

Activation of Macrophages by High Aspect Ratio Nanostructures.

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Concerns have been raised about the potential health risks of nanoparticles at large. It has long been known that asbestos fibres are carcinogenic and since several types of engineered nanostructures have similar dimensions and/or aspect ratios it could be that these man-made structures are also toxic. Considering the increased use of engineered nanoparticles, including carbon nanotubes, we face an increased risk of environmental, occupational and public exposure¹. It is in this context interesting that it may take decades before asbestos related symptoms appear. It is thus pivotal that the toxicity and potential health hazards of nanostructures are carefully scrutinized.

Nanoparticles may enter the body through the skin, the lungs or the digestive tract although these structures have barrier functions and elaborate systems to prevent the entry of foreign particles like viruses and bacteria. Still, if the barriers are passed there is a second line of defence comprised of professional phagocytes including macrophages which belongs to the innate immune system. These cells can engulf, and destroy foreign particles. If they fail, chronic inflammation may occur and the risk for the development of more serious conditions increases. Asbestosis is one such condition.

In the present study we investigated what would happen if we exposed isolated macrophages to nanoparticles with high aspect ratios.

Mouse macrophages were obtained using tissue culture medium to obtain an intraperitoneal (i.p.) lavage. Red blood cells were removed by treatment with ammonium chloride. The macrophages were subsequently collected by centrifugation and seeded onto glass slides. In some experiments nanowires (see below) were injected i.p. 3 days before preparation of the macrophages.

GaP nanowires (50 nm diameter and 2.5 μm long) were prepared by epitaxial² while matrix assisted casting (polystyrene) or electrodeposition (nickel) was used to produce several μm long wires with a diameter of 200 nm (Fig 1.)

Macrophages which had attached to the glass were subsequently exposed to nanowires at a concentration of around per 20×10^6 wires/ml. Within minutes the macrophages attacked the wires and tried to engulf them (fig.2 and 3). This occurred independent of the chemical composition or stiffness of the wires. Addition of nanowires to the macrophages induced cell death as measured by the cellular uptake of propidium iodide which does not enter living cells. The extent of cell death was related to the nature of the wires. Cells recovered from animals injected with nanowires prior to macrophage preparation attached better to glass slides than sham injected animals, a classical sign of macrophage activation.

We conclude that nanowires are rapidly attacked and internalized by macrophages and that nanowires can activate these cells and thus the innate immune system. The toxicity of the nanowires appears to be related to their physical and mechanical properties

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1. Helland et al, Environmental health Perspectives **115** 1125 (2007).
2. Hällström et al, Nano Lett. **10** 2960 (2007).

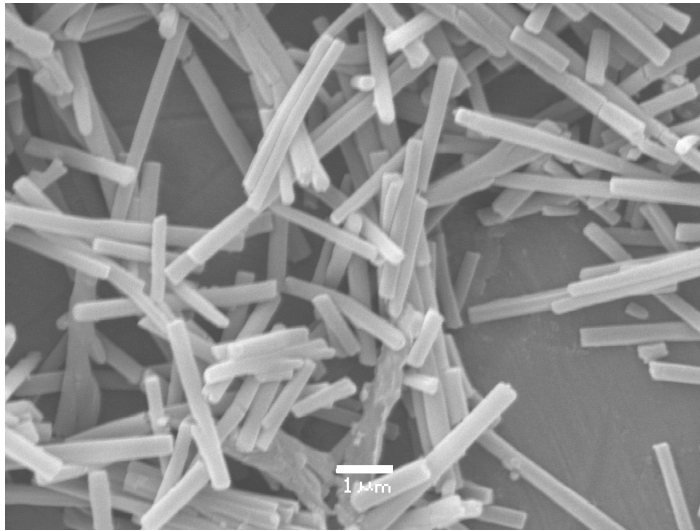


Fig.1: *Plastic nanowires* :Scanning electron micrograph of plastic nanowires. The wires were produced by casting polystyrene into Anopore (aluminium oxide) filters. The filters were then dissolved in NaOH to liberate the wires.

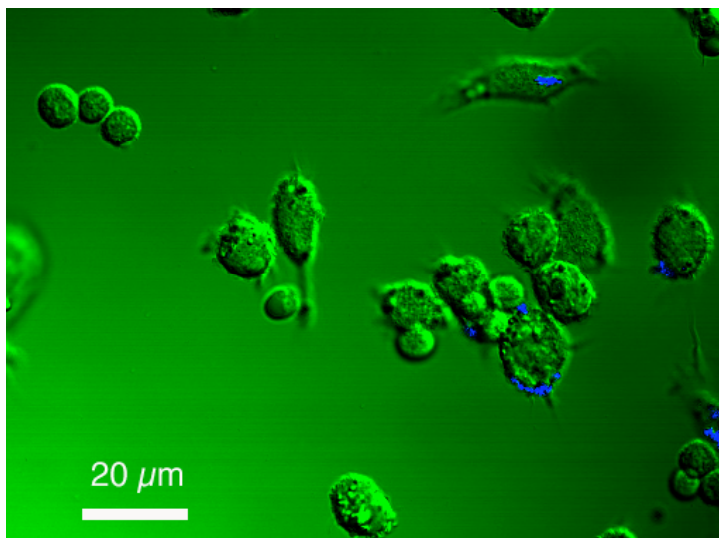


Fig. 2: *GAP nanowires phagocytosed by macrophages*: Confocal image of macrophages which have engulfed GaP nanowires (blue).

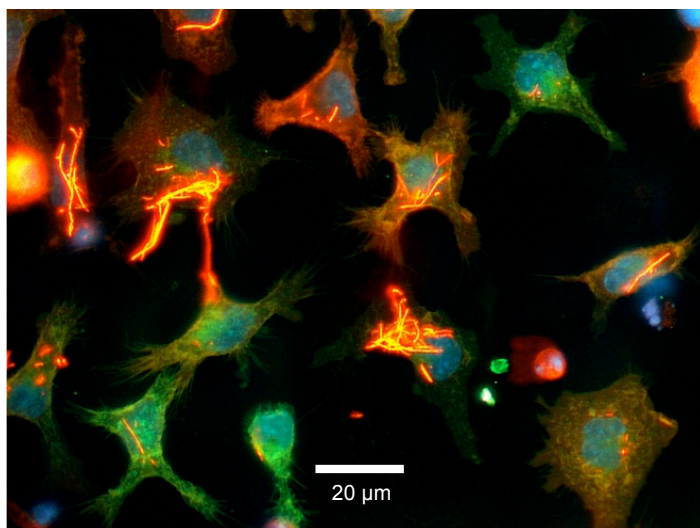


Fig. 3: *Macrophages that have engulfed fluorescently labelled nanowires* : Wires are orange and macrophage nuclei appear blue.