Ultrasound-modulated hydrogel for precision bone regeneration: on-demand release for enhanced healing

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Introduction

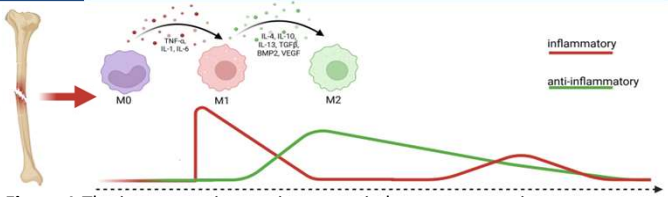


Figure 1: The immune microenvironment in bone regeneration

Bone is a dynamic tissue with strong regenerative capacity, but largescale defects often result in non-union fractures and permanent loss. Macrophages play a pivotal role in bone healing, transitioning from a pro-inflammatory (M1) to anti-inflammatory (M2) phenotype. Both stages are essential, yet the precise timing for this transition remains unclear. This project aims to develop an ultrasound-responsive bone implant capable of offering spatiotemporal control over immune activation to optimize bone regeneration.

Materials and methods

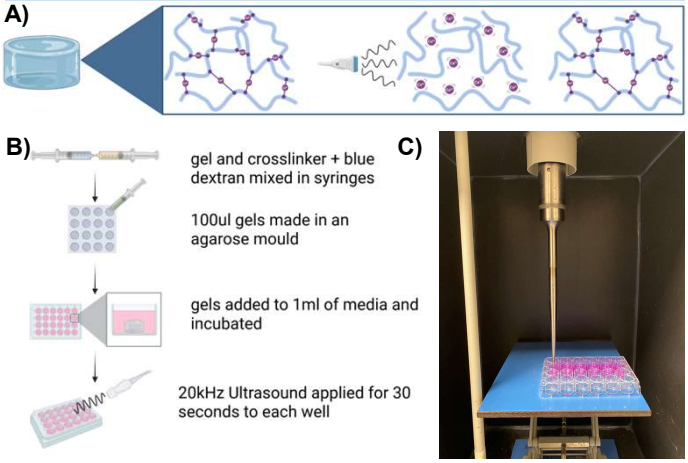


Figure 2: **A)** schematic illustrating the mechanism of action of Sodium alginate under ultrasound **B)** Gel fabrication Process **C)** Ultrasound application setup

Sodium alginate hydrogels were fabricated using the above technique, at 3.5% w/v with a crosslinking ratio of 25:9 using $CaCl_2$ and $CaSO_4$. Fluorescent beads of 3 different sizes and with positive and negative surface charges were added at a concentration of 100µl per 1ml of gel and the hydrogels were allowed to set for 30 minutes before incubating in media under normoxic conditions at 37.5°C for 3 weeks. Ultrasound was applied at each timepoint under the following conditions: 20kHz for 30 seconds total with a 50% duty cycle, with the media removed post treatment. Fluorescence intensity assays were used to determine concentration of beads that was released. Electrospinning optimisation was carried out at 10kV with a 30g needle and a 10mm dropping distance, and flow rate and gel composition were varied (as in fig.4). ImageJ analysis was used to determine microsphere size and distribution

Results

Ultrasound modulates alginate degradation

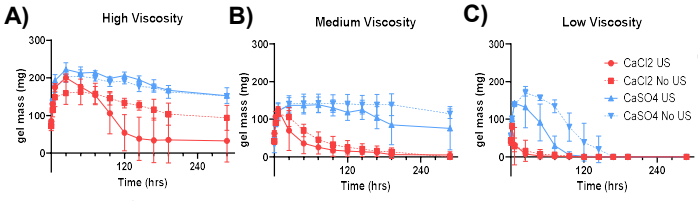
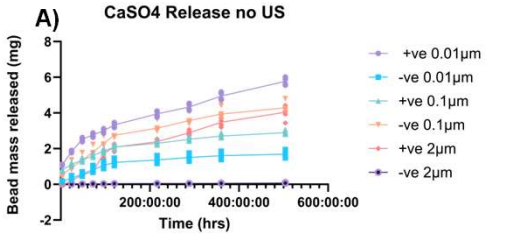
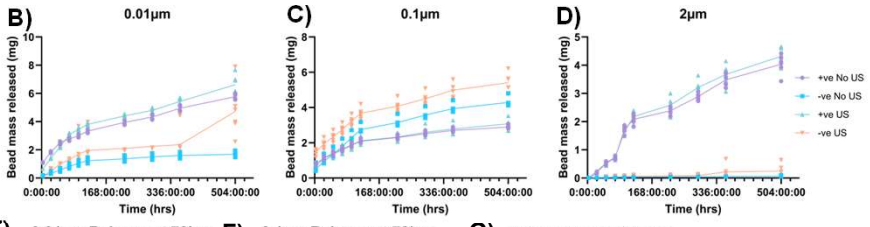


Figure 3: A-C) Degradation of Sodium alginate gels over time, with and without ultrasound at varying viscosities

Particle size and charge will impact the rate of drug release



Ultrasound increases drug release



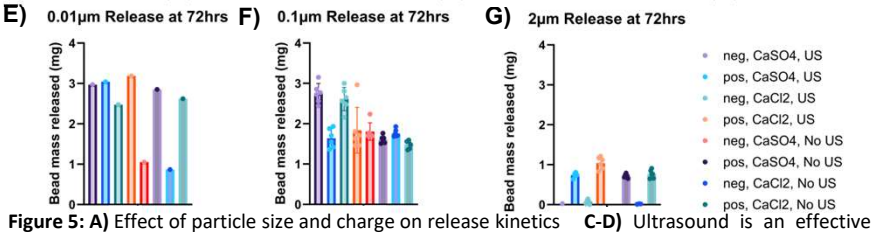


Figure 5: A) Effect of particle size and charge on release kinetics **C-D)** Ultrasound is an effective modulator of drug release, increasing release **E-G)** Drug quantity released at 72hrs

Alginate Microsphere Fabrication Using electrospinning for drug delivery

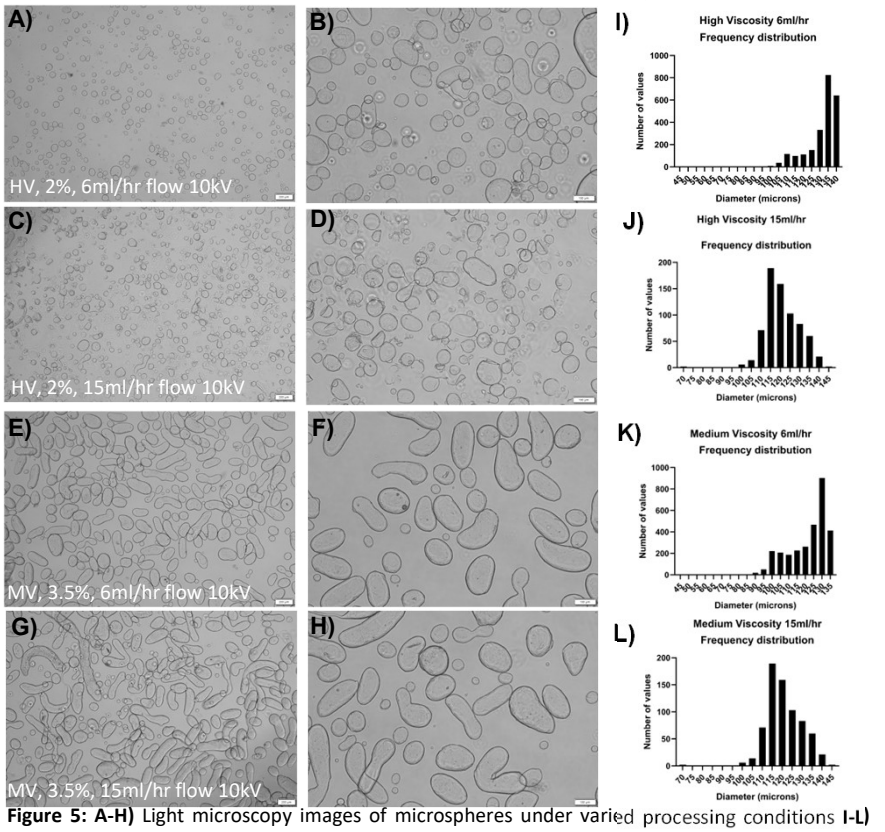


Figure 5: A-H) Light microscopy images of microspheres under varied processing conditions **I-L)** Particle size and distribution graphs

Discussion

The above results demonstrate that sodium alginate is highly responsive to ultrasound, and that ultrasound has a significant effect on gel degradation. Both particle size and charge has a significant effect on the release profile of drugs from the gel, while crosslinker has very little effect. The optimisation of electrospinning parameters demonstrated that this is a suitable processing method for alginate and identified the optimal processing parameters as 2% HV, which is validated by its degradation properties.

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