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Jean-Baptiste Masson, Jean-Louis Martin

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J.-B. Masson[a] and J.-L. Martin

Laboratoire d'Optique et Biosciences, École Polytechnique, CNRS UMR 7645, INSERM U696, 91128 Palaiseau, France

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Abstract. In most organs, depending on the scale, the nature of the heart pump, the geometry and topology of the organ, some of the blood vessels tend to exhibit sinuous trajectories. We describe a part of this sinuous behavior, including partial biological and strong physical effects in a global physical framework. We will voluntarily focus on physical and topological effects. This study is performed on the vitelline membrane of the chicken embryo. Crossing angles, sinuosity, and the oscillation amplitude of the vascular system are analyzed. Surprisingly, the equation of river meandering dynamics is found to model the sinuosities in the vascular system, and an extension of this equation to non planar case is able to explain the effect of tissue global curvature on the vascular system. Results of this study could lead to a new understanding of the interplay between biological signaling and physical effects in determining the vascular pattern in different tissues.

PACS. 87.18.La Morphogenesis – 87.19.Rr Mechanical properties of tissues and organs – 87.19.St Movement and locomotion

1 Introduction

Blood vessels are a complex network of tubes that carry oxygenated blood and nutrients, but also evacuate cellular waste and exchange thermal energy throughout our bodies. The process of growing new blood vessels, known as angiogenesis [1], is a fundamental biological mechanism that results in serious disease when it is not functioning properly. Angiogenesis is an essential process during both development and the restoration of blood flow to injured tissues. This process is regulated by interplay of growth factors, inhibitors, shear stress and transmural pressure [2–4]. It is also involved in tumor growth.

Nevertheless, the structure of the vascular system should have a major influence on the biological activity of the cells nourished by the blood circulating in the network. For example, the spatial structure of coronaries (Fig. 1A) exhibits sinuosities that will have strong consequences on both the blood flow and the atheromatous plaques accumulation within the artery. Furthermore, some organs having strong global curvature seem to have a strong oscillatory vascular structure. So, curvature may be an important (among others) parameter acting on blood vessel sinuosity. Surprisingly, the sinuosities of the vascular structure have not yet been investigated, even though the geometry of the vascular structure will have strong consequences on the blood flow, the tissues irrigation and the physical properties of the biological tissue. Therefore, characterizing and understanding some of the mechanisms involved in this oscillatory-like behavior, could lead to interesting models as well as a better understanding of vascular behavior.

In this paper, we give a physical framework to the oscillatory like behavior of the vascular system, by including both the physical and the biological effects that act on it. The study is performed on the membrane surrounding the chicken embryo, the vitelline membrane [5]. Crossing angles, sinuosity, and amplitude of oscillation of the vascular system are analyzed. The equation of river meandering dynamics is found to model very well the sinuosities of the vascular system. Finally, the effect of global curvature of the vitelline membrane on the blood vessels is investigated. The extension of the river meandering dynamics equation to non planar space is partially able to explain the effect of global curvature on the vascular system.

2 First experiment

In brief, the embryo development [5] can be described as follow: during the first two days of incubation the embryo develops, generating a capillary plexus, i.e. capillaries without blood flow, on the vitelline membrane. Around 35 h after incubation the heart begins to beat, and a complete new vascular system is created on the vitelline membrane. This study is focused on this new vascular system.

Experiments began 35 h after incubation, at 37 °C, by the removal of 5 ml of eggs white and a part of the shell. In order to avoid drying of the eggs, 5 ml of Phosphate Buffered Saline (PBS) with calcium and magnesium were

[a] e-mail: jean-baptiste.masson@polytechnique.fr

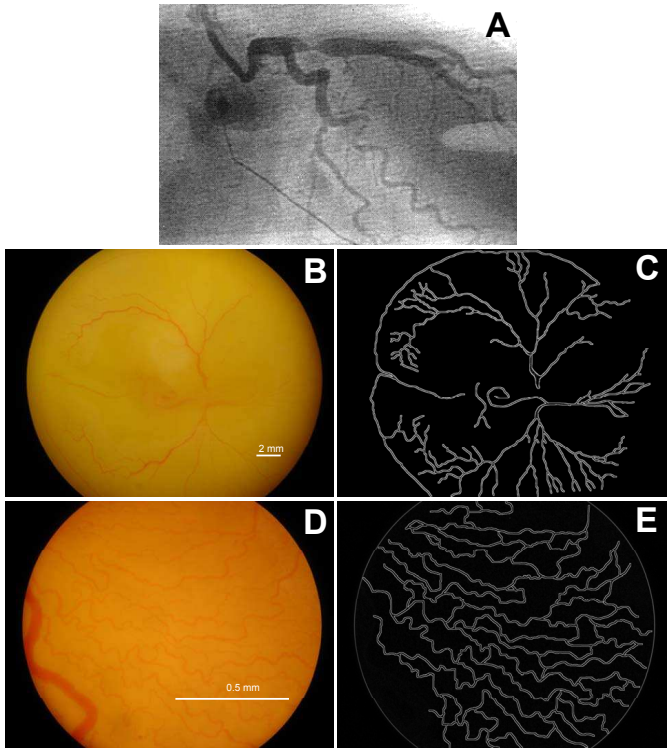

Fig. 1. A X-ray photo of a coronary. B Photo of the vascular structure of the vitelline membrane of the chicken embryo (main vascular structures visible are in the zona opaca vasculosa and sinus terminalis) and D photo of the vascular structure near the head of the chicken embryo (in the zona pellucida). C and E results of the numerical procedure for photos B and D.

added to the egg, and the removed shell was replaced by transparent adhesive paper. All eggs were studied between approximatively 35 and 96 h. After 96 h new processes take place, such as the change of yolk structure [5], which could add artifacts to the study. The experiment was performed on 70 eggs.

The first kind of experiment consisted in a continuous imaging of the vitelline membrane. Analysis was performed on the pictures taken between the first heart beat and 90 h after incubation. To each blood vessel first appearance was associated its first geometry, not all blood vessels just after the heart begins to beat are straight lines, and the time at which its evolution has been studied. Imaging was performed using a CCD captor put on a microscope.

3 Numerical analysis

Representative pictures of the vascular system that can be treated by the numerical procedure are given in Figures 1B, 1D. Our interest is focused on the large blood vessels which are clearly visible on the images. All images were converted into black and white images. All treatments (Fig. 2) were done in MATLAB. Each image was sharpened a first time, and then the contrast was enhanced. The algorithm of contrast enhancement was the one used in Image J [6]. Contrast enhancement was made with 10% of saturated pixel, and the histogram of pixel brightness was equalized. Major part of the background was then removed using the rolling ball algorithm developed by Sternberg [7] with a 25 pixel radius. Then the image was "thresholded". The minimum part of the threshold was not changed, only the upper part was adjusted in order to get most of the vascular network. After this thresholding, the Sobel Find Edges algorithm was used. The result was then cleaned with a denoising algorithm and solitary white pixels were removed. At the end of this procedure, the edges of the capillary network were known. Most of vascular structures extracted by this procedure (Figs. 2C, 2E) had the same size, which was not an issue for this study as vascular width was not the main object of the study. By filling the edge result with white pixels, corresponding to the inner parts of the blood vessels, and then skeletonizing it, the topology of the network was extracted. The vertexes were easily extracted, as they were points with at least three neighbors. To extract angles between blood vessels the direction of blood circulation were added to the network. The angle was therefore defined by blood circulation, without any uncertainty. Finally, the angle was extracted as the angle of the curves tangent to the blood vessel at the vertex. We define the sinuosity of a curve as the ratio of the curved distance between the vertexes (distance along the trajectory of the vessel), and the distance between the extrapolated Bezier curve (second order) between each vertex. The use of Bezier curves allows the comparison of several different parts of the vitelline membrane and eliminate some global motions of the blood vessels. The sinuosity takes values superior or equal to 1, high values of sinuosity indicate a tortuous trajectory. Finally, the amplitude of sinuosities are the local maximum deviation between the blood vessel and the extrapolated Bezier curve joining two vertices, and its extraction is performed with a mean squared derivative algorithm is used.

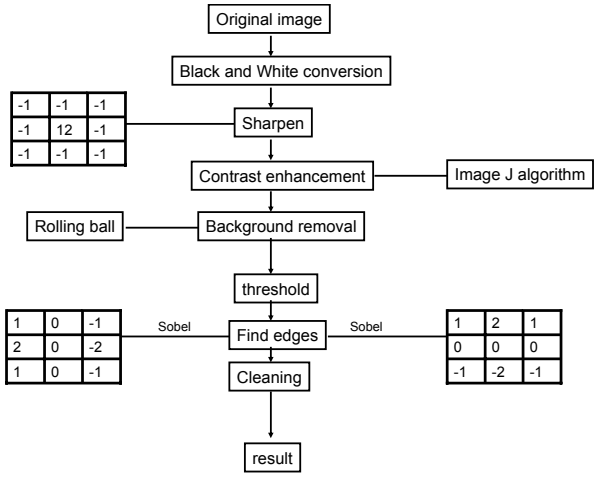

Fig. 2. Schema of the image treatment program.

In some cases, for angle detection or sinuosity measurements, the algorithms were not able to give a reasonable

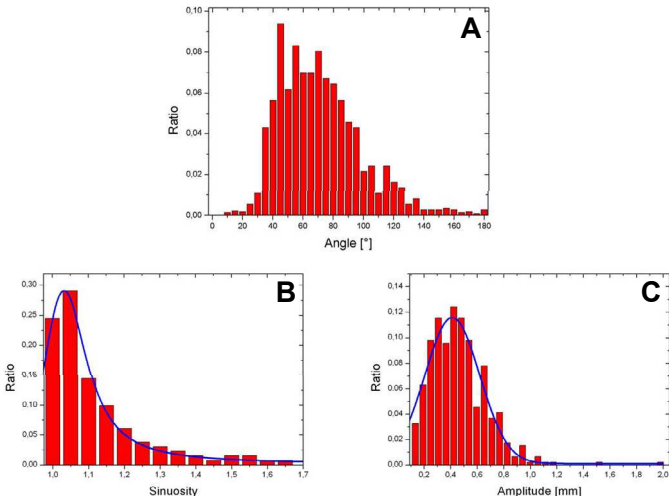

Fig. 3. Results of the first experiment. All histogram values are divided by the global number of data considered N. A Histogram of crossing angles of blood vessels ($N = 9844$). B Histogram of sinuosity ($N = 11003$), in Blue the fit extracted from the equation of vascular motion. C Histogram of the amplitude of sinuosity, in Blue the fit extracted simulation ($N = 11003$).

result, therefore, theses angles or sinuosity were removed from the statistics. It adds a small error to the results, but we found that for angle measurements, there is an error every 150 angles measurements, and for sinuosity measurement there is an error every 100 measures.

4 Results and model

The first result extracted from numerical treatments was the statistic of angle crossing between blood vessels (Fig. 3A). Significant angle values are found between 30° and 140°, and angles mean value is 73° with a standard deviation of 26°. It is a wide repartition of angle. One of its consequences is to reduce possible instabilities in blood repartition [8], which could lead to under oxygenation and underfeeding of some parts of the biological tissues. Furthermore, from direct analysis of the motion of the red blood cells, the flow can be immediately extracted, and one might notice that at vascular crossing the flow is not shared equally between the two new vessels, because of the constant changes in diameter and flow rate. Finally, at this scale and at these times of the embryo development, it will be interesting to investigate [9] whether this vascular network minimizes the global resistance to flow with respect to neither the volume nor the surface of the network [8].

As it can be seen in Figures 1B, 1D, blood vessels tend to oscillate around a mean trajectory. In Figure 3B, the statistic of sinuosity can be seen. Sinuosity mean value is 1.07 ± 0.005. Furthermore, almost 20% of the blood vessels have a sinuosity superior to 1.25 ± 0.005, which means a strong lateral space extension. This sinuosity should play a major role in tissue irrigation, by allowing single blood vessels to cover more area.

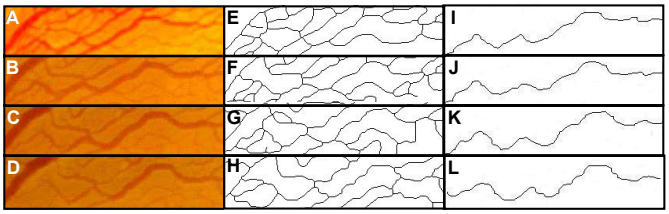

Fig. 4. Example of blood vessels trajectory evolution. The image is 1.7 mm by 0.4 mm. A–D are direct images of the blood vessels; the first image was taken at 40 h, and then other images were taken every 8 h after the first one. E-H are results from the numerical procedure. I–L Only one of the blood vessel skeleton is displayed.

In order to describe the behavior of the blood vessel, it is modeled as an infinitely thin string of length L, and its motion is bidimensional. Furthermore, its motion is considered to be the deviation from the Bezier curves used to extract data from experiments. The evolution of this string is described with curved coordinates (coordinates along the trajectory), s, so its position is defined by $\hat{r}(s)$, and usual moving basis of vectors $[\hat{S}, \hat{N}]$ where $\hat{S}$ is the unit vector tangent to the string, and $\hat{N}$ is the unit vector normal to $\hat{S}$. We focus here on the evolution of the trajectory of the blood vessel, which is modeled by the string. Therefore, only the motion of the resulting string from the numerical analysis procedure of the blood vessels is analyzed.

The first step in describing the blood vessel behavior is the local observation of its trajectory. An example of evolution may be seen in Figures 4A–4L. From direct analysis of the evolution of the local deviation of the Bezier trajectories of the blood vessels in the vitelline membrane extracted from the numerical procedure, and for low in-plane curvature $\kappa(s)$, the rate of normal migration (migration along $\hat{N}$) of the blood vessel trajectory can be expressed by the relationship:

$$M_{sts} = \alpha\kappa(s), \tag{1}$$

where α is not considered here to be a function of s. We stress here that this relation is extracted from experimental data. It is a function of biological activity and of several physical interactions such as constrains and stresses. In fact, even with the Bezier curve procedure it may still be a function of s. Yet, as a first attempt of modeling, α is considered to be a constant. So, with this hypothesis the analysis of the data has been performed again and optimized for a constant α. Therefore the description of the sinuosity dynamics will give global general results averaging result from different vitelline area not having the exact same dynamics. Furthermore, because the experimental recording can be considered to be relatively short, α is considered to be time independent. Nevertheless, it is sure (and for most vascular structure of organs) that α may have significant variation with time. Thus, blood vessel changing size or having a higher blood flow, with a straightening dynamics may also be modeled by

the diminution of α, even the negativity of α. Finally, this relation is an approximation of the true relation; nevertheless as the string is continuous, locally smooth and never exhibits any sharp changes, this migration can be written as a series of power of $\kappa(s)$. We may therefore consider M_{sts} expression as a first order approximation with curvature. The zeroth order approximation is a rate of migration M_{sts} is equal here to 0 (because of the Bezier deviation). Therefore, the relation above summarizes the effect of local biological and physical activity that act on the blood vessel trajectory within the description of this model.

Interestingly, there is a similarity here with the local meandering dynamics of rivers [11–13]. The physical process underlying the meandering of the river channel is an outbalance equilibrium of erosion and deposition from the sides of the channel. The erosion and deposition are supposed to be proportional to the normal velocity of the water on the edge of the channel. Furthermore, the flow in the river has high Reynolds number [11,12], and so its velocity in the channel u can be approximated by writing:

$$u(n) \approx C\left(1 + n\kappa\left(s\right)\right), \tag{2}$$

where C is a constant, and n is the coordinate along $\hat{N}$. Therefore, the rate of normal migration of the channel is also proportional to the local planar curvature. It is just an interesting comparison, there are no erosion-deposition dynamics in the cells motion. In the model used to study the blood vessel sinuosity motion we are just dealing with a string dynamics.

If this migration term was the only one, or even with an added noise migration, blood vessels will tend to have divergent trajectories. A small variation of the planar curvature $\kappa(s)$ will be indefinitely amplified. During blood vessel migration, several mechanical stresses act on endothelial cells. Therefore, the response of the string model to physical stresses can be included in the minimizing of this free energy F:

$$F[r] = \int ds\left|\kappa\left(s\right)\right|^2. \tag{3}$$

Furthermore, experiments show that blood vessels have smooth trajectories with no sharp changes. The minimization of F and the smoothness of trajectories can be included in another rate of migration term [10]:

$$M_{Fss} = \beta\left(\frac{\partial^2\kappa\left(s\right)}{\partial s^2} - \kappa^3\left(s\right)\right), \tag{4}$$

where β is modeled to be not a function of s. β is modeled in the same way than α, i.e. β is a function of biological and physical activity, nevertheless we chose to model it as a constant. This migration is the consequence of strong global physical considerations. Finally, all other parameters local or global, that we did not modeled or linked to true biological noise, are inserted into a Gaussian noise:

$$M_{noise} = \chi(s,t). \tag{5}$$

The noise is chosen to be Gaussian as a hypothesis. The standard deviation D may also be a function of time, biological and physical activity, yet it is modeled to be constant.

An other remark can be made in order to get the equation of motion of the blood vessel: in the same way than in river meandering dynamics the dominant local velocity of motion is the one in the normal ($\hat{N}$) direction. Thus, as this equation is dealing with a noise term, the tangential velocity (along $\hat{S}$) of the curve can be replaced by its average value, which leads to the meandering river equation [11]:

$$\frac{\partial \boldsymbol{r}}{\partial t} = -\alpha\frac{\partial^2 \boldsymbol{r}}{\partial s^2} - \beta\frac{\partial^4 \boldsymbol{r}}{\partial s^4} + \chi\left(s,t\right) - \left\langle \int_0^s ds^o\kappa\left(s^o\right)\left(\alpha\kappa\left(s^o\right) + \beta\left(\frac{\partial^2\kappa\left(s^o\right)}{\partial s^2} - \kappa^3\left(s^o\right)\right) + \chi(s^o,t)\right)\right\rangle\hat{S}. \tag{6}$$

Interestingly, this equation is able to model both the effects of high Reynolds number flow on river meandering dynamics, and the effects of low Reynolds number flow on the endothelial cells and so on the trajectories of blood vessels. Nevertheless, a major difference between the two physical processes is that in meandering river dynamics, small-wavelength meander bends tend to decay due to the Bernoulli shear [13]. In the vascular system all wavelengths are authorized and they are no specific processes acting on small-wavelength bends. Another major differences between the two dynamics is that there are no endothelial cells detachment and migration inside the blood vessel. Furthermore, the model does not comment at all the motion of endothelial cells close to the blood vessel.

In order to model both the sinuosity statistics and the sinuosity amplitude statistics, we have implemented a program to solve the equation of motion of the blood vessel curve. The statistics of length observed in the biological samples were added to the program to adjust the length of the string. Furthermore, the initial geometry of the non straight blood vessel at initial time of recording were added also to the numerical statistic calculation. The number of iterations was scaled to the time of experimental measurements, for each blood vessel. As all blood vessels are not visible at the same time, experiments give access to a statistic of recording times. A large number of simulations were performed, and result was extracted after a spatial rescaling (Figs. 3B, 3C). The correlation between experimental results and simulations is good, and the distribution of meandering amplitude seems to follow a Gaussian like statistics. Nevertheless, it should be pointed out that there are three parameters in the equation of motion: α, β, D, where D is the standard deviation of the noise and that clearly the solution extracted here is not unique and some values of the parameters give similar results. Nevertheless, results of the model give quantitative results and further experimental work will give more results and help find the exact relations linking the parameters to both physical and biological data. Furthermore,

experimental work should be focus to give three parameters value to specific zone of the vitelline membrane and specific zone of the chicken embryo and watch the relation between these zones.

In this numerical treatment the blood vessels crossing problem has been voluntarily avoided. In the simulation, a probability of crossing could have been added and then the decomposition of the first string into two new ones could have been modeled. Nevertheless, because this problem does not deal with river meandering dynamics, such assumptions can not easily be made. Complex biological rules govern the connection properties of blood vessels, especially about artery-vein differentiation. Complete theory is not known but a very promising one known as the "go with the flow" [14,15] seems to give very good experimental results. Therefore, and because of this complexity, we have chosen to add a length distribution to the program.

5 Second experiment and extension of the model

The second experiment, was performed to investigate global curvature effects on the vascular structure, it began 46 h after incubation. The purpose was to give an almost constant global curvature to the vitelline membrane, in order to study its effect on the vascular structure. Several balls of glass, of different sizes, were designed. They were washed successively with bleach, alcohol and water to be finally exposed to UV light for 15 min. All this procedure was performed in order to diminish the risks of infections of the vitelline membrane. Half of the balls were put directly on the vitelline membrane, the other half, under this membrane by pushing the yolk. Imaging was continuously performed between 48 h and 75 h after incubation. There are different kinds of issues for these two experiments: in the first kind, there is the risk of injuring the membrane, in the second kind, injuring the yolk will cause the quick death of the embryo. All experiments were performed far from the embryo itself, so on the external parts of the vitelline membrane.

The global tissue curvature was investigated, because several biological tissues having a non negligible curvature tend to have a strongly oscillating vascular structure. In this study, the vascular network was on a planar field, which was in three dimensional spaces. It is important to see the effect of global curvature on blood vessel dynamics for a better understanding of the vascular structures on the surface of some organs such as the heart. Evolution of the mean value of sinuosity with the variation of the curvature is exposed in Figure 5. A clear increase in the sinuosity is observed with the rise of curvature. Results of statistics of amplitude (an example is exposed in Fig. 6) showed modifications and distortions that did not seemed to be well fitted using modified values of the couple (α, β). There is an intrinsic effect of the change of curvature, it is the change of metric. Derivation in curved space is not similar to derivation in flat space [16]. Therefore, In order

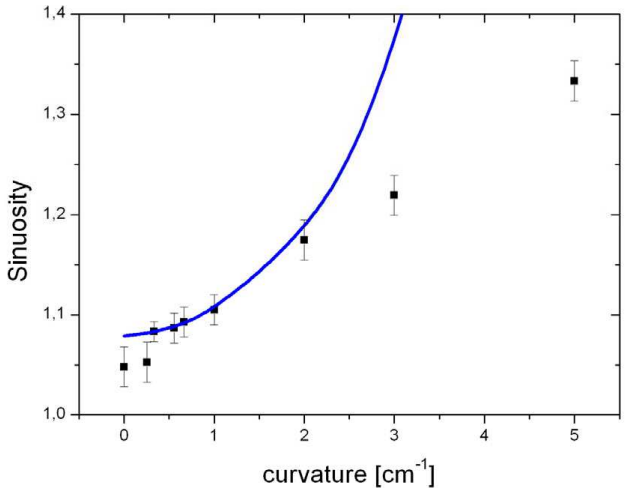

Fig. 5. Results of the second experiment. The histogram values are divided by the global number of data considered N. Evolution of the mean value of sinuosity versus global curvature ($N = 4532$), in Blue the fit of the modified equation of vascular motion, where the normal derivatives have been replaced by their covariant equivalent.

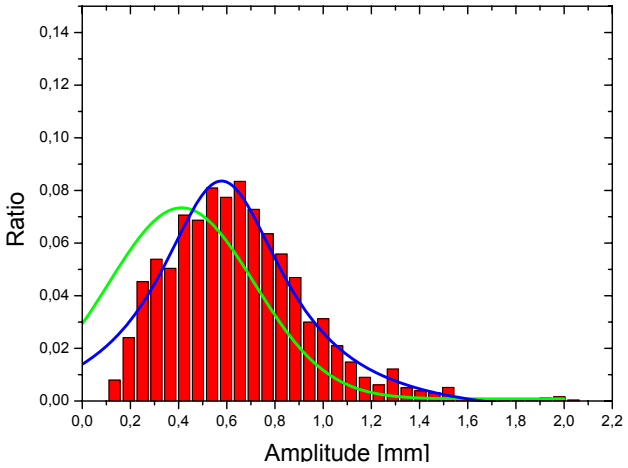

Fig. 6. Example of statistics of amplitude of sinuosity for a global curvature of 1 cm. The green curve is a fit obtained by varying the couple of parameters α and β. The blue curve is a fit obtained with the same value of α and β than in the first part of this paper but with the covariant derivatives.

to model the effect of the global curvature of the vitelline membrane, we focused on the effect of the change of the space metric. All derivatives used in equation (5) were replaced by their curved space equivalent:

$$\nabla_j \boldsymbol{r} = \left(\frac{\partial r^i}{\partial x^j} + r^k \Gamma^i_{jk} \right) e_i, \tag{7}$$

where Γ^i_{jk} are the Christoffel's symbols [16], e_i are the local unit vectors, x^j are the local coordinates, and where we have used implicit summation notation.

Replacing the classic derivatives with covariant derivatives modifies the local in-plane values of $\kappa(s)$ and so

modifies the dynamics of the blood vessel motion. Simulations were performed with the same three parameters α, β, D used to fit the first experiment data (Figs. 3B, 3C). The result is exposed in Figure 5. We see that for small global curvature the fit is in good agreement with experimental data. Clearly, for these curvatures there is only a pure metric effect, that acts locally on the curve of the blood vessel by changing $\kappa(s)$. We can see a difference between the fit and experimental data growing with the rise of the global curvature. Clearly, other biological processes are acting on the system due to the geometry modifications, and to the mechanical constrains that act on the vitelline membrane. These experimental observations seem in good agreement with the surface vascular structures and in fact with the global vascular structure observed in many organs such as the heart, the liver or the kidney, all three of them having a complex curved geometry.

6 Conclusion

In this paper we focused our analysis on the unusual subject of sinuosities in the vascular system. We developed an image analysis procedure, allowing the direct study of the evolution of the vascular structure. The equation of river meandering dynamics has been able to model the statistics of both sinuosity and amplitude of sinuosity of the embryo vascular system. The model has been used to globally model the sinuous behavior of the blood vessel, leading to a general description of the meandering dynamics of the vitelline membrane. Furthermore, it has been shown that for small global curvature, the rise of sinuosity of the blood vessel network is mainly the consequence of the change of space metric.

Finally, in this paper we have focused the study on physical effects. A development of the model should investigate the dependency of the three parameters α, β and D with biological and physical activity. The equation found to model this behavior can be extended and developed in order to better match new discoveries in angiogenesis research. Clearly, studying the structure and properties of the vascular system will lead to a better understanding of the function and structure of some organs.

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