Crosslinking & biopolymers in tissue engineering

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Crosslinking & biopolymers in tissue engineering /2008 to 2017

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Introduction

tissue engineering
Crosslinking biopolymers for biomedical applications

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Biomaterials made from proteins, polysaccharides, and synthetic biopolymers are preferred but lack the mechanical properties and stability in aqueous environments necessary for medical applications. Crosslinking improves the properties of the biomaterials, but most crosslinkers either cause undesirable changes to the functionality of the biopolymers or result in cytotoxicity. and in many instances the stability in aqueous and physiological environments required for medical applications [6]. For instance, films and electrospun structures made from proteins disintegrate at high humidities or in aqueous solutions [7,8]. Crosslinking has been the most common approach to overcome the limitations of biomaterials [9,10]. Crosslinkers interconnect molecules, increase mo-
Chemical crosslinking

Physical crosslinking

Enzymatic crosslinking
Introduction

Cross linker

- Glutaraldehyde
- EDC/NHS
- Epichlorohydrin
- Glyoxal
- Genipin

Hydrogels

Sponges

Films

Micro, nanoparticlel

Electrospun fibers

Micro fibers
Introduction

Proteins
- Gelatin
- BSA
- Collagen
- Zein
- Keratin

Biomaterials
- DNA
- Cellulose
- Starch
- Carbohydrate
- HA
- Alginate
- Chitosan
Cross linker
Glutaraldehyde

**Discussion**

**Advantage**

- functional groups with proteins & carbohydrates (1)
- improvement in tensile properties (1)
- improvement in mechanical properties (1)
- accelerate the calcification (9)
Discussion

Glutaraldehyde

Advantage

- flexible & transparent (10)
- in many application biodegradable (5)
- a low cost cross linker
Discussion Glutaraldehyde

Disadvantage

- Cytotoxicity (non-cytotoxic: up to 8\%) (1)
carboxylic acids

- crosslink both polysaccharides and proteins
- biocompatible

- 2 carboxylic groups can crosslink biopolymer in wet and dry conditions

- fibers, fils, electrospun crosslinked with citric acid.(1)
Discussion

- Crosslinked in dry & wet condition
- Improvement in tensile properties
- Stability under aqueous condition (1)
Citric acid crosslinked β-cyclodextrin/carboxymethylcellulose hydrogel films for controlled delivery of poorly soluble drugs

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ABSTRACT

Citric acid crosslinked β-cyclodextrin–carboxymethylcellulose (BCD-CMC) hydrogel films were prepared by esterification-crosslinking method for the controlled release of ketoconazole (model drug). The hydrogel films were prepared using different crosslinker: monomer ratios and the effects of different parameters on drug release were studied. The results showed that the film prepared with 60% crosslinker exhibited best release of ketoconazole. The results indicated that the hydrogel films are a potential therapeutic matrix for the controlled release of ketoconazole.
Citric acid

**Advantage**

- improve the mechanical properties (1)
- stability of biomaterials (1)
- Provides pendant functionality (1)
- formation of ester bonds better compatibility (1)
Discussion

Citric acid ...

Advantage

- Up to the 80% of citric acid in the human body is found in bones
- good tensile properties
- Water stability
**Discussion**

**Advantage...**

- Successfully used as a cross linker
- Cheap
- Non-toxic(6)

**Citric acid...**

![Citric acid structure](image)
Decreased crosslinking using 10% ≠ using lower citric acid

the fibers were crosslinked with citric acid to improve tensile properties and water stability
Discussion

Advantage

- Easiest biomaterial to be fabricated (1)
- Controlled release (1)
- Poor dissolve in water
Films & EDC/NHS

- tensile strength
- improvement in mechanical properties
- aqueous stability (1)

EDC/NHS: N-Ethyl-N0-(3-(Dimethylamino)propyl) Carbodiimide/N-Hydroxyl Succinimide
Discussion

- Mechanical properties were improved
- Tensile properties
- Better stability in water (13)
a) Non-cross-linked

b) Cross-linked with EDC/NHS
EDC/NHS+…

- **Carbodiimide** crosslinking with:
  - Type A gelatin
  - Type B gelatin
  - Collagen

- **Amide bond** found in carbodiimide-cross-linked product.
Discussion

Scaffold: sponge & fibres ....
Sponges

- 3D porous structures
- used as scaffolds for culturing of osteoblasts
- tooth tissue engineering
Discussion

Sponges…

Disadvantage

- Poor mechanical properties
- Poor stability in water
Discussion

Sponges & EDC/NHS

- Sponges
- EDC/NHS
- lysine
- glutamic acid
- glycine

improved thermal stability
&
lower rate of biodegradation
Hydrogel

**Advantage**
- capacity to retain large amounts of water (1)
- Delivery of drugs, peptides, and proteins (1)
- usable for in vitro and in vivo applications (1)
- high swelling & maintain morphology useful for ophthalmic, wound healing (1)
Hydrogel Wound Dressing

The Japan Science and Technology Corporation contracts research on this material to the private sector.

Merits:
1. Speeds healing
2. Painless removal of the dressing
3. No residue
4. Transparency enables observing the healing process

Use: 
1. Skin burns  
2. Bedsores  
3. Pharmaceutical chemical for wet cloth  
4. Facial pac

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Hydrogel...

- **Advantage....**
- Called “smarts materials” (4)
- highly porous structure (6)
- Composed of natural polymers
- Can enhance specific cellular functions
- excellent biological functions
- maintain cell viability
Discussion

Hydrogel...

Fig. 1: Swelling and deswelling behaviour of interpenetrating hydrogel network with the variation in temperature and pH.
Hydrogel

Discussion

Advantage....

- ability to swell in water without getting dissolved in it(6)
Development of a novel pH sensitive silane crosslinked injectable hydrogel for controlled release of neomycin sulfate

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ABSTRACT

Silane crosslinked biopolymer based novel pH-responsive hydrogels were fabricated by blending the cationic (chitosan) and anionic (alginate) polymers with poly(vinyl alcohol). Tetraethoxysilane (TEOS) was used as a crosslinker in different amounts due to its nonhazardous nature, to study its impact on the swelling and controlled drug release properties. The hydrogel exhibited a pH-sensitive swellability. These hydrogels were injected into the muscle tissue of mice to study their pharmacokinetics. Neomycin was encapsulated in the hydrogel core and released over a period of 7 days. A controlled drug release matrix was obtained with a controlled release over a period of 7 days. The in vivo study showed that this system had the potential for sustained delivery of neomycin in the targeted region.

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Hydrogel...

- **Advantage**
- Hydrophilic
- Superabsorbent
- Appear like a living tissue.
- Are prone to sense a minor change in external stimuli(7)
Discussion

Hydrogel…

Advantage….

- Better growth of cancer cells in the gelatin hydrogels
- Cells were successfully encapsulated
Hydrogel…

- Ecofriendly, biodegradable, biocompatible
- Nontoxic
- Cost effective

To be used in:

- Biomedical, cosmetics, biotechnology mainly for drug delivery and agricultural applications (7)
Histological observation of abdominal walls treated with modified hydrogel at 14 days after surgery
Discussion

fibers

- Number of layers
  - Braiding angle
  - Fiber diameter

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fibers

- as sutures & tissue engineering scaffolds
- made fibers: collagen, wheat & gluten & soy proteins

use as:

- tissue engineering
- drug delivery
- Scaffolds(1)
Advantage

- good mechanical properties under dry conditions

Disadvantage

- poor stability under aqueous conditions
fibers

- Fibers + gelatin + genipin → delivery of NGF

- Considered ideal for regeneration of nerve (1)

NGF = Nerve Growth Factor
Electrospun fibers

- Made of: biopolymers resemble the ultrafine fibrous network in ECM
- Can promote the attachment
- Proliferation of cells(1)

ECM = Extracellular Matrix

- Last updated: 04/05/2017
Discussion

Electrospun fibers…

- mechanical properties similar to of native tissue

Disadvantage

- poor water stability
Electrospun fibers…

electrospun fibers $\rightarrow$ saturated glutaraldehyde vapor

- tensile strength
- resist collagenase

- Porosity
- Cytotoxicity

- citric acid $\rightarrow$ electrospun collagen fibers $\rightarrow$ glycerol
Electrospun fibers…

- Electrospun fibers + zein
  - Weak tensile properties
  - Rapidly dissolve in aqueous solutions

- Electrospun fibers + zein + citric acid
  - Attachment
  - Proliferation of fibroblasts(1)
Discussion

Effects of crosslinking:
- Reinforced stability and delayed drug release;
- Prolonged blood circulation;
- Improved safety and tolerability;
- Optimized biodistribution;
- Reduced systemic toxicity;
- Enhanced antitumor efficacy.
Discussion

micro- and nanoparticles

Calcification Targeting
micro- and nanoparticles

- in vivo delivery of drugs
- biopolymers & metallic & synthetic polymers

Limitation biopolymers:
- Poor stability
- Agglomeration
- Particle size
- Relatively quick degradation(1)
Discussion

micro- and nanoparticles…

- Chitosan nanoparticles control release of drugs
- Protein nanoparticles:
  - unstable under physiological environment
  - accumulate in the kidneys (1)
Discussion

Biomaterial
Discussion

Biomaterial

C + H + O = Carbohydrates
Advantage

- natural bioadhesive (1)
- biocompatible
- is being used as wound covering
- Drug delivery
- Tissue engineering scaffolds
Discussion
Discussion

HYALURONIC ACID

\[
\begin{align*}
&\text{OH} \\
&\text{O} = \text{C} = \text{O} \\
&\text{OH} \\
&\text{O} - \text{HO} \\
&\text{OH} \\
&\text{O} \\
&\text{OH} \\
&\text{O} - \text{NH} \rightarrow \text{O} \\
&\text{O} \\
&\text{O} \\
&\text{n}
\end{align*}
\]
Discussion

Biomaterial
Advantage

- most widely used for medical applications (1)
- intended for controlled release of bovine serum albumin (BSA) (1)
- stimulate the migration cells
- Proliferation cells (14)
- major protein in ECM (14)
Discussion

Collagen...

Disadvantage

- poor mechanical properties
- unstable
- dissolve in water (1)
Discussion

- Gelly in water
- Cartridge capsule
- Drug delivery
- Tissue engineering scaffolds
Discussion
Discussion

- as a new material for biomedical applications. (11)
- DNA hydrogels prepared for chemical & physical cross-linkers.


Discussion

Advantage

- Natural
- Biocompatible
- Biodegradable
- as drug delivery (11)

DNA…
Despite improvement in resistance to degradation after crosslinking, collagen do not have adequate properties (1)
blend of different polymers

- collagen/chitosan + glutaraldehyde → improve their mechanical properties & stability
- collagen/chitosan + genipin → degradation & swelling
- Collagen/hyaluronic acid/poly capro lactone + EDC/NHS + UV wound dressings (1)
blend of different polymers…

- Glutaraldehyde/malic acid + collagen → degradation & growth of L 929 cells (1)
- Collagen hydrogels + EDC → resistance to collagenase
Electrospun fibers

- Swelling
- Promoted viability of human dermal fibroblasts
- Actin fiber formation

- Suggesting for tissue engineering
carboxylic acids

- Crosslink both polysaccharides and proteins
- Biocompatible

- 2 carboxylic groups can crosslink biopolymer in wet and dry conditions

- Fibers, fils, electrospun crosslinked with citric acid (1)
carboxylic acids…

- Crosslinked in dry & wet condition
- Improvement in tensile properties
- Stability under aqueous condition(1)
carboxylic acids…

- Improvement in properties
- Promote the attachment
- Proliferation of cells
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THANK YOU
AND THIS
IS END OF MY
POWERPOINT PRESENTATION