12,13] the most efficient path to more effective bone repair intervention strategies is expected to involve extensive virtual experimentation [17]: many cycles on the right side of Figure 1 coupled with the occasional but essential cycle through the left side. Each Fig. 1 cycle illustrates key features of the scientific method. That vision is enticing. However, we are very early stage. We face a series of significant, multifaceted technical and scientific barriers. Because of those barriers, even today, essentially all of biomedical research is confined to the left side of Fig. 1. This project focuses on identifying right-side barriers, discovering software solutions, developing means to implement those solutions, and then revise those means iteratively and easily as mechanistic insight improves.

1.4 Barriers

The right side of Fig. 1 requires utilizing software devices that can be adapted easily to the demands of current cycle and the new demands of the next several cycles. We need to discover possible mechanisms yet, as is often the case with medical problems, we must start with vague, coarse grain ideas about mechanisms. *That is a barrier.* We need the right side framework to facilitate mechanism discovery. Left side biological mechanisms, even when unknown, are real and concrete. The mechanisms on the right side will need to be biomimetic and concrete, yet there are no guidelines for specifying the requirements for software devices that can both facilitate discovery and, at a different level, function as concrete biomimetic mechanisms. *That is the second barrier.* The best documented scientific protocol for discovering mechanisms is broadly recognized to require transitioning through three critical stages: instantiating a candidate mechanism schema (hypothesis; Fig. 1a), modular subassembly (Fig. 1b), which for us depends on well-designed software components, and forward/backward chaining [6], which for us requires in silico experimentation (Fig. 1c). *The latter is the third barrier*: the right side components must be sufficiently adaptable and flexible so that we can easily revise and reassemble (reuse and repurpose) them as insights into plausible mechanistic features evolves. Influencing all of the preceding is the process by which we specify and select phenomena to be explained (Fig. 1d).

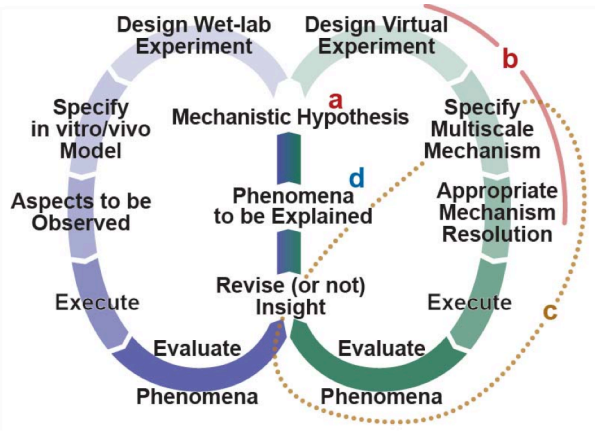

Figure 1. Coupled use of wet-lab and virtual experiments. Both sides are characterized by a scientific flow from design through interpretation. Iteration is essential to gradual success on both sides. Today, it takes much longer to complete a right side cycle: it is easier (but more costly) to complete a left side cycle. An additional focus of the research is reversing that relationship. **a**, **b**, **c** & **d** are discussed in the text.

2 Approach

Computer scientist and software engineers occasionally draw inspiration from how biology solves problems and overcomes seemingly daunting obstacles. We are exploring the reverse process: we anticipate that we can adapt advances in software development and requirements engineering to demonstrate progress in eroding the above barriers. The tasks described above (**1.4**) clearly require multiple different features. We are exploring adapting aspects of feature-oriented software development [5]. Because the right side method will be perpetually iterative, we are also exploring adapting aspects of the iterative criteria-based approach to engineering the requirements of software development [23].

2.1 Targeted Attributes

Requirements of software mechanisms are influenced by the type and granularity of wet-lab data that will serve as validation targets. Consequently, identifying the attributes to be targeted is a necessary and essential first step, and that is discussed in section **3.2**. A validation target is a wet-lab measured bone-healing attribute, a targeted attribute (TA), coupled with prespecified "similarity meaure" (SM). We anticipate that a networked set of analog mechanisms (Fig. 1, right side) capable of generating phenomena similar to a prespecified set of bone-healing attributes, the phenomena to be explained—Fig. 1d, will stand, at least temporarily, as a theory of bone healing. The approach is somewhat analogous to the following: We taste a slice of cake and ask, what were the ingredients; what recipe was used? If you can make a cake that tastes like the original slice, then we can claim that we chose the right ingredients.

Our approach to achieve an initial, working bone-healing theory requires moving forward in small steps, and each step is one completed cycle on the right side of Fig. 1. A single cycle, described below under Iterative Refinement Protocol, advances in stages that combine the scientific method and good software engineering practices.

2.2 Agent-based Models

Salient characteristics of referent wet-lab experiments include pervasive uncertainty, sparse system information, and considerable variability [12]. They make distinguishing causes from effects difficult. Agent-based methods provide the flexibility, extensibility, and generality needed to assemble software mechanisms that are increasingly biomimetic during execution. We use ***analog*** to identify variants of an agent-based model (ABM) in order to emphasize that simulated mechanisms are intended to be analogous to a particular biological counterpart. The biology of bone healing is somewhat compartmentalized. Analogs need to be similarly compartmentalized. Many biological processes are analogized as logical statements; for instance, if protein A binds to protein B; then protein B is activated. Agents will implement similar rule-base behavior when mediating interacting components. We require analogs to be multiscale to mimic biological phenomena believed to involve components interacting at different functional levels. An analog-to-wet-lab mapping has a spatial and temporal scale. Biological spatial scales typically correspond to functional "levels" being observed. A wet-lab experiment's temporal scales typically correspond to sets of measurements at intervals. Likewise, the state of different analog components (often at different spatial scales) must update at different time steps/simulation cycles. Our goal is that analog measurements made at different spatial and temporal scales during the same simulation can be mapped separately and quantitatively to wet-lab measurements—TAs—also spanning different spatial and time scales. Achieving that goal requires relational grounding throughout the analog [14]. Consequently, specific mapping models are expected to vary.

3 Methods

3.1 The Iterative Refinement Protocol

Our use cases are in silico experiments that mimic the wet-lab experiments from which the TAs were selected. Our core method is the Iterative Refinement (IR) Protocol [22,24]. We approach credible validation by achieving validation targets, and a validation target is achieved by cycling many times through the IR Protocol.

1) Gather possible TAs from the literature and recent experiments, taking into account the mechanistic granularity that may be required to validate and select any one TA. Choices are constrained by a strong parsimony guideline and the requirement that we do not disrupt already achieved TAs.
2) Initially specify, and later increase mechanistic granularity parsimoniously. Mechanistic over-granularization can greatly expand analog behavior space and the set of parameterizations that enable validation. A good practice is to take smaller steps that mostly fail (Step 7). In doing so we accumulate evidence for how and why we are shrinking possible mechanism space.
3) Update similarity measures (SMs), their target values, and how they are used.
4) Specify a mechanism revision hypothesis. Typically, there are multiple, equally possible or plausible options.
5) Specify an analog revision plan. So doing may include (or not) revising modules, components, model use case(s), parameters, rules, and parameterization ranges. It is most common to modify parameterizations. Random sampling of a relatively small region of parameter space enables us to observe a consistent analog phenotype.
6) Conduct and measure simulation experiments.
7) A failed mechanism (cannot achieve the validation target), even when coarse grain, provides new knowledge and shrinks plausible mechanism space. If it fails, then return to Step 4 or 5. If successful, then we have achieved a degree of validation. We anticipate cycling through Steps 4-6 several dozen times before achieving a particular validation target.
8) Afterward, we have two options: a) increase stringency of one or more SMs; so doing may falsify the mechanism. Or b) select another TA from the list. During previous projects, it was not unusual for insights achieved or observations made during an IR Protocol cycle to alter opinions about and priorities of listed TAs.

3.2 Toward an In-Silico Analog of Bone Fracture Healing

At the most coarse grain, we can specify two equally plausible mechanism schemas for fracture healing based on the common pathway characterized by this sequence of transformations (state changes): progenitor cell → chondrocyte (C) → hypertrophic chondrocyte (HC) → mineralized HC (MHC) → bone (B). *Mechanism 1*: some HCs undergo apoptosis (programmed cell death), and then osteoprogenitor (OP) cells migrate into their empty lacunae; subsequently, they differentiate into osteoblasts and begin laying down mineralized bone matrix. *Mechanism 2:* HC are triggered to transform into osteoblasts and generate their own mineralized bone matrix.

The wet-lab system is bone healing in mice over four weeks. The system attributes—TAs—undergoing change can be assigned to seven categories: 1) vascularization, 2) innervation, 3) apoptosis, 4) mechanical properties, 5) cell differentiation, 6) immune system involvement, and 7) bone specification (e.g. mineralization). Others may be added as mechanistic insight improves. Clearly, the analog system will need software components (initially coarse grain) that map to these seven categories. Measurement of molecular markers is a standard means to distinguish between different cell types and thus distinguish the above two mechanisms. In fracture repair, cellular phenotype distinction is currently infeasible because hypertrophic chondrocytes and osteoblasts exhibit extensive overlap in molecular markers [9,11,19,25].

The local microenvironment of the above cell types is heterogeneous and dynamic, a consequence of multiplexed changes occurring throughout the observation interval. To start, our working hypothesis is that the nature of those multiplexed changes within a large enough space, spanning an "adequate" interval, produces a *tipping point*, which enables the unfolding of the transdifferentiation that characterizes *mechanism 2*. Absent an "adequate" *tipping point*, fractures fail to heal. We need an in silico framework and software components that enables building evidence for or against that scenario.

4 Requirements

To enable the variety of analog use cases needed to explore and challenge tipping point mechanisms, we maintain that analog systems must meet these five requirements.

1. An analog's components and spaces will be concrete (enabling knowledge embodiment and facilitating falsification), wherein its details will be directly defined by its use cases. Analog components will be somewhat modular, in schedule as well as state. So doing helps accomplish the following activities.
   a. Defining and annotating component- and module-to-biological counterpart mappings during experimentation, by making them explicit, intuitive, and easily understood.
   b. Making analog modules and their mechanisms quasi-autonomous and thus more biomimetic.
   c. Making it increasingly easy to adapt, reuse, and repurpose components to represent different past and future experiment designs, protocols, and phenomena.
   d. Making it straightforward to change mechanistic detail (granularity, resolution) to simulate additional attributes or experiments.
   e. Facilitating verification through unit testing, where each component can be tested in isolation as well as in the composed analog context.
   f. Facilitating versioning, where each component can evolve independent of other components.
   g. Building trust in surviving analogs by accumulating direct in silico-to-wet-lab validation evidence, where measures taken during in silico experiments are mapped quantitatively to counterpart measures taken during wet-lab experiments.
   h. Facilitating archiving analog and mechanism evolution along with in silico experiment successes and failures within the framework. The latter is important because when an in silico experiment fails in some way, we acquire new knowledge, e.g., a feature of an analog mechanism thought to have a particular in vitro biological counterpart, does not. However, in a different context, that mechanism or some variant may prove useful.
2. Components and spaces can be assembled easily to simulate current, past, and future laboratory or clinical experiments. Generating many alternative, plausible, testable (through in silico experimentation), components for each function/structure and then selecting against those that fail, is needed to achieve our goal. So doing helps accomplish two activities.
   a. It becomes increasingly easy to construct (plug together) and explore alternative mechanistic hypotheses and intervention scenarios. It facilitates contrasting their predictions during simulation.
   b. It becomes increasingly easy to construct multiscale, multiresolution, multi-attribute analogs (eventually individualized virtual patients) composed of heterogeneous (form, function, methods, formalisms, etc.) components.
3. Simulation experiments are feasible in the presence and absence of chemical entity objects (hereafter, CE-objects). They are also feasible in the presence of multiple CE-objects. Components within analogs can recognize different CE-objects and adjust their response accordingly.
4. Coarse grain (from the perspective of biological organization) phenomena will derive mostly from local component interactions at a finer grain (local includes a living entity's immediate environment). When required, finer grain mechanisms can respond to coarser grain phenomena.
5. Semi-automated modeling methods are needed to more

rapidly complete two critical activities:

a. Use in silico experimentation to explore and shrink spaces of competing mechanistic hypotheses.
b. Use cross-model validation methods to discover parsimonious options to increase and decrease component and analog granularity when milestones change and when new questions require new use cases, which necessitates changing targeted attributes and/or shifting attention to new aspects and phenomena.

5 Conclusions

Requirements for most software engineering projects anticipate a final product that is typically maintained and incrementally improved thereafter. That is not the case here. Our software analogs will be perpetual works in progress. Despite the case presented is section **2.2** for relying on agent-based methods, the discussion above about evolving TAs and requirements demonstrates that simulating the variety of measurement methods, data structures, experimental observations, and bone healing phenomena may be beyond the scope of any one model of computation (MoC), such as continuous-time systems, ABMs, ODEs, finite element, systems and process networks, and Stream X-Machines. Depending on the particular use case—a virtual experiment intended to achieve a particular validation target, we can anticipate that our analogs and their mechanistic components will require drawing on different MoCs. We also anticipate that incrementally improving mechanistic insight will require the co-existence of multiple, occasionally inconsistent, yet equally plausible, explanations of some attributes. Somewhat analogous situations have been confronted successfully within other domains [7]. Inconsistency robustness [4], for example, is recognized within computer science domains as being important to the aspect-oriented nature of scientific modeling and simulation. Faced with a variety of attributes and phenomena, we cannot restrict ourselves to one MoC, to one modeling method, as is typically done in biomedical research. We need to be open to the idea that achieving a variety of attribute targets may require utilizing a variety of MoCs, and that will require learning from efforts in other domains to develop strategies for integrating MoCs within our bone healing analogs as they evolve and mature.

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