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RGD immobilization on PLLA membranes to promote biocompatibility

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1. Introduction

Polylactide (PLA) has been widely employed to fabricate scaffolds for tissue engineering. To improve cell attachment on such a material, various approaches have been conducted. However, adsorption of adhesive proteins and traditional chemical modification to the PLA scaffolds were all confront with some difficulties for practical applications. Plasma treatment is another method to improve the biocompatibility of PLLA. It was manifested to be an efficient way to modify the surface of scaffolds with little effect on the porous structure and bulk properties. The plasma treatment can improve the surface hydrophilicity of a material more easily than other modification processes. The plasma technique could also induce active functional groups onto the polymer chains, and by reacting with them a variety of biomolecules, can be successfully immobilized on the material surface. Therefore, in this study, plasma treatment was employed to immobilize RGDS on PLLA scaffold to improve their cell affinity, which was evaluated by culture of osteoblast-like cells.

2. Results and discussion

The porous scaffolds used in this study were prepared by the so-called freeze–extraction methods [1]. Quantitative analysis, to determine the amount of the peptides on the PLLA scaffolds, was carried out with a HPLC and the amount of the immobilized peptides per unit surface area was calculated to be in the order of 10^{-11} mol cm^{-2}. It is known that a RGD density of 1×10^{-12} mol cm^{-2} is sufficient to support the adhesion and spreading of osteoblastic cells, clustering integrin receptors, and organization of actin stress fibers [2]. Obviously, with plasma treatment, RGD can be successfully immobilized on PLLA scaffolds and the immobilized amount is high enough to enhance cell adhesion.

The number of the attached ROS cells at various culture times on PLLA films was measured. It has been proved in this study that

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the attachment of ROS cells to the RGDS-immobilized PLLA film is obviously the fastest one, suggesting that the cell affinity of PLLA is strongly improved with the immobilization of RGDS. Tat improvement on ROS attachment rate can also be accomplished with immobilization of RGES, but the degree of enhancement is much less compared with the immobilization of RGDS. The effect of the immobilization of RGD on cell attachment stems from the recognition of the immobilized RGDS sequence by the adhesion receptor on the cell membrane. On the basis of the mechanism of peptide recognition, the immobilization of RGES should not have effect on cell attachment since it cannot be recognized by the adhesion receptors.

RGDS was immobilized to PLLA films after plasma treatment either under vacuum or not under vacuum. The results indicated that the attachment rate of ROS cells to the RGDS-immobilized film (not under vacuum) was a little lower than that to the RGDS-immobilized film (under vacuum). The difference between theses two immobilization processes will be discussed in more detail later. Besides, the cell attachment and proliferation on the RGDS-immobilized PLLA without plasma treatment are as poor as the unmodified ones, so the data about cell culture are not shown in this paper.

Von Kossa staining was performed to investigate if the cultured ROS cells (osteoblastic-like cells) can be preceded to form bone like tissues. It can be seen that, with longer time of mineralization, the area containing calcium salt (black spot) increases, indicating a higher degree of mineralization. Besides, with the same mineralization time, the RGDS-immobilized PLLA scaffold has larger area of black spots, suggesting that more cells be mineralized. Obviously, with the same culture time, more cells tended to form bone-like tissues on the RGDS-immobilized scaffold than on the unmodified and RGES-immobilized scaffolds.

3. Conclusions

RGDS was successfully immobilized on the PLLA scaffolds via plasma treatment. It was observed that the immobilized RGDS promoted attachment of ROS cells to PLLA. Also, more cells were found in the RGDS-modified scaffold than in the unmodified scaffold, tending to form mineralized foci. The results suggest that the immobilization of RGD makes PLLA scaffolds more suitable for culture of osteoblast-like cells and facilitates their application in bone regeneration.

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