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Full Length Research Paper

Effect of storage media and time on fin explants culture in the goldfish, *Carassius auratus*

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The effect of storage media and time was investigated on fin explants culture in the goldfish (*Carassius auratus*). Fin explants under sterile conditions were able to produce cells at different storage media and time. On the outgrowth of cells, fin explants stored for seven days before culturing showed significantly higher growth ($P<0.05$) as observed on the fin explants stored in Dulbecco's modified Eagles medium (DMEM, 84.44%), phosphate buffered saline (PBS, 62.42%) and in control/fresh fin explants (100%), compared with the explants with no storage medium (25.56%) at day three of culture. From day seven to 14, all caudal fin explants exhibited a 100% outgrowth of cells regardless of treatment. However, caudal fin explants kept for 10 days in 4°C showed a significant difference ($P<0.05$) from fin explants stored in DMEM (96.67%), PBS (75.57%) and the control (100%), as compared with no storage medium (no growth) on day three. Moreover, only 6.67% of no storage medium group could maintain outgrowth of cells, while other treatment group reached 100% of outgrowth after 14 days of culture.

Key words: *Carassius auratus*, fin explants, goldfish, storage.

INTRODUCTION

Establishment of genome resource banks with assisted breeding technologies has the potential for preserving genetic diversity of many endangered species (Harnal et al., 2002). Tissue samples can be used to reintroduce lost genes back into the breeding pool by somatic cloning and skin cryobanking can be performed more conveniently (Silvestre et al., 2004). In the case of fish, it is essential to recover somatic cells from fin explants (Mauger et al., 2006). Fins explants are also easy to sample and they have natural regenerative capacities (Akimenko et al., 2003). In the perspective of genome cryobanking, an efficient strategy for genetic conservation requires cryopreservation of the greatest initial number of cell tissue samples from every original genotype of interest (Silvestre et al., 2002). To some extent, cryobanking of somatic tissues is a technique to keep the genetic records of fish valuables for biodiversity conservation and animal breeding when gametes and embryos are not available (Moritz and Labbe, 2008).

Piscine cell cultures are good models for *in vitro* studies of the extracellular factors that influence cell proliferation, differentiation and propagation of pathogenic fish viruses (Hashimoto et al., 1997). While many fish cell lines have been established mainly for use in fish pathology, the basic techniques have mostly been adapted from those of mammalian cells (Shima et al., 1980; Wakamatsu et al., 1984). Recent studies showed the potential of using somatic cells from fins for fish somatic cell nucleus transfer (Cardona-Costa et al., 2006). Fish cloning studies have been conducted previously on Cyprinidae species including the zebrafish and the goldfish.

In this study, the goldfish (*Carassius auratus*) was chosen as a model species because it can be considered the aquatic animal equivalent of the laboratory rat mainly

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Abbreviations: DMEM, Dulbecco's modified Eagles medium; **PBS,** phosphate buffered saline.

due to its size and abundance (Rougée et al., 2007). In addition, goldfish is one of the few fish species that have been domesticated (Balon, 2004) and is an ideal species for laboratory research involving reproduction (Stacey et al., 2001). Furthermore, goldfish is also a representative of cyprinids (Sokolowska et al., 1984; Bandyopadhyay et al., 2005). This study aimed to assess the ability of fin explants to produce cells stored at 4°C using different storage media for seven and 10 days before culturing under field conditions.

MATERIALS AND METHODS

Experimental animal

The goldfishes, *C. auratus,* used in this study were purchased from a fish pet supplier, acclimatized and then reared in a glass aquarium (30 L) in the laboratory.

Chemicals

All chemicals were purchased from Invitrogen Corporation (Carlsbad, CA, USA), unless otherwise stated.

Explants collection and culture

The method of Choresca et al. (2009) for establishing the goldfish explants was followed with some modifications. Briefly, goldfish caudal fins were wiped with sterile cotton swabs immersed in 70% alcohol to remove the mucus, and then a portion of the caudal fin was cut with sterile scissors to establish fin explants. Fin explants (10 to 15 cut explants) from goldfish caudal fins were stored either in Dulbecco's modified Eagles medium (DMEM) or phosphate buffered saline (PBS) or without any storage medium as experimental design. Fresh sample without storage was also used as a control. After storage, fin tissue samples were rinsed and washed in DMEM medium and then immediately placed in culture medium containing DMEM supplemented with 1% (v/v) P/S and 10% fetal bovine serum (v/v) in 60 mm culture dishes. A 10% (v/v) collagenase type I solution was added to the culture media for enzymatic digestion in establishing the primary culture and removed after three days of culture. Culture dishes were placed in an incubator at 26°C under a humidified atmosphere of 5% CO_2 and medium was changed every three days over the experimental period. The ability of fin explants to produce cells was assessed after three to 14 days of culture.

Experimental treatments

In experiment 1, the effect of different storage media on the ability of the plated explants to produce cells in culture stored at 4°C in seven days was determined and in experiment 2, the effect of different storage media on the ability of the plated explants to produce cells in culture stored at 4°C in 10 days was evaluated. Fin explants from goldfish caudal fin were stored either in DMEM, PBS, with no storage medium and control/fresh explants.

Explant rating and statistical analysis

Percentage of cell donor explants was expressed by number of explants surrounded by growing cells divided by total plated explants multiplied by 100. All treatments were replicated three times. Results of the experiments were analyzed using one-way analysis of variance (ANOVA). Percentages were arcsine transformed prior to analysis. Comparisons of mean values among treatments were performed using Duncan's multiple range test (DMRT). All statistical analyses were performed using Statistical Program for Social Sciences (SPSS Inc., Chicago, IL, USA). Differences were considered significant at a P value of < 0.05.

RESULTS AND DISCUSSION

Obtaining fresh tissue samples and establishing cell lines under laboratory conditions is manageable, but doing this under field conditions is totally different and significantly more challenging with the need for materials and equipment to maintain the status of tissues in a sterile environment. Mauger et al. (2006) reported that sampled fins can be successfully stored up to three days at room temperature in a sterile environment. However, in field conditions the facilities can be far from the laboratory and there are many samples that need to be processed for culture. In this experiment, we tested different storage media and time by keeping the goldfish fin explants stored at 4°C for seven and 10 days.

It was observed that the fresh sample without storage showed 100% of outgrowth cell culture in both experiments. In experiment 1, results show that fin explants under sterile conditions were able to produce cells in culture regardless of storage media and exhibited 100% outgrowth of cells after seven days of culture. However, cell outgrowth were significantly reduced in no storage medium group (25.56%) compared to control/fresh fin explants (100%) or DMEM (84.44%) and PBS (62.42%) treated groups (Figure 1). However, in Figure 2, fin explants kept for 10 days at 4°C, showed a significant difference (P<0.05) compared to fin explants stored in DMEM (96.67%), PBS (75.57%) and in control (100%), compared with no storage medium (no growth) on day three. Also, no increase of outgrowth of cells was observed in the non-storage medium (6.67%) compared with the other treatments (100%) on day 14.

In addition, it was noted that explants stored in DMEM and PBS produced a high number of outgrowing cells. This may be attributed to the intervention and composition of storage media like vitamins, amino acids and sugars, as in the case of DMEM. Moreover, in the case of PBS it was attributed to the saline that keeps preserving the sample. It was reported that various media like DMEM, PBS, Ham's F-12 and other medium have been used in the past as preservation media in skin grafts (Tosun et al., 2001; De et al., 2008). In addition, human skin samples can be conserved in saline solutions (Cetin et al., 2000) or in culture medium (Rosenquist et al., 1988) for 30 days at 4°C. Storage at 4°C is favorable for maintaining the quality of fin explants and this is consistent with the work of Moritz and Labbe (2008), where storage of the fins at 4°C prevented tissue damage and maintained fin ability for cryopreservation.

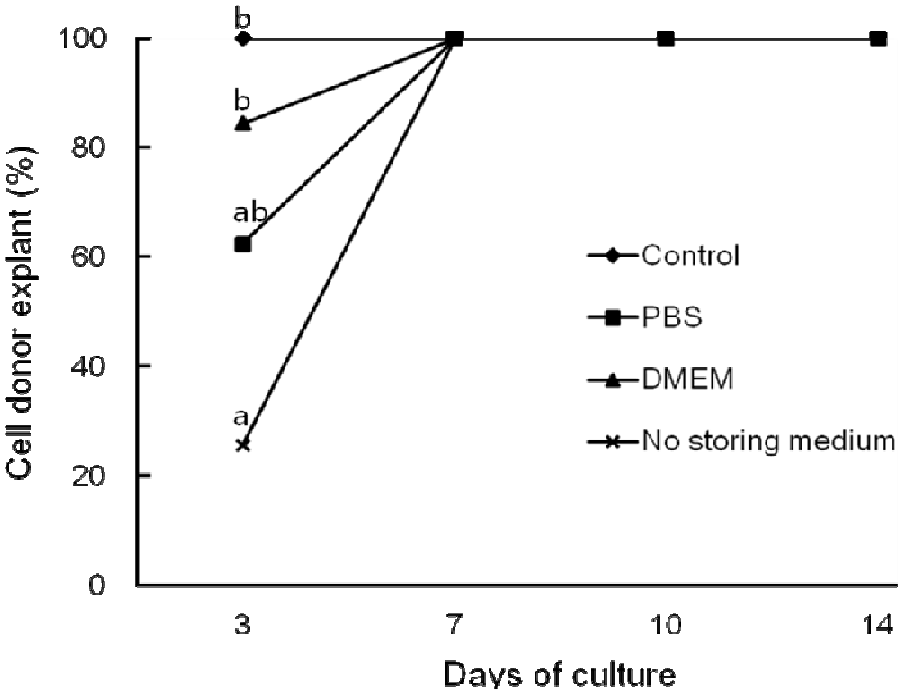

Figure 1. Effect of different storage media on the ability of the plated explants to produce cells in culture stored at 4°C in seven days. Significant differences (P<0.05) are indicated by different superscripts.

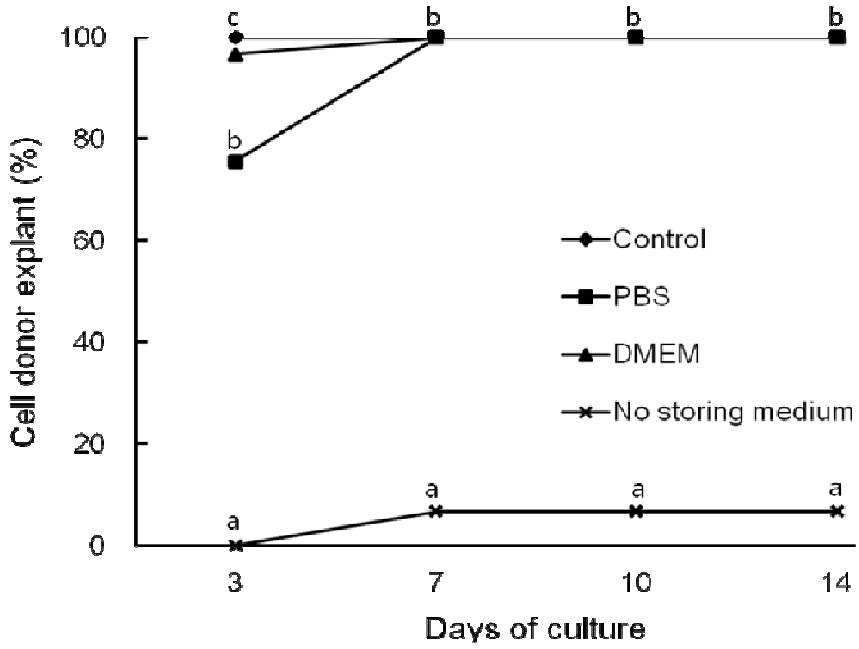

Figure 2. Effect of different storage media on the ability of the plated explants to produce cells in culture stored at 4°C in 10 days. Significant differences (P<0.05) are indicated by different superscripts.

Based on the results of this study, we concluded that caudal fin explants under sterile conditions were able to produce cells at different storing media kept for seven or 10 days at 4°C. This shows that explants can be main-

tained in storage medium in a week prior to culture and can be applied under field conditions.

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REFERENCES

Akimenko MA, Mari-Beffa M, Becerra J, Geraudie J (2003). Old questions, new tools and some answers to the mystery of fin regeneration. Dev. Dyn. 226: 190-201.

Balon EK (2004). About the oldest domesticates among fishes. J. Fish Biol. 65: 1-27.

Bandyopadhyay P, Swain SK, Mishra S (2005). Growth and dietary utilization in goldfish (*Carassius auratus*) fed diets formulated with various local agro- produces. Bioresour. Technol. 96: 731-740.

Cardona-Costa J, Roig J, Perez-Camps M, Garcia–Ximenez F (2006). Vitrification of caudal fin explants from zebrafish adult specimens. Cryoletters. 27(5): 329-332.

Cetin C, Kose AA, Aral E, Ercel C, Tandogdu O, Karabagli Y, Ozyilmaz M (2000). The effects of saline and plasma on skin graft keratinocyte viability. Br. J. Plast. Surg. 53: 418–419.

Choresca Jr. CH, Koo OK, Oh HJ, Hong SG, Gomez DK, Kim JH, Lee BC, Park SC (2009). Different culture conditions used for arresting the G0/G1 phase of the cell cycle in goldfish (*Carassius auratus*) caudal fin-derived fibroblasts. Cell Biol. Int. 33: 65-70.

De A, Mathur M, Gore MA (2008). Viability of cadaver skin grafts stored in skin bank at two different temperatures. Indian J. Med. Res. 128: 769-771.

Harnal VK, Wildt DE, Bird DM, Monfort SL, Balou JD (2002). Computer simulations to determine the efficacy of different genome resource banking strategies for maintaining genetic diversity. Cryobiology. 44: 122-131.

Hashimoto H, Toyohara H, Yokoyama Y, Sakaguchi M, Ozato K, Wakamatsu Y (1997). Effect of carp serum on the growth of the goldfish fin cells in early passage. J. Fish Biol. 50: 201-207.

Mauger PE, Le Bail PY, Labbe C (2006). Cryobanking of fish somatic cells: optimizations of fin explants culture and fin cell cryopreservation. Comp. Biochem. Physiol. B. 144: 29-37.

Moritz C, Labbe C (2008). Cryopreservation of goldfish fins and optimization for field scale cryobanking. Cryobiology. 56: 181-188.

Rosenquist MD, Cram AE, Kealey GP (1988).Skin preservation at 4°C: a species comparison. Cryobiology, 25: 31-37.

Rougée L, Ostrander GK, Richmond RH, Lu Y (2007). Establishment, characterization, and viral susceptibility of two cell lines derived from goldfish, *Carassius auratus* muscle and swimbladder. Dis. Aquat. Org. 77: 127-135.

Shima A, Nikaido O, Shinohara S, Egami N (1980). Continued *in vitro* growth of fibroblast-like cells (RBCF-1) derived from the caudal fin of the fish, *Carassius auratus*. Exp. Gerontol. 15: 305-314.

Silvestre MA, Saeed AM, Escriba MJ, Garcia-Ximenez F (2002). Vitrification and rapid freezing of rabbit fetal tissues and skin samples from rabbits and pigs. Theriogenology, 58: 69-76.

Silvestre MA, Sanchez JP, Gomez EA (2004). Vitrification of goat , sheep, and cattle skin samples from whole ear extirpated after death and maintained at different storage times and temperatures. Cryobiology, 49: 221-229.

Sokolowska M, Peter RE, Nahorniak CS, Pan CH, Chang JP, Crim LW, Weil C (1984). Induction of ovulation in goldfish, *Carassius auratus*, by pimozide and analogues of LHRH. Aquaculture, 36: 71-83.

Stacey N, Fraser EJ, Sorensen P, Vander Kraak G (2001). Milt production in goldfish: regulation by multiple social stimuli. Comp. Biochem. Physiol. 130: 467-476.

Tosun Z, Aktan TM, Hosnuter M, Savaci N, Duman S (2001). Investigation of skin graft viability with DMEM and Ham's F-12 fluids. Ann. Plast. Surg. 47: 217-218.

Wakamatsu Y, Oikawa A, Obika M, Hirobe T, Ozato K (1984). Fish hereditary melanoma cell lines of different degrees of cell differentiation. Dev. Growth Differ. 25: 503-513.