CHAPTER 4

Results and Discussion

This chapter can be separated into two categories which are the results and the discussion.

4.1. Results

4.1.1. Characterization of scaffolds with AFM

Both gelatin scaffolds and blended scaffolds were shown in Fig 4.1. Young's modulus of dried scaffold was tested by AFM and the result was shown in Fig 4.2. Young's moduli of gelatin and blended scaffolds were 53.30 ± 26.80 kPa and 98.01 ± 17.50 kPa, respectively. The blended scaffolds had higher Young's moduli than gelatin scaffolds, which implied that the blended scaffolds possessed stronger structure than gelatin scaffolds.

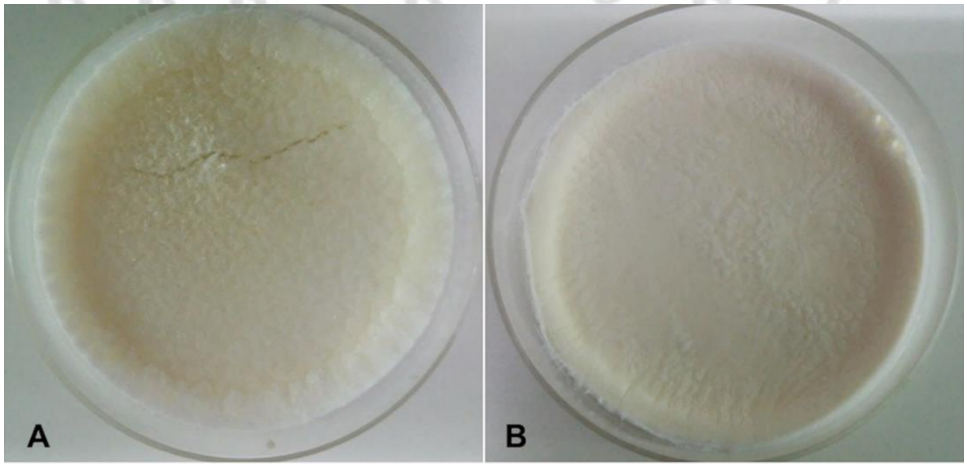

Figure 4.1 (A) Dried gelatin scaffolds. (B) Dried blended scaffold

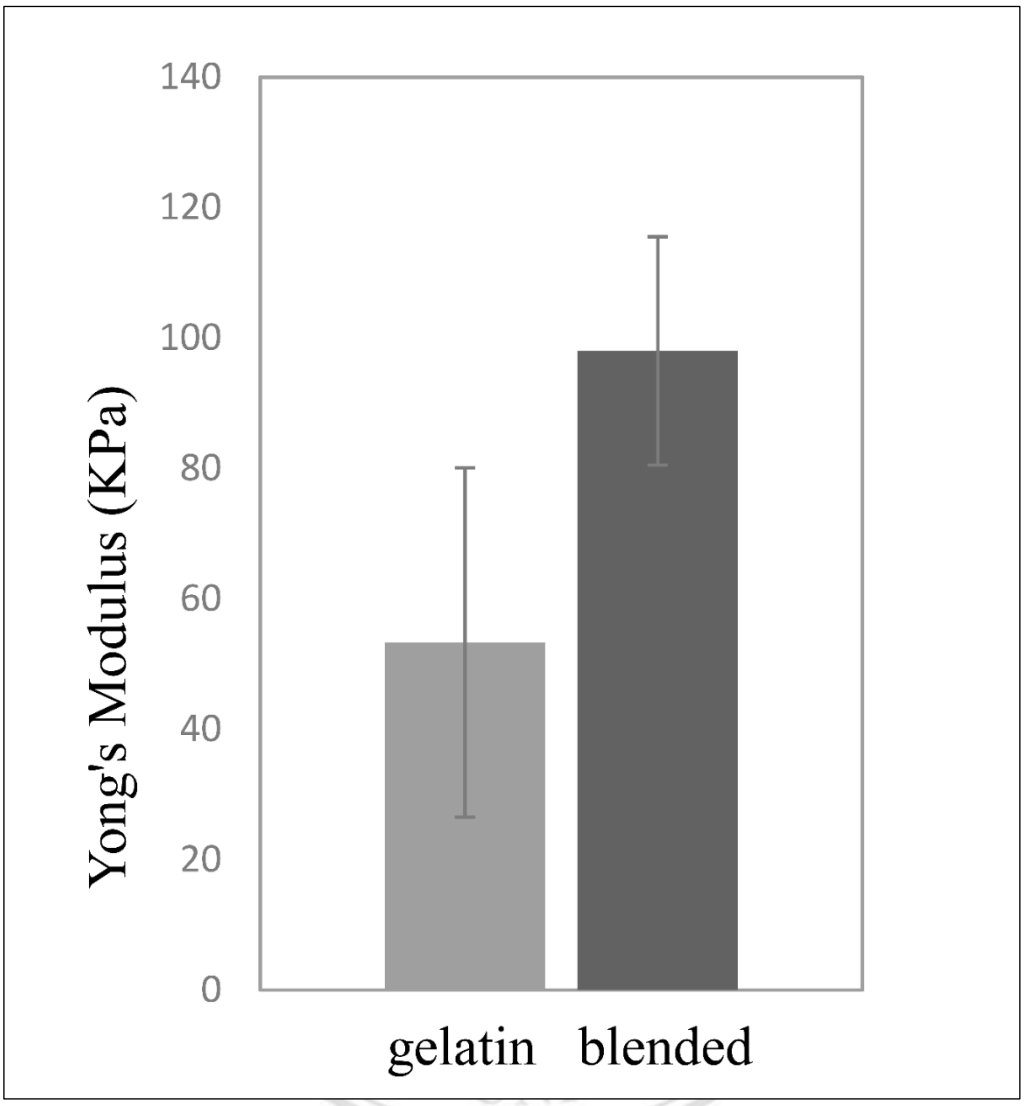

Figure 4.2 Young's moduli of gelatin scaffolds and blended scaffolds. The blended scaffolds had higher Young's modulus compared to gelatin scaffolds.

4.1.2. Porosity measurement

Porosity was tested by using water displacement method according to the different weights between dry and wet scaffolds. Fig 4.3 showed that gelatin scaffolds presented higher porosity than blended scaffolds. The porosity of blended scaffolds was 21.48 ± 1.01% while the porosity of gelatin scaffolds was 85.41 ± 2.11%.

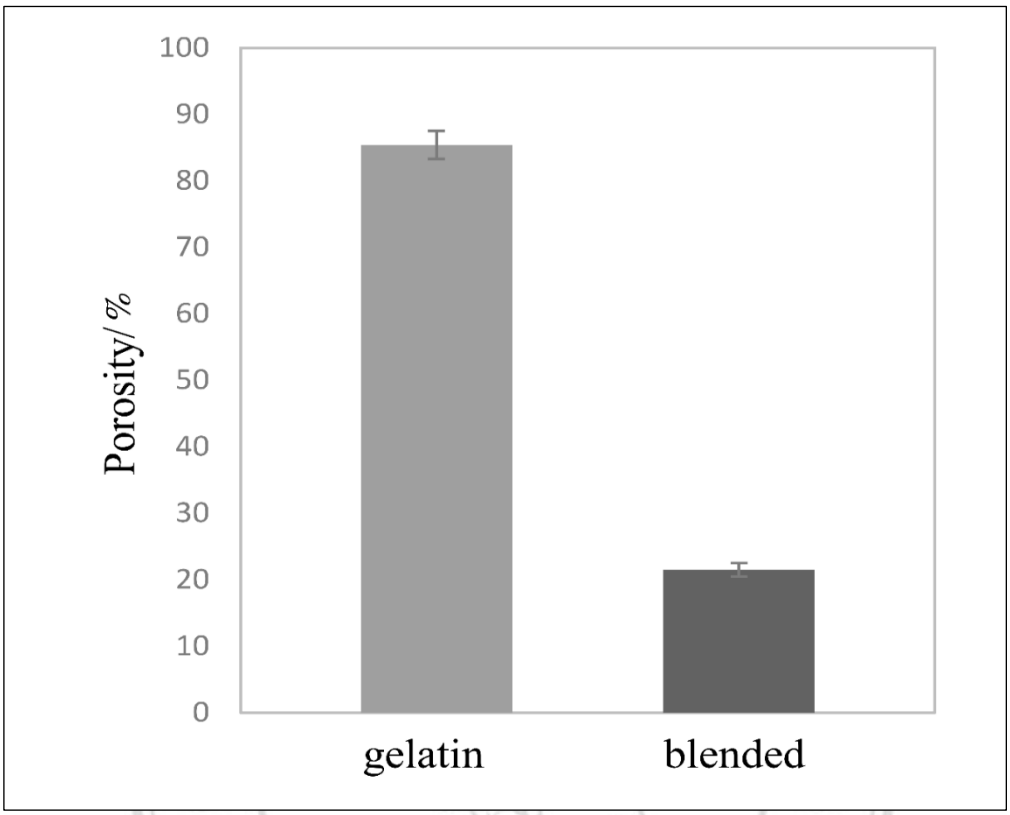

Figure 4.3 Porosity of gelatin scaffolds and blended scaffolds. Gelatin scaffolds possessed higher porosity compared to blended scaffolds.

4.1.3. Swelling ratio measurement

Table 4.1 showed the swelling ratios of both gelatin and blended scaffolds at 3 h, 7 h, and 24 h, respectively. The result showed that both blended scaffolds and gelatin scaffolds had very good swelling capacity. However, gelatin scaffolds swelling capacity was higher than blended scaffolds. In both types of scaffolds, swelling capacity was increased from 3 h to 24 h.

Table 4.1: Swelling capacity of gelatin scaffolds and blended scaffolds were investigated at different time interval: 3 h, 7 h and 24 h.

Swelling ratio	3 h	7 h	24 h
gelatin scaffold	1189.56 ± 147.23	1442.28 ± 143.65	1904.81 ± 166.06
blended scaffold	863.84 ± 97.32	1126.65 ± 58.52	1474.59 ± 65.50

4.1.4. In vitro degradation rate test

The degradation rate was tested at 0, 3, 7 and 10 days; and the result was shown in Fig 4.4. The degradation rate of gelatin scaffolds was higher than blended scaffolds.

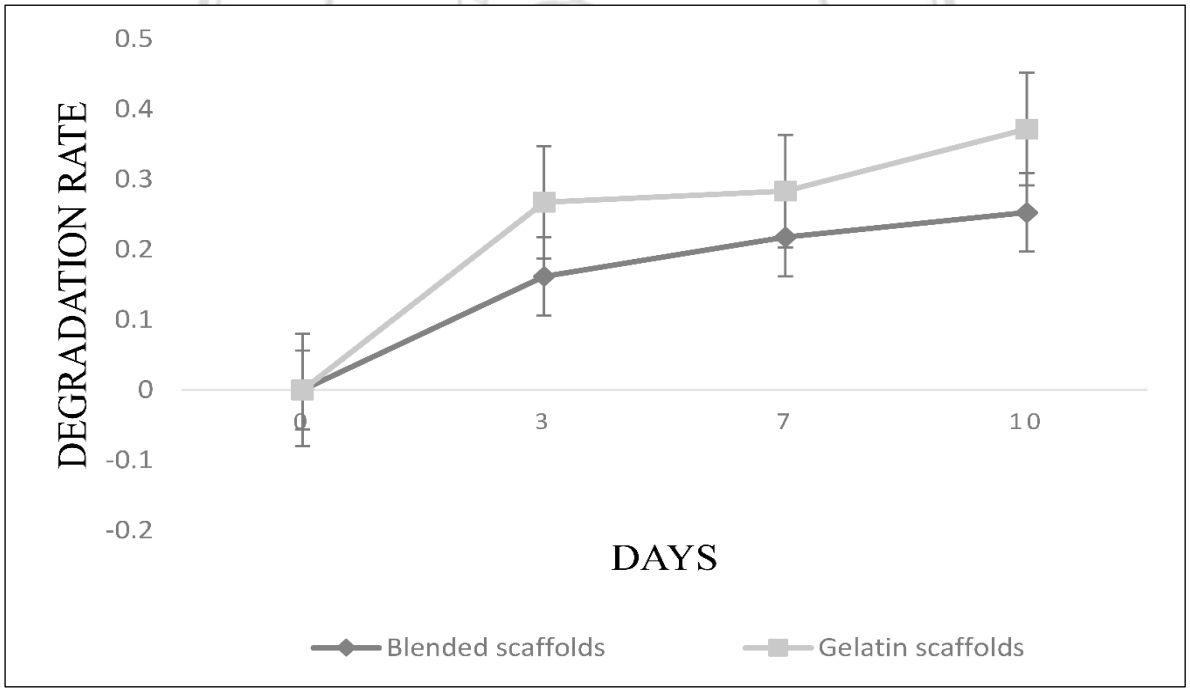

Figure 4.4 Degradation rate of gelatin scaffolds and blended scaffolds from 0 day to 10 days. Gelatin scaffolds showed higher degradation rate compared to blended scaffolds.

4.1.5. Cell culture

NIH/3T3 cells cultured on gelatin and blended scaffolds for 24 h were shown in

Fig 4.5. Cells were aggregated on gelatin scaffolds while they were spread on blended scaffolds. At 4 days culture, NIH/3T3 cells cultured on gelatin and blended scaffolds were observed with SEM and the result was shown in Fig 4.6. The result showed that the average pore size of gelatin scaffolds was 336.3 3 ± 52.25 µm, while the average pore size of blended scaffolds was 68.17 ± 8.91 µm. NIH/3T3 proliferated and covered the surfaces of both scaffolds at 4 days culture.

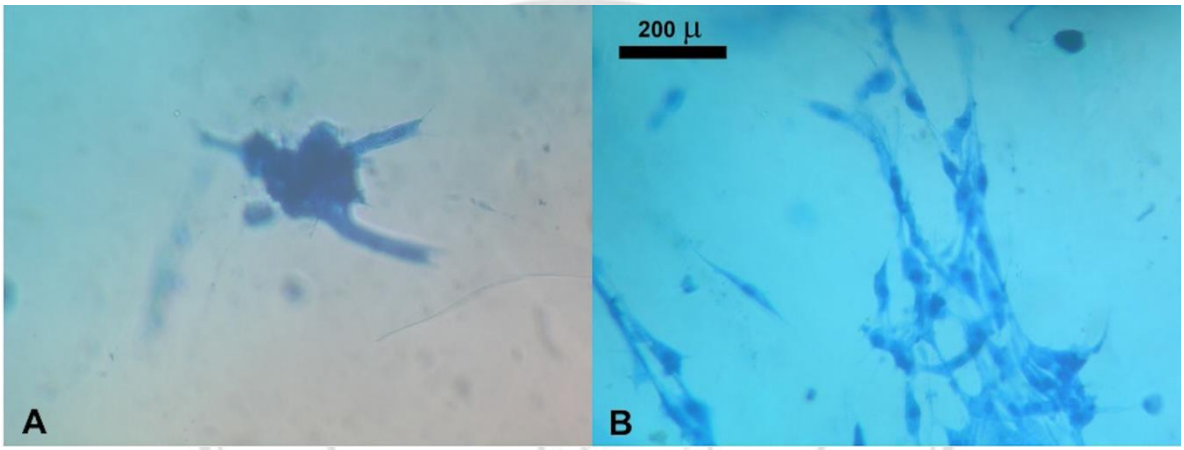

Figure 4.5 Morphology of NIH/3T3 fibroblasts cultured on gelatin scaffolds and blended scaffoldsfor 24 h. Cells were spread with good morphology on blended scaffolds but they were aggregated on gelatin scaffolds.

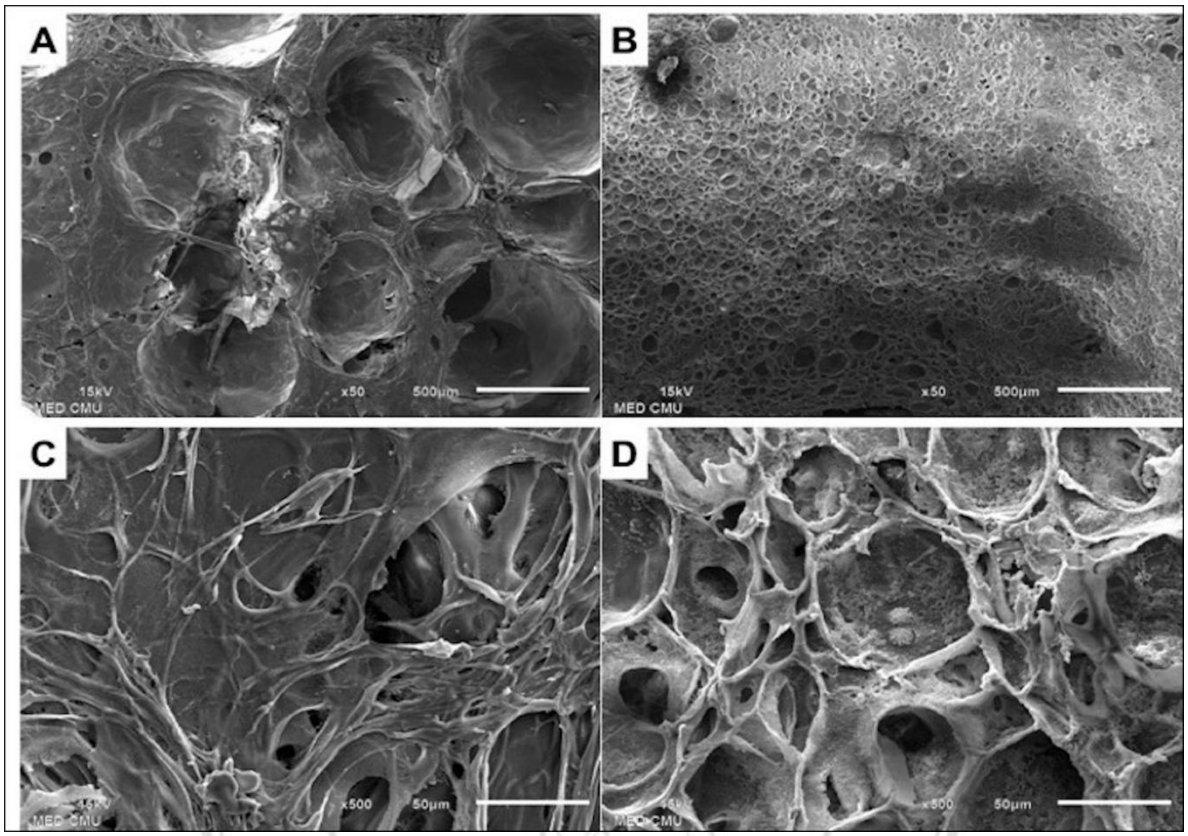

Figure 4.6 SEM imaging of gelatin scaffolds and blended scaffolds seeded with NIH/3T3 fibroblasts. (A&B) SEM imaging of gelatin scaffolds and blended scaffolds at 50×magnification showed that pore size of gelatin scaffolds was bigger than blended scaffolds. (C&D) At 4 days cultivation NIH/3T3 fibroblasts could proliferate and cover the surfaces of both gelatin scaffolds and blended scaffolds (500×magnification).

4.1.6. In vitro relative cell viability study

The result of MTT assay was shown in Fig 4.7. The %viability of gelatin and blended scaffolds were compared to control cells on tissue culture plates. The relative cell viability of gelatin and blended scaffolds were 12.82% and 30.16%, respectively. The color of formazan product produced on control group, gelatin and blended scaffolds was correlated with the result of %viability as shown in Fig 4.8.

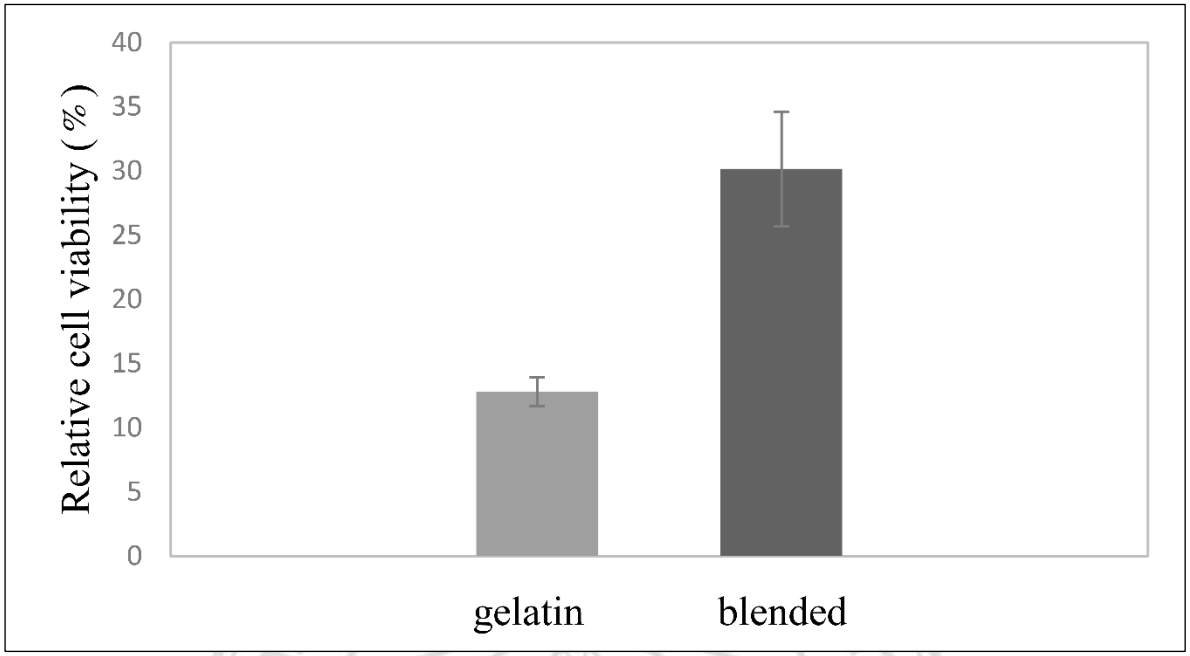

Figure 4.7 Relative cell viability of NIH/3T3 fibroblasts on gelatin scaffolds was lower than that of blended scaffolds.

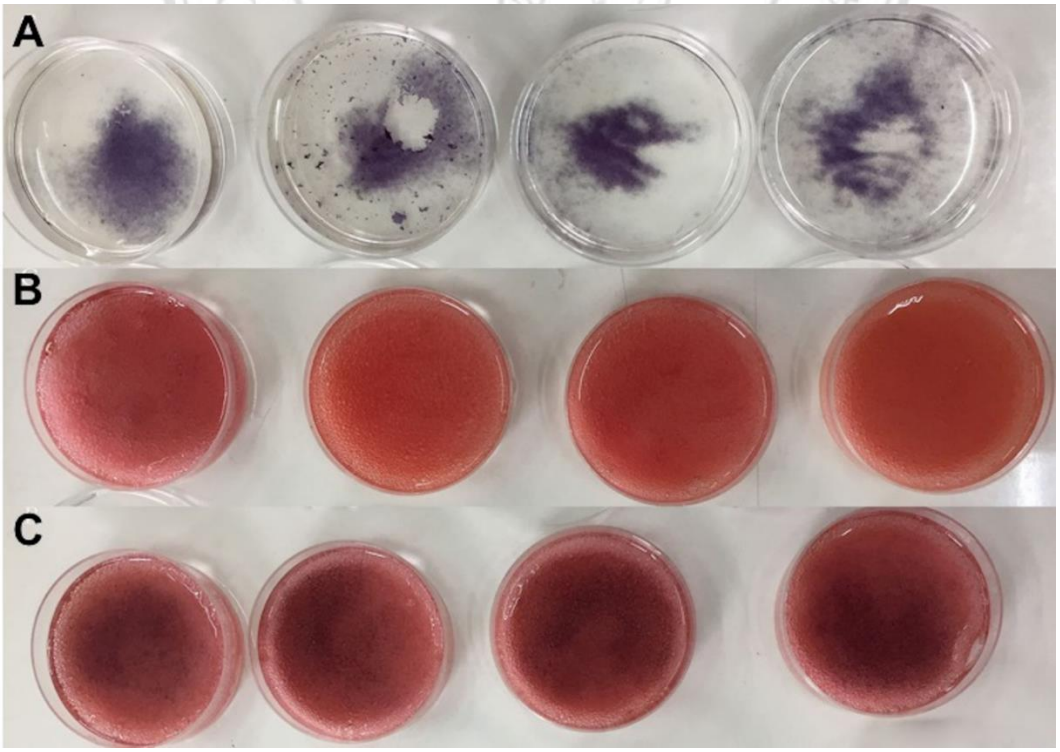

Figure 4.8 The purple color of formazan product on (A) tissue culture plates, (B) gelatin scaffolds and (C) blended scaffolds. The color of formazan product was correlated to the %cell viability.

4.1.7. Cell relative gene expression

Fig 4.9 presented the relative expression of collagen type 4 in NIH/3T3 cells. At 10 days culture, blended scaffolds showed higher expression of collagen type IV than gelatin scaffolds. Cells on both types of scaffolds showed higher collagen type IV expression when compared to cells on tissue culture plates.

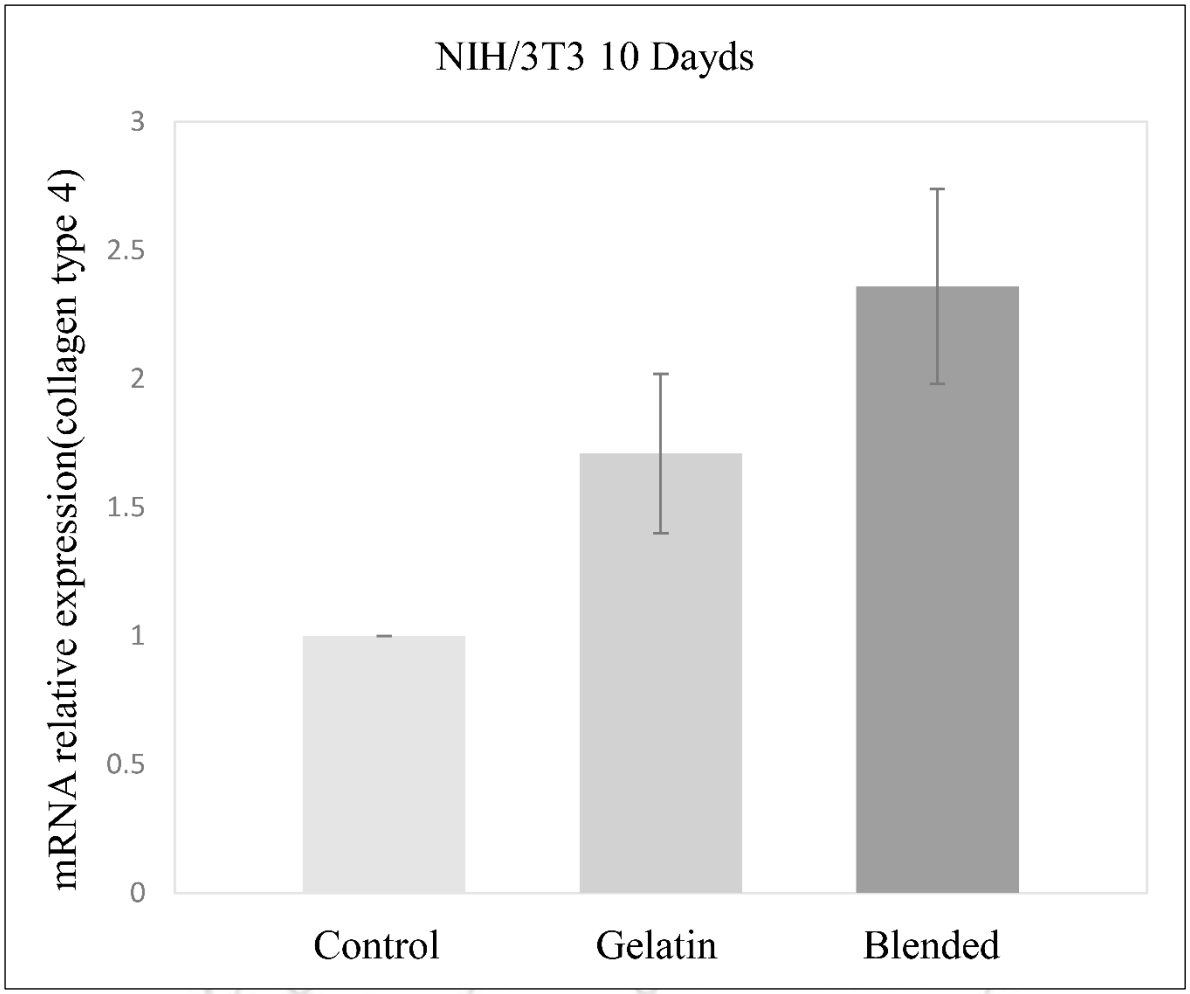

Figure 4.9 The relative expression of collagen type4 at 10 days culture. NIH/3T3 fibroblasts on blended scaffold showed higher collagen type4 expression than that of gelatin scaffolds.

4.6. Discussion

In this study, the result has shown that the porosity of gelatin scaffolds is higher than blended scaffolds, then obviously the swelling ratio of gelatin scaffolds is higher too. Besides, the higher porosity and larger pore size also allowed more water to enter the scaffold (Liu, Huang et al. 2014, Song, Li et al. 2015). The higher porosity and larger pore size contributed to lower Young's modulus of gelatin scaffolds compared to blended scaffolds (Yu, Matthew et al. 2008). The swelling capacity of gelatin scaffolds is higher than blended scaffolds. The previous study mentioned that the molecular chains movement in blended scaffold was more limited so lesser amount of water could be absorbed when the degree of cross-linking was higher (Song, Li et al. 2015). One of the most important criterions of a scaffold is to maintain an optimal degradation rate so that the scaffold could be able to facilitate the growth of cells and tissues (Ye, Mohanty et al. 2014). This study used lysozyme because lysozyme is an enzyme present in certain human body fluids so lysozyme played a very important role in biodegradation of scaffolds (Baniasadi, Ramazani S A et al. 2015). The degradation rate of gelatin scaffolds was higher than the blended scaffolds because the bigger pore size allowed more solution to pass into the gelatin scaffolds and promoted the degradation. More importantly, the higher content of hydrophilic glycolic acid in gelatin facilitated the absorption and diffusion of water and promoted the hydrolysis (Wu and Ding 2004, Liu, Huang et al. 2014). The blended scaffolds were able to provide the more stable environment to cell growth since cells prefer a harder structure to proliferate. Herein this study, the final concentration of 7% gelatin, 0.5% PVA and 0.1% chitosan was chosen as the optimal ratios. If the higher chitosan and PVA concentration were used, the cross-linking of gelatin would be insufficient (Song, Li et al. 2015). Previous research mentioned that the swelling ratio of hybrid hydrogel scaffold is related to not only the degree of cross-linking but also proportional to the total concentration of hydrogel scaffold. Molecular chain' s movement was more limited in blended scaffolds so lesser amount of water could be absorbed when the degree of cross-linking is higher (Song, Li et al. 2015). The blended scaffolds possessed higher degree of cross-linking, which contributes to the lower swelling ratio compared to gelatin scaffolds.

The previous study reported that there was specific integrin–ligand interactions between the cell and the surrounding ECM that can influence the cell attachment and migration. Therefore, the scaffold that possessed a relatively high surface area was very important for optimal cell attachment. Numerous researchers had found that the specific surface area decreases with increasing pore size. As a result, it was hypothesized that cell attachment would decrease linearly with increasing pore size (O'Brien FJ 2005, Murphy, Haugh et al. 2010). Because of those reasons, the surface area of gelatin scaffolds decreased with increasing pore size which lead to lower degree of cell survival than blended scaffolds. The collagen type IV was used as a biomarker of skin formation because Type IV collagen is a type of collagen found primarily in the skin within the basement membrane zone or dermal–epidermal junction, where it is mostly found in the lamina densa (Abreu-Velez and Howard 2012, Matsuura-Hachiya, Arai et al. 2017). The previous study showed that reasonable designed scaffold should be able to regulate cell morphology which in turn regulate cellular functionality, such as proliferation and differentiation (Kumar, Tison et al. 2011).

The blended scaffold had more surface area and more porosity which facilitated the transportation of nutrients and metabolites and supported cell adhesion. While the larger pore size of gelatin scaffold lead to the lesser surface area which contributed to the limitation of cell adhesion, and the cells simply migrated through the scaffold. Then the level of cell-to-cell contact were low which resulted in the low proliferation rate (Murphy, Haugh et al. 2010, Tsai, Hung et al. 2014) which could be confirmed by MTT assay. The relative cell viability of blended scaffolds was much higher than gelatin scaffolds. For these reasons, the blended gelatin-PVA-chitosan scaffold got a better result of collagen type IV expression than the gelatin scaffold at 10 days. Thus, the blended gelatin-PVA-chitosan scaffolds have the better conditions for cells survival than gelatin scaffolds. This research demonstrated that the blended scaffolds had better properties than pure gelatin scaffolds, which implied that the blended scaffolds could provide better environment for cell proliferation and differentiation.