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**Original Article**

# Alterations in the transmural gradient of ventricular repolarization with different pacing sites in normal and heart failure canines

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**Abstract:** Alterations of the transmural gradient of repolarization may contribute to the increase of transmural dispersion of repolarization and ventricular arrhythmias. The transmural gradient of repolarization may play an important role in sudden death associated with left ventricular epicardial pacing. To investigate the changes of transmural gradient dispersion of ventricular repolarization with different pacing sites in heart failure (HF) canines, 8 mongrel dogs were randomized into healthy group and HF group ($n$ = 4). We mapped the monophasic action potential duration (MAPD) in the subendocardial, subepicardial and mid-myocardial layers of the left ventricle (LV) in canines of healthy and HF groups during right atrium (RA) pacing, right ventricular apical endocardial ($RV_{Endo}$) pacing, left ventricular lateral epicardial ($LV_{Epi}$) pacing and biventricular (Biv) pacing respectively. The results showed that in the healthy group, the MAPDs were significantly different among the three layers during RA pacing (all $P < 0.05$). The MAPD was longer in the mid-myocardial layer compared with those in the subepicardial and subendocardial layers during $RV_{Endo}$, $LV_{Epi}$ or Biv pacing ($P < 0.05$). However, there was no significant difference in MAPD between the subendocardial and subepicardial layers during $RV_{Endo}$, $LV_{Epi}$ or Biv pacing ($P > 0.05$). In the HF group, the MAPDs in all three layers were prolonged compared with those in the same locations in the healthy group (all $P < 0.05$). However, there were no differences in MAPD among the three layers during RA, $RV_{Endo}$, $LV_{Epi}$ or Biv pacing (all $P > 0.05$). By MAP recording with our new mapping electrode, we found a transmural MAPD gradient among the three layers of the LV during RA pacing and the gradient between the subendocardial and subepicardial layers vanished during $RV_{Endo}$, $LV_{Epi}$ or Biv pacing in healthy dogs. In contrast, there was no transmural MAPD gradient during RA, $RV_{Endo}$, $LV_{Epi}$ or Biv pacing in HF dogs. These results are helpful to understand the mechanism of ventricular arrhythmias in patients with HF.

**Key words:** heart failure; monophasic action potential; repolarization; transmural gradient; pacing

# 不同部位起搏对正常和心力衰竭犬心室跨壁复极梯度的影响

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**摘 要**：跨壁复极梯度的改变可能导致跨壁复极离散度的增加和室速的增多，跨壁复极离散可能在左室心外膜起搏相关猝死中发生重要作用。本研究对心力衰竭(心衰)犬进行心室不同部位起搏，观察左心室跨壁复极梯度的变化。选用8条健康杂种犬，随机分成健康对照组和心衰组($n$ = 4)，心衰组4条健康犬经快速右心室心内膜心尖部(right ventricular apical endocardium, $RV_{Endo}$)起搏4~5周建立慢性充血性心衰模型。健康对照组和心衰组分别在右心房(right atrium, RA)起搏、$RV_{Endo}$起搏、左心室心外膜(left ventricular lateral epicardium, $LV_{Epi}$)起搏及双心室(biventricular, Biv)同步起搏的条件下，应用自制的跨室壁单相动作电位(monophasic action potential, MAP)记录电极，于左心室同步记录和测量三层心肌(内层、中层、外层)的MAP时程(MAP duration, MAPD)。结果显示，健康对照组窦性心律时左心室三层心肌MAPD比较：中层 > 内层 > 外层，各层之间比较均有

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统计学差异(均$P < 0.05$)；$RV_{Endo}$、$LV_{Epi}$、Biv起搏时MAPD仍为中层 > 内层 > 外层，心外膜层与心内膜层MAPD比较无统计学差异($P > 0.05$)；每种起搏时中层与心外膜层及心内膜层的MAPD比较均有统计学差异(均$P < 0.05$)。与健康对照组比较，心衰组不同起搏模式(RA起搏、$RV_{Endo}$起搏、$LV_{Epi}$起搏、Biv起搏)时左心室心肌各层的MAPD均延长(均$P < 0.05$)。心衰组RA、$RV_{Endo}$、$LV_{Epi}$及Biv起搏时左心室三层心肌MAPD均表现为中层 > 内层 > 外层，但各层心肌MAPD均无统计学差异(均$P > 0.05$)。通过应用改良标测导管记录MAP的方法，我们发现健康犬RA起搏时左心室心内膜层、中层及心外膜层存在明显的跨壁梯度，$RV_{Endo}$、$LV_{Epi}$、Biv起搏时左心室心内膜与心外膜间跨壁梯度消失；然而心衰犬左心室的三层心肌在RA、$RV_{Endo}$、$LV_{Epi}$、Biv起搏时均不存在跨壁梯度，这些结果有助于加深对心衰患者室性心律失常发生机制的理解。

**关键词**：心力衰竭；单相动作电位；复极；跨壁梯度；起搏
**中图分类号**：Q25

Sudden cardiac death (SCD) is the most severe manifestation of heart disease and presumably caused by ventricular tachyarrhythmias in patients with heart failure (HF) [1]. Pacing from the left ventricle (LV) or biventricular (Biv) pacing can increase the repolarization dispersion and may increase ventricular tachyarrhythmias [2]. Transmural dispersion of repolarization plays an important role in the genesis of polymorphic ventricular tachycardia in HF patients with the LV or Biv pacing. Although previous studies focused on changes in transmural dispersion of repolarization in different animal models of HF [3, 4] and different ventricular pacing modes [5], the mechanisms for cardiac electrical heterogeneity resulting from pacing at different sites are poorly understood. In this study, we developed a new kind of mapping electrode based on previous studies for recording monophasic action potentials (MAPs) from subendocardial, subepicardial and mid-myocardial layers of the LV [6], and we mapped the LV of healthy and HF dogs *in vivo* by recording MAPs in order to investigate the changes of transmural gradient dispersion of ventricular repolarization with different pacing sites in HF canines.

## 1 MATERIALS AND METHODS

### 1.1 *Animal preparation*

Eight mongrel dogs were randomized into two groups: healthy group [$n = 4$, body weight (23.6 ± 1.3) kg] with normal hearts, and HF group [$n = 4$, (24.1 ± 2.9) kg] in which congestive HF was induced by rapid ventricular pacing (240 beats/min for 4 to 5 weeks) [7]. In the HF group, an 'Animal Pacer' model EP 6877 was implanted subcutaneously at the back of the dog and the lead (5076, Medtronic) implanted in the right ventricle (RV) apex. Cardiac function was evaluated before and after HF by ultrasonic cardiography (UCG) [8]. The study was approved by Ethical Committee of Dalian Medical University.

To record MAPs, the dogs were anesthetized with sodium pentobarbital (30 mg/kg, IP), and then were intubated and ventilated with 50% $O_2$/50% $N_2O$ using a constant volume ventilator (Simens 900 C). Arterial oxygen saturation ($SAO_2$) was monitored and kept above 95% during the study. A 6 Fr catheter was inserted into the femoral artery for continuous arterial pressure monitoring. In the healthy group, after the normal structure and function of the dog heart was established by UCG, a quadripolar pacing catheter (Cordis Webster, 6 Fr) was positioned in the right atrium (RA) appendage and RV apex via the right external jugular vein under fluoroscopy. In the HF group, the lead implanted in the RV apex for chronic ventricular pacing was disconnected from the pacemaker and used for RV apex pacing during the acute study. A median sternotomy was performed, and the pericardium was opened with a base-to-apex incision in both two groups. The epicardial pacing electrode was sutured to the posterolateral wall of the LV for LV lateral epicardial ($LV_{Epi}$) pacing.

### 1.2 *Electrodes for recording ventricular repolarization*

A plunge needle contained 3 pairs of silver bipolar electrodes was used for MAP recording from the subepicardium, mid-myocardium, and subendocardium. The six 0.18 mm-diameter silver electrodes and the 1.1 mm-diameter needle were insulated by polytetrafluoroethylene. The three pairs of electrodes were inserted in the needle, and the recording ends of each pair were exposed at two apertures on the needle wall at three levels (1 mm, 5 mm, and 11 mm distal to the needle tip). We also injected glue into the plunge needle in order to stabilize the positions of the electrode pairs (Fig. 1*A*).

The electrode was inserted into the muscle of the LV (Fig. 1*B*). The monophasic action potential duration

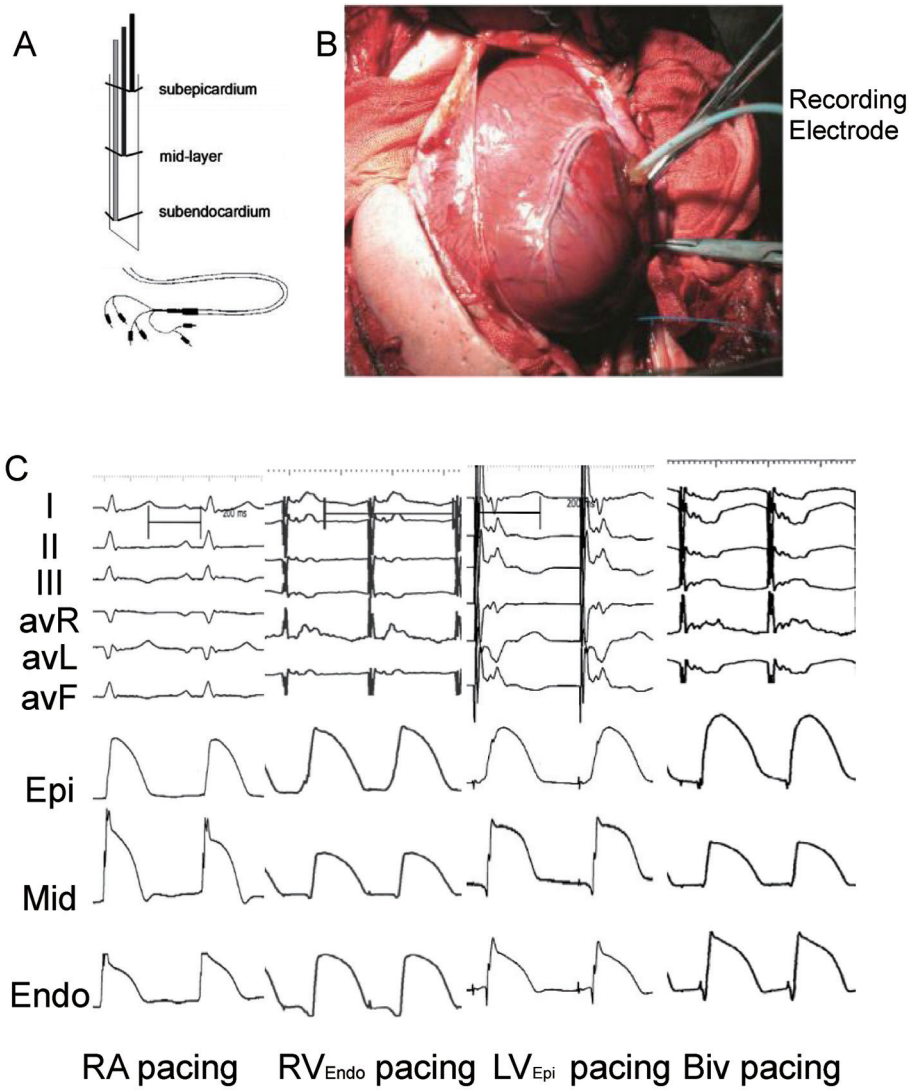


Fig. 1. Method to evaluate the transmural dispersion of repolarization in canines. *A*: Schematic representation of the experimental recordings. A plunge needle containing 3 bipolar silver electrodes was used to record monophasic action potentials (MAPs) from the subepicardium, mid-myocardium and subendocardium. *B*: The heart was explored by incision via a median sternotomy. The epicardial pacing electrode was sutured to the posterolateral wall of the left ventricle (LV), and the mapping electrode was inserted in the anterior free wall of the LV. *C*: MAP duration (MAPD) of subendocardial (Endo), subepicardial (Epi) and mid-myocardial (Mid) layers during right atrium (RA) pacing, right ventricular apical endocardial ($RV_{Endo}$) pacing, left ventricular lateral epicardial ($LV_{Epi}$) pacing and biventricular (Biv) pacing in a healthy canine.

(MAPD) of the subendocardial, subepicardial and mid-myocardial layers were recorded at six locations on the LV (anterior base, posterior base and lateral free wall halfway between apex and base, mid-anterior free wall, and LV apex).

1.3 *Experimental protocol*

MAP signals were recorded at a filter bandwidth of 0.05–400 Hz. The MAP electrogram was recorded during pacing from the RA, endocardium of the RV apex ($RV_{Endo}$), $LV_{Epi}$ and Biv respectively. MAPD recordings were made during stable pacing at a constant rate. The paced voltage was twice diastolic threshold and the pacing cycle length was 335 ms for 1∶1 capture at each site. There was an interval of 5 min between pacing from each site. The MAPs recorded from the distal, mid and proximal electrodes were considered to reflect electrograms from the subendocardial, mid-myocardial and subepicardial layers, respectively (Fig. 1*C*).

1.4 *MAPD analysis*

An independent investigator performed MAPD analysis

off-line. The MAPD was defined as the time interval from the earliest point of the upstroke to 90% repolarization. The MAPD was measured from a recording displayed at 100 mm/s.

1.5 *Statistical analysis*

All data are presented as the mean ± SD. Differences of MAPD between the two groups and among the 4 pacing sites were analyzed with a Kruskal-Wallis test, and Dunn's multiple comparison test was used for *post hoc* comparisons. A value of $P < 0.05$ was considered statistically significant.

## 2 RESULTS

HF was successfully induced by rapid ventricular pacing in 4 dogs in HF group, and the cardiac function was decreased by measuring UCG (Fig. 2).

### 2.1 The transmural MAPD in the healthy group

In healthy dogs, the MAPD was the longest in the mid-myocardial layer and shortest in the subepicardial layer, and there were significant differences among all three layers during RA pacing (Mid *vs* Endo, $P < 0.001$; Mid *vs* Epi, $P < 0.001$; Endo *vs* Epi, $P = 0.038$). The MAPD was longer in the mid-myocardial layer compared with the subepicardial and subendocardial layers during $RV_{Endo}$ pacing (Mid *vs* Endo, $P = 0.005$; Mid *vs* Epi, $P = 0.001$), $LV_{Epi}$ pacing (Mid *vs* Endo, $P = 0.001$; Mid *vs* Epi, $P < 0.001$) or Biv pacing (Mid *vs* Endo, $P = 0.016$; Mid *vs* Epi, $P = 0.002$). However, there were no differences in MAPD between the subendocardial and subepicardial layers at the three different ventricular pacing sites ($RV_{Endo}$ pacing, $P = 0.465$; $LV_{Epi}$ pacing, $P = 0.095$; Biv pacing, $P = 0.443$) (Fig. 3*A*).

### 2.2 The transmural MAPD in the HF group

In the HF group, the MAPD was not significantly different among the three layers during RA pacing (Mid *vs* Endo, $P = 0.312$; Mid *vs* Epi, $P = 0.057$; Endo *vs* Epi, $P = 0.369$), $RV_{Endo}$ pacing (Mid *vs* Endo, $P = 0.122$; Mid *vs* Epi, $P = 0.098$; Endo *vs* Epi, $P = 0.911$), $LV_{Epi}$ pacing (Mid *vs* Endo, $P = 0.674$; Mid *vs* Epi, $P = 0.184$; Endo *vs* Epi, $P = 0.363$) or Biv pacing (Mid *vs* Endo, $P = 0.122$; Mid *vs* Epi, $P = 0.060$; Endo *vs* Epi, $P = 0.735$) (Fig. 3*B*) .

The MAPD of the subendocardial layer in the HF group was prolonged compared with that in the healthy group during RA pacing ($P < 0.001$), $RV_{Endo}$ pacing ($P < 0.001$), $LV_{Epi}$ pacing ($P < 0.001$) or Biv pacing ($P < 0.001$) (Fig. 3*C*a). The differences between the

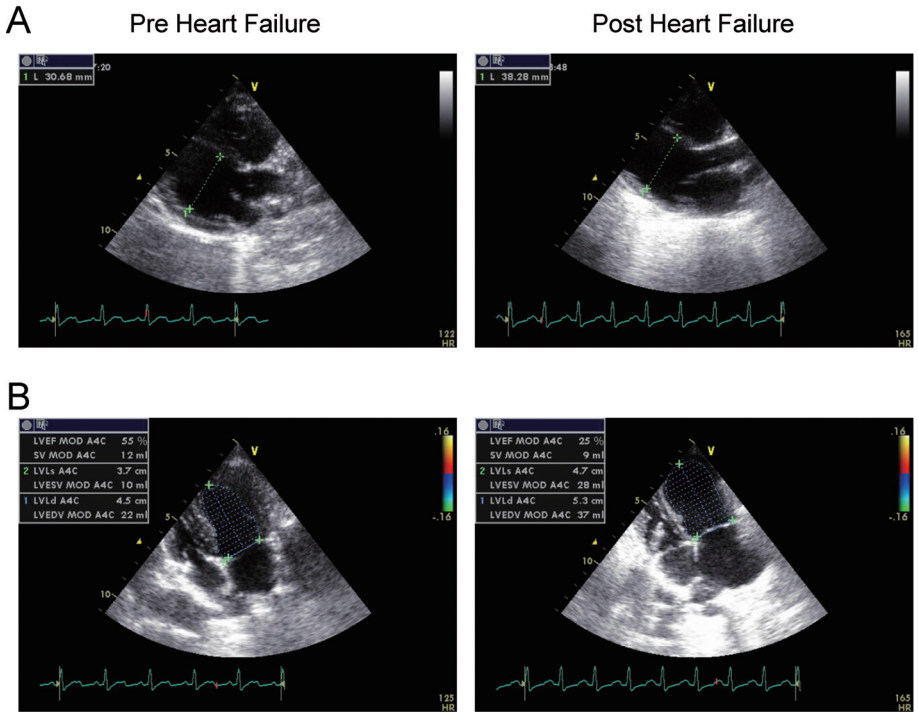


Fig. 2. The cardiac function of canines was decreased after heart failure (HF) by measuring ultrasonic cardiography (UCG). *A*: The left ventricle (LV) end-diastolic diameters were 30.68 mm and 38.28 mm before and after HF respectively. *B*: The LV ejection fractions (EF, %) were 55% and 25% before and after HF respectively.

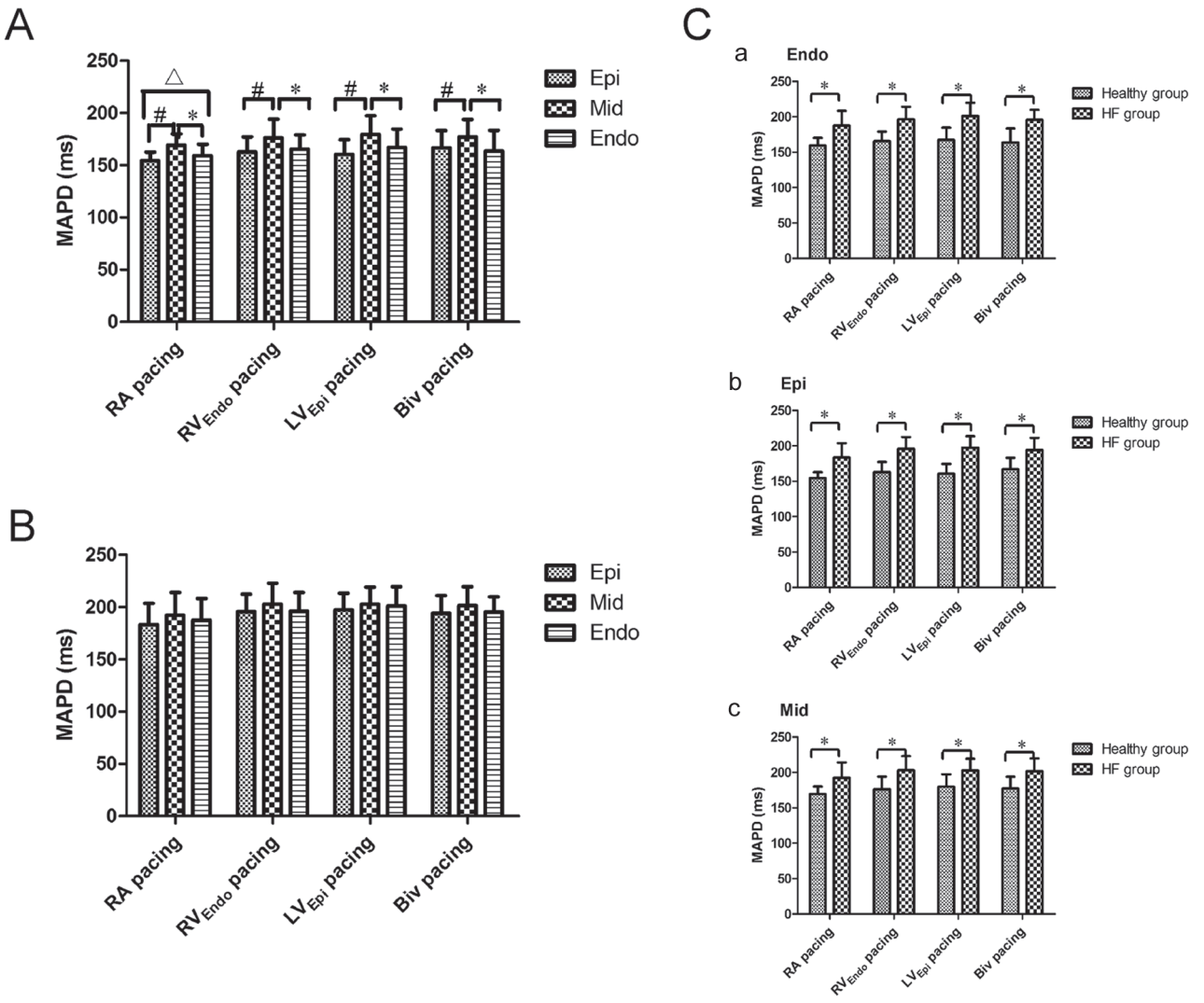


Fig. 3. Results of the transmural dispersion of repolarization on canines. *A*: MAPD in the healthy group. Comparison of MAPD (subendocardial, subepicardial and mid-myocardial layers) during pacing at various sites in the healthy group. The MAPD was the longest in the mid-myocardial layer and the shortest in the subepicardial layer at different pacing sites. *B*: MAPD in the HF group. Comparison of MAPD (subendocardial, subepicardial and mid-myocardial layers) during pacing at various sites in the HF group. There were no statistically significant differences among the subendocardial, subepicardial and mid-myocardium layers at different pacing sites. *C*: Comparison of MAPD of the subendocardial layer (a), subepicardial layer (b) and mid-layer (c) between the healthy group and HF group during different myocardial pacing sites. Epi, subepicardial layer; Mid, mid-myocardial layer; Endo, subendocardial layer; RA pacing, pacing in the right atrium; $RV_{Endo}$ pacing, pacing in the endocardium of the RV apex; $LV_{Epi}$ pacing, pacing in the epicardium of the LV posterolateral wall; Biv pacing, biventricular pacing. $^{\#}P < 0.05$, $^{*}P < 0.05$, $^{\triangle}P < 0.05$; Mean ± SD, $n = 4$, 6 points per dog.

failing and non-failing hearts were most remarkable in the subepicardial layer (during RA pacing, $P < 0.001$; during $RV_{Endo}$ pacing, $P < 0.001$; during $LV_{Epi}$ pacing, $P < 0.001$; during Biv pacing, $P < 0.001$) (Fig. 3*C*b) and mid-myocardial layer (during RA pacing, $P < 0.001$; during $RV_{Endo}$ pacing, $P < 0.001$; during $LV_{Epi}$ pacing, $P < 0.001$; during Biv pacing, $P < 0.001$) (Fig. 3*C*c).

## 3 DISCUSSION

The MAP can be recorded from beating hearts *in vivo* and can accurately indicate the time of activation and the entire repolarization time course of the transmembrane action potential [9]. For our *in vivo* study, we developed a new kind of mapping electrode based on previous studies and the boundary current hypothesis

for recording MAPs from different myocardial layers of the LV. The mapping electrode was similar to custom-made electrodes reported by Zhao *et al.* [10]. We made several changes. Each needle included 3 pairs of bipolar recording electrodes and the distance between the proximal and distal pairs was similar to the thickness of the LV in the dog. We recorded MAPs in different myocardial layers of the LV perspicuously, and our results showed the presence of a transmural repolarization gradient during RA pacing in the healthy heart. Importantly, this normal gradient disappeared due to the heterogeneous prolongation of MAPD in HF dogs. We also found that a greater increase of the MAPD in the subepicardial layer was the main reason for the reduced transmural gradient among the three myocardial layers during RA pacing. These results are similar to those of Glukhov *et al.* [11]. We found normal gradient disappeared in HF canines by MAPD recording with our new mapping electrode, which was much more convenient than the action potential duration mapping in isolated wedges of human ventricular wall for evaluating the a transmural repolarization gradient [12]. Previous studies [13] have suggested that calcium overload may be the main reason for the HF-induced prolongation of ventricular repolarization time observed in the present study. The precise mechanism responsible for the dispersion of repolarization in HF is unknown, and more studies about this issue are needed.

Pacing in ventricle alters the sequence of ventricular activation and repolarization and induces electrical remodeling [14, 15]. In our study, pacing at all three ventricular sites resulted in prolonged MAPD in all three layers in healthy and HF dogs, which may increase the risk of ventricular arrhythmias. Cardiac resynchronization therapy (CRT) has been widely adopted as a treatment for HF since it improves exercise tolerance and New York Heart Association (NYHA) functional class, and reduces HF-related arrhythmogenesis deaths [16, 17]. HF patients needing long-period ventricular pacing adpoted CRT first. However, pacing from the ventricle including Biv pacing may increase ventricular tachyarrhythmias and the prolonged MAPD in all three layers in our study may be a potential mechanism, which was similar to the study of Glukhov *et al.* [11]. A new method of transseptal LV endocardial pacing is associated with significant reduction in transmural dispersion of repolarization characteristics compared to epicardial pacing in CRT [18, 19], which still need further studies to determine whether these effects may contribute to reduction of arrhythmias in patients with CRT.

There are several limitations in our study. First, we did not ablate the left bundle branch, so the animals did not show left bundle branch block which is typically observed in patients that get CRT. Second, we only observed the transmural gradient in the three myocardial layers during acute pacing modes and long-term pacing may have different effects. Third, we did not perform RV MAP mapping, so were not able to evaluate transmural repolarization gradients in the whole heart. We will do further studies to try to address all of the above issues.

In summary, by MAP recording with our new mapping electrode, we found a transmural MAPD gradient among the three myocardial layers of the LV during RA pacing, and the gradient between the subendocardial and subepicardial layers vanishes during $RV_{Endo}$, $LV_{Epi}$ or Biv pacing in healthy dogs. In contrast, there was no transmural MAPD gradient during RA, $RV_{Endo}$, $LV_{Epi}$ or Biv pacing in HF dogs.

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