Dendrimer crosslinked collagen hydrogels modified with YIGSR peptide and their effects on cellular behaviors of human corneal epithelial cell line and nerve regeneration

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Introduction

The extracellular matrix (ECM) is the natural scaffold for the cells. It acts not only as a mechanical support, but also creates microenvironments to which the cells respond. Constructing a matrix or scaffold simulating the ECM environment is regarded as a general strategy in tissue engineering to promote cell growth and restore key functions to damaged tissues and organs. Among the macromolecules in the ECM, laminin, a complex trimer glycoprotein, is a major component of the basement membrane and has a neurite-promoting activity, stimulates Schwann cell mitosis. The presence of nerves in corneal equivalents is important for functional restoration. The specific amino acid sequences involved in receptor interaction include RGD and IKVAV of the laminin α-chain, YIGSR of the laminin β1-chain, and RNIAEIIKDI of the laminin γ-chain [1]. The YIGSR sequence of laminin has been incorporated in biomaterials to promote peripheral [2], central [3] nerve regeneration. Grafted YIGSR was also found to promote human corneal stratification and neurite in-growth [4]. Our objective therefore was to examine the introduction of YIGSR into novel dendrimer crosslinked collagen [5] by way of the multifunctional dendrimers, and to examine the effects of the incorporated YIGSR on the behaviour of corneal epithelial cells behaviour and on nerve regeneration.

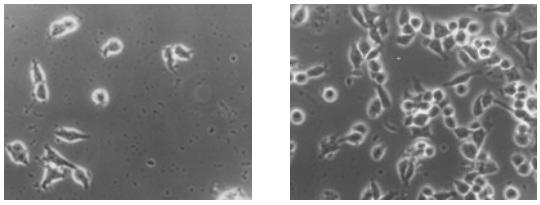
Methods

In a modification to our previous method for synthesis of collagen scaffolds [5], YIGSR, EDC and NHS were added to aqueous dendrimer solutions and the mixture reacted overnight at room temperature. The molar ratio of YIGSR to dendrimer was 1:1. EDC:NHS:COOH of YIGSR was 5:2:1. The product was purified by dialysis (Spectra/Por membrane (MWCO 500)). Formation of YIGSR modified dendrimer was confirmed by H-NMR and MALDI-TOF. The YIGSR containing dendrimers (YIGSR-m-dendrimer) were then used for collagen crosslinking. A series of YIGSR modified collagen gels with different amounts of YIGSR were prepared by using various YIGSR-m-dendrimer percentages. To quantify the peptide content in the collagen, YIGSR was radiolabeled with ^{125}I. Denaturation temperatures and mechanical properties were examined to investigate the effects of YIGSR incorporation on collagen crosslinking and properties. Immortalized human corneal epithelial cells (HCEC) were used to evaluate the effects of YIGSR on cell adhesion, proliferation and stratification. Axonal growth from chick embryo dorsal root ganglia (DRG) was examined to study the effect of YIGSR incorporation on nerve regeneration.

Results and Discussion

H-NMR and MALDI confirmed the successful reaction of YIGSR with dendrimers. YIGSR content in the collagen gels was found to be 3.1×10^{-2}mg/mg collagen, meaning that 24 to 26% of initial YIGSR present in the reaction mixture was incorporated into the gel. Similar results were obtained by radiolabeling and H-NMR. Slightly decreased denaturation temperatures and mechanical properties compared to unmodified collagen gels suggested the possible interference of YIGSR incorporation to crosslinking of collagens.

The presence of YIGSR improved the adhesion and proliferation of HCEC on collagen gels (Fig.1). HCEC stratification was promoted by incorporated YIGSR resulting more cell layers and increased layer thickness ($P<<0.05$). The extent of stratification, low on the base gels, increased with small amounts of YIGSR incorporation but then decreased as a function of the amount of incorporated YIGSR (Fig. 2). The extension and density of nerves growing into the YIGSR modified collagen gels was significantly higher than that in unmodified collagen gels.



(a) (b)

Figure 1. Adhesion of HCEC on (a)control and (b) YIGSR modified (1.6×10^{-2} mg YIGSR/mg collagen) collagen samples (40x, culture time 120min).

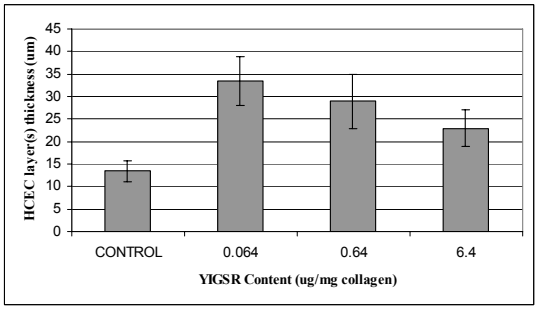


Figure 2. Stratification of HCEC on control and YIGSR modified collagen gel surfaces

Conclusions

The results of the current study suggest that cell adhesive peptides can be incorporated into dendrimer crosslinked collagen gels by way of the dendrimers with only minor interference to crosslinking, producing transparent, mechanically strong and more biocompatible collagen gels. Human corneal epithelial cell adhesion, proliferation, stratification and nerve extension were promoted by YIGSR modification.

References

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