

Short Communication

Body extract of tail amputated zebrafish promotes culturing of primary fin cells from glass catfish

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The most spectacular regenerative events in vertebrates are epimorphic regeneration. In this study, interestingly, a whole-body extract 24 h after tail amputation enhanced primary cell growth and viability compared to that of a non-tail amputated body. Additionally, these effects of extract treatment *in vitro* were dose-dependent occurring at concentrations of 0.02, 0.05 and 0.1 mg/ml. This is the first *in vitro* study on the interaction between primary fin cells from glass catfish and tail amputated body extracts of zebrafish. These results provide an essential knowledge base for rational approaches to tissue and organ regeneration.

Key words: Cell growth, cell viability, extract, glass catfish, regeneration, zebrafish.

INTRODUCTION

All organisms have a biological response to damage, but their ability to recover varies extensively. Mammals contain several organ systems capable of regeneration, such as the blood and liver, but are somewhat disadvantaged when compared with amphibians and teleost fish, which have a remarkable capacity to regenerate damaged organs including heart, spinal cord, retina, and limbs/fins (Akimenko et al., 2003; Brockes and Kumar, 2002).

After Broussonet (1786) reported that an adult fish could completely regenerate its fins after amputation, many studies focusing on fish fin regeneration have been conducted to examine the regeneration mechanism (Akimenko et al., 1995; Poss et al., 2000). In particular, small teleost fish such as the zebra fish (*Danio rerio*) have emerged as powerful animal models for under-

standing epimorphic regeneration for the following reasons: (1) the comparatively simple structure of the fin means fewer cell types are involved in the regeneration process, (2) much quicker regeneration times and ease of raising a large number of animals in the laboratory, (3) an expanding body of useful molecular reagents are available for extensive research on zebrafish embryology, and (4) the availability of genetic approaches (Johnson and Weston, 1995).

In this study, we describe that *in vitro* primary cell growth and viability was increased by a zebrafish body extract after tail (caudal fin) amputation. As stated, fish fins can fully regenerate their missing structures in several days after amputation, so we questioned whether this *in vivo* regenerative action could also affect *in vitro* cell growth. It has long been suggested that an adequate nervous network is required to regenerate limbs and fins (Singer, 1952; Goss and Stagg, 1957; Stocum, 2004).

It has also been suggested that blood vessels play a role in regeneration (Peadar and Singer, 1966; Huang et al., 2003). Here, we did not focus on specific factors required for regeneration but used a whole-body extract, because fin regeneration cannot be modulated by one factor.

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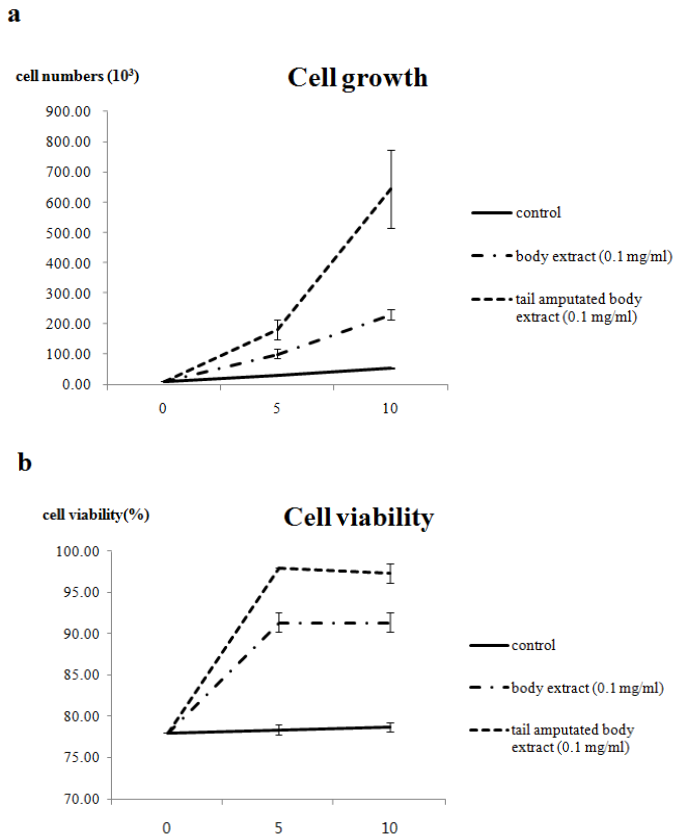


Figure 1. Compared to a non-tail amputated body extract, whole-body extract 24 h after tail amputation enhanced (a) cell growth and (b) cell viability of primary glass catfish cells (means \pm S.E.M).

MATERIALS AND METHODS

Fifty (50) zebrafish (three months old) were reared in a 70 L glass aquarium equipped with aeration at a water temperature of 26°C (Yin et al., 2008). Twenty-five (25) fish were anesthetized by immersion in water containing 0.6 mg/ml ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich, St. Louis, USA), and amputations were made using a razor blade to remove one-half of the caudal fin as described previously (Akimenko et al., 1995; Poss et al., 2000). Fish were returned to their tanks and reared without feed.

Several reports have described that the blastema, a hallmark of epimorphic fin regeneration, is formed, and that migration of epithelial cells and disorganization of mesenchymal tissue also begins within 24 h after fin amputation (Poss et al., 2003; Poleo et al., 2001). Therefore, 24 h after amputation, whole-body extracts were prepared as described by McGann et al. with some modifications (McGann et al., 2001) in both tail amputated and non-tail amputated zebrafish. Briefly, fish were sacrificed and whole bodies were kept in a -80°C freezer overnight, collected in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Eggenstein, Germany) with 1% (v/v) penicillin-streptomycin (P/S, Gibco), and hand-homogenized for 10 min. Cell debris that remained insoluble in DMEM was removed by centrifugation at 15000 \times g for 25 min. The supernatant was filter sterilized through a 0.22- μ m syringe filter.

Caudal fin cells of glass catfish established in the Laboratory of Aquatic Animal Medicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University

(Han et al., 2011) were used to generate primary fin cells. Glass catfish has a genetic resource value because of their naturally transparent body and limited availability (Lim, 1999). The cells were cultured in DMEM with 1% P/S and 10% (v/v) fetal bovine serum (Gibco) in an incubator at 26°C with a humidified atmosphere of 5% CO₂.

Viable cells (1.2×10^4 cells/ml) were replated and incubated in the whole-body extracts containing culture medium for 5 days. The composition of the extract in culture medium was as follows: Group 1 was non-tail amputated zebrafish extract at a final concentration of 0.1 mg/ml; group 2, tail amputated zebrafish extract at a final concentration of 0.1 mg/ml, group 3 was tail amputated zebrafish extract at a final concentration of 0.05 mg/ml, and group 4 was tail amputated zebrafish extract at a final concentration of 0.02 mg/ml. The cell number before plating was normalized with a hemocytometer, and each treatment was performed in triplicate. Cell proliferation and viability of different groups of cells were assessed after a 5 day incubation using an automated cell counter (Invitrogen, Carlsbad, Ca, USA) after 0.4% trypan blue staining (Cell viability % = the number of viable cells \times 100 / the number of total cells).

RESULTS AND DISCUSSION

Interestingly, *in vitro* cell growth and viability were affected by both whole-body extracts from amputated and non-amputated fish (Figures 1a and b). Thus, we speculated that the whole-body extract of zebrafish acted as an enhancer of primary cell culture. However, cell proliferation and viability were promoted more after treatment with tail amputated zebrafish extract for five days compared to treatment with non-tail amputated whole-body extract. Although not as high as the early treatment, cell proliferation and viability were also significantly enhanced by treatment with tail amputated zebrafish extract at days 5 to 10 corresponding to the result from days 0 to 5. Thus, it was clear that some major factors expressed at 24 h after fin amputation could affect *in vitro* cell cultures for at least 10 days, although the molecules and signals implicated in regeneration may vary widely.

Our studies also revealed that cell growth and viability were influenced by the concentration of tail amputated body extract and that growth and viability increased gradually depending on extract concentration (0.02, 0.05, or 0.1 mg/ml). However, no significant difference in cell proliferation was observed between the 0.05 and 0.1 mg/ml concentrations (Figures 2a and b).

So far, no report has conducted an *in vitro* study on the interaction between primary fin cells and whole-body extracts of zebrafish. Moreover, the whole-body extract of tail amputated fish, which was suspected to have regeneration ability, promoted *in vitro* cell growth and viability compared with a non-tail amputated fish extract. We cannot directly say this study is related to research mammalian tissue and organ regeneration but it could provide essential knowledge for rational approaches to tissue and organ regeneration in mammals, which have low regeneration ability. Most spectacular regenerative events in vertebrates represent epimorphic regeneration,

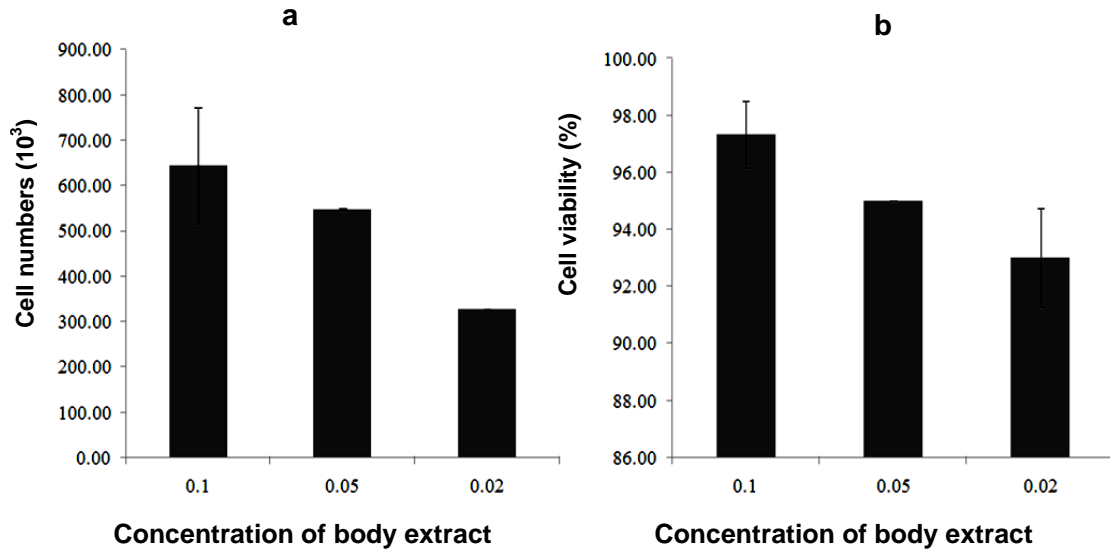


Figure 2. Effects of the *in vitro* extract treatment were dose-dependent at concentrations of 0.02, 0.05 and 0.1 mg/ml: (a) cell growth and (b) cell viability (means \pm S.E.M.).

and many recent studies have reported this ability in small teleosts such as zebrafish. However, the exact mechanism of epimorphic regeneration in teleosts has not been discovered. Future studies will be required to apply these aspects in other species and to identify specific molecules that modulate regeneration activity.

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