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Developmental stage dependent neural stem cells sensitivity to methylmercury chloride on different biofunctional surfaces

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ABSTRACT

Sensitivity of neural stem cells viability, proliferation and differentiation upon exposure to methylmercury chloride (MeHgCl) was investigated on different types of biofunctional surfaces. Patterns of biodomains created by microprinting/microspotting of poly-L-lysine or extracellular matrix proteins (fibronectin and vitronectin) allowed for non-specific electrostatic or specific, receptor mediated interactions, respectively, between stem cells and the surface. The neural stem cell line HUCB-NSC has been previously shown to be susceptible to MeHgCl in developmentally dependent manner. Here we demonstrated that developmental sensitivity of HUCB-NSC to MeHgCl depends upon the type of adhesive biomolecules and the geometry of biodomains. Proliferation of HUCB-NSC was diminished in time and MeHgCl concentration dependent manner. In addition, the response to MeHgCl was found to be cell-type dependent. Undifferentiated cells were the most sensitive independently of the type of bioactive domain. Significant decrease of GFAP+ cells was detected among cells growing on poly-L-lysine, while on fibronectin and vitronectin, this effect was observed only in the highest (1 μM) concentration of MeHgCl. β-Tubulin III expressing cells were most sensitive on fibronectin domains. In addition, limited bioactive domains to μm in size, as compared to non-patterned larger area of the same adhesive substrate, exerted protective role. Thus, the surface area and type of cell/biofunctional surface interaction exerted significant influence on developmental stage and cell-type specific response of HUCB-NSC to MeHgCl.

1. Introduction

New therapeutics or chemical compounds designed for clinical applications need to be thoroughly investigated in appropriate experimental models that provide an evidence that these chemicals are safe and nontoxic for humans.

Most of the toxicological tests currently in use are performed *in vivo* and based on animal models. The use of *in vitro* methodologies for toxicity testing is gaining increasing acceptance, in particular to support mechanistic studies. However, the most commonly used culture models are immortalized cell lines or primary cultures, mainly derived from animal tissues (Hogberg and Bal-Price, 2011). Models based on stem cells, either pluripotent embryonic stem cells (Kuegler et al., 2010; Visan et al., 2012) or somatic stem cells (Buzanska et al., 2009a; Fritsche et al., 2011), were proposed to be a good alternative. Embryonic/pluripotent or fetus-derived somatic stem cell lines can be applied to investigate *in vitro* the toxic effects of compounds and constitute a good model of prenatal human organisms (Nerini-Molteni et al., 2012). However, species differences in sensitivity to toxic chemicals have been systematically documented in the 3-dimensional neurospheres culture derived from rodent and human neural progenitors (Moors et al., 2009; Fritsche et al., 2011) indicating an emerging need for the application of human stem cell models.

The adverse effects of chemicals can be assessed in variety of cellular assays. Such assays include: identifying the mode of cell death (Orrenius et al., 2011) altered cell proliferation (Skalamera et al., 2011), genotoxicity (Speit et al., 2009; Kirsch-Volders et al., 2011), teratogenicity (Colleoni et al., 2012), cytochrome P450-dependent monooxygenases (CYPs) (Guengerich, 2006) or using the modes of action approach (Tegenge et al., 2011; Zimmer et al., 2012), which give a better insight into the molecular mechanisms underlying cellular toxicity pathways. Emerging technologies, such as global gene expression profiling, epigenetic analysis and proteomics analysis, combined with bioinformatics are used to investigate such mechanisms (Meganathan et al., 2012).

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The nervous system is very sensitive to xenobiotics and the degree of this susceptibility depends on its developmental stage. Toxic compounds can disrupt the symmetric and asymmetric self-renewal divisions of neural stem cells, as well as neuronal and glial differentiation, migration, synaptogenesis or myelinization, and development of the blood–brain barrier (Coecke et al., 2007). Recently, a developmental neurotoxicity testing (DNT) strategy was set up to guide scientists how to assess the susceptibility of the developing nervous system to the exposure to harmful toxicants (Crofton et al., 2010, 2012).

From about 80 thousands of existing chemical compounds, only few are considered as developmental neurotoxins. One of these is methylmercury (MeHg) of which neurotoxic effects were well established based on epidemiological data and widely studied in animal or human experimental models. Several cases of mass pollution by MeHg were reported in the past, in particular from water contaminated by industrial waste (Grandjean and Landrigan, 2006). Methylmercury bioaccumulates in the sea animals (especially in fish), reaching there high concentrations. Consumption of contaminated fish can cause MeHg poisoning. Methylmercury present in food can be easily absorbed through the gut into the circulating blood and can cross the blood-brain barrier and placenta (Clarkson, 1997).

The degree of neurotoxic insults caused by MeHg depends upon the developmental stages of the nervous system, the duration of exposure (chronic vs. acute) and the accumulated dose (Rodier, 1995). It was demonstrated that neuronal cells at the earliest stages of their differentiation are more susceptible to MeHg treatment (Tamm et al., 2006; Buzanska et al., 2009a). Methylmercury can cause neurotoxic effects on children as well as in adults, but in the latter the extent and severity of the damage is less pronounced. The fetal brain can be affected while there are no signs of MeHg poisoning in the mother's organism. In adults, MeHg poisoning can cause vision and hearing deficiencies, as well as other neurological disorders (Hunter and Russell, 1954; Newland et al., 2008). In the case of fetuses, exposure to high concentration of MeHg causes widespread damage of the Central Nervous System (CNS) which may lead to cerebral palsy, blindness, deafness and severe mental retardation. In children, repeated exposure to low concentrations of MeHg may lead to problems with vision, hearing, walking, speech and signs of mild retardation (Castoldi et al., 2001).

Several cellular mechanisms were described to be involved in neuronal cell death caused by MeHg, including disturbance of calcium intracellular level, oxidative stress induction and interaction with sulfhydryl groups of proteins (Tamm et al., 2006; Farina et al., 2011) that leads to structural and functional protein modification (Castoldi et al., 2001). Additionally, it has been shown *in vitro* that MeHg causes perturbation in the formation and disruption of microtubule assembly (Sager et al., 1983). MeHg can induce apoptotic or necrotic cell death and this effect is concentration dependent: high concentration of toxicant (5–10 μM) causes necrotic cell death, while upon exposure up to 1 μM MeHg cells are dying mainly by apoptosis, as shown in primary rat neuronal cell cultures (Castoldi et al., 2001). Such concentration-dependent sensitivity to MeHg was confirmed in other *in vitro* experiments with rodent embryonic stem cells differentiated into neural progenitors (Tamm et al., 2006; Visan et al., 2012) and human neural stem cells derived from human cord blood (Buzanska et al., 2009a).

Application of neural stem cells (NSC) in DNT testing is a very promising alternative to conventional *in vitro* models, but it requires understanding of the complex biology of these cells and their interaction with the natural environment, which may greatly influence cell susceptibility to toxic compounds (Bal-Price et al., 2012). These cells are found *in vivo* in a specific microenvironment, called niche (Riquelme et al., 2008), where the fate of a neural stem cell depends on interaction with niche components including extracellular matrix elements (Scadden, 2006). Therefore, also the mode of interaction of NCS with their microenvironment *in vitro*, may greatly influence cells response to toxic compounds, when applied for neurotoxicity testing. For this purpose it is important to evaluate *in vitro* the influence of different extracellular matrix components on stem cell developmental processes. Using different biofunctionalized surfaces *in vitro*, we were able to mimic *in vivo* conditions, in order to test the main developmental processes of NCS such as adhesion, proliferation, differentiation and apoptosis. Modulation of the composition of biofunctionalized surfaces allows investigating the way of interaction of cells with the substrate and the activation of intracellular signaling pathways (Falconnet et al., 2006; Buzanska et al., 2010). The biofunctionalized surfaces can be applied in toxicological or diagnostic tests based on interaction of immobilized cells with the microenvironment resembling *in vivo* cell niche (Falconnet et al., 2006).

Neural stem cells from human umbilical cord blood (HUCB-NSC) cell line have been tested for their sensitivity to organic and inorganic chemicals in the context of developmental neurotoxicity (Buzanska et al., 2009a). Using this cell line we were able to discriminate between neurotoxic and non-neurotoxic compounds and have shown developmental stage and cell type specific response to MeHg. HUCB-NSCs have been also widely investigated for their potential to adhere, proliferate and differentiate into neural lineages on the pattern of biofunctional domains of different shape and composition. In experiments using nano/micropatterned biofunctionalized surfaces, we have shown that modification of these surfaces may direct developmental decisions of neural stem cells. This response was related to the composition of biofunctional domains allowing for either electrostatic (on poly-L-lysine) or specific receptor-mediated (on fibronectin) type of adhesion (Buzanska et al., 2009a; Zychowicz et al., 2011). However the geometry of investigated pattern of biofunctional domains has also exerted significant influence on the cellular response of HUCB-NSC (Buzanska et al., 2009a; Zychowicz et al., 2012). In this work, we test the hypothesis that the type of biofunctional surface and its size limitation to μm domains may affect sensitivity of neural stem cells to developmental neurotoxicants. We were investigating whether microenvironmental interactions on different type of biofunctional surfaces can influence human neural stem cell response to different concentrations of methylmercury chloride (MeHgCl, which by dissociation produces toxic methylmercury cation). For that purpose the influence of MeHgCl on developmental processes, such as survival, proliferation and differentiation of HUCB-NSC have been studied on the active biofunctional surfaces of different content, representing either non-specific, electrostatic, or specific, receptor mediated type of interactions on the cell membrane/adhesive surface interface. Moreover, the implication of the patterned biofunctional domains of micrometer size on developmental processes of HUCB-NSC was investigated.

2. Materials and methods

2.1. Design and fabrication of biofunctional surfaces

2.1.1. Microcontact printing

Molds for microcontact printing process were fabricated by casting polydimethylsiloxane (PDMS) silicone elastomer against silicon master, hardened at 65 °C for 4 h and used as stamps for direct printing of adhesive substrates: poly-L-lysine (PLL, 25 μg/ml), fibronectin (FN, 42 μg/ml) or vitronectin (VN, 42 μg/ml) (all from Sigma–Aldrich) on Petri dishes (3 cm in diameter) coated with a cell repellent plasma polymerized polyethylene oxide-like (PEO-like) film (Ruiz et al., 2008). Former results show that the adhesive