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Marine mammal cell cultures: To obtain, to apply, and to preserve

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ABSTRACT

The world's oceans today have become a place for the disposal of toxic waste, which leads to the degradation of marine mammal habitats and populations. Marine mammal cell cultures have proven to be a multifunctional tool for studying the peculiarities of the cell physiology and biochemistry of these animals as well as the destructive effects of anthropogenic and natural toxicants. This review describes the sources of marine mammal live tissues and the methods required for establishing cell cultures, their use, and long-term storage. Approaches to conserving rare animal species by applying cell biology methodologies are also discussed.

Contents

1. Introduction

The extinction of some mammal species is a natural and inevitable evolutionary process. But today it prevails over speciation, mainly due to human activities such as the destruction of mammalian habitats, industrialization, anthropogenic pollution, excessive

Abbreviations: iPSC, induced pluripotent stem cells; MICA, major histocompatibility complex class I chain related protein A.

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hunting, and so forth. (Holt and Pickard, 1999; Kasuya, 2008). The conservation of every single species is required to maintain biodiversity, because eliminating any species can result in the malfunction of an entire ecosystem (Myers et al., 2000). The world's oceans are becoming a place for the toxic waste disposal of hydrocarbons (mainly petroleum-derived), organochlorine and organophosphorus derivatives (pesticides, fungicides, herbicides, and polychlorinated biphenyls), heavy metal compounds (mercury, cadmium, chromium, lead and so on), and radionuclides. Most of these are relatively stable chemically, essentially being non-biodegradable. The ocean's top predators such as marine mammals appear to be the most susceptible to these xenobiotics, which increase in concentration with every step up the food chain (Fossi et al., 2000; Whittle et al., 1977). Many toxins have a lipophilic nature and are accumulated in the substantial fat tissues of these animals (Aguilar and Borrell, 1991; Corsolini et al., 1995; Marsili, 2000). In addition, the sensitivity of some marine mammals to toxic agents increases due to the specificity of the cytochrome-oxidase systems functioning in their cells (Tanabe and Tatsukawa, 1992; Watanabe et al., 1989), and consequently, their inability to quickly neutralize the xenobiotic. Toxins provoke disorders in the endocrine, reproductive (Corsolini et al., 1995), immune, and nervous systems (Geyer et al., 1984), increasing the risk of cancer (Gauthier et al., 1998) and early death, and thereby dramatically decreasing the abundance of mature marine mammal individuals, populations, and species, in general (Fossi and Marsili, 2003; Kasuya, 2008; Pomeroy, 2011).

Before the middle of the 1990s, most ecotoxicological studies for assessing the negative effects of xenobiotics on marine mammals were conducted on dead animals; about 40% of research sacrificed the animals, while 60% was performed on stranded animals or those who accidently died in captivity (Fossi and Marsili, 1997; Fossi et al., 1997a). Today, chemical tests on live marine mammals are considered highly unethical and are prohibited by international laws. Maria Cristina Fossi and Letizia Marsili and their coauthors have often suggested using only “non-destructive tools and biomarkers” (Fossi, 1994; Fossi et al., 1999; Fossi and Marsili, 2003; Fossi et al., 1997b, 2000, 2003, 2010). The general idea is to develop methods for collecting, processing, and comprehensively analyzing biological material from marine mammals with no or minimal harm to the animals as a means of continuously monitoring their health and to identify the species and populations at risk. Almost any tissue from a dead mammal (by accident, illness or stranded) or blood, skin, and feces samples from living animals can be used to gather extremely valuable data on the current status of xenobiotic pollutants (Fossi et al., 2000, 2003, 2007, 2013; Marsili et al., 2014), their accumulation (Marsili, 2000; Marsili et al., 2014; Sorensen et al., 2008) and influence on metabolism within different organs (Fossi and Marsili, 1997, 2003; Sakai et al., 2004; Sorensen et al., 2008; Stegeman and Hahn, 1996), the variations marine mammal feeding (Marcoux et al., 2007), and the expression of genes involved in the immune response to contaminants (Lunardi et al., 2016; Mancia et al., 2014). However, once isolated from the living biological material and properly stored, cell cultures can be used as a model system of an entire animal, organ, or tissue for an almost unlimited number of tests, whenever necessary, worldwide, and under precisely controlled conditions. These advantages are turning marine mammal cell cultures into an irreplaceable multifunctional instrument in physiological, biochemical, genetic, and ecotoxicological studies, replacing the use of whole animals.

2. Establishing the cell culture

2.1. Collection of biological material

The less invasive approaches to taking biological material from living marine mammals are skin biopsy (Ellis et al., 2009; Jin et al., 2013; Yu et al., 2005) and blood collection (Bogomolni et al., 2016a; Frouin et al., 2008). A punch biopsy tool (2–6 mm in diameter and 2–4 mm deep) or scalpel is used to take skin samples from an animal that is caught, sampled, marked (if needed), and released (Boroda et al., 2015; Lahvis et al., 1993; Mollenhauer et al., 2009; Wang et al., 2011). Before biopsy, the skin must be disinfected with 70% ethanol, and after sampling, the wound must be treated with anti-inflammatory ointment (Jin et al., 2013). In the case of wild animals, darts equipped with sterilized stainless steel collecting tips of different diameters (4–10 mm) and lengths (10–40 mm) are used depending on the animal species and size; the dart is fired with a crossbow or a pneumatic gun or used by mounting the tip on a pole (Marsili et al., 2000). A detailed illustration of the dart has been published by Mathews and colleagues (Mathews et al., 1988). However, obtaining sterile tissue samples from live marine mammals is almost impossible. Thus, once taken, the skin pieces must be briefly rinsed in 70–72% ethanol (Mathews et al., 1988; Sweat et al., 2001) and/or submerged into a mixture of concentrated antibiotics and antimycotics and stored at a temperature near 0 °C from several hours to days while it is shipped to the laboratory (Mathews et al., 1988). The mixture, which is prepared on a nutrient medium (Spinsanti et al., 2008; Wang et al., 2011; Wise et al., 2008) or in a buffer (Yu et al., 2005), should contain at least two antibiotics to efficiently suppress bacterial contamination in the event there are microorganisms present that are resistant to one of them (Lincoln and Gabridge, 1998). The commonly used antibiotics are penicillin (with the final concentration 100–500 units/ml), streptomycin (100 μg/ml) (Wise et al., 2008), gentamycin (50–100 μg/ml) and neomycin (20 μg/ml) (Lambertsen et al., 1988; Sweat et al., 2001; Wise et al., 2008; Yu et al., 2005). Amphotericin B (1–5 μg/ml) or nystatin (up to 100 units/ml) is added to prevent fungal growth (Jin et al., 2013; Marsili et al., 2000; Mathews et al., 1988; Sweat et al., 2003). The types of antibiotics and antimycotics, their concentrations, and the incubation times should be determined based on the individual animal species (*i.e.*, the skin's bacterial flora, if it is known) and the sterility of the biopsy to be conducted (Lincoln and Gabridge, 1998). We usually place the skin sample into phosphate buffered saline with 10% fetal bovine serum, 200 units/ml penicillin, and 200 μg/ml streptomycin immediately after obtaining it and transport it to the laboratory on a wet ice during 1–24 h. The sample is then transferred to a mixture of penicillin (500 units/ml), streptomycin (500 μg/ml), and the antifungal agent Primocin™ (100 μg/ml) for 20 min at room temperature. The antibiotics are removed by incubating the sample in phosphate buffered saline three times for 10 min before either isolating the cells or cryopreserving the skin sample.

Blood can be collected from superficial vessels on the ventral aspect of the tail or the flipper of both free-ranging (Lahvis et al., 1993; Ross et al., 1993) and well-trained captive marine mammals (Sorensen et al., 2008). The blood should be injected into tubes containing anticoagulants (ethylenediaminetetraacetic acid or sodium heparine) and kept cool during shipment to the laboratory within 24–30 h for further processing.

A deceased animal can also be a source of tissues for deriving viable cell cultures from any organ depending on the time passed since death: from several minutes to 6 h for blood, bone marrow, and lungs (Frouin et al., 2010; Hymery et al., 2013; Larsen et al., 2013); up to 12 h for kidney, bronchial epithelium, and testes (Carvan et al., 1994; Sweat et al., 2001, 2003; Wise et al., 2008); and up to 24 h or more for skin and blubber (Mancia et al., 2012; our unpublished data).