

Evaluation of the potential cytotoxicity of some paramagnetic material used in measurements of the concentration of oxygen

Mao-xin Wu, Harald M. Swartz

The measurement of the concentration of oxygen ($[O_2]$) has received increased attention recently and several promising new methods have been developed, especially those based on electron paramagnetic resonance. The latter technique requires the addition of paramagnetic materials which are sensitive to $[O_2]$. We report here the potential cytotoxicity of some of the key paramagnetic materials, using assays of clonogenicity and exclusion of trypan blue in CHO cells. The results with the new solid particles, fusinite and lithiumphthalocyanine, were essentially similar. No cytotoxicity was found with any size of particles when the excess were removed from the media after 24 hours. With large ($>10\mu m$) particles which remain extracellular, there was no cytotoxicity found under the experimental conditions, even for long term exposures and low cell densities. Cytotoxicity was found with high concentrations of small particles which were in the media for the full time (nine days) of the assay. The mechanism for the later appears to be physical crowding due to continuous ingestion of particles rather than direct chemical effects. Typical nitroxides, Cat¹, PCA, and Tempol were not found to be cytotoxic under the conditions of these experiments, which included conditions exceeding those usually used in the measurement of $[O_2]$. Two transition element complexes, $K^3Fe(CN)_6$ and potassium tri(oxalate) chromate or (CrO_x) which are used in some studies employing nitroxides were cytotoxic, with the effects increasing with concentration and exposure time. For shorter times, 10-20 min., and at concentration levels (<50 mM) that are compatible with experimental procedures to measure $[O_2]$, their cytotoxicity may be tolerable. These results indicate that under experimental conditions compatible with a wide range of applications, oxygen sensitive paramagnetic materials can be used without unacceptable toxicity. Experiments which require the use of paramagnetic broadening agents, however, need to be scrutinized carefully. The use of lithium phthalocyanine and fusinite seem to be especially promising because of the high sensitivity to $[O_2]$ and low cytotoxicity.