Letter to the Editor

Reply to Mühlfeld & Brandenberger

Quantification of nanoparticle 'dose'

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We agree with much of the substantial letter of Mühlfeld and Brandenberger, submitted to *Nanomedicine* in response to our paper on nanoparticle (NP) quantification (Elsaesser A, Barnes C, McKerr G *et al.* Quantification of nanoparticle uptake by cells using an unbiased sampling method and electron microscopy. *Nanomedicine (Lond.)* 6(7), 1189–1198 (2011)). In particular we concur with the conclusion that design-based stereology is the approach of choice for NP quantification in biological substrates, precisely the conclusion in our invited Technical Review covering all methods of NP quantification, published in *Nanomedicine* [1].

The main body of their letter covers many of the points that we have already made in the two papers cited above. In drawing attention to their own work in this field, which we have acknowledged in our paper, Mühlfeld and Brandenberger stress the importance of the 'relative deposition index', which is also delivered as a by-product of the method described in our current paper. They make the additional point that if you cannot distinguish individual NP, either because of agglomeration or electron microscopy resolution problems, then you cannot count them. We completely agree. This was ever thus in the real world of microscopy and nanobiology and if it occurs you probably need to consider one of the alternative methods discussed in Elsaesser *et al.* [1].

Our current paper requires the estimation of two quantities, the total number of nanoparticles and the total number of cells within which they are contained. We employed the fractionator for the first task, of which Mühlfeld and Brandenberger approve. We presented three independent methods for estimating the total number of cells, which appear to correlate well. There does seem to be a bone of contention with the use of the DeHoff and Rhines [2] method, which requires an assumption of cell convexity (quite reasonable with the cells we used), of which Mühlfeld and Brandenberger do not approve.

No less an authority on the theoretical basis of stereological relations, Baddeley and Vedel-Jenson discuss DeHoff and Rhines' method in their book and provide mathematical proof. On the following page they mention [3]:

"How, then, can we estimate N_V from plane sections? In fact this is not possible, in complete generality, from a plane section alone. The equation:

$$N_V = Q_A / E[H]$$

involves the mean particle height $E[H]$. [N_V is the numerical density (number of cells per unit volume), and Q_A is the areal density (number of cell transects per unit area).] Since particle heights are not directly observable in the plane section, $E[H]$ cannot generally be determined. Therefore, N_V cannot be generally determined.

This problem can be circumvented only when we have extra information: where there is *a priori* knowledge about particle shape, or where particle heights can be measured, or when some form of 3D observation is possible."

In fact we have obtained that *a priori* knowledge using a direct method of estimating $E[H]$ from the cell counter. We are content that it is reasonable to use DeHoff and Rhines in this manner. Mühlfeld and Brandenberger in their letter recommend the use of the optical fractionator, instead of DeHoff and Rhines's method. However, that method itself is not without its practical problems, as has been emphasized in Section 5.10 of the book *Unbiased Stereology* [4]. To apply the optical disector/fractionator method it is a practical requirement that the objects to be counted need to be convex and indeed near spherical, so that a plane of maximal clarity of the enclosing membrane can be determined. Departures from sphericity make this very difficult, or impossible. There

is a universal 3D counting rule, the ‘unbiased brick’ [5,6], which will cope with arbitrarily shaped particles. However, with closely packed objects use of the ‘unbiased brick’ becomes very difficult and time consuming to apply. It can be done [7], but requires analysis on registered serial sections, which would make the method impractical (which is supported by our own experience). Of course our current paper does not preclude the use of the optical fractionator if researchers wish to use it.

In conclusion, we feel that we have developed an approach to NP quantification that is both practical and efficient, qualities that are very important when using electron microscopy, which is very time consuming. Crucially, it delivers estimates of absolute numbers of NP per cell per cellular subcompartment. In nanomedicine this equates to ‘dose’, a critical metric. We think this will prove to be of considerable benefit to the nanomedicine community and look forward to collaborating with colleagues in its future use.

Financial & competing interests disclosure

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