Applications of Alginate Hydrogels and Porous Silicon in Drug Delivery and Tissue Engineering

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I. Introduction

Tissue engineering involves the repair and regeneration of various tissues throughout the human body that have been adversely affected by disease or injury. Through combining the body's cells with synthetic scaffolds that mimic the extracellular matrix, tissue engineering promotes proliferation of cells at damaged sites (**Figure 1**).[1] Recent advances have demonstrated that using biocompatible materials such as alginate hydrogels (**Figure 2)**—polymer networks derived from brown algae—are a cheap and environmentally-friendly approach to this.[1]

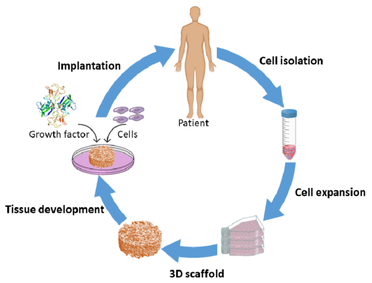


Figure 1. Diagram of Tissue Engineering[2]

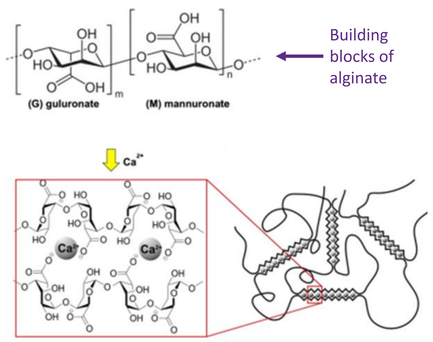


Figure 2. Egg-box structure of Ca^{2+} ionically crosslinking alginate[3]

One necessary modification to these scaffold materials is to load them with drugs that can facilitate healing. In addition to hydrogels, drugs can also be loaded into a material known as porous silicon (pSi). pSi nanoparticles or membranes, created from etching crystalline silicon, can be physically entrapped inside alginate hydrogels to create a two-system drug delivery mechanism with sustained release (**Figure 3**). This allows drugs such as growth factors, substances that stimulate cell growth, to be released at different times as the pSi and alginate hydrogel degrade.

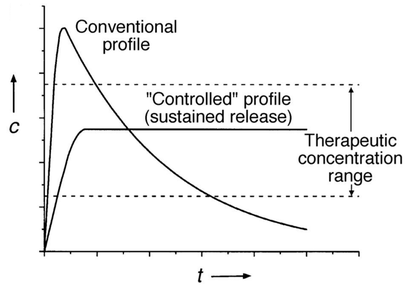


Figure 3. Sustained (Controlled) vs. Burst (Conventional) release in drug delivery[4]

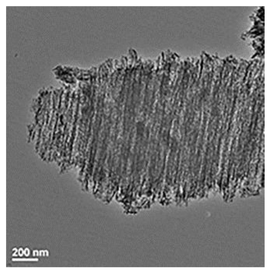


Figure 4. Representative TEM image of pSi nanoparticle

This project entails the construction of alginate hydrogels that incorporate model dye-loaded porous silicon (pSi) particles. The release of a model dye known as rhodamine 6G (R6G) was monitored to assess the efficacy of the two-system drug delivery mechanism.

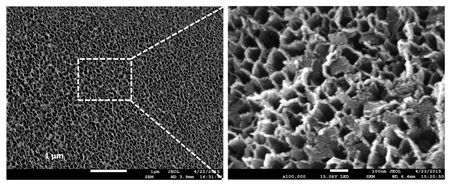


Figure 5. Representative SEM image of pSi nanoparticle

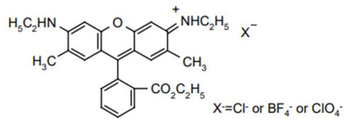


Figure 6. Structure of Rhodamine (R6G)

II. Experimental

A. Loading R6G into pSi Nanoparticles

- 20 mg pSi placed in a 10 mL beaker
- 2 mg/mL solution of rhodamine in ethanol was made
- Hot plate set at 37°C (incubate both pSi and rhodamine solution prior to loading for 10 mins at 37°C)
- 20 additions of 100 µL of 2 mg/mL rhodamine in ethanol
- R6G loaded pSi dried in vacuum overnight and stored in desiccator

Figure 7. 20 mg pSi in 10 mL beaker

Figure 8. 2 mg/mL R6G in ethanol

B. Loading R6G into pSi Membranes (Box & Tube Methods)

- The mass of the pSi membrane in either box (**Figure 9**) or tube (**Figure 10**) was obtained
- The number of additions of dye solution needed to obtain around a 20% loading was determined
- Added the dye in ethanol dropwise to the membrane, allowing the solvent to evaporate in between each addition
- R6G loaded pSi membranes dried with lid open overnight

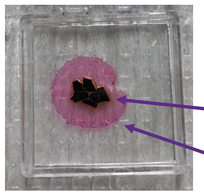


Figure 9. Box Loading Method

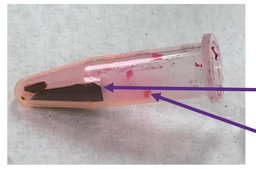


Figure 10. Tube Loading Method

C. Fabrication of Alginate Hydrogels

400 µL 2% w/v alginate
100 µL 10 mg/2.5 mL $CaCl_2$
100 µL 1.5 g/25 mL glucono-δ-lactone (GDL)

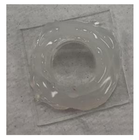


Figure 11. Mold constructed using plastic tube top hot glued onto a 1 cm x 1 cm glass slide

1 week of gel formation

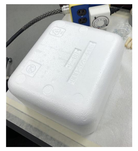


Figure 12. Humidity box constructed out of a Styrofoam box with a large surface area petri dish filled with water placed under it

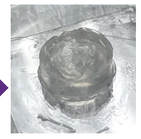


Figure 13. Plain alginate hydrogel after removal from mold

D. Physically Entrapping pSi into Alginate Hydrogels

"Quick aqueous" method

- Loaded pSi nanoparticles mixed in with the alginate prior to pipetting the pSi/alginate slurry into the molds (**Figure 14**)

"pSi membrane" method

- Loaded pSi membranes inserted into plain alginate gels using a scalpel after their gelation and removal from molds (**Figure 15**)

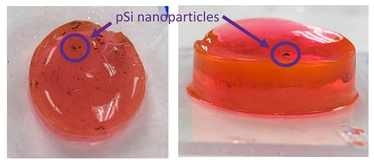


Figure 15. "Quick Aqueous" Method – Dark specs indicate pSi nanoparticles (top view & side view)

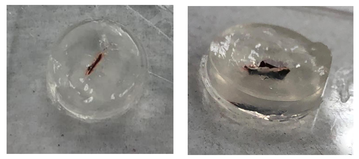
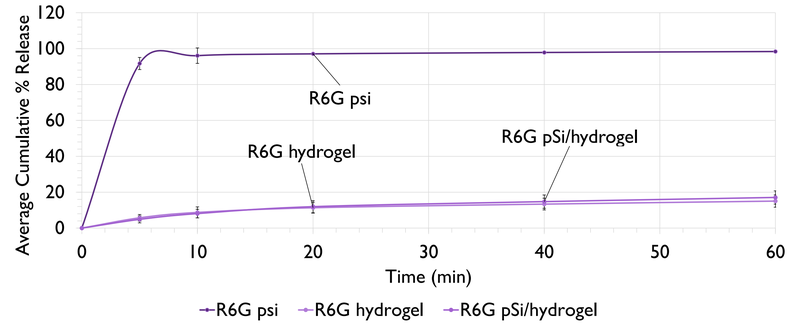


Figure 16. "pSi Membrane" Method (top view & side view)

III. Results

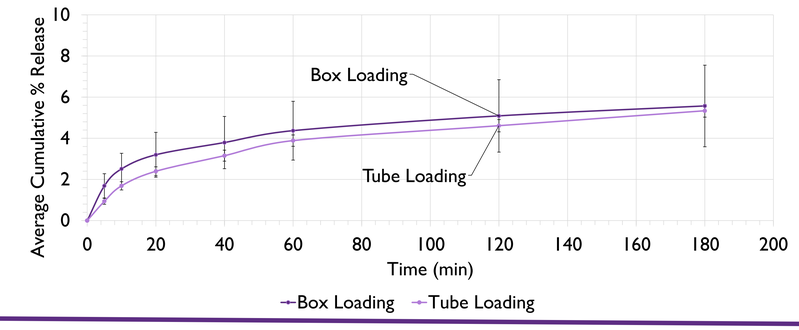
A. R6G Loaded & Released in Water from pSi Nanoparticles in an Alginate Hydrogel vs. from Alginate Hydrogels only vs. from pSi Nanoparticles only

- When R6G is released in water from pSi nanoparticles only, the average cumulative release was observed to be 91.7% after the first 5 minutes, indicating a burst release
- The "quick aqueous" loading method results in quick release of R6G from pSi – switched to pSi membranes with a lower internal surface area to theoretically slow R6G release from pSi into the hydrogel



B. R6G Loaded & Released in Water from pSi Membranes Loaded in Box vs. Tube

- Both R6G loading methods for pSi membranes resulted in an average cumulative release in water over a 2-hour period of less than 6% (5.3% for tube and 5.6% for box)



IV. Conclusions

- Release of R6G from pSi nanoparticles physically entrapped in alginate hydrogels and from alginate hydrogels only prevented the burst release of the model dye that was observed when releasing R6G from pSi only
- Localization of pSi in alginate hydrogels was achieved through physically entrapping model dye-loaded pSi membranes instead of pSi nanoparticles. However, the loading of rhodamine in pSi membranes was not observed to result in high payloads of the model dye when released in an aqueous environment

V. Future Work

- The mechanism of loading pSi membranes which achieves the highest payload and most sustained release of the model dye over time needs to be determined
- The release of a therapeutic drug or growth factor from pSi membranes physically entrapped in alginate hydrogels will be monitored for potential use for *in vivo* tissue engineering applications

VI. References

1. Sahoo, D.R.; Biswal, T. Alginate and its application to tissue engineering. *SN Applied Sciences*. **2021** *3*(30).
2. Asadian, M; Chan; Norouzi; Grande; Silvia; Cools; Pieter; Morent; Geyter, D. Fabrication and Plasma Modification of Nanofibrous Tissue Engineering Scaffolds. *Nanomaterials*. **2020**.
3. Shaari, N.; Kamarudin, S.K; Recent Advances in Additive-Enhanced Polymer Electrolyte Membrane Properties in Fuel Cell Applications: An Overview. *Int J Energy Res*. **2019**; 1-39.
4. Santini, JT Jr; Richards, AC; Scheidt, R; Cima, MJ; Langer, R. Microchips as Controlled Drug-Delivery Devices. *Angew Chem Int Ed Engl*. **2000.** *39*(14), 2396-2407.

VII. Acknowledgments

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