Nanoparticles 'Clicked' onto Nanofibrous Scaffolds for Meniscal Repair

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Click chemistry is a class of reactions wherein molecular pieces are easily joined together, like buckling two pieces of a seat belt.[1] In the context of tissue engineering, click chemistry can serve to fabricate innovative delivery mechanisms capable of regenerating damaged or diseased tissues via spatiotemporal-controlled release. Dense connective tissues, such as knee menisci, sustain demanding load-bearing functions via a complex arrangement of extracellular matrix proteins that surrounds and protects tissue specific cell types. Following injury, endogenous cells within these tissues are unable to promote tissue regeneration and instead form less functional scar tissue. Thus, therapies that activate and recruit endogenous cells to promote tissue regeneration may significantly improve treatment and quality of life for millions of patients annually. Click chemistry-based delivery systems present a novel way of achieving these therapeutics. We previously showed that activation of Hedgehog signaling via a small molecule agonist, Purmorphamine (Pur), accelerated wound closure and attenuated cartilage erosion that results from meniscus injury.[2] Here, we overcome challenges to Pur delivery, such as rapid clearance and off-target toxicity, via delivery from polymeric nanoparticles (NPs) localized to the injury site by immobilizing NPs on a nanofibrous repair scaffold via click chemistry.

Rather than employing copper-catalyzed azide-alkyne cycloaddition as done by Lancuski et. al.[3], we utilized strain-promoted azide-alkyne cycloaddition (SPAAC) to functionalize electrospun polycaprolactone (PCL) nanofibrous scaffolds with azide groups that click with PEG-PCL NPs (prepared via oil-in-water emulsion) containing the alkyne counterpart (DBCO). These azide-modified scaffolds, when compared to electropsun PCL controls lacking azide groups, selectively reacted with DBCO-conjugated fluorophores, validating the successful fabrication of a 'clickable' scaffold. Scratch assays using murine and porcine meniscus fibrochondrocytes revealed that Pur-loaded, DBCO-conjugated NPs increased the migration of meniscus fibrochondrocytes compared to free Pur delivery. We then implanted NP-conjugated scaffolds in vivo using a nude rat xenotransplant model. For this, adult porcine meniscal explants were incised to create a horizontal defect that was either left unfilled or filled with NP-conjugated scaffolds (Pur-loaded and empty). All groups were evaluated at 3 and 14 days and analyzed for cell invasion via staining of nuclei and matrix deposition with H&E. We observed increased cell infiltration from day 3 to 14. More notably, there was a marked increase in cell number with implantation of Pur-loaded NPs compared to empty controls.

This study validates a new approach to produce nanofibrous azide-functionalized PCL scaffolds via SPAAC click chemistry. The reaction between azide-modified PCL nanofibers and fluorophore-DBCO was highly specific, fast, repeatable, and stable over the long-term after repeated washes. This indicates that azide-modified PCL scaffolds may be used to immobilize DBCO-modified NPs. When tested in vivo, Pur delivery via NP-conjugated scaffolds accelerated meniscal repair via increased cell infiltration. These data support the novel use of click chemistry-based approaches for sustained and localized delivery of small molecule drugs for dense connective tissue repair. Clinically, this delivery method has the potential to overcome many challenges in musculoskeletal tissue repair by providing sustained and local release. More generally, this study demonstrates the applicability of click chemistry-based mechanisms in biomedical therapeutics.

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References

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