PRESENTATION
ON

Evaluation of a Collagen Type II-Targeting Peptide-Drug Conjugate for Rheumatoid Arthritis Treatment

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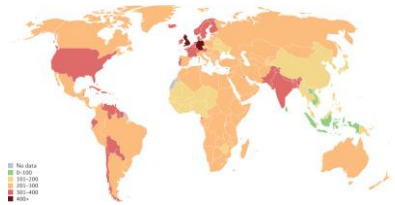
NEXT-GENERATION DELIVERY INNOVATIONS

What is Rheumatoid Arthritis (RA) ?

Chronic inflammatory form of arthritis and an autoimmune disorder



Affects ~1% of global population (approx. 75 million)

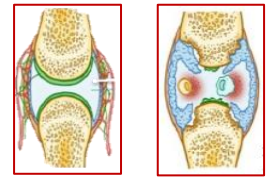
Onset is typically between 30 and 60 years of age

Women are 2-3 times more likely to develop RA than men

Up to 50% of RA patients are unable to work within 10 years of onset of disease

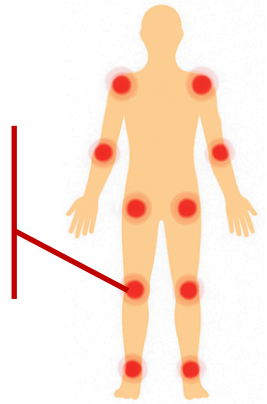
Inflammopharmacology, 31(1), 253-264
Nature Reviews Rheumatology, (2022), 18(10), 591-602.
The Lancet Rheumatology, (2023), 5(10), e594-e610.
medbroadcast.com

How RA progresses?

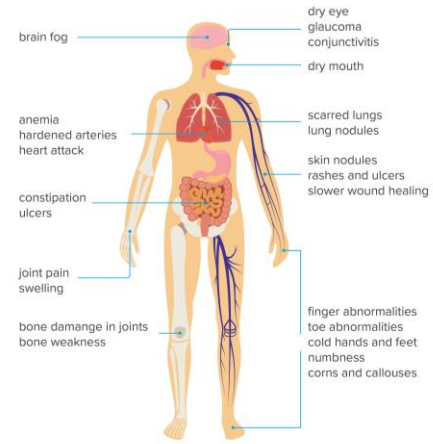


Before

After

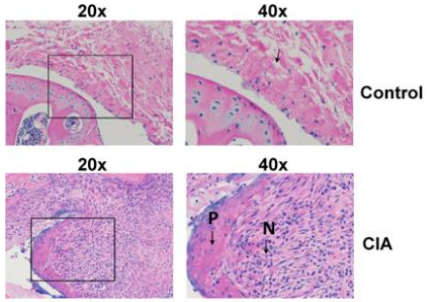


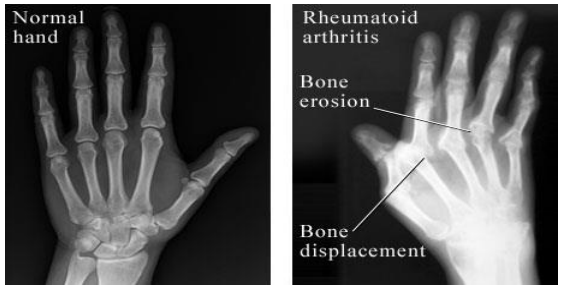
Localized



Systemic

Irreversible joint damage within 8-10 years of onset





What is the current treatment regime?

To control the symptoms	To prevent pain and inflammation
Glucocorticoids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)	Disease Modifying Anti-Rheumatic Drugs (DMARDs) (Synthetic, Biologic)

Limitations

- Rapid clearance
- Dose escalation
- Systemic toxicity

What is missing in current RA therapies?

Target molecules for drug delivery in the treatment of RA

Limited specificity to joint cavity
-
Off-target toxicity

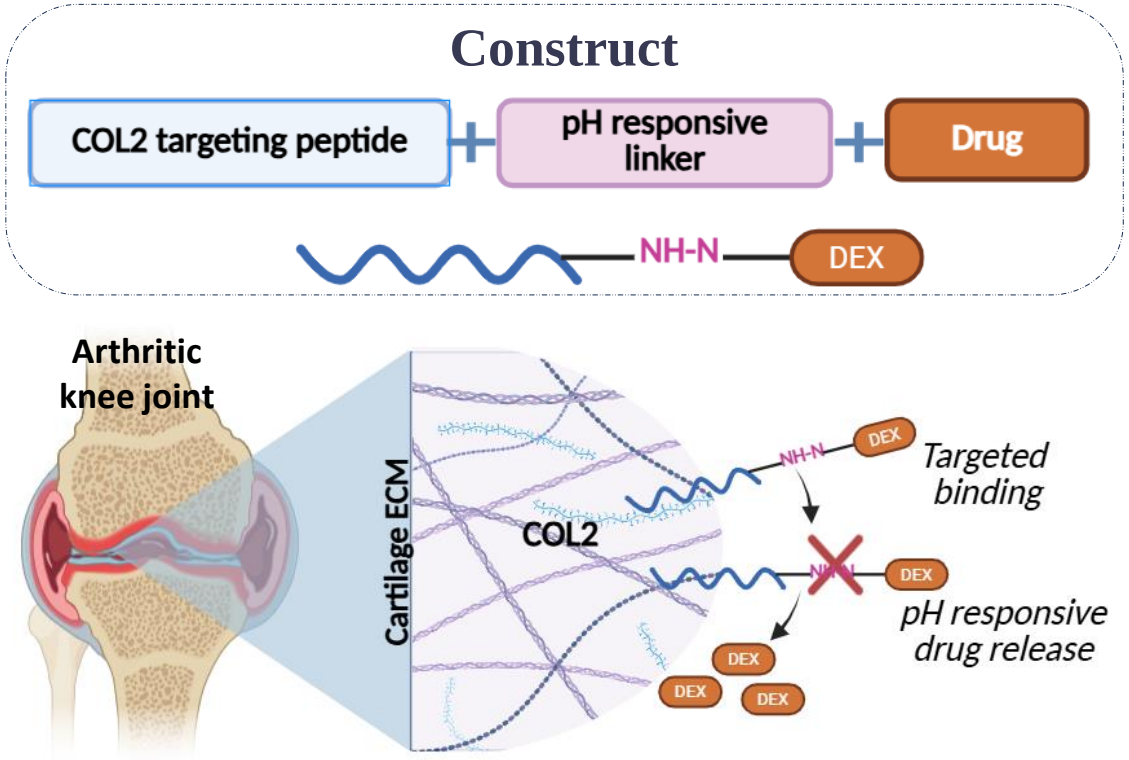
Collagen type II as target for drug delivery : Peptide-based drug delivery system

Therapeutic delivery, (2020), 11(4), 269-284

Hypothesis: A self-assembling, collagen type II-targeted peptide–drug conjugate with pH-sensitive release can enable systemic delivery of drug with localized activation

Approach

- ✓ **PDC Composition**: Peptide (targeting Collagen II) + pH labile linker + Anti-inflammatory Drug
- ✓ **Targeting Mechanism**: Peptide which specifically binds to exposed collagen type II in inflamed joints
- ✓ **pH-Sensitive Release**: Hydrazone bond cleavage and drug release in the mildly acidic pH (6.5–6.8), characteristic of inflamed synovial fluid, minimizing release in healthy tissues (pH 7.4)



Peptide — Linker — Drug

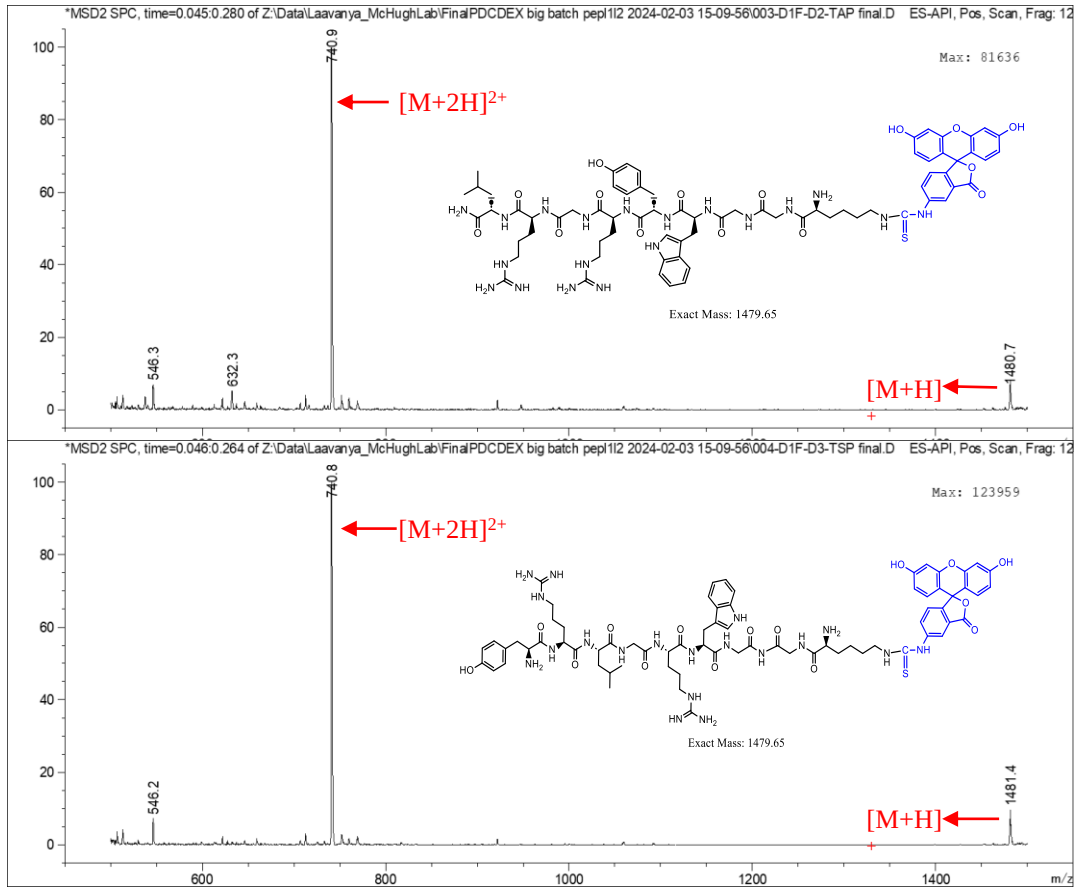
a. Confirmation of binding potential of peptide to target

- Method: Synthesis of actual and scrambled peptides through the manual solid phase peptide synthesis process

✓Tagged actual peptide (AP)
✓Tagged scrambled peptide (SP)

Characterization: Evaluation of fluorescence quenching upon binding, ITC, Binding on cartilage explants

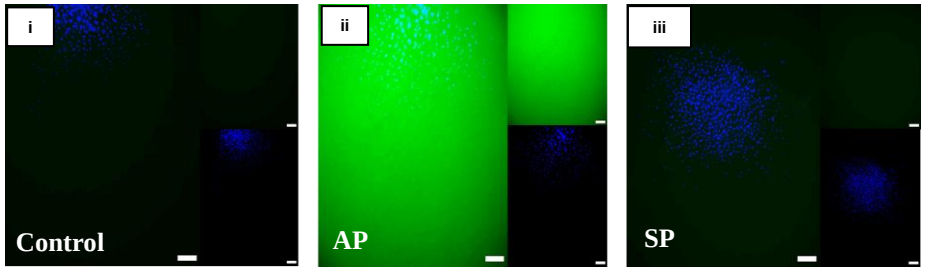
Confirmation of mass: ESI-MS



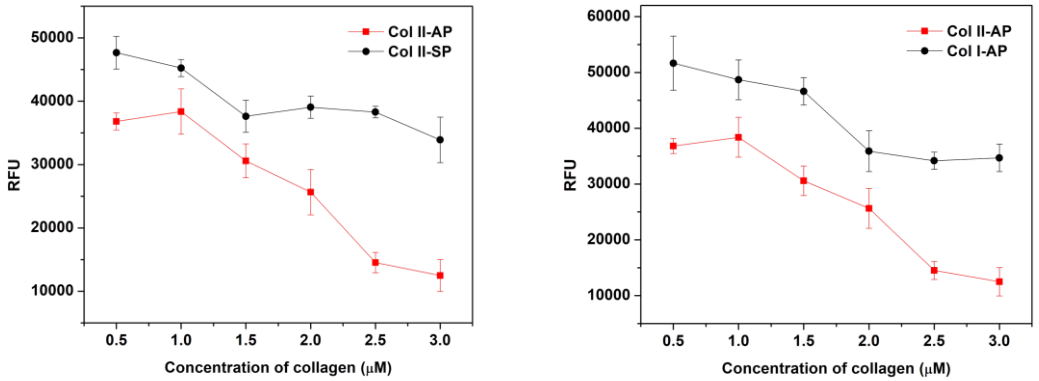
Peptide — Linker — Drug

a. Confirmation of binding potential of peptide to target

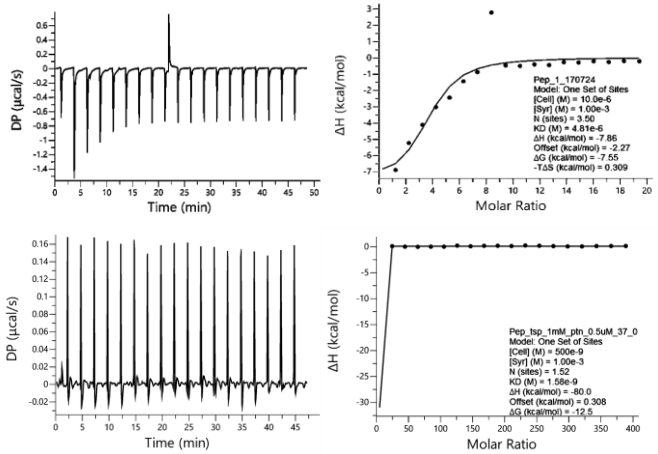
Binding study on cartilage explant



Fluorescence quenching upon binding



K_d value: Isothermal titration calorimetry



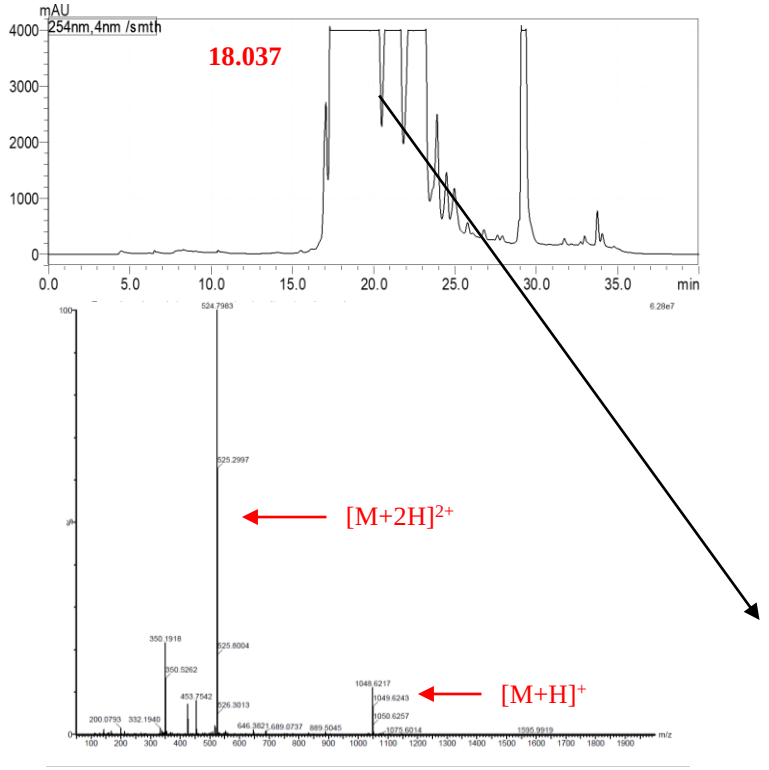
Observation: Actual peptide demonstrated a high affinity for the COL2 protein compared to the scrambled peptide and has a K_d value of 4.81μM

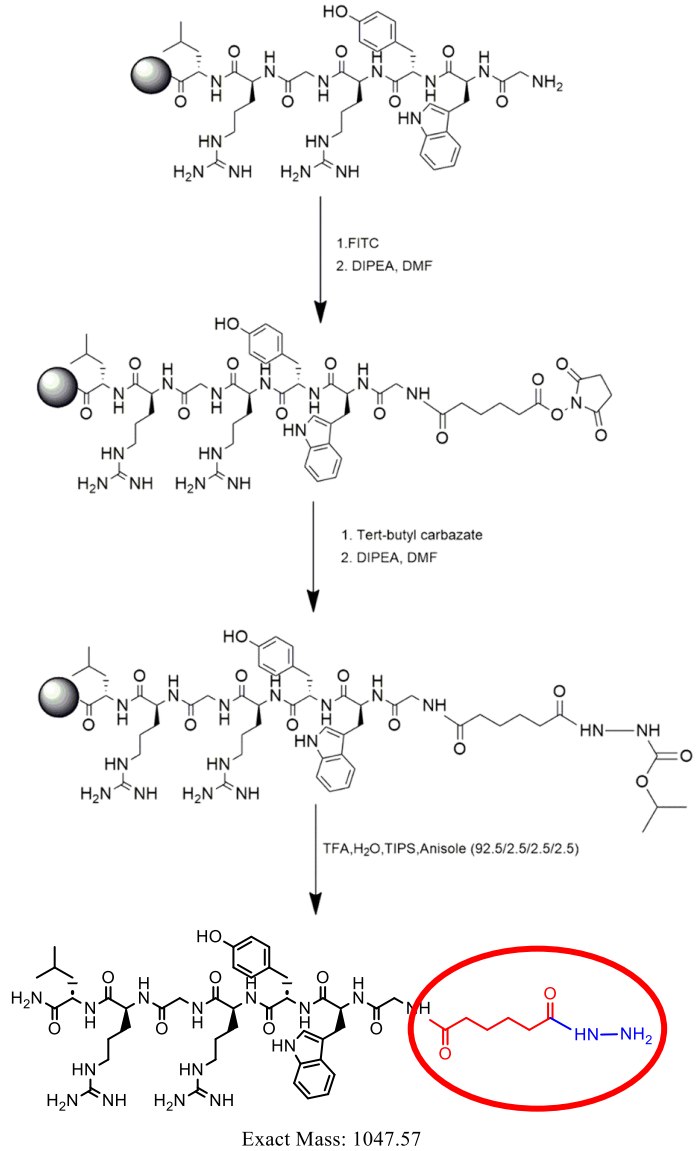
Peptide — **Linker** — Drug

a. Confirmation of binding potential of peptide to target

b. Functionalized peptide synthesis (P-Hy)

- Method – Solid phase peptide synthesis method with low loading Rink amide MBHA resins
- Characterization : Preparative HPLC for purification, Analytical HPLC to check purity, HNMR and mass spectrometry for structural and molecular confirmation





Exact Mass: 1047.57

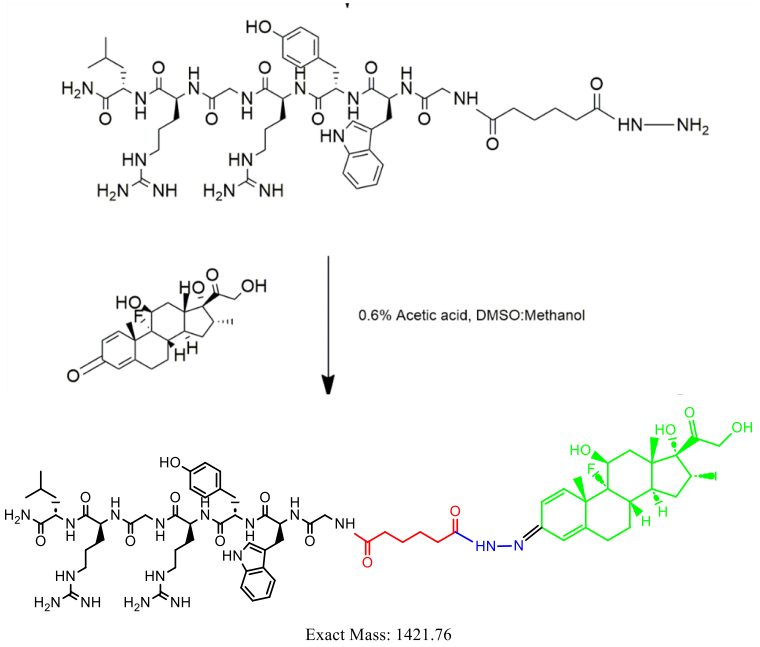
Peptide — **Linker** — **Drug**

a. Confirmation of binding potential of peptide to target

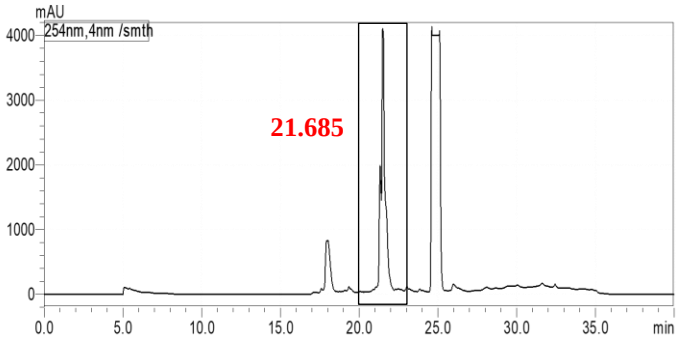
b. Functionalized peptide synthesis (P-Hy)

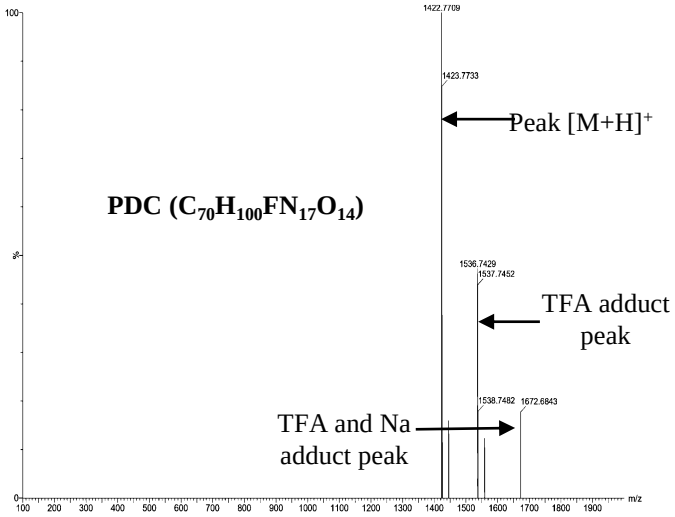
c. Peptide-drug conjugate (PDC) synthesis and characterization

- Method – In solution
- Characterization : Preparative HPLC for purification, Analytical HPLC to check purity, HNMR and mass spectrometry for structural and molecular confirmation



Exact Mass: 1421.76





Peptide — **Linker** — **Drug**

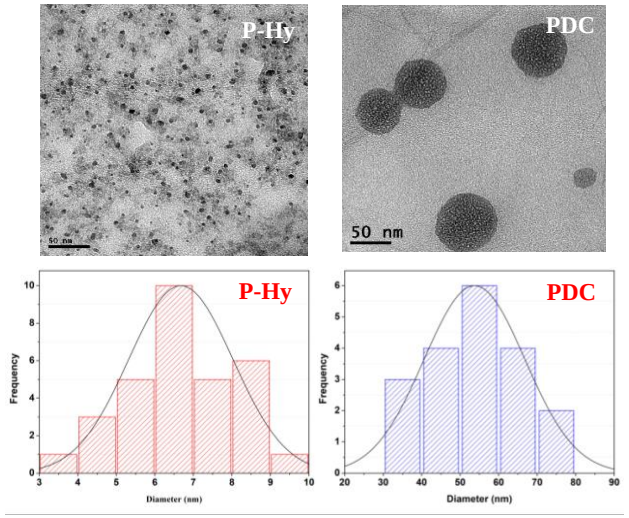
a. Confirmation of binding potential of peptide to target

b. Functionalized peptide synthesis (P-Hy)

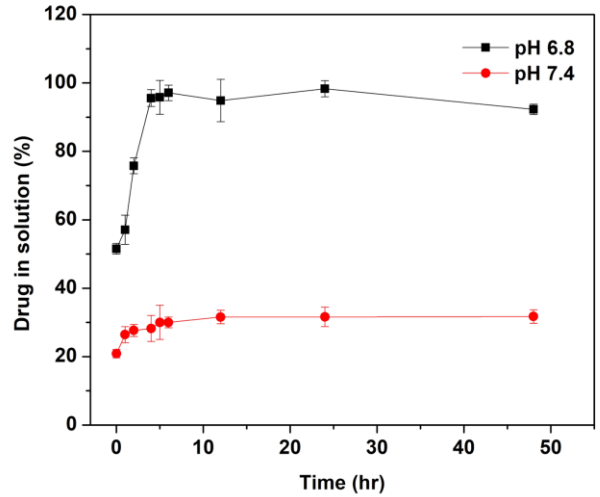
c. Peptide-drug conjugate (PDC) synthesis and characterization

Morphology, self-assembling property and stability

Transmission Electron Microscopy



PDC stability in different pH buffers



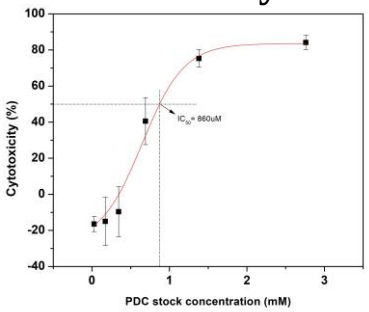
Observation: PDC exhibited a hydrodynamic size of 210 nm (DLS) and a core particle size of 50–60 nm (TEM). Drug release reached 30% at pH 7.4 and almost 95% at acidic pH within 48 hours.

Peptide — Linker — Drug

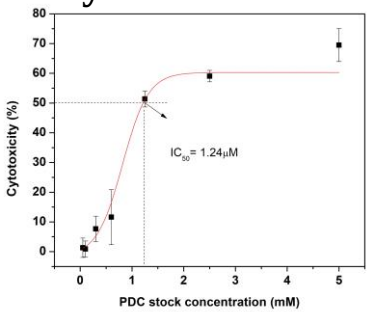
In vitro biocompatibility and hemocompatibility

IC_{50} of PDC with different cell types

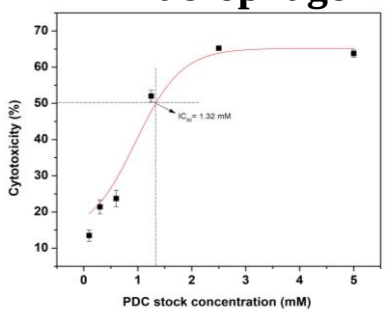
Isolated goat knee chondrocytes



SW 982 human synovial sarcoma

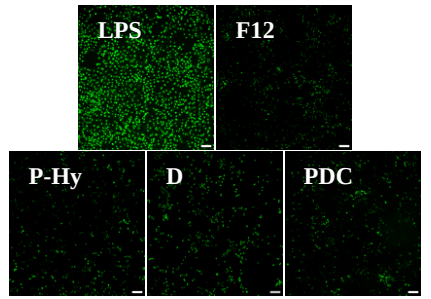


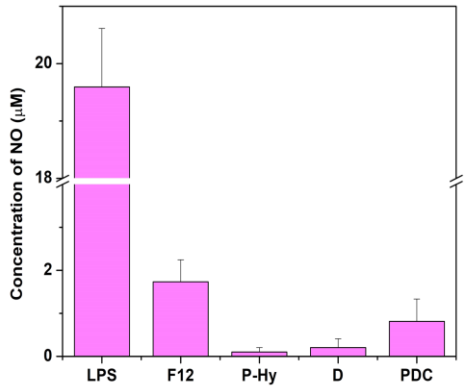
RAW 264.7 murine macrophage



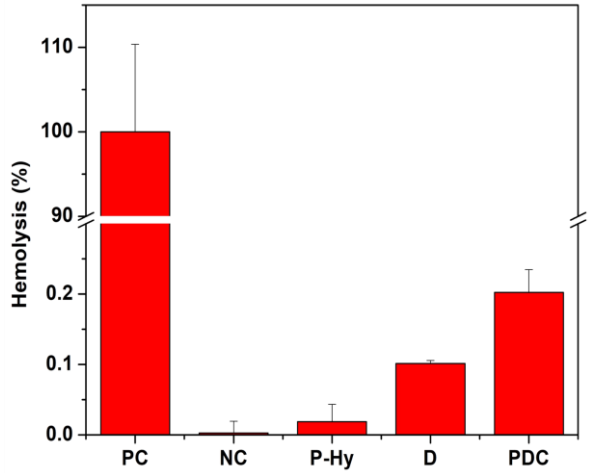
Observation: IC_{50} of the PDC :1.32 mM in RAW 264.7, 0.86 mM in primary chondrocytes, and 1.24 mM in SW982.

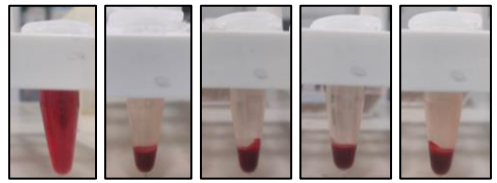
DCFDHA and Griess assay for ROS estimation





Hemolysis assay





Observation: Systemic safety criteria of PDC was confirmed and it showed no hemolytic activity or induction ROS synthesis

Summary

- ✓ Developed a collagen type II-targeting, self-assembling peptide–drug conjugate (PDC) designed for systemic delivery in rheumatoid arthritis.
- ✓ Confirmed the targeting capability of the selected peptide sequence toward collagen type II.
- ✓ Demonstrated the pH-responsive behavior of the PDC, enabling controlled drug release under physiological and pathological conditions.
- ✓ Preliminary studies confirmed the in vitro biocompatibility of the PDC with relevant cell lines.

Future work

- ✓ Evaluation of biological stability and anti-inflammatory efficacy *in vitro* on going.
- ✓ Further validation of safety, targeting, and therapeutic effect in a collagen-induced arthritis (CIA) animal model will be performed.

Thank You

Acknowledgement:

- Dr. Vignesh Muthuvijayan
- Dr. Kevin McHugh
- Lab members
- Mehta Rice Engineering Scholars Program – Internship (MRSEP)
- Prime Minister's Research Fellowship (PMRF)

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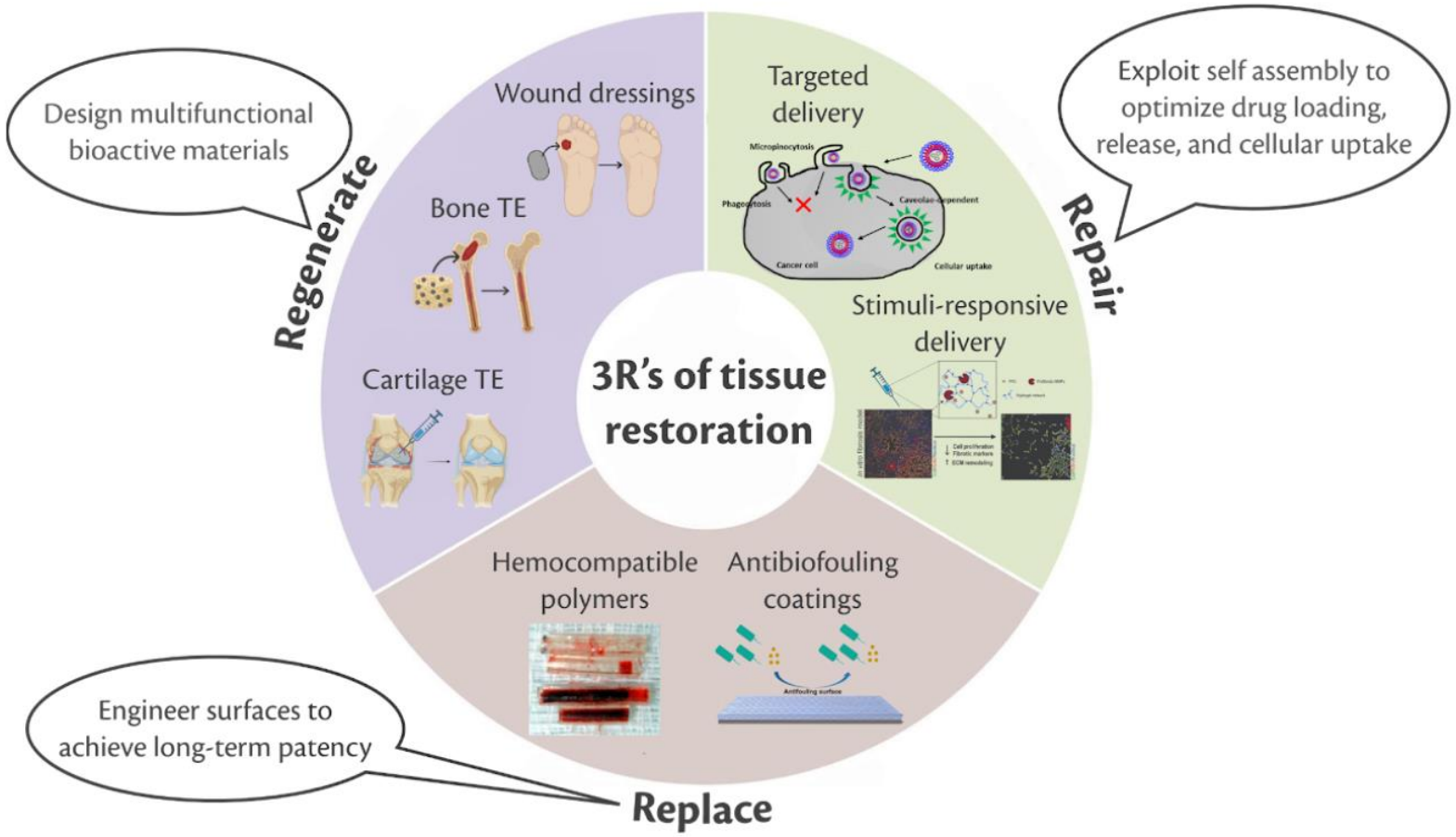
Lab's focus


Design multifunctional bioactive materials
Regenerate
Wound dressings
Bone TE
Cartilage TE
3R's of tissue restoration
Targeted delivery
Micropinocytosis
Phagocytosis
Caveolae-dependent
Cancer cell
Cellular uptake
Stimuli-responsive delivery
Repair
Exploit self assembly to optimize drug loading, release, and cellular uptake
Hemocompatible polymers
Antibiofouling coatings
Engineer surfaces to achieve long-term patency
Replace