Identification and Effects of Bioactive Factors in Decellularized Collagen Biomatrices

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Introduction

Decellularized collagen matrices derived from the small intestine or the bladder submucosa (BSM) have been used clinically in several clinical applications, including bladder reconstruction, hypospadias and urethral stricture repair. These matrices are biocompatible, and have appropriate characteristics needed for tissue regeneration. In addition, bioactive factors existing in the matrix are believed to be responsible for enhanced tissue regeneration. In this study specific growth factors (GFs) present in the BSM collagen matrices were identified to better define their potential regenerative characteristics.

Materials and Methods

Decellularized porcine bladder submucosa was lyophilized, pulverized, and suspended in five different extraction buffer systems. The extraction mixture was centrifuged, and the supernatant collected, filtered and concentrated using molecular weight cut-off (MWCO) of 9K and 3K. The total protein quantity was measured by DC protein assay and the protein bands were obtained by silver and Coomassie stain methods. A variety of growth factors including VEGF, TGF-α, FGF-2, EGF, KGF, IGF-I, PDGF, NGF, and BMP-4, were analyzed using ELISA and Western blot. Histological and immunohistochemical examination were performed to confirm the presence and distribution of extracellular matrix (ECM) and GFs. The effect of GFs on cell proliferation was tested using fibroblasts. The cells were cultured with various concentrations of total protein (0, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10μg/ml) in 1% fetal bovine serum for 36 hours. The protein extract was supplied to cells by three different routes; the protein was mixed with cells and plated, the protein was added to the culture media after cell attachment on the culture plate and the protein was coated on the plate followed by cell attachment. Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl) -2-(4-sulfophenyl)-2H-etrazolium (MTS) assay.

Results and Discussion

The total protein content of the decellularized collagen matrix increased with time, and the optimum extraction period was 72 hours (79.4μg / 1mg of BSM). The urea and acetic acid extract of BSM produced a higher activity. Silver and Coomassie stain bands showed the matching size with VEGF(42kDa), BMP4(33kDa), KGF(28kDa) and PDGF(16kDa) in BSM. Western blot and ELISA proved the existence of VEGF(42 kDa), BMP4(33kDa), PDGF(28 kDa), KGF(19 kDa), EGF(6kDa), and TGF-a(5.5kDa) (Table.1). Histological and immunohistochemical examination demonstrated the distribution of collagen, elastin and TGF-2 within the matrix. The fibroblast proliferation was shown to be dose dependent from 0.1 to 6 μg/ml (Fig. 1). High concentrations of total protein inhibited cell proliferation. The cells plated simultaneously with the protein extract showed an increased proliferation as compared to the addition of the extracts after cell attachment on the plate. The plates pre-coated with ECM proteins showed marked increase in cell proliferation under 6μg/ml of protein.

Table 1. Growth factors identified in the protein extract of bladder submucosa matrix using ELISA and western blot.

Growth factor	MW(kDa)	Result
VEGF	42	++
BMP4	33	++
PDGF	28	+++
KGF	19	+
EGF	6	+
TGF-α	5.5	+

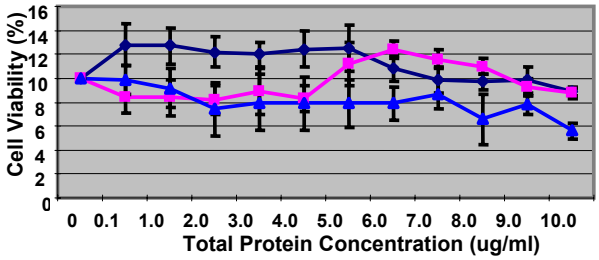

Figure 1. Cell proliferation in response to the total protein extracted from bladder submucosa matrix. Fibroblasts were incubated for 36 hours, and the cell proliferation was determined by MTS assay (◆, protein coated; ■, protein was added simultaneously to cells; ▲, protein was added to the medium after cell attachment on the plate).

Conclusion

This study demonstrates that bladder submucosa collagen matrix preserves many growth factors after the decellularization process. The mixture of growth factors present in BSM enhanced cell proliferation. These findings suggest that GFs contained within the matrix contribute to the tissue regeneration process when implanted in vivo. Further understanding of activity and interaction of these bioactive factors would result in maximizing the tissue regeneration potential of these matrices.

References

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