# Identification of the presence of carbon nanotubes in bovine embryo by Raman spectroscopy

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**Abstract** – This study aimed to evaluate whether mutiwalled carbon nanotubes (MWNT) pass through the zona pellucida. Embryos were examined by Raman analysis using confocal microscopy coupled spectrometer equipped with a CCD detector. Figure 1 and 2 shows the presence of nanotubes inside the embryo, confirmed by G-band characteristics of carbon nanotubes. The embryo zona pellucida can be penetrated by nanotubes.

Carbon nanotubes have emerged as a new and efficient alternative for the transport and translocation of therapeutic molecules and for gene delivery. Zona pellucida consists of glycoproteins that acts as a protective barrier against virus in mammalian embryos and its integrity is used as a criterion for international trade. However, it is unclear whether carbon nanotubes pass through the zona pellucida. This study aimed to detect the presence of mutiwalled carbon nanotubes (MWNT) inside bovine embryos with intact zona pellucida. Non-functional and non-purified MWNT (size: 40 to 100 µm, diameter: 20 nm) produced by catalytic vapour deposition (ferrocene as catalyst) were used. The embryos were produced after *in vitro* maturation and fertilization of bovine oocytes obtained of ovaries collected from slaughtered cows. At day seven post-fertilization embryos at blastocyst stage were randomly distributed into two culture groups: control group (without MWNT; n=5) and treated group (with 0.2µg/ml MWNT; n=5). Embryos in both groups were cultured in CR2aa medium, supplemented with 10% of fetal calf serum and granulosa cell monolayer, for 72h in microdrops covered by mineral oil and under 5% $CO_2$ at 38.5° in air. Embryos from were washed with phosphate buffered saline (PBS) and dehydrated in lamina at room temperature before analysis. Raman spectra were collected at room temperature in air at various points along the MWNT using a spectrometer (Andor™ Technology – shamrock sr-303i). Confocal Raman measurements were performed on an inverted optical microscope (Nikon – Eclipse TE2000-U) with the addition of an x,y-stage for raster-scanning samples. Light from a He-Ne laser (632.8 nm) was reflected by means of a beam splitter and then focused onto the surface of the sample using an oil-objective with 60× magnification with numerical aperture NA = 1.4. Raman scattered light was collected by the same microscope objective and recorded using either a single-photon counting avalanche photodiode (APD – Perkinelmer Optoelectronics – model: SPCM-AQR-14) or a spectrograph with an air charged-coupled device (CCD – Andor™ Technology - iDus). Figure 1 shows the presence of nanotubes inside the embryo confirmed by G-band characteristics of carbon nanotubes [1]. Although the embryos were washed MWNT were trapped on outside surface of their zona pellucida. Dehydration may have increased the concentration of MWNT inside the embryo, resulting in a more intense staining (Figure 1A; point 4). In conclusion, MWNT can penetrate the zona pellucida of *in vitro* fertilized bovine embryos and, thus, it can be used for delivering genes inside embryos. However, its embryotoxic effect, if any, need to be established.

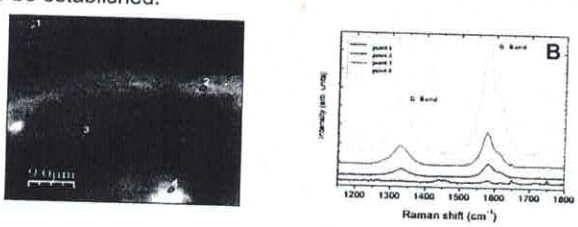


**Figure 1. A:** Embryo with carbon nanotubes (point 1: outside of embryo; point 2: interface zona pellucida and air; point 3: zona pellucida; point 4: carbon nanotubes into embryo); **B:** G-band characteristics of carbon nanotubes.

## References

[1] M.S. Dresselhaus, A. Jorio, M. Hofmann, G. Dresselhaus and R. Saito. Nano Letters, 10 (2010): 751-758.

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