In Vivo Translation of an Injectable Chondrocyte-Laden Micro-Scale 'Noodle' to Promote Cartilage Repair

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Introduction

Microfracture (MFx) is one of the most common surgical procedures used to promote cartilage repair. In this process, bone marrow is released into a defect site through mm-sized drill holes into the subchondral bone. Mesenchymal stem cells (MSCs) within this marrow colonize the defect and form a fibrocartilaginous tissue over time. While common, outcomes for MFx are suboptimal in terms of restoration of native tissue structure, function and durability, and the procedure is contraindicated in older persons whose MSC population has dwindled. To address this limitation, co-culture systems have been developed for in vitro culture in which factors released from a small fraction (<20%) of co-localized chondrocytes (CH) are used to promote the chondrogenesis of MSCs from older donors. Our recent work showed that the intercellular communication that occurs between CHs and MSCs in co-culture is mediated by extracellular vesicles (EV)[1]. EVs from CHs promoted MSC chondrogenesis, proliferation and matrix formation in 3D culture, while at the same time reducing MSC apoptosis, inflammatory signaling, and osteo/adipogenic differentiation. Of note, the distance over which this intercellular communication occurred was very small, and thus close proximity of the two cell types is critical for successful outcomes[2-4]. To take advantage of this co- culture effect, and to translate these findings towards clinical application, we introduced a CH-laden micro-scale 'noodle' ('micro-noodles'; Ø250μm) that provides 10 times greater surface area across which encapsulated cells can communicate compared to a conventional cylindrical construct (Ø4×2.25mm) of the same volume. Within these micro-noodles, CHs readily take up nutrients and secrete factors over a small path length. Here, we investigated the chondro-inductive capacity of these micro-noodles in vitro in a marrow-mimicking environment, and then tested their impact in a large animal model of cartilage repair, where the recipient MSCs in this in vivo co-culture system were those present in the defect after a MFx procedure.

Methods

Study 1

Adult porcine CHs and MSCs were obtained from articular cartilage and bone marrow, respectively, and were expanded through passage[1-2]. CHs were labeled with CellTracker and suspended at 20 or 60 × 10^6 cells in 1% w/v methacrylated hyaluronic acid (MeHA) (Lifecore Biomedical)[3]. Micro-noodles were fabricated by UV crosslinking using a custom-built micro-bore tubing system[5]. Formed micro-noodles (Ø 0.25 × 70 mm) were cultured in a defined medium with TGF (CM+; 10ng/mL) for 2 weeks. To investigate the chondro-inductivity of CH-laden micro-noodles on MSCs in a bone marrow-like environment, pre-cultured micro-noodles were mixed with MSCs (CH:MSC ratio = 1:4) in fibrin gel (Tisseel) for an additional 2 weeks.

Study 2

To enable tracing of micro-noodles after implantation, MeHA was mixed with methacrylated rhodamine B and/or radiodense zirconium (IV) oxide nanoparticles. Micro-noodles loaded with methacrylated rhodamine B and/or zirconium nanoparticles were visualized on a fluorescent/confocal microscope or via microCT (Fig 1C-D). For the in vivo study, three adult Yucatan mini-pigs (18 mos.) were used, with the surgical protocol as previously described[6]. Four chondral defects (Ø4 mm) were created in the trochlear groove in a unilateral procedure. After MFx, micro-noodles with or without cells were loaded into the defects. Animals were euthanized after 1 week, the trochlear groove was grossly inspected and imaged, and individual cartilage defects (including the underlying bone) were isolated for histology/immunohistochemistry and infrared imaging. Significance was determined by two-way ANOVA with Tukey's post hoc ($p<0.05$).

Results

Study 1

In vitro, CH-laden micro-noodles promoted MSC chondrogenesis in fibrin at sites adjacent to noodles, while constructs with only MSCs showed little matrix formation, primarily in the periphery (Fig 1A-B). Constructs with micro-noodles showed dense proteoglycan, Collagen I and II deposition (not shown).

Study 2

When micro-noodles were injected into the in vivo defect, they remained in place and

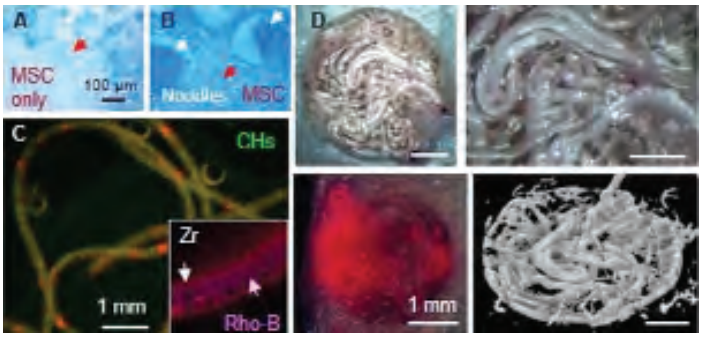

Figure 1. Fabrication and validation of micro-noodles *in vitro.* **(A-B)** Influence of CH-laden micro-noodles on MSC chondrgeneis in fibrin gel. Aldan blue staining for PGs **(A)** MSC only (red arrows), **(B)** micro-noodles (white arrows)} (2w culture+2w pre-culture of micro-noodles in CM+) **(C)** Rhodamine B and Zr nanoparticle-labeled micronoodles. (Inset=micro-noodle; Zr nanoparticle=black. speckles). **(D)** Osteochondral Unit with Zr nanoparlicle-modified micro-noodles **(Top left)**, Zoomed view **(Top right)**, Micro-noodles detected by fluorescent scope **(Bottom left)**, μCT imaging of Zr nanoparticle-modified micro-noodles **(Bottom right)**.

retained their fluorescent characteristics after one week, as was visualized by fluorescent/confocal microscopy (Fig 2A-C). CHs remained within the micro-noodles and maintained their rounded cell morphology. Histological analysis showed that injected micro-noodles were randomly distributed throughout the defect and promoted matrix deposition. Matrix staining was greatest in regions in which micro-noodles and bone marrow were in close contact with one another, while regions filled only with marrow resulted in little cartilage-like matrix production (Fig 2D-F).

Discussion

In this study, we developed a 'micro-noodle' system to harness the chondro-inductivity of CH-secreted factors on MSCs both in vitro as well as in a large animal model of cartilage repair. MSCs in fibrin gel that were adjacent to micro-noodles at the center of the construct produced robust proteoglycans whereas those cultured alone produced little matrix. After confirming the potential for tracking rhodamine B- and zirconium nanoparticle-modified micro- noodles using various imaging modalities, we demonstrated that CH-laden micro-noodles remained in place for at least 1-week post-implantation in a large animal cartilage defect model. Histological analysis showed that these CH-laden micro-noodles interdigitated into the bone marrow and fibrous tissue

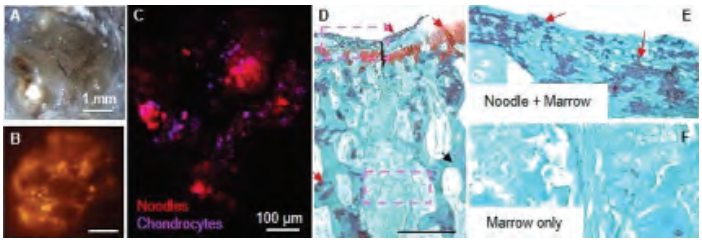

Figure 2. Retention of micro-noodles in a large animal cartilage defect model *in vivo.* **(A)** Gross image of defect filled with micro-noodles, **(B)** Micro-noodles detected by fluorescent scope, **(C)** Micro-noodles remained in the defect after 1 week post surgery , **(D)** Cell-laden micro-noodles combined with microfracture (Safi-O slaining Red/dark red staining (red arrow) indicate PGs, **(E)** Zoomed view: Matrix deposition shown in dark red on the wound site (red arrow}, where micro-noodles and bone marrows were mixed in, **(F)** Microfracture mediated defect area at the bottom, where bone marrows were mostly present.

filling the defect after MFx, and that a greater amount of matrix was formed in these regions. Despite the promise of these early findings, some micro-noodles were dislodged from some defects, potentially due to their low stiffness and the presence of synovial fluid. Ongoing studies are focused on enhancing the delivery and adhesion of these micro-noodles within the defect as well as designing a fully arthroscopic system for delivery of autologous chondrocyte-seeded micronoodles in a one-step, minimally invasive procedure. If successful, this technology has the potential to dramatically improve cartilage repair therapeutics.

Clinical Relevance

This novel micro-noodle system provides an efficient means by which to promote intercellular communication between chondrocytes and bone marrow MSCs in the context of microfracture. If successful, this in vivo co-culture approach will enhance functional cartilage formation with microfracture procedure and expand its indications into older patient populations.

References

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