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A Twofold Usage of an Agent-Based Model of Vascular Adaptation to Design Clinical Experiments

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Abstract

Several computational models of Vein Graft Bypass (VGB) adaptation have been developed in order to improve the surgical outcome and they all share a common property: their accuracy relies on a winning choice of their driving coefficients which are best to be retrieved from experimental data.

Since experiments are time-consuming and resources-demanding, the golden standard is to know in advance which measures need to be retrieved on the experimental table and out of how many samples. Accordingly, our goal is to build a computational framework able to pre-design an effective experimental structure to optimize the computational models setup.

Our hypothesis is that an Agent-Based Model (ABM) developed by our group is comparable enough to a true set of experiments to be used to generate reliable virtual experimental data.

Thanks to a twofold usage of our ABM, we created a filter to be posed before the real experiment in order to drive its optimal design.

This work is the natural continuation of a previous study from our group [1], where the attention was posed on simple single-cellular events models. With this new version we focused on more complex models with the purpose of verifying that the complexity of the experimental setup grows proportionally with the accuracy of the model itself.

Keywords

Agent-Based Model; Experiment Planning; Virtual Dataset

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1. Introduction

Peripheral Arterial Diseases (PADs) are the leading cause of morbidity in the Western Society with a 13% people over 50 years old affected [2]. Among the various surgical techniques aimed to restore the physiological circulation, the most performed one consists into bypassing the occlusion by using an autologous saphenous vein graft, a procedure known as Vein Graft Bypass (VGB) [3–5].

Despite years of surgical improvements, the rate of VGBs failure on a medium term follow-up remains unsatisfactorily high, with an 18% of grafts failing within just 2 months from the original operation, a percentage that ramps up to 40% on a longer follow-up [6, 7].

The failure of VGBs is mainly identifiable with the re-occlusion of the graft and it is attributable to its adverse adaptation to the new environmental conditions [8–11]. By switching from venous to arterial regime, the graft simultaneously faces two phenomena, which balance determines the success or the failure of the procedure: i) Intimal Hyperplasia (IH) and ii) Wall Remodeling (WR) consisting in the thickening of the innermost and outermost layer of the graft respectively.

Our group of investigators, also along with others [12, 13], studied vascular adaptation both on an experimental [11] and on a computational perspective, from deterministic differential equation systems [14] to Multi-Scale Models (MSMs) [15] to Agent-Based Models (ABMs) [16] regulated by cellular automata principles based on Monte Carlo simulations.

Their driving coefficients were retrieved directly from rabbit-based experimental data, a detailed description of which is offered in [9, 11, 17]. In the process it has been observed that the level of detail carried by the experimental measures drives the resources needed for their retrieval. Simple geometrical measures, e.g. compartments' radii or thicknesses, can be easily evaluated within a week, while more complex measures like cellular density maps can require a year of work.

As experiments are consuming in terms of time and resources, the golden standard is to know in advance how to design the best experimental setup in order to retrieve the desired coefficients' value. Accordingly, the goal of this paper is to build a computational framework able to preidentify which are the measurements and which is the size of the specimen suitable enough to explore the experimental observations in a way that allows to effectively setup a computational model of vascular adaptation.

Our hypothesis is that the ABM previously introduced [16] is comparable to a real experiment in a manner that it can be used to generate virtual experimental data. Indeed, on one end the model is relatively accurate in a given coefficients' domain, so much to mimic the fundamental biological functions of the VGB's adaptation with high credibility, an example of which is shown in Fig. 1. On the other end, seen its stochastic nature, the ABM shares the same level of noise of a true experiment.

The rationale of this work stands on the fact that if an experimental setup is not proved to be effective computationally, then there is no point to test it on a real experimental table,

because it will certainly result in a failure. This is why the current work wants to be a filter to be placed before the real experiment in order to drive its optimal design.

Accordingly, the computational framework presented is based on a twofold usage of our ABM. The model is used on one end to generate virtual experimental data and on the other end as a true computational model and the two resulting outputs are compared in order to solve the inverse problem established between them. The solution of the latter depends on the winning choice of the measure to be monitored as output of the models, that represent the experimental measure, and on the number of independent simulations, that in turn represents the size of the experimental specimen.

In the previous work from our group of investigators [1], we demonstrated how a simple onecoefficient implementation of the ABM requires an experimental setup relatively easy to prepare and not particularly resources demanding. In addition, the robustness of the experimental setup can be improved by simply using a larger number of samples. However, this has been proved false for multi-coefficients models. The un-verified hypothesis we left behind was that more complex models also require more informative measurements, generally complex and time-demanding to retrieve.

The current work wants to offer a more complete analysis by solidifying the previous evidences and by verifying the cited hypothesis. Accordingly we challenged the presented framework with an increasing degree of complexity of the ABM, by studying first singular cellular events, i.e. intimal/medial mitosis in order to replicate IH and WR respectively, and then by coupling them in order to verify that indeed the complexity of the experimental measurements and/or the size of the specimen grow proportionally with the complexity of the ABM.

Finally, the robustness of our basic assumption relates on an accurate setup of the coefficients driving the ABM and especially on an effective choice of their range of perturbation, as fluctuations of the ABM can lead to large output variations that would be hard to catch with this method. Both the required features are guaranteed by an accurate study of the experimental data [11] preceding the model's implementation and by a thorough validation of the ABM on them in order not to run into unrealistic states potentially given by the model. This is a standard procedure adopted in the development of a computational model and it has been followed in the previous work by our group [16] the basic structure of the ABM is taken from.

2. Methods

Fig. 2 shows the backbone of the computational framework proposed that will be common for all the virtual experiments that will be described. The methodology presented is based on a twofold usage of our ABM [16] that, depending on its implementation, acts both as virtual experimental dataset generator, labeled as ABM1, and as a true computational model, labeled as ABM2.

The left part of Fig. 2 refers to the generation of the virtual experimental data, which are assumed to be comparable to true experimental data seen the level of accuracy of the ABM

and its experiments-like level of noise. Standard methods have been used both to restrict the analysis to a minimum set of coefficients and to reach a high level of confidence with our ABM, respectively by performing a non-linear stability analysis [18] and by cross-validating it on phenomenological models [16].

For the purpose of this work, the ABM can be thought as a black box driven in input by hemodynamic perturbation, ε and χ respectively referring to shear stress and wall tension, and by constant coefficients, organized in vector $\vec{\alpha}$, that define the level of activity of the cellular events of interest, mainly intimal and medial cellular mitosis in our case study.

The output of ABM1 is the generic virtual measurement S^k which relation follows:

$$S^k = \frac{\sum_{n=1}^N S_n^k}{N}. \quad (1)$$

k is the label that identifies the entity of the measurement (e.g. $k = 1$ for intimal area, $k = 2$ for medial area, *et cetera*), and N is the number of independent simulations run with ABM1 that corresponds to the specimen's size in a true experimental setup. The fact that the ABM has a strong stochastic nature makes necessary to run a certain number of independent simulations and to consider as output the average of them in order to evaluate a result free from causalities. It is easy to notice a parallelism with a true experimental setup, where a certain number of independent subjects is studied and the average trend is considered as output. Also, like for true experiments, the number of independent simulations of ABM1 is decided in order to minimize as much as possible the standard deviation.

As it will stand out, the nature of S^k can be either of a constant measure, e.g. intimal/medial area, or of a distance-depending one, e.g. cellular density across a graft's layer.

In turn, the right part of Fig. 2 represents how the ABM is used as a true computational model. In order to simulate the decisional process referred to vector $\vec{\alpha}$, a browsing of the coefficients' space was performed in order to set an inverse problem which solution defines specimen's size and entity of measurements for our analysis.

More precisely, starting from the same setup of ABM1, the entity of which will be specified case-by-case, a perturbation vector $\vec{\delta} = \{-0.5, -0.375, \dots, 0, \dots, +0.5\}$ was applied to the driving coefficient vector ($\vec{\alpha}$). At each δ_i corresponds a new input and so a new output of ABM2. While the output of ABM1, S^k , is a unique measurement, the output of ABM2 is a vector of measurements $\vec{M}^k$, where its size is equal to the size of $\vec{\delta}$, and it corresponds to $P=9$, and the measurements are of the same kind identified by label k . For a matter of completeness and as an anticipation, while facing two-coefficient models, also the inverse problem will become twodimensional, turning the vector $\vec{M}^k$ into a matrix $\underline{\underline{M}}^k$.

Remaining for this general description in the one-dimensional case, to solve the inverse problem consists in comparing S^k with $\vec{M}^k$, i.e. in finding the global minimum of the objective function φ_k defined as follows:

$$\varphi_k = \sqrt{\frac{1}{P} \sum_{p=1}^P (S^k - M_p^k)^2}. \quad (2)$$

If the entity of the measurement is suitable enough, and if the number of samples guarantees a robust analysis, the vector resulting from the minimization of φ_k , indicated with $\vec{\alpha}_E^N$, will correspond coefficient-by-coefficient to the vector of input coefficients for ABM1, i.e. the equality $\vec{\alpha}_E^N = \vec{\alpha}$ will be respected.

As counter-proof, another independent virtual dataset is generated by running the ABM on the base of N simulations and by setting the input to be $\vec{\alpha} = \vec{\alpha}_E^N$. The output is measured as done for ABM1, and labeled as S_E^k and the percentile error between S^k , the previous reference, and S_E^k , the new reference is evaluated by representing the distance between the original dataset and the prediction based on our experimental design, and it writes

$$\Xi_k = \frac{|S^k - S_E^k|}{S^k} * 100. \quad (3)$$

The rationale is that when the Ξ_k vs. N plot goes below an acceptable threshold of error, N can be considered as a suitable number of samples to build an effective experimental setup, and at the same way, k can be considered as a suitable measurement.

The framework presented focuses on two different aspects of an experimental setup and it has to be expected that the prevalence of one aspect rather than the other might be different case-by-case. The real challenge is to tackle this variability and to be able to predict the best decisional process. In order to prove this concept a rigorous analysis must be conducted by clearly distinguishing the effect of increasing the specimen's size from pursuing a more accurate measurement while dealing with an increasing complexity of the computational model. A clear separation between the two procedural choices is important especially because they typically require different resources.

As mentioned, an increasing level of complexity of the ABM has been investigated by first turning on singular cellular events one at the time, and this is the case of intimal mitosis to simulate IH and medial mitosis to simulate WR, and then by turning on the same cellular events simultaneously.

For each of the cases studied, the computational framework above described was applied and its peculiar response to different measurements and specimen's sizes was evaluated in order to design the best experimental plan for the coefficients' retrieval.

2.1 Intimal Hyperplasia

IH is mostly driven by Smooth Muscular Cells (SMCs) division within the tunica intima fostered by a decrease in shear stress from the baseline condition, which corresponds to the healthy vein at time of implant. In it, assuming for simplicity a unitary velocity, and being $\mu = 3.2 \text{ cP}$ the blood viscosity, the shear stress (τ) was recorded to be 6.4 Pa and its value assumed to be uniformly distributed along the luminal interface. This simplification allows to maintain radial symmetry and so to deal with an easier problem that better suits the purpose of this work. In an optical of defining a more realistic shear stress distribution, this assumption should be abated in favor of a non-uniform stress along the interface that would allow the intima to encroach the lumen in a non-regular way, closer to the physiological reality indeed.

As already mentioned, the ABM is stochastic and so the related formula corresponds to a density of probability, a concept true for WR as well, which writes

$$p_{div}^{int} = \alpha_1 \left(1 + \alpha_3 \frac{\tau(t) - \tau_0(1 + \varepsilon)}{\bar{\tau}} \right), \quad (4)$$

where $\alpha_1 = \frac{1}{T_{cell}} = 0.5$ drives the baseline of cellular division being $T_{cell} = 12 \text{ hours}$ the standard cellular cycle time, $\tau(t)$ is the shear stress recorded at t-th time point, τ_0 is the baseline value of shear stress recorded at time of implant, $\bar{\tau} = 0.25$ is a normalization constant that maintains the discrete probability value between 0 and 1 for the Monte Carlo method requirements, $\varepsilon = 0.75$ represents a 75% drop that is imposed to the shear stress value at time of implant in order to simulate the reduction of shear stress occurring at the lumen's interface that promotes SMCs division [19, 20]. $\alpha_3 = 0.2$ is the constant coefficient that drives SMCs intimal division, representing in this case study the coefficient to design an experiment for its retrieval. Finally, the model was run for a follow-up time of 4 months, a timeframe that allows a clear cellular pattern formation. Cited timeframe will be maintained across all the experiments performed.

The measurement tested in first instance is the intimal area, labeled as $k = 1$, while the robustness of the virtual experimental setup was tested with an increasing number of samples, starting from $N = 10$ up to $N = 100$ with an interval of 10 samples. Our hypothesis is that in order to retrieve a single coefficient on the base of experimental data, a measurement that is relatively simple to obtain is enough in order to appreciate a satisfying outcome, which can always be further improved by progressively increasing the size of the specimen up to reach the desired level of accuracy and confidence.

For this case study, to solve the inverse problem is equivalent to retrieve for α_3 the value of 0.2 by minimizing the objective function φ_1 that corresponds to the Root Mean Square

(RMS) deviation between the outputs of ABM1, S^1 , and ABM2, $\vec{M}^1$. Finally, the addressed problem is one-dimensional as a single coefficient is retrieved out of the input vector $\vec{\alpha}$.

2.2 Wall Remodeling

The framework for WR is similar to the one described for IH, with the difference that WR is fostered by SMCs division within the tunica media favored by an increase of wall tension from the baseline condition. The related density of probability writes

$$p_{div}^{med} = \alpha_1 \left(1 + \alpha_4 \frac{\sigma(t) - \sigma_0(1 + \chi)}{\bar{\sigma}} \right), \quad (5)$$

where $\sigma(t)$ is the wall tension recorded at t -th time point, σ_0 is the baseline value of wall tension recorded at time of graft's implant, $\bar{\sigma} = 40$ is the normalization constant with same purpose of $\bar{\tau}$, $\chi = -0.008$ represents a 0.8% increase in wall tension to promote SMC division, which represents the typical response of the tunica media to the venous/arterial switching regime [21]. Finally $\alpha_4 = 0.3$ is the constant coefficient that drives SMCs medial division and it here represents the coefficient to design an experiment for its retrieval.

The measurement chosen is the medial area, labeled as $k = 2$, while the number of samples is again investigated between $N = 10$ and $N = 100$ with an interval of 10 samples. The hypothesis is that measuring the area of the media is enough to accurately retrieve α_4 and, in addition, a higher level of confidence can be reached by incrementing the number of samples used in the experiment.

To solve the inverse problem for WR is equivalent to be able to retrieve for α_4 the value of 0.3 by minimizing the objective function φ_2 , i.e. the RMS deviation between the output of ABM1, S^2 and ABM2, $\vec{M}^2$.

Finally, as for IH, and for the same reasons, the problem presented in this sub-section is still onedimensional.

2.3 Coupled Intimal Hyperplasia and Wall Remodeling

A more realistic implementation of the ABM considers the simultaneous study of IH and WR which maintains the same setup for both the cellular events, described respectively with Eq. (4) and Eq. (5).

A model closer to the physiological reality implies a higher level of complexity of the ABM and consequently a more articulated analysis to design an effective experimental setup. Three different experiments for the simultaneous retrieval of α_3 and α_4 have been designed and tested computationally and they are listed below:

1. **Experiment #1:** Intimal area ($k = 1$) and medial area ($k = 2$) measured on a base of $N = 10$ samples.

2. **Experiment #2:** Intimal area ($k = 1$) and medial area ($k = 2$) measured on a base of a progressively increased number of samples, from $N = 10$ to $N = 100$ with an interval of 10 samples.
3. **Experiment #3:** cellular density map across the wall ($k = 3$) evaluated on a base of $N = 10$ samples.

2.3.1 Experiment #1—The first experiment is the easiest to conceive both in terms of complexity of the measurements and in terms of number of samples.

The size of the specimen is $N = 10$, while the measurements chosen to solve the inverse problem are intimal area and medial area. If with a single cellular event the analysis was one-dimensional, with two cellular events acting simultaneously the problem becomes two-dimensional. Vector $\vec{\delta}$ still represents the level of perturbation carried on $\vec{\alpha}$ in order to simulate the browsing of the coefficients' space and it is specifically applied both to α_3 and α_4 .

Accordingly, the output of ABM2 is no more a simple vector, but a $\langle PxP \rangle$ square matrix, where $P = 9$ corresponds to the size of $\vec{\delta}$ and it writes

$$\underline{\underline{M}}^k = \begin{pmatrix} m_{11}^k & \cdots & m_{1P}^k \\ \vdots & \ddots & \vdots \\ m_{P1}^k & \cdots & m_{PP}^k \end{pmatrix}. \quad (6)$$

The generic m_{ij}^k stands for the measurement uniquely individuated by i and j , respectively referring to the degree of perturbation employed on α_3 and α_4 . This makes that the i -th row of $\underline{\underline{M}}^k$ includes the values for a fixed α_4 at the variation of α_3 , while the j -th column includes the values for a fixed α_3 at the variation of α_4 .

The objective function $\underline{\underline{\phi}}^k$, with $k = 1, 2$ respectively for the retrieval of α_3 and α_4 , is also in the form of a $\langle PxP \rangle$ square matrix, and it writes

$$\underline{\underline{\phi}}^k = \begin{pmatrix} \phi_{11}^k & \cdots & \phi_{1P}^k \\ \vdots & \ddots & \vdots \\ \phi_{P1}^k & \cdots & \phi_{PP}^k \end{pmatrix}, \quad (7)$$

where ϕ_{ij}^k is the distance between m_{ij}^k and S^k , with $i = 1, 2, \dots, 9$ and at the same way $j = 1, 2, \dots, 9$, in formula:

$$\phi_{ij}^k = \|m_{ij}^k - S^k\|. \quad (8)$$

As a consequence, each of the two coefficients will be retrieved by minimizing a 2D surface instead of a 1D curve seen in the single coefficient case.

To solve the inverse problem for IH and WR combined is equivalent to be able to retrieve at the same time for α_3 the value of 0.2 and for α_4 the value of 0.3 by minimizing the objective function $\underline{\underline{\phi^k}}$ defined with Eq. (7) and Eq. (8).

As final remark, it has to be expected that by adding more cellular events to the analysis, the complexity and the dimensionality of the inverse problem increase proportionally with the closeness of the model to the reality.

2.3.2 Experiment #2—A second experimental setup was tested in order to appreciate the effect of an increased specimen's size. The same analysis proposed for experiment #1 is here repeated by progressively increasing the number of samples from $N = 10$ to $N = 100$ with a 10 samples increasing step, while the measurements tested are the same, i.e. intimal area and medial area for the simultaneous retrieval of α_3 and α_4 respectively.

If the setup presented in this second experiment offered a better solution for the inverse problem, it would be a clear sign that an increased complexity of the computational model can be handled by proportionally increasing the size of the experimental specimen.

On the contrary, if the analysis did not benefit from a bigger N , our hypothesis is that the winning strategy to design an effective experiment is to choose a different measure, able to bring a more peculiar level of detail on the cellular events under study.

2.3.3 Experiment #3

The purpose of the third experiment is to verify the latter hypothesis by investigating the effect on the inverse problem's solution of a different experimental measurement, specifically the cellular density map across the graft's wall, labeled as $k = 3$. Consequently, this last analysis deals with the output of ABM1 that is now $\mathcal{S}^3$ and the output of ABM2 that is now $\underline{\underline{M^3}}$.

About $\mathcal{S}^3$, it is important to specify that this is no more a constant value like $\mathcal{S}^1$ or $\mathcal{S}^2$, but a distance dependent dynamic that tracks the number of cells across the graft wall, where the distance is measured from the lumen edge and normalized on the wall's thickness. For the reader's convenience, we will keep referring to the density map with the simple $\mathcal{S}^k$ notation, even though the most accurate one would be (d) seen its dependence on the distance from the lumen.

In order to better specify the nature of $\mathcal{S}^3$, let us refer to Fig. 3, where a one-dimensional segment of the ABM is represented for a given radial angle ϑ with SMCs represented with black ovals and ECM portions with dark grey rectangles. The figure only serves as an example, so, for simplicity, the ABM sites are here represented as rectangles even though the model lays on a hexagonal grid-based structure and the distribution of SMCs vs. ECM is chosen randomly, without representing any of the patterns studied so far.

For each segment, the number of SMCs is recorded and the entire wall is investigated with a resolution of 1 degree. The density map for the single ABM run was evaluated as the mean of the segments analyzed and the virtual experiment conducted on the base of 10 independent simulations ($N = 10$ indeed), such as the final relation for S^3 writes:

$$S^3 = \frac{1}{N} \sum_{i=1}^N \frac{1}{N_a} \sum_{a=1}^{N_a} S_j^3 \Big|_{\vartheta_i} \quad (9)$$

where $S_j^3 \Big|_{\vartheta_i}$ is the number of SMCs for a generic ϑ_i retrieved for the j-th individual of the virtual experiment (j-th independent simulation as output of the ABM), with $\vartheta_i \in \vec{\vartheta} = \{1^\circ, 2^\circ, \dots, 360^\circ\}$ and $N_a = 360$ that corresponds to the number of segments, and finally $N = 10$ corresponding to the number of samples. Finally, the density map is normalized on the wall thickness in order to retrieve a standardized analysis.

The output of ABM2, $\underline{\underline{M}}^3$ has still the shape of a $\langle PxP \rangle$ square matrix, like the one described with Eq. (6) with the difference that $m_{ij}^3 \in \underline{\underline{M}}^3$ individuates the cellular density map, and not intimal/media area, retrieved out of ABM2 with a level of perturbation still identified by i and j respectively for IH and WR leading coefficients.

As a natural consequence, the objective function $\underline{\underline{\phi}}^3$ to be minimized has the same shape of the one described with Eq. (7). However, ϕ_{ij}^3 , differently from ϕ_{ij}^1 and ϕ_{ij}^2 , is now the distance between two curves, instead than two constant values and the RMS deviation is considered as an effective indicator for said distance.

Finally, to solve the inverse problem here presented is equivalent to be able to minimize $\underline{\underline{\phi}}^3$ in correspondence of $\{\alpha_3 = 0.3; \alpha_4 = 0.4\}$, at the intersection of the straight lines individuated by α_3 and α_4 , differently from what seen for $\underline{\underline{\phi}}^1$ and $\underline{\underline{\phi}}^2$ where the inverse problem was minimized along a straight line.

The experiment tested constitutes the antithesis of the previous one described, indeed a successful solution of the inverse problem with the current setup would imply that a more complex computational model should be tackled by retrieving an experimental measurement able to bring more significant details more than with a bigger specimen's size.

3. Results and Discussions

For the reader's convenience the results are presented with separate subsections that follow the scheme reported below:

1. Virtual dataset
2. Single Intimal Hyperplasia analysis

3. Single Wall Remodeling analysis
4. Coupled Intimal Hyperplasia/Wall Remodeling analysis

3.1 Virtual Experimental Dataset

Fig. 4 offers a general overview of the different virtual experiments performed with ABM1 and assumed to be comparable to a real experiment. The analysis of the different density maps, here represented as a histogram with 5 steps of discretization for each compartment, offers a nice quantitative and visual comparison between the different cases.

The method used to retrieve the density maps represented in Fig. 4 corresponds to the one described in section 2.3.3. In each panel, the number of SMCs across the wall is represented against the normalized wall thickness, making so both intima (in red) and media (in blue) to share the same unitary dimension.

The results reported are coherent with the well-known experimental reality [9, 11]. Fig. 4(A) represents the baseline condition in absence of any environmental perturbation that corresponds to a healthy vein at the time of implant.

By fostering intimal SMCs division, a noticeable increase in intimal SMCs number is recorded in Fig. 4(B), well representing IH, while no substantial variations are recorded in the number of medial SMCs.

Similar considerations can be made for Fig. 4(C), where SMCs division within tunica media generates an increase in SMCs medial number, while no variation from the baseline is recorded for intimal SMCs, typical of the WR phenomenon.

Results appreciated in Fig. 4(B) and 4(C) are coherent with the single cellular event nature of the experiments performed so far, while a different subject is valid for Fig. 4(D), where SMCs division is active both in intima and in media, doing so both the number of SMCs in intima and in media are prone to increase.

Despite the setups to simulate the WR in Fig. 4(D) and Fig. 4(B) are the same, a lower increase in medial cell numbers is recorded in Fig. 4(D) than in 4(B). This is due to the fact that IH and WR act simultaneously in the last experiment performed, and accordingly, the IH phenomenon accelerates the wall relaxation toward the baseline value and this reduces the SMCs division in the media. This is the perfect example of how cellular events can generate noise at experimental level when they act simultaneously, making the identification process way more challenging. In our specific the specific case this happens by affecting the identification of α_4 , an aspect that will be especially clear while studying the simultaneous retrieve of α_3 and α_4 through the experiments outlined in section 2.3.

3.2 Intimal Hyperplasia

The inverse problem to singularly identify α_3 was successfully solved and the related analysis is reported in Fig. 5.

By monitoring the intimal area as the mean value over 100 samples, a precise identification of $\alpha_3 = 0.2$ was obtained. This is clear from Fig. 5(A) where the intimal area as output of ABM1 (S^I) is plotted in solid red, along with its standard deviation in dashed red, against the intimal area as output of ABM2 ($\overrightarrow{M^I}$) in solid blue, again along with its standard deviation. The fact that the perturbation level-dependent blue curve intersects the constant red curve right in correspondence of $\alpha_3 = 0.2$ is a clear indicator of the goodness of the analysis, also corroborated by Fig. 5(B), where φ_1 , standing for the distance between S^I and $\overrightarrow{M^I}$, is precisely minimized in correspondence of $\alpha_3 = 0.2$.

Fig. 5(C) shows the impact of a bigger specimen on the analysis. From the comparison between the minimization of φ_1 by using $N = 10$ (blue dashed line) and $N = 100$ (solid red), it is easy to deduce how the number of samples used is pivotal in order to precisely identify the right value of α_3 . One can indeed simply appreciate how, by using only 10 samples, φ_1 is not uniquely minimized and not even in correspondence of the right value. Finally, the non-suitability of a low number of samples is further sustained with Fig. 4(D) that shows the trend of Ξ_1 against N . If an error lower than 2% is considered as acceptable, then at least 50 samples are needed to obtain a realistic analysis, a number that increases if a lower error is desired.

Summing up, according to the successful minimization results, the intimal area can be considered as a solid measurement to prepare the experimental setup aimed to retrieve the single α_3 , and as its measuring does not pose any significant issue, other potential measurements have not been investigated. Also, a specimen size of $N = 50$ samples is acceptable to ensure the robustness of the experiment.

3.3 Wall Remodeling

Very similar considerations can be retrieved from the identification of α_4 , which results are reported in Fig. 6 with the same fashion of Fig. 5.

The accuracy of this identification may be not as high as the one appreciated for α_3 , but it is still reasonable enough to be considered as suitable for a modeling purpose. Indeed, if on one side from Fig. 6(A) emerges that the computational model curve, in blue, does not perfectly intersect the virtual dataset curve, in red, in $\alpha_4 = 0.3$, on the other side it is clear from Fig. 6(B) how φ_2 is still minimized around 0.3, even though not with the same sharpness of φ_1 in correspondence of $\alpha_3 = 0.2$ - see Fig. 5(B).

What already appreciated for α_3 about the increased confidence along with an increased number of samples is confirmed for α_4 by observing Fig. 6(C), where φ_2 does not reach a global minimum when minimized on the base of 10 samples. The analysis of Fig. 6(D) not surprisingly confirms the need of a conspicuous sample numbers to ensure the reproduction of the virtual experimental data free from a high error.

It is interesting to notice how not even a high number of samples like $N = 100$ ensures to reach a $\Xi_2 < 2\%$ like instead happened for Ξ_1 . However, a relative plateau is reached with N

= 60 that can be considered as a number beyond that the goodness of the analysis does not significantly increase.

Accordingly, if someone assumes a $\Xi_2 \approx 2.6$ as an acceptable error, the medial area can be considered as an appropriate measure to setup the experiment out of 60 independent samples.

A comparison between the two analyses already suggests how IH is an event more easily manageable than WR in terms of experimental planning and it has to be expected that this trend is further accentuated when turning both the cellular events on simultaneously as the two of them do not act independently.

3.4 Coupled analysis

Our expectations were confirmed with the inverse problem solution analysis for the coupled coefficients.

Fig. 7 reports the results for the first experiment described in section 3.3 for the coupled IH and WR, where a qualitative more than a quantitative analysis is appreciable. It is important to remark how to solve the inverse problem for the coupled IH and WR by monitoring intimal/medial area means to minimize a surface around a straight line instead of a curve around a single point. Also, the number of samples was chosen to be $N = 10$ for testing this experiment. Fig. 7(A) and Fig. 7(B) present the results of the φ_1 -surface minimization in a 3D and 2D view respectively for readers' convenience. It results clear how φ_1 is minimized in correspondence of the straight line individuated by $\alpha_3 = 0.2$. This is a remarkable result, indeed even though the level of complexity of the system has increased of one order of magnitude, by maintaining the same experimental setup a satisfying result is still achievable.

However, the same cannot be stated about the minimization of φ_2 , for which Fig. 7(C) and Fig. 7(D) present the results again in a 3D and 2D view respectively. In this case a clear minimization is not visible around the straight line identified by $\alpha_4 = 0.3$, being the surface affected by sparse local minima which do not even correspond to the target value for α_4 .

This results can have two implications that do not necessarily exclude each other: i) in order to setup an experiment able to replicate the coupled IH and WR mechanisms, the number of samples must be incremented to reach a better convergence, or ii) that the medial area is not a suitable measurement to correctly tune α_4 .

This was actually to be predicted. As anticipated while discussing Fig. 4, the IH had some kind of noisy influence on the WR, while the *vice versa* was not valid. The resulting limited variation in the number of medial SMCs, at least for the coupled IH and WR respect to the single WR, makes that a different measurement, still representative of WR but not as much influenced by IH, might be more suitable to setup α_3 and α_4 simultaneously.

The aim of the second experiment is to verify if an increased number of samples, e.g. from $N = 10$ to $N = 100$, was or was not a winning choice in order to improve the results obtained with the first one. Accordingly, Fig. 8 offers the same study provided with Fig. 7, with the difference that the analysis is now conducted out of $N=100$ samples.

From Fig. 8, the accuracy of the analysis does not benefit from an increased N . The focal point was in particular to improve the results appreciated in Fig. 7(C) and Fig. 7(D), a goal that is not achieved with Fig. 8(C) and Fig. 8(D), where the objective function is not minimized along the straight line individuated by $\alpha_4 = 0.4$ and more in general, a global minimum cannot be retrieved out of the surface represented. The accuracy of α_3 identification is not affected by a bigger N , not improved nor worsened.

As the second experimental setup did not bring any improvement, the attention was brought on the third experiment that aims to demonstrate how the wall density map should be considered as an effective measurement to be retrieved at experimental level for the simultaneous retrieval of α_3 and α_4 independently by the number of samples the analysis is carried out from.

Fig. 9(A) and Fig. 9(B) show the minimization of the related objective function on a 3D and 2D view respectively. The data presented demonstrate how the objective function is minimized right at the intersection of $\alpha_3 = 0.3$ and $\alpha_4 = 0.4$, which means that the inverse problem is solved in correspondence of both the targeted coefficients. This shows how measuring the wall cellular map on the basis of 10 samples is a choice suitable to retrieve the values of coefficient α_3 and α_4 acting simultaneously. However, it has not to be excluded that a higher samples number improved the robustness of the analysis.

Table 1 offers an overview of the experiments computationally tested, the ensemble of which draws a clear path for the experimental planning. The level of model's complexity, proportionally increasing with the number of coefficients acting together, differently impacts the experimental design, either in terms of entity of the measurements to retrieve or in terms of number of samples to be employed to set up the experiment.

On one end, a single-coefficient computational model can be adequately informed by focusing on the size of the specimen more than on the entity of the measurement. Indeed, both for IH and WR acting singularly the associated inverse problem is solved by evaluating a measure relatively easy to retrieve at experimental level, i.e. intimal and medial area respectively. For both, an analysis out of 10 samples did not give the expected results and this is why the output of the same framework has been tested for both the cellular events by increasing the samples from $N = 10$ to $N = 100$ with a 10 samples step. A reasonable compromise between accuracy of the solution and maintaining N reasonable low was found for IH and WR respectively with 50 and 60 samples.

On the other end, a two-coefficient model requires to monitor a measure that carries a more detailed level of information. Results of experiment #1 and experiment #2 described in section 3.4 clearly verified this hypothesis. Indeed, intimal and medial area did not prove themselves effective in solving the inverse problem associated to coupled α_3 and α_4 and this is true both on the base of 10 samples and on the base of 100 samples, showing that this kind of inverse problem is not sensitive to the specimen's size if tackled with a non-suitable measure.

However, the last experiment studied in the same section showed how a detailed analysis of the cellular density map across the wall is able to deliver the desired amount of information

in order to setup an effective experiment. This is not surprising if someone thinks about the peculiar role that cellular motility has during wall remodeling [22, 23]. The increased level of complexity of the model can only be handled with a measurement able to deliver some kind of spatial information, a characteristic that simple measures like intimal or medial area lack, while measures like density maps are perfect for. It is also interesting to notice how already a reduced virtual dataset, i.e. just 10 samples, is already enough to reach a reasonable output.

Finally, it has to be expected that i) an increased number of samples can only further improve the outcome and ii) moving toward a model closer to the physiological reality, realistically including more than 2 cellular events acting together, 10 samples will not be a solid specimen's size anymore, while certainly cellular density map should be the first choice as measure to be tracked in an experimental setup.

4. Conclusions

This work represents an agile framework to pre-design the experiments needed to setup computational models of vascular adaptation able to reproduce as accurately as possible the biological reality. With an *ad hoc* use of our ABM [16], the minimum number of samples needed for the analysis can be easily predicted and, not less important, the suitability of the biological measurements verified *a priori*.

With the current implementation, we demonstrated how increasing the complexity of our ABM impacts the entity of the measurement to be retrieved experimentally more than the size of the specimen required for its setup. This is the answer to the pending question we left behind in the prior work by our group [1], where it was not clear whether the size of the specimen or the entity of the measurement was the key player to set up the model effectively. However, whether a further increased complexity implied an even more accurate measure or a higher number of samples, or both, remains to be verified in future developments of this work.

Further on the next steps to address, a particular attention will be dedicated to additional cellular events like apoptosis, ECM deposition/degradation, and especially cellular motility, the study of which has been demonstrated to be pivotal for this field in a recent study from our group of investigators [22]. The real challenge on this will be to always verify that the main assumption holds in order to deal with a realistic analysis. The always actual goal for computational scientist is after all to advance the model closer to the physiological reality. Along this line, an additional further development will focus on moving from a 2D space to a 3D space. This is a task that should obviously be performed at the level of ABM first and then applied to the presented framework with the same modalities here described. One possible approach could be to re-think the whole structure as a 3D grid and not anymore as a 2D, also re-implementing the fundamental laws in a 3D space. However, an intermediate 2.5D simulation can also represent a more agile way to move toward the third dimension. Given a segment of graft of interest, several cross sections corresponding to as many ABMs can be studied separately and the 3D anatomy reconstructed by interpolation.

A particular importance should also be given to the validation of the presented framework that can be performed with an a posteriori set of experiments aimed to prove that the entities of the measurements and the size of the specimen predicted by it, fit indeed the requirements to effectively tackle the problem under analysis.

A framework like the one presented can certainly represent a great turning point in the field of computational models, because it will give the researchers a feeling of the resources needed before conducting the actual experiment, limiting in this way any kind of wastefulness in terms of equipment and of resources, especially animal resources speaking about medical research field.

Finally, the flexibility of the framework presented allows it to be easily re-shaped for problems of different nature and consequently applied to several fields of research.

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Biography

Marc Garbey, PhD is the scientific director of MITIE and chair of the Center for Computational Surgery at Houston Methodist Research Institute. He former was a professor in biology, mathematics and computer science at University of Houston. He received his PhD from Ecole Centrale de Lyon, France in applied mathematics in 1984. His work in computational surgery is funded by NSF and NIH. He has about 200 referred publications published mainly in journal of mathematics, computer science, mechanic, biology and medicine.

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Stefano Casarin, PhD is a postdoctoral fellow with the Center for Computational Surgery at Houston Methodist Research Institute. He received his MS in biomedical engineering from Politecnico di Milano, Italy in 2013 and his PhD in fluid mechanics from Université de La Rochelle, France in 2017. His work, under the mentorship of dr. Marc Garbey and dr. Scott Berceci, mostly focuses on the development of computational models to improve the outcome of vascular therapies and treatments in response to arterial occlusive diseases. His work is supported by NIH and he has been publishing in journals in the field of computational science and theoretical biology.

References

1. Casarin S, Berceci SA, Garbey M, A Computational Model-Based Framework to Plan Clinical Experiments – An Application to Vascular Adaptation Biology Shi Y. et al. (eds) Computational Science – ICCS 2018. ICCS 2018. Lecture Notes in Computer Science, vol 10860 Springer, Cham, 2018.
2. Morley R, Sharma A, Horsch AD, Hinchliffe RJ, Peripheral artery disease. *BMJ*, 2018 360: p. j5482.
3. Mirza A, Bower T, Endoscopic versus open great saphenous vein harvesting for femoral to popliteal artery bypass. *Journal of Vascular Surgery*, 2018 67(4): p. 1199–1206. [PubMed: 29102747]
4. Villemoes M, Lindholt JS, Houliand KC, Gottschalksen B, Petersen NP, Rasmussen M, et al., Cost-Effectiveness Evaluation of Heparin Coated Versus Standard Graft for Bypass Surgery in Peripheral Artery Disease Alongside a Randomised Controlled Trial. *European Journal of Vascular and Endovascular Surgery*, 2018 56(1): p. 87–93. [PubMed: 29622512]
5. Zhu Y, Valley MP, Bilateral Versus Single Internal Mammary Artery Use in Coronary Artery Bypass Grafting: A Propensity Matched Analysis. *Heart, Lung and Circulation*, 2018.
6. Goldman S, Zadina K, Moritz T, Ovitt T, Sethi G, Copeland JG, et al., Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: results from a Department of Veterans Affairs Cooperative Study. *J. Am. Coll. Cardiol*, 2004 44(11): p. 2149–2156. [PubMed: 15582312]
7. Samano N, Souza D, The no-touch saphenous vein for coronary artery bypass grafting maintains a patency, after 16 years, comparable to the left internal thoracic artery: A randomized trial. *The Journal of Thoracic and Cardiovascular Surgery*, 2015 150(4): p. 880–888. [PubMed: 26282605]
8. Davies M, Hagen PO, Pathophysiology of vein graft failure: A review. *European Journal of Vascular and Endovascular Surgery*, 1995 9(1): p. 7–18. [PubMed: 7664016]
9. Jiang Z, Wu L, Miller BL, Goldman DR, Fernandez CM, Abouhamze S, et al., A novel vein graft model: adaptation to differential flow environments. *Heart and Circulatory Physiology*, 2004 286(1): p. H240–H245. [PubMed: 14500133]
10. Kudo F, Muto A, Maloney SP, Pimiento JM, Bergaya S, Fitzgerald TN, et al., Venous Identity Is Lost but Arterial Identity Is Not Gained During Vein Graft Adaptation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2007 27: p. 1562–1571.
11. Klein B, Destephens A, Dumeny L, Hu Q, Yong H, O'Malley K, et al., Hemodynamic Influence on Smooth Muscle Cell Kinetics and Phenotype During Early Vein Graft Adaptation. *Annals of Biomedical Engineering*, 2017 45(3): p. 644–655. [PubMed: 27624660]
12. Donadoni F, Almarza-Pichardo C, Bartlett M, Dardik A, Homer-Vanniasinkam S, Zuccarini-Diaz V, Patient-Specific, Multi-Scale Modeling of Neointimal Hyperplasia in Vein Grafts. *Front. Physiol*, 2017 8.

13. Manini S, Passera K, Huberts W, Botti L, Remuzzi A., Computational model for simulation of vascular adaptation following vascular access surgery in haemodialysis patients. *Comput. Methods Biomech. Biomed. Engin*, 2014 17: p. 1358–1367. [PubMed: 23281788]
14. Garbey M, Berceci SA, A dynamical system that describes vein graft adaptation and failure. *J. Theor. Biol*, 2013 336: p. 209–220. [PubMed: 23871714]
15. Casarin S, Berceci SA, Garbey M, Linking gene dynamics to vascular hyperplasia –Toward a predictive model of vein graft adaptation. *PLoS ONE*, 2017 12(11): p. e0187606. [PubMed: 29190638]
16. Garbey M, Casarin S, Berceci SA, Vascular Adaptation: Pattern Formation and Cross-Validation Between an Agent Based Model and a Dynamical System. *J. Theor. Biol*, 2017 429: p. 149–163. [PubMed: 28645858]
17. Fernandez C, Goldman DR, Jiang Z, Ozaki CK, Tran-Son Tay R, Berceci SA, Impact of Shear Stress on Early Vein Graft Remodeling: A Biomechanical Analysis. *Annals of Biomedical Engineering*, 2004 32(11): p. 1484–1493. [PubMed: 15636109]
18. Marino S, Hogue IB, Ray CJ, Kirschner DE, A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J. Theor. Biol*, 2008 254: p. 178–196. [PubMed: 18572196]
19. Meyerson S, Schwartz LB, The effects of extremely low shear stress on cellular proliferation and neointimal thickening in the failing bypass graft. *Journal of Vascular Surgery*, 2001 34(1): p. 90–97. [PubMed: 11436080]
20. Liu M, Roubin GS, King SB, 3rd, Restenosis After Coronary Angioplasty – Potential Biologic Determinants and Role of Intimal Hyperplasia. *Circulation*, 1989 79: p. 13741387.
21. Owens C, Adaptive changes in autogenous vein grafts for arterial reconstruction: Clinical Implications. *J. Vasc. Surg*, 2010 51(3): p. 736–746. [PubMed: 19837532]
22. Garbey M, Casarin S, Berceci SA, A Versatile Hybrid Agent-Based, Particle and Partial Differential Equations Method to Analyze Vascular Adaptation. *Biomech. Model Mechanobiol*, 2018 to appear.
23. Tardy Y, Resnick N, Nagel T, Gimbrone MA, Jr, Dewey CF, Jr, Shear stress gradients remodel endothelial monolayers in vitro via a cell proliferation-migration-loss cycle. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 1997 17(11): p. 3102–3106.

Highlights

- A computational framework is realized in order to pre-design experiments for the setup of computational models of Vein Graft Bypasses (VGBs) adaptation.
- An Agent-Based Model (ABM) is assumed to be comparable to a true experiment and used both to generate virtual experimental data and to browse the driving coefficients' space, generating two separate threads of outputs.
- The inverse problem set between the latters is solved in order to predict the entity of the experimental measure and the specimen's size.
- While basic one-coefficient models require measurements relatively simple to retrieve, e.g. compartments' areas, more complex models imply a bigger effort to explore spatial informative measurements, e.g. cellular density maps. As general trend, the bigger the samples' number, the more robust the analysis.

Fig. 1 –. Agent-Based Model.

The ABM (right panel) replicates clinical evidences of a 6 months VGBs follow-up (left panel) [16].

Fig. 2 –. Conceptual Scheme.

A twofold usage on our ABM generates both the reference measurements (S^k) intended as experimental data to be retrieved, and the perturbed output ($\vec{M}^1$) to be compared with the reference in order to solve the inverse to optimally plan the experiment [1].

Fig. 3 –. Density Map.

One-dimensional segment of the ABM, where each site is represented with a rectangle. The empty sites represent the lumen of the graft, while the wall is populated by SMCs (black ovals) and ECM (gray rectangles).

Fig. 4 – Virtual Experimental Dataset.

Starting from a basic solution (A) that represents a healthy vein at the time of implant, ABM1 is setup in order to simulate the hyperplasia of the tunica intima (B) and tunica media (C), both acting singularly (B)-(C) and coupled (D) [1].

Fig. 5 –. Intimal Hyperplasia.

The interval of confidence (A) and the value (B) of α_3 , being the coefficient driving intimal mitosis, are retrieved on the base of $N=100$ samples by measuring the intimal area as output of the virtual experiment. The impact of a larger specimen is evaluated by comparing the solution of the inverse problem retrieved with 10 and 100 sample (C) and by studying the N -dependent dynamic of percentile error between virtual reference (ABM1) and computational model (ABM2) (D) with $N \in \{10, 20, \dots, 100\}$ [1].

Fig. 6 –. Wall Remodeling.

The interval of confidence (A) and the value (B) of α_4 , being the coefficient driving medial mitosis, are retrieved on the base of $N=100$ samples by measuring the medial area as output of the virtual experiment. The impact of a larger specimen is evaluated by comparing the solution of the inverse problem retrieved with 10 and 100 sample (C) and by studying the N -dependent dynamic of percentile error between virtual reference (ABM1) and computational model (ABM2) (D) with $N \in \{10, 20, \dots, 100\}$ [1].

Fig. 7 – Coupled Analysis: Experiment #1.

The value of α_3 , being the coefficient driving intimal mitosis, through intimal area measurement and α_4 , being the coefficient driving medial mitosis, by medial area measurement are identified with IH and WR acting simultaneously out of $N=10$ independent simulations. The minimization of φ_1 and φ_2 is represented both in 3D (A)(C) and in 2D view (B)-(D) respectively.

Fig. 8 –. Coupled Analysis: Experiment #2.

The value of α_3 (intimal mitosis) through intimal area measurement and α_4 (medial mitosis) by medial area measurement are identified with IH and WR acting simultaneously out of $N=100$ independent simulations to verify the effectiveness of increasing the specimen's size to improve α_3 and α_4 retrieval. The minimization of φ_1 and φ_2 is represented both in 3D (A)-(C) and in 2D view (B)-(D) respectively.

Fig. 9 –. Coupled Analysis: Experiment #3.

The value of α_3 (intimal mitosis) and α_4 (medial mitosis) are identified with IH and WR acting simultaneously by monitoring the cellular density map across the graft's wall on the base of $N=10$ independent simulations. Both the 3D view (A) and the 2D view (B) are offered for the reader's convenience.

Table 1.
Virtual Experiments Summary.

Each virtual experiment is described by the cellular event tracked, the corresponding coefficient on the ABM, the experimental measure tested along with the number of samples and the suitability of the experimental setup.

Cellular Event	Coefficient	Experimental Measurement Tested		Number of Samples		Experimental Setup Suitable (Y/N)			
Intimal Hyperplasia(IH)		Intimal Area		10		N			
				50		Y			
Wall Remodeling (WR)		Medial Area		10		N			
				60		Y			
Coupled IH/WR		Intimal/M edial Area		10		N			
				100		N			
		Wall Cellular Map		10		Y			