The Role Of Macrophages In Tissue Reactions To Implanted Plastics:
A Study In Rodents And Humans

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by

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Macrophages are widely distributed in the body and are involved in various physiological functions, especially those related to both innate and adaptive immunity. Moreover, they have been shown to play a pivotal role in healing and repair. Their role in the latter has been studied using several experimental systems including the implantation of foreign material into various tissues. In this presentation I shall concentrate on the tissue reactions to the implantation of plastic polymers (polyethylene, polypropylene) into rodents (mice and rats) and, to a lesser, degree, humans.

In early experiments plastic discs measuring 5mm in diameter were implanted into the subcutaneous tissues of mice and then removed at 4 days, 1 week, 2 weeks and 4 weeks after surgery. By 4 days the discs were almost completely covered by a layer of macrophages. These macrophages were most likely derived from the sustained exudation of monocytes and their attachment to plastic surfaces was not unexpected since it has been known for many years that mononuclear phagocytes have the propensity to adhere preferentially to plastics and glass, and the process is probably enhanced by vitronectin. By one week some 8-10% of cells attached to the plastic disc were multinucleated giant cells which are known to form by fusion of newly emigrated monocytes (Fig 1).

![Fig 1 Macrophages and multinucleated giant cells](image)

The number of multinucleated cells attaching onto the disc rose to 22-26% by the end of 2 weeks after implantation and their proportions varied but little after that.

Injection with tritiated thymidine followed by autoradiography showed that 3-8% of macrophages were actively dividing and even a few multinucleated giant cells were also in the synthetic phase of the cell cycle. (Fig 2).
Obviously then the source of the mononuclear phagocytes and their derivatives that were adhering to the surface of the plastic were not just from exuded monocytes but also from the active division of the emigrated cells.

Scanning electron microscopy showed that all the cells adhering to the plastic surface were highly irregular and malleable, possessing many surface flaps, projections, flat lamellipodia and long fine filopodia (Figs 3, 4 and 5).
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Fig 4 Many flaps and projections characterise the malleable macrophage surface

Fig 5 Multinucleated giant cell displaying much surface irregularity
In addition both adherent macrophages and multinucleate giant cells were often exceptionally flattened, measuring only a few micrometers in height (Fig 6,7).

Fig 6 Flattened macrophage

Fig 7 Flattened multinucleate giant cell
Moreover, trails of fibrillar material were seen on the surface of the substratum which probably reflected secretion of fibronectin and/or matrix associated protein IG-H3, both of which contribute to the formation of extracellular matrix (Fig 8).

**Fig 8 Trail of matrix left by a macrophage**

Transmission electron microscopy displayed the extensive cytoplasmic development present in both macrophages and multinucleated giant cells including the formation of membranous labyrinths into which lytic enzymes as well as the products of their action are likely to be present (Figs 9 and 10).

**Fig 9 Multiple surface projections emanating from macrophages**
In most cases the cytoplasmic flaps and projections prevented the macrophages from close apposition (probably surface charge related) but in some instances close apposition and interdigitation had occurred reflecting the “epithelioid” change (Fig 11).
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Microorganisms were not detected on any of the samples examined. Presumably constant patrolling by these mononuclear phagocytes together with the NO radicals that they generate renders unlikely bacterial colonisation after the surgical procedure of implantation.

Interestingly chemotactic experiments using macrophages have shown that these cells can traverse tunnels measuring 5-8 micrometers in diameter. Scanning electron microscopy shows that thin pseudopodia from the cells emerge from these small orifices, and are then followed by the main cell mass (Fig 12).

![Fig 12 Macrophage projection protruding from a small channel](image1)

This ready deformability of mononuclear phagocytes is also demonstrated by the examination of post capillary venules in the vicinity of the implants. In several instances monocytes were seen traversing the open interendothelial junction – often a space no more than 1-2 micrometers in width (Figs 13 and 14).

![Fig 13 Monocyte emigrating through an interendothelial junction](image2)
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Surrounding the layer of macrophages attached to the implanted disc varying numbers of fibroblasts and myofibroblasts are seen (Figs 15, 16 and 17).

Fig 14 Monocyte squeezing through an interendothelial junction

Fig 15 Fibroblasts in scar tissue
These are attracted by the fibronectin secreted by macrophages and are encouraged to grow and replicate by Fibroblast Growth Factor and Platelet Derived Growth factor, both again secreted by macrophages. These fibroblasts initially (especially in the first week after surgery) secrete collagen type III and later collagen type I and the synthesis of collagen is encouraged by the ornithine secreted macrophages. As already indicated above macrophages contribute to the extracellular matrix components that are produced by fibroblasts. Interestingly, fibroblasts secreted Macrophage Colony Stimulating Factor which in turn stimulates macrophage division.
Macrophages also secrete Vascular Endothelial Growth Factor as well as Fibroblast Growth Factor, both of which encourage the growth and replication of endothelial cells which then form capillary buds and are attracted to areas where fibronectin concentration is high (Figs 18 and 19).

It seems, therefore, that macrophages have a major role in orchestrating the process of repair in healing tissues and there is evidence of efficient dialogue between the cells involved in the process.
To examine such tissue reactions in a situation reflecting the use of such material in patients the following experiments were performed. Multifilamentous polypropylene mesh was implanted into the subcutaneous region of the dorsum of mice and rats and the mesh and surrounding tissues were removed, 2, 4, 6 and 8 weeks after implantation and then examined by light microscopy. By two weeks macrophages and, to a lesser degree, multinucleated giant cells had invaded the interfibrillar spaces and they or their cytoplasmic extensions were abutting onto the filaments of the mesh (Figs 20, 21).

Occasionally an eosinophilic coagulation (which stained for glycans) occupied the interfilamentous spaces while in some areas fibrovascular connective tissue had separated the individual filaments of the mesh. (Fig 22).
Fibrovascular connective tissue surrounded the mesh and this appeared dense and more compacted in samples removed eight weeks after implantation. Many collagen fibres were argyrophilic (type 3 collagen) in specimens removed at two weeks but were less prominent in implants removed at eight weeks after surgery. Gram stains did not reveal the presence of microorganisms.

The histological appearances of the mesh implanted in animals were compared with those of implanted multifilamentous and monofilamentous mesh taken from a 58 year old woman two years after surgery. The tissue reaction surrounding the multifilamentous mesh was similar to what was seen in the animal experiments. Macrophages and multinucleated giant cells were closely opposed to the filaments of the mesh while dense connective tissue surrounded the implant with only a few scattered inflammatory cells punctuating the scar tissue. Figs 23, 24, 25, 26).

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**Fig 22** Scar tissue and matrix proteins invading multifilamentous tape

**Fig 23** Scar tissue surrounding multifilamentous tape
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Fig 24 Macrophages and multinucleate giant cells surrounding individual filaments

Fig 25 Matrix proteins and macrophages abutting onto individual filaments
A similar reaction was seen around the components of the monofilamentous mesh but in this instance the connective tissue was not as densely compacted, the collagen fibres were not as thick while inflammatory cells (mainly mononuclear) were somewhat more prominent. (Figs 27, 28 and 29).
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Fig 28 Multinucleated giant cells and macrophages surrounding individual filaments.

Fig 29 Multinucleated giant cells, macrophages and loose connective tissue surround individual filaments.
No difference in the concentration of argyrophilic fibres was discerned in the tissues surrounding the types of mesh while Gram stains did not reveal the presence of microorganisms.

The preliminary studies described above have demonstrated that there are close similarities in the tissue reactions to implanted plastics in rodents and humans. The sustained macrophage and multinucleated giant cell adhesion onto the plastic surface may be related to the physiochemical properties of plastics and also reflects continuing monocytic emigration and local division. The ready deformability of mononuclear phagocytes probably accounts for infiltration by these cells of the interfilamentous spaces while the more densely compacted connective tissue around multifilamentous tapes in the one human sample examined may reflect the presence of a greater number of macrophages. Further investigations are needed to fully elucidate the precise tissue reactions around different types of mesh.