Math. Model. Nat. Phenom.
Vol. 2, No. 2, 2007, pp. 142-149

Typical Atherosclerotic Plaque Morphology Produced in Silico by an Atherogenesis Model Based on Self-Perpetuating Propagating Macrophage Recruitment

R.N. Poston[a 1] **and D.R.M. Poston**[b]

[a] School of Medicine, Guy's Campus, King's College London, United Kingdom
[b] Ideaworks3D, London EC1, United Kingdom

Abstract. Atherosclerosis always develops in plaques, and the reasons are not clear. We test the hypothesis that plaque morphology results from a self-perpetuating propagating process driven by macrophages (Mphs). A computer model of atherogenesis was written in which the computer screen represents a surface view of a flattened area of an arterial wall on which greatly accelerated atherogenesis is depicted. Rate of Mph recruitment from blood monocytes is set as a steeply rising function of the number of Mphs locally present. Smooth muscle accumulation depends on Mph number, Mphs have a probability of death/loss, and lipid accumulation results directly from the death of Mphs. The program runs in reiterative cycles.
From an initially normal wall, fatty streak-like foci of Mphs form at random sites, which may progress or regress. Some develop into progressive focal lesions resembling advanced plaques, which are Mph-rich and have a central fibrous cap-like central region of smooth muscle cells. Lipid accumulates centrally in them. To investigate a fetal origin of atherosclerosis, the simulation was initially loaded with Mphs: lesion development was greatly enhanced. These results strongly resemble atherosclerosis in vivo, and support the Mph-dependent hypothesis of spreading plaque growth.

Key words: atherosclerosis, atherosclerotic plaque, macrophage, smooth muscle cell, endothelium, lipid, fetus, computer simulation
AMS subject classification: 92C50, 92-04

1. Introduction

Atherosclerosis always develops in focal plaque lesions, even though the stimulus may be uniform, e.g. hypertension (Figure 1). No satisfactory hypothesis for this morphology has been established. The precursor lesion, the fatty streak, is an accumulation of leukocytes, mainly foamy Mphs, in the arterial intima. A major feature of the advanced lesion is the massive accumulation of Mphs. Entry is via the arterial endothelium, where monocytes, which are the precursor cells to the macrophages, can be seen binding to and entering the lesions. This is known to occur through a highly regulated process of cell adhesion to the artery wall.

[1]Corresponding author. E-mail: robin.poston@kcl.ac.uk

We propose that the lesions prior to complications comprise a self-perpetuating propagating form of chronic inflammation, with the Mph being the principal cell type involved. Several lines of well-established evidence support this hypothesis.

1. Monocyte adhesion to an endothelium can enhance further leukocyte adhesion and transmigration [1, 2].
2. Monocytes adherent to an endothelium can induce monocyte binding adhesion molecules on it [1, 3].
3. Atherosclerotic plaque endothelium shows localised expression of monocyte-binding adhesion molecules, for which there is functional evidence of their activity [4, 5].
4. Mphs are potent at oxidising LDL, and this species is found in lesions. Ox-LDL and its derivatives can activate endothelium to enhance the specific adhesion of monocytes [6].
5. Mphs in the lesion produce endothelial activating cytokines, such as TNFα [7]. They also produce the MCP-1 monocyte chemotactic factor [8], which can bind to proteoglycans of the endothelial luminal surface and enhance monocyte adhesion.
6. Mphs produce enzymes that can modify LDL so that it has complement activating activity. Activated complement is found in lesions and has endothelium activating and monocyte chemotactic properties [9].

Figure 1: Aorta of an elderly woman with hypertension showing multiple focal mild raised advanced atherosclerotic plaques (down arrow) and fatty streaks (up arrow).

7. Maternal hypercholesterolaemia has been associated with increased fatty streak formation in the fetus, and an accelerated development of atherosclerotic vascular disease in later life [10]. Self-perpetuating development of the early lesions may explain the subsequent disease.

Smooth muscle cells (SMC) accumulate in the atherosclerotic arterial intima, where they proliferate and secrete extracellular matrix to form a fibrous cap. Growth factors from Mphs, including foam cells, are probably a major cause, although the activated endothelium may also contribute [11]. Extracellular lipid accumulates in the outer part of the intima in advanced atherosclerotic plaques. Death of foamy lipid-containing Mphs in that region, where they become anoxic and apoptotic, is likely to give rise to much of it [12].

To study the morphogenesis of the atherosclerotic plaque, we have investigated the kinetics of Mph recruitment, smooth muscle cell accumulation and extracellular lipid deposition by a computer simulation which uses a set of algorithms based on the interactions described above.

2. Materials & Methods

The 2D computational domain represents the luminal surface of a flattened area of the wall of a large artery. It comprises an array of computational cellular automata, each numerical cell encoding the content of the arterial wall. At each point, the content of biological cells in the arterial wall beneath is simulated, and has a content of Mph , SMC and extra cellular lipid. In the arterial surface view, the numbers of cells are coded by the brightness of the pixel colour for Mphs (yellow) and smooth muscle cells (SMC) (green); initially it is normal, free of cells and shown as red. The display of SMC predominates over Mph. In addition, a simulated cross-section can be shown through the thickness of the arterial wall along any user-defined line on the main display. In this view, the components are stacked quantitatively and arbitrarily in the arrangement usually seen in vivo, with smooth muscle cells at the inner aspect, Mphs in the middle and extracellular lipid at the outer aspect. The profiles of the cell types are smoothed by quadratic interpolation.

The program, written in Microsoft Visual C++, operates by running repeated cycles in which monocyte recruitment can occur at multiple randomly chosen sites, analogous to collisions of monocytes with the artery wall, and governed by probability functions dependent on cell numbers already present at that site and adjacent points. All adjacent points are weighted equally. The programmed probability relationships are as follows:

1. Mph recruitment probability depends on Mph numbers, and is initially at a very low percentage value, which rises steeply with numbers of Mph already present (Figure 2).

2. Mphs have a death probability, which can be a function of the pre-existing Mph number (Figure 2).

3. Mph death results directly in extra-cellular lipid accumulation: the quantity of lipid deposited per Mph death can be preset.

4. Mphs have a probability of recruiting smooth muscle cells as a function of their cell number. A logarithmic function is used (straight line on a similar log-linear graphical interface to Figure 2.).

5. SMC can inhibit recruitment of Mphs by reducing recruitment probability by a preset linear cell number dependent factor.

The results at individual points are controlled by random number functions reflecting the prevailing probabilities. The quantitative values of the probability functions are programmed manually using graphic interfaces (Figure 2). The very small initial value of macrophage recruitment is inserted numerically. The values required to give realistic results were found by experiment with the program, and were not taken from in-vivo data, as inadequate information is available. It operates in 256 colour mode.

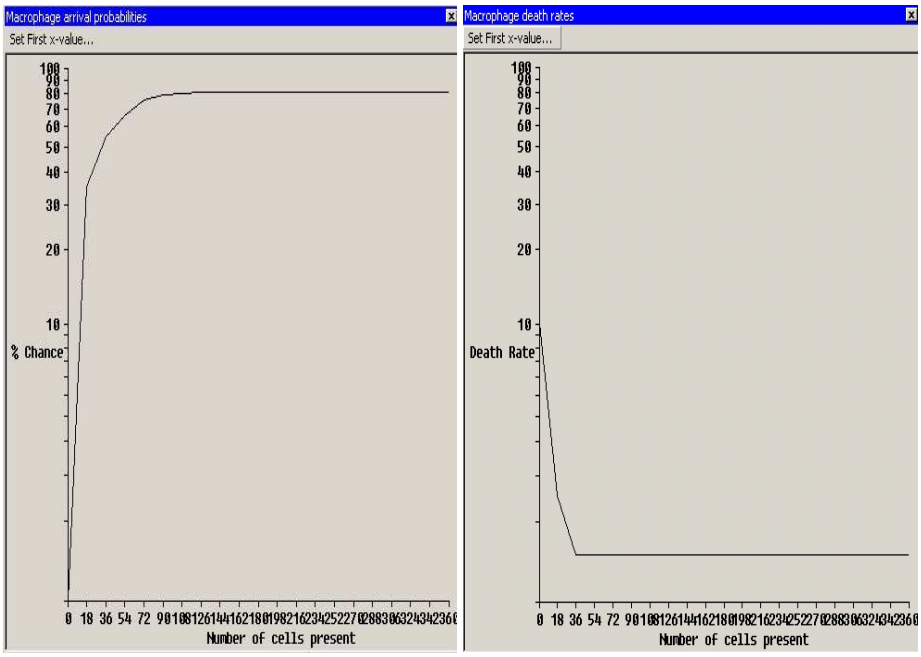

Figure 2: Left: macrophage arrival probability - graphical interface, right: macrophage death probability - graphical interface.

Use is made of Direct3D software (Microsoft) to derive an alternative 3D rendered display of a tubular artery. The position of the arterial luminal surface is displayed so that the wall is apparently thickened and the lumen decreased in relation to the total quantity of cells and lipid accumulated in the arterial wall. The software simulates visualisation of the artery from a range of cursor-controlled viewing positions.

3. Results

The simulation runs at high speed, over 10 cycles/second, and the lesions shown (Figure 3) developed in 30 seconds. Initial adjustment of parameters showed that to obtain focal lesions it was necessary for the probability of Mph recruitment to rise steeply with recruited cell number from an initial very small value. The smallest possible initial value of 0.018 % per cycle in the absence of recruited Mph generates adequate lesions, and was used as the default. This then rises to around 80 % when multiple Mphs are present locally. Equally, it was essential to have a rate of Mph death/ loss from the intima to avoid progressive accumulation of cells everywhere. On running the simulation under appropriate conditions, Mphs appear initially at random over the screen, but many are lost through programmed death. Soon small collections develop, resembling fatty streaks, which are metastable, and last for several cycles, because recruitment

balances loss (Figure 3). Some disappear, while others progress to foci resembling advanced lesions. The balance between Mph recruitment and death parameters was critical to the number and extent of lesion development.

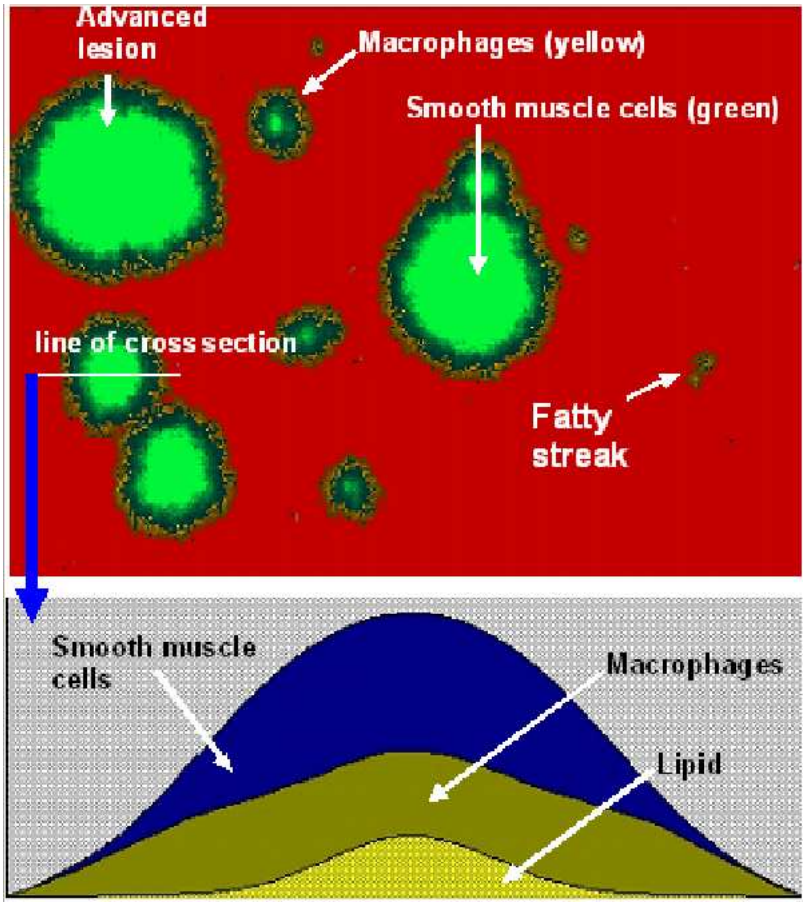

Figure 3: Upper part:- Simulation of arterial wall showing development of fatty streaks and advanced atherosclerotic plaques. Normal arterial wall -red, macrophages - brown, smooth muscle cells - green. Simulation run for 300 cycles with default parameters. Note smooth muscle cell -free borders of plaques. Lower part:- cross section of lesion as indicated by line in upper part. Components are stacked arbitrarily. Smooth muscle cells - blue, macrophages - green, lipid - yellow. Note central location of fibrous cap and lipid.

The simulated advanced lesions grow progressively in size, and resemble those in vivo in that they have a central mass of SMC, and Mph throughout, also that at the lesion margins the Mph are without SMC (Figure 3., Videos 1:-early lesions and 2:- late lesions and cross section). Lipid derived from Mph death accumulates the centre of the lesions, as found in vivo. The typical cross section shown has a good resemblance to a lesion in vivo. Each run produces results that differ in position and detail of the plaques, due to their dependence on random events. The run shown in Figure 3 was done without SMC inhibition of Mph recruitment. If it is included, the number of Mph decreases centrally, as often found *in vivo.*

To simulate the fetal origin of atherosclerosis by fatty streak formation in utero, the program was run

with an initial loading of Mphs, by greatly increasing the probability of recruitment for an initial 10 cycles. With a loading of 750 Mphs, the final atherosclerosis was severely exacerbated, and the number of cells on the screen was increased from a mean of 89,000 to over 560,000 (10 runs) after 300 cycles.

The 3D rendered display provides a quite realistic image of an atherosclerotic artery (Video 3). The program can be run in this mode to give an accelerated simulation of atherogenesis.

4. Discussion

This is the first simulation of atherogenesis *in silico* producing a plaque distribution of atherosclerosis by a propagating phenomenon in a 2D image of the arterial surface, and further deriving 3D rendered images where the thickness of the diseased wall is taken into account. It develops atherosclerotic plaque - like lesions with a strong resemblance to those found *in vivo.* It therefore supplies good supporting evidence for the hypothesis that focal atherosclerotic plaques develop through self-perpetuating propagating Mph recruitment, and by Mph-dependent smooth muscle accumulation. Furthermore, it suggests that these hypotheses are sufficient to explain the plaque nature of atherosclerosis.

The model makes the assumption that the arterial wall is entirely uniform before the disease commences. This is a simplification, as it is well known that haemodynamic changes at branch points predispose to atherogenesis. Nevertheless, even at these sites, the lesions still have plaque morphology. It therefore seems likely that the local factors are principally modulators of the initial conditions before the plaque-generating positive feedback process ensues. It would however be easy to vary the initial conditions locally to simulate wall heterogeneity.

The positive feedback mechanism may include additional components apart from Mphs, and the simulation does not distinguish between the direct effects of Mphs and those of other related phenomena, e.g. the associated T lymphocyte infiltration. It is clear that unusual circumstances must be present in the arterial wall to allow self-perpetuating inflammation to develop. The arterial intima is unlike most tissues as it is not normally vascularised, and is surrounded by the relatively impermeable barrier of the arterial media. It may therefore be difficult for oxidised LDL, Mphs and their inflammatory products to be removed from the intima, and their ability to interact with the arterial endothelium may therefore be greater than in thinner walled vessels.

At present little information is available on the lifespan of atheroma Mphs, but it is likely to be less than the protracted period over which atherosclerosis develops. The simulation suggests that there is a critical balance between monocyte recruitment and Mph death/ inactivation that determines the growth of the plaques. Furthermore Mphs close to the endothelium would be anticipated to have the greatest effect on it, and a role for monocytes is possible when adherent within the lumen. It is also possible that inactivation through differentiation to lose an inflammatory phenotype may be important, in addition to elimination by death. For simplicity these factors have not been considered separately in the simulation. Local factors may modulate viability: plaque Mph produce cytokines capable of autocrine stimulation, for example M-CSF [13] suggesting that when clustered together their viability might be increased, and this has been reproduced in the simulation. By contrast, apoptosis occurs in atheroma Mphs, so the situation is complex, and Mph viability may deserve further investigation as risk factor both in vivo and in silico. Interestingly, Mph viability in the simulation altered the shape of the lesions, as they are centrally thicker when it is high (not shown).

Development of a smooth muscle cell mass in the centre of the lesion is seen in the simulation and resembles the fibrous cap in vivo. The model shows clearly that it arises as a consequence of the centrifugal spreading development of the lesion, as the SMC accumulate in the central region where the integral of the

Mph number and duration is greatest. The central SMC are found in the inner intima, and tend to separate the Mph from the endothelium. The relatively fewer Mph sometimes found in the central region of a fibrous-cap rich lesion could result from a lesser activating effect on the endothelium, and this was reproduced in the simulation by the inclusion of an inhibitory effect of SMC on Mph recruitment. By contrast, in the recently formed Mph-rich, but smooth muscle-poor, margin, it can be seen that the accumulation of SMCs lags behind the Mphs, as the total numbers and duration of Mph presence are much less. It is often in this zone that a plaque becomes unstable, through the weakening of the arterial wall by Mph enzymes, and thrombosis follows.

Self-perpetuating Mph dependent lesion growth readily explains the fetal origin of atherosclerosis, as seen by its potentiation by a small initial Mph infiltrate in the simulation. Although the effects of maternal hypercholesterolaemia in producing fatty streaks in the fetus is well documented, a mechanism for the demonstrated accelerated development of atherosclerosis in affected children has not so far been established [10].

The self perpetuating hypothesis has consequences for the therapeutics of atherosclerosis, as the events in the Mph-dependent positive feedback loop will have their effects greatly amplified by its action. Inhibition of these critical factors is therefore likely to be effective in treating the disease.

Acknowledgement

We thank Dr. John McGregor for his collaboration, and for his support of this study.

References

[1] M. Takahashi, U. Ikeda, J. Masuyama, S. Kitagawa, T. Kasahara, M. Shimpo, placeS. Kano, K .Shimada. Monocyte-endothelial cell interaction induces expression of adhesion molecules on human umbilical cord endothelial cells. Cardiovasc. Res., 32 (1996), 422-9.

[2] A. Tsouknos, G.B. Nash, G.E. Rainger. Monocytes initiate a cycle of leukocyte recruitment when cocultured with endothelial cells. Atherosclerosis, 170 (2003), 49-58.

[3] K.E. Noble, P. Panayiotidis, P.W. Collins, A.V. Hoffbrand, K.L. Yong. Monocytes induce E-selectin gene expression in endothelial cells: role of CD11/CD18 and extracellular matrix proteins. Eur. J. Immunol., 26 (1996), 2944-51

[4] R.N. Poston, D.O. Haskard, J.R. Coucher, N.P. Gall, R.R. Tidey. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. Amer. J. Pathol., 140 (1992), 665-73.

[5] R.N. Poston, R.R. Johnson-Tidey. Localised adhesion of monocytes to human atherosclerotic plaques demonstrated in vitro. Implications for atherogenesis. Amer. J. Pathol., 149 (1996), 73-80.

[6] J.A. Berliner, M.C. Territo, A. Sevanian, R. Soroush, J.A. Kim. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. J. Clin. Invest., 85 (1990), 1260-1266.

[7] P.G. Tipping, W.W. Hancock. Production of tumour necrosis factor and interleukin-1 by macrophages from human atheromatous plaques. Amer. J. Pathol., 142 (1993), 1721-1728.

[8] S.D. Cushing, A.M. Fogelman. Monocytes may amplify their recruitment into inflammatory lesions by inducing monocyte chemoattractant protein. Arterioscler. Thromb., 12 (1992), 78-82.

[9] J. Torzewski, D.E. Bowyer, J. Waltenberger; C. Fitzsimmons. Processes in atherogenesis: complement activation. Atherosclerosis, 132 (1997), 131-138.

[10] W. Palinski, C. Napoli. The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. FASEB. J., 16 (2002), 1348-1360.

[11] A.D. Hughes, G.F. Clunn, J. Refson, C. Demoliou-Mason. Platelet-derived growth factor (PDGF): actions and mechanisms in vascular smooth muscle. Gen. Pharmacol., 27 (1996), 1079-1089.

[12] M.M. Kockx, G.R. De Meyer, J. Muhring, H. Bult, J.Bultinck, A.G. Herman. Distribution of cell replication and apoptosis in atherosclerotic plaques of cholesterol-fed rabbits. Atherosclerosis, 120 (1996), 115-124.

[13] S.K. Clinton, R. Underwood, L. Hayes, M.L. Sherman, D.W. Kufe, P. Libby. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. Amer..J. Pathol., 140 (1992), 301-306.