Delivery of Alpha-Ketoglutarate from Hyaluronic Acid Hydrogels for Bone Repair Applications

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Research Question

How can the release of alpha-ketoglutarate from polymer microparticles in a hyaluronic acid hydrogel be controlled for increased bone formation?

Background

Approximately 1.6 million people in the United States undergo bone graft surgery every year to treat bone loss.[1] Currently, autograft and allograft bone tissues are the only options for treating bone loss, however, they pose many limitations.[1] Therefore, there is a pressing clinical need to create a novel treatment that will promote bone repair. To accomplish this, the immunomodulating molecule alpha-ketoglutarate (aKG), which stimulates cell metabolism and modulates osteoclast function, can be used.[2] The goal of this project is to create a biomaterial scaffold that will allow control over bone formation through the delivery of aKG in the form of a microparticle.

Polyester of Alpha-Ketoglutarate Microparticle (paKG MP) Synthesis

To control the release of aKG it was modified into microparticles (MPs). Hydrolytically degradable aKG polymers (paKG) were first created by reacting aKG with 1,10 decanediol (Figure 1).[3] paKG was purified and formed into MPs through a standard oil in water emulsion technique.[3]

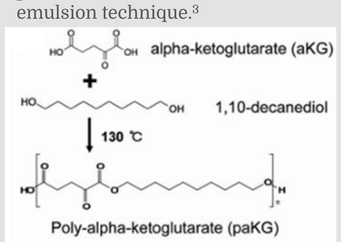


Figure 1: Chemical structure of alpha-ketoglutarate and 1,10-decanediol to form poly(alpha-ketoglutarate).[3]

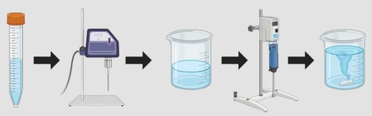


Figure 2: Oil in water emulsion used to create paKG MPs by sonicating and homogenizing paKG in PVA and then lyophilizing.

paKG MP Characterization

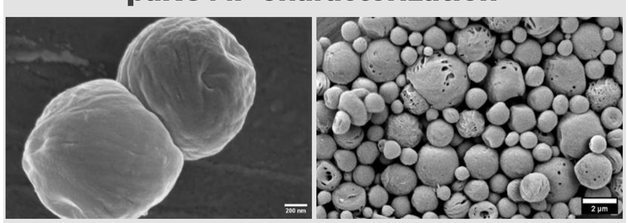


Figure 5: The synthesized paKG MPs were visualized using Scanning Electron Microscopy (SEM), showing mostly uniform particle sizes with slight variations.

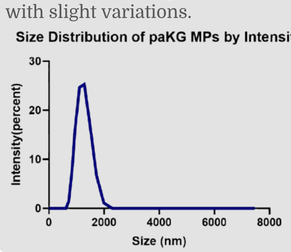


Figure 3: Dynamic Light Scattering (DLS) was run on the MPs, showing a size distribution around 1000 nm.

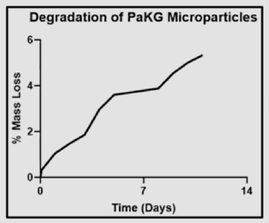


Figure 4: Percent mass loss of paKG MPs over a 12-day period. An increase in mass loss is seen, which corresponds to the degradation of the paKG MPs.

Maleimide Functionalized Hyaluronic Acid (MaHA) Hydrogel

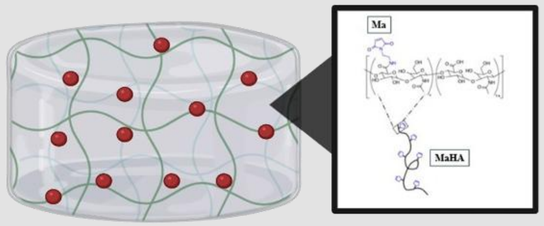


Figure 6: MaHA hydrogel delivery system for paKG MPs. Maleimide was functionalized to a hyaluronic acid backbone as a crosslinking mechanism to form a polymer hydrogel.

Maleimide Functionalized Hyaluronic Acid (MaHA) Characterization

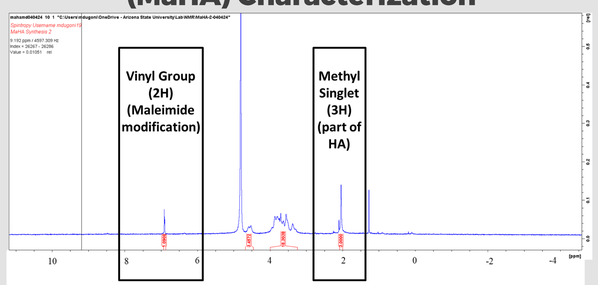


Figure 7: Proton nuclear magnetic resonance (^{1}H NMR) run on Maleimide functionalized hyaluronic acid (MaHA).

MaHA Hydrogel Synthesis

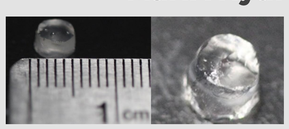
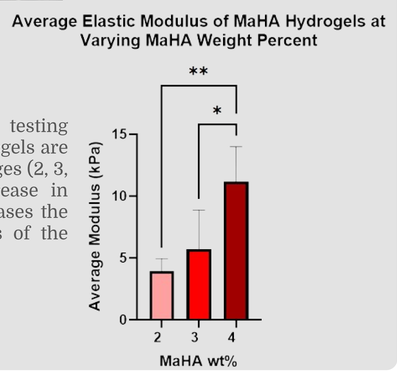


Figure 8: MaHA hydrogels without paKG MPs.

Figure 9: Compression testing data run on MaHA hydrogels are varying weight percentages (2, 3, 4wt%) showing an increase in weight percentage increases the average elastic modulus of the MaHA hydrogel.



MaHA Hydrogel Synthesis with paKG MPs

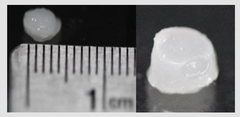


Figure 10: MaHA hydrogels with paKG MPs.

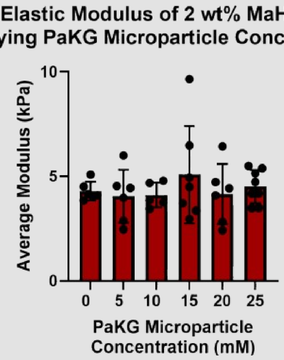


Figure 11: Compression testing data run on 2 wt% MaHA hydrogels with varying amounts of paKG MPs (0, 5, 10, 15, 20, 25mM) showing the addition of paKG does not impact the average elastic modulus of the MaHA hydrogel.

Osteoblast Differentiation and Investigation

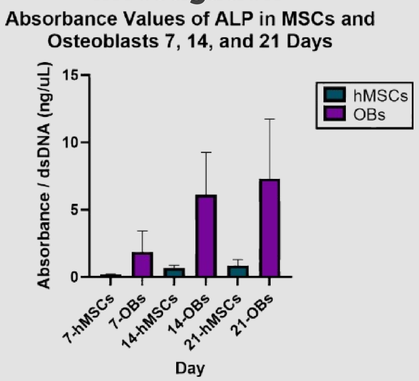


Figure 12: Absorbance values correlating to the concentration of ALP over a twenty-one-day period in hMSCs treated with hMSC media or osteo media. The concentration of ALP increased over time in cells treated with osteo media, proving that osteoblasts were synthesized.

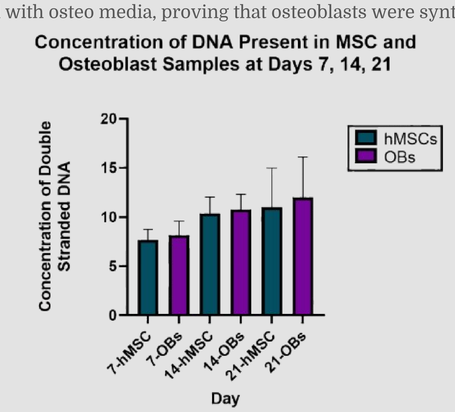


Figure 13: PicoGreen dsDNA assay for hMSCs treated with base and osteo media. Increase in the number of osteoblasts and hMSCs over a twenty-one-day period.

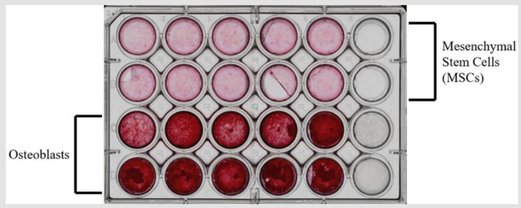


Figure 14: Results of the Alizarin Red S Stain shows calcium was present in the wells with osteoblasts (hMSCs in osteo media), an indicator healthy osteoblasts capable of mineralization were cultured.

Osteoclast Differentiation and Investigation

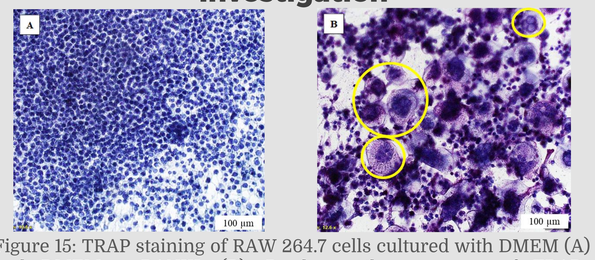


Figure 15: TRAP staining of RAW 264.7 cells cultured with DMEM (A) and DMEM + RANKL (B). B shows the presence of TRAP+ multinucleated (3+ nuclei) osteoclasts outlined in yellow circles.

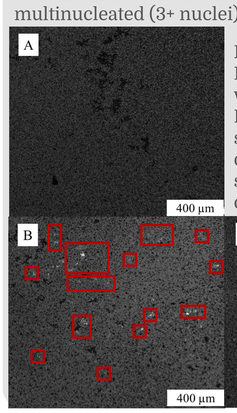


Figure 16: pit resorption assay and Von Kossa staining of RAW 264.7 cells cultured with DMEM (A), DMEM + RANKL (B), and DMEM + RANKL + paKG MPs (C). Pits can be seen in B and C, proving the presence of osteoclasts. There are fewer pits in C, showing the osteoclast inhibiting properties of aKG.

Conclusion & Future Work

- **paKG MPs** exhibited the desired spherical **morphology** and **size** distribution, and their **degradation** was confirmed.
- **MaHA hydrogels** were successfully **synthesized** at **multiple weight percentages**, demonstrating **tunable elastic moduli**.
- The addition of **paKG MPs** did **not impede** hydrogel **crosslinking** or alter the **mechanical properties**.
- **Differentiation** of healthy **osteoblasts** and **osteoclasts** was observed, validating the in vitro cell model.
- The cell-based **assays** used can now be applied to evaluate the **bioactivity** of **aKG** released from the MPs.
- **Release kinetics** studies will be conducted to quantify **aKG release** from **hydrogels** at varying paKG concentrations
- The **impacts** of **MaHA** hydrogels with **paKG MPs** on **osteoblasts** and **osteoclasts** will be evaluated.

Acknowledgments

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Figures 2, 4 and 6 were created with BioRender.com