The Optimum Resistance of Small Intestine Fusion by Pulse Source

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Research article

Keywords: Small intestine fusion, Pulse source, Burst pressure, Resistance

Posted Date: January 28th, 2020

DOI: https://doi.org/10.21203/rs.2.21965/v1

Abstract

Background

The radiofrequency (RF)-induced intestine-fusion, as a replacement method for traditional suture in surgery, has been studied for years. The present study reports the optimum resistance of small intestine fusion. As the feedback signal, resistance will be the indicator of the fusion completion for device design of intestine-fusion, and in-depth study of microstructure change.

Materials and methods

A self-design pulse source is used for small intestine fusion with adjustable voltage, duty ratio, frequency and output time. In this study, the frequency is 440 kHz; voltage, output time and compression pressure (CP) on the small intestine are independent variables for the experiment. The differences of hematoxylin-eosin (H&E) staining slices are discussed. The real time current is measured and recorded during the fusion for calculation of resistance. The burst pressure (BP) is measured with a pressure gauge and a peristaltic pump after the fusion completion.

Results

The highest BP is 38.9 mmHg with CP of 900 kPa, voltage of 50 v and time of 5 s. The optimum resistance is 71.1 Ω.

Conclusions

The quality of fusion is a result of combined influence of CP, voltage and time. The optimum resistance of 71.1 Ω is proposed for the highest BPs and automatically fusion.

Background

In the most intestinal cancer surgery, the success of the surgery is dependent on the integrity of the anastomoses in the inflammation phase [1]. Hand suturing was the main method for intestinal anastomoses in the past. However, the bleeding and the leaks with potential inflammation reduce the success rate of the operation, the long suturing time increases the operation risk. After the emerging of the laparoscopic surgery, stapling has become the main method for intestinal anastomoses as the convenience accelerates surgical procedure and the reliability reduces bleeding and leaks. Nowadays, stapling has been large-scale applicated in the intestinal surgery, but the failure exists because of the technical shortcomings and the low degree of surgeons' proficiency. Surgeons even have to do suture after stapling to make sure of the reliability. the high price of stapling also increased the surgery cost [2].

Throughout the years, there have been various attempts to explore alternative anastomotic methods based on RF tissue-fusion as its natural advantages: it does not leave foreign material which may induces inflammation and leads to infection, short operation time and low cost.

In 2007, Ligasure Anastomotic Device was developed and used for intestinal tissue of porcine (4 pigs, 2 anastomoses each) and all seals were macroscopic intact both immediate after creation and healing at the 7th postoperative day. This result confirms the feasibility to create experimental intestinal anastomoses using RF power source [3]. In 2010, the BP was used to evaluate the quality of colonic anastomoses in vitro. An optimal interval of CP (CP = 1.125 N/mm^2) in respect of a high amount of BP was detected. Further studies exploring the main effects and interactions of tissue and process parameters to the quality of the fusion site still need to follow [4]. In 2013, the study shows that both bipolar RF energy and optimal CPs are needed to create strong intestinal seals. This finding suggests that RF fusion technology can be effectively applied for bowel sealing and may lead to the development of novel anastomosis tools [5]. In 2014, a novel concave-convex electrode for colonic anastomoses by RF tissue-fusion was proposed to reduce thermal damage essential for anastomotic healing and was verified effectively [6].

Besides the macroscopic aspects, some researchers proposed optic measurements for diagnosing quality of anastomoses and exploring the insight mechanism. In 2008, real-time optical measurements was applied to improve understanding of the tissue modifications induced by RF fusion. An algorithm was proposed based on the measurement of the absolute transmittance of the tissue, making use of the modified Beer-Lambert law. Optical measurements show considerable potential as a modality to investigate the process of RF fusion and as feedback to control RF delivery in real time so that optimal transformations are achieved [7]. In 2014, the first optical-Raman-spectroscopy study on porcine small intestine was proposed in vitro. This study provides direct insights into tissue constituent and structural changes on the molecular level, exposing spectroscopic evidence for the loss of distinct collagen fiber rich tissue layers as well as the denaturing and restructuring of collagen crosslinks post RF fusion. These findings open the door for more advanced optical feedback-control methods and characterization during heat-induced tissue fusion, which will lead to new clinical applications of this promising technology [8].

Great progress has been made in the RF intestinal fusion field. However, the above studies don’t provide the direct method for evaluate quality of fusion in clinical application. The most important point is that how to create the automatic procedure for anastomoses and how to verify the accomplishment of the procedure. As in the clinic application, measuring BP is not possible; optic measurements need extra devices which increases complexity of surgery. In fact, the impedance measurement is a feasible method for evaluate quality of fusion, but there are few reports to study the relation between impedance and the quality of fusion. In this study, the optimum resistance is proposed for accomplishment of small intestine fusion which reaches the high BP based on the large-scale experiments in vitro. Fusion with different voltage levels, CPs and fusion times has been verified.

Methods

Pulse source

A self-design constant voltage pulse source is used for the experiment. The bipolar square wave is generated by a full bridge converter. The voltage value, duty ratio, frequency and output time are all adjustable. In this study, the duty ratio is set as 100% and frequency is set as 440 kHz. The output voltage is measured by a differential probe RP2015D made by Rigol and an oscilloscope 6403D made by PicoScope. The rise time (10%-90%) from negative 50 v to positive 50 v is 24 ns and the fall time (10%-90%) is 45 ns under the same condition for each pulse. In this study, different voltage values from 20 v to 80 v and output time of 5 s, 10 s and 15 s are applied to the small intestine.

Compression device and BP measurement

The compression machine ZQ-990A-1 is made by Zhiqu Precision Instruments with a pressure from 0-200 N (Fig. 1). The applied pressure can automatically adapt to the varying thickness during the fusion for keeping constant pressure. Small intestine is placed on the bottom clamp, and then the top clamp is pushed down for clamping small intestine. After several seconds, the pressure is constant then the pulse source outputs the voltage. In this study, the six CPs are 36, 45, 54, 63, 72, 81 N and the fusion time is 5 s, 10 s or 15 s. The electrode material is Nickel-chromium alloy with a surface size of 3*40 mm. As the width of small intestine is around 30 mm, So fusion surface size is 3*30 mm. Intensity of pressures are calculated by CP/fusion surface size, which are 400, 500, 600, 700, 800, and 900 kPa.

The peristaltic pump BT-100CA is made Jihpump with a flow rate from 0.07-79 ml/min (Fig. 1). In this study, the flow rate is set as 5 ml/min. The pressure gauge YK-100 is made by Shileke Technology with a measuring range from 0-1500 mmHg with an accuracy of 0.4 mmHg (Fig. 1). A three-terminals of the T-tube were connected separately to the pressure gauge, peristaltic pump and one end of the small intestine(another end of the small intestine has been sealed by fusion). The BP is defined as the maximum pressure measured during infusion. Once the fused anastomotic line starts to leak, the pressure begins to drop.

Impedance measurement

Pearson current monitor 4100 and oscilloscope 6403D are used for measuring current. As the voltage is constant, impedance is calculated by voltage/current.

Preparation of fresh small intestine

A complete porcine small intestine is harvested from pig at slaughterhouse. After cleaning, the small intestine is immersed in 0.9% saline and delivered at 0–4 ℃ to the laboratory for the experiments. Before the experiment, a secondary cleaning is applied to the small intestine. Then, the small intestine is cut into 70 mm segments and immersed in 0.9% saline until fusion. All the prepared small intestines were used for fusion experiments within 24 h after harvest. Mucosa-to-mucosa fusions are formed on porcine small intestine segments in vitro by the pulse source mentioned above.

Experiment process

Small intestines are harvested from pig and cleaned at the slaughter. After delivered to the laboratory, small intestines are cut into 70 mm segments. Each small intestine is placed on the bottom clamp, then the pressure machine begins to push top clamp down for applying pressure. Usually this process takes several seconds and then the pulse source is turned on for generating pulse square voltage, which is transferred by wires to clamps. Oscilloscope 6403D record the complete waveform of current during the fusion. small intestines are tested for BP after the fusion (Fig. 2).

Histology

Fused small intestines are fixed in formalin immediately after fusion. The tissue is processed in paraffin wax and prepared in slices, then cut transversely to the seal and stained with hematoxylin-eosin (H&E) staining.

Results

Burst pressure

As the existence of biological tissue heterogeneity and the measuring error, the BP of the adjacent segments are usually different. In order to get the accurate data with less sampling error, each BP is the average value of the ten experiments under the same condition. For example, the BP is 28.1 mmHg under the condition of 50 v, 700 kPa and 5 s (Fig. 3). The highest 35.8 mmHg and lowest 24.7 mmHg are removed while calculating average value for less sampling error.

In this study, a total of six group small intestines are tested for BP, including 106 BP values, which means 1060 segments are tested for BP.

For CP less than 400 kPa, the BP is lower than 15 mmHg and most fusions fail. So the above data only include the fusion with CP greater than or equal to 400 kPa. CP, voltage and fusion time are all the influence factor of BP. For relatively low CP, voltage and relatively short fusion time, the fusion may failed; for relatively high CP, voltage and relatively long fusion time, the temperature is too high for optimum fusion, so the fusion also failed. In each graph, the BPs usually increase at first and then decrease except the 10 s and 15 s in Fig. 4 (a) and Fig. 5 (b). For CP of 400 kPa or 500 kPa, time of 10 s or 15 s, the highest BP need high voltage of 70 v or 80 v. For high CP of 900 kPa and long time of 15 s, the highest BP need only voltage of 20 v. With the increase of CP, the optimum time decreases from 15 s to 5 s.

The transverse slice and H&E stain of fusion samples are showed in the Fig. 5. With the increase of CP, the width of fusion line decreases and the tightness of fusion line increases (from a to e). With the CP of 100 kPa and 300 kPa, there is obvious gap in fusion line (d and e). So the BPs of fusion with 300 kPa or below are relatively too low and not included in the above discussion. For Fig. 5 (b), (f) and (g), the fusion time and CP are the same, with the increase of voltage, the width of fusion line decreases and the tightness of fusion line increases. However, the high level of voltage and CP is adverse to the fusion, showed in the Fig. 5 (h). The fracture at the fusion line indicates the fusion failed, which is also consistent with the BP results.

Impedance

For analyzing optimum fusion quality, the peak current of each cycle (440 k cycles/s) is recorded by the oscilloscope (Fig. 6). The current rises at the beginning and then decreases. The beginning current is 3.86 A, so the impedance is 12.95 Ω,the maximum current is 6.26 A, so the minimum impedance is 7.99 Ω, after which the current begins to decrease. The final current is 1.77 A and the impedance is 28.25 Ω excluding the oscillation. The current waveforms of 10 s and 15 s are not recorded, as the depth of the oscilloscope 6403D is 1 G, which means for current waveform of 5 s, the sampling rate is around 19 ns. The increased time will increase the sampling rate and decrease the accuracy of measurement, thus the data are uncomparable. Another reason is that the slope of current after 5 s does not change much.

Figure 7 shows the impedance of different voltages with 700 kPa and 5 s. The change of the impedance increases as the voltage increases. At the end of 5 s, the impedance of small intestine is larger if greater voltage applied.

Figure 8 shows the impedance of different CPs with 50 v and 5 s. There is not obvious change rule for impedance of different CPs. However, the resistance actually differs of different CPs, which will be discussed in the next section.

Resistance

Resistance is real part of the impedance. In the last section, the resistance is not separated from the impedance. In this section, the resistance of the fusion end is recorded.

At the beginning of the fusion, the output current is square waveform, usually after around 1 s (depends on voltage and CP), the current overshoot appears (Fig. 9). An equivalent circuit model of the small intestine consists of a capacitance and resistance. For a circuit of paralleled relatively smaller capacitance and relatively bigger resistance, the main current is on the capacitance at the beginning. With the increase of current on the capacitance, the current on the resistance also increases. There is a time that the current of capacitance begins to decrease and at last the current on the capacitance is zero and the whole current is on the resistance. So there is a peak current at the beginning for each pulse, as the exist of capacitance, the overshoot current is actually a charging process. After that, the current of smooth interval is the actual resistance of the small intestine. The median value of each cycle is extracted as the resistance in this study. The resistance at the end of fusion is recorded for each small intestine. In order to get the accurate data with less sampling error, each resistance at the end of the fusion is the average value of the ten experiments under the same condition. The highest and lowest resistance are removed while calculating average value.

Figure 10 shows the relation of BP and resistance under different conditions. For CP of 400 kPa, the highest BP is 28.5 mmHg with a resistance of 80.0 Ω; for CP of 500 kPa, the highest BP is 30.9 mmHg with a resistance of 65.6 Ω; for CP of 600 kPa, the highest BP is 32.5 mmHg with a resistance of 69.0 Ω; for CP of 700 kPa, the highest BP is 32.5 mmHg with a resistance of 86.2 Ω; for CP of 800 kPa, the highest BP is 33.6 mmHg with a resistance of 64.9 Ω; for CP of 900 kPa, the highest BP is 38.9 mmHg with a resistance of 61.0 Ω. For each line, the resistance increases as the voltage increases, so the voltage is the lowest for the first one on the left and the highest for the first one on the right on each line.

Discussion

In this study, the BP of small intestine fusion is measured based on a large scale experiments. Voltage, CP and fusion time are all factors affecting the BP. The maximum BP is 38.9 mmHg when the parameters are 50 v, 900 kPa and 5 s. The high fusion quality needs appropriate parameters. Low voltage, CP or short fusion time can not provide high seal strength; high voltage, CP or long fusion time may cause thermal damage to small intestine and is actually adverse to fusion. Small intestine fusion needs suitable parameters. The maximum BP is 38.9 mmHg when the parameters are 50 v, 900 kPa and 5 s. In 2013, a research group reaches a maximum BP of 27.56 mmHg under the condition of 150 kPa. For other parameters, the study only mentions that the frequency is 473 kHz [4]. For other similar studies, usually only one parameter is discussed. The combined influence of voltage, CP and time are analyzed for the first time. The optimum fusion quality is the combined action of CP, voltage and fusion time.

The transverse slice and H&E stain of fusion samples are showed nextly. This result is matched to the BP result: the tight fusion line with appropriate parameters. The tissue structure from microscopic aspect explained the relationship between BP and parameters. Low voltage or CP can not provide tight fusion line; high voltage or CP induces fracture at the fusion line, which makes the fusion failed.

The current of during the fusion is recorded and impedance/resistance is calculated for the first time in the tissue fusion field. In Fig. 6, current waveform of 700 kPa, 50 v, and 5 s is recorded. However, the curve is not smooth. When the fusion begins, small intestine deforms, so the top clamp accelerates moving down for keeping constant pressure. In a very short time the compression pressure is lower than the preset value. When the pressure reaches the preset value, the speed of the top clamp begins to decrease and in this very short time, the actual pressure is greater than the preset value as the top clamp is still moving down. Lower pressure decreases the tightness of the small intestine, so conductive channel is weakened and current decreases; greater pressure increases the tightness of the small intestine, so conductive channel is enhanced and current increases. If the response speed is quick enough, the current should be a smooth curve. The current waveforms of 10 s and 15 s are not recorded, as the depth of the oscilloscope 6403D is 1 G, which means for current waveform of 5 s, the sampling rate is around 19 ns. The increased time will increase the sampling rate and decrease the accuracy of measurement, thus the data are uncomparable. Another reason is that the slope of current after 5 s does not change much.

The difference between impedance and resistance is explained by Fig. 9. Due to the high bandwidth and storage depth, the oscilloscope can record the current of long time and high precision. The current recorded in the last section is impedance, not the resistance actually, as the exist of capacitance of the small intestine. There is abvious relationship between impedance and voltage, but no relationship between impedance and CP. However, there is obvious relationship between resistance and all parameters.

In the Fig. 10, even the resistance at the end of the fusion differs a lot from 7.6 to 421.1 Ω, the optimum resistance is concentrated. Based on this large-scale experiment, there is an optimum resistance range of 61.0 to 86.2 Ω, the average of these six resistance is 71.1 Ω. With a preset voltage and CP, the resistance decreases at the beginning and then increases as the time increases. Regardless of the value of voltage and CP, small intestines will be sealed at a time with the optimum resistance. For pulse source design, the current at this moment will be the best feedback signal as the end of fusion. In the last section, the difference of impedance under different CPs is small, so impedance is not a possible way to verify the fusion quality. However, resistance is a meaningful parameter for verifying the fusion quality.

This is a macroscopical result for small intestine fusion, as the microcosmic change need to be verified by more experiments to explain this optimum resistance. What kind of chemical reaction or physical reaction occurred in cells or tissues during the fusion need more experiments. In our view, the small intestine will be in the optimum fusion quality with a certain microcosmic structure, and the resistance in this condition should also be a certain value. The microscopic studies need extra experiments to be verified.

Based on our experiments, the proper temperature is also essential for the fusion. The temperature is actually monitored during the fusion by an infrared thermometer. For most conditions, the temperature is beyond 100 ℃, unless the voltage or the compression pressure is too low, and the rise of temperature is very fast, usually it takes only 2 s or 3 s from 0 to 100 ℃ (depends on voltage and CP). The long time of high temperature is actually bad for the fusion, as the best BP is the time of 5 s; but the high temperature is also essential for the fusion. Considering the cell viability, we proposed the short fusion time is the future research direction. In addition, more experiments about the synergistic effect of temperature and current need to be designed.

Conclusions

In this study, a total of six group small intestines are tested for BP, including 106 BP values, which means 1060 segments are tested for BP. The combined influence of voltage, CP and time is discussed. The highest BP is 38.9 mmHg with 900 kPa, 50 v and 5 s. The transverse slice and H&E stain of fusion samples explained the relationship between BP and parameters from microscopic aspect. Then the impedance and resistance is measured and discussed for the optimum resistance. A resistance of 71.1 Ω is proposed as the optimum resistance for the end of fusion, which will be an indicator for automatical fusion with best fusion quality.

Abbreviations

RF
Radiofrequency
CP
Compression pressure
H&E
Hematoxylin-eosin
BP
Burst pressure

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

The work was supported by the Academy for Engineering and Technology, Fudan University (JIH2310003). The funders had no role in the design, data collection, data analysis, data interpretation or writing of this paper, nor in the decision to submit the paper for publication.

Authors' contributions

KL applies for the project and establishes experimental direction; YW designs the pulse source, experimental scheme and accomplishes the fusion experiments. CZ and HW Wang assists YW measure the BP. XX assists YW make transverse slice and H&E stain of fusion samples. YW drafted the manuscript. All authors reviewed the final manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

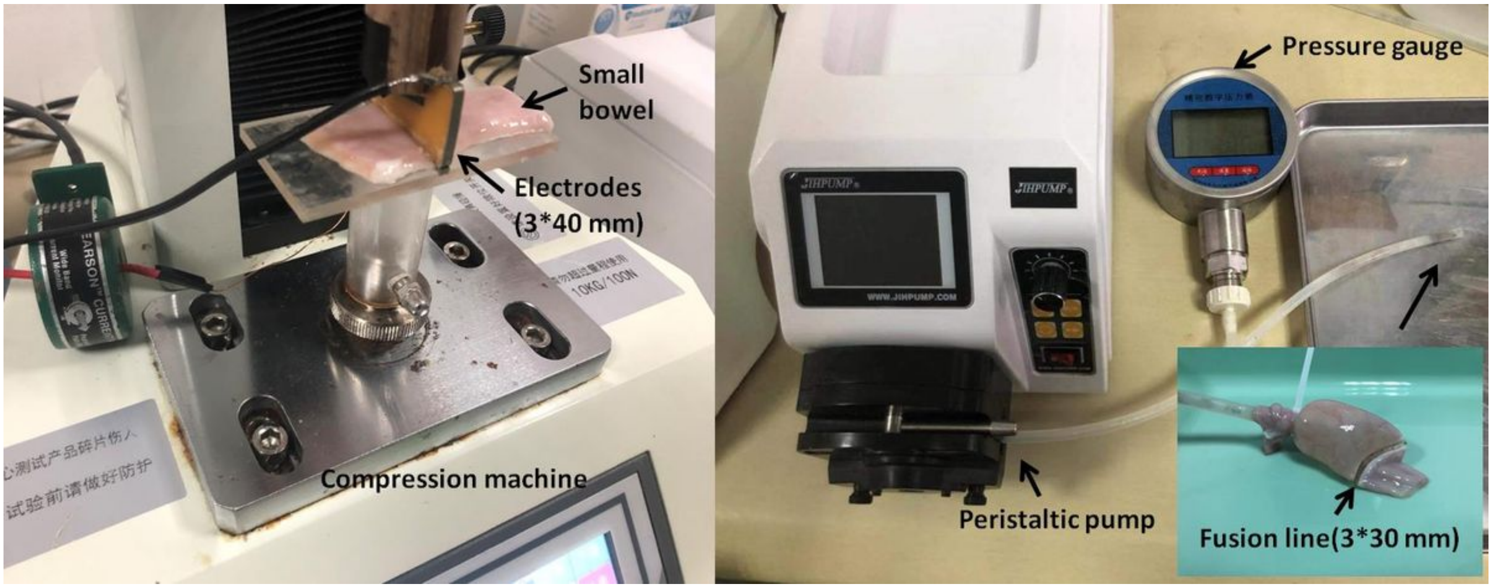

Figure 1

Compression machine, peristaltic pump, pressure gauge and BP measurement

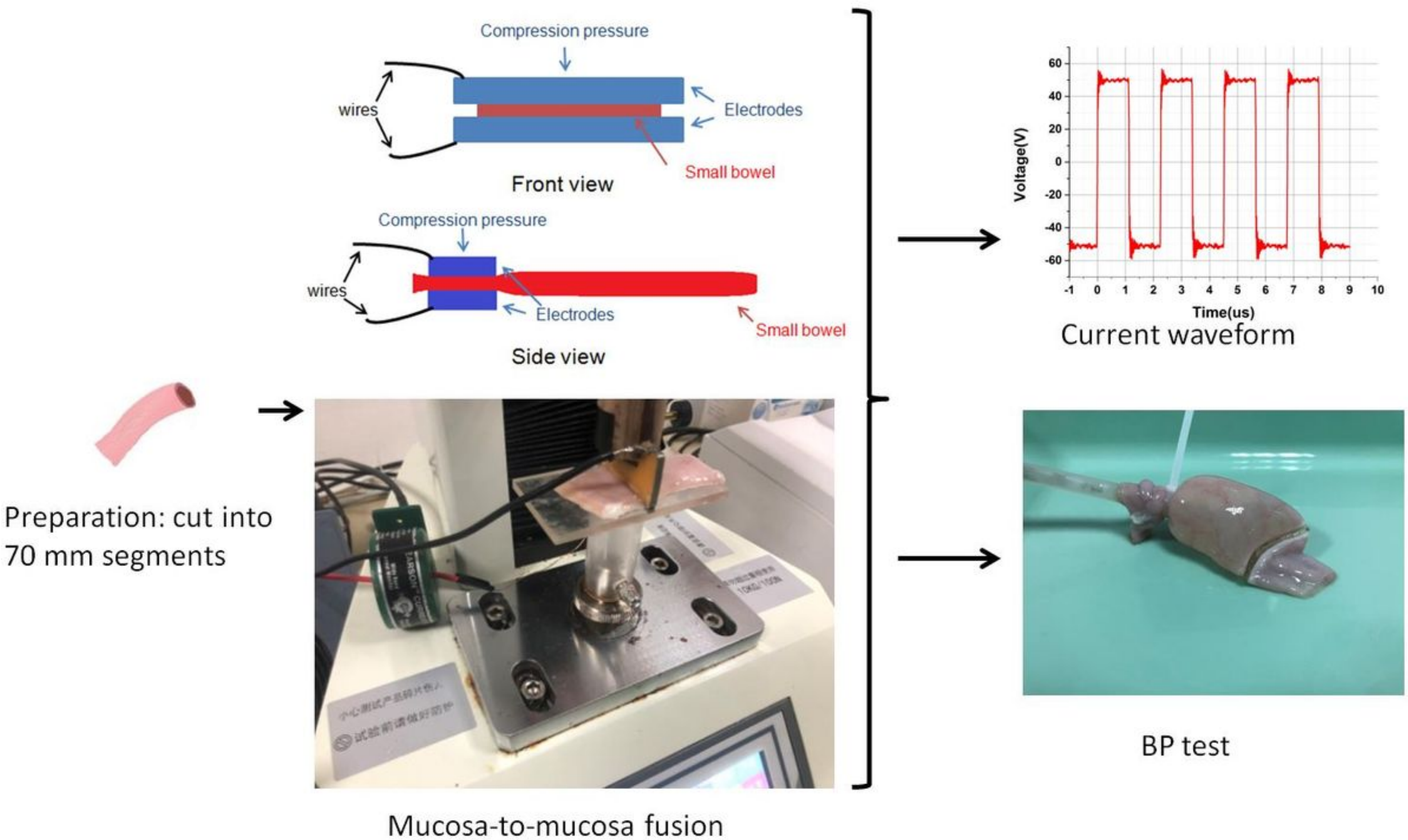

Figure 2

Experiment process

	50 v, 700 kPa, 5 s				
BP(mmHg)	35.8	25.1	30.6	26.1	28.3
	30.9	31.5	24.7	26.3	25.8
Average(mmHg)	**28.1**				

Figure 3

The BP of the small bowel fusion under the condition of 50 v, 700 kPa and 5 s

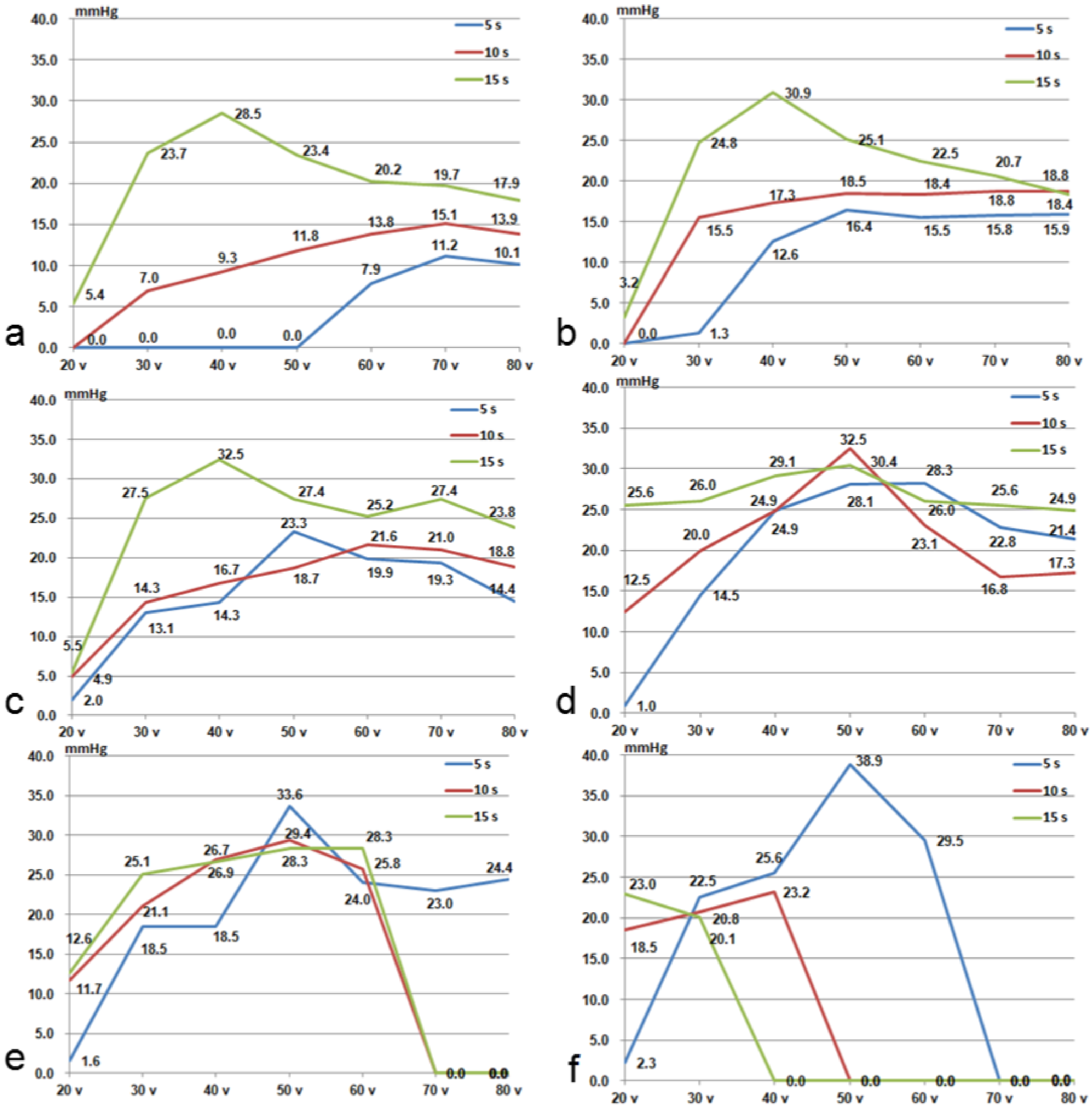

Figure 4

The BP of small bowels with different CP, voltage and time. The horizontal axis is voltage and the vertical axis is BP for each graph; the blue line is the BP with 5 s, the red line is the BP with 10 s and the green line is the BP with 15 s: a, CP= 400 kPa; b, CP= 500 kPa; c= 600 kPa; d= 700 kPa, e= 800 kPa; f= 900 kPa.

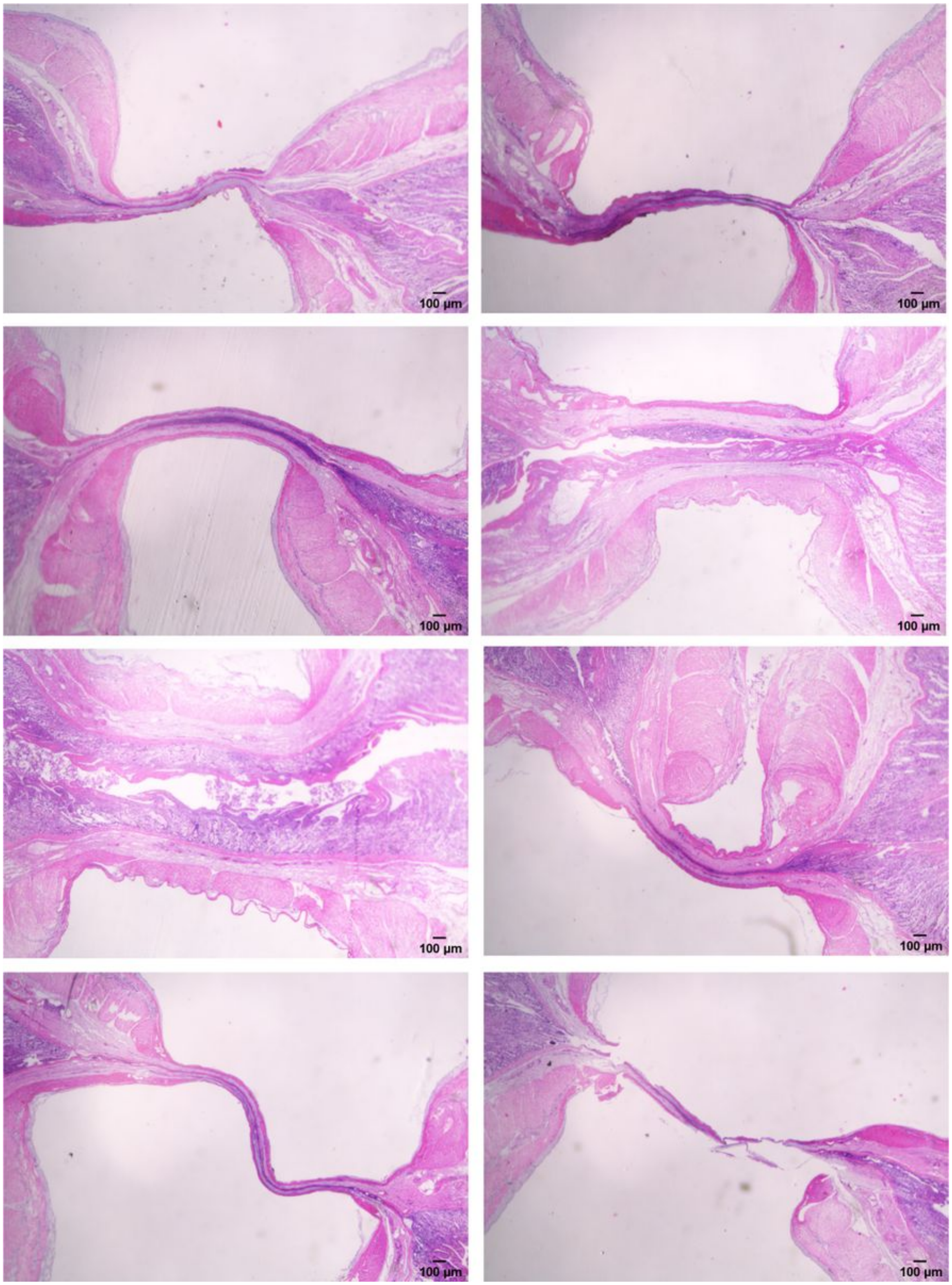

Figure 5

Transverse slice and H&E stain of fusion samples of 50 v and 5 s for: (a)CP= 900 kPa; (b)CP= 700 kPa; (c)CP= 500 kPa; (d)CP= 300 kPa; (e)CP= 100 kPa; (f)transverse slice and H&E stain of fusion samples of 30 v, 5 s and CP= 700 kPa; (g)transverse slice and H&E stain of fusion samples of 70 v, 5 s and CP= 700 kPa; (h) transverse slice and H&E stain of fusion samples of 70 v, 5 s and CP= 900 kPa

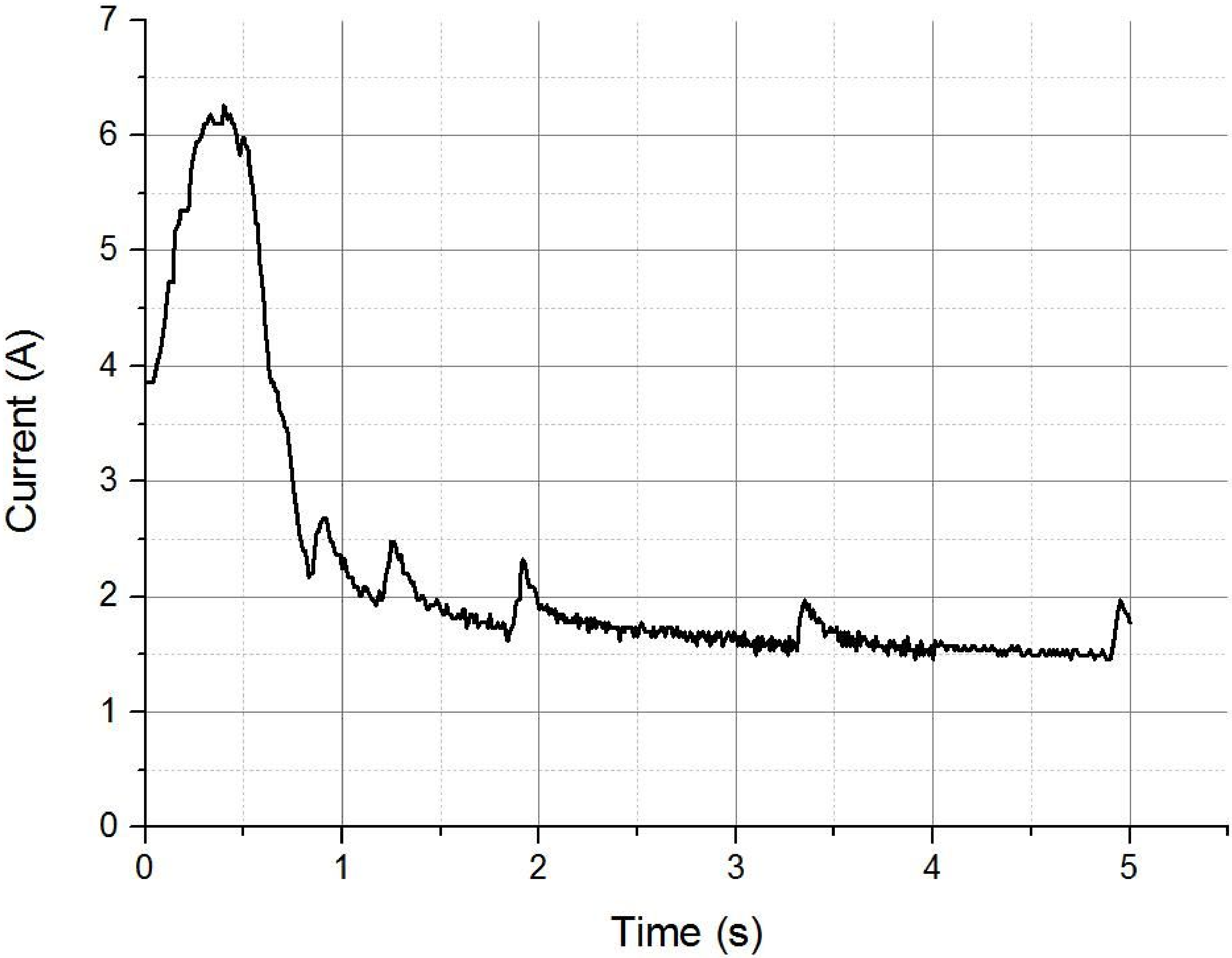

Figure 6

The current waveform of 700 kPa, 50 v, and 5 s during the fusion

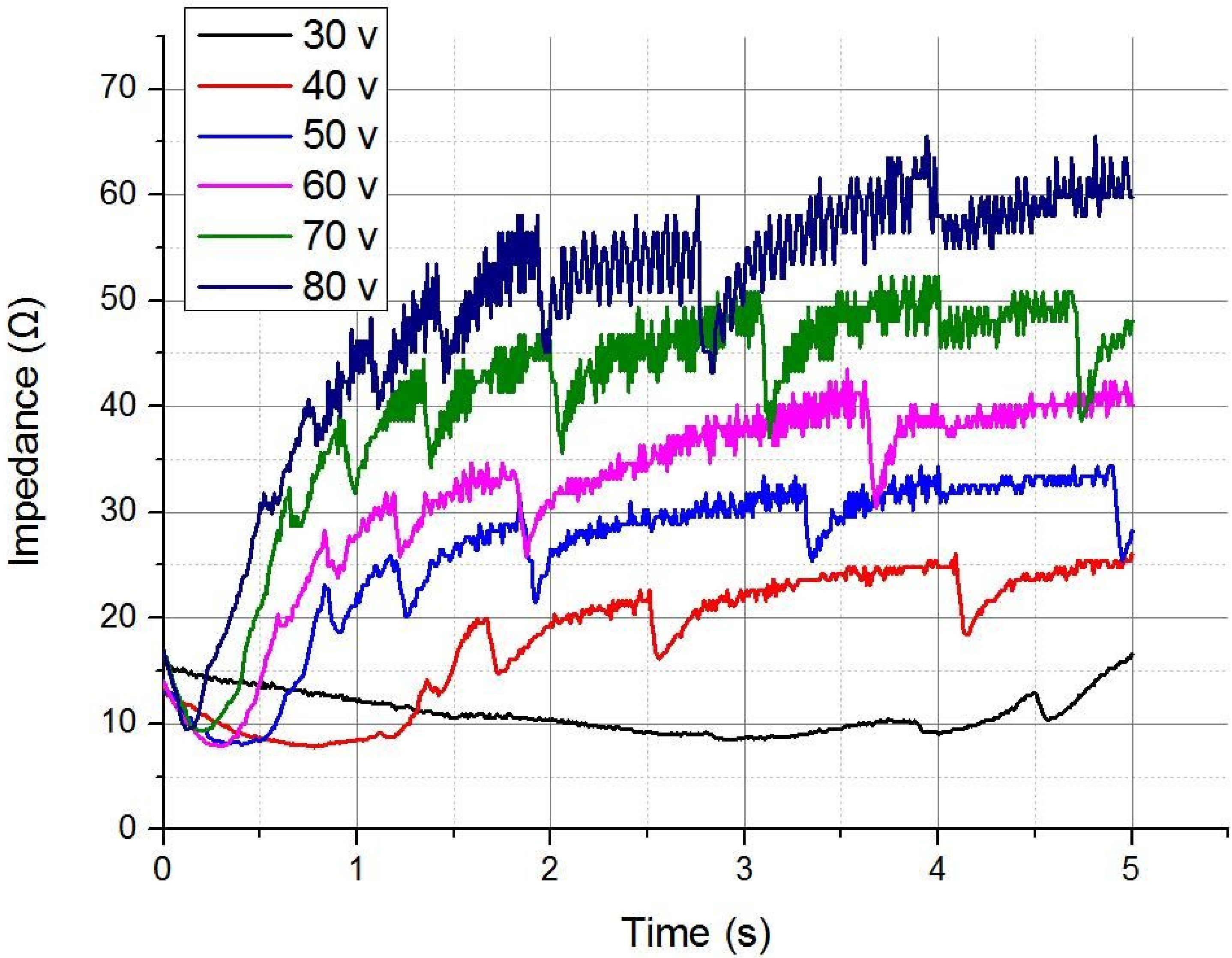

Figure 7

The impedance of different voltages with 700 kPa and 5 s

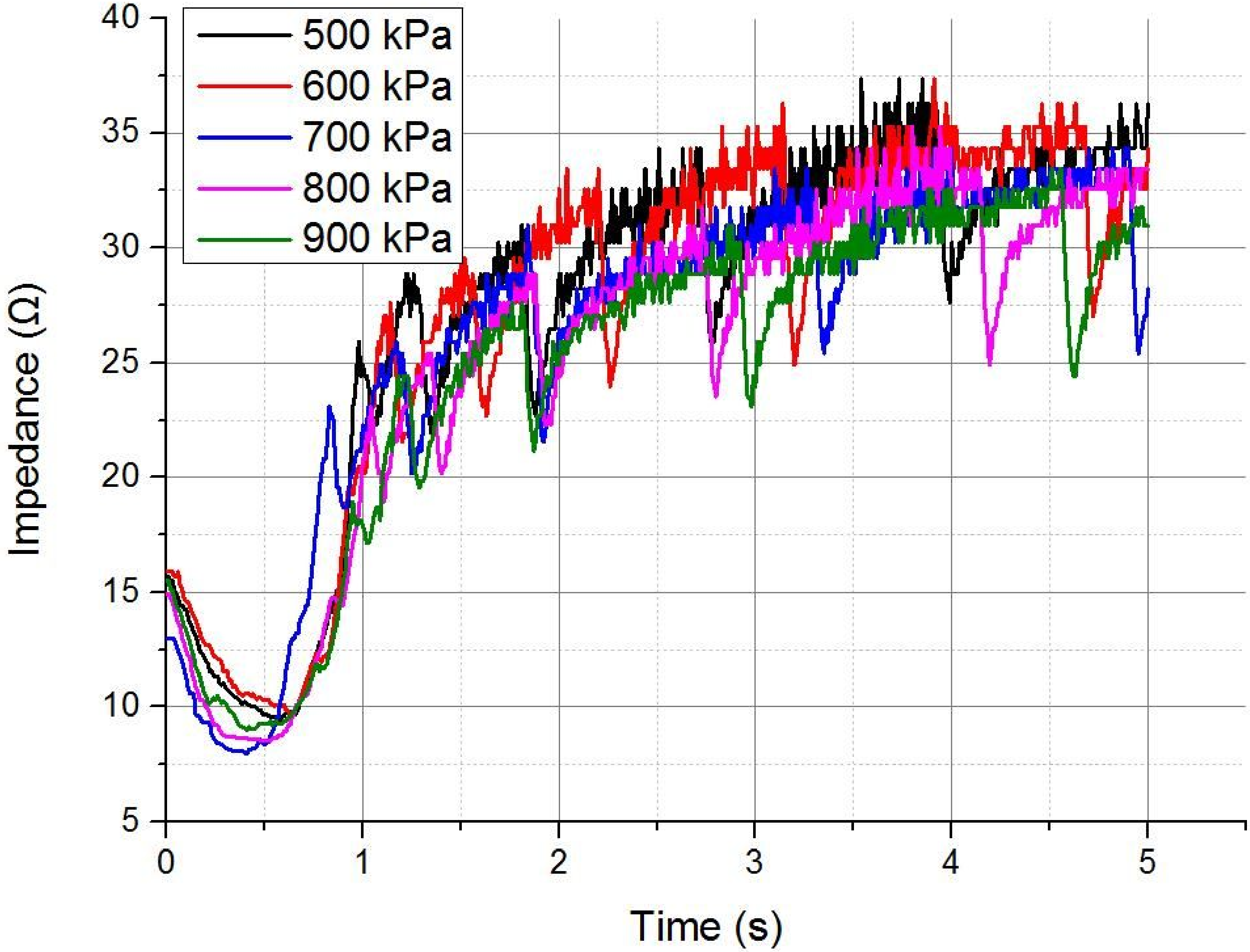

Figure 8

The impedance of different CP with 50 v and 5 s

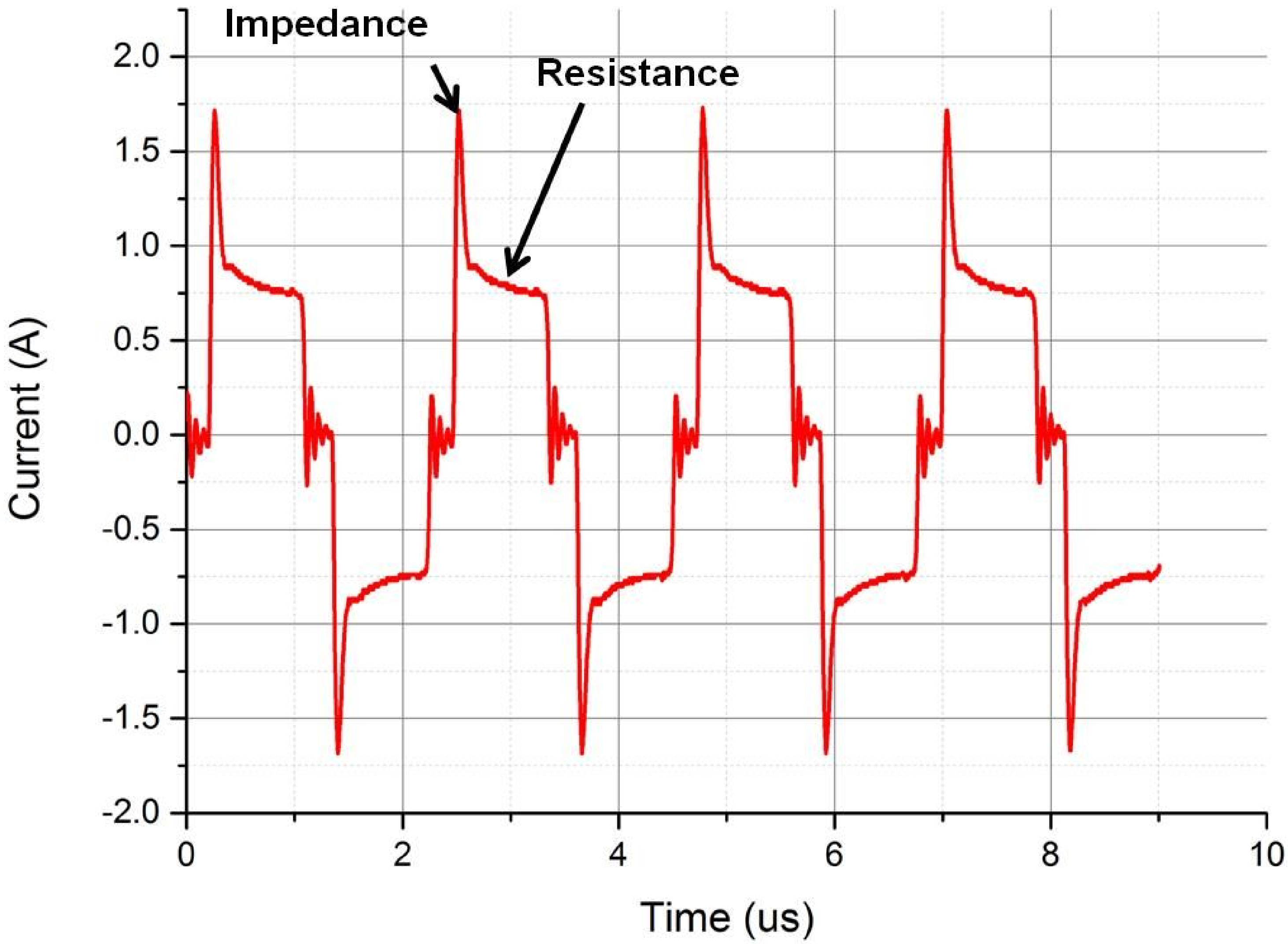

Figure 9

The current waveform of 700 kPa, 50 v and 5 s at around time 1s

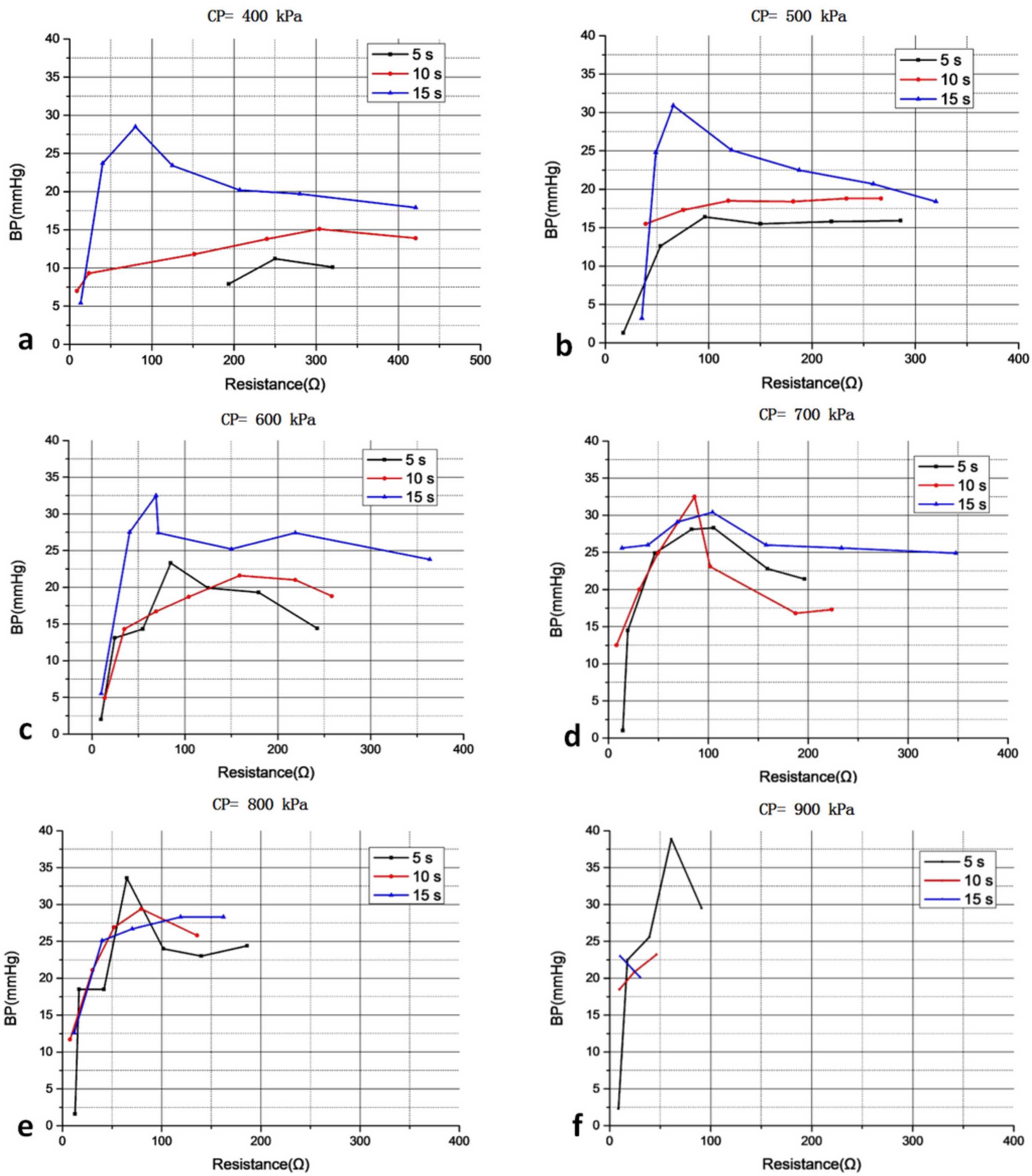

Figure 10

The relation of BP and resistance under different conditions: (a)CP= 400 kPa; (b)CP= 500 kPa; (c)CP= 600 kPa; (d)CP= 700 kPa; (e)CP= 800 kPa; (f)CP= 900 kPa. In each graph, the black line is the results of 5 s, the red line is the results of 10 s and the blue line is the results of 15 s. For each dot on the same line, the voltage is different, the resistance increases as the voltage increases.