Hearing Research xxx (2018) 1–14

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research Paper

Microenvironmental support for cell delivery to the inner ear

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ARTICLE INFO

Article history:
Received 12 December 2017
Received in revised form
10 April 2018
Accepted 19 June 2018
Available online xxx

Keywords:
Heparan sulfate
Cochlear implantation
Neuroprotection
Spiral ganglion
Cell therapy

ABSTRACT

Transplantation of mesenchymal stromal cells (MSC) presents a promising approach not only for the replacement of lost or degenerated cells in diseased organs but also for local drug delivery. It can potentially be used to enhance the safety and efficacy of inner ear surgeries such as cochlear implantation. Options for enhancing the effects of MSC therapy include modulating cell behaviour with customized bio-matrixes or modulating their behaviour by *ex vivo* transfection of the cells with a variety of genes. In this study, we demonstrate that MSC delivered to the inner ear of guinea pigs or to decellularized cochleae preferentially bind to areas of high heparin concentration. This presents an opportunity for modulating cell behaviour *ex vivo*. We evaluated the effect of carboxymethylglucose sulfate (Cacicol®), a heparan sulfate analogue on spiral ganglion cells and MSC and demonstrated support of neuronal survival and support of stem cell proliferation.

1. Introduction

1.1. Cell transplantation to the inner ear

Cell transplantation presents a promising approach not only for the replacement of lost or degenerated cells in diseased organs but also for local drug delivery. Transplanted cells may release a variety of factors such as cytokines or growth factors for the support of the organ, supporting its cells and their functional integrity. Several types and sources of cells have been considered for transplantation. Genetically engineered fibroblasts and stem cells have been used for growth factor delivery to the brain (Makar et al., 2014). Haematopoietic stem and progenitor cells are attractive vectors for gene delivery since they are easily obtained from the recipient and allow autologous transplantation (Bordignon and Roncarolo, 2002). Alternate strategies include using embryonic stem cells (ESC) and mesenchymal stromal cells (MSC) derived from various sources such as bone marrow, adipose tissue or umbilical cord.

Cell transplantation to the inner ear has been addressed experimentally in many studies (reviewed in (Warnecke et al., 2017)). Depending on the therapeutic purpose, cells need to reach structures in both, the perilymphatic and endolymphatic space, which can potentially negatively impact cell survival. Mesenchymal stromal cells are multipotent cells that can be isolated from a variety of adult tissues. Most intensively investigated are MSC derived from bone marrow. Their low immunogenicity has made them attractive for clinical application (Lee et al., 2016). Intravenous administration of MSC resulted in recruitment and engraftment into the inner ear to various degrees (Dai et al., 2010; Choi et al., 2012). In addition, local administration of MSC to the inner ear leads to the regeneration of the fibrocytes in the lateral wall and enhanced functional recovery of the cochlea (Kamiya et al., 2007). Engraftment and differentiation of MSC to fibrocytes was shown preferentially in paediatric animal models rather than in adults after perilymphatic administration of the cells (Kasagi et al., 2013). Most of their actions are believed to be due to their secretome (D'souza et al., 2015; Tran and Damaser, 2014), and this has been also demonstrated recently for the treatment of the inner ear (Yoo et al., 2015). Given that many of the beneficial effects of these cells are related to production of growth factors and miRNAs, a novel functional description for MSC has been proposed: "*medicinal signalling cells*" emphasizing their paracrine effects (Girolamo et al.,

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https://doi.org/10.1016/j.heares.2018.06.015
0378-5955/

2013). There is increasing evidence that other populations of the bone marrow such as hematopoietic stem cells may be involved in cochlear repair (Tan et al., 2008; Yoshida et al., 2007), especially the repair of the lateral wall.

1.2. Matrix support of transplanted cells

The extracellular matrix (ECM) provides structure, mechanical integrity and biochemical activity in a three dimensional complex (Guvendiren and Burdick, 2013). In addition, it hosts soluble signalling molecules including growth factors (Guvendiren and Burdick, 2013). In a diseased organ, the native ECM is altered and does not offer physiological support. Thus, cells transplanted to a diseased organ can potentially sense and response to such deviations. The ECM of the cochlear microenvironment offers important cues for stem cell behaviour and differentiation (Mellott et al., 2017). As has been recently demonstrated for the brain (Kabu et al., 2015), cochlear insult may also create a hostile microenvironment that could affect survival and integration of transplanted cells. Pharmacological agents that mimic the effects of the ECM could therefore be utilized to support not only transplanted but also residual cochlear-residing cells.

Heparan sulfate is a multi-disaccharide-chain located in the ECM. In interaction with various other proteoglycans, it forms the structure of the ECM and induces signal transduction (Häcker et al., 2005). Heparan sulfate can bind various growth factors and thereby enabling a change to their active state (Okolicsanyi et al., 2014). Heparan binding growth factors (HBGF) are known to support neural survival (Yu et al., 2011). Regenerating agents (RGTA) are heparan sulfate analogues that substitute the natural proteoglycans as they mimic their abilities with a minimized vulnerability to proteolysis making them ideal for translational research (Rouet et al., 2007). In addition, RGTA provide growth factors, cytokines, interleukins, colony stimulating factors, chemokines and neurotrophic factors (Barritault and Caruelle, 2006). After cell death, a liberation of glycanases and proteases occurs that degrades heparan sulfate. This leads to a release of the cytokines, especially the heparan binding growth factors (HBGF), which are then soluble and therefor unprotected from proteolysis. RGTA rebind the HBGF and thus protect them from proteolysis. Therefore, they preserve the natural signal cascade and as a result support tissue regeneration. Previous studies demonstrated their promising implementation in wound healing including chronic corneal (Kymionis et al., 2014) and dermal ulcers (Groah et al., 2011).

The aim of the present study was to evaluate the migration of MSC *in vivo* and evaluate potential matrix factors that impacted transplanted MSC. Heparin and heparan sulfate proteoglycans have been shown to affect MSC growth and differentiation (Ling et al., 2016; Titmarsh et al., 2017). Heparan sulphate is present in the lateral wall of the cochlea (Cosgrove et al., 1996), where prior studies have demonstrated engraftment of stem cells (Lang et al., 2006). In the following study, the question whether the cochlea and especially the scala media provides adequate scaffold for integration of MSC was investigated in an animal model using cell transplantation to the scala tympani of guinea pigs. In addition, engraftment of MSC after their perfusion into decellularized cochlea scaffolds was investigated in order to identify putative binding preferences based on the presented extracellular matrix of the endolymphatic or perilymphatic space. Finally, carboxymethylglucose sulfate (Cacicol®), a heparan sulfate analogue and RGTA, was tested *in vitro* for its ability to function as a supportive biomimetic matrix for spiral ganglion neurons and for MSC.

2. Materials and methods

2.1. Ethical statement

Human bone marrow was obtained during routine orthopaedic procedures from otherwise healthy donors after approval of the institutional ethical committee of Hannover Medical School as described previously (Roger et al., 2016). Written informed consent was obtained from all donors (Roger et al., 2016). All personal information apart from age and gender was deleted. The use of the cells for research purpose was approved by the Ethics Committee of Hannover Medical School (2080–2013). For the experiments with the decellularized cochleae, MSC were isolated from human umbilical cords according to the protocols approved by the University of Kansas Human Subjects Committee (KU-Lawrence IRB approval #15402). The *in vivo* study on guinea pigs was permitted by the local government (LAVES, registration no. 05/1053) and was conducted in accordance with the German “Law on Protecting Animals”.

For mouse studies, C57BL/6 female mice (~6 weeks old) were purchased from Charles River and euthanized according to approved IACUC protocol (ACUP #2014–2234) at the University of Kansas Medical Center (KUMC).

Spiral ganglion cells (SGC) were dissected from neonatal Sprague-Dawley rats of both sexes (P3-5). This procedure was approved by the Institutional Animal Care and Research Advisory Committee and by the local state authorities. The study was conducted in accordance with the German ‘Law on Protecting Animals’ and with the European Communities Council Directive 86/609/EEC for the protection of animals used for experimental purposes. The euthanasia for the *in vitro* experiments is registered (no.: 2013/44) with the local authorities (Zentrales Tierlaboratorium, Laboratory Animal Science, Hannover Medical School, including an institutional animal care and use committee) and is reported on a regular basis as demanded by law. For exclusive euthanasia of animals for tissue analysis in research, no further approval is needed if no other treatment is applied beforehand (German Animal Welfare Act, §4). The rats (breeding stock was supplied by Charles River (Charles River, Wilmington, USA)) were bred and born for research study purposes.

2.2. Isolation and cultivation of human MSC

Human MSC were prepared from fresh bone marrow aspirates by density gradient centrifugation and subsequent plastic adhesion of mononuclear cells as described previously (Schäck et al., 2013). Heparin-containing bone marrow diluted with phosphate buffered saline (PBS) was layered onto a Biocoll density gradient (Biochrom AG, Berlin, Germany) and centrifuged for 30 min at 500 g. The mononuclear cell fractions at the interface was obtained and seeded in cell culture flasks in MSC medium at 37 °C with 5% CO_2 at 85% humidity (DMEM FG 0415; Biochrom, Berlin, Germany supplemented with 10% v/v fetal bovine serum; Thermo Fisher Scientific, Schwerte, Germany, 20 mM HEPES, 1% 100 U/ml/100 g/ml penicillin/streptomycin; Biochrom AG, Berlin, Germany and 2 ng/mL human recombinant FGF2; PeproTech, Hamburg, Germany). The cells were passaged at a density of around 70% by the use of 0.025% Trypsin-EDTA solution (Biochrom AG, Berlin, Germany) and seeded at a density of 2*103 cells per cm (Bordignon and Roncarolo, 2002) for transfection. Plastic adhesion and expression of surface markers such as CD73, CD105, and CD90 were used as criteria to define MSC. Analysis of presumptive osteogenic or adipogenic differentiation and stemness of cultured MSC was randomly performed using

Please cite this article in press as: Schulze, J., et al., Microenvironmental support for cell delivery to the inner ear, Hearing Research (2018), https://doi.org/10.1016/j.heares.2018.06.015