Covalent Immobilization of Heparin on Gelatin Methacryloyl as a Platform for Sustained Drug Delivery

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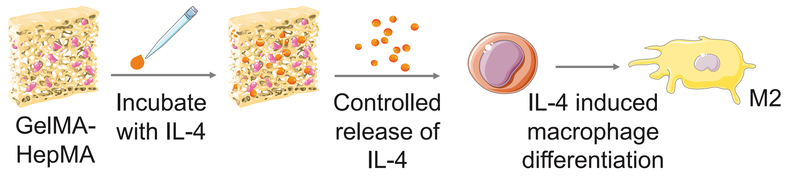
INTRODUCTION

- The inflammatory response to the biomaterials or scaffolds is critical to the outcome of tissue engineering. A prolonged inflammation will retard the tissue regeneration process, whereas, by modulating macrophages from the M1 "pro-inflammatory" phenotype to the M2 "pro-regenerative" phenotype, the tissue regeneration process can be accelerated and promoted.
- Heparin has been implicated to have an affinity for a variety of cytokines. For instance, interleukin-4 (IL-4) modulates macrophages to a pro-regenerative phenotype, and also promotes tissue regeneration.

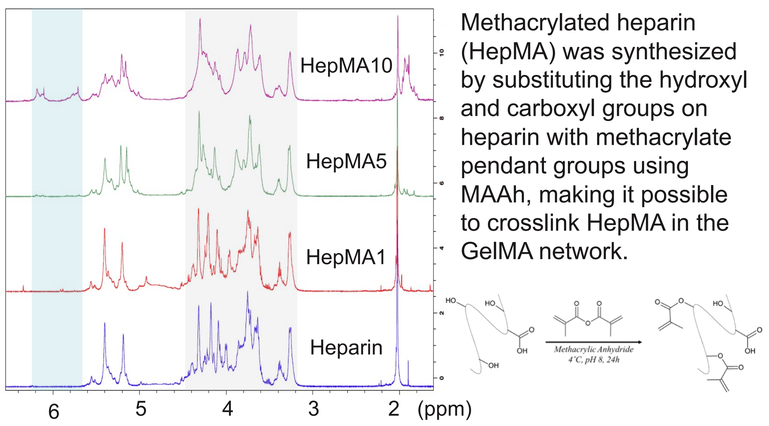
MATERIAL & METHODS



- In this study, we chemically modified heparin using methacrylate anhydride (MAAh) to covalently immobilize heparin to a gelatin methacryloyl (GelMA) hydrogel.
- The degree of modification on heparin molecules was measured using NMR, and the retention of heparin in the hydrogel was measured using a dimethylmethylene blue (DMMB) assay.
- The scaffold was designed to sustain the release of IL-4, aiming at modulating macrophages to a pro-regenerative phenotype and promoting tissue regeneration [1]. The macrophage response was evaluated *in vitro*.

RESULTS

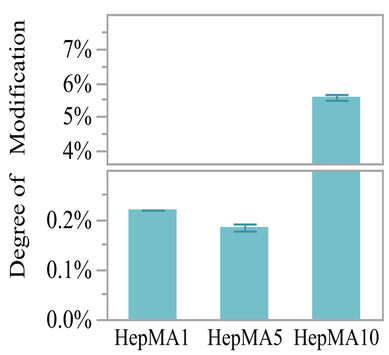
Synthesis of Methacrylated Heparin (HepMA)



Methacrylated heparin (HepMA) was synthesized by substituting the hydroxyl and carboxyl groups on heparin with methacrylate pendant groups using MAAh, making it possible to crosslink HepMA in the GelMA network.

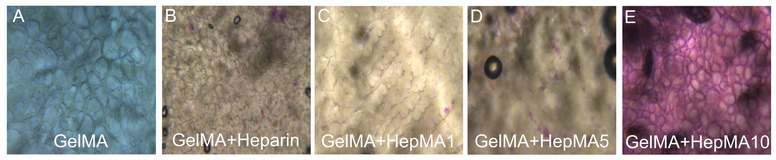
Here, heparin was chemically modified using 1, 5 and 10 fold molar excess of MAAh compared to the hydroxyl groups in heparin. They are referred to as HepMA1, HepMA5, HepMA10, respectively.

The degree of methacrylation on heparin was measured using NMR and calculated by comparing methacrylate vinyl protons (highlighted in blue in the spectra above) to the protons of the repeating disaccharide unit of heparin (grey). As shown in the figure on the right, a higher level of heparin functionalization was achieved by adding a higher quantity of MAAh to the reaction.

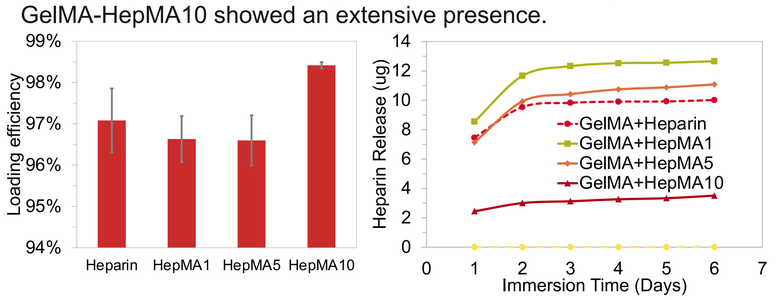


RESULTS

The Retention of HepMA in the GelMA Hydrogel

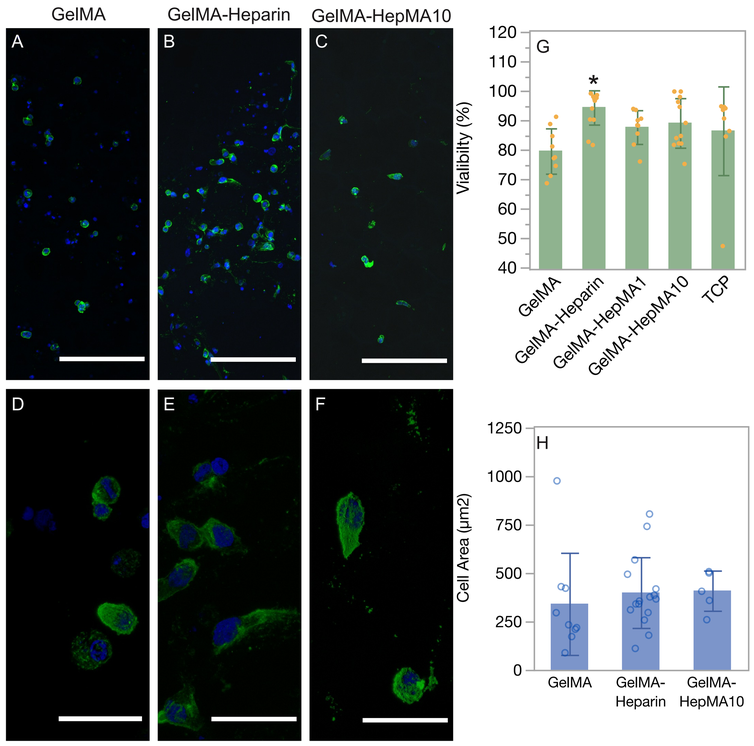


After successful synthesis of HepMA, we incorporated it into the GelMA hydrogel. The GelMA-HepMA prepolymer solution contained 10% GelMA, 1% HepMA, 0.25% IC2959 dissolved in 0.1M PBS. The GelMA-HepMA scaffolds were stained with DMMB reagent as shown above. Blue indicates the absence of heparin while pink confirms the presence of heparin. The GelMA scaffold, as the negative control, showed solid blue staining. The GelMA-heparin, GelMA-HepMA1, GelMA-HepMA5 showed only a limited amount of heparin, whereas GelMA-HepMA10 showed an extensive presence.



After gelation, the hydrogel was washed in PBS and the loading efficiency of heparin on the scaffold was calculated by comparing the amount of heparin detected in the wash solution to the total amount loaded previously. As shown in the figures above, HepMA10 had a higher retention compared to other scaffolds.

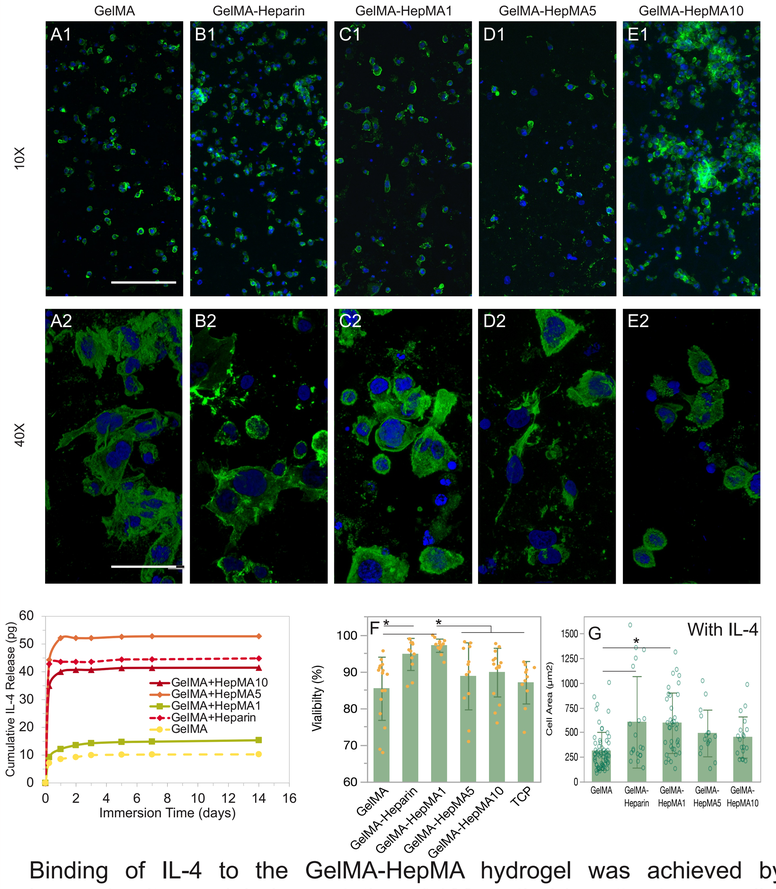
The presence of HepMA in GelMA did not affect the macrophage viability and morphology



THP-1-cell-induced macrophages were cultured on the GelMA-HepMA hydrogel to evaluate the macrophage response *in vitro*. In the figures, (E & F) macrophages showed an elongated morphology on the (E) GelMA-heparin and (F) GelMA-HepMA10 samples, which shows a similar morphology to M2 macrophages [4]. There was no significant difference in cell viability and cell area between the different groups, except that the GelMA-heparin scaffold increased the cell viability compared to the GelMA hydrogel.

RESULTS

The release of IL-4 from GelMA-HepMA hydrogel ameliorated macrophage viability and altered macrophage morphology



Binding of IL-4 to the GelMA-HepMA hydrogel was achieved by immersing it overnight in a solution of 100 ng IL-4 in cell culture media. The scaffolds were washed three times in PBS to wash off unbound IL-4. The release of IL-4 from the hydrogel was measured by ELISA.

The hydrogel containing unmodified heparin resulted in a burst release of IL-4, while all the other scaffolds showed an initial burst release followed by a gradual release for at least 14 days.

The GelMA hydrogel control and the low-level-modified HepMA1 showed the slowest release of IL-4. They also improved cell viability compared to the other hydrogels. Unmodified heparin and HepMA1 with the addition of IL-4 also caused larger single cell size compared to the GelMA control. Further study is needed to evaluate the gene expression profile of the macrophages.

CONCLUSIONS

In this study, we used MAAh to chemically modified heparin so as to covalently immobilize it to a GelMA hydrogel scaffold and provide sustained release of IL-4. Increasing the degree of modification enhanced the loading efficiency and the retention of heparin on the scaffold. The GelMA-HepMA hydrogel showed no toxicity and no significant effect on the morphology of the THP-1 cell induced macrophages. The gene expression of the THP-1 cells will be undertaken to evaluate the phenotype change of the macrophages in response to the IL-4 loaded GelMA-HepMA hydrogel.

REFERENCES

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