II. Tissue Engineering

VIABILITY OF SMOOTH MUSCLE CELLS CULTURED ON COLLAGENOUS SCAFFOLDS FOR TISSUE ENGINEERING OF BLOOD VESSELS

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Summary

To characterize cell cultures for tissue engineering purposes, a new method is developed to study cell viability. Markers of both proliferation and apoptosis were analyzed in one single assay with use of a semiquantitative real-time RT-PCR method. In this way no influence on growth behaviour of crosslinking of collagenous scaffolds was observed. These results resemble (qualitative) results of histology. We suggest that this test is suitable to standardize cell culturing for tissue engineering applications.

Introduction

The final aim of this study is to produce a tissue-engineered (TE) autologous vascular prosthesis, exclusively composed of biological materials and vascular cells, which is compatible with blood flow and can be used as a graft in vascular surgery especially for small-diameter applications (<6 mm). Our approach to make such an artificial blood vessel is that it will be characterised by the three-layered structure of a natural blood vessel: the intima, the media and the adventitia. The first step of this project includes culturing human vascular smooth muscle cells (SMCs) in a biodegradable tubular scaffold composed of collagen and elastin to obtain a media of a TE blood vessel. Here, static culturing of SMCs on top of flat porous scaffolds composed of collagen and elastin and the development of a new method to test viability of these cell cultures are described. To measure viability of the cell cultures, proliferation and programmed cell death (apoptosis) were analysed by measuring cyclin E [1] and tissue transglutaminase (tTG) [2] mRNA expression levels with use of a semiquantitative RT-PCR. This method can be used to test the phenotypic state of SMCs to compare growth behaviour of cells of different batches/sources and to compare growth behaviour of cells cultured on (and in) different collagenous scaffolds.

Experimental methods

SMCs were isolated from umbilical cord veins and identified with use of a specific monoclonal antibody against alpha-smooth muscle actin (alpha-SMA) and vimentin [3]. Cells were seeded and subsequently cultured on flat porous films composed of type I insoluble collagen (derived from bovine achilles tendons) and insoluble elastin (from equine ligamentum nuchae). Cross-linking of the films was performed either with a water-soluble carbodiimide or with a diamine, in presence of the carbodiimide. These scaffolds were optimised from a physical and chemical point of view. Cell attachment and growth were verified by histology using a standard Elastic Von Gieson staining procedure. Proliferation and apoptosis of the cells were determined by measuring cyclin E and tTG mRNA expression levels respectively. Cells were lysed; total RNA contents were isolated and cyclin E and tTG mRNA expression levels were determined by using a semiquantitative RT-PCR method on a real-time TaqMan analyser [4].

To set up this method for SMC cultures, cells were cultured on gelatin-coated tissue culture polystyrene (g-TCPS) for up to 36 h. Culture medium supplemented with 20% serum was used as a control and medium without serum (serum starvation) was used to induce apoptosis. mRNA expression levels of the two proteins were normalised to mRNA expression levels of porphobilinogen deaminase (PBGD) and compared to the mRNA expression levels at time point 0 (t=0). Ratios of cyclin E and tTG mRNA expression levels were calculated to make a quantified comparison of proliferation vs. apoptosis. Results are shown as mean ± standard errors of the mean of separate triplicate experiments. Cyclin E and tTG mRNA expression levels of cells cultured on native and crosslinked collagenous scaffolds were analysed in the same way. These levels were also normalised to PBGD mRNA expression levels, but for this purpose, expression levels of cells cultured on crosslinked scaffolds were compared with expression levels of cells cultured on native ones. Ratios of cyclin E and tTG mRNA expression levels were calculated and preliminary results are shown as mean ± standard errors of the mean of duplicate experiments.

Results and discussion

Human umbilical vein SMCs, were cultured on porous films composed of collagen and elastin. Cells adhered and grew in multilayers on top of the porous films and (to a lesser extent) in between the fibres of collagen and elastin after 14 days of static culturing. No influence of crosslinking of the scaffolds on growth behaviour of SMCs was observed by histology (see Fig. 1).

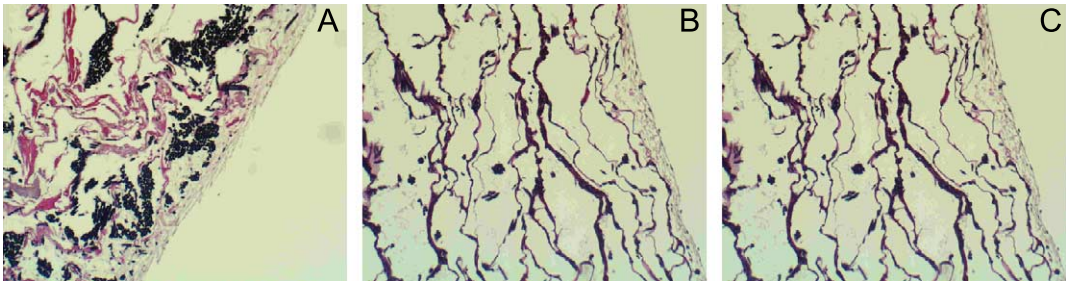

Fig. 1. Histology (EG) of human umbilical vein SMCs cultured for 14 days on porous scaffolds composed of collagen and elastin. Scaffolds were noncrosslinked (A) or crosslinked with a carbodiimide (B) or crosslinked with a diamine in the presence of a carbodiimide (C).

When SMCs were cultured on g-TCPS, tTG mRNA expression levels increased after 12 h in SMCs cultured in medium without serum as shown in Fig. 2A. Control cell cultures did not show this increase. Cyclin E mRNA expression levels were less influenced by serum starvation (Fig. 2B). mRNA expression levels of cyclin E and tTG were nearly constant in cells cultured on g-TCPS in medium with 20% serum. Ratios of cyclin E and tTG mRNA expression levels showed a significant reduction during growth of cells in medium without serum compared to medium with 20% serum as expected (Fig. 2C).

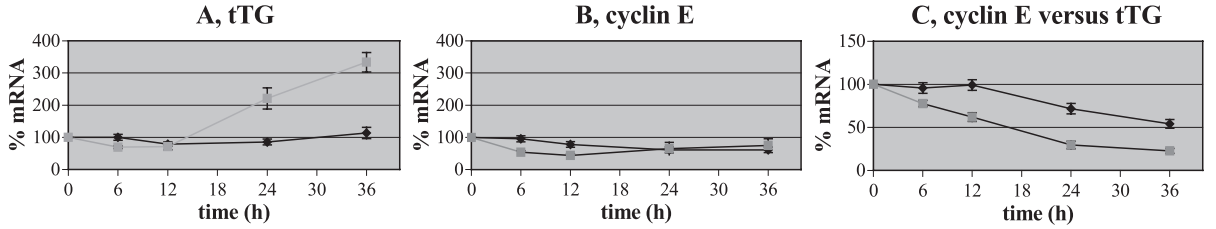

Fig. 2. mRNA expression levels of tTG (A) and cyclin E (B) and ratios of cyclin E and tTG mRNA expression levels (C) compared to levels at t=0 of umbilical vein SMCs plotted against time of culturing. Cells were cultured in medium with (control, black) and without serum (serum starvation, grey) on gelatin coated TCPS for up to 36 h.

When SMCs were cultured on TE collagenous scaffolds, no influence of crosslinking of the scaffolds on growth behaviour was observed after 7 days of culturing (Fig. 3).

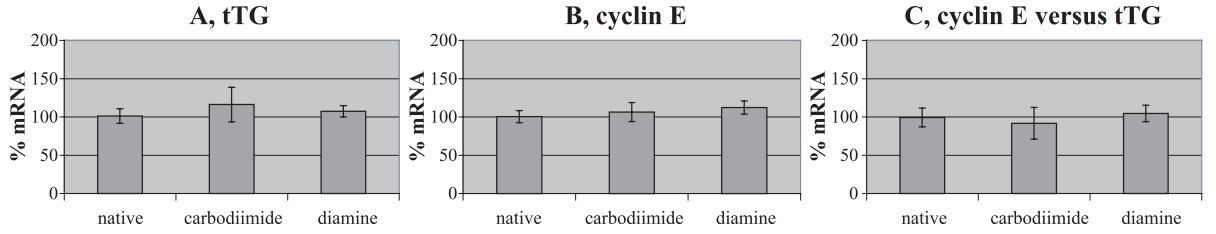

Fig. 3. mRNA expression levels of tTG (A) and cyclin E (B) and ratios of cyclin E and tTG mRNA expression levels (C) of SMCs cultured for 7 days on TE scaffolds. mRNA expression levels of cells cultured on native scaffolds were compared to expression levels of cells cultured on crosslinked scaffolds. Crosslinking was performed either with a carbodiimide or with a diamine.

Conclusions

Human vascular SMCs of mesenchymal origin adhere and proliferate on flat scaffolds composed of insoluble collagen and elastin. A new method is developed to characterize and compare cell growth behaviour of SMCs cultured on and in TE scaffolds by measuring and comparing proliferation vs. apoptosis in one single assay. Serum starvation can be used in this test as a positive control for tTG mRNA expression levels. Crosslinking of TE scaffolds either with a water-soluble carbodiimide or with a diamine, in presence of the carbodiimide does not influence SMC cell behaviour. In addition this new method can be used to characterize and compare cell growth behaviour of different batches of cells. We suggest that with this approach, it will be possible to culture cells in a standardized way not only for obtaining an artificial media of a TE blood vessel using SMCs but also for all kinds of tissue-engineering purposes using other cell types.

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ELECTROSPINNING COLLAGEN AND ELASTIN FOR TISSUE ENGINEERING SMALL DIAMETER BLOOD VESSELS

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Summary

Nonwoven fibrous matrices of collagen and elastin have been prepared by means of electrospinning. Variations in the morphology of the scaffolds have been evaluated as a function of the ratio of the two proteins. Scaffolds having an architecture resembling the media of natural blood vessels have been obtained. Crosslinking has been used as a valuable method to improve the stability of such matrices. The obtained nonwoven matrices can be used for tissue engineering applications.

Introduction

Tissue engineering of small diameter (<6 mm) blood vessels is regarded as an excellent opportunity to overcome the problems and poor performance of synthetic artificial blood vessels. Our approach comprises the design and development of a biodegradable porous three-layered tubular scaffold. Electrospinning is used to reach this aim. In this technique, a polymer solution is subjected to an electric field that permits the formation of a fibre from a charged jet. An advantage of this technique is that fibres having a diameter in the range of a few hundred nanometres can be produced. This permits to obtain scaffolds with a large surface area and a high porosity, two essential characteristics for cell culturing.

Experimental methods

Solutions of collagen/elastin/PEO (C:E:P) are prepared in hydrochloric acid, containing NaCl and are then charged in a syringe connected to a syringe pump in a horizontal mount. The solution is directed to a capillary blunt needle tip through a silicone tube. An electronic potential is applied to the needle by an electrode connected to a high-voltage supply. Fibres are collected either on a rotating mandrel placed between the capillary tip and a grounded aluminium plate or on the grounded aluminium plate itself. A charged steel ring is placed perpendicular to the jet at the end of the capillary tip to stabilize the jet and to direct it downwards (Fig. 1).