Developing a Targeted Nanomedicine to Alleviate Lumbar Radiculopathy via Systemic Delivery

Li Xiao[1], Tinghui Li[2], Yi Zhang[3], Jun Dai[1], Francis H. Shen[1], Li Jin[1], Harry C. Dorn[2],[4] Xudong Li[1, 5]

Departments of [1]Orthopaedic Surgery, and [5] Biomedical Engineering, University of Virginia, Charlottesville, Virginia; [2]Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia; [3]Biomedical Imaging Research Institute, Cedars-Sinai Medical Center, Los Angeles, California; [4]Virginia Tech Carilion Research Institute, Roanoke, Virginia

Introduction

Low back pain is the most common health problem with a prevalence of over 80%, and an estimated annual cost of $100 billion in U.S. **Intervertebral disc degeneration** is a major cause of low back pain. Current symptomatic relief interventions cannot improve long-term outcomes of discogenic low back pain, *as no disease-modifying medications are yet available* [1].

Inflammation has been revealed to play a crucial role in disease progression. We have demonstrated anti-inflammatory **nanomedicine candidate C_{60}** and its chemical derivatives as a new class of **pleiotropic therapeutic** agent to alleviate **lumbar radiculopathy** (**Fig. 1**) [2-4]. We also discovered a formyl peptide receptor 1 (FPR-1) specific peptide cFLFLF with robust **targeting** property towards infiltrated **inflammatory cells,** *e.g.* macrophages, near herniated disc (**Fig. 1**) [5-7].

Pleiotropic therapeutic agent (nanofullerol C_{60})

FPR-1 targeted cFLFLF binds w/ infiltrated inflammatory cells near disc herniation

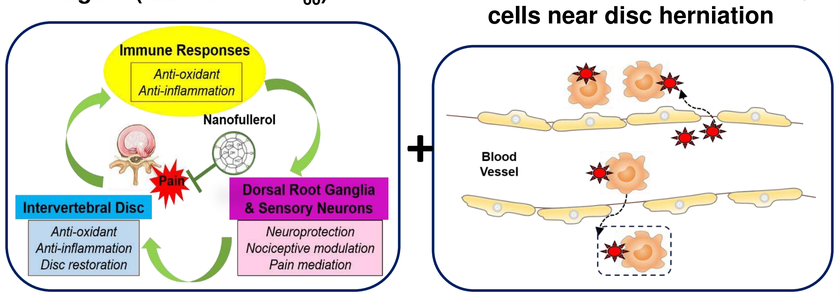

Figure 1. Schematic illustration on therapeutic function of nanofullerol C_{60} class and FPR-1 targeting mechanism of cFLFLF-based peptides [2-7].

Core Strategy—Systemic & targeted delivery

Here, we aim to develop a targeted nanomedicine (**F**luorescence labeled **T**argeted **C_{60}** derivative, "**FT-C_{60}**") to alleviate lumbar radiculopathy via systemic delivery. **FT-C_{60}** has a modular design, including a functionalized C_{60} as therapeutic moiety, a polyethylene glycol (PEG) and lysines (K) as linker and spacer, a cFLFLF peptide as targeting moiety, and a near infrared fluorescence dye Cyanine 5 (Cy5) for characterization, *in vitro* and *in vivo* detection (**Fig. 2**).

***First-of-its-kind* targeted theranostic agent FT-C_{60} for systemic treatment of discogenic back/leg pain**

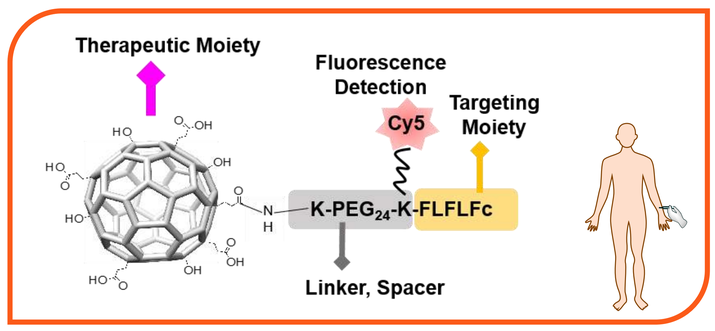

Figure 2. Illustration of our core strategy in newly developed *first-of-its-kind* theranostic agent FT-C_{60}, empowering systemic delivery of nanomedicine to treat discogenic back/leg pain.

References

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Preferential binding & anti-inflammation

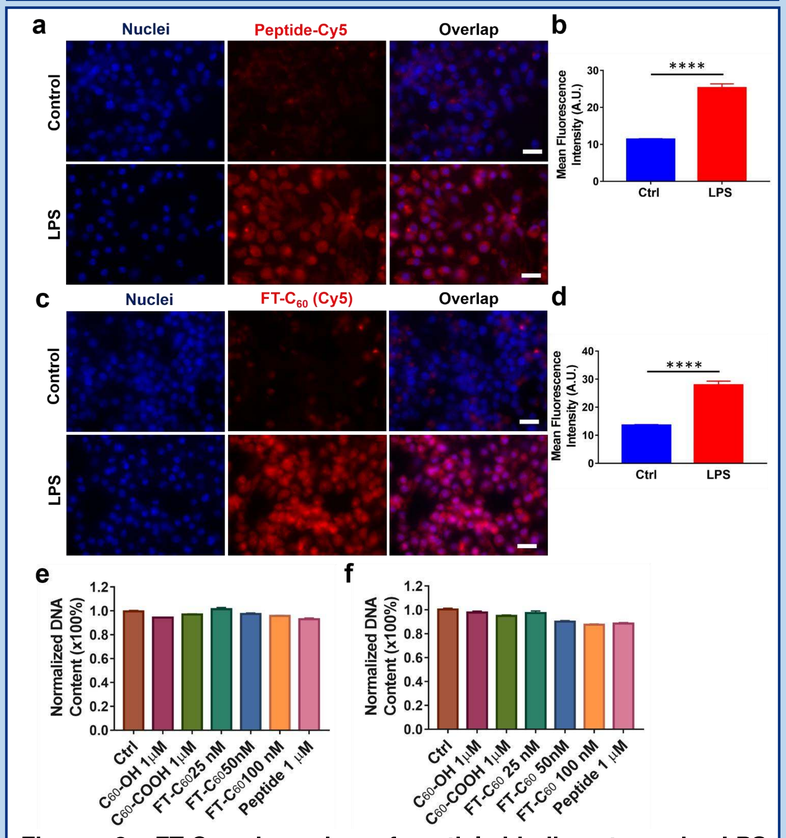

Figure 3. FT-C_{60} showed preferential binding towards LPS stimulated FPR-1 presenting macrophages with negligible cytotoxicity *in vitro*. Fluorescence cell images showed both **(a)** peptide (1μM) and **(c)** FT-C_{60} (100 nM) possessed preferential binding to LPS-stimulated FPR-1 presenting macrophages *v.s.* controls. Images were taken at a magnification of ×600 using Cy5 and DAPI channels. Scale bar=20 μm. Mean fluorescence intensity per cell of **(b)** peptide and **(d)** FT-C_{60} cell binding corroborated visual observation (****p<0.0001, *t*-test). FT-C_{60} at various doses exhibited negligible cytotoxicity in macrophages after **(e)** 1 day and **(f)** 3 days.

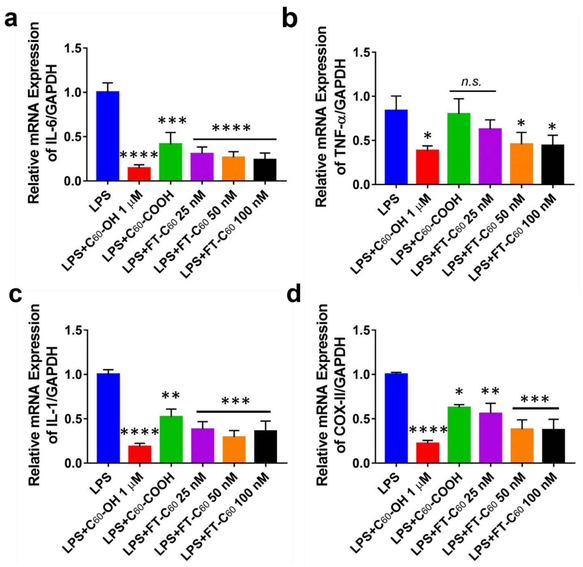

Figure 4. FT-C_{60} effectively inhibited LPS-induced pro-inflammatory genes in macrophages in a dose-dependent manner. FT-C_{60} protected macrophages from LPS stimulation via preventing mRNA expression of **(a)** IL-6, **(b)** TNF-α, **(c)** IL-1, and **(d)** COX-II, with similar protective and anti-inflammatory effects of C_{60}-OH and C_{60}-COOH. Cells were pre-treated with nanomedicine candidates overnight before LPS (100 ng/mL, 5 hr) stimulation. *p<0.05, **p<0.01,***p<0.001, ****p<0.0001 *v.s.* LPS treated groups; *n.s.,* not significant.

Acknowledgement

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In vivo targeted delivery and therapeutics

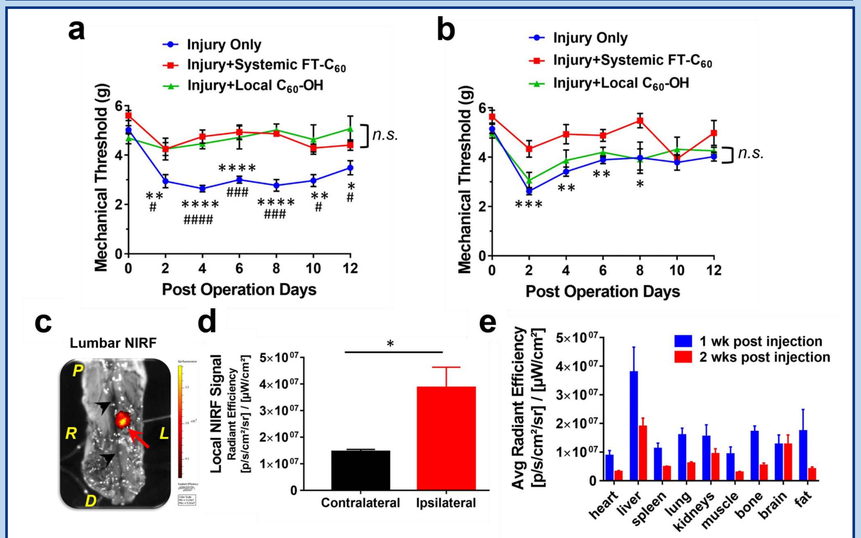

Figure 5. Systemic delivery of newly developed targeted nanomedicine candidate (FT-C_{60}) effectively attenuated pain in a mouse model of lumbar radiculopathy. (a) Single injection (*i.v.*) of FT-C_{60} (10 nmol per 20 g mice) effectively attenuated mechanical hyperalgesia in mouse up to POD 12, similar to intra-operative local administration of C_{60}-OH (1 μM , 10 μL). **(b)** Neither injury nor injury+treatments (systemic & local) group showed significantly altered contralateral mechanical threshold. **(c)** *Ex vivo* near infrared fluorescence imaging (NIRF) of mouse spine depicted targeting property of FT-C_{60} towards inflammatory infiltration of injured discs at POD 7 (also 7 days after *i.v.* injection of FT-C_{60}), evidenced by NIRF signal registration with microscopically confirmed disc herniation site (red arrow). P, proximal; D, distal; L, left (ipsilateral); R, right (contralateral); Black arrowheads, mid-plane of spine. **(d)** Semi-quantitative analysis supported significantly higher (~3-fold) NIRF signal (targeted FT-C_{60} accumulation) at ipsilateral disc herniation site, compared to contralateral side (*p<0.05). **(e)** Organ distribution of FT-C_{60} illustrated dynamic hepatobiliary clearance of systemic delivered FT-C_{60} for up to 2 weeks , grounding for further pharmacodynamic/kinetic studies. Note: One way ANOVA with multiple comparison and multiple t-test were used in (a) and (b). For Injured Only group, *p<0.05, **p<0.01,***p<0.001, ****p<0.0001 *v.s.* Injury+Systemic FT-C_{60}, and #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 *v.s.* Injury+Local C_{60} group. *n.s.* indicated not significant between two groups.

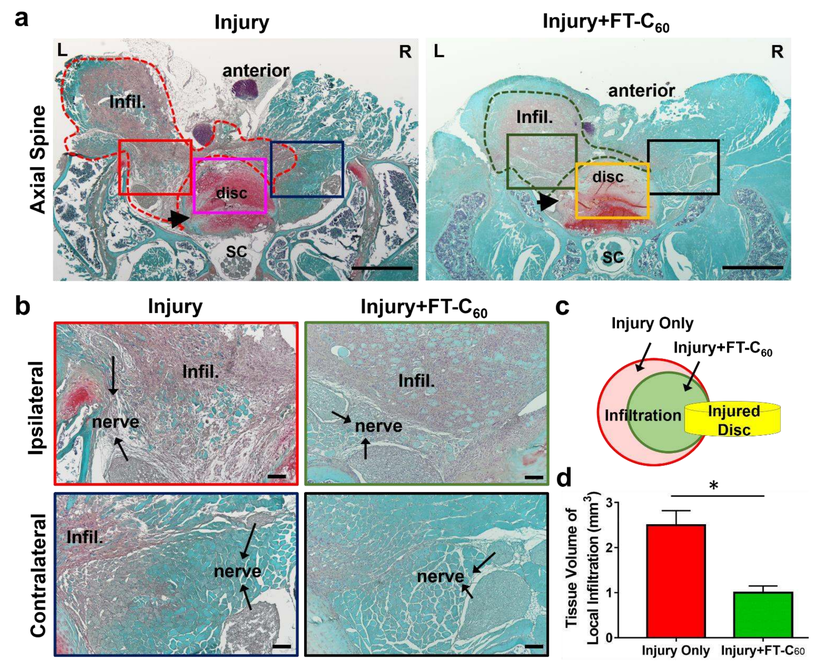

Figure 6. Safranin-O/fast green staining showed attenuated infiltration tissue volume near disc herniation site at POD7. (a) Low (×40) and **(b)** high (×200) magnification axial spine images. **(c), (d)** Customized quantitative analysis of anterior infiltration tissue exhibited that systemic administered FT-C_{60} reduced calculated tissue volume of inflammatory infiltration for more than 50%. Infil, inflammatory infiltration; SC, spinal cord; nerve, spinal nerve; AF, annulus fibrosus; NP, nucleus pulposus; Scale bar represents 500 μm in (a) and 100 μm in (b).

Summary & Significance

- **We developed a *first-of-its-kind* systemic and targeted nanomedicine candidate to treat lumbar radiculopathy.**
- **Our promising *in vitro* and *in vivo* data warrants further structure-activity relationship, PK/PD, and mechanistic studies for translational applications.**