Treatment of disc degeneration by nano-fullerenes: an in vitro study

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INTRODUCTION
Low back pain (LBP) is the leading resource of physical disability and results in high cost of healthcare among people under 45 years of age. It has been shown by lots of studies that LBP is closely related to the disc degeneration, and an efficient pain relief can be achieved after treatment of disc degeneration. Currently treatment of disc degeneration still remains a great challenge to both clinical physicians and basic scientists. Oxidative stress is regarded to play a crucial role in disc degeneration. Fullerenes, with a unique chemical structure, have been proven very powerful antioxidants. However, therapeutic strategies using antioxidative reagents have received little attention, and in particular, there are no reports on the effects of fullerenes on disc degeneration yet. In this study, degenerative changes in human nucleus pulposus (NP) cells were induced by IL-1β, and the protective activity of a nanoparticle suspension of a polyhydroxylated fullerene, fullerol (nano-fullerol) was investigated against the matrix destruction.

METHODS
Aqueous nano-fullerol suspension was prepared by dissolving fullerol (MER, Tuscon, AZ) in water. UV-Vis spectrum, size distribution and surface charge of nano-fullerol particles were characterized by the spectroscopy, dynamic light scattering and zeta potential analyses. The scavenging activities of nano-fullerenes against superoxide anion and hydroxyl radicals were determined by pyrogallop auto-oxidation and Fenton-type reaction methods, respectively. We obtained NP tissues of non-degenerated intervertebral discs from six individuals (age 13-16 years) who had undergone discectomy for surgical management of scoliosis at University of Virginia Hospital, approved by the IACUC. NP cells were isolated by an enzymatic digestion method previously described, and cultured in alginate bead in the presence of IL-1β and/or nano-fullerols. Cellular mRNA levels of target genes were determined by real-time RT-PCR. Cellular sulfated glycosaminoglycan (sGAG) were measured colorimetrically and normalized to total DNA. Data from 4 repeats were expressed as mean ± SD, and Student’s t-test was used for data statistical analysis.

RESULTS
In aqueous suspension fullerene nanoparticles were of negative charge of -60~40 mV (Fig. 1C), with their aggregate sizes ranging from 25~45 nm (Fig. 1B). Their characteristic peak in UV-Vis spectrum was around 290 nm (Fig. 1A). They were confirmed to have strong scavenging abilities against superoxide anion (Fig. 2A) and hydroxyl radicals (Fig. 2B). It was revealed that nano-fullerol elicited little cytotoxicity (Fig. 3) and could reduce degenerated changes in IL-1β-induced matrix destruction in alginate bead cultures of NP cells, both in mRNA (Fig. 4) and protein level (Fig. 5).

DISCUSSION
The present data provided evidences showing beneficial effects of nano-fullerol on disc degeneration, and shed light on its potential clinical use for novel chemotherapy of disc degeneration. Further efforts are being made to investigate in vivo treatment of nano-fullerol by intradiscal injection with a rabbit disc degeneration model generated through annulus needle puncture, as well as the detailed mechanisms of nano-fullerol such as cell uptake, cellular redox status and so on.

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