In Silico White Blood Cell: A Synthetic Model of Leukocyte Rolling, Activation, and Adhesion During Inflammation

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Abstract

We have constructed a synthetic in silico model for representing the dynamics of leukocyte rolling, activation, and adhesion on substrate-coated flow chambers. Software components were designed, instantiated, verified, plugged together, and then operated in ways that can map concretely to mechanisms believed responsible for leukocyte rolling, activation, and adhesion. Here, we show our model's ability to represent data from in vitro flow chamber studies of leukocyte rolling, activation, and adhesion on P-Selectin, ICAM-1 and CXCL1 substrate-coated surfaces.

Keywords: leukocyte, rolling, adhesion, inflammation, synthetic model

1 INTRODUCTION

Leukocyte recruitment during inflammation is normally a beneficial process, as it plays an essential role in the body's first major defense against infection. However, it is also associated with diseases such as asthma, rheumatoid arthritis, and atherosclerosis [1]. Discovery of new and improved therapeutics will be facilitated by having a better understanding of how this complex process works and by being able to predict consequences of interventions. Making such predictions requires having a model with a large, explorable behavior space. Traditional mathematical models are insufficient for the task.

Advanced modeling and simulation methods are needed that can be used to discover, clarify, and challenge plausible design plans for how components at various systems levels are thought to fit and function together. Demonstrating that a design plan is functionally plausible requires assembling individual components according to that design plan, and then showing that the constructed device, an analogue – on its own – exhibits behaviors that match those observed in the original biological system.

Here we describe the development and usage of a new class of models that can meet those needs. Using the synthetic modeling approach, we have developed an in silico model that can be used as an experimental system for testing hypothesized design plans of the molecular-level events that are thought to mediate leukocyte rolling, activation, and adhesion to endothelial substrate during inflammation. Object-oriented software components were designed, instantiated, verified, plugged together, and then operated in ways that can map concretely to mechanisms believed responsible for leukocyte rolling, activation, and adhesion. The result is an in silico analogue of the wet-lab experimental systems to study leukocyte rolling and adhesion; the experimentally measured phenotypic attributes can be compared and contrasted to those of leukocytes from referent systems. The expectation is that increasing behavioral similarity between actual leukocytes in context and our analogue systems will require, and can be achieved in part through, similarities in design plans and in generative mechanisms.

We previously constructed, verified, and validated an in silico synthetic model of leukocyte rolling, activation, and adhesion on P-Selectin, VCAM-1 and GRO-α substrate coated surfaces [2]. Here, we report progress towards extending the phenotypic overlap to include rolling, activation, and adhesion on P-Selectin, ICAM-1 and CXCL1 substrate-coated surfaces.

2 BACKGROUND

The interactions between leukocytes and endothelial cell surfaces during rolling, activation, and adhesion are complex and involve concurrent ligand-binding events between several combinations of receptor-ligand pairs (Fig 1). The selectin family of receptors mediates the initial rolling interactions. The most important is P-selectin, which bind to carbohydrate ligands, such as PSGL-1. The integrin family of receptors exclusively mediates adhesion. They exist largely in non-adhesive, low-affinity states in order to prevent non-specific adhesion to vessels. VLA-4 and LFA-1 are the predominant integrins for adhesion. VLA-4 is distinct from other integrins in that it can also participate in rolling when in its low affinity state.

Upon detection of chemokine, spatially restricted signaling events initiate conformational changes of nearby integrins such that they are able to form high-affinity bonds with their ligands. These high-affinity bonds are

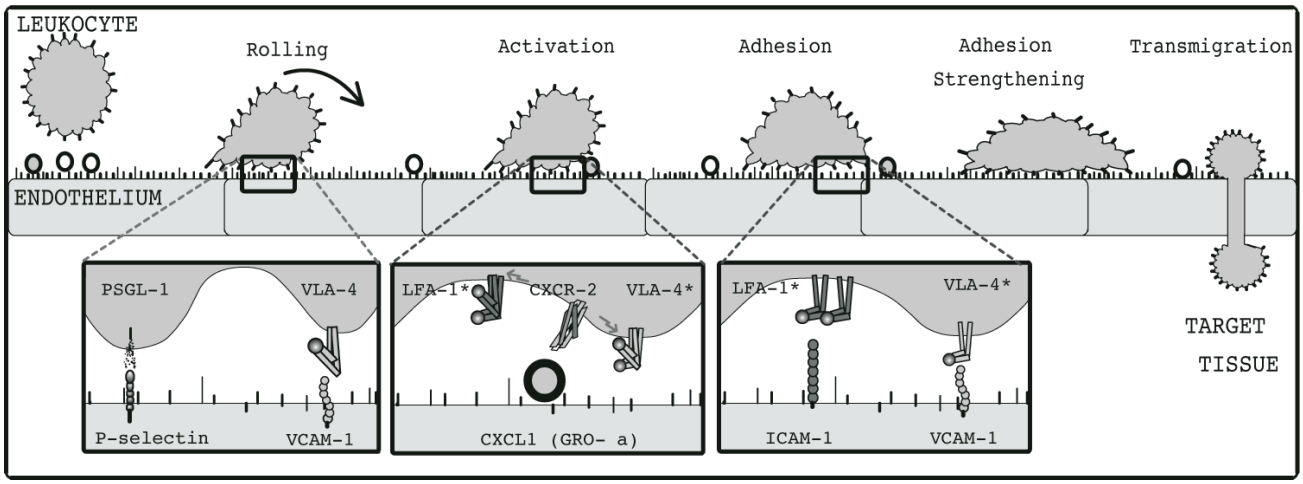

Figure 1. Cartoon Depicting the Roles of the Relevant Receptor-Ligand Pairs During Rolling, Activation, and Adhesion During Inflammation. Asterisks, as in LFA-1* and VLA-4*, indicate integrins in a high-affinity conformational state.

strong enough to enable the leukocyte to remain adherent and resist the shear force from the blood flow. The LFA-1 integrin has also been hypothesized to undergo clustering events upon binding multivalent ligand. This may aid in maintaining adhesion. After adhesion, the leukocyte transmigrates through the endothelial layer and into the target site [3].

3 METHODS

The in silico system we have created is a discrete event, discrete space, and discrete time analogue of the entire parallel plate flow chamber system for studying leukocyte rolling and adhesion during inflammation (Figure 2A-D). We found that an agent-oriented approach was needed to achieve the capabilities required for this project [2]. To avoid confusion and clearly distinguish in silico components, features, measurements, and events from their *in vitro* counterparts, such as a leukocyte and bonds, we use SMALL CAPS when referring to the in silico analogues (Table 1). We used RePast as our modeling and simulation framework. It is a java-based software toolkit developed at the University of Chicago for creating and exercising agent-based models [4].

3.1 The In Silico Analogue

ISWBC experiments are analogous to those performed using an in vitro flow chamber system. While on the SURFACE, LEUKOCYTES use their RECEPTORS to interact and form BONDS with RECEPTORS. Those interactions are recorded and measured. The decisional process used to determine behaviors is summarized in Figure 3. The ISWBC consists of components having three levels of spatial resolution: LEUKOCYTE-level, MEMBRANE/SURFACE UNIT-level, and LFA1 grid/ICAM1 grid-level (Figure 2B-D). High-level behaviors are dependent upon the collective operation of agents contained within each of the lower levels. For example, the behavior of MEMBRANE and SURFACE UNITS are emergent properties of the RECEPTOR objects contained within each. Similarly, the behavior at the LEUKOCYTE-level is dependent upon the collective events that occur within the underlying MEMBRANE/SURFACE UNITS. Conversely, events at the highest level impose constraints upon allowed lower level behaviors. For example, the positioning and movement of the LEUKOCYTE on the SURFACE dictate which MEMBRANE and SURFACE UNITS are overlapping and can interact.

The receptors found at the tips of microvilli (PSGL-1, VLA-4, and CXCR-2) and their counter-receptors (P-Selectin, VCAM-1, and CXCL1) are represented by PSGL1, VLA4, CXCR2, PSELECTIN, VCAM1, AND CXCL1. Each one maps to several binding molecules of the same type that may be found within a discrete area within the referent. For example, a PSGL1 RECEPTOR mapped to several PSGL-1 adhesion molecules found within a specified area. The number represented by that object is determined by its parameter, *TotalNumber*.

Distinct from the other RECEPTORS, each LFA1 represented single LFA-1 molecules found on membranes between microvilli. Similarly, each ICAM1 represented a single ICAM-1 molecule. Clustering of LFA-1 on the membrane upon binding to multivalent ligand has been hypothesized to play a role in adhesion. We chose to represent LFA-1 at this level of resolution in order to explore the role of LFA-1 integrin clustering events.

3.2 MEMBRANE/SURFACE UNIT-level Behaviors (BOND Formation and Dissociation)

BOND formation events occur when a RECEPTOR in a MEMBRANE UNIT encounters its partner in an overlapping SURFACE grid subsection in the CONTACT ZONE. For each potential BOND, the value of parameter *Pon* for that

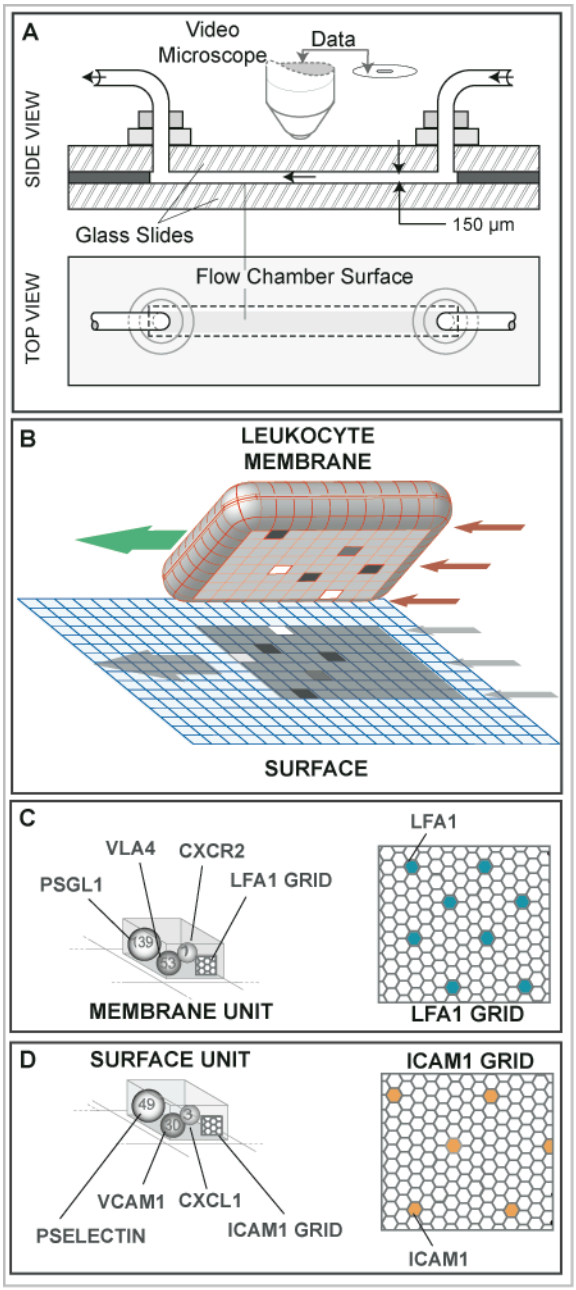

Figure 2. Sketches of in vitro and in silico experimental system components. (A) A sketch of a typical parallel plate flow chamber used for in vitro studies of leukocyte rolling and adhesion. A video microscope is used to record leukocyte behaviors. (B) A LEUKOCYTE is shown pulled away from the simulated flow chamber surface to which it was attached. The left arrow indicates ROLL direction; the three right arrows indicate SHEAR resulting from the simulated flow. The simulated flow chamber surface is discretized into independent units of function called SURFACE UNITS. The LEUKOCYTE'S MEMBRANE is similarly discretized into matching units of function called MEMBRANE UNITS: 600 total (20 x 30). The 8 x10-shaded region on the SURFACE and on the underside of the LEUKOCYTE identifies the CONTACT ZONE. The UNITS within the CONTACT ZONES that are shaded differently indicate different numbers of BONDS had formed between LIGAND–LIGAND pairs in overlapping UNITS; otherwise, no BONDS formed. ROLLING is the result of a sequence of forward ratchet events. One ratchet event is the result of one row of MEMBRANE UNITS being released at the rear of the CONTACT ZONE along with engagement of a new row of at the front of the CONTACT ZONE. One ratchet event maps to a leukocyte rolling 1 µm (relative to the flow chamber surface). (C) A MEMBRANE UNIT is illustrated. Each MEMBRANE UNIT is simulated using a software object functioning as a container. All leukocyte membrane functionality (relevant to these studies) within each UNIT is represented by four objects functioning as agents: PSGL1, VLA4, CXCR2, and LFA1. The microvillar receptors PSGL1, VLA4, CXCR2 are shown as spheres, where each number on the sphere indicates the number of receptors each agent represents. (D) A SURFACE UNIT is illustrated. Similar to MEMBRANE UNITS, each SURFACE UNIT is simulated using a software object functioning as a container. All flow chamber surface functionality (relevant to these studies) within each UNIT is represented by four objects functioning as agents: PSELECTIN, VCAM1, CXCL1, and ICAM1.

Biological Aspects	Model Components	Description
Substrate-Coated Surface	SURFACE	2D Square Lattice
Functional Unit of the Surface	SURFACE UNIT	Grid Unit of the SURFACE
Surface area containing ICAM-1	ICAM1GRID	2D Hexagonal Lattice
Leukocyte	LEUKOCYTE	Object
Leukocyte Membrane	MEMBRANE	2D Square Lattice
Functional Unit of Leukocyte Membrane	MEMBRANE UNIT	Grid Unit of the MEMBRANE
Membrane area containing LFA-1	LFA1GRID	2D Hexagonal Lattice
Chemokine	CXCL1	Object
Chemokine Receptor	CXCR2	Object
Adhesion Molecule	ADHESION MOLECULE	Object
Tensile Force on Rear Bonds	*RearForce*	Parameter
Hypothesized Biological Mechanisms	Operating Mechanisms	Algorithms

Table 1. Biological aspects from the in vitro flow chamber system and their in silico analogue counterparts.

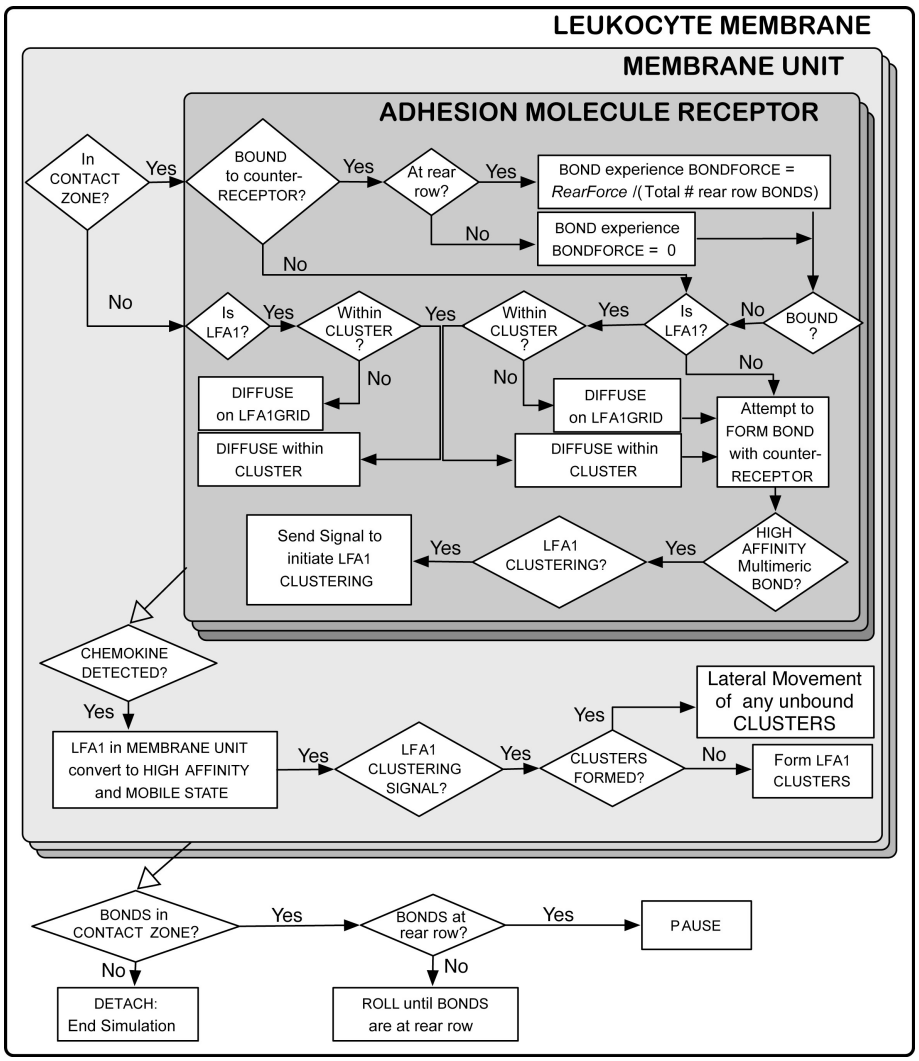

Figure 3. The decisional process for the LEUKOCYTE MEMBRANE, each MEMBRANE UNIT, and each ADHESION MOLECULE during a simulation cycle. The LEUKOCYTE steps through its decisional process only once during a simulation cycle. At the start of the cycle, the MEMBRANE instructs all MEMBRANE UNITS and all ADHESION MOLECULES contained within to follow the decisional process shown. Once that process is complete, the MEMBRANE completes its process by selecting and following the one applicable action option. The state of each MEMBRANE UNIT depends on the properties of the RECEPTOR objects contained within. During each simulation cycle, each MEMBRANE UNIT, selected at random, uses this decisional process to update its status relative to the SURFACE UNIT over which it is positioned. The LIGAND-BINDING properties and spatial movement of LFA1S are dependent on its state, which may change upon local ACTIVATION events. White arrows indicate changing to a different level of resolution after all objects within the current level in the model have completed their decisional process.

RECEPTOR-LIGAND pair is compared to a randomly generated number between 0 and 1 to determine if that potential BOND becomes an actual BOND. The effect of shear on the rear of a leukocyte is represented by the variable *RearForce*. BONDS at the rear experience a *bondforce* that is calculated each simulation cycle by dividing the *RearForce* value by the total number of BONDS in the rear row of the CONTACT ZONE. BONDS within the rest of the CONTACT ZONE experience no *bondforce*. Drawing from in vitro data, we have assumed a simple linear relationship between *bondforce* and the probability of BOND dissociation. It is calculated as (probability of dissociation) = b0 + (*bondforce*) x b1, where b1 and b0 are the slope and intercept, respectively, of the line segment associated with a specific *bondforce*. Each type of simulated adhesion molecule pair uses a unique set of b0 and b1 values.

Local ACTIVATION of INTEGRIN agents occurs when a CHEMOKINE RECEPTOR in a MEMBRANE UNIT detects a CHEMOKINE in an overlapping SURFACE UNIT. An ACTIVATION SIGNAL is sent to local INTEGRINS.

3.3 LFA1Grid/ICAM1Grid-level Behaviors

If an ACTIVATION SIGNAL is detected, the LIGAND-BINDING and DISSOCIATION properties of a local LFA1 changes from a state with low affinity properties to one of higher affinity through a change in the parameter values of *Pon*, b0, and b1.

The DIFFUSIVE/CLUSTERING properties of LFA1 are also dependent on its state. The parameter, *moveNum*, which differs for each integrin activation state, determines the number of moves an LFA1 makes within the LFA1 grid at each simulation cycle. LFA1 has an equal probability of moving into any of its six neighboring spaces, but cannot move into an already occupied grid space.

Upon binding multivalent ICAM1, LFA1 CLUSTERS are formed by randomly choosing non-overlapping regions on the LFA1GRID, and filling each with all the LFA1 in that MEMBRANE UNIT. The number of CLUSTERS per LFA1GRID is specified by the parameter *NumLFA1Clusters*. During a simulation, CLUSTERS with unbound LFA1 randomly move to new and unoccupied locations within the LFA1GRID, a

process that maps to molecular diffusion within a region of membrane. An LFA1 CLUSTER on the LFA1GRID is specified by a diameter, which in turn is specified by the parameter *LFA1ClusterDiameter*. The latter determines the length and width of the region that LFA1S must stay within while DIFFUSING on the LFA1GRID.

3.4 LEUKOCYTE-level Behaviors (LEUKOCYTE ROLLING)

LEUKOCYTE behavior is determined by the number and location of BONDS at the MEMBRANE UNIT-level. If there are BONDS within the rear column of the CONTACT ZONE**,** the LEUKOCYTE PAUSES, or remains STATIONARY, until the next simulation cycle. If there are no BONDS within the rear column of the CONTACT ZONE, the LEUKOCYTE, influenced by the simulated shear force, performs a forward ROLLING movement. ROLLING is the result of a sequence of forward ratchet events. The process involves removing a column from the rear of the rectangular interaction zone of the MEMBRANE while a new one is placed at the front of the zone above the SURFACE.

3.5 The In Silico Experimental Method

An in silico experiment consists of building the software into an executable, creating and editing the parameter vector, and beginning the simulation. An experiment on an individual leukocyte consists of a single run with the output representing the results of the experiment. An experiment on a population of leukocytes can also be executed by performing a batch run [2]. Depending on the experiment type, simulation output can consist of LEUKOCYTE positions, BONDS and RECEPTOR information at each time step. At the end of the experiment, one of several data reduction scripts may be run to process and analyze the simulation results. Examination of data from simulations used a combination of Matlab (7.0.0) and Microsoft Excel.

3.6 ISWBC Development

Code is managed using CVS with a single HEAD branch and ChangeLogs for each commit. Experiments are conducted using the last stable version of the HEAD branch. As an experiment runs, simulation data is kept in memory until the end of the experiment, at which time it is written to comma separated files indexed by filename.

Development follows a loose and iterative specification, implementation, test, and commit process. Each specific change is documented in the ChangeLog. The changes are then committed to CVS; there is one CVS module for the entire framework.

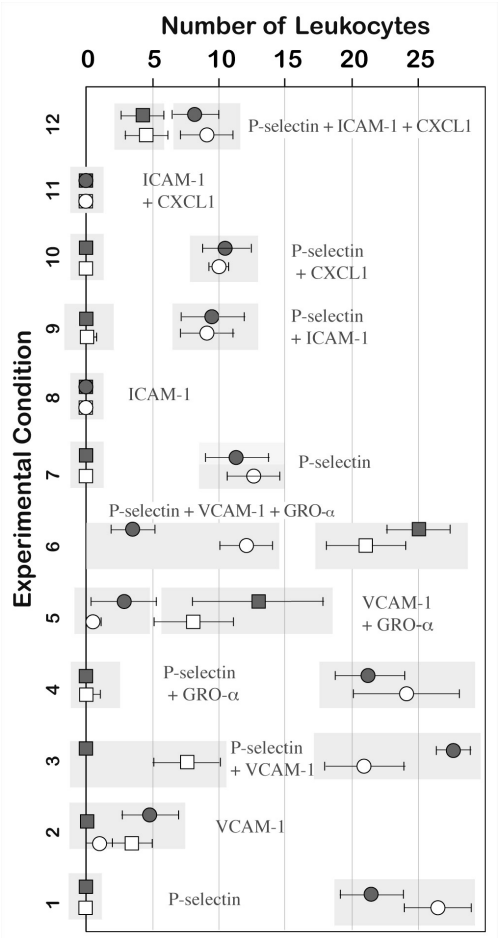

Figure 4. Comparison of in vitro and in silico results for twelve different experimental conditions. The first six in vitro conditions (from [6]): the flow chamber surface was coated with P-selectin and/or VCAM-1 with or without immobilized GRO-α chemokine. In silico data for first six experimental conditions were previously reported in [2], and are shown for comparison with new results. The last six in vitro conditions (from [5]): the flow chamber surface was coated with P-selectin and/or ICAM-1 with or without immobilized CXCL1 chemokine. The average number of leukocytes that rolled and adhered within each field of view were recorded for a 30-second observation interval. In vitro: white circles: average number of rolling leukocytes; white squares: average number of adherent leukocytes; error bars: ± 1 SD. The data are clustered and plotted for each of twelve conditions as labeled. The in silico experiments mimicked the in vitro experimental conditions and also the results: the results are averages from 20 sets containing 30 LEUKOCYTES each; each simulation ran for 300 simulation cycles (equivalent to 30 seconds). In silico: dark circles: average LEUKOCYTES that ROLLED; dark squares: average LEUKOCYTES that ADHERED; error bars: ± 1 SD. Each light gray box contains the two sets of observations (in vitro and in silico) that should be compared.

4 RESULTS

Smith et al. observed leukocyte rolling, activation, and adhesion in blood-perfused flow chambers coated with P-selectin, and/or ICAM-1, with or without CXCL1 [5]. Nine one-minute recordings of a field of view were taken of the center of each chamber under each experimental condition. Population-level measures of rolling and adhesion were taken by averaging the number of rolling cells and adherent cells, respectively, for each condition. Arrested cells were defined as those cells that were adherent for at least 30 s. We simulated analogous experimental conditions. The data are averages from 20 sets of experiments containing 30 LEUKOCYTES each, with the duration of each run being 600 simulation cycles (equivalent to 1 minute). The number of ROLLING and ADHERING LEUKOCYTES for each batch were counted and averaged. LEUKOCYTES that remained stationary on the SURFACE for at least 300 simulation cycles (about 30 seconds) were classified as ADHERED. Figure 5 (conditions 7-12) shows that for all ligand combinations, both the LEUKOCYTE ROLLING and ADHESION in silico data matched that from the flow chamber experiments fairly well.

5 DISCUSSION

It is expected that increasing behavioral similarity between leukocytes in vitro and our analogues will require, and can be achieved in part through, similarities in design plans and in generative mechanisms (Fig 5). Therefore, it has been an early goal to produce increasing overlap between ISWBC behaviors, properties, and characteristics with those of leukocytes in vitro. We follow an iterative refinement protocol to systematically and sequentially extend the overlap of model and referent system phenotypes [2].

Briefly, the protocol is: for the wet-lab systems being studied, create a list of attributes to be targeted. Design, construct, and enable the analogue to exhibit the targeted phenotypic attributes. Iteratively falsify and revise the operating mechanisms until the analogue exhibits the targeted phenotypic attributes within a pre-specified level of similarity, thereby achieving a level of validation. The process is then repeated with the addition of more phenotypic attributes to the targeted list.

We previously reported progress validating against our initial set of targeted attributes [2]. The ISWBC was able to successfully represent the dynamics of individual leukocytes rolling separately on P-selectin and VCAM-1, along with the transition from rolling to adhesion on P-selectin and VCAM-1 in the presence of GRO-α chemokine. Additionally, the individual in silico and in vitro behavioral similarities translated successfully to population-level measures. Here we report progress towards extending the model further by expanding the set of targeted phenotypic attributes to include rolling, activation, and adhesion on P-Selectin, ICAM-1 and CXCL1 substrate-coated surfaces.

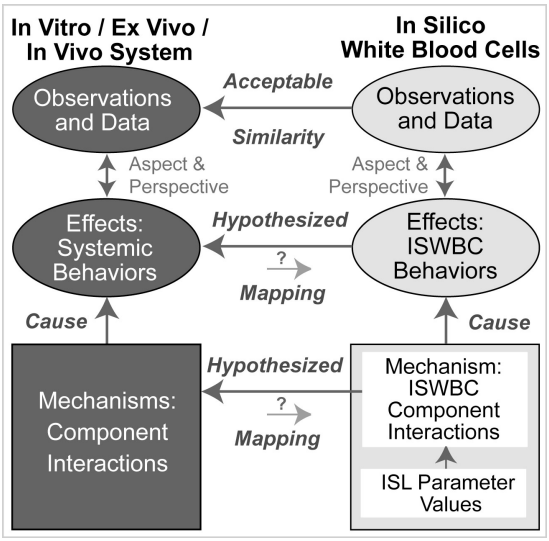

Figure 5. Relationship between the wet-lab flow chamber experiments and the ISWBC analogues. A plausible, abstract mechanistic description is hypothesized and specified. Software components are designed, coded, verified, assembled, and connected according to the mechanistic specifications. The result is a collection of micro-mechanisms rendered in software. Execution through a simulation gives rise to a working analogue. Its dynamics are observable and intended to represent the corresponding dynamics believed to occur between the leukocyte and the in vitro substrate-coated flow chamber surface during an experiment. Simulation measures provide time series data that are intended to mimic corresponding leukocyte behavior measurements, which can then be compared.

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