Published in final edited form as:
Cardiovasc Intervent Radiol. 2018 November ; 41(11): 1773–1778. doi:10.1007/s00270-018-2034-9.

Evaluation of the heat sink effect after transarterial embolization when performed in combination with thermal ablation of the liver in a rabbit model

Charles J Puza, BA[1], **Qi Wang, MD, PhD**[2], and **Charles Y Kim, MD**[1]

[1]Division Interventional Radiology, Duke University Medical Center, 2311 Erwin Road, Durham, NC 27710

[2]Department of Radiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Road, Wuhan, 430022, People's Republic of China.

Abstract

Purpose: To assess the contribution of the heat sink effect when combining thermal ablation with transarterial embolization(TAE).

Materials and Methods: Radiofrequency ablation(RFA) or microwave ablation(MWA) were performed in the liver of non-tumor bearing rabbits. Three perfusion groups were used: rabbits that were sacrificed then immediately ablated (non-perfused liver group to simulate embolized tumor with no heat sink), rabbits that underwent hepatic TAE followed by ablation (embolized liver group), and rabbits that underwent ablation while alive (normally perfused liver control group). For each perfusion group, 8 RFAs and 8 MWAs were performed. Probes were inserted using ultrasound guidance to avoid areas with major blood vessels. During ablation, temperatures were obtained from a thermocouple located 1 centimeter away from the ablation probe to assess heat conduction. With MWA, temperatures were also measured from the antennae tip.

Results: For RFA, embolization of normal liver did not increase temperature conduction when compared to the control group. However, temperature conduction was significantly increased in the nonperfused group (simulating embolized tumor) compared to controls($p=0.007$). For MWA, neither embolization nor nonperfusion increased temperature conduction compared to controls. With MWA, the probe tip temperature was significantly higher in the nonperfused group compared to the control and embolized group.

Conclusions: In nonperfused tissue simulating tumor, RFA demonstrated modest enhancement of temperature conduction, whereas MWA did not. Embolization of normal liver did not affect RFA or MWA. Findings suggest that heat sink mitigation plays a limited role with combination embolization-ablation therapies, albeit more with RFA than MWA.

Corresponding author: Charles Y. Kim MD, Duke University Medical Center, Department of Radiology - Box 3808, 2301 Erwin Road, Durham, NC 27710, office phone: 919-684-7424, fax: 919-613-2680, charles.kim@duke.edu.

Conflicts of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

NOTES
The authors declare that they have no conflict of interest.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Keywords

microwave ablation; radiofrequency ablation; heat sink; combination therapy; embolization

INTRODUCTION

While percutaneous thermal ablation is highly effective for hepatic tumors measuring 3 centimeters in diameter or less, its efficacy is limited by tumor size, as evidenced by a decreasing recurrence-free survival rate with larger tumors [1, 2]. Effective treatment of tumors in the 3–5 centimeter range has been achieved with the combination of transarterial embolization (TAE) or transarterial chemoembolization (TACE) with radiofrequency ablation (RFA) or microwave ablation (MWA). When compared to thermal ablation alone, the combination of TACE with thermal ablation results in an increased ablation zone size [3, 4], decreased local recurrence rate [5–8], and improved overall survival [9, 5, 6, 10, 7, 8]. In fact, TACE combined with RFA has been shown to be as effective as surgery for both small (<3 cm) and intermediate-sized (3–5cm) HCC's [11, 12]. The efficacy of RFA and MWA in combination with TACE has been shown to be similar [13, 14]. The ablation zone size has been shown to be similar when RFA is combined with either TACE or TAE [4].

The reason for this benefit with combination therapy is not well understood, as the underlying potential mechanisms have not been scientifically explored. It is uncertain whether the combination of embolization with ablation synergistically enhances ablation effects, or whether they provide simply additive effects, since TAE and TACE are well known to induce significant HCC necrosis [15, 16]. The most popular theory for synergistic potentiation of ablation is via mitigation of the "heat sink" effect [17, 18]. The heat sink effect describes the phenomenon whereby flowing blood adjacent to or within tissues being targeted for ablation results in relative tissue cooling due to heat transfer by convection [19]. Thus, by diminishing arterial blood flow within the target tissue via embolization, there is a theoretically decreased heat sink, which may result in increased conduction of heat to a wider volume of tissue. Additional theoretical factors may include the physiologic impact of hypoxia on tissue viability and interaction of heat with the chemotherapeutic agent with TACE. The purpose of this study was to assess the degree of contribution of the heat sink effect when combining thermal ablation with transarterial embolization in a rabbit model.

MATERIALS AND METHODS

Approval was obtained from the local institutional animal care and use committee. Female New Zealand White rabbits (Charles River Laboratories, Chicago, Illinois) were obtained at the age of 11–17 weeks. Animals were allowed to acclimate for at least 48 hours prior to use. Both RFA and MWA were tested. For each modality, animals were divided into three groups. The control group consisted of rabbits undergoing thermal ablation of the untreated rabbit liver while alive with normally perfused livers. The embolization group consisted of rabbits that first underwent particle embolization of the proper hepatic artery to stasis followed by ablation of the liver while alive. While there is no hepatic arterial flow in rabbits that underwent embolization, there is expected to be normal perfusion of the liver parenchyma by the portal venous system. The non-perfused group consisted of rabbits that were sacrificed followed by immediate ablation of the untreated liver. Since HCC derives nearly all of its blood flow from the hepatic arterial system, embolized tumor would be expected to have little to no perfusion; therefore, animal sacrifice was performed to create a non-perfused parenchyma environment to model embolized tumor without any heat sink effect due to flowing blood. In total, there were 6 groups, with 8 ablations performed per group. For these non-survival surgeries, ketamine (22mg/kg IM) and xylazine (5–10mg/kg IM) were utilized for induction. After intubation, general anesthesia was maintained with isofluorane (0.5–5%). Animal sacrifice was performed with potassium chloride (5–10mg/kg IV) with bilateral thoracotomy.

Embolization technique:

After removal of hair from the right inguinal region and administration of local anesthetic, a 1 centimeter incision was made. The femoral artery was dissected free of the surrounding tissues then accessed with a 21 gauge needle, allowing insertion of a 5 French vascular sheath (Glidesheath Slender, Terumo, Somerset, NJ). Using a 5 French angled catheter and an angled hydrophilic guidewire (Terumo), the celiac artery was accessed and digital subtraction angiography was performed to delineate anatomy. The angled catheter was then advanced into the proper hepatic artery over a guidewire. In order to minimize confounders, particles without adjunct chemotherapeutic agents were used so that the actual heat sink effect could be analyzed. The entire liver was then embolized with 40 micrometer microspheres (Embozene, Boston Scientific, Natick, MA). Embolization was performed to stasis.

Thermal Ablation and Temperature Measurement:

After removal of hair from the epigastrium and administration of local anesthetic, a 5–8 centimeter midline incision was made. The peritoneum was carefully incised, allowing exposure of the liver.

For animals undergoing RFA, a 7 millimeter active tip RFA electrode (Cool-Tip, Medtronic, Minneapolis, MN) and a 16 gauge thermocouple (Zoro, Buffalo Grove, IL) were secured to a plastic spacer in a parallel fashion at a distance of 1 centimeter apart. The water cooling system was not utilized. The ablation electrode and thermocouple were jointly advanced into a suitable site within the liver at a site at least 2 centimeters in thickness using ultrasound guidance. The electrode and thermocouple were positioned to be at least 1 centimeter from the liver surface, remote from major portal and hepatic veins, and remote from the hepatic hilum and inferior vena cava. Ablation was performed for 10 minutes. The power output level was constantly adjusted to keep the probe tip temperature between 90 and 99 degrees Celsius while keeping the impedance under 999 Ohms. The temperature of the thermocouple was recorded just prior to ablation and upon termination of the ablation (measured at the exact last second of the ablation). Up to three ablations were performed per liver, with no more than one ablation in each lobe.

For animals undergoing MWA, a PR-15 microwave ablation antennae (NeuWave Medical, Madison, WI) was utilized. Experiments were performed in an analogous fashion as for

RFA. Microwave ablation was performed at 30 W for 60 seconds. The antennae tip contains a thermocouple at the margin of the active segment that conveys real-time temperature measurements. Thus, temperatures were measured at all time points from the thermocouple 1cm away from the antennae as well as from the antennae tip itself. The temperatures were recorded as measured with RFA.

Statistical Analysis:

Based on preliminary experiments with MWA, the mean increase in temperature at 1 cm away from the MWA probe after 1 minute at 30 Watts was 4°C. Using the observed standard deviation of 1.3°C, a sample size of 8 ablations in each group was needed to detect a 2 degree difference at an alpha of 0.05 and power of 80%. Thus, a sample size of 8 was used for each group. RFA performed for 10 minutes resulted in an increase in temperature of 4 degrees Celsius at a distance of 1 cm, and thus this parameter was used for RFA, as was a sample size of 8. A more remote distance with a lesser degree of temperature change was chosen over a closer distance with higher temperature changes, due to a greater expected level of temperature variability.

For each experimental condition, the temperature immediately before and immediately after ablation were compared using the paired t-test. The net change in temperature immediately before and immediately after ablation as measured 1 cm away from the RFA electrode or MWA antennae was calculated and compared between groups using the unpaired t-test, as were differences in antennae tip temperatures. Data were analyzed using SPSS version 22 (IBM, Chicago, IL). Differences were considered significant at a P value less than 0.05.

RESULTS

Temperature increases at 1 cm away from the ablation probe

Measurement of the temperature 1 centimeter away from the RFA probe revealed significant increases in temperature with radiofrequency ablation in the control group, embolization group, and non-perfused group ($p=0.001$, 0.012, and <0.001 respectively, Table 1). With MWA, the temperature 1 centimeter away from the MWA antennae was also significantly increased with ablation in all 3 groups ($p<0.001$ for all).

Differences in temperature at 1 cm away from the ablation probe comparing groups

For RFA, the relative amount of temperature increase 1 centimeter away from the RFA electrode was not significantly different comparing the control and embolization groups ($p=0.421$) (Table 1). However, the temperature change in the non-perfused group was significantly greater than both the control and embolization groups ($p=0.007$ and $p=0.002$, respectively). For MWA, the temperature increase at 1 centimeter away from the MWA antennae was not significantly different between any of the groups.

Analysis of MWA probe tip temperatures

As the antennae tip temperature during MWA is not manually controlled as during RFA, MWA antennae tip measurements were compared between groups, revealing similar temperatures between the control and embolization group (87.9 ± 2.7 versus 82.0 ± 8.2, p=0.07). However, the antennae tip temperature during ablation of the non-perfused livers (93.3 ± 4.9) was significantly higher than both the control and embolized livers (p=0.019 and 0.005, respectively).

DISCUSSION

The combination of embolotherapies with thermal ablation has emerged as an important strategy for the treatment of intermediate sized tumors, which results in high response rates in intermediate-sized tumors that are typically achievable only with smaller tumors. While it is widely assumed that the heat sink effect is the reason for increased ablation zone sizes after embolotherapy, our results suggest that its actual role may be somewhat limited. Neither RFA nor MWA demonstrated increased heat conduction after TAE of normal parenchyma when compared to controls. However, there was significantly increased heat conduction with RFA but not MWA in animals with nonperfused livers. These findings suggests that the heat sink effect may play a role in the improved ablation efficacy achieved with RFA (but not MWA) in tumoral tissue that has minimal to no perfusion after TAE. However, the higher temperatures of the MWA probe tip in the nonperfused groups compared to the embolized and control groups suggests that the heat sink does have some degree of impact on temperatures achieved with MWA.

Transarterial embolization of HCC results in a varying degree of perfusion alteration when comparing tumor tissue versus normal parenchyma due to the differential blood supply from the hepatic arterial system. Since tumors derive the vast majority of their blood supply from the hepatic arterial circulation, TAE is expected to result in nearly complete abrogation of its blood supply. To simulate embolized tumors with minimal to no residual perfusion, the current study utilized rabbits that were sacrificed immediately prior to ablation. While arterial embolization of HCC may obstruct all perfusion to the tumor, there may be only a 20–25% decrease in perfusion within normal hepatic parenchyma [20]. Thus, it may not be surprising that TAE of normal hepatic parenchyma did not result in enhanced heating of parenchyma near the ablation probe, for both RFA and MWA, when compared to control livers. However, in the non-perfused group, there was significantly enhanced heat conduction with RFA compared to controls, which implies that there is sufficient mitigation of the heat sink effect to modulate temperature conduction. These findings suggest that embolization likely enhance RFA of tumor tissue by diminishing the heat sink effect within the tumor with little to no change in the heat sink of normal parenchyma. However, the heat sink effect appears to have a more minor role with MWA, and thus the improved efficacy observed with combination embolization-MWA therapies may be related to alternative mechanisms.

The finding of a heat sink effect with RFA but not MWA with combination embolization-ablation is in line with several studies that have shown that the heat sink effect plays a significantly greater role for RFA than MWA when performed adjacent to major vessels [21–23]. One possible explanation is the physics of these two modalities; RFA depends on current flow, which can be affected by varying resistances of local structures, such as blood vessels. In contrast, MWA generates heat via oscillation of molecules without reliance on current flow. However, the finding that the antennae tip temperature was higher after MWA of non-perfused liver parenchyma than the control or embolization group suggests that there may be some degree of heat sink effect. However, it should be noted that the ablation times were markedly different in the current experiments (10 minutes for RFA and 1 minute for MWA), which were based on the differences in ablation speed and magnitude, in order to keep the ablation zone sizes of appropriate size for the rabbit liver. Also, there are likely to be additional physical and physiologic factors that contribute to combination therapy effectiveness. Finally, it is possible that there is no actual synergism between embolization and MWA, with improved responses being simply due to overlapping effects (i.e. viable cells after embolization may be destroyed with ablation).

One of the main limitations of this study is the analysis of combination therapy on non-tumor bearing rabbits. The VX2 rabbit tumor model is known for its tumor size variability, tumoral heterogeneity, variable tumor hypervascularity, and unpredictable degrees of spontaneous necrosis, all of which would confound heat conduction with ablation [24]. For these reasons, we felt that nonperfused normal parenchyma would reasonably simulate the effect of tumor embolization on tumor perfusion while avoiding the numerous confounders introduced with tumor models. Another limitation is the use of TAE instead of TACE. While TACE has been utilized in the vast majority of literature on combination therapies, TAE was utilized in in this study to study the heat sink effect because the presence of chemotherapeutic agent should not affect heat conduction. Another limitation is the potential impact of medium and large vessels on the conduction of temperatures and the heat sink effect in treated areas of the liver [19]. Attempts were made to minimize this confounding effect by avoiding areas of liver with sizable vessels. Finally, ablation zone diameter measurements and histopathologic analysis were not performed because it has already been established that ablation and infarction zone size are increased with combination therapy [4]. Instead, the purpose of this study was instead to assess alterations in temperature conduction related to the heat sink effect caused by changes in tissue perfusion. Furthermore, the physical zone of ablation or necrosis could be affected by hypoxia-related cell death or other yet-unknown effects of combination therapy.

In summary, the findings from the current study suggests that the heat sink effect likely plays a limited role in potentiating combination therapies involving embolization and thermal ablation. Our results suggest that embolization does not alter the heat sink effect occurring with ablation of normal liver. However, the enhanced heat conduction in nonperfused tissue with RFA but not MWA suggests a synergistic mechanism when combining embolization with RFA but not MWA for treatment of tumors. Our findings suggest that mitigation of the heat sink effect with embolization contributes to enhanced tumoral destruction after RFA but only minimally with MWA, and without significantly affecting normal parenchyma. Given this overall modest contribution of the heat sink effect with combination embolization-ablation, further studies are needed into potential factors that may contribute to the ablation zone potentiation achieved with combination therapies.

Acknowledgements:

This study was funded by the National Cancer Institute (grant number 5R03CA191978), Society of Interventional Radiology (Dr. and Mrs. W.C. Culp Student Research Grant), Radiologic Society of North America (Medical Student Research Grant), and the Duke University Department of Radiology (Charles Putnum Vision Grant).

REFERENCES

1. Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y et al. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer. 2005;103(6):1201–9. doi:10.1002/cncr.20892. [PubMed: 15690326]
2. Benson AB, 3rd, D'Angelica MI, Abbott DE, Abrams TA, Alberts SR, Saenz DA et al. NCCN Guidelines Insights: Hepatobiliary Cancers, Version 1.2017. J Natl Compr Canc Netw. 2017;15(5):563–73. [PubMed: 28476736]
3. Yamanaka T, Yamakado K, Takaki H, Nakatsuka A, Shiraki K, Hasegawa H et al. Ablative zone size created by radiofrequency ablation with and without chemoembolization in small hepatocellular carcinomas. Jpn J Radiol. 2012;30(7):553–9. doi:10.1007/s11604-012-0087-2. [PubMed: 22610876]
4. Mostafa EM, Ganguli S, Faintuch S, Mertyna P, Goldberg SN. Optimal strategies for combining transcatheter arterial chemoembolization and radiofrequency ablation in rabbit VX2 hepatic tumors. J Vasc Interv Radiol. 2008;19(12):1740–8. doi:10.1016/j.jvir.2008.08.028. [PubMed: 18951042]
5. Peng ZW, Zhang YJ, Chen MS, Xu L, Liang HH, Lin XJ et al. Radiofrequency ablation with or without transcatheter arterial chemoembolization in the treatment of hepatocellular carcinoma: a prospective randomized trial. J Clin Oncol. 2013;31(4):426–32. doi:10.1200/JCO.2012.42.9936. [PubMed: 23269991]
6. Peng ZW, Zhang YJ, Liang HH, Lin XJ, Guo RP, Chen MS. Recurrent hepatocellular carcinoma treated with sequential transcatheter arterial chemoembolization and RF ablation versus RF ablation alone: a prospective randomized trial. Radiology. 2012;262(2):689–700. doi:10.1148/radiol.11110637. [PubMed: 22157201]
7. Kim JH, Won HJ, Shin YM, Kim SH, Yoon HK, Sung KB et al. Medium-sized (3.1–5.0 cm) hepatocellular carcinoma: transarterial chemoembolization plus radiofrequency ablation versus radiofrequency ablation alone. Ann Surg Oncol. 2011;18(6):1624–9. doi:10.1245/s10434-011-1673-8. [PubMed: 21445671]
8. Kang SG, Yoon CJ, Jeong SH, Kim JW, Lee SH, Lee KH et al. Single-session combined therapy with chemoembolization and radiofrequency ablation in hepatocellular carcinoma less than or equal to 5 cm: a preliminary study. J Vasc Interv Radiol. 2009;20(12):1570–7. doi:10.1016/j.jvir.2009.09.003. [PubMed: 19879777]
9. Yan S, Xu D, Sun B. Combination of radiofrequency ablation with transarterial chemoembolization for hepatocellular carcinoma: a meta-analysis. Dig Dis Sci. 2012;57(11):3026–31. doi:10.1007/s10620-012-2212-6. [PubMed: 22585384]
10. Lu Z, Wen F, Guo Q, Liang H, Mao X, Sun H. Radiofrequency ablation plus chemoembolization versus radiofrequency ablation alone for hepatocellular carcinoma: a meta-analysis of randomized-controlled trials. European journal of gastroenterology & hepatology. 2013;25(2):187–94. [PubMed: 23134976]
11. Lee HJ, Kim JW, Hur YH, Shin SS, Heo SH, Cho SB et al. Combined Therapy of Transcatheter Arterial Chemoembolization and Radiofrequency Ablation versus Surgical Resection for Single 2–3 cm Hepatocellular Carcinoma: A Propensity-Score Matching Analysis. J Vasc Interv Radiol. 2017. doi:10.1016/j.jvir.2017.05.015.
12. Takuma Y, Takabatake H, Morimoto Y, Toshikuni N, Kayahara T, Makino Y et al. Comparison of combined transcatheter arterial chemoembolization and radiofrequency ablation with surgical resection by using propensity score matching in patients with hepatocellular carcinoma within Milan criteria. Radiology. 2013;269(3):927–37. doi:10.1148/radiol.13130387. [PubMed: 24086071]
13. Ginsburg M, Zivin SP, Wroblewski K, Doshi T, Vasnani RJ, Van Ha TG. Comparison of combination therapies in the management of hepatocellular carcinoma: transarterial chemoembolization with radiofrequency ablation versus microwave ablation. J Vasc Interv Radiol. 2015;26(3):330–41. doi:10.1016/j.jvir.2014.10.047. [PubMed: 25534635]
14. Vasnani R, Ginsburg M, Ahmed O, Doshi T, Hart J, Te H et al. Radiofrequency and microwave ablation in combination with transarterial chemoembolization induce equivalent histopathologic coagulation necrosis in hepatocellular carcinoma patients bridged to liver transplantation. Hepatobiliary Surg Nutr. 2016;5(3):225–33. doi:10.21037/hbsn.2016.01.05. [PubMed: 27275464]

15. Tsochatzis E, Garcovich M, Marelli L, Papastergiou V, Fatourou E, Rodriguez-Peralvarez ML et al. Transarterial embolization as neo-adjuvant therapy pretransplantation in patients with hepatocellular carcinoma. Liver Int. 2013;33(6):944–9. doi:10.1111/liv.12144. [PubMed: 23530918]
16. Riaz A, Lewandowski RJ, Kulik L, Ryu RK, Mulcahy MF, Baker T et al. Radiologic-pathologic correlation of hepatocellular carcinoma treated with chemoembolization. Cardiovasc Intervent Radiol. 2010;33(6):1143–52. doi:10.1007/s00270-009-9766-5. [PubMed: 19967371]
17. Higgins MC, Soulen MC. Combining locoregional therapies in the treatment of hepatocellular carcinoma. Semin Intervent Radiol. 2013;30(1):74–81. doi:10.1055/s-0033-1333656. [PubMed: 24436520]
18. Stone MJ, Wood BJ. Emerging local ablation techniques. Semin Intervent Radiol. 2006;23(1):85–98. doi:10.1055/s-2006-939844. [PubMed: 21326723]
19. Yu NC, Raman SS, Kim YJ, Lassman C, Chang X, Lu DS. Microwave liver ablation: influence of hepatic vein size on heat-sink effect in a porcine model. J Vasc Interv Radiol. 2008;19(7):1087–92. doi:10.1016/j.jvir.2008.03.023. [PubMed: 18589324]
20. Bierman HR, Byron RL, Jr., Kelley KH, Grady A. Studies on the blood supply of tumors in man. III. Vascular patterns of the liver by hepatic arteriography in vivo. J Natl Cancer Inst. 1951;12(1):107–31. [PubMed: 14874125]
21. Bhardwaj N, Strickland AD, Ahmad F, Atanesyan L, West K, Lloyd DM. A comparative histological evaluation of the ablations produced by microwave, cryotherapy and radiofrequency in the liver. Pathology. 2009;41(2):168–72. doi:10.1080/00313020802579292. [PubMed: 19152189]
22. Pillai K, Akhter J, Chua TC, Shehata M, Alzahrani N, Al-Alem I et al. Heat sink effect on tumor ablation characteristics as observed in monopolar radiofrequency, bipolar radiofrequency, and microwave, using ex vivo calf liver model. Medicine (Baltimore). 2015;94(9):e580. doi:10.1097/MD.0000000000000580. [PubMed: 25738477]
23. Primavesi F, Swierczynski S, Klieser E, Kiesslich T, Jager T, Urbas R et al. Thermographic real-time-monitoring of surgical radiofrequency and microwave ablation in a perfused porcine liver model. Oncol Lett. 2018;15(3):2913–20. doi:10.3892/ol.2017.7634. [PubMed: 29435018]
24. Parvinian A, Casadaban LC, Gaba RC. Development, growth, propagation, and angiographic utilization of the rabbit VX2 model of liver cancer: a pictorial primer and “how to” guide. Diagn Interv Radiol. 2014;20(4):335–40. doi:10.5152/dir.2014.13415. [PubMed: 24834491]

Table 1.

Temperature measurements in degrees Celsius 1 centimeter away from the ablation probe after 10 minutes of RFA and 60 seconds of MWA (mean ± standard deviation).

	RFA				MWA			
	pre	**post**	**change**	**p-val**	**pre**	**post**	**change**	**p-val**
Control	37.8±1.9	42.1±2.4	4.3±2.3	0.001	38.6±0.8	42.6±1.9	4.0±1.3	<0.001
Embolized	38.1±0.6	41.4±2.9	3.3±2.7	0.012	39.0±1.7	43.2±2.6	4.2±1.5	<0.001
Non-perfused	35.9±0.5	43.1±1.2	7.1±1.0	<0.001	36.3±0.9	40.1±1.3	3.8±1.3	<0.001
Control vs embolized			P=0.421				P=0.76	
Control vs non-perfused			P=0.007				P=0.79	
Embolized vs non-perfused			P=0.002				P=0.58	