In vivo biodegradation of graphene: A Confocal Raman Microscopy study

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Abstract

The use of graphene based nanomaterials for biomedical applications is gaining huge interest. For its meaningful clinical translation, a proper knowledge about toxico-kinetics of graphene and its interaction with biological components is highly essential. In this study, using confocal Raman microscopy, we have investigated the fate of graphene *in vivo*, in intravenously injected mouse models up to 3 months. 3D Raman images of graphene localized in tissue sections from organs such as lung, liver, kidney and spleen were created by k-means cluster analysis (KCA) imaging, which revealed the size of aggregated graphene nanoparticles in tissues as 1-10 µm. Raman spectroscopic profile of graphene related structural disorder occurred over 3 months from tissue embedded graphene were monitored by analyzing the formation of defect related D' band, line broadening of D and G bands (Γ_D, Γ_G) , increase in I_D/I_G ratio and overall intensity reduction. KCA imaging enabled to observe the defects in graphene aggregates within a spatial resolution of ~312 nm and showed that the structural disorders were mostly evident on the edges of graphene aggregates from 8th day onwards and grew towards inner regions over 3 months. Immuno-histopatholgy (CD68 macrophage specific antibody) analysis reveals that defective graphene aggregates were found from tissue bound macrophages of lung, liver and spleen. This suggests the possible enzymatic biodegradation of graphene caused by the breakage of sp^2 C-C network in graphene leading to more number of amorphous or sp^3 carbons. Furthermore, *in vitro* studies conducted on macrophage cell lines showed similar defect related spectral characteristics from macrophage engulfed graphene that confirms the possible macrophage mediate biodegradation of graphene.

References

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Figures

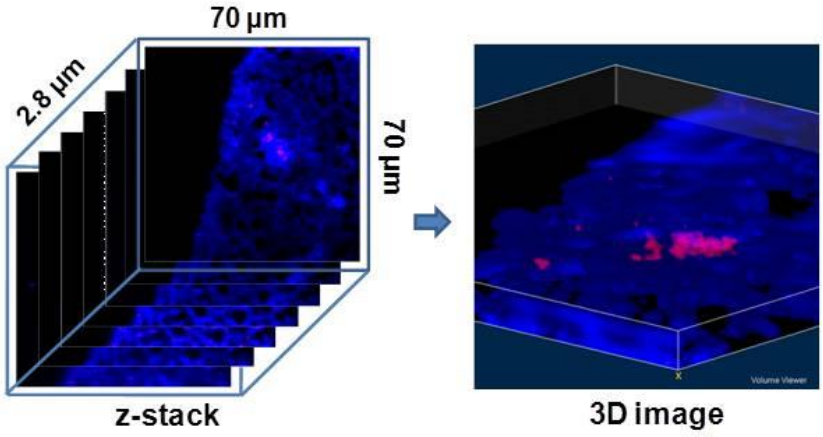

Fig 1: 3D image of graphene embedded liver tissue section. z-stack images (x,y,z: 70,70,2.8 µm) from 7 planes with an inter-planar distance of 400 nm were acquired and created using KCA (graphene in red and tissue in blue).

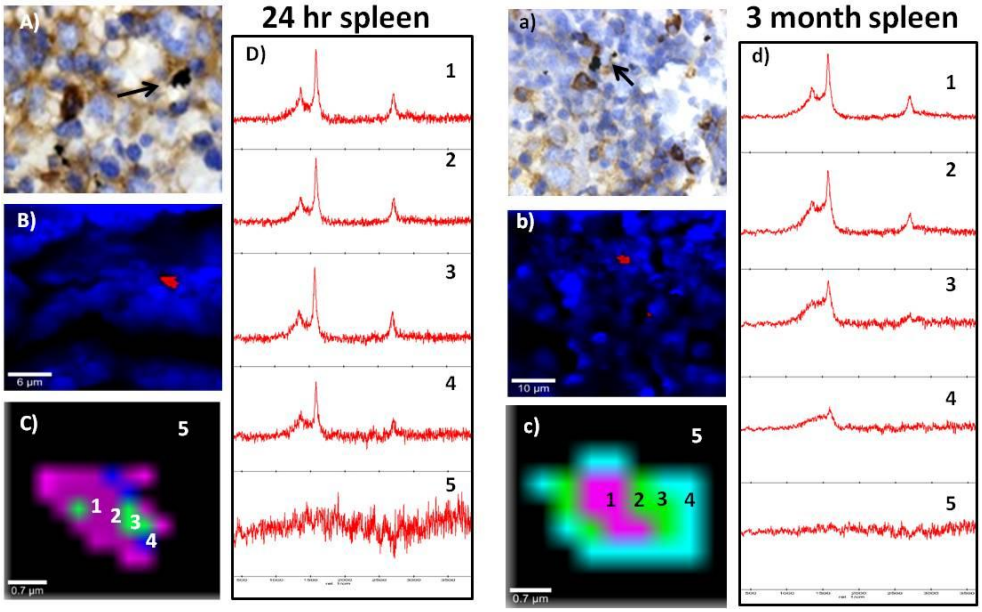

Fig 2: CD68 stained images (A,a), Raman images (B,C,b,c) and Raman spectra (D, d) of 5 different spots on graphene aggregate from tissue after 24 hour (A,B,C,D) and 3 months (a,b,c,d) of intravenous administration of graphene. Raman spectra from the edges of graphene aggregates from 3 months tissue section clearly indicates the spectral features related to biodegradation of graphene.