NANO-TIO$_2$ HYDROSOL/COLLAGEN-CHITOSAN COMPOSITE SCAFFOLD FOR WOUND REPAIRING

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Collagen-Chitosan (COL-CS) porous scaffolds have been widely used as a dermal equivalent to induce fibroblasts infiltration and dermal regeneration. To improve the anti-bacterial properties, nano-TiO_2 hydrosol was introduced into COL-CS scaffolds. The TiO_2/COL-CS composites scaffolds were prepared through freeze-drying. Their possible application in wound healing was tested in vitro. Scanning electron microscopy (SEM) was employed to study the micro-structure of the scaffolds. The swelling property and porosity of the composite were investigated. The results showed that the scaffold may provide good permeability and humid environment for wound healing. The SEM images of the scaffold showed a porous feature which would be favorable for cell migration and rapid ingrowth of host fibroblasts and endothelial cells. The nano-TiO_2/COL-CS composite scaffolds could be a promising candidate for wound healing dressing.

Keywords: collagen; chitosan; wound healing; nano-TiO_2

INTRODUCTION

Skin substitution with skin replacement materials can be a lifesaving measure in the treatment of acute burns and scald (Dainiak, 2010). Biologically active scaffolds used in skin-replacement play a critical role in wound healing. Their function is to provide mechanical support; to direct the growth of cells, either seeded onto the scaffold or migrating from surrounding tissue; and to enhance angiogenesis (MacNeil, 2007).

Collagen is the most abundant ECM constituent of nature. Dermis and scaffolds made from collagen exhibit weak antigenicity, biodegradability, and superior biocompatibility (haemostatic and cell-binding properties) (Lee, 2001; Chen, 2005). Chitosan, a natural-based polysaccharide, is a versatile biopolymer usually derived by partial deacetylation of chitin. It has been proved to possess variety of fascinating biological properties such as biodegradability, biocompatibility, non-antigenicity, nontoxicity, antimicrobial activity and hemostasis, making it a potential candidate in the wound management area (Ong, 2008; Ishihara, 2002; Anilkumar, 2011).

During the wound healing process, an appropriate dressing to cover the wound is required to avoid loss of heat and reduce the wound infection rate. However, most commercially available dressings do not have active antibacterial capabilities, which results in increased infection rates and ulcer formation. Moreover, the wound dressing must be frequently replaced to avoid inducing a secondary injury that would inevitably prolong the healing time (Yu, 2014). In recent years, various bacterial infection become more serious, inhibiting the growth and reproduction of bacteria has also become increasingly important (Zhu, 2011). TiO_2 nano-particles have been widely applied in biomedical and bioengineering fields, owing to their strong oxidizing properties, chemical inertness, anti-bacterial and non toxicity (Yan, 2011).

The main purpose of this study is to develop a TiO_2/COL-CS composite scaffold for wound repairing. Nanometer titanium dioxide was prepared by sol-gel method (Mao, 2005; Venkatachalam, 2007; Macwan, 2011). COL-CS composite scaffold supplemented with different ratios of TiO_2 was prepared by freeze-drying technique and characterized by using SEM.

MATERIALS AND METHODS

Materials

Chitosan (>90% deacetylation) was from Jinhu Crust Product Co., LTD., China. Tetrabutyl titanate (M=340.36) was made by Tianjin Hedonghongyan reagent factory. Fresh pigskin was commercially available. All other reagents used were of analytical grade.

Collagen Extraction

Type I collagen used here was extracted from fresh pigskin. The extraction was performed according to the procedure of Feng Wen-po et al (2010). The purified collagen was freeze-dried and stored at − 20°C for subsequent use.

Nano-TiO_2 Hydrosol Preparation

Tetrabutyl titanate (TBT) was used as a raw material. The molar ratio of TBT, distilled water, ethanol, hydrochloric acid was 1:200:16:0.3. Firstly, hydrochloric acid (0.22g) was dissolved in distilled water(73.52g) to yield solution A. TBT (6.95g) was added to anhydrous alcohol (15.05g) while stirring and the pH was adjusted to 3.0 by HCl, yielding solution B. Solution B was added dropwisely to solution A while vigorously stirring for 2 h at 40°C.

TiO_2/COL-CS Composite Scaffold Fabrication

The blend of collagen and chitosan was prepared by mixing the solutions of collagen (0.5%) and chitosan (0.5%) in 0.5 M acetic acid at the ratio of 1:1. Similarly nano-TiO_2 blended with COL-CS composite was prepared by mixing different amounts of nano-TiO_2 hydrosol with the COL-CS solution to reach the concentrations of 0, 1, 3, 5, and 7% (w/w), respectively. The mixtures were then poured into a six-well culture plates and frozen overnight at − 30°C, after lyophilization dried for 24h. All the samples were stored at − 20°C for subsequent use.

Micro Structure of TiO_2/COL-CS Composite Scaffold

The cross sectional morphology of the TiO_2/COL-CS composite scaffold was characterized by a SEM (Quanta200). The cross sections were prepared by cryogenically fracturing the films in liquid nitrogen and then coated with aurum before observation.

PBS Solution Adsorption of TiO_2/COL-CS Composite Scaffold

The water absorption and equilibrium water content of TiO_2/COL-CS composite scaffold were determined. The samples were put in pH=7.4 phosphate buffered saline (PBS) at 35.0 ± 0.5^0C. The water absorption and equilibrium water content of the samples in the PBS media were calculated according equations (1) and (2) (Yan, 2011):

$$A(\%) = \left(\frac{W_S - W_0}{W_0} \right) \times 100 \quad (1)$$

$$B(\%) = \left(\frac{W_S - W_0}{W_S} \right) \times 100 \quad (2)$$

where *A*=Water adsorption, *B*=Equilibrium water content, W_s is the weight of swollen sample and W_0 is the initial sample weight. Three tries were done and the average data was reported.

Porosity Studies of TiO_2/COL-CS Composite Scaffold

The initial weights of scaffolds with the same size were examined by analytic balance, and then soaked in dehydrated alcohol at room temperature for 24 h. After the excess ethanol was carefully wiped from the surface, the scaffolds were weighted again. The interval porosity of the samples were calculated according to equation (3):

$$C(\%) = \left(\frac{W_t - W_0}{\rho_0 V_0} \right) \times 100 \quad (3)$$

where *C*=Porosity, W_0 is the initial weight of the scaffold, W_t is the swollen weight, V_0 is the initial volume of the scaffold, and ρ_0 is the density of dehydrated alcohol (0.79 g/ml).

RESULTS AND DISCUSSION

Micro Structure of TiO_2/COL-CS Composite Scaffold

The microstructure such as pore size and its distribution, porosity as well as pore shape prominently affect the cell attachment, proliferation, function and migration in tissue engineering. The cross section of scaffolds of COL-CS and COL-CS-TiO_2 (1,3,5,7%) was studied by SEM and the images are shown in Figure 1.

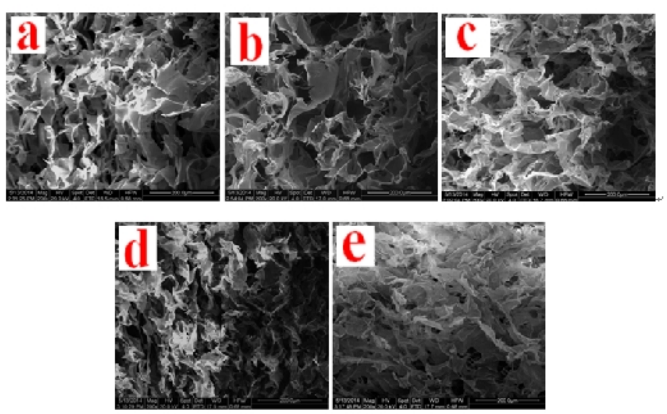

Figure 1. SEM images of cross sections of COL-CS scaffolds with various concentration of nano-TiO_2 (0,1,3, 5 and 7% represented as a, b, c, d, e, respectively)

From Figure 1, it was shown that the TiO_2/COL-CS has good pore size distribution, and there is a relation between the pores and aperture. The scaffold formed lamellar structure which may be conducive to cell attachment, proliferation, function and migration. No obvious change was found in the three-dimensional morphology of scaffold with the addition of TiO_2. With increasing the TiO_2 content, the pore structure becomes uniform and the density of the aperture also increases. At the TiO_2 content of up to 5%, the pore size and its distribution are better than others. Up to 7%, the aperture decreases and lamella structure has a portion of stack, which is not conducive to cell adhesion, migration and growth.

PBS Solution Adsorption of TiO_2/COL-CS Composite Scaffold

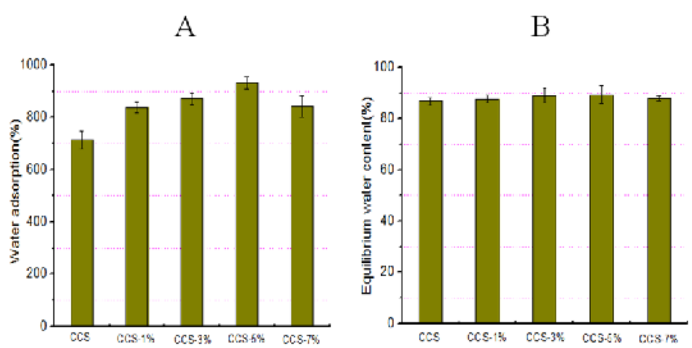

Figure 2. Water adsorption and equilibrium water content of COL-CS (CCS) scaffold containing different concentration of nano-TiO_2 (0,1,3,5,7%)

High water keeping ability of scaffold is favorable for cell adhesion and growth. It would make it easy to transport nutrients from the scaffold to cells in the culture system. In wound healing, it would prevent the fluid loss from the body while applied on the wound. The water keeping ability of the TiO_2/COL-CS scaffold could be attributed to both of their hydrophilicity and maintenance of their three-dimensional structure. From Figure 2, after being immersed in the PBS media for 24h, the water adsorption reach up to 715%, and the water adsorption gradually increased with increasing the nano-TiO_2 concentration from 1% to 5%, this may be due to the hydrophilic property of nano-TiO_2 that was included in the COL-CS composite scaffold. When reach out to 7%, little decrease was found in the water adsorption. The nanometer titanium dioxide is easy to reunite, which decreases its hydrogen forming ability. As a result, the water adsorption of the scaffold decreased.

Porosity of TiO_2/COL-CS Composite Scaffold

From Figure 3, the porosity of the COL-CS scaffold was about 82.07%, and the porosity of the TiO_2/COL-CS scaffold with different nano-TiO_2 concentrations was about 88.21%, 92.76%, 95.02%, 88.23%. With the addition of TiO_2, the porosity of the scaffold increases gradually to reach the maximum at 5%. Pore size and its uniformity, porosity determine the mechanical properties and the water retention. Thus the scaffold can easily absorb the culture medium to facilitate the cells for migration, adherence and proliferation in the porous structure.

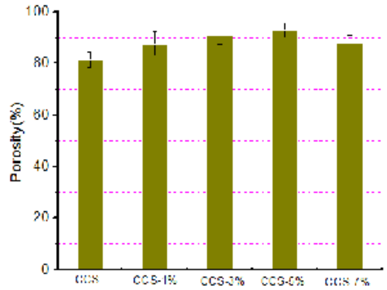

Figure 3. Porosity of COL-CS (CCS) scaffold with different concentration of nano-TiO_2 (0,1,3,5,7%)

CONCLUSIONS

Porous TiO_2/COL-CS scaffold was prepared by freeze-drying. Nano-TiO_2 affects the water adsorption and the porosity of the scaffold. No significant differences were found in the morphology. The SEM images of the scaffold showed a porous feature, favorable for cell migration and rapid growth of host fibroblasts and endothelial cells. The water adsorption of the TiO_2/COL-CS scaffold reached the maximum of about 1200%. The porosity of all COL-CS scaffold is more than 80%, with the maximum of about 93%, The scaffold may provide good permeability and humid environment for wound healing. The nano-TiO_2/COL-CS composite scaffolds could be a promising candidate for wound healing dressing.

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