INTRODUCTION. Over the recent years methods to grow cartilage tissue in vitro from isolated chondrocytes and mesenchymal stem cells have been successfully developed. In both systems monitoring cell chondrogenic capacities is of crucial importance. Cell expansion in monolayers (2D) is a necessary step to enrich the autologous cell population prior to injection in the patient or for an in vitro cartilage implant production. Culturing cells in tissue-like systems (3D) however represents a more physiological microenvironment for the study of cartilage biology and pathology. We combined recombinant gene technology, tissue engineering and screening platforms to develop an assay where cartilage specific marker gene expression can be monitored in 2D and 3D tissue-like cultures. So far our efforts focused to optimize transfection efficiencies, to establish and characterize 2D and 3D cell cultures, and to setup a non-destructive fluorescent measurements in 96 well plate format.

METHODS. Human cartilage collected at autopsy in accordance with ethical regulations of Canton Bern, Switzerland was enzymatically digested and cultured in 2D or 3D in media supplemented with 1µg/ml of insulin and 50µg/ml ascorbic acid. hMSC were isolated from a donor (with approval) and cultured in 2D or 3D in serum free media containing 10 ng/ml TGFβ1 and 100 nM dexamethasone. Different transfection methods were performed according to manufacturer’s protocols. TaqMan real-time PCR was performed and monitored using the ABI Prism 7700 Sequence Detection System. Comparative Ct method was used to evaluate results and 18S RNA was used as the internal control for cDNA input. EGFP expression was measured with the standard fluorescent reader.

RESULTS. Different methods were tested to optimize transfection of primary human chondrocytes. Lipid-mediated FuGENE6, JetPEI, and Metafectene transfection methods resulted in less than 10% of EGFP positive cells. The Amaza nucleoporation technology resulted in transfection efficiency of 60%, while transfection using Adenoviral system resulted in the transfection efficiency of over 90% (Figure 1). To ensure that our culture conditions for chondrocytes and hMSC were appropriate for the validation of our promoter-reporter constructs (see Discussion) we performed real-time PCR analysis to determine endogenous expression levels for the collagen I, collagen II and COMP (Figure 2). Obtained results show that COMP expression follows collagen II expression during the expansion of freshly isolated chondrocytes as well as during the propagation of hMSC. Thus COMP can be used as another valid marker to monitor redifferentiation process in both cell systems. Significant differences in gene expression in 2D versus 3D indicate that hMSC represent an excellent cell system for monitoring changes in cartilage specific gene expression.

To establish a non-destructive screening system, we setup an agarose 3D culture system in the 96 well format. After transient transfection with the CMV-EGFP adenoviral vector, fluorescence was measured in a non-destructive manner for more than 10 days (Figure 3). The initial high transient EGFP expression was observed which subsequently decreased to a stable level after 8 days. The fluorescence was significantly higher when compared to the untransfected control cells, and stable over time.

DISCUSSION. Development of a cell-based assay to monitor changes in 2D and 3D chondrocytes and hMSC cultures is an important step towards a more efficient study of cartilage-modifying molecules such as growth factors, hormones or small molecule drugs. We have established the most efficient transfection protocol, 2D and 3D cell culture systems from isolated chondrocytes and hMSC, and an accurate and reproducible fluorescent read-out. Our ongoing work focuses on validation of our promoter-reporter constructs, consisting of EGFP expression plasmids with cartilage-specific promoters including collagen I, collagen II, SOX9, Cartilage Oligomeric Matrix Protein (COMP), matrix metalloproteinase 2 (MMP2) and ADAMTS4 (aggrecanase). Potential applications of our assay will include evaluation of the effects of signalling molecules, growth factors and drugs on cell proliferation and differentiation. In clinic, the assay may be a valuable quality control tool for assessing patients chondrogenic cell potential prior to implantation or in vitro cartilage growth. Furthermore, the high throughput non-destructive assay may represent a fast and efficient screening platform for drugs on micro 3D cultures to study processes in degenerative cartilage diseases such as osteoarthritis and rheumatoid arthritis.

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