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New biological assay to test viability of cultured cells for in vitro research applications

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Introduction

The balance between apoptosis and proliferation of vascular smooth muscle cells (SMCs) is responsible for mediating profound changes in vascular architecture in development and disease. New insights in the biology of SMCs can be important to our understanding of (patho) physiological mechanisms and for tissue-engineering (TE) applications. Here the development of a new method to characterise SMCs regarding proliferation versus apoptosis is described and the application of this method for TE purposes is showed. Proliferation and programmed cell death (apoptosis) of human umbilical vein SMCs were analysed by measuring cyclin E (1) and tissue transglutaminase (tTG) (2) mRNA expression levels with use of a semi-quantitative real-time RT-PCR method. Ratios of cyclin E and tTG were calculated to make a quantified comparison of proliferation versus apoptosis. In this way the viability of SMCs cultured on standard culture systems and on TE scaffolds for small diameter blood vessel constructs were analysed.

Methods

Umbilical vein SMCs (3) were used as a model to study the balance between proliferation and apoptosis in vitro. Cells were cultured on gelatin-coated tissue culture polystyrene (g-TCPS) in medium with 20% serum (control) and in medium without serum to induce apoptosis for up to 36 h.. For TE of small diameter blood vessels, cells were seeded by filtration 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 flat scaffolds (to increase the physical and mechanical properties) was performed either with a water-soluble carbodiimide or with a diamine. Cell attachment and growth were verified by histology. Proliferation and apoptosis of the cells were determined by measuring cyclin E and tTG mRNA expression levels respectively using a semi-quantitative RT-PCR method on a real-time TaqMan analyser (4). mRNA expression levels of the two proteins were normalised to mRNA expression levels of porphobilinogen deaminase (PBGD). mRNA expression levels of cells cultured on g-TCPS were compared to levels at time point 0 (t = 0). mRNA expression levels of cells cultured on crosslinked scaffolds were compared to levels of cells cultured on non-crosslinked (native) scaffolds. Ratios of cyclin E and tTG mRNA expression levels were calculated to make a quantified comparison of proliferation versus apoptosis and results are shown as mean ± standard errors of the mean of separate triplicate experiments.

Results

tTG mRNA expression levels increased in human umbilical vein SMCs cultured on g-TCPS in medium without serum, whereas control cell cultures did not show this increase. Cyclin E mRNA expression levels were less influenced by serum starvation. Ratios of cyclin E and tTG mRNA expression levels showed a significant reduction during growth of cells in medium without serum compared to control cell cultures as shown in figure 1A.

SMCs cultured in the porous tissue engineered flat scaffolds adhered and grew in multi layers on top of and 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. To the contrary, with the new semi-quantitative real-time RT-PCR method, an enhanced viability of cells cultured on crosslinked scaffolds was observed compared to native scaffolds as shown in figure 1B.

Conclusions

A new method is developed to characterise cell growth behaviour of SMCs by measuring and comparing proliferation versus apoptosis in one single assay. Serum starvation can be used in this test as a positive control for tTG mRNA expression levels. This new method can be used to characterise and compare cell growth behaviour of different batches of cells since standardisation of cell cultures with in vitro research is often difficult. In addition this test

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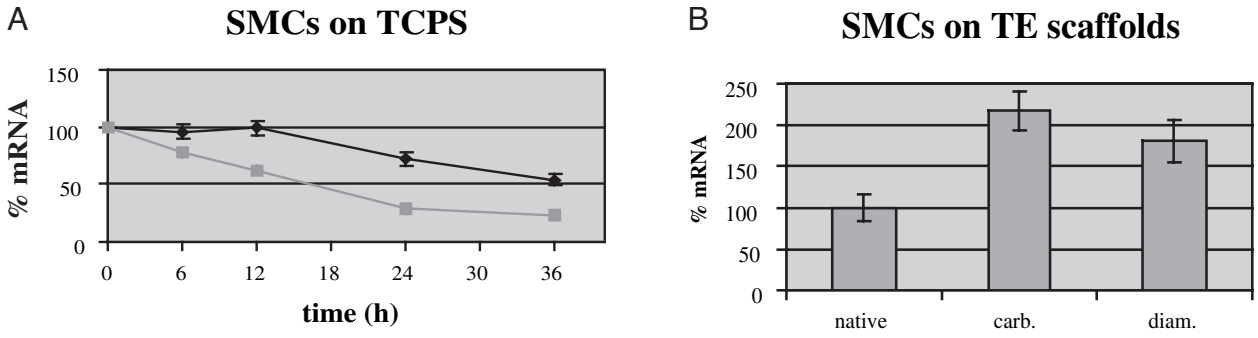

Figure 1. Ratios of cyclin E and tTG mRNA expression levels of umbilical vein SMCs cultured on g-TCPS (A) and on TE scaffolds (B). A: Cells were cultured in medium with 20% serum (–●–) and without serum (–■–) on gelatin coated TCPS for up to 36 h and mRNA expression levels were compared to time point zero. B: Cells were cultured in medium with 20% serum on native and crosslinked scaffolds and mRNA expression levels of cells cultured on crosslinked scaffolds (either with a carbodiimide (carb.) or with a diamine (diam) were compared to levels of cells cultured on native scaffolds.

can be used to compare growth behaviour of cells cultured on (and in) different scaffolds for TE applications. Crosslinking of TE scaffolds composed of collagen and elastin for small diameter blood vessel applications with a carbodiimide or with a diamine seems to be beneficial to SMC behaviour. We suggest that with this approach it will be possible to culture cells in a standardised way not only for obtaining an artificial media of a TE blood vessel using SMCs but also for all kinds of TE purposes using other cell types.

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An ELISA for the determination of lipoprotein (a) with careful accuracy targeting

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Introduction

Recently, much interest has been focused on lipoprotein(a) (Lp(a)), since there is a lot of evidence that its circulating level represents an independent risk factor for ischemic heart disease even after correction for the contribution of the classical risk factors (1). Results that Lp(a) is a strong indicator for cerebrovascular disease have also been reported (2). Because of its independent athero-thrombotic risk, a rapid, accurate and simple method for its quantification is desired. Recently, the interlaboratory-CV for Lp(a) measurement was found to be large. It could be reduced to a few percent by the conversion of mg/l units to nmol/l units (3). Consequently, an ELISA in which the monoclonal antibody associates with an epitope on the unique fragment V is now in use as a reference method (3). The conversion to the nmol/l units has the drawback that most epidemiological studies in which mg/l units are used are no longer clinically interpretable. We here describe efforts to settle the accuracy of our Lp(a) method which may give insight into the relation of old and new standardization criteria.

Materials and methods

Lp(a) (d: 1.045-1.063 g/ml) was isolated by density gradient ultracentrifugation and heparin-sepharose chromatography. By immunisation of a rabbit, specific antibodies to apo(a) were obtained not cross reacting with apolipoprotein B or plasminogen. With the IgG fraction a sandwich ELISA was developed

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