



Review

Can gap junctions deliver? ☆

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ABSTRACT

In vivo delivery of small interfering RNAs (siRNAs) to target cells via the extracellular space has been hampered by dilution effects and immune responses. Gap junction-mediated transfer between cells avoids the extracellular space and its associated limitations. Because of these advantages cell based delivery via gap junctions has emerged as a viable alternative for siRNA or miRNA delivery. Here we discuss the advantages and disadvantages of extracellular delivery and cell to cell delivery via gap junction channels composed of connexins. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

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1. Introduction

The selectivity inherent in single gene silencing by small interfering RNAs (siRNAs) carries great therapeutic potential [1,2]. There has nonetheless been slow progress towards clinical application of siRNA because of a number of limitations, including triggering of immune responses by both viral vectors, and liposomal vehicles [3,4] and ineffective delivery of therapeutic concentrations to target cells resulting from (for example) the inability of delivery systems to specifically select target cells from their surroundings [5]. All the *in vivo* therapeutic approaches tested to date incorporate as their final common pathway

the extracellular space. This is a significant limitation because the extracellular volume is large, leading to dilution. It is a space shared by target cells and surrounding cells alike, which limits specificity and presents the potential for immune interactions.

To avoid the issues inherent in delivery via the bloodstream or by direct interstitial delivery, cells have been suggested as an alternative delivery mechanism. It was recently speculated that this could be accomplished utilizing pinocytotic delivery of exosomes [6]. This approach, however, still involves the extracellular space.

An alternative is provided by gap junction channels [7] that avoids the extracellular space by moving siRNA from the interior of the delivery cell to the interior of the target cell through an intercellular channel. In this brief review, we first describe the evidence for cell based delivery of siRNA mediated by gap junction channels. We follow this description with an evaluation of a pinocytotic-based pathway. Finally, we describe a rationale for using adult mesenchymal stem cells (MSCs) as the cell of choice for focal or systemic delivery of siRNA.

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2. Gap junctions: an intercellular pathway for siRNA delivery

Gap junction channels form intercellular conduits between adjacent cells exclusive of the extracellular space, allowing easy transfer of solutes from one cell interior to another [8,9]. In mammals the connexin is the predominant subunit protein that forms gap junction channels. There are more than 20 identified connexins in the human genome and the majority of human cell types express connexins, the only notable exception being adult skeletal muscle. Many tissues express multiple connexins [10]; as a result three generic channel types are possible, homotypic, heteromeric and heterotypic [11–15]. Homotypic channels are composed solely of one type of connexin while heteromeric and heterotypic channels incorporate at least two connexin types. The literature is replete with examples of *in vitro* homocellular coupling in which the pair is composed of two identical cells [14–17] and *in vitro* heterocellular coupling, such as a stem cell and a cardiac myocyte cell pairs, mediated by the formation of functional gap junction channels [18,30]. *In vivo* evidence also demonstrates heterocellular coupling, an example being endothelial cells and vascular smooth muscle [19] and, in the extreme, xenographic heterocellular coupling [20,21]. One essential feature for the formation of gap junction channels is close apposition of cells while a second is the expression of cellular adhesion molecules like the cadherin family of proteins [22]. The expression of adhesion molecules such as cadherins is robust within all the tissues [23,24] and should be assessed when considering the selection of delivery and target cells.

2.1. Essential properties for a cell based, gap junction mediated delivery system

There are three necessities for an intercellular delivery system utilizing gap junctions: (1) adequate expression of connexins to sustain a sufficient pool of active channels; (2) adequate patency or channel open probability to facilitate rapid transfer; (3) sufficiently permissive permeability/selectivity properties. Fig. 1A illustrates connexin protein expression and distribution in the form of green fluorescence at cell boundaries and within perinuclear intracellular compartments.

The original studies that determined connexin turnover rate by Fallon and Goodenough [25] revealed a half-life for connexin32 of 4–5 h. Subsequent studies have generated similar results for other connexins including Cx43 [26,27]. Despite the rapid turnover rate for connexins dual voltage clamp/dual whole cell patch clamp has shown gap junction mediated coupling to be common to most cell types [7,9,28–30] and has allowed the determination of open probability and permeability of gap junction channels for the more ubiquitously expressed connexins. By far the best example is Cx43 which is expressed robustly in many cell types [10]. Open probability of homotypic Cx43 channels ranges from 0.5 to nearly 1.0 when transjunctional voltage is small and the percentage of time occupied by open subconducting states (lower than the maximal conductance value) is less than 5% of the total open time [31,32]. Fig. 1B illustrates symmetric voltage dependence typical of homotypic Cx43 and Fig. 1C shows typical multichannel activity indicative of Cx43.

Using dual whole patch clamp, different monovalent cations and anions can be introduced into a cell pair and their relative permeability assessed. In general, gap junctions tend to be weakly selective for monovalent cations over anions [29,33,34]. The cation sequence often follows an Eisenmann series I or II sequence, suggesting a solvent environment within the pore similar to that in the bulk solution [35,36]. Recent studies by Mathias et al. [37] and Gao et al. [38] have modeled and experimentally demonstrated bulk fluid flow through gap junction channels which is consistent with the Eisenmann series data, and provides additional evidence that gap junction channels are a solvent-filled space.

The type I or II sequence is most consistent with a poorly-selective channel, but not all connexins are the same in this regard. Consider

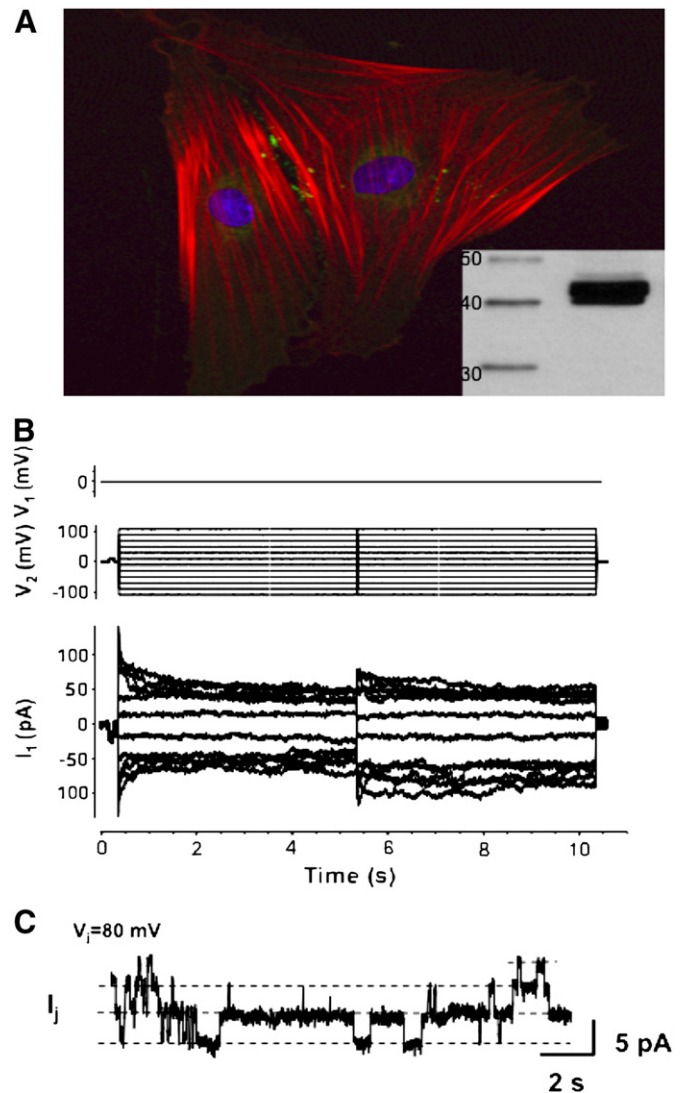


Fig. 1. Fig. 1A is a micrograph showing Cx43 in rat mesenchymal stem cells [5] at cell–cell interfaces. Red = phalloidin staining, Green = Cx43, Blue = DAPI. The insert is a western blot showing robust expression of Cx43 and molecular markers, 50, 40 and 30 kD, in the left lane. Fig. 1B shows a typical macroscopic record of junctional currents taken at different voltages. Fig. 1C shows multichannel activity obtained from human MSCs. Insert: horizontal bar = 2 s, vertical bar = 5 pA.

that Cx43 has a lower unitary conductance than Cx40 or Cx37 but is less selective for anions relative to cations. One possible explanation is that Cx40 or Cx37 might have a lower access resistance but a smaller pore diameter and/or a different distribution of fixed charge sites along some portion of their lengths [33,34,36,39].

Other tools useful in assessing the permeability and selectivity characteristics of connexins are fluorescent and radiolabeled probes of larger size than monovalent cations and anions [40–43]. Studies which use exogenous probes or monitor reporter gene activity [44] resulting from transfer of endogenous solutes (e.g., second messengers) into recipient cells, generally are consistent with a poorly selective channel type. A general rule which has emerged for all connexins thus far studied is: Only when the minor diameter of the solute probe begins to approximate the functional diameter of the pore wall does selectivity in the form of size and charge affect solute/probe permeability [43,44]. As previously mentioned the least selective gap junction channel is one composed of Cx43, whose properties make it the most likely channel to allow the passage of large solutes. In 2005 Neijssen and collaborators [45] asked if a fluorescently labeled polypeptide of up to 8 amino acids (~1.8 kD) in length could pass from

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