Emerging Technologies in Cardiac Pacing From Leadless Pacers to Stem Cells

Michael Lawren Co, MD, MSc[a], John Paul Khouzam, BS[b], Issa Pour-Ghaz, MD[c], Sheharyar Minhas, MD[d], and Indranill Basu-Ray, MD, FACP, FACC[e,*]

From the [a] Department of Cardiology, Loma Linda University Medical Center, Loma Linda, CA, [b] University of Notre Dame College of Medicine, Notre Dame, IN, [c] Department of Cardiology, University of Tennessee Health Science Center, Memphis, TN, [d] Department of Internal Medicine, Baptist Memorial Hospital, Memphis, TN and [e] Arrythmia Service, Department of Cardiology, Memphis VA Medical Center, The University of Memphis, Memphis, TN.

Abstract: Modern pacemakers can sense and pace multiple chambers of the heart. These pacemakers have different modes and features to optimize atrioventricular synchrony and promote intrinsic conduction. Despite recent advancements, current pacemakers have several drawbacks that limit their feasibility. In this review article, we discuss several of these limitations and detail several emerging technologies in cardiac pacing aimed to solve some of these limitations. We present several technological advancements in cardiac pacing, including the use of leadless pacemakers, physiologic pacing, battery improvements, and bioartificial pacemakers. More research still needs to be done in testing the safety and efficacy of these new developments. (Curr Probl Cardiol 2021;46:100797.)

Introduction

Today, modern pacemakers (PMs) can both pace and sense multiple chambers of the heart, including the right atrium, right ventricle, and left ventricle, depending on the patient's clinical

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need. Different pacing modes, smart algorithms, and features have been designed to optimize atrioventricular (AV) synchrony, promote intrinsic conduction while preserving energy to prolong battery life. Furthermore, intelligent algorithms can adjust heart rate to physical demands and predict impending heart failure exacerbation. Some PMs are now even MRI-conditional, thereby allowing patients to undergo imaging when indicated safely.[1]

Despite these advancements, modern PMs still come with several significant limitations. First, pacemaker generators have a finite battery life, which can last somewhere between 6 and 15 years depending on multiple patients and device factors that affect energy consumption, such as pacing requirements and capture thresholds. This means that patients, particularly younger ones, will require more than one invasive procedure in their lifetimes, leading to cumulative procedure-related risks. Second, PMs are foreign objects (generators and leads) located inside the human body, and they can potentially cause bloodstream infections that can predispose to endocarditis and damage cardiac structures. Third, the same pacemaker leads can also develop lead fracture and insulation breakdown causing pacemaker malfunction. Finally, the pacemaker implantation can be associated with a spectrum of complications, including pocket hematoma, infection, and wound dehiscence. A laundry list would further include pneumothorax, cardiac tamponade, lead dislodgment, venous thromboses and obstruction, and tricuspid insufficiency. The rate of reported postoperative adverse events has been estimated to be as high as 10%.[2] Other limitations of modern PMs are the inability to mimic physiological autonomic responsiveness, metal allergy, and electrical interference.

Extensive research and development aimed at supplanting these deficiencies are either on the table to be incorporated now or soon. This review article reviews some of the emerging technologies in cardiac pacing and discusses their putative role in the near future.

Leadless PMs

Leadless cardiac pacemakers (LCP) are PMs that do not have the traditional cardiac pacemaker components, particularly the transvenous leads. Here the generator and the tines connected to the tissue are one assembly. The size is that of a large multivitamin capsule. These devices are implanted directly inside the heart. Eliminating the need for a surgical pocket for generator implantation eradicates any form of pocket complications, including infection, hematoma, and fluid collection. This ensures

that attendant symptoms, including pain, generator migration, lead connection failures, are automatically absent. Similarly, the absence of transvenous leads appropriately abolishes chances of lead complications, including lead infection and or lead damages, like insulation failures.

The concept of a self-contained intracardiac "leadless" pacemaker was first proposed in 1970, but it was not clinically used initially due to battery longevity concerns.[3] Since then, 2 different LCPs from two different manufacturers have been developed, marketed, and become clinically available: the NanoStim Leadless Pacemaker System (LPS) developed by St. Jude Medical (now Abbott Laboratories) and the Micra Transcatheter Pacing Systems (TPS) by Medtronic. Unfortunately, St. Jude Medical recalled the NanoStim device in 2016 after a higher than expected rate of critical battery failure, so currently, only the Micra TPS (and its last reiteration Micra AV, see below) is clinically available.[4]

Indications for implantation of an LCP include:

Chronic atrial fibrillation with AV block or significant pauses;
Sinus rhythm with high-grade AV block with a low level of physical activity;
Sinus bradycardia with infrequent pauses, and
Unexplained syncope with abnormal electrophysiological findings such as prolonged HV interval.

LCPs have also been reported to be a viable option for appropriately selected adult patients with congenital heart diseases when transvenous pacing is not a suitable option, such as in some patients with intracardiac shunts or tricuspid valve disorders.[5,6] The contraindications to implant include a mechanical tricuspid valve, preexisting endocardial pacing or defibrillation leads, inferior vena cava filter, and hypersensitivity to dexamethasone acetate. Unfavorable femoral venous anatomy, morbid obesity preventing the implanted device from obtaining telemetry communication, pacemaker syndrome, and preexisting severe pulmonary hypertension are some of the other limitations.[7-9] Micra is a single component LCP implanted into the right ventricle (RV) percutaneously through the use of a catheter delivery system inserted into the femoral vein. It is secured into the RV myocardium via passive fixation through four self-expanding electrically inert nitinol tines. Its tip is steroid eluting designed to reduce inflammation and scarring. The other end of the device has a mechanism that allows it to be retrievable if necessary. Estimated battery longevity based on nominal settings is about ten years, similar to conventional PMs. Micra features pacing modes identical to a single chamber pacemaker (VVI, VVIR, VOO, OVO, OFF).[9,10] These devices can detect and adjust to physical activity through a 3-axis accelerometer-based adaptive pacing feature.[11]

Clinical experience with Micra has so far been positive. The LEADLESS II trial,[8] a prospective randomized, multicenter clinical study that enrolled a total cohort of 526 patients with an indication for permanent VVI pacing, showed that the Micra TPS was able to achieve both its primary efficacy endpoint (acceptable pacing threshold and R-wave amplitude) and primary safety endpoint (freedom from device-related serious adverse events at 6 months of follow-up). Implant success was reported to be at 96%, with the median procedural time of 29±18 minutes. About 6.7% of patients had serious adverse events that included cardiac perforation, device dislodgement, elevated pacing thresholds requiring retrieval and replacement, and vascular complications. Subsequent worldwide, Micra Post-Approval Registry[12] demonstrated excellent early and intermediate-term safety and efficacy, closely reflecting previously reported data. In this registry, the device was successfully implanted in 99.1% of patients, and significant complications occurred 63% less than transvenous systems. Another large postapproval study[13] involving 720 patients showed that LCPs resulted in improved health-related quality of life at 3 and 12 months, a high level of satisfaction, and less activity restriction than traditional pacemaker systems.

The Micra system's safety profile has been corroborated by other studies that showed lower complication rates than conventional PMs.[14] Major complications are rare, but these most commonly include pacing issues such as elevated thresholds, dislodgment, battery failure, and femoral access site complications such as hemorrhage, hematoma, or pseudoaneurysms. Some other rare complications include procedure-related cardiac injuries such as cardiac perforation, tamponade, or pericardial effusion.[15] However, more rare complications such as the development of malignant ventricular arrhythmias, possibly related to traumatic myocardial inflammation, have also been reported and need to be looked further.[16-18] While there was an initial concern of possible LCP dislodgment, a large meta-analysis involving 2116 patients implanted with LCPs showed a low rate of dislodgment of about 0-1% compared to conventional PMs with 1%-2.69%.[19] In cases of device end-of-life/premature battery depletion or when there is a requirement for a system upgrade, the implanted LCP can simply be turned off or abandoned. However, there are instances when it has to be extracted, such as in cases of infection or dislodgment. A systematic review by Li et al[20] found that available data demonstrated that chronic retrieval could be performed up to 4-5 years after implantation with high success and low complication rates. A small single-center study in Europe showed that patients with a Micra LCP could safely and effectively undergo cardiac magnetic

resonance imaging at either 1.5 T or 3.0 T with no relevant changes in device parameters within three months of follow-up.[21]

A key limitation of the original Micra TPS is the lack of atrial sensing. It does not have atrial tracking capabilities, which is helpful on some occasions to augment cardiac output. Lack of atrial sensing can also cause AV-desynchrony and lead to poor outcomes. Patients with sinus rhythm and AV block have been shown to benefit from dual-chamber PMs that can provide AV synchrony.[22-24] More recently, customized software was developed and downloaded for already implanted Micra devices to detect atrial contraction using the devices' built-in 3-axis accelerometers. Early feasibility studies showed that this new algorithm could detect intracardiac accelerations and improve AV synchrony in patients with AV block and a single chamber LCP.[25,26] The prospective MARVEL 2 (Micra Atrial Tracking using a Ventricular accelerometer 2) study involving 75 patients from 12 centers assessed this new algorithm's performance and found that it significantly improved AV synchrony in patients with sinus rhythm and AV block without any pauses or episodes of oversensing-induced tachycardia.[27] Most recently, a case report showed that atrial pacing from a preexisting pacemaker could be mechanically sensed by an LCP with this downloaded algorithm and thus present a potentially novel method to treat heart block in patients with concomitant sinus node dysfunction.[6] Even though this new Micra LCP with atrial sensing can promote AV synchrony, it is still limited because it only offers pacing in the right ventricle. Studies have previously shown that ventricular contractions from RV pacing are less physiologic than those from the native intrinsic conduction system.[28] High RV pacing burden is associated with the development of heart failure. Promotion of intrinsic conduction is therefore preferred. While this may be achieved through programming, a pacing rate lower than intrinsic rate, those patients with concomitant sinus node dysfunction can expect a high RV pacing burden. Hence, LCPs with multichamber pacing are preferred. In the future, these are expected to be developed and perhaps wholly obviate the need for transvenous leads.

EBR systems designed the WiSE CRT system to provide cardiac resynchronization therapy (CRT) via a leadless pacing electrode in the left ventricle in conjunction with a previously implanted dual-chamber right-sided pacemaker/Intracardiac Defibrillator (ICD) (coimplant). This device consists of an external transmitter and an internal receiver electrode. The external transmitter detects RV pacing from the coimplant. It then converts this signal to ultrasound (US) energy that gets transmitted to the receiver electrode. The receiver electrode then provides a pacing

stimulus to the left ventricle by converting ultrasound energy into an electrical impulse. Current CRT technology relies on a transvenous lead implanted into the coronary sinus or one of its branches to provide epicardial pacing to the left ventricle. This can be associated with implant difficulties in those with unfavorable coronary sinus anatomy, suboptimal pacing related to epicardial pacing site, and limited locations for implantation, thereby affecting optimal ventricular synchronization. WiSE CRT system solves these issues by direct implantation of receive electrode in the left ventricular wall endocardium thought to be the optimal site for CRT pacing. Both the First-In-Man study and the nonrandomized SELECT-LV study showed promising results of the WiSE CRT system in terms of clinical response.[29,30] A real-world registry involving 90 patients in 14 centers showed excellent technical success, but complication rates were still relatively high.[31] The original WiSE CRT is not a fully leadless pacemaker system. Recently, there was a case report of a patient with a fully leadless pacemaker system that combined the Wise CRT and Micra systems.[32]

While the use of leadless PMs may be promising, there are still some issues that can be of concern for these devices. First, implantation of these devices requires an invasive procedure that may have rare but devastating complications. Second, except for the WiSE CRT, these devices come with limited battery life. Once the Pacemaker's battery gets depleted, it may be possible to extract the old device and replace it with a newer one. However, this adds 2 additional extraction and reimplantation procedures, thereby adding to the cumulative procedural risk. The advent of bioartificial Pacemaker possibly solves this problem (see below).

Physiologic Pacing

As the name implies, physiologic pacing (PP) utilizes the intrinsic conduction system to pace the cardiac chambers. PP is not a new concept in electrophysiology (EP) and has been the goal since the earlier days of device implantation and development. However, PP's idea has undergone dramatic changes as our knowledge regarding cardiac pacing has expanded.[33] Though not physiological biventricular pacing is associated with lesser risk than RV pacing. Chronic RVA pacing has been shown to lead to left ventricular remodeling, left ventricular systolic and diastolic dysfunction, valvular pathologies, and ventricular arrhythmias.[28] CRT is recommended for patients with an indication for synchronized pacing due to conditions such as atrial fibrillation or bradycardia. Best candidates for CRT are patients with a wide QRS complex, left bundle branch block (LBBB), and nonischemic

etiology of heart failure; however, CRT is not recommended in patients with a QRS duration of less than 120 ms.[34] Classic Physiological pacing existent today is His-bundle (HB) and left-bundle-branch (LBB).

HB Pacing

HB is responsible for synchronous activation of the right and left ventricles under a normal conduction pathway, leading to the heart's efficient pumping function. His-bundle pacing (HBP) has been utilized for the past several decades. Traditionally, the use of HBP was cumbersome due to a lack of development in tools and techniques. Recent developments in EP have enabled a more robust and mainstream HBP application with promising results.[35,36] For HBP, identifying His potential is critical in the lead and device delivery.[37] The most common pacing lead used is the 3830 Select Secure MRI SureScan HIS lead (Medtronic, Minneapolis, MN) with an outer lead diameter of 4.2 French (F) and is 69 cm in length. The lead has a 1.8 mm exposed active helix part and requires an outer sheath for placement. There are 2 sheaths used for placement. C315 His sheath (Medtronic) is a non-deflatable sheath with an outer 7.0 F diameter and an inner 5.5 F diameter. It is 43 cm long with a primary curve to providing the ability to reach the tricuspid annulus and a secondary cure that can reach the septum. C304-69 sheath (Medtronic) is a deflatable sheath with a 5.7 F inner diameter, and an outer diameter of 8.4 F.[38] Intracardiac electrograms (EGM) are recorded using a pace-sense analyzer. Usually, an electrophysiology recording system is used to help with EGM analysis during the procedure. Mapping catheter (unipolar at the time of mapping) is used to locate the HB.[39]

For HBP implantation, after vascular access is obtained (cephalic, axillary, or subclavian), C315 His sheath is inserted over a guidewire. The pacing lead is advanced after the guidewire removal. A unipolar connection is made between the lead and the tissue allowing EGMs to be recorded. Once an atrial to ventricular EGM ratio of 1:2 or greater is seen, the sheath is pointed toward the superior-anterior septum or mid-posterior septum to achieve a high-frequency near-field HB recording, indicating a good contact. Unipolar pacing is initiated, and the QRS morphology is monitored. The response can be selective HBP (SHBP) or non-selective HBP (NSHBP). In SHBP, the paced QRD morphology is identical to the intrinsic narrow QRS complex. In NSHBP, stimulus-ventricular activation is shorter than the HV interval. In this case, the pacing output is gradually decreased to observe the QRS morphology and determine the appropriate pacing location. Once the site is confirmed, the pacing lead is fixed while leaving a loop in the atrium.[39-41]

Long-term outcomes of HBP have shown promising results comparable with CRT and RVA. HBP is successful in 76% of patients with infranodal block and successful in 93% of patients with AV nodal block. QRS morphology has remained relatively stable without progression. HBP observational studies have shown a reduction in the combined endpoint of heart failure hospitalization than RVA pacing. This benefit was more in patients with higher ventricular pacing burden. Studies have shown improved hemodynamics with HBP resulting in improved systolic blood pressure and stroke volume.[42,43] These findings were also demonstrated by Lustgarten et al, who found improvements in clinical endpoints of New York Heart Association class, 6-minute walk test, quality of life, and ejection fraction.[44] One of the significant drawbacks of HBP is the difficulty in the procedure, and advanced centers with highly specialized teams are required for successful procedural outcomes.[45] HBP shows great promise in reducing morbidity and mortality by offering proper physiological pacing utilizing the native cardiac conduction system, and with advancements in the technology, better outcomes are expected.

LBB Pacing

The LBB pacing (LBBP) concept arises from the idea that pacing at a location distal to the block should resolve the block, which in turn would translate into a low pacing threshold. Huang et al first reported direct LBBP during HBP when high output was required to correct the LBBB. By advancing the pacing tip toward the ventricle, LBBB resolved, and there was a low pacing capture threshold.[46] The outcome included improvements in both left ventricular ejection fraction and heart failure symptoms.

In the LBBP method, similar leads and delivery systems to the HBP are used. The pacing lead used is a SelectSecure (Model 3830 69 cm; Medtronic Inc, Minneapolis, MN) with an exposed 1.8-mm active helix. The sheath used is a Select Site fixed curve sheath (Model C315 HIS; Medtronic Inc). An electrophysiological multichannel recorder is used for intracardiac EGMs together with a 12-lead electrocardiogram. pace-sense analyzer is used to test the pacing parameters and obtain EGMs. LBBP uses a transventricular-septal approach. It is critical to use a temporary pacing wire for back-up pacing for possible complete heart block secondary to mechanical injury. Pacing and recording are done with a unipolar configuration. There are 2 main methods used for LBBP lead placement: single-lead method (SLM) and dual-lead method (DLM).[48,49]

In SLM, 2 main approaches are used to locate the pacing site. HB is used as an anatomic marker in the first method, and the pacing lead is

placed in the HB under fluoroscopic guidance. Images are obtained, and the sheath and pacing lead is advanced toward the interventricular septum below the septal leaflet of the tricuspid valve on the right side. This location is usually 10-20 mm away from the HB region. In the second approach, the tricuspid valve annulus is used as an anatomic marker where the pacing lead and sheath are advanced directly across the valve. A pacing site is identified on the right side of the interventricular septum. The sheath is placed perpendicular to the septum, and the pacing lead is advanced. Paced QRS morphology is observed, which shows an LBBB morphology with a QRS duration of less than 150 ms, positive QRS in the lead II, biphasic QRS in the lead III, and a notched QRS in lead V1. After ensuring the location is correct with different fluoroscopic views, the lead is fixated with intermittent pacing to ensure successful delivery. ECG is monitored for the presence of RBB delay on V1 and V2. After all criteria for LBBP are met, the process is considered complete.[47,49,50]

In DLM, there are also 2 approaches possible. DLM uses 2 sets of 3830 leads and C315 delivery sheaths. In the first approach, the first pacing lead is placed in the HB and used as an anatomic marker to assess the block site if there is LBBB present. The second delivery sheath with the pacing lead for LBBP is advanced inferior to the septal leaflet of the tricuspid valve in the ventricular septum, approximately 10-20 mm from the HB. Fixation of the lead is done similarly to the SLM. In the second approach, after an initial attempt to perform LBBP with one pacing lead fails, a second pacing lead is used to locate a more optimal pacing site while the first lead is used as an anatomical marker. This method is mainly used in patients with enlarged right atrium or ventricle. When the location is identified, the first lead is moved to the right atrium and placed there.[46,47,51]

One advantage of the LBBP compared to the HBP is the relatively large area available for selection. LBBP has good safety outcomes and clinical feasibility. Lead fixation is more easily achieved than HBP, and there is less risk of lead dislodgment as seen in HBP.[52] Studies have further shown that LBBP can achieve a low and stable capture threshold compared to SHBP NSHBP (20).[50] However, conditions such as severe fibrosis makes implantation more difficult. In patients with a dilated cardiomyopathy or mechanical desynchronization, LBBP might not be the optimal choice.[45] Although LBBP provides a superior method for pacing compared to HBP, theoretically, it is not without limitations, and thus, clinical assessment for each technique is vital. One central area that LBBP is lacking is long-term safety results. With more studies on these two promising methods, we can see the real clinical significance of HBP and LBBP.

Battery Improvements

Modern PMs require an electrical power source mainly provided by batteries enclosed in the pacemaker unit. However, a significant drawback of such systems is the limited capacity and lifetime due to the finite amount of energy stored in the unit. When devices approach the end of life for the battery, unit replacement is required. This may embroil the patient with complications associated with the procedure, such as possible infection, bleeding, and extra cost. To overcome these limitations, various methods of energy-harvesting are currently being investigated. These methods include solar energy, piezoelectric energy, and transcutaneous US energy harvesting triboelectric units.

Solar-Powered PMs

One promising area is the use of solar energy for energy harvesting. The use of solar power has become mainstream in modern days, and since near-infrared light can easily penetrate the human skin, this can provide a pathway for energy harvesting. It has been shown that even a few minutes of direct sunlight may provide enough energy for a pacemaker to function for 24 hours.[53] However, the fundamental limitation is the need for the person to be exposed to direct sunlight, which can have various limitations based on location, climate, and lifestyle. A recent study by Haeberlin et al investigated the feasibility of battery-less, solar-powered cardiac PMs in animal models.[53,54] Their group demonstrated that in ex vivo conditions, it is possible to implant a fully functioning PM using ambient light as the energy source with good functional outcomes. They measured the amount of energy harvestable under direct sunlight and established that a few minutes of direct sunlight could provide enough energy for the PM to function for one day. Under ambient light, they witnessed the possibility of harvesting a considerable amount of energy, which at its lowest levels was at least approximately twice the housekeeping power consumption needed by a modern PM. In vivo, they were able to see that a 1 cm^2 subcutaneous solar module exposed to direct sunlight for 1 minute could harvest enough energy for several hours of PM function. They also showed that even with a 9 mAh accumulator without any energy input, the device lasted 40 days. Song et al have investigated the feasibility of such devices in human cadaver tissues and have established that such harvesting is possible, and the amount of energy captured is dependent on the size chosen.[55,56] To maximize harvesting, the device needs to in a location that would guarantee higher solar irradiation, such as the lateral neck, and the depth should be kept at a level to maximize light penetrance and minimize the likelihood of skin erosion. Also, a method

of notifying the patients that the battery level is low is critical. Nevertheless, fundamental questions need to be answered before this technology is ready for human use.

Conformal Piezoelectric Energy Harvesting

Another method of harvesting energy uses biomechanical energy by using wearable or implantable PMs that can convert mechanical energy using piezoelectric crystals to electrical energy for powering the device.[57,58] The main issue in this form of energy harvesting is the type of device used. These devices need to have excellent electromechanical coupling ability and be flexible. The development of nanowires that could enable such a task is critical. These devices need to be biocompatible and withstand various mechanical wear and tear levels while not causing immune reactivity. Dagdeviren et al were able to show that piezoelectric devices can yield significant electrical power by harvesting energy from motions of internal organs.[59] Li et al applied an implantable piezoelectric energy generator to directly power a PM by harvesting the natural energy of the heartbeat in vivo bovine model.[60] Thus, with recent developments in piezoelectric powered PM, implantation of such devices is a promising area in the field of EP.

Capacitive Triboelectric Technology

Triboelectric generators is another promising area of research. These generators use the triboelectric effect, an electrically charging phenomenon using frictional energy when various materials are in contact.[61] These generators can be used for harvesting energy by using US generators as the source for transferring energy in vivo. In this technology, high frequency vibrating and implantable generator devices harvest the US energy in vivo. These devices are thin and implanted under the skin at around 10 mm depth. These devices have a thin and large perfluoroalkoxy membrane, which contracts, causing the triboelectric phenomenon to generate negative charges on the membrane's surface, creating a current. The generated current is then used to charge a capacitance that can be used to power a PM.[62] This triboelectric signal depends on the acoustic radiation pressure and decreases with an increased distance. Ex vivo studies have shown this technology's capability to charge capacitors and batteries, allowing PM to work with such energy sources.[63,64] With more developments in this field, this form of PM technology can provide a promising future path.

Bioartificial PMs

As eluded earlier, present-day PMs, though very intelligent machines, come with many risks and compromises while mimicking the conduction biology.[65,66] Biological PMs have emerged in current research to address these limitations, but more research that attends to ethical and reliability problems must be done before cell-based, and gene-based therapy can be applied clinically.

Prerequisites for Biological PMs

The sinoatrial node (SAN) spontaneously depolarizes during diastole and is governed by many underlying currents, most notably the If current and IK1 current. The If (funny) current is generated by sinoatrial nodal cells and flows through hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, cation channels that are activated by hyperpolarization (voltages lower than -50 mV). It is mainly involved in diastolic depolarization, therefore playing an essential role in spontaneous automaticity.[67,68] HCN4 is one of four isoforms and is highly expressed in the SA node, and it accounts for more than 81% of total HCN mRNA in the rabbit SA node.[69,70] Mutations in HCN4 are implicated in sinus node dysfunction.[71,72] The inward rectifier potassium (Kir) channel current, also known as IK1, is mainly involved in maintaining prolonged action potentials during diastole. IK1-enhanced human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) acquire stable resting membrane potentials.[73] In contrast, guinea pigs were transduced with dominant-negative Kir 2.1, and their left ventricular cardiomyocytes showed spontaneous action potentials.[74]

Therefore, the biological approach to improve cardiac automaticity lies in enhancing the If current and in attenuating the IK1 current to generate a region of automaticity in the heart that functions as a substitute for a dysfunctional SAN.[75]

Cell-Based Approaches

Biological PM involves the introduction of a cluster of spontaneously beating cells into the heart. The earliest reported case of a cell-based system utilized fetal cardiomyocytes injected into the left ventricle in canines with AV block. This resulted in ventricular escape rhythms in the 2 transplanted dogs that were not observed in control dogs.[76] This approach has been further used with various spontaneously beating cell types. Human embryonic stem cells (hESCs) readily differentiate into

spontaneously beating cardiomyocytes, making them of interest in producing ectopic pacemaker regions. In vivo transplantation of hESC-derived cardiomyocytes into the left ventricle of guinea pigs resulted in biological pacemaker activity in the form of a successful spread of membrane depolarization from the site of injection confirmed by optical mapping.[77] The transplantation of hESCs into the hearts of pigs with complete AV block resulted in significant persistent pacing for weeks, confirmed by optical mapping and histopathological examination.[78] However, this approach has limitations in the form of ethical dilemmas (the controversial origin of the cells), poor engraftment, and transplantation requires extensive immunosuppression.

An alternative approach that addresses these limitations is to use induced pluripotent stem cells (iPSCs), created from previously differentiated adult cells obtained from skin or hair. These iPSCs are then further differentiated into SAN-like cardiomyocytes, which can be used in vitro or in vivo. A study used iPSC-derived cardiomyocytes in dog hearts by open thoracotomy, but biological pacemaker activity of rates 40-50 bpm was only seen in 50% of the animals.[79] Though the strategies address hESC limitations, iPSCs still produce mixed populations of cells with various phenotypes, which leaves the possibility of immature cells migrating or differentiating into different cell types. It has been shown that overexpressing a transcription factor-like Shox2 (specific to SAN development during differentiation) can highly favor cardiac PM cell population from iPSCs.[80]

Gene-Based Approaches

The gene-based approach to induce biological pacing involves identifying a gene of interest involved in PM activity of the heart and delivering (through a vector) a short nucleic acid sequence to manipulate the expression of the gene. The earliest gene therapy approach targeted $\beta 2$ adrenergic receptors found in increased density at the SAN. This approach suggested that these receptors are involved in regulating the funny current.[81] By overexpressing the $\beta 2$ adrenergic receptors, studies were able to significantly increase the endogenous SAN rate of mice and pigs compared to controls.[82,83] Though these studies did not technically create a biological pacemaker, it was shown that gene therapy could have a profound impact on sinus node pacing.

Another approach attempts to suppress and downregulate IK1 currents, involved in action potential repolarization and maintaining resting potential in diastole. Suppression of this current and similar potassium currents

can therefore generate automatic rhythms because they release the IK1 current's so-called "electrical brake." A study successfully reduced the number of functional Kir channels by overexpressing Kir2.1 AAA, a dominant-negative construct, in the left ventricular myocardium of guinea pig hearts.[84] The overexpression of the construct allowed cardiomyocytes to depolarize spontaneously by suppression of the IK1 current. However, because IK1 was downregulated, an essential factor in repolarization is eliminated, leading to prolonged repolarization, increasing the risk of torsades de pointes.

As previously mentioned, HCN channels generate the funny current during the hyperpolarization phase of the action potential. Therefore, another approach is to overexpress HCN to generate functional pacing. Qu et al overexpressed HCN2 in dogs, which was a more promising target because of its better activation kinetics than the other HCN isoforms. Spontaneous rhythms were found 4 days after delivery in the left atria, and heart rate showed autonomic responsiveness.[85] Follow-up studies injected HCN2 construct into the LBB of canines with AV block, introducing biological pacemaker function. However, basal and maximal rates were significantly slower.[86]

Hybrid Gene-Cell Approach

A hybrid gene-cell approach involves overexpression of pacemaker genes in cells before transplanting them in vivo. Plotnikov et al overexpressed HCN2 in human mesenchymal stem cells and injected them into canine hearts with complete heart block.87 Animals showed biological pacemaker activity without cellular rejection. This approach avoided using viral vectors and the necessity for immunosuppression, but heart rates were comparatively low (50-60 bpm).[87]

Somatic Reprogramming

Somatic reprogramming involves reactivating developmental pathways studied in embryonic development to reprogram adult cardiomyocytes into pacemaker-like cells by overexpressing transcription factors.

TBX18 is a vital transcription factor shown to be relevant in the embryonic differentiation of SAN cells.[88] By overexpressing TBX18, Kapoor et al reprogrammed ventricular cardiomyocytes into SAN-like cells, creating a biological pm rhythm that corrected the bradycardic disease model in guinea pigs.[89]

Another approach identified pair-related homeobox 1 (prrx1), a transcription factor, as a way to differentiate stem cells into sinus node-like cells. Yin et al overexpressed prrx1 in brown adipose-derived stem cells and found it current in altered cells only and none in the control group, showing successful induction of sinus-node-like cells.[90] They also found that prrx1 was coexpressed with other transcription factors and genes involved in SAN-like cell development, such as TBX18, ISL-1, and HCN4.

Conduction Tissue

Another biological pacing approach is to engineer conduction tissue in vitro and precisely transplant it in vivo for therapeutic results. Zhang et al seeded cardiac progenitor cells into a collagen sponge and transplanted them into rat hearts to determine if they could act as an AV conduction pathway.[91] Staining revealed that 60 days after transplantation, a large amount of myocardial tissue formed and that HCN2 and other connexins were present. This suggested that the engineered conduction tissue formed gap junctions in the surrounding myocardium, and the EKG confirmed clear pre-excitation in the transplanted rats. Within one hour following AV block, transplanted rats had a recovery rate of 61.54% with normal heart rhythms in contrast to only 4.17% in the control group. This study shows that engineered conduction tissue can be a potential therapy for AV block and could potentially function as a method to introduce biological pacing clinically.

Conclusions

PMs have come a long way since the first one was implanted over half a century ago.[92] Recent developments in pacemaker technology have led to improvements in its efficacy profile. Despite such achievements, many compromises are inevitable while implanting a pacemaker compared to the body's pacing mechanism. Leadless PMs have circumvented certain complications associated with transvenous pacing but do carry its limitations. With research involving HB and left bundle pacing, options for PP have emerged. Improvements in the pacemaker battery and energy harvesting may address the issue of limited battery life. Finally, the advent of bio-PMs may be a solution to complications related to a traditional cardiac pacemaker. However, despite substantial developments in the last one decade, the technology is far from prime. Given the fast pace of research, it is tenable to dream of one in the near future.

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