

Stem cells from human exfoliated deciduous teeth differentiate into functional hepatocyte-like cells by herbal medicine

Wen-Ta Su* and Xiao-Wei Chen

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, 1 Sec. 3, Chung-Hsiao E. Rd, Taipei 10608, Taiwan

Abstract. Stem cells from human exfoliated deciduous teeth (SHEDs) are mesenchymal stem cells isolated from the exfoliated human deciduous incisor that can differentiate into a many cell types. In this study, we evaluated the effect of liquorice or angelica extracts on the hepatic differentiation potential of SHEDs cells. SHEDs cells cultured in medium containing liquorice extracts were analyzed for 1) changes in cellular morphology, 2) changes in hepatic gene expression, AFP (Alpha-fetoprotein) and ALB (Albumin), and 3) albumin secretion and urea synthesis activity. Our data show that the hepatic differentiation potential of SHEDs cells is enhanced by the presence of liquorice or angelica extracts in the culture medium. Our findings present new therapeutic possibilities for liver damage repair.

Keywords: SHEDs, liquorice, angelica, differentiation, hepatocytes

1. Introduction

Liver is a primary internal organ involved in almost all physiological activities directly or indirectly. Chronic hepatic failure usually requires organ transplantation, which is limited by donor shortage and high mortality rates. Hepatocyte transplantation is simpler and less expensive than whole liver transplantation. However, this form of therapy is limited by the low availability of mature functional hepatocytes. The therapeutic potential of stem cells in liver diseases and regeneration has been confirmed *in vitro* and *in vivo* [1,2]. These stem cells can differentiate into hepatocyte-like cells capable of performing routine liver functions such as albumin production and urea metabolism [3]. Adult stem cell is an appropriate cell source with plasticity and has no ethical issues attached. Therefore, SHEDs cells are an appropriate choice for hepatic cell therapy [4,5].

SHEDs cells may serve as a suitable cellular source in tissue engineering [6]. SHEDs cells offer the advantage of rapid proliferation, easy isolation, and vast differentiation, making them a valuable resource of stem cells for the regeneration and repair of craniofacial defects, tooth loss, and bones [7].

Herbal medicine has been used to treat or prevent liver disease in Eastern countries. More recently,

*Corresponding author: Wen-Ta Su, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, 1 Sec. 3, Chung-Hsiao E. Rd, Taipei 10608, Taiwan. Tel.: +886-2-27712171 ext. 2554; Fax: +886-2-27519204; E-mail: f10549@ntut.edu.tw

standardized herbs have been prescribed for patients with liver dysfunctions. The extracts of liquorice and angelica are used in traditional Chinese medicine and have many medicinal functions. This study examines the effect of liquorice or angelica extracts on the hepatic differentiation potential of SHEDs cells.

2. Materials and methods

2.1. Isolated process of stem cells and routine culture

SHEDs cells were first isolated from three different individuals according to the procedure outlined by Miura et al. [8]. Cells were then suspended in α MEM medium (Gibco) supplemented with 10% FBS, 20.0 ng/mL of BFGF and 100.0 μ M Ascorbic acid for cellular proliferation. Cell surface marker measurements were determined using flow cytometry (BD-FACS, Taiwan) and analyzed using the WinMDI software.

2.2. Cellular morphology observation

SHEDs cells seeded on tissue culture petri dishes were cultured in the presence of liquorice (1 mg/mL) or angelica (0.1 mg/mL) extracts in the growth medium. Control cells received no herbal supplement. Changes in cellular morphology were examined using fluorescence microscopy (Zeiss Axioplan 2; Axiovision software).

2.3. Immunocytochemistry and functional evaluation by ELISA performing

SHEDs cells grown on petri dishes were fixed with 4.0% formaldehyde solution for 0.5 h, followed by washes with Phosphate-Buffered Saline (PBS). Cells were then incubated with anti-AFP (1:100) or anti-ALB (1:100) antibodies for 1 h, washed three times with PBS, followed by the addition of secondary antibody Alexa Fluor (1:100) for 1 h.

As a measure of hepatocyte function, albumin secretion and urea synthesis were determined using the Albumin assay kit (DIUR-250) and Urea assay kit (DIUR-500) respectively. Glycogen accumulation was determined by Periodic acid-Schiff staining.

Table 1

The sequences of primers used for the real time PCR

Gene name	primer sequences
AFP	F: 5'- CACTGCTGCAACTCTTCGTA-3'
	R: 5'- CTTTGGACCCTCTTCTGTGA-3'
ALB	F: 5'- TCTTCGTCTCCGGCTCTG-3'
	R: 5'- CTGGCAACTTCATGCAAAT-3'
GAPDH	F: 5'-ATGAGAAGTATGACAACAGCC-3'
	R: 5'-AGTCCTTCCACGATACCAA-3

2.4. Gene expression by RT-PCR

Total RNA from SHEDs cells were extracted using TRIzol. 500 ng of RNA was used per cDNA synthesis reaction using SuperScript® III Reverse Transcriptase kit according to manufacturer's instructions (Invitrogen). RT-PCR was performed using target genes and GAPDH as a control. The sequences of PCR primers are listed in Table 1.

3. Results and discussion

3.1. Characterization of SHED cells

As determined by flow cytometry, SHEDs cells expressed markers of mesenchymal stem cells, such as CD90 (99.3%), CD73 (99.2%), OCT-4 (96.4%), CD105 (44.5%) and SSEA4 (64.2%). However, isolated cells do not express CD34 or CD45. These results suggest that like mesenchymal stem cells, SHEDs cells have the ability to differentiate into multiple cell lineages, making them attractive candidates for tissue engineering [9]. The cellular morphology after 21 days of herbal induction is shown in Figure 1.

Immunocytochemistry and functional evaluation In order to verify the differentiation potential of SHEDs cells into liver cells [10], SHEDs cells were incubated in culture medium containing extracts of liquorice or angelica, followed by examination of hepatic markers. According to Figure 2, cultured

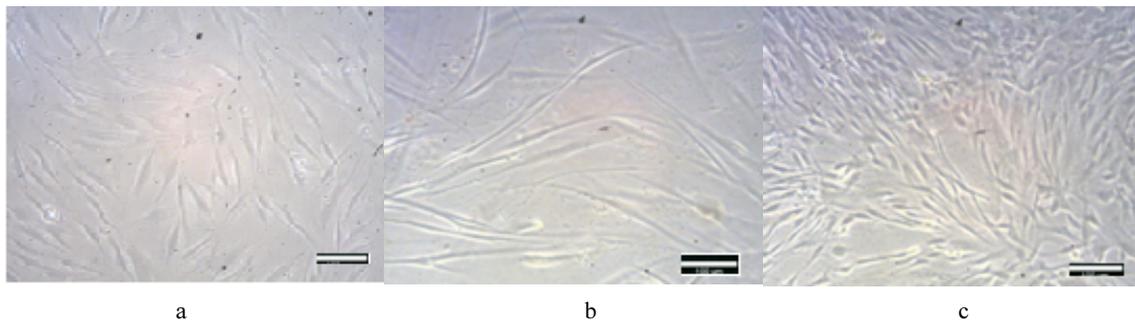


Fig. 1. Cellular morphology of non-induced (a) and induced SHEDs cells after 21 days culture (b: liquorice inducing, c: angelica inducing). Scale bar 100 µm.

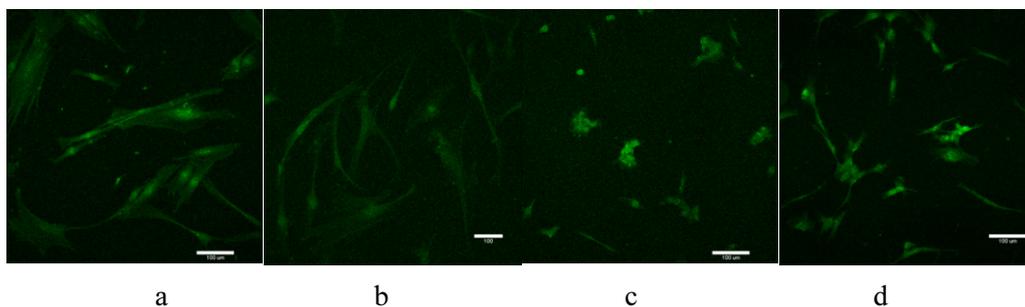


Fig. 2. The AFP (a) and ALB (b) shown by liquorice inducing, and AFP (c) and ALB (d) represented by angelica inducing. Scale bar 100 µm.

SHEDs cells show positive staining for hepatic markers such as AFP and ALB after 21 days in culture. These results show that in the presence of liquorice or angelica, SHEDs cells can readily differentiate into liver cells in vitro.

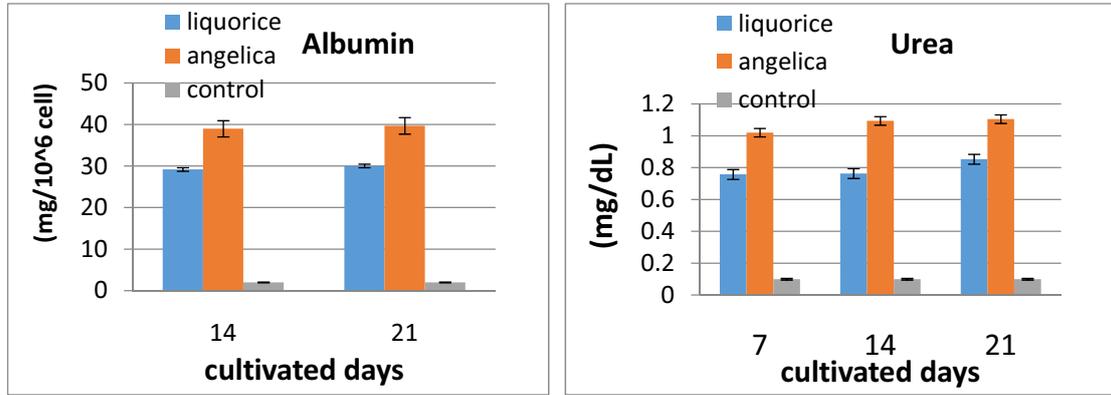


Fig. 3. The urea synthesis capacity and albumin secretion activity of differentiated cells. Values are expressed as mean ± SD (n=3), * p<0.05

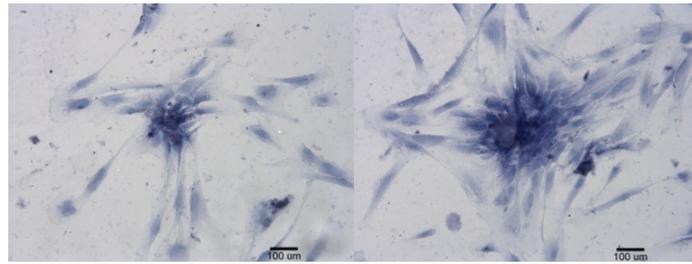


Fig. 4. The staining of glycogen accumulation by liquorice inducing (left) and angelica inducing (right). Scale bar 100 μm.

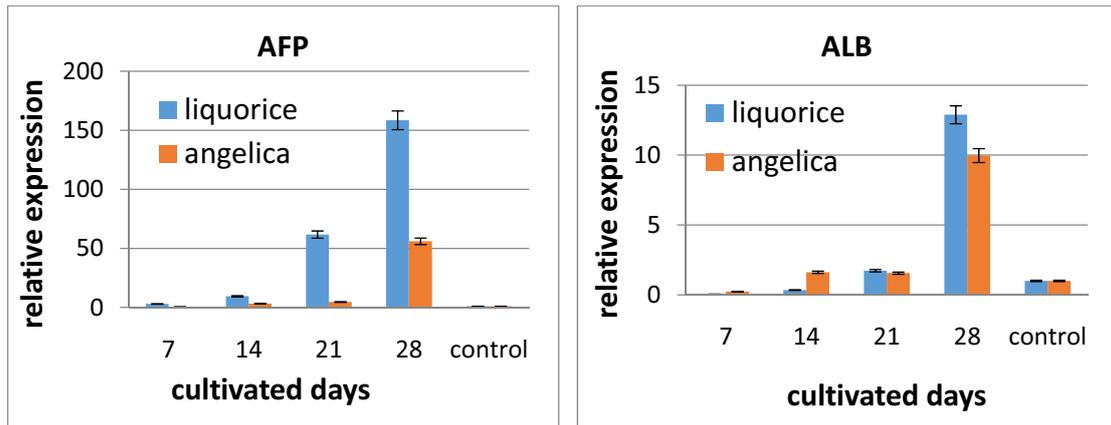


Fig. 5. The gene expression of AFP and ALB after liquorice and angelica inducing. Values are expressed as mean ± SD (n=3), * p<0.05

The albumin secretion and urea synthesis activity of differentiated cells are shown in Figure 3. Angelica extracts induced both higher albumin secretion and urea synthesis than liquorice extracts at various culturing times. Figure 4 show herbal induced glycogen accumulation

3.2. Gene expression

Herbal induction of AFP and ALB gene expression was observed in Figure 5 [11]. Notably, liquorice induction was higher than that of angelica for all the time points tested. Based on these results, both the extracts of liquorice and angelica can induce hepatic differentiation from SHEDs cells. While liquorice extracts induced higher expression of AFP and ALB, angelica was better at promoting albumin secretion and urea synthesis.

4. Conclusion

This study confirmed that herbal medicines such as liquorice and angelica can induce the hepatic differentiation of SHEDs cells, which may provide a cellular based therapy for hepatic failure.

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