Chitosan grafted with fibronectin as a building unit for bioactive thermosensitive hydrogels

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Injectable hydrogels for cell encapsulation and delivery

- Cell suspension in a polymer solution
- Polymer in good solvent
- Heating at 37°C
- Polymer phase separation
- Macroporous cell-laden hydrogel
- Encapsulated cell
- Physiological environment
  - Macroporous structure
  - Fast gelation properties
- Water-rich macropore
- Polymer-rich scaffold
Chitosan/Phosphate salts thermosensitive solutions

Chitosan (CS)  

Beta-glycerophosphate (βGP)  

Ammonium hydrogenophosphate (AHP)

pH = 7.0-7.4 ; 300 mOsm.L⁻¹

Quick gelation  

Macroporosity  

Cytocompatibility

Dang et al., Carbohydrate Polymers, 2022
Improving bioactivity in our chitosan/phosphate salts hydrogel

Hydrogel network

Poor bioactivity
Weak cell adhesion

Biofunctionalization of pore surfaces to promote adhesion and confer bioactivity
Incorporation of an adhesion protein: the fibronectin

Fibronectin (Fn) 550kDa

Chitosan (CS) 250kDa

Carbodiimide coupling

Chitosan-grafted-Fibronectin CS-\textit{g}-Fn

C5H11O2N2\textsubscript{92} H\textsubscript{2}O\textsubscript{8} C5H7O2N\textsubscript{8} H\textsubscript{2}O\textsubscript{6} C5H7O3N\textsubscript{4} H\textsubscript{2}O\textsubscript{4}

30 μm

CS* channel (AlexaFluor488)

CS*-g-Fn*

Fn* channel (AlexaFluor568)

Merged channels
Amide linkage

**Context**
Thermosensitive hydrogels with a gelation transition at 37°C are promising tools for cell encapsulation. Their ability to reverse liquid at body temperature allows to mix with cells and form a solid gel when cooled. These materials can be used as drug carriers and protect cells from harsh external conditions. The following criteria are respected:
- pH and osmolarity close to physiological values
- Controlled gelation properties enabling cell encapsulation
- Microstructure compatible with nutrient diffusion and cell migration
- Gel-polymer matrix interactions for cell adhesion

**Chitosan with phosphate salts**
- Acrylamidophosphonate (AAPM)
- Control of (CS) [CS] and (CS) [CS]

**One-pot** synthesis of Chitosan grafted with Fibronectin (CS-g-Fn) and encapsulation procedure

- Fibronectin (Fn)
- Cross-linking
- Carbodiimide crosslinking EDC, Sulfo-NHS

**Significant difference in $M_w$**

Effect of fibronectin on ...

Macroporosity?

Gelation properties?

Cell metabolism?

**References**