

## Editorial

# Recreating the Sinus Node by Somatic Reprogramming: A Dream Come True?



## Recreación del nódulo sinusal mediante reprogramación somática: ¿un sueño hecho realidad?

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Electronic pacemakers have been the standard of care for over 50 years<sup>1</sup> for patients with symptomatic bradycardia caused by conditions such as sick sinus syndrome, complete heart block, and congenital heart block.<sup>2</sup> Effective, electronic devices are subject to hardware malfunction, recalls of generators and leads, implant-related complications and infections.<sup>3</sup> Although the incidence is not high, device-related infections continue to rise, affecting over 2% of device implants<sup>4</sup> and often requiring complete hardware removal and treatment with systemic antibiotics, hospitalization, and placement of a backup temporary pacemaker wire in pacemaker-dependent patients.<sup>5</sup> Another important at-risk population that could benefit from biological pacemakers are fetuses with congenital heart block (frequently related to maternal anti-Ro/SSA antibodies).<sup>6</sup> Those patients cannot receive intra-uterine electronic devices and frequently develop fatal hydrops-fetalis because of their inability to maintain an adequate heart rate to support the circulation.<sup>7</sup>

As an alternative to electronic devices, our group<sup>8</sup> and others<sup>9</sup> have been working for over a decade to develop biological alternatives to electronic pacemakers. Many important contributions have been made to the field, and, despite positive popular press (eg, Briggs<sup>10</sup>), skeptical opinions have been expressed by experts in the field<sup>11</sup> about the prospects of clinical application. The purpose of the present editorial is to review the different biological pacemaker strategies focusing on the potential for human translation.

### BIOLOGICAL PACEMAKERS: WHICH STRATEGY IS PREFERABLE?

Different approaches have been studied preclinically including: a) stem-cell based approaches<sup>12</sup>; b) hybrid (cell- and gene-based approaches);<sup>12</sup> c) gene therapy over-expressing a single or a combination of ion channels<sup>12</sup> (known as functional reengineering), and more recently, d) gene therapy to achieve somatic reprogramming.<sup>13,14</sup>

Different stem-cell based approaches have been used such as embryonic stem cells,<sup>15,16</sup> or induced pluripotent cells capable of

differentiating into spontaneously-beating heart cells, and generate short-term biological pacemaker activity. While generally effective, they are not free of problems: they can have limited engraftment and rejection (requiring immunosuppressive therapy) and be proarrhythmic, given the heterogeneity of the cell population obtained with present isolation and purification techniques. As a result of this heterogeneity, different action potential durations (affecting myocardial repolarization) can create a substrate for functional reentry-induced arrhythmias.

Hybrid approaches, in which stem cells are “loaded” with ion channel genes, have also been used to deliver the gene of interest without a viral vector.<sup>12</sup> The greatest experience with this approach has been obtained using human mesenchymal stem cells that have been “loaded” with one of the HCN (hyperpolarization activated cyclic nucleotide-gated) family of genes (responsible for generating the pacemaker funny current or  $I_f$ ). The major advantage of this approach is that human mesenchymal stem cells are somewhat nonimmunogenic. On the other hand, they tend to migrate from the injection site with the potential to create multiple ectopic foci of automaticity.<sup>17</sup>

The first de novo biological pacemaker by gene therapy was created by Miake et al<sup>18</sup> in 2002. Over-expressing a dominant negative mutant (Kir2.1AAA) of the inward rectifier current ( $I_{K1}$ ) converted a normally-quiescent myocyte into an oscillating one, capable of generating spontaneous action potentials and biological pacemaker activity in vivo. While this approach was effective, the use of a mutant gene could represent a problem for human translation due to safety and regulatory concerns. Other approaches focused on overexpressing wild-type or mutant forms of the HCN family of genes (responsible for the pacemaker current or  $I_f$ ) have been developed in different small- and large-animal models. While this approach offered the advantage of expressing an endogenous gene, the heart rates achieved were not optimal and the delivery methods were highly invasive (open-chest or left-sided/arterial approaches).<sup>19</sup>

In contrast to functional reengineering approaches, somatic reprogramming seeks to create genuine sinus node tissue from ordinary heart muscle. The approach involves reexpressing a gene, *TBX18*, that figures prominently in the development of the sinoatrial node (SAN) during embryonic life; this suffices to create a biological pacemaker in situ.<sup>13,14</sup> Initially, in vitro experiments

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demonstrated the ability of the human *TBX18* gene to reprogram normal working myocytes into pacemaker cells or induced SAN cells. Moreover, when injected into a rodent model of heart block, *TBX18*-transduced animals exhibited biological pacemaker activity that originated from the injection site, as evidenced by QRS morphology and axis.<sup>13</sup> When induced SAN were analyzed by morphometry and molecular techniques, they resembled native SAN cells. Induced SAN cells have a characteristic long lean shape, increased levels of *HCN2* (the pacemaker channel gene) and connexin 45 (the predominant gap junction in the SAN), and decreased levels of connexin 43 (the predominant gap junction in the normal working myocardium) and *I<sub>K1</sub>*.<sup>13</sup> All these features of *TBX18*-induced SAN cells mimic the signature characteristics of the native SAN.

To further characterize the effects of *TBX18*-induced biological pacemaker when delivered in a clinically-realistic fashion, we implemented a modification of a protocol we had previously described.<sup>14,20</sup> In brief, *TBX18* (or control) was injected through a venous catheter into the high posterior septum. The entire procedure was performed by minimally-invasive methods limited to the venous side. The catheter used for *TBX18* delivery was advanced through the femoral vein, and has been used extensively in human stem cell trials.<sup>21</sup> Interestingly, those animals that received *TBX18* exhibited physiologically relevant biopaced rhythms that allowed them to achieve higher levels of physical activity compared with age-matched controls. Additionally, those animals that received the biopacer required minimal backup electronic pacemaker utilization, unlike controls, which were largely pacemaker-dependent.

#### DELIVERY SYSTEMS: READY FOR THE CLINIC?

A fundamental consideration when delivering a biological therapy (or any therapy) to patients is the invasiveness of the method. No physician will consider offering patients a treatment that requires open-chest delivery (unless there is an additional indication to perform surgery), or even a left-sided approach with the consequent risks of hematoma and stroke. As described above, we have recently developed a minimally-invasive (venous catheter-based system) to deliver our biopacer gene without the need for open-heart surgery or access to the left-sided circulation.<sup>14,20</sup> This technique is a modification of a cardiac catheterization technique routinely used by clinicians in the human electrophysiology laboratory. The genes are delivered by a commercially-available catheter (NOGA Myostar™; Biological Delivery Systems, Diamond Bar, California, United States) that can be advanced to the heart, construct a 3-dimensional electroanatomic map and inject the biopacer with a high level of precision by a retractable needle.

Other groups have proposed injection into the left bundle (via the arterial circulation) in the setting of device-related infections.<sup>22</sup> Accessing the left-sided heart can have catastrophic consequences, such as stroke and vascular complications. A recent editorial by Rosen<sup>11</sup> speculates that there may be more risks when injecting in the right side in the setting of a device infection, but this is difficult to rationalize given the systemic nature of blood-borne infections. In our opinion, a right-sided minimally-invasive delivery technique would be optimal when translating this technology to the clinic.

#### FIRST-IN-HUMAN TRIAL: WHAT WOULD BE THE IDEAL POPULATION?

As clinician-scientists, when developing a new class of therapeutic agent to better treat our patients, we must target

first those patients at risk that have no good prognosis, or no good alternatives with currently available therapies. Three groups of patients satisfy these criteria: a) patients with pacemaker-related infections who are pacemaker-dependent and need their hardware to be removed to more effectively treat the infection<sup>3</sup>; b) pediatric congenital heart disease patients who have unfavorable anatomy for endovascular devices and often need multiple system changes when they grow into adult life, and c) fetuses with intrauterine congenital heart block, who often develop hydrops fetalis and stillbirth due to the lack of reliable therapy.<sup>7</sup>

#### CONCLUSIONS

The thought of recreating a sinus node by a single human gene injection, avoiding the need for implanting electronic devices, may sound like one of those stories that we read in science fiction novels.<sup>23</sup> With the advent of minimally-invasive delivery systems, novel biological agents, and accumulating efficacy and safety data in preclinical models of human disease, we may soon see this dream come true.

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#### CONFLICTS OF INTEREST

None declared.

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