

## Preparation conditions affect the pore size, water vapor permeability and other physical characteristics of resulting chitosan- $\gamma$ -polyglutamic acid complex scaffolds

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### Abstract

The objectives of the study are to explore the preparation conditions affect the pore size, water vapor permeability, and other physical characteristics of resulting chitosan- $\gamma$ -polyglutamic acid ( $\gamma$ -PGA) complex scaffolds. The results obtained showed that pore size of chitosan-  $\gamma$ -PGA complex scaffolds differed with different DDs of chitosan used, the lower the DDs of chitosan used, the smaller the pore size of complex scaffolds resulted. The pore size of chitosan-  $\gamma$ -PGA complex scaffold differed also with the concentration of  $\gamma$ -PGA used, the higher the concentration of  $\gamma$ -PGA, the smaller the pore size of complex scaffolds. The pore size also differed between different molecular weight (MW) of  $\gamma$ -PGA used, the higher the MW of  $\gamma$ -PGA, the larger the pore size of complex scaffolds resulted.

The water vapor permeability of chitosan- $\gamma$ -PGA complex scaffolds were functions of the pore size of the resulted complex scaffolds. The larger the pore size, the higher the water vapor permeability of complex scaffolds. The water vapor permeability of the complex scaffolds depended also on the hydrophilicity of complex scaffolds. The higher the hydrophilicity of complex scaffolds, the lower the water vapor permeability. Physical properties such as weight loss, swelling ratio, tensile strength, tensile strain etc...are depended also on the DDs of chitosan, Mw of  $\gamma$ -PGA and the weight ratio of chitosan to  $\gamma$ -PGA used in preparation of complex scaffolds

**Keywords:** Chitosan,  $\gamma$ -polyglutamic acid, complex scaffold, pore size, water vapor permeability, physical characteristic.

### Results and Discussions

#### 1. SEM observation of the scaffolds microstructure and their pore size

##### 1.1 Pore structure of chitosan- $\gamma$ -PGA complex scaffolds

Fig. 1 shows the SEM pictures of the chitosan- $\gamma$ -PGA complex scaffolds at 60 X magnifications. The pore sizes of the scaffolds were calculated from the pictures with an image analysis system. Three dimensional and interconnected porous structure with pronounce wall were observed for the chitosan only scaffolds especial for those prepared from lower DD chitosan. Structure of complex chitosan- $\gamma$ -PGA scaffolds prepared from high Mw  $\gamma$ -PGA were smooth and less shrinkage, it is consistent with the results of Hsieh et al. [1]. They reported that with an increasing in the amount of  $\gamma$ -PGA in the complex scaffolds, the surface structure of the matrices became smoother and less shrinkage. SEM pictures showed that the higher the molecular weight of  $\gamma$ -PGA used, the closer the shape of spherical pores.

## 1.2 Pore size of chitosan- $\gamma$ -PGA complex scaffolds

Results in Table 1 indicated the pore size of scaffolds prepared from 60% DD chitosan only was about  $185 \pm 26.45$  nm and those prepared from 80% DD chitosan only was about  $165 \pm 17.32$  nm.

The pore size of chitosan- $\gamma$ -PGA complex scaffolds prepared from 5 or 8 parts of chitosans of the same DD of 60% or 80% to one part of low or high molecular weight  $\gamma$ -PGA were showed in Table 1. Pore size of those complex scaffolds are between  $52 \pm 10.75$  nm and  $176 \pm 18.17$  nm with narrow pore size distribution. The results indicated that the pore size of chitosan- $\gamma$ -PGA complex scaffolds decreased with increasing concentration of  $\gamma$ -PGA; the pore size increased with increasing the molecular weight of  $\gamma$ -PGA. The pore size of chitosan-  $\gamma$ -PGA complex scaffolds increased with increasing DD of chitosan used. This may be related to the number of ionic interactions between chitosan and  $\gamma$ -PGA. The higher the DD of chitosan, the higher the number of the protonated amino group available to interact with carboxylic group of  $\gamma$ -PGA thus resulted in larger pore size of complex scaffolds.

## 2. The water vapor permeability

Results in Table 1 indicated the water vapor transmission rate of chitosan only scaffolds did not differ with DD of chitosan used. However, water vapor transmission rate of the complex scaffolds blended 60% DD chitosan with high Mw  $\gamma$ -PGA were higher than that blended with low Mw  $\gamma$ -PGA. However, complex scaffolds blended 80% chitosan with different Mw  $\gamma$ -PGA did not different significantly. This may be due to water vapor transmission rate of the scaffolds are depend on the pore size of the scaffolds mainly. The water vapor permeability of scaffolds increased with increasing pore size of scaffolds. For those scaffolds prepared from the same concentration of  $\gamma$ -PGA but the different DD of chitosan, difference in water vapor permeability may be due to the following reasons. The water vapor permeability depends not solely by the pore size of the scaffolds, but also on the hydrophilicity of the prepared scaffolds. The scaffolds prepared from DD80 chitosan containing more amino groups on the backbone of the molecules thus increasing the hydrophilicity of the prepared scaffolds. This may prevent the water vapor escape out of the scaffolds therefore, lower the water permeability. Water vapor permeability is controlled by the ratio of vapor pressure of water molecules in the scaffolds to the vapor pressure of the air. If air vapor pressure is saturated then the water vapor in the scaffolds will be condensed. The mechanism of water vapor permeability beginning from the water molecule changes from liquid phase to vapor phase, the vapor phase molecules might be absorbed by the scaffolds then diffused from the scaffolds to the open air. Those results are similar to Mi et al. [2]. The larger the pore size, the higher the water vapor transmission rate of the scaffolds.

## 3. Weight loss percentage during immersion in phosphate buffer

Results in Fig. 2 show that the weight loss percentage of the chitosan-  $\gamma$ -PGA complex scaffolds increased over time. The weight loss percentage were lower for those chitosan- $\gamma$ -PGA complex scaffolds prepared from chitosan with higher DD chitosan and also for those chitosan-  $\gamma$ -PGA complex scaffolds prepared from higher MW  $\gamma$ -PGA. It implied that the uncross-linked chitosan or  $\gamma$ -PGA molecules should be dissolved in phosphate buffer during test thus resulted in weight loss percentage. The higher weight loss percentages were observed for those chitosan-  $\gamma$ -PGA complex scaffolds that contain higher concentration of  $\gamma$ -PGA. Therefore, the molecules leached out might be mainly  $\gamma$ -PGA.

## 4. Degradation during shaking in phosphate buffer

Results in Fig. 3 showed that the degradation rate of the chitosan-  $\gamma$ -PGA complex scaffolds increased over time during shaking in phosphate buffer. The degradation rate were higher for those chitosan-  $\gamma$ -PGA complex scaffolds prepared from chitosan with higher DDs and also for those chitosan-  $\gamma$ -PGA complex scaffolds prepared from lower MW  $\gamma$ -PGA. It implied that the uncross-linked chitosan or  $\gamma$ -PGA molecules should be leached out during shaking in phosphate buffer.

#### 5. Degradation by lysozyme

Results in Fig.4 showed that the degradation rate of the chitosan-  $\gamma$ -PGA complex scaffolds by lysozyme in phosphate buffer increased over time. The degradation rates were lower for those chitosan-  $\gamma$ -PGA complex scaffolds prepared from chitosan with higher DDs. This may be due to the ionic interactions between chitosan and  $\gamma$ -PGA is the prominent forces that facilitate the scaffold formation. Therefore, the higher the DD of chitosan, the better the structure the scaffold will be formed. Another reason might be due to that the higher DD chitosan, the less the N-acetyl group should have that rendered the less degradation chance by lysozyme. The degradation rates were higher for those chitosan-  $\gamma$ -PGA complex scaffolds prepared from higher MW  $\gamma$ -PGA and/or lower proportion ratio of  $\gamma$ -PGA. It implied that the higher proportion ratio of chitosan in the chitosan-  $\gamma$ -PGA complex scaffolds facilitated the degradation and rendered the release of  $\gamma$ -PGA molecules.

#### 6. Swelling percentage

Results in Fig. 5 show time-depend-swelling ratios of chitosan only scaffold or different complex scaffolds in PBS at 37 °C between the first and 72 h. The swelling percentages of complex scaffolds of chitosan- $\gamma$ -PGA complex scaffolds were higher than that of chitosan only. Swelling percentages that measured between 12 to 24 h were higher than those measured at other periods. Swelling percentage was higher for those complex scaffolds containing higher ratio of  $\gamma$ -PGA. The results indicated that swell percentage of scaffolds was affected by the hydrophilic groups of its components. The reasons for higher swelling percentages of complex scaffolds prepared from higher ratio of  $\gamma$ -PGA may be due to higher hydrophilicity of  $\gamma$ -PGA. Therefore, the swelling percentage increased with increasing ratio of  $\gamma$ -PGA in complex scaffolds. Taghizadeh et al. [3] reported used of chitosan of higher DDs, lower crystallinity, and the lower Mw increased the swelling percentage.

#### 7. Tensile strength and tensile strain of scaffolds

##### 7.1 Effect of DD of chitosan in the dry state

Results in Fig. 6 show the tensile elongation (A) and tensile strength (B) of different types dry scaffolds. Tensile strains of chitosan only scaffolds that are prepared from higher DD chitosan were higher than that of lower DD chitosans. However, after blended with  $\gamma$ -PGA, tensile strain of complex scaffolds were higher for those containing lower DD chitosans. Higher tensile strain indicated higher flexibility. Tensile strength of chitosan- $\gamma$ -PGA complex scaffolds did not differ with the DD of chitosan used. Tensile strength of chitosan- $\gamma$ -PGA complex scaffolds depend on the Mw of  $\gamma$ -PGA used. Complex scaffolds prepared from higher Mw  $\gamma$ -PGA were higher than that prepared from low Mw ones.

##### 7.2 Effect of DD of chitosan in the swollen state

Results in Fig. 7 show the tensile elongation (A) and tensile strength (B) of different

types wet scaffolds. Tensile strains of chitosan only scaffolds that are prepared from lower DD chitosan were higher than that of higher DD ones. However, after blended with  $\gamma$ -PGA, tensile strain of complex scaffolds did not differ with the DD of chitosan used. Tensile strength of chitosan only scaffolds or those blended with  $\gamma$ -PGA were higher for those prepared from lower DD chitosan.

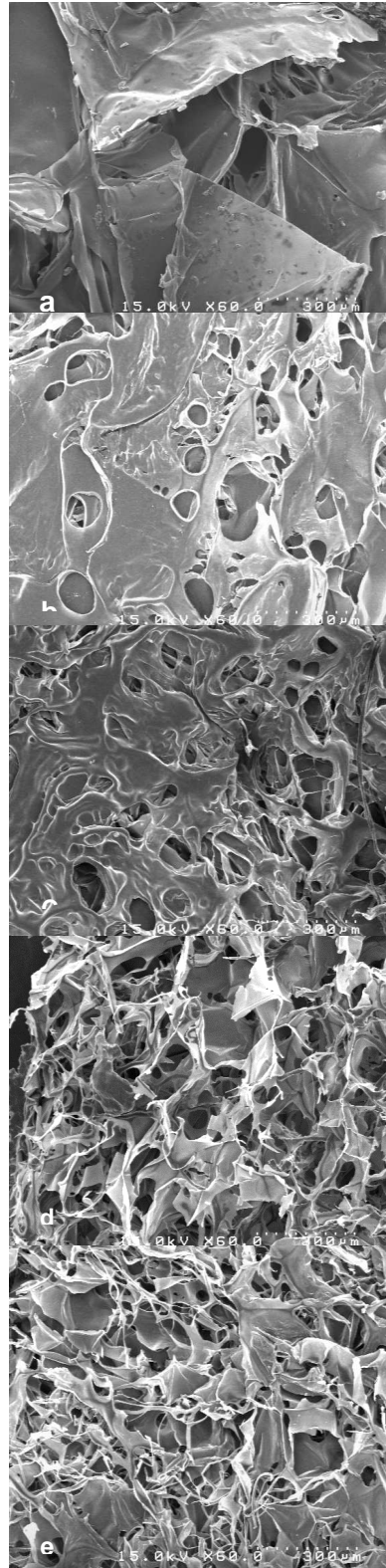
### Conclusion

1. Chitosan- $\gamma$ -PGA complex scaffolds showed better swelling ratio and tensile strain than that prepared from chitosan alone. The improvement were more pronounced for those using higher concentration and/or higher Mw - $\gamma$ -PGA.
2. Chitosan- $\gamma$ -PGA complex scaffolds was formed via ionic interactions between cationic chitosan and anionic  $\gamma$ -PGA, however, intermolecular entanglement also an important factor that influence the physical properties of the scaffolds prepared.
3. The porous scaffolds prepared via freezing drying were simple and fast, however, the homogeneity of large size chitosan-  $\gamma$ -PGA complex scaffold are not good. The inhomogeneity might result in difference in the characteristics observed

### Reference

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(A) DD60



(B) DD80

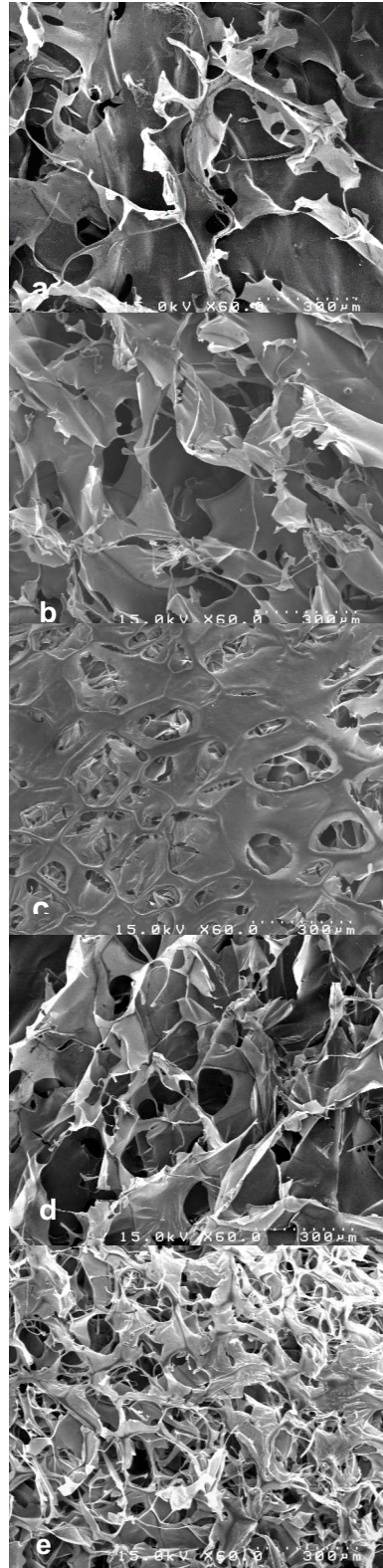


Fig. 1. Scanning electron micrograph of chitosan- $\gamma$ -pga scaffolds. (A) 60%DD chitosan. (B) 80%DD chitosan; a chitosan, b 8:1(volume ratio) chitosan: high molecular weight  $\gamma$ -pga, c 5:1(volume ratio) chitosan: high molecular weight  $\gamma$ -pga, d 8:1(volume ratio) chitosan: low molecular weight  $\gamma$ -pga, e 5:1(volume ratio) chitosan: low molecular weight  $\gamma$ -pga.

Table 1. Mean pore size ( $\mu\text{m}$ ) and water vapor transmission rate of scaffolds prepared from DD 60% or DD 80% chitosan alone or prepared from chitosans mentioned and  $\gamma$ -PGA of low or high Mw at different volume ratios.

Scaffolds	Mean pore size ( $\mu\text{m}$ )	Water vapor transmission rate ( $\text{g}/\text{m}^2/\text{day}$ )
DD60	$185 \pm 26.45$	$3953.6 \pm 113.9$
Chit8-Hp	$128 \pm 15.00$	$3555.9 \pm 74.3$
Chit5-Hp	$118 \pm 23.62$	$3189.0 \pm 39.2$
Chit8-Lp	$73 \pm 16.36$	$3024.5 \pm 34.1$
Chit5-Lp	$52 \pm 10.75$	$2964.2 \pm 78.0$
DD80	$165 \pm 17.32$	$3904.3 \pm 25.4$
Chit8-Hp	$176 \pm 18.17$	$3184.6 \pm 87.2$
Chit5-Hp	$135 \pm 13.43$	$3126.8 \pm 25.8$
Chit8-Lp	$164 \pm 23.02$	$3233.1 \pm 36.2$
Chit5-Lp	$118 \pm 19.23$	$3107.4 \pm 52.8$

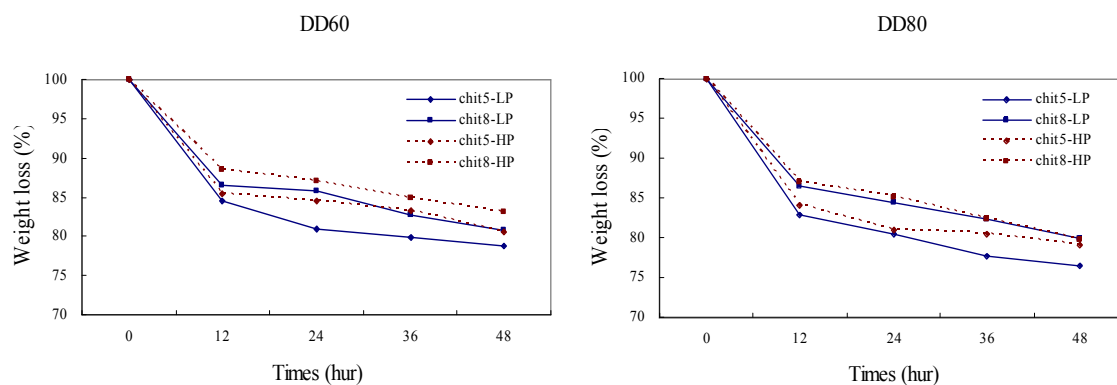


Fig. 2. Weight loss over time of different types of scaffolds in physiologic solution at 37°C and pH 7.4. (Chit5-Lp: chitosan with low molecular weight  $\gamma$ -pga in 5:1 volume ratio, Chit8-Lp: chitosan with low molecular weight  $\gamma$ -pga in 8:1 volume ratio , Chit5-Hp: chitosan with high molecular weight  $\gamma$ -pga in 5:1 volume ratio, Chit8-Hp: chitosan with high molecular weight  $\gamma$ -pga in 8:1 (volume ratio). (\* $p < 0.1$ ,  $n=3$ ).

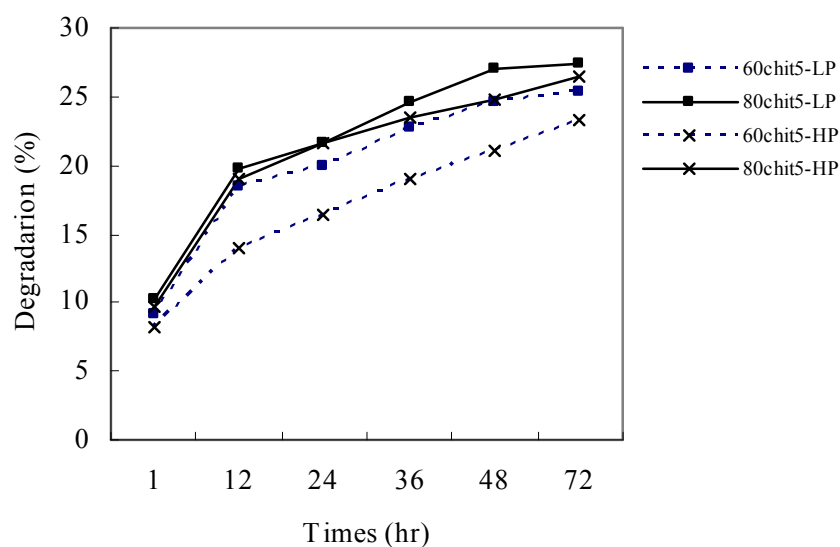


Fig. 3. The degradation percentage of different types of scaffolds in PBS solution. (60 Chit5-Lp: 60% DD of chitosan with low molecular weight  $\gamma$ -pga in 5:1 volume ratio, 80Chit5-Lp: 80%DD of chitosan with low molecular weight  $\gamma$ -pga in 5:1 volume ratio, 60Chit5-Hp: chitosan with high molecular weight  $\gamma$ -pga in 5:1 volume ratio, 80Chit5-Hp:



chitosan with high molecular weight  $\gamma$ -pga in 5:1 (volume ratio). (\* $p < 0.1$ ,  $n=3$ ).

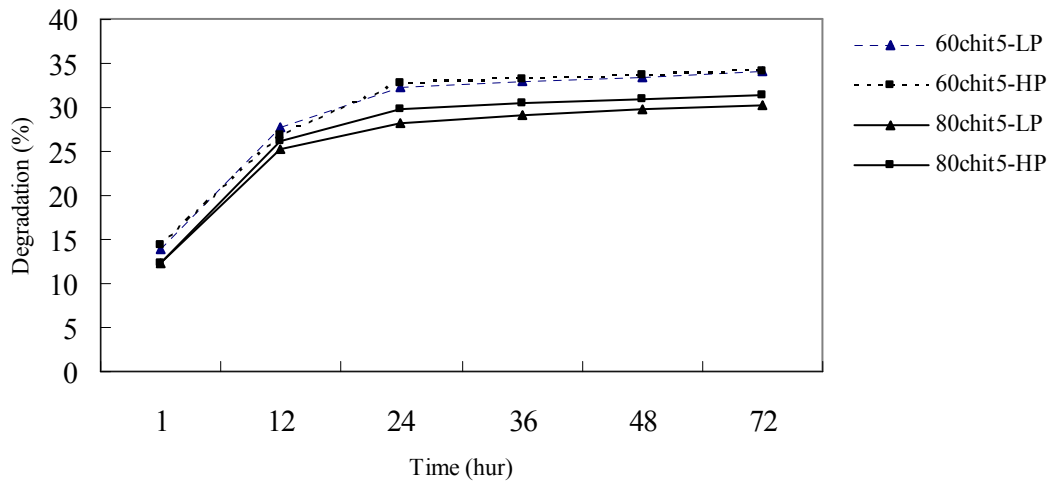


Fig. 4. The degradation percentage of different types of scaffolds by lysozyme. (60Chit5-Lp: 60%DD of chitosan with low molecular weight  $\gamma$ -pga in 5:1 volume ratio, 80Chit5-Lp: 80% DD of chitosan with low molecular weight  $\gamma$ -pga in 5:1 volume ratio, 60Chit5-Hp: chitosan with high molecular weight  $\gamma$ -pga in 5:1 volume ratio, 80Chit5-Hp: chitosan with high molecular weight  $\gamma$ -pga in 5:1 (volume ratio). (\* $p < 0.1$ ,  $n=3$ ).

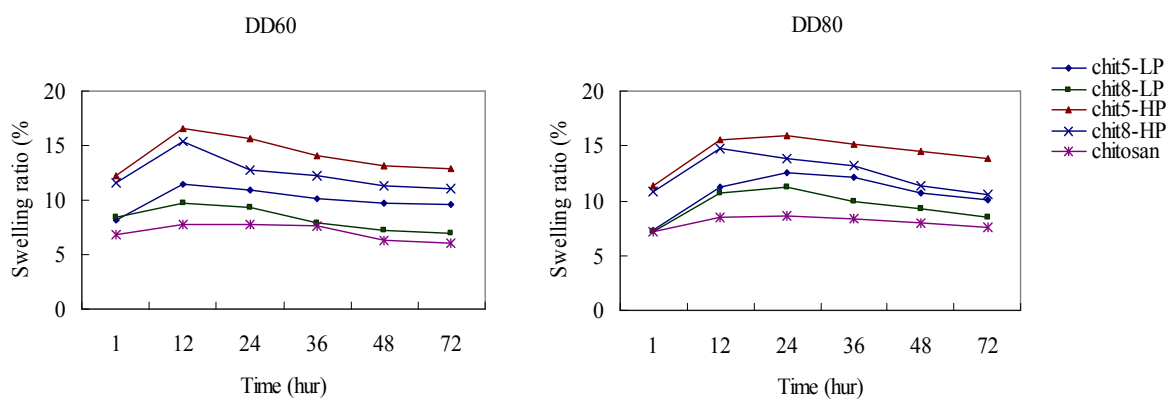


Fig. 5. The swelling ratio over time in physiologic solution at 37°C and pH 7.4. (Chit5-Lp: chitosan with low molecular weight  $\gamma$ -pga in 5:1 volume ratio, Chit8-Lp: chitosan with low



molecular weight  $\gamma$ -pga in 8:1 volume ratio , Chit5-Hp: chitosan with high molecular weight  $\gamma$ -pga in 5:1 volume ratio, Chit8-Hp: chitosan with high molecular weight  $\gamma$ -pga in 8:1 (volume ratio). (\* $p < 0.1$ ,  $n=3$ ).

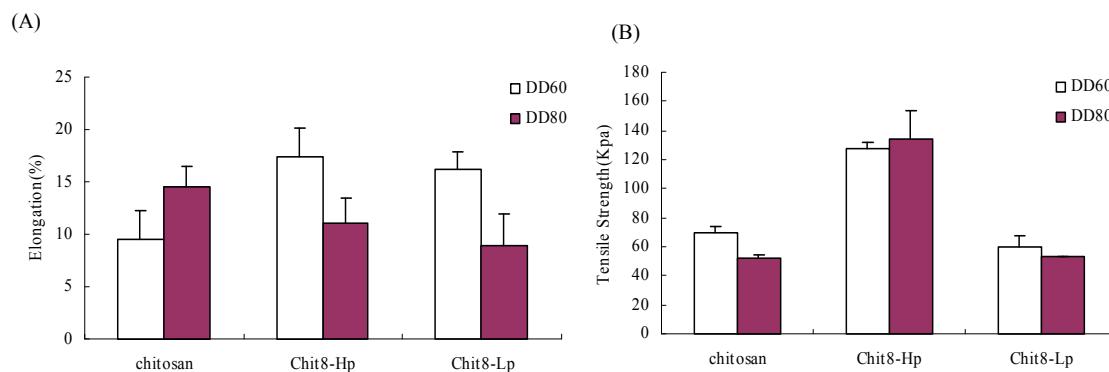


Fig. 6. Mechanical properties of different types of scaffolds in dry state. (A) Elongation (%), (B) Tensile Strength (Kpa). (Chit8-Hp: chitosan with high molecular weight  $\gamma$ -pga in 8:1 volume ratio ; Chit8-Lp: chitosan with low molecular weight  $\gamma$ -pga in 8:1 (volume ratio). (\* $p < 0.1$ ,  $n=3$ ).

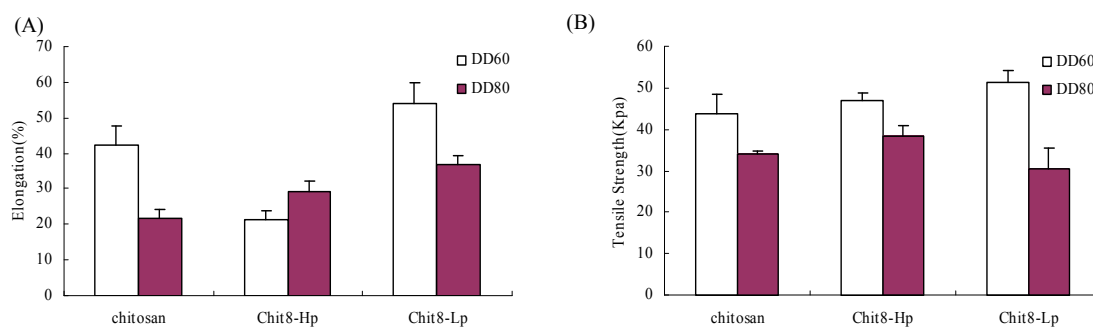


Fig. 7. Mechanical properties of different types of scaffolds in the wet state. (A) Elongation (%), (B) Tensile Strength (Kpa). (Chit8-Hp: chitosan with high molecular weight  $\gamma$ -pga in 8:1 volume ratio; Chit8-Lp: chitosan with low molecular weight  $\gamma$ -pga in 8:1 volume ratio) (\* $p < 0.1$ ,  $n=3$ ).