Title. A-model, a deep look into the atherogenesis onset.

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Introduction.

The term "*atherosclerosis*" was introduced by Marchand to describe the association of fatty degeneration and vessel stiffening (Aschoff, 1935; Crowther, 2005). Atherosclerosis, as it has been described in Lusis (2000), is a "*progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries*". Its initial phase, *atherogenesis*, consists in the sub-endothelial accumulation of cholesterol-engorged macrophages, called *foam cells* (FC). FC are the precursors of more advanced lesions characterized by the accumulation of lipid-rich necrotic debris and smooth muscle cells. Atherosclerosis is a major health problem (e.g. cardiovascular disease accounts for approximately 29% of all deaths worldwide (HWH, 2009)) having special higher prevalence in western countries due to inappropriate diet habits.

The classical paper from Anitschkov and Chalatov (1913), dating back to the beginning of the previous century, demonstrated that the development of an atherosclerotic lesion and consumption of fat were related; the conclusion from this result stated the relation between diet, metabolism and atherosclerotic cardiovascular disease (CAD) (Hansson, 2007). This classical work defined the view of the dynamics of the plaque evolution for the most of the rest of the century and yet, just no more than three decades ago, the accepted theory was that the lesion was caused by proliferative expansion of smooth muscle cells, driven by growth factors released from platelets (Schaffner et al, 1980). This theory was modified in the eighties when it was observed that, although smooth muscle cells pre-dominate in progressive human and rhesus monkey plaques, many cells in lesions of other species appear to be of a different type (Schaffner et al, 1980); more specifically there were observed large, round, markedly vacuolated cells that were named "foam cells". Later it was shown that those cells were macrophages of monocytic origin (Adams & Bayliss, 1976) At that time the key elements considered in plaque development were fat, platelets, smooth muscle cells and macrophages (MP), but the role of MP was of the lipid-accumulating histiocytes rather than immune cells.

However, a first hint that the immune system was involved in CAD was the observation of INFα in the atherosclerotic plaque (AP), when the only –at that time- known source of INFα were T cells (Johansson et al., 1985). A more concrete proof that the immune system was involved came after the mapping of T cells, macrophages, smooth muscle and endothelial cells in the human AP (Johansson et al, 1986); a later strong inditia was the direct immune-fluorescent localization of T cells in the lesion and producing INFα (Hansson et al., 1989).

Several models explaining different stages of atherosclerosis has been developed in the recent years. In (Khatib, 2007) a mathematical model that considers atherogenesis as a inflammatory process is described. The inflammatory response is considered as the higher concentration of monocytes within the intima. A one- and two- spatial dimension models based on reaction-diffusion systems are described. These model provides first insights on the disease-on-set assumption as they prove a bi-stable system dependent on oxidized-LDL concentrations. An extension of this model was presented in (Calvez et al, 2010) where a simple lesion growth model relying on the biomolecular processes that take place in the intima is coupled with blood flow dynamics and mass transfer. Simulations within a 2d geometry of the carotid artery were compared with experimental data. In (Ibragimov et al., 2005) the initial development of AP was modeled as the interplay between chemical and cellular species in the human artery and bloodstream.

There are also mathematical models describing specific bio-entities of relevance for atherosclerosis. An interesting example is studied in (Prosi et al. 2005) where the behavior of LDL in the blood stream and the arterial walls is studied. A more recent model (Little et al, 2009), that extends (Prosi et al., 2005), simulates the early stage atherosclerotic lesion formation by describing atherogenesis as a spatial reaction-diffusion model. This model is the first atherosclerosis model that addresses clinical usability.

We describe the creation and quantitative assessment of a new atherogenesis model (to be refereed to as the A-model), which considers specifically the different elements within the immune system and proposes a modeling framework that allows the system to find the correct dynamics. A-model aims to be clinically relevant as it can assess the identification of targets to reduce and/or stop plaque formation at different stages of the disease.

Model.

Most of the atherosclerosis (and atherogenesis) models reviewed are based on single key-reviews, specific references and/or single visions such as the consideration of oxidized LDL effects omitting the immune system´s effects. However atherogenesis is a most complicated system and the state-of-art shows that no reference is complete. We considered it necessary to extensively review most key references in the field and to summarize three key aspects: (a) the key bio-entities to be included, (b) how do they interact, and (c) the biological processes underlying the interactions. For the interested reader, **some** of what we considered as "most relevant references in the development of this model" were (Lusis, 2000; Libby et al. 2011).

To answer (a), the first step was to decide the scale of the model. Despite the evidence of a genomic effect, there is little mechanistic knowledge of how the atherosclerosis is regulated at the transcriptomic level; furthermore AP development is a process that is many weeks long, when the genetic regulation works in a different time-scale. For those reasons we decided to generate a model that includes "cell to cell" interactions and "molecule-cell" interactions within the intima.

The second step was to select the entities to be included. To this end we first enumerated those entities that have strong qualitative evidence of their relevance in AP development; secondly, we selected 8 entities (state variables, SV) taking into account the necessity of having a sufficiently small model and the necessity of including elements from both leading theories in AP development: lipid-driven and immune-driven theories. Three SV are molecules: Low Density Lipo-protein (LDL), oxidized LDL (oxLDL) and High Density Lipo-protein (HDL). Four are cell types: macrophages (MP), T pro-inflammatory cells (T-INF), T anti-inflammatory cells (T-AINF) and B cells. By this selection we state the necessity to consider that the T-cell related immune system has both anti-AP and pro-AP effects (Hanson et al., 2011). One entity represents the plaque size (PLA). Those entities will be referred to as state-variables of the model; their specific meaning is explained in Table 1. Within the model a cell is described by its grade of activation. The activation of the endothelium (mediated by oxLDL) is not considered a state variable, but a value that can be computed from the state variables of the model; Figure 1 (c) describes the process.

Table 1. Entities included in A-model.

Name	Description	Model approach
LDL	Low-density-lipoprotein in the intima. Proportion of low-density-lipoprotein (LDL) in the intima respect to LDL in the blood.	Value related to the concentration in the intima.
oxLDL	Oxidized low-density-lipoprotein in the intima.	Value related to the concentration in the intima.
HDL	High-density-lipoprotein in the intima.	Value related to the concentration in the intima.
MP	Macrophages	Level of activation and cell density
PLAQUE	Plaque. It contains foam cells and necrotic skeletons from foam cells, macrophages and T pro-inflammatory cells.	Percentage of plaque compared to the final size.
T-INF	T inflammatory cells; we consider Tc and Th1 types.	Level of activation and cell density
T-AINF	T anti-inflammatory cells; we consider for instace Treg and Th17.	Level of activation and cell density
B-cells	B cells.	Level of activation and cell density

The selection of the entities was also affected by the PA's biological processes of interest (BPoI). Four BPoI have been widely discussed in the literature: LDL oxidation (LDLoxid), Foam Cell Formation (FCF), Endothelium Activation (EA) and Reserve Cholesterol Transfer (RCT); see Figure 1 for a graphical description. The rest of BPoI are related to fluxes (LDL and HDL moving in and out of the intima) and cell processes such as recruitment, activation and (necrotic and apoptotic) death.

Each biological process is defined by its substrate(s), product, the stimulatory entities, the inhibitory entities and the mathematical formulation (described in the next sub-section). A summary for the system is provided in Table 2.

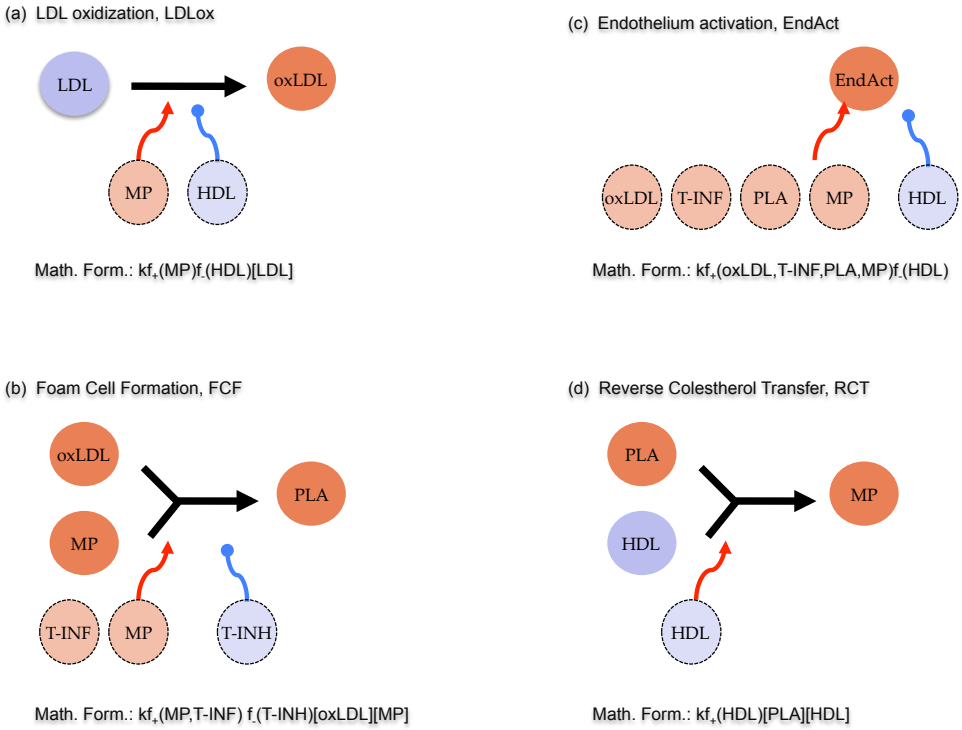

Figure 1. Graphical description of selected biological process. (a), (b) (c) and (d) graphically describe a selection of biological processes included in the model. Each sub-plot describes a unique biological process. Dotted circles denote entities estimulating (red-line) or inhibiting (blue-line) a process. Math. Form. Describes the mathematical formulation. *f* denotes a sigmoid function which is described by three parameters.

Table 2. Processes described in A-model.

Processes	Substract	Product	Stimulus	Inhibition
Recruit(MP)		MP	MP, T-INF, ENDA, oxLDL	MP, T-AINF
Recruitment(T-INF)		T-INF	T-INF, MP, oxLDL, Bcells, EndA	MP, T-AINF
Recruitment(T-AINF)		T-AINF	T-AINF, MP	T-INF
Recruitmen(Bcells)		Bcells	T-AINF, MP, ENDA, T-INF, PLAQUE	
Oxidation of LDL	LDL	oxLDL	MP, EndA	HDL
Plaque formation	MP, oxLDL	PLAQUE	T-INF, MP	
Reverse Cholestherol Transfer	PLAQUE, HDL	MP		
Endothelium Activation		EndA	MP, oxLDL,T-INF, PLAQUE	HDL
Clear Dead Cells	PLAQUE, Bcells			
Digestion of oxLDL	oxLDL, Bcells			
Influx HDL	HDLblood	HDL		
Efflux HDL	HDL			
Influx LDL	LDLblood	LDL		
Efflux LDL	LDL			
Degradation of MP	MP	PLAQUE		
Degradation of T-INF	T-INF	PLAQUE		
Degradation of T-AINF	T-AINF			
Degradation of B-cells	B-cells			

Our A-model is encoded in SBML format Hucka et al (2003); it will be publicly available and its identifier is MODEL1002160000 at http://www.ebi.ac.uk/biomodels-main.

Analysis of the Model.

We analyze the model by using our previously developed work-flow which allows us to, despite the

parameter uncertainty, to compute a reduced set of possible mechanistic explanations for AP growth Gomez-Cabrero et al. (2011). The workflow was previously successfully applied in computational neuroscience as a tool to answer a specific question in selective attention by exploring the feasible parameter space (Ardid et al., 2010) and to find different qualitative behaviors (Gomez-Cabrero et al. 2009) in a working memory model. We show that our approach provides plausible hypotheses for the mechanisms underlying atherogenesis and predicts how the reduction of LDL levels affects the plaque formation.

Conclusions.

The proposed models allows to investigate in detail the following questions: (1) To evaluate which hypothesis, a "sigmoid" plaque growth, (that states a initial slow growth, followed by a fast growth, and finally achieves a steady state in the plaque size); or a "linear" plaque growth, is correct; (2) to quantify the effects of the different biological processes during the plaque growth; (3) to evaluate the behavior of the state variables during the plaque growth; (4) to quantify the effects of LDL reduction over different stages of the plaque growth; (5) and to find biological elements to target in order to reduce the Plaque formation when LDL reduction is not enough.

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