Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
Instructors: Xiufang Zhang, Nanming Zhao, Yandao Gong

Physical and chemical modification and evaluation of chitosan nerve conduit material

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Background

1. In China, the number of patients with nerve trauma caused by various injuries or diseases is 1-3 million per year.

2. Without proper therapy in time, these patients will loss corresponding physiological functions permanently.

3. Few of the patients can be treated properly: difficulties in technology and economy

Different choices of nerve recovery

1）Surgical suture end-to-end

Disadvantage: suitable only for small nerve gap

2） Graft as a guide for axon regeneration

Large gaps can be repaired with a graft inserted between the proximal and distal nerve stumps as a guide for the regenerating axon

- **Autograft**

nerve removed from another part of the body, blood vessels or muscle fibres

Disadvantages:

∗need for second surgical treatment;

∗limited availability;

∗denervation of the donor site

- **Allograft,**

nerve removed from other persons

Disadvantage:

*immune rejection;
*low success rate;
*limited availability

- **Heterograft**

nerve from animals such as pig

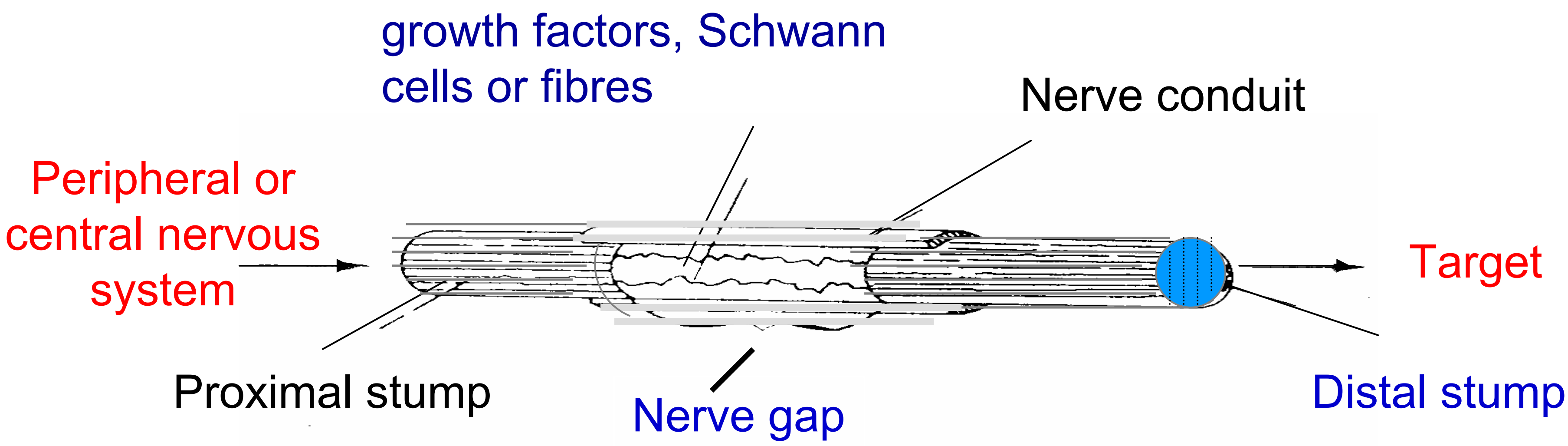
Disadvantage:

* **immune rejection;**
* **low success rate;**
* **some other risk**

- **Artificial nerve conduit**

Nerve conduit is an artificial graft that bridges the gap between the nerve stumps and directs and supports regenerating axon.

* **Hollow conduit;**
* **Conduit filled with growth factors, Schwann cells or fibres.**



The function of nerve conduit

Nerve conduit bridges the gap between the nerve stumps and forms an environment suitable for nerve regeneration

Advantages

- Concentrating neurotrophic factors;
- Reducing cellular invasion and scarring of the nerve;
- Providing guidance to prevent neuroma formation and excessive branching.

Nerve conduit biomaterials

1) Nonresorbable materials such as silicone rubber

Disadvantage： need for second surgical treatment

2) Biodegradable or resorbable biomaterials

- **Synthetic:**

*poly-lactic acid (PLA);

*polyglycolic acid (PGA);

...

Disadvantages：

*expensive;

*acidic degradation product

- **Natural:**

*chitosan;

*collagen,

...

Chitosan, the fully or partially deacetylated form of chitin, is a positively charged polymeric saccharide with (1,4)-linked D-glucosamine repeat units

Advantages:

- **biocompatible,**
- **biodegradable,**
- **plentiful in nature,**
- **cheap**

Disadvantages

- Low mechanical strength and toughness;
- Low solubility;
- Difficulty in manufacturing and shaping;

Work in our lab

- •Improvement of mechanical properties of chitosan;
- •Improvement of chitosan biocompatibility;
- •Control of chitosan biodegradability;
- •Conduit making — chitosan shaping;
- •Preliminary functional evaluation.

Chitosan modification

- **Blending or chemical linking with gelatin, collagen and polylysine;**
- **Surface coating with laminin, fibronectin, serum and polylysine;**
- **γ-ray irradiation;**
- **Alkylation;**
- **Crosslinking with different reagent;**
- **Modulation of deacetylation degree.**

Evaluation of chitosan-derived materials

- Biocompatibility evaluation
- Biodegradability evaluation
- Physical property evaluation
- Functional evaluation

Biocompatibility evaluation

- Contact angle
- Protein adsorption
- Cell affinity:
  - ➢Attachement of cultured cells;
  - ➢Proliferation: MTT measurement
  - ➢Differentiation: neurite length and other morphological features

- **Water contact angle**

- **The hydrophilicity of a biomaterial is a determinant of the material's biocompatibility.**
- **Hydrophilicity of a biomaterial is dependent on its surface contact angle.**

•Protein adsorption

- ➢The adsorption of proteins, such as some ECM molecules, onto material surface, is an important determinant of biocompatibility of biomaterials
- ➢The laminin and fibronectin adsorption on film surface was investigated using ELISA and desorption method.

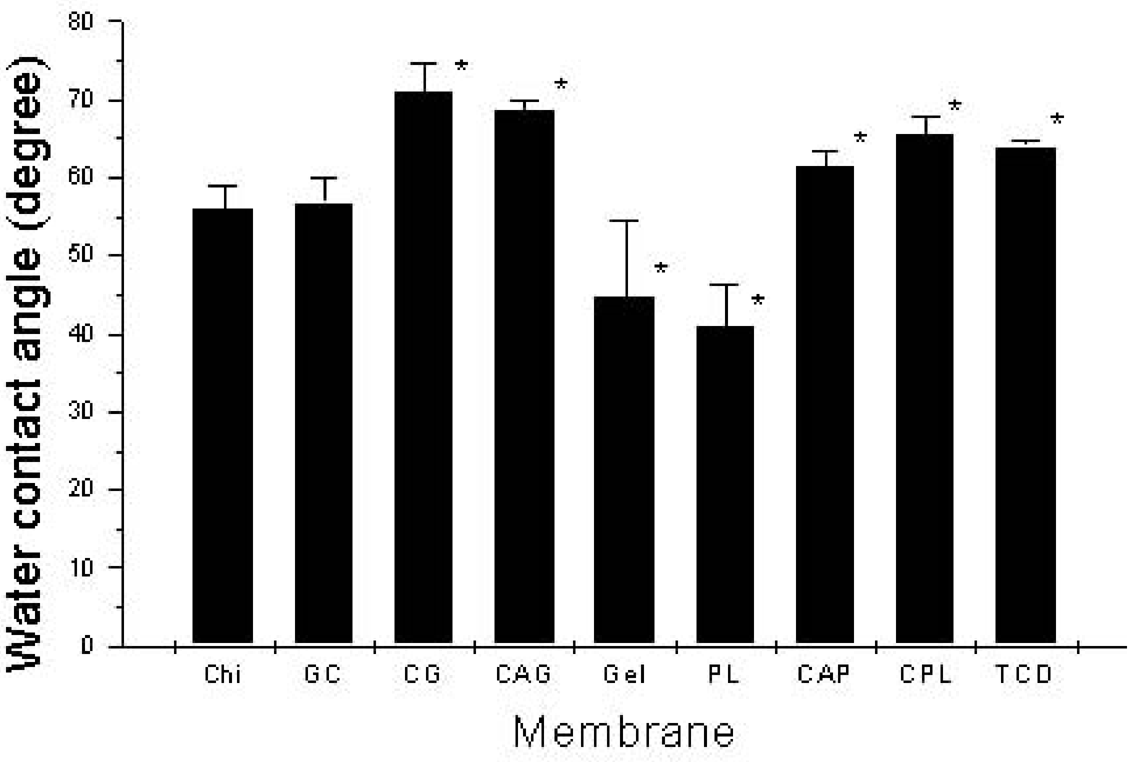
- **Biodegradability evaluation**

- **Evaluation of other physical property**
  - **Solubility**
  - **Crystallinity**
  - **Mechanical properties**

Chitosan modification (1)

- Blending or chemical linking with gelatin, collagen and polylysine;

- Surface coating with laminin, fibronectin, serum and polylysine



Chi: chitosan;

GC: glutaraldehyde-crosslinked chitosan;

CG: glutaraldehyde-crosslinked chitosan-gelatin conjugate;

CAG: chitosan-gelatin mixture;

Gel: gelatin;

PL: polylysine;

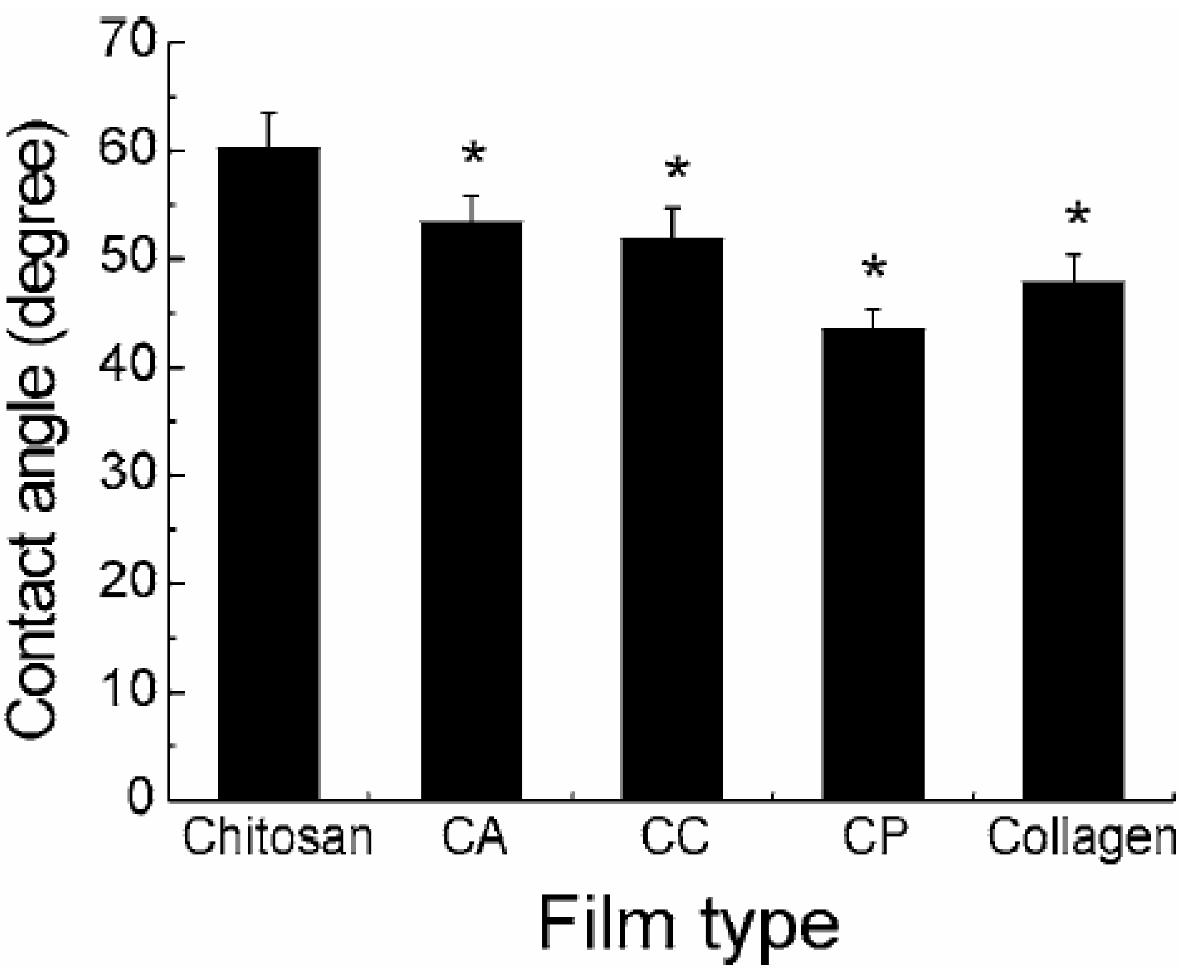
CAP: chitosan coated with polylysine;

CPL: chitosan-polylysine mixture.

Water contact angle of 8 chitosan derived materials and tissue culture dish (TCD)

The contact angle value is the average of 6 measurements.

*: $p<0.05$ relative to Chitosan.



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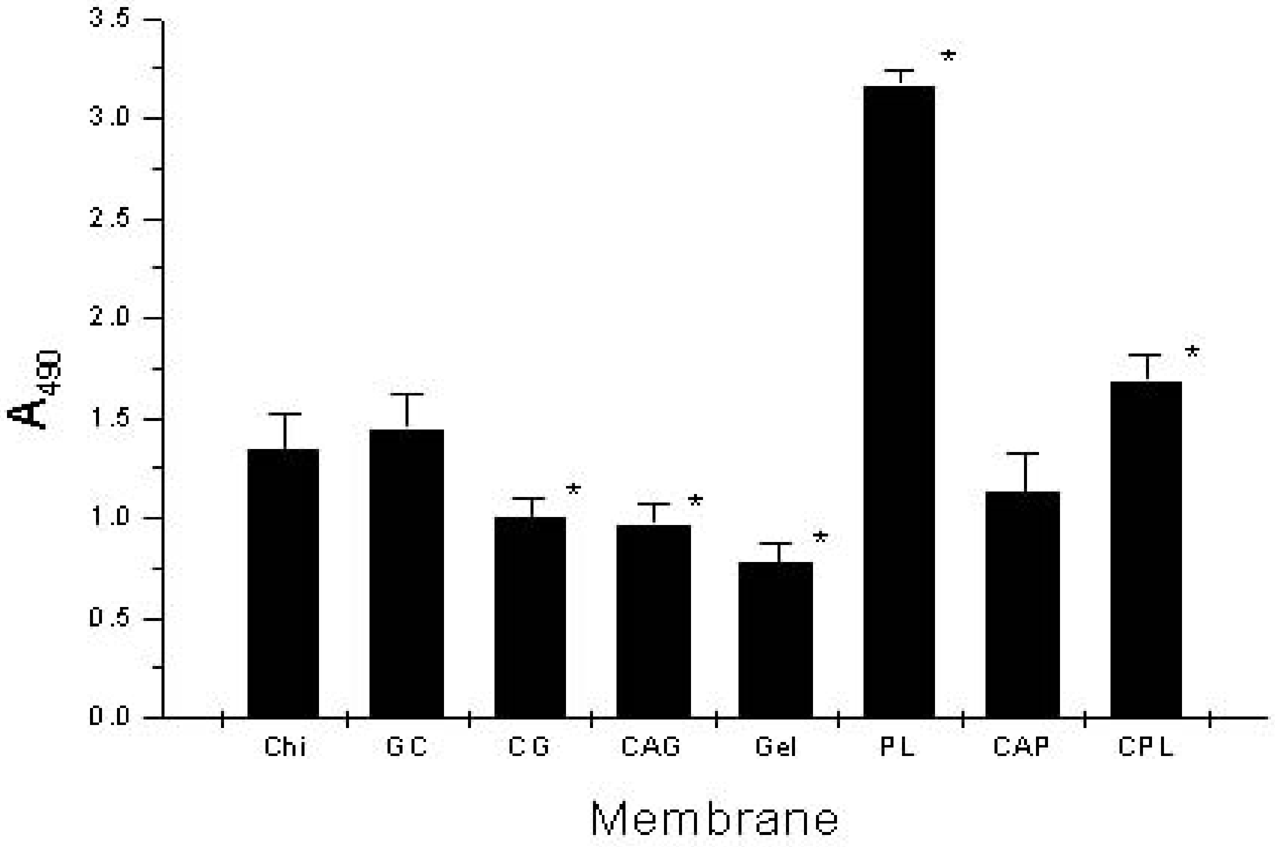
CC: collagen-blended chitosan film;

CP: poly-L-lysine-blended chitosan film

Water contact angle of five types of material measured at pH 7.4

* : Statistically significant lower contact angle (P < 0:05) compared to chitosan.

Adsorption of laminin in laminin solution on 8 chitosan derived materials



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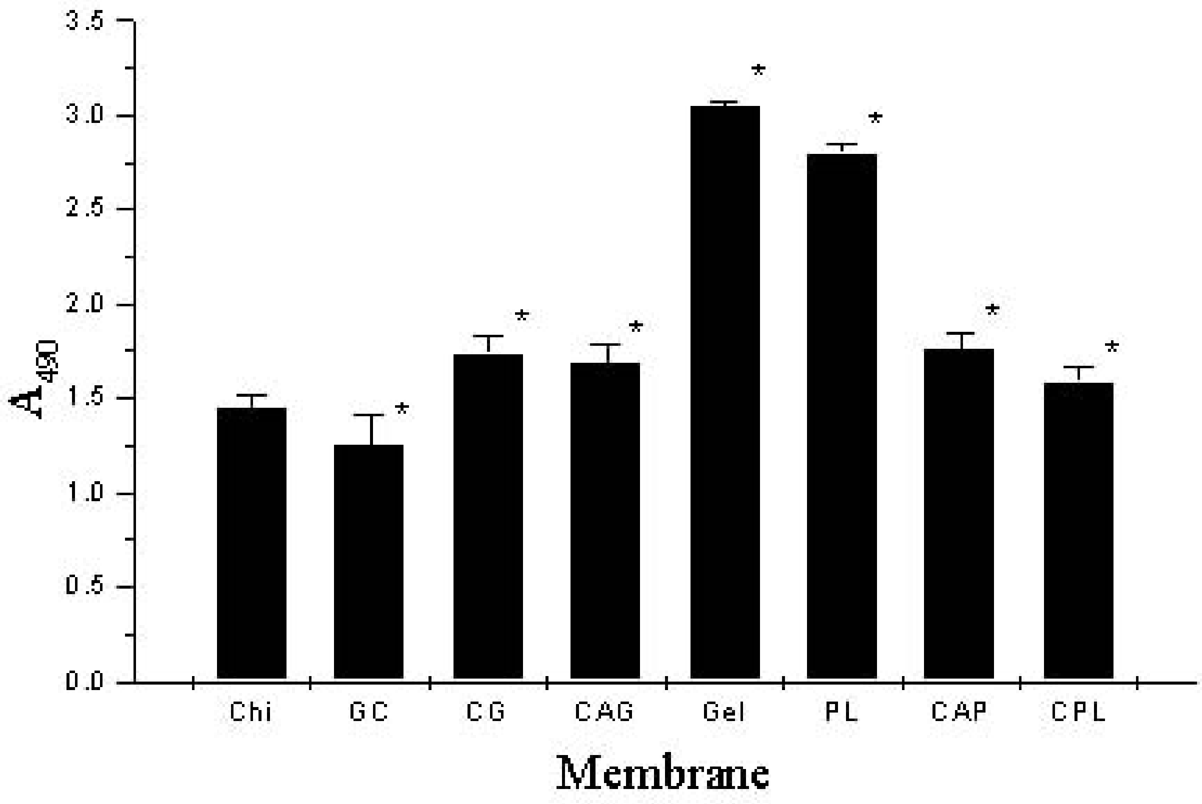
Adsorption of fibronectin in fibronectin solution on 8 chitosan derived materials



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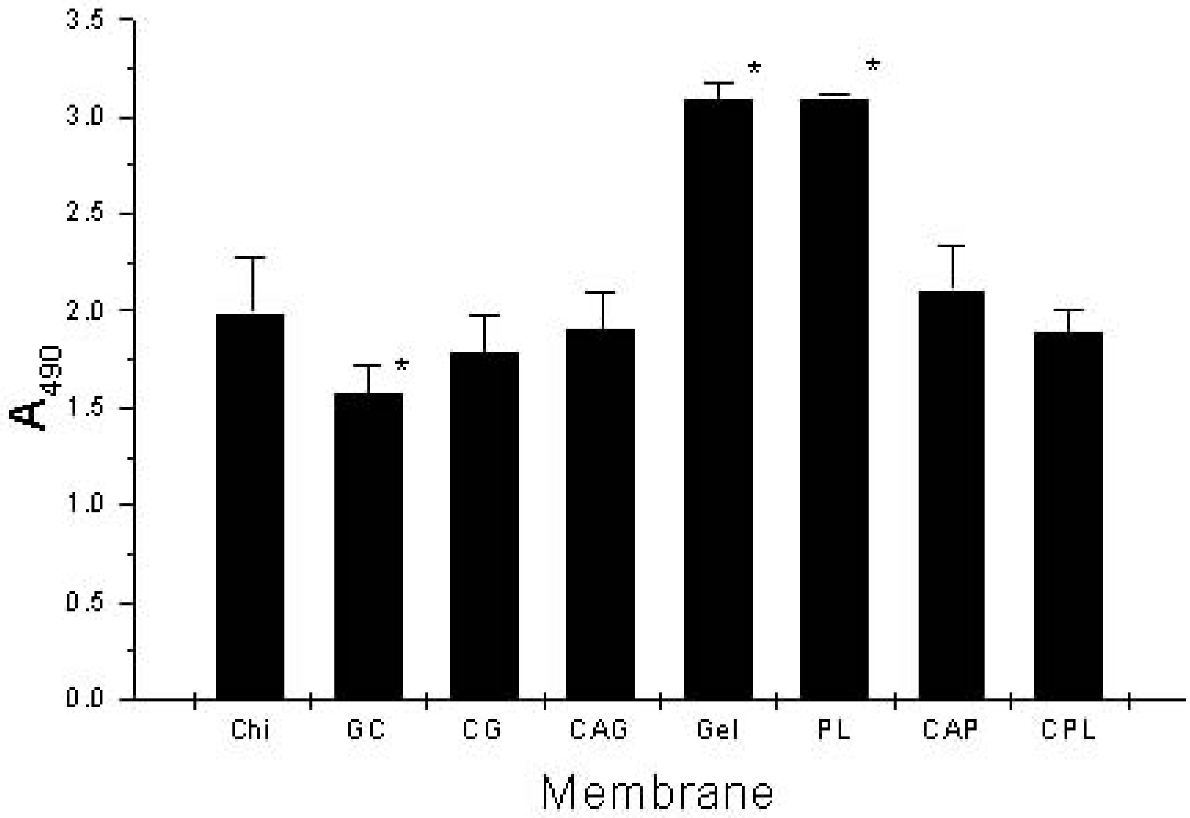
Adsorption of fibronectin in 5% serum on 8 chitosan derived materials



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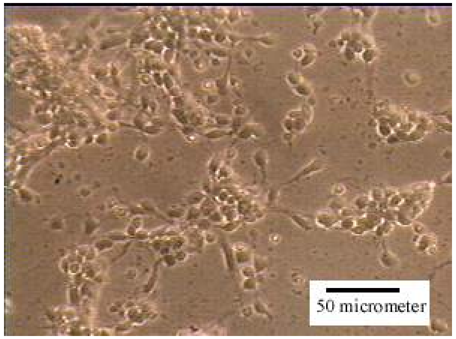
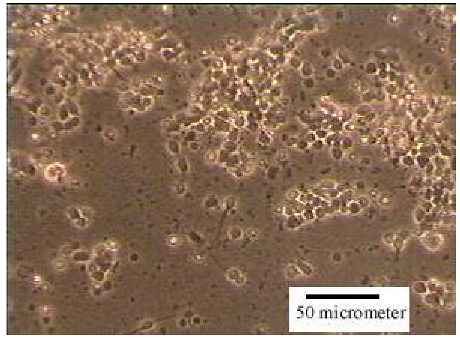
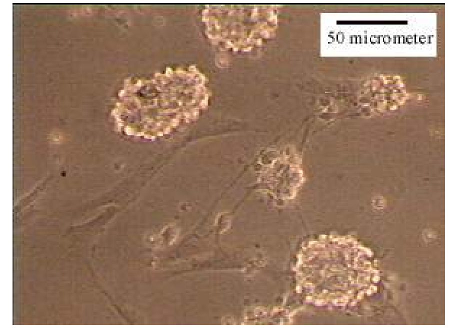
FMCC (fetal mouse cerebral cortex) cells cultured for 1 day



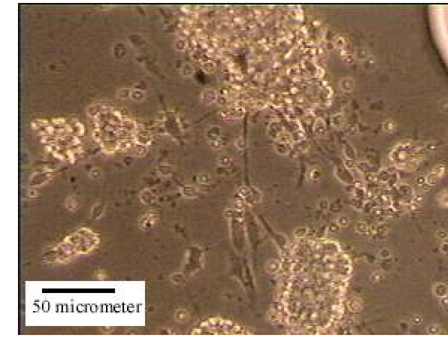
Chi



GC



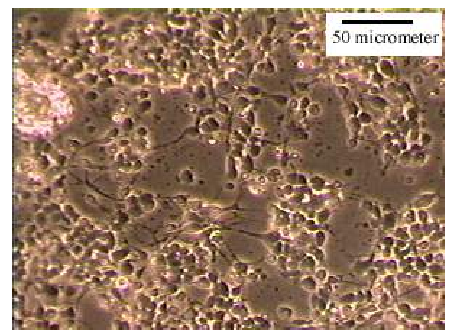
CG



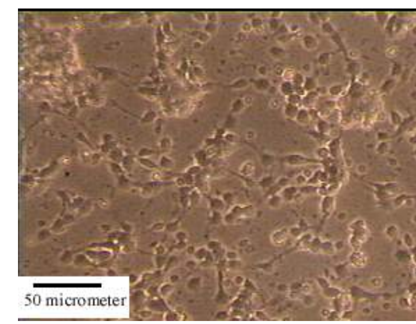
CAG

Gel

PL



CAP



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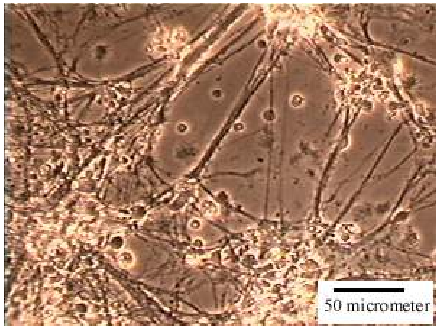
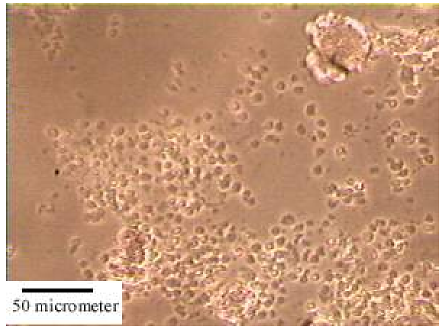
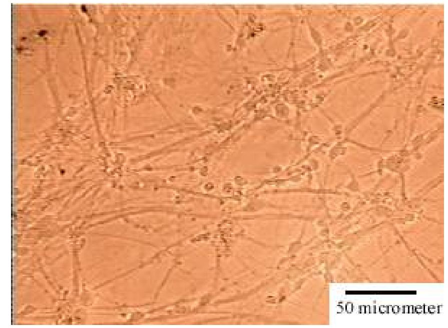


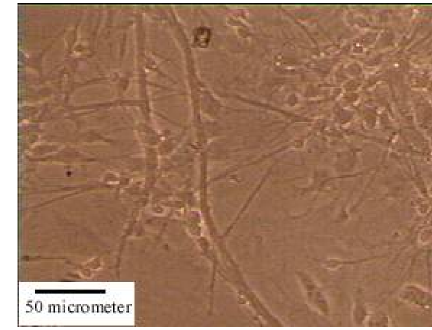
Chi



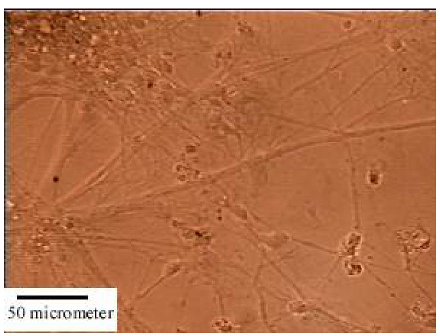
GC



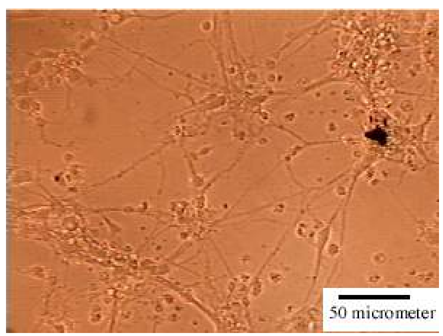
CG



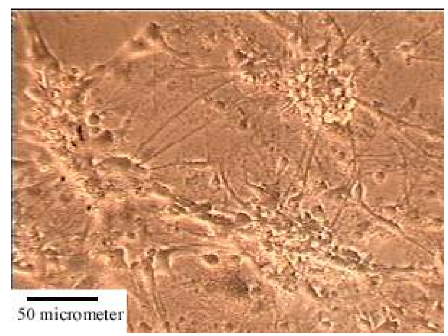
CAG



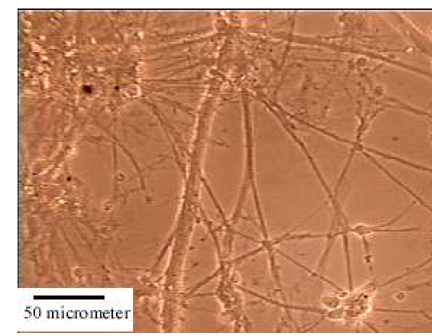
Gel



PL



CAP



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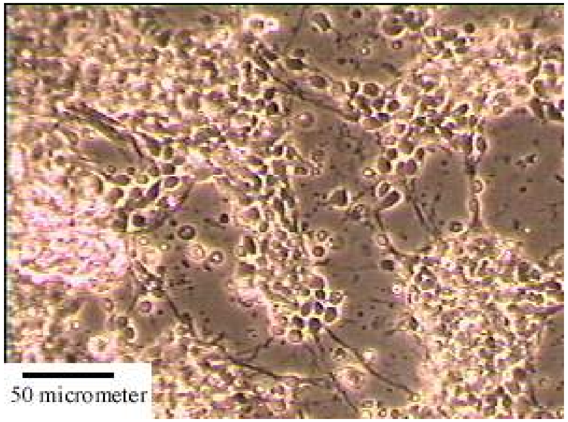
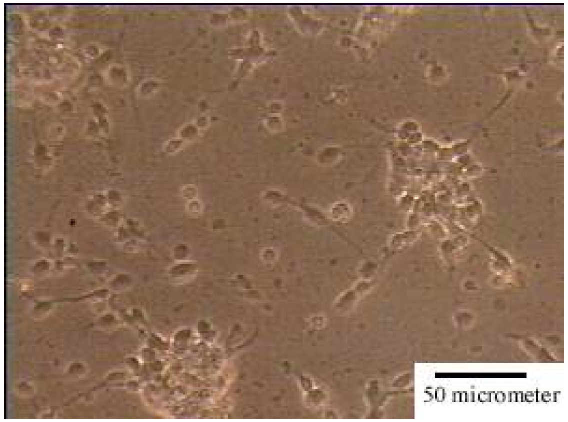
FMCC cell culture (1 day) on chitosan-derived materials precoated with proteins



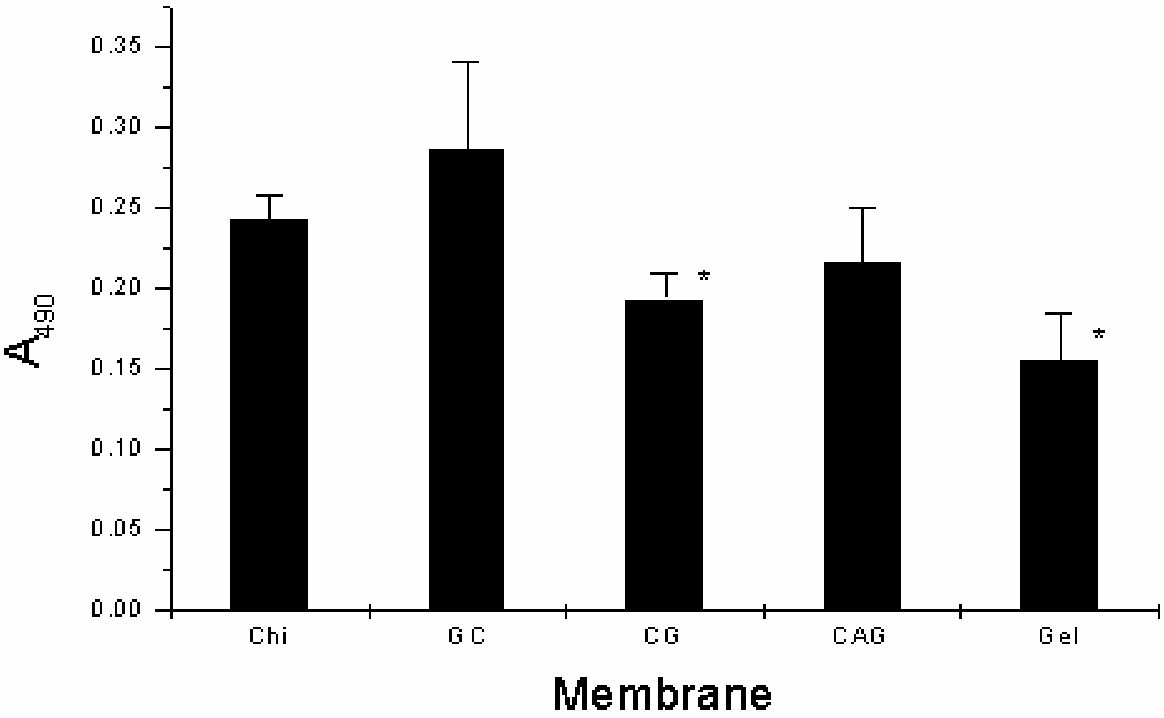
chitosan film coated with **laminin**



chitosan film coated with **fibronectin**

chitosan film coated with **serum**

Growth of gliosarcoma (9L) cells on chitosan-derived materials



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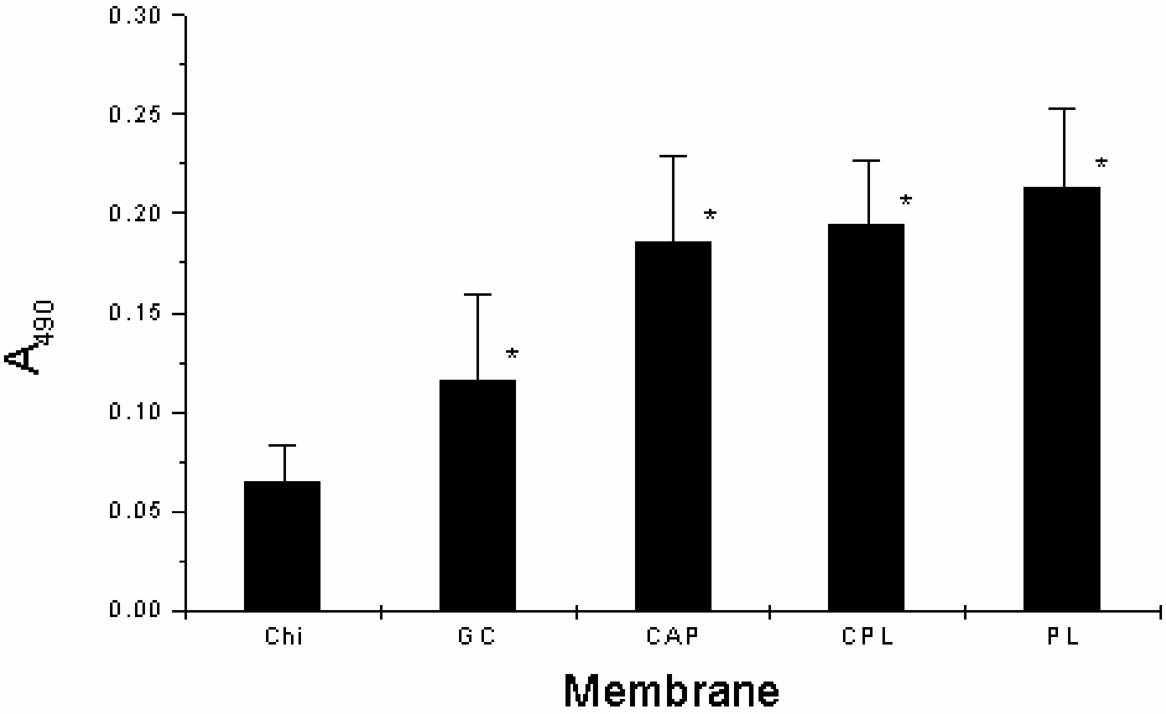

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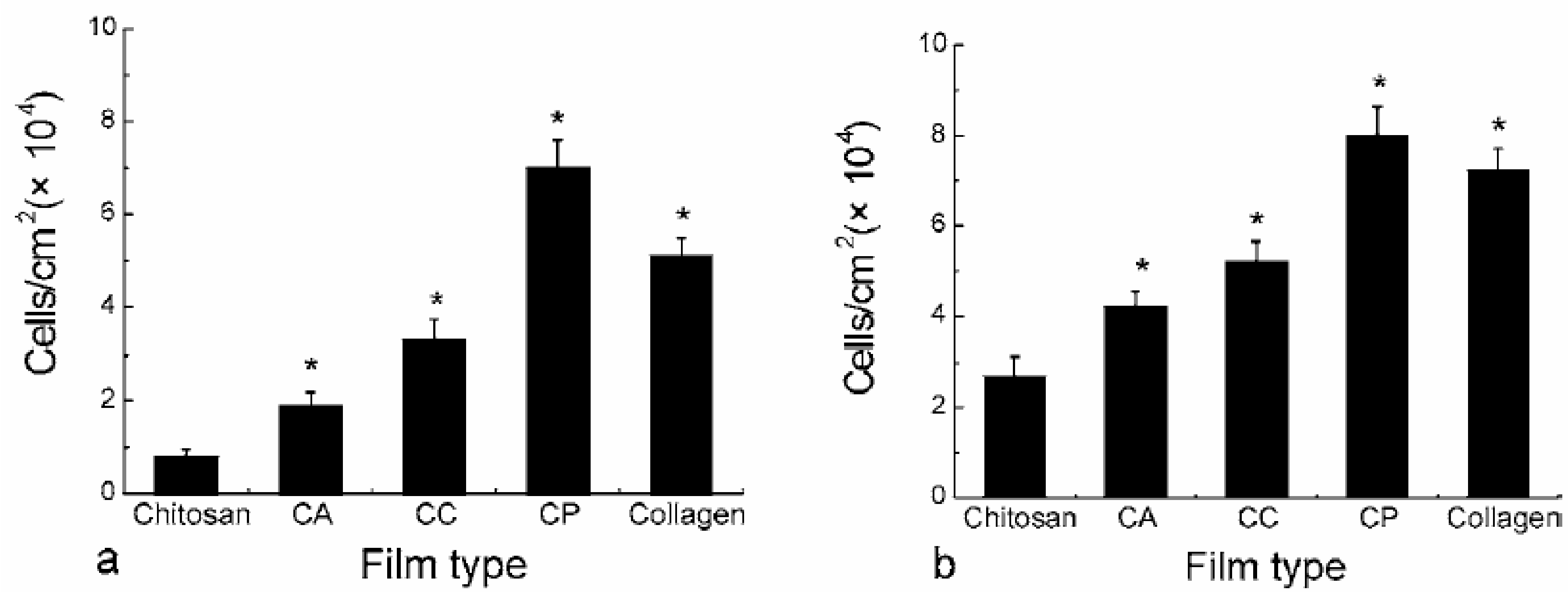
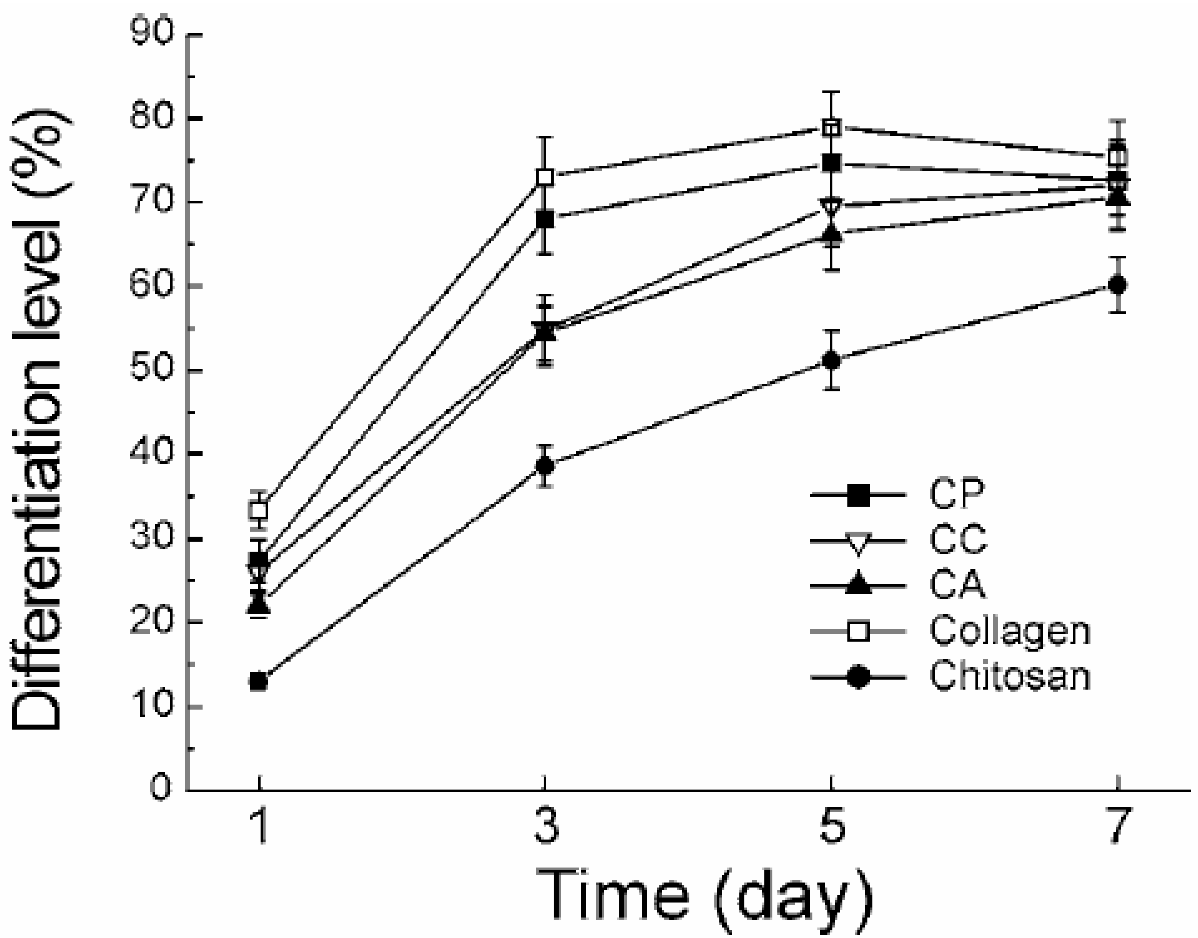
Attachment of PC12 cells to the five types of material



(a) Serum-free medium, (b) in medium containing 5% serum
CA: albumin-blended chitosan film;
CC: collagen-blended chitosan film;
CP: poly-L-lysine-blended chitosan film.
Initial seeding density was $1x10^5$ cells/cm^2
* Statistically significant greater number of attached PC12 cells (P < 0:05) compared to chitosan.



CA: albumin-blended chitosan film;

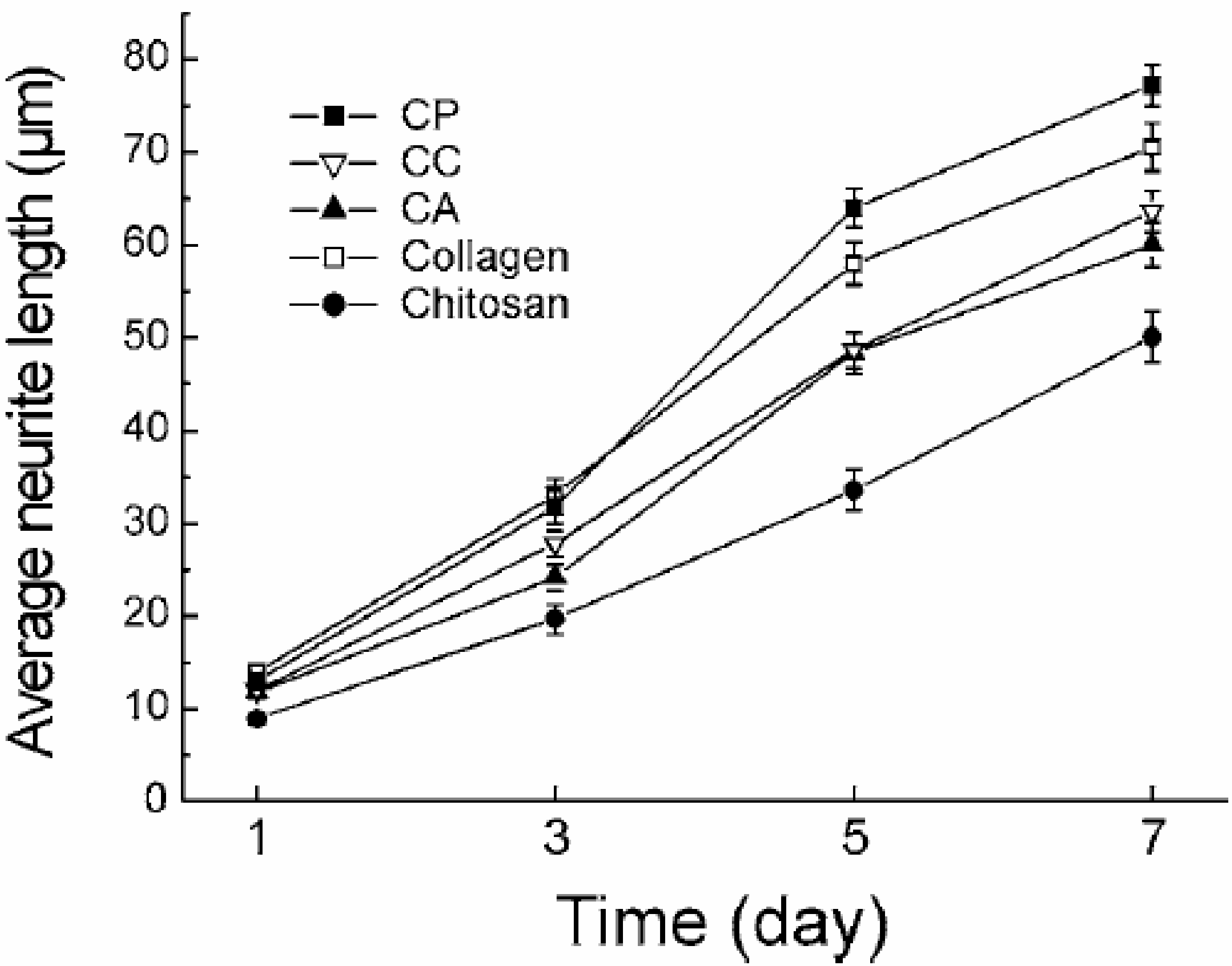
CC: collagen-blended chitosan film;

CP: poly-L-lysine-blended chitosan film;

Differentiation level = (n/N)x100%

N: total cell number on the film;
n: number of cells in which the neurite was longer than 10 mm.

Differentiation level of PC12 cells cultured on the five types of material



CA:

albumin-blended chitosan film;

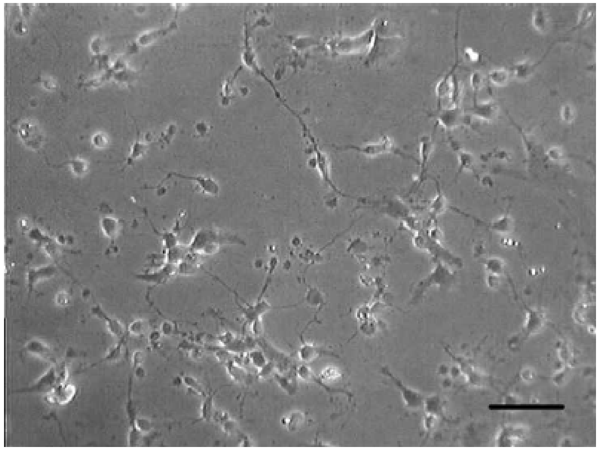
CC:

collagen-blended chitosan film;

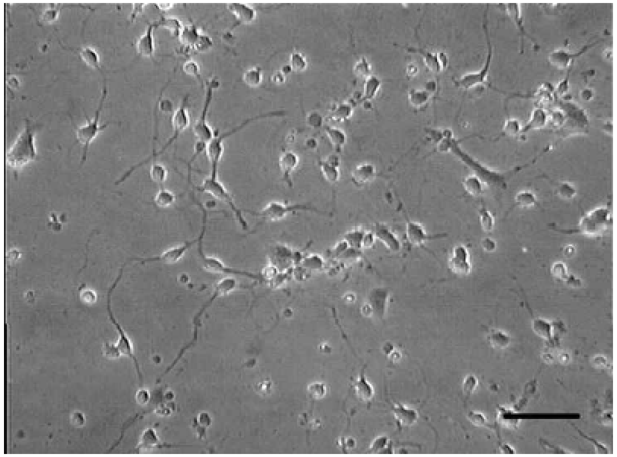
CP:

poly-L-lysine-blended chitosan film.

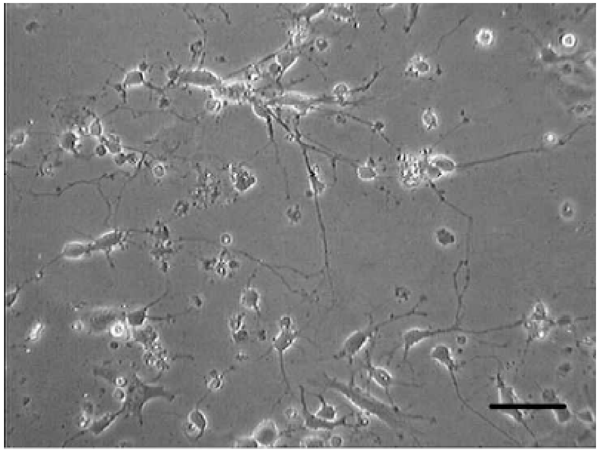
Average neurite length of PC12 cells cultured on the five types of material



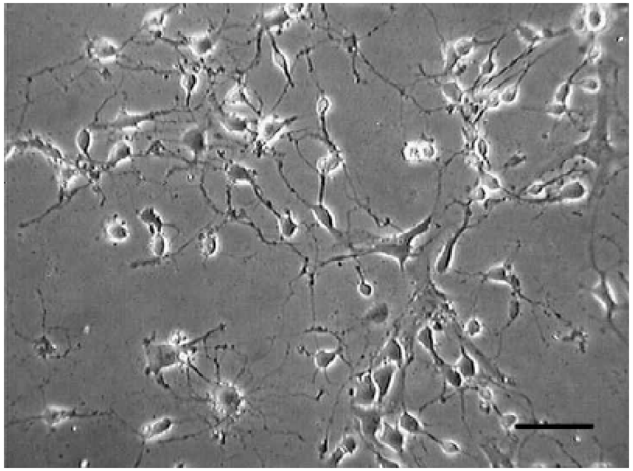
chitosan



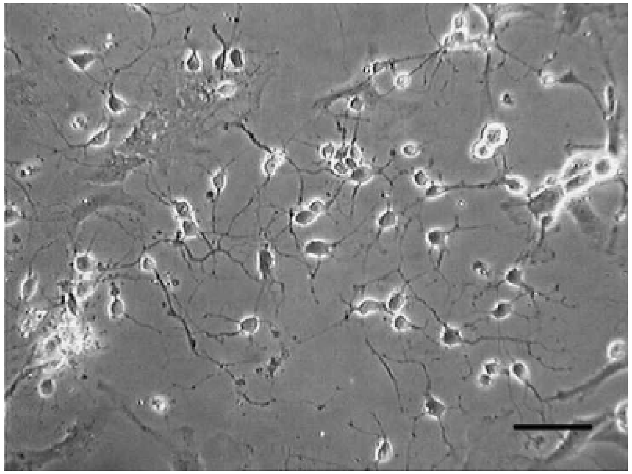
albumin-blended chitosan



collagen-blended chitosan



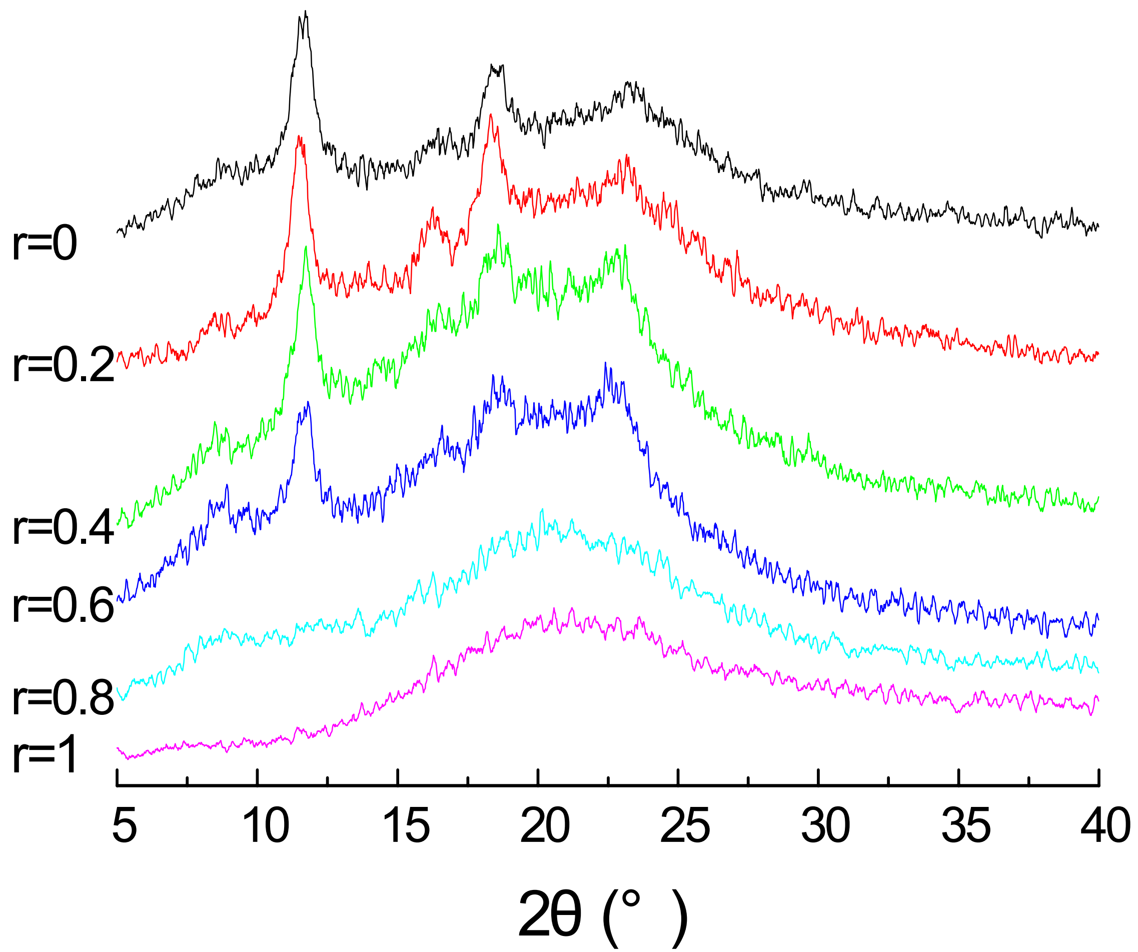
poly-L-lysine-blended chitosan



collagen

Fetal mouse cerebral cortex (FMCC) cells cultured (for 3 days) on five types of materials (Bar: 50 μm)

Effect of gelatin content on the biological and physicochemical properties of chitosan-gelatin composite

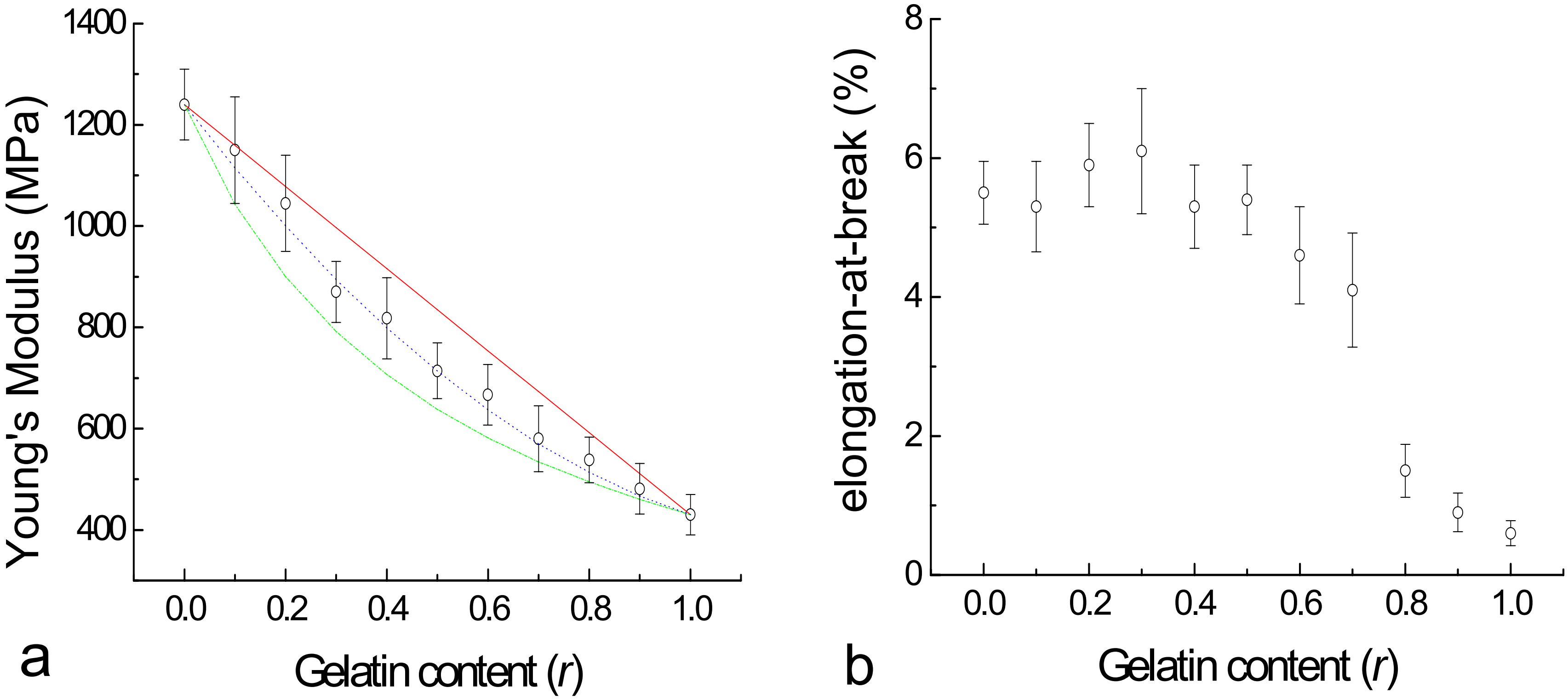


X-ray diffraction patterns of chitosan-gelatin composite films with different gelatin content r.

The crystallinity of the composite film decreased with increasing gelatin content r

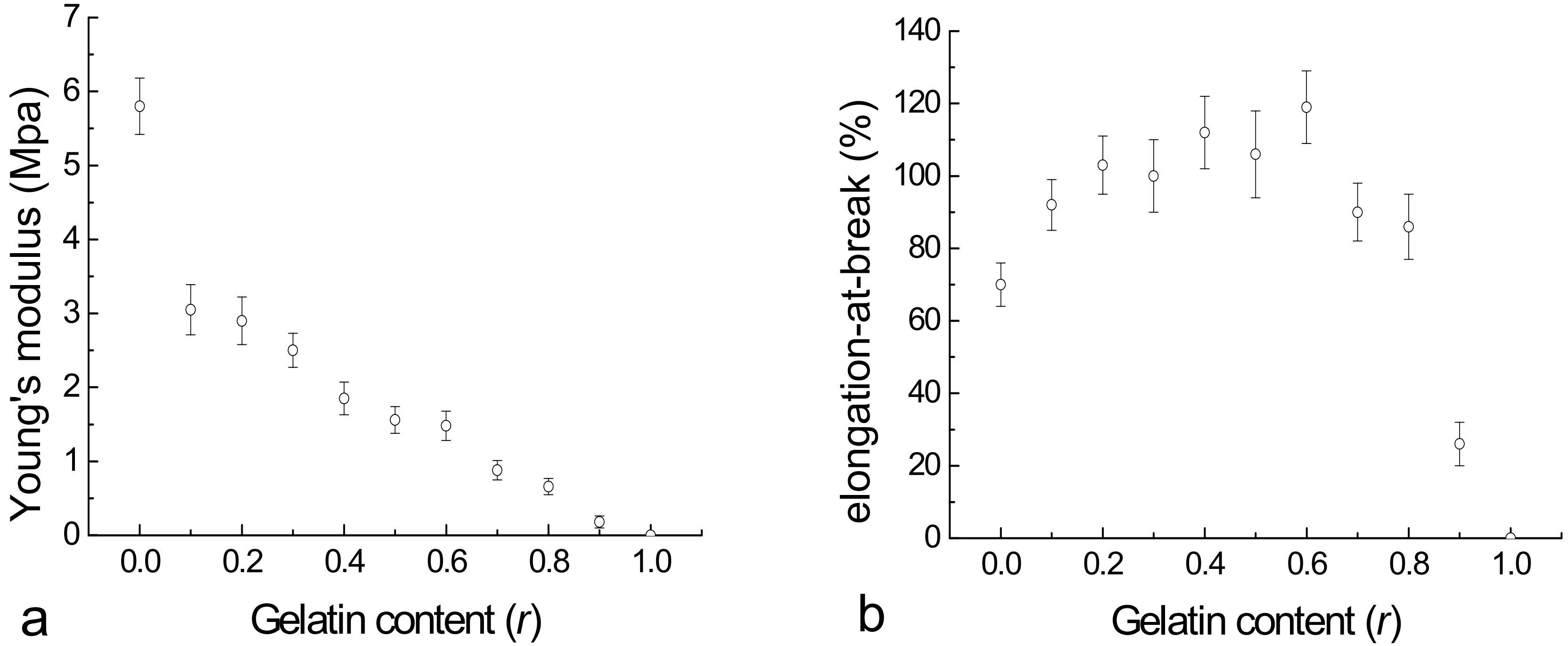
Crystallinity and rupture strain maximum of chitosan-gelatin composite films

gelatin content (r)	crystallinity (Xc, %)	rupture intensity (MPa)	
		dry	wet
0. 0	19. 8	68. 6±2. 8	5. 50±0. 67
0. 2	14. 4	68. 2±3. 9	4. 20±0. 48
0. 4	6. 5	62. 4±3. 4	2. 80±0. 42
0. 6	4. 2	51. 6±2. 2	2. 30±0. 28
0. 8	0. 0	43. 3±2. 5	1. 40±0. 18
1. 0	0. 0	36. 7±1. 3	0. 0



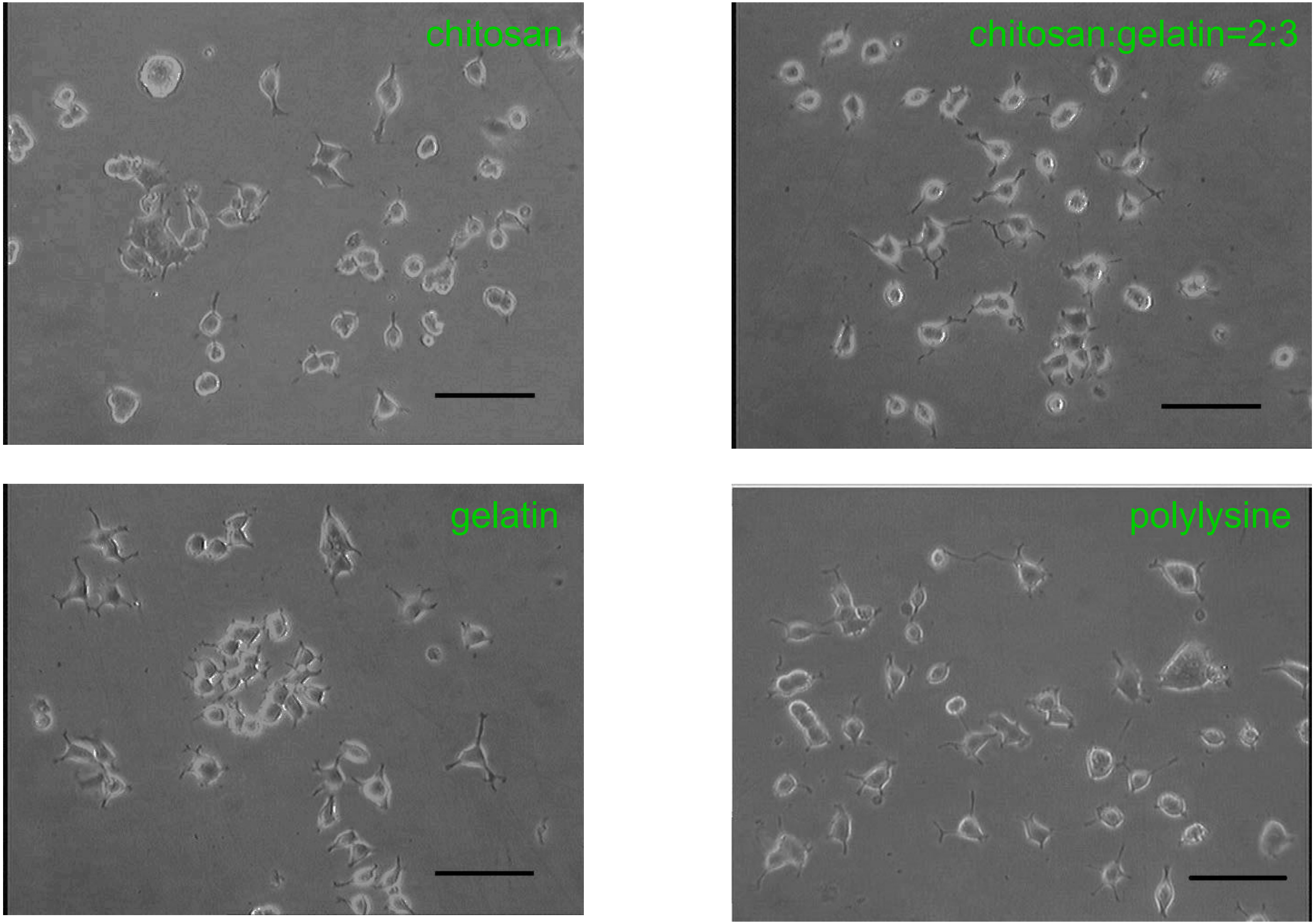
Young's modulus E (a) and percentage of elongation-at-break ε_B (b) of composite films in dry state as a function of gelatin content r

Both the Young's modules and percentage of elongation-at-break of the composite films decreased with increasing gelatin content r



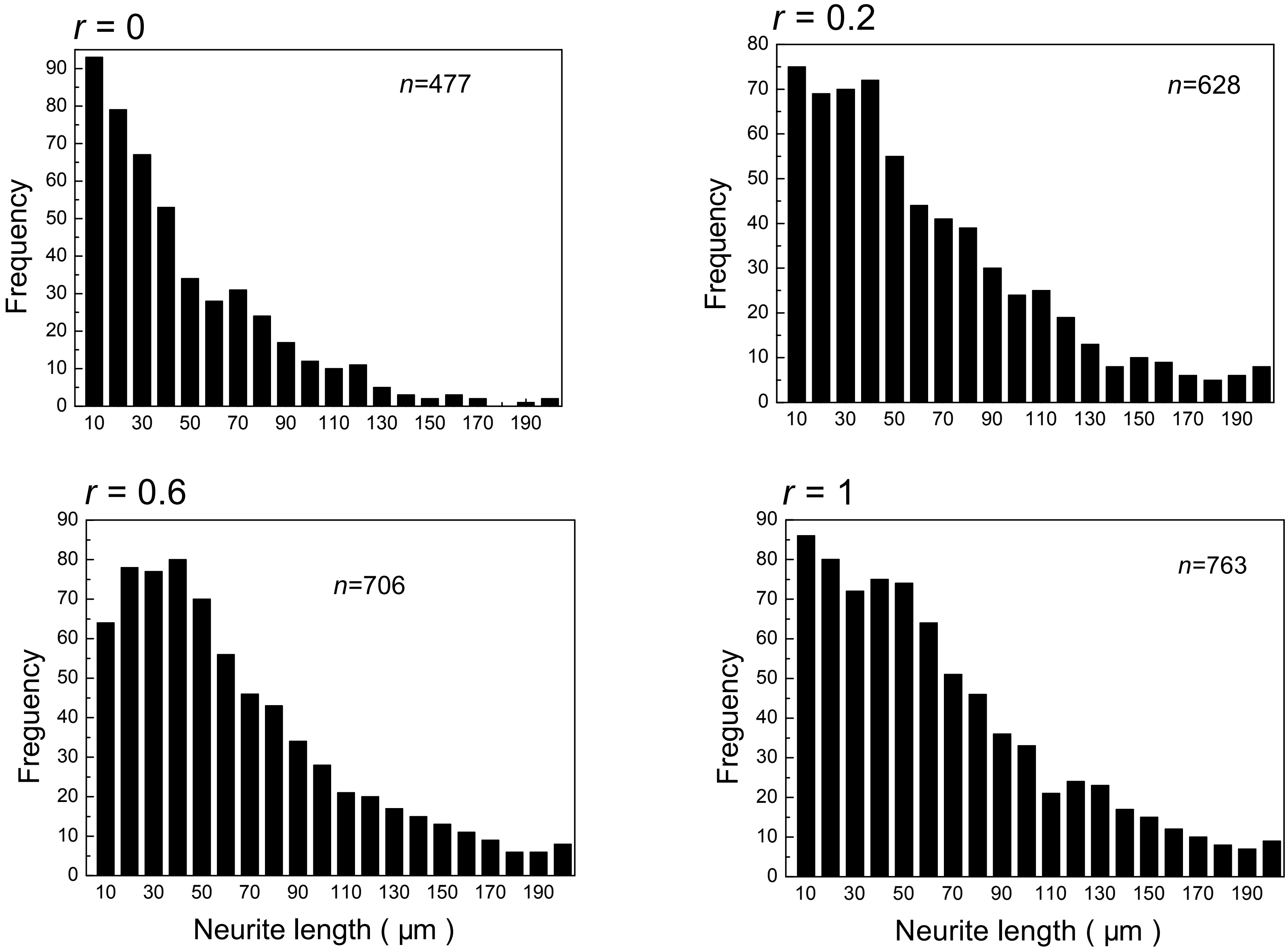
Young's modulus E (a) and percentage of elongation-at-break ε_B (b) of composite films in wet state as a function of gelatin content r.

With increasing gelatin content r, the Young's modules of the composte films in wet state decreased and the percentage of elongation-at-break increased at first, then decreased when r > 0.6.



1-day cultured PC12 cells on different films. Bar=100 μm.

Blending chitosan with gelatin improved the attachment and growth of the cells.



Histograms for neurite length of 6-day cultured PC12 cells on 4 kinds of materials.

r : gelatin content

The median neurite length increased with increasing gelatin content r.

Conclusion

- Proper physical blending or chemical linking with gelatin, collagen and polylysine can improve the biocompatibility of chitosan and keep its physical properties reasonable.
- Even a simple coating with laminin, fibronectin, serum and polylysine is also of help for chitosan biocompatibility.