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Comparison of Helzel and OxyLite Systems in the Measurements of Tumor Partial Oxygen Pressure (pO_2)

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Abstract

It has been demonstrated in both experimental and human malignancies that hypoxic tumor cells are linked with aggressive disease phenotype. One of the methods to identify these cells is by direct physical measurement of tumor pO_2 . This study compared pO_2 values measured with two systems, the Helzel Hypoximeter (successor of the polarographic Eppendorf electrode) and the Oxford-Optronix OxyLite (fiber-optic probe), in R3327-AT and R3327-AT/tkeGFP tumors. Partial oxygen pressure was measured in individual tumors with either system or in the same tumor with both systems. The similarities and discrepancies in pO_2 measurements between the two systems were also investigated when tumor-bearing animals were breathing pure oxygen. Our data showed a considerable heterogeneity in pO_2 values in each tumor using both the Helzel and OxyLite systems. Similar results were obtained with both systems for the mean and median pO_2 values, and the distributions of pO_2 values within the interval $0 < pO_2 < 40$ mmHg (the range important for defining tumor hypoxia) were found to be statistically equivalent. However, the frequencies of high pO_2 values (>40 mmHg) and zero values measured by the two systems were statistically significantly different.

INTRODUCTION

Hypoxia is commonly found in solid tumors of various origins. The growth of a solid tumor is limited by neovascularization, which is required for oxygen and nutrient supply. Tumors become hypoxic because of uncontrolled proliferation of tumor cells and insufficient neovascularization. Tumor cells that are deprived of oxygen and nutrients could result in a more aggressive tumor phenotype. It has been reported that tumor cells in neuroblastoma, prostate androgen-sensitive adenocarcinoma, and breast ductal carcinoma *in situ* lose differentiation characteristics during proliferation under hypoxia and develop more traits of malignancy (1–3). Hypoxic tumor cells are more likely to be resistant to ionizing radiation and chemotherapeutic agents and possess increased potential for invasion, metastasis and patient mortality (4–10). These findings suggest the importance of recognizing oxygen status in tumors before the initiation of cancer therapy.

Various methods have been developed to identify and quantify tumor hypoxia, including invasive techniques such as polarographic needle probes (e.g. Eppendorf pO_2 histogram) (8),

fluorescence-based fiber-optic probes, (e.g. OxyLite™) (11,12), the comet assay (13), the *in vivo-in vitro* clonogenic assay (14,15), and nitroimidazole-based hypoxia markers (e.g. pimonidazole, EF5) (16–18). In addition to these invasive techniques, positron emission tomography (PET) (19,20) and single-photon emission computed tomography (SPECT) with CT image fusion (21) hold promise for identifying tumor hypoxia non-invasively at both the global and local level. Many hypoxia-targeting molecules labeled with positron-emitting radionuclides have been developed (19,20,22–26). In imaging tumor hypoxia in the clinic, non-invasive methods such as PET have many advantages. They can be used serially to observe changes as the tumor grows, can provide three-dimensional distributions for radiotherapy treatment planning, and can be used during and after treatment for evaluation of therapeutic response.

In our laboratory investigations, endeavors have been made to measure tumor tissue pO_2 with the OxyLite system as a means to validate images of tumor hypoxia obtained with PET hypoxia radiotracers. A disadvantage of the OxyLite system is that the probe must stabilize for 1–2 min at each individual measurement location, thereby limiting the amount of data that can be acquired in each animal. In 2003, the Helzel Hypoximeter, a successor of the Eppendorf system, became available commercially. Although tumor pO_2 measurements with the Eppendorf and OxyLite systems have been compared by other investigators (27,28), additional systematic and detailed analyses of the similarities and differences between pO_2 measurements obtained with each systems, in particular in the same tumors, is required. In this work, the two probe systems were compared systematically in tumors of different sizes under both normal air-breathing and pure oxygen-breathing conditions so that their performance could be evaluated in tumors with widely disparate hypoxic cell fractions.

MATERIALS AND METHODS

Animals and Tumor Models

Eight- to 10-week-old male BALB/c nude mice purchased from National Cancer Institute Animal Production (Frederick, MD) were housed five per cage in small animal facilities in our Institute. A constant temperature and humidity were maintained. Animal pellets and acidified water were provided *ad libitum*. Experiments were conducted according to the principles outlined in the Guide for the Care and Use of Laboratory Animals prepared by the Institutional Animal Care and Use Committee and Research Animal Resource Center of MSKCC.

Experimental tumors were derived from R3327-AT Dunning rat prostate anaplastic adenocarcinoma R3327-AT cells and stably transduced cells that constitutively express the tkeGFP reporter gene, R3327-AT/ tkeGFP. This latter cell line showed less hypoxia in our previous study, as shown by ^{18}F -FMISO PET and autoradiography (22) as well as by pimonidazole immunohistochemical staining and OxyLite probe measurements (data not shown). The tumor cells were maintained *in vitro* in DMEM (Mediatech, Inc., Herndon, VA) supplemented with 10% fetal calf serum (Gemini Bio-Product, West Sacramento, CA) and an antibiotic mixture of penicillin and streptomycin (Mediatech). Near-confluent cells were trypsinized using 0.05% trypsin plus 0.54 mM EDTA (Mediatech), and single cell suspensions containing 2.0×10^7 cells /ml were prepared. Then 2.0×10^6 cells in 100 μ l were transplanted subcutaneously into the right hind limb of each animal. When the tumors became palpable, tumor size was measured by a caliper and the volume was calculated as $\pi abc/6$, where a , b and c are three mutually perpendicular diameters. Tumors of 5–12 mm average diameter were used for experiments. The number of tumors studied in each experiment is summarized in Table 1.

Tissue pO_2 Measurement Systems

The first tumor tissue pO_2 measurement device was the polarographic probe system, the Phoenix Tissue Hypoximeter (Helzel Medical Systems, Kaltenkirchen, Germany). This system uses recessed-tip O_2 microelectrodes (29). The probe is 300 μm in diameter at the tip and 500 μm in diameter at the shaft with a glass-insulated gold micro-cathode (17 μm in diameter) that is recessed and covered with a Teflon membrane (50 μm) (30). The probe was calibrated before each experiment according to the manufacturer's recommendation. Briefly, the probe was placed into a phosphate-buffered saline (PBS)-filled chamber equilibrated with air and nitrogen to achieve the desired partial oxygen pressure to test and validate the probe calibration.

The second tumor tissue pO_2 measurement device was a four-channel fiber-optic oxygen-sensing device (OxyLite™4000, Oxford Optronix, Oxford, UK). The OxyLite probe consists of the dye ruthenium chloride held in a polymer matrix 230 μm in diameter at the tip. It operates on the principle of oxygen-induced quenching of the light emitted by the fluorescent dye, with the quenching being dependent on pO_2 . Each OxyLite probe was calibrated by the manufacturer prior to its delivery, and the calibration data were used in the data acquisition. The calibration data were checked by the same procedure described for the Helzel probes.

Anesthetics

The use of halothane, including isoflurane anesthetics, is contraindicated for the Helzel system. Thus we used intraperitoneal injections of an anesthetic mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) for all pO_2 measurements. Comparison of pO_2 measurements using the OxyLite system and isoflurane inhalation from our previous study (11) with those using ketamine/xylazine in this study showed no significant differences in the average pO_2 values and in the pO_2 distributions between these two approaches.

pO_2 Measurements using the Helzel and OxyLite Systems in Different Tumors during Air Breathing

In the first study, measurements were performed with both pO_2 probe systems in separate tumor-bearing animals. Anesthetized animals were placed in the prone position on a temperature-regulated heating pad that maintained the core (rectal) temperature at approximately 37°C. To facilitate probe penetration, a catheter with a 24G3/4 gauge needle (Introcan® Safety™, B. Braun Medical Inc., Bethlehem, PA) was used to pierce through the skin where the probe was to be inserted.

For measurements with the Helzel system, when the calibration was completed, the probe was attached to the motor connector, which was affixed to the micromanipulator stand. The electrode probe was also introduced vertically into the tumor via the catheter described earlier. Advancing a probe through the tissue was controlled by computer and typically consisted of a 1.0-mm forward and 0.3-mm retraction motion, so as to decompress the tissue at the measurement site. The probe automatically performs multiple measurements depending on the tumor size. Measurements were made along five to eight parallel tracks in each tumor.

For the OxyLite system, a probe attached to a micromanipulator was inserted into the tumor through the catheter. Measurements were made by advancing the probe in incremental steps of 1.0 mm each. Tissue compression was minimized by advancing the probe 0.2 mm deeper than the measurement step (a total of 1.2 mm) and retracting it 0.2 mm. At the first measurement point after the probe insertion, pO_2 readings were often unstable, and they became stable only after 1–5 min (11). Fluctuations also occurred as the probe advanced; thus measurements were recorded when the readings became stable for >10 s. Similar to the Helzel system, measurements were made along five to six parallel tracks in each tumor. The mean and median pO_2 values were calculated for the set of values for each individual tumor.

pO₂ Measurements using the Helzel and OxyLite Systems in the Same Tumors during Air Breathing

In the second experiment, *pO₂* values were measured in the same tumors using both systems. For this purpose, the measurement procedure was modified slightly. Each anesthetized animal bearing either an R3327-AT or an R3327-AT/tkeGFP tumor was placed on the temperature-regulated heating pad. The tumor size was measured and a line was drawn along the largest longitudinal dimension. The *pO₂* measurements were performed with the Helzel and OxyLite systems in parallel trajectories along the line in an interleaved manner, and four trajectories were made with each system on each tumor. The order of the measurements was reversed for each subsequent animal to avoid possible bias. Measurements were made by advancing the probe in incremental steps of 0.5 mm each. Nine R3327-AT and 19 R3327-AT/tkeGFP tumors were used to compare the two systems.

pO₂ Measurements using the Helzel and OxyLite Systems in the Same Tumors in Pure Oxygen-Breathing Animals

In a third experiment, we attempted to evaluate the behavior of both probes under conditions in which the animals were switched to breathing 100% oxygen, since this had been shown to significantly reduce tumor hypoxia in other studies (11,31). Our pilot study in which the tumor *pO₂* was measured continuously illustrated that carbogen or pure oxygen breathing increased tumor *pO₂* readings and that this increase reached a plateau within 45 min. Thus, in these experiments, *pO₂* measurements were started 1 h after the initiation of oxygen breathing with i.p. ketamine/xylazine anesthesia. Measurements using both the OxyLite and Helzel systems were performed on a total of six R3327-AT tumor-bearing mice breathing oxygen.

Statistical Methods

In experiment 2, a series of *pO₂* measurements were obtained for each of the two systems along parallel tracks every 0.5 mm for up to 10 mm depth. The scattergram data are shown in a dot plot using an indexing set, calculated as $[40 \cdot \text{mouse} + 20 \cdot (\text{track} - 1) + \text{depth} - 20]/40$, on the *x* axis with the *pO₂* values on the *y* axis. For the air-breathing mice, a large proportion of the recorded *pO₂* values are zero plus the measurement error. Since the measurement errors depend on the system, a system-dependent threshold was used to designate these low values as zeros. For each mouse the proportion of values that were zero was calculated and the two systems were compared using the Wilcoxon signed-rank test. The larger values were pooled and their distribution was obtained using density estimation (32). These were compared using the Wilcoxon rank-sum test. For the pure oxygen-breathing mice in experiment 3, there were relatively few zero values, thus allowing for an assessment of the correlation between the two systems by comparing the measurements from the same depth along the parallel tracks for the two systems. These data are shown in a scatterplot. The OxyLite system saturates near 100, and thus high values get truncated and are flagged using a threshold. The values within the low and high thresholds were used to obtain the distribution of *pO₂* using density estimation.

For comparisons with previously published data from our group and others, the mean and median *pO₂* values ± 1 SD were calculated separately for each experimental group. During the data processing to determine the mean or median *pO₂* values, *pO₂* readings of less than zero were treated as zero.

RESULTS

Histogram of pO_2 Measurements using the Helzel and OxyLite Systems in R3327-AT Tumors of Different Sizes

To compare the two systems, pO_2 distributions were measured in R3327-AT tumors of different sizes. In experiment 1, a total of 19 and 21 R3327-AT tumors were used for measurements with the Helzel and OxyLite systems, respectively. The total numbers of measurement points with the Helzel and OxyLite were 1341 and 900, respectively. Table 2 shows the percentages of pO_2 readings below 2.5, 5.0 and 10.0 mmHg, the range of pO_2 readings, and the mean and median pO_2 values of all the tumors measured separately with either the Helzel or the OxyLite system.

Histograms of pO_2 readings measured by the two systems are shown in Fig. 1a (Helzel) and b (OxyLite) for five tumor size groups. The data presented are for tumor groups that are fairly well matched in size and number. A comparison of that two panels shows that the two pO_2 measurement systems yielded similar data for the distributions of frequency (percentage of readings). As expected, the percentage of readings below 2.5 mmHg increased from 15–20% for the smallest sizes to above 80% for the largest tumors, and this was true for both pO_2 systems. The increase was most drastic from the smallest tumor size group to the next size group, concomitant with a decrease in the incidence of higher pO_2 readings.

Comparison of pO_2 Measured in the same Tumor with both Helzel and OxyLite Systems during Air Breathing

In experiment 2, pO_2 levels were measured with both systems in the same tumor. In total, nine animals with R3327-AT tumors (ranging from 360 mm³ to 930 mm³) and 19 animals with R3327-AT/tkeGFP tumors (ranging from 170 mm³ to 1500 mm³) were studied. In each tumor, eight trajectories were used, four each for the Helzel and the OxyLite, with a total of 52–84 measurements per system in each tumor.

Figure 2 shows the data acquired with both the Helzel and OxyLite systems in the form of dot plots, which allow all of the measured data to be plotted on a single graph. The data for the mice with R3327-AT tumors are shown on the top row and those for the mice with R3327-AT/tkeGFP tumors on the bottom row. The results for the Helzel system (panels a and c) are noisier around zero than those for the OxyLite system, and thus a threshold of 2.5 and 1, respectively, was used to identify the zeros. There were significantly more zero pO_2 values for the R3327-AT tumors measured with the OxyLite system than for those measured with the Helzel system ($P < 0.01$).

Figure 2c and d shows the results for the 19 R3327-AT/tkeGFP tumor-bearing animals for the Helzel and OxyLite probes. The mean percentages ($\pm$ SD) of readings both <2.5 and <5.0 mmHg, stratified according to tumor volume, using the Helzel and OxyLite systems are given in Table 3 for the R3327-AT tumors. No statistically significant differences were observed between the two probe systems in R3327-AT/tkeGFP tumors ($P = 0.138$).

The majority of the data points are zero or insignificantly above or below zero for all but the smallest xenografts. Therefore, the statistical analysis was divided into an analysis of the percentage of measurements at zero for each of the pO_2 measurement devices and an analysis of whether the distribution of non-zero values was significantly different. The frequency of zero values with the OxyLite probe was significantly greater than that obtained with the Helzel probe system for measurements on the R3327-AT tumors ($P = 0.039$), but no significant difference was found for the R3327-AT/tkeGFP tumor ($P = 0.798$). There are differences in the histology between the two R3327-AT tumor cell lines, with slightly more hypoxia and necrosis being observed in tumors derived from the parental line. The results of the second part

of the analysis are shown in Fig. 3, which shows the distribution density of non-zero pO_2 values for the R3327-AT and R3327-AT/tkeGFP tumors. In this analysis, we also found a small significant difference between the distributions of pO_2 values for the R3327-AT tumors ($P < 0.01$), but no significant difference was observed for the distribution measured by the two systems for the larger data set obtained for the R3327-AT/tkeGFP tumors ($P = 0.138$).

Comparison of pO_2 Measurements with the Helzel and OxyLite Systems in the Same Tumors during Pure Oxygen Breathing

In experiment 3, both pO_2 probe systems were evaluated in tumors to compare their performance upon perturbation of the pO_2 gradients by performing measurements in the same tumors with both probes 60 min after the initiation of oxygen breathing. The measurements were made in alternating tracks with both the Helzel and OxyLite systems in six R3327-AT tumors ranging from 180 to 360 mm³ (median of 232 mm³ and mean of 250 mm³). Figure 4a and b shows dot plots of the individual measured values for animals 1–6 obtained with the Helzel and OxyLite probes, respectively. The tumor pO_2 values are distributed more broadly, with only a small percentage of zero readings for both measurement systems when the mice were breathing pure oxygen. The range of values was from 1.6 to 153 mmHg for the Helzel probe and from 0 to 99 mmHg for the OxyLite probe. The OxyLite probe saturates at 100 mmHg, which accounts for the large number of values between 98–100 mmHg in panel b and the lack points beyond that value. The mean and median pO_2 values measured by the Helzel polarographic electrode were 47.7 and 46.6 mmHg, similar to the mean and median of 44.3 and 53.0 mmHg measured with the OxyLite fiber-optic probe. The percentages of pO_2 readings during pure oxygen breathing that were less than 2.5, 5.0 and 10.0 mmHg were 5.3, 5.8 and 10.6 for the Helzel system and 7.9, 9.2 and 13.6 for the OxyLite system. The distributions of pO_2 values measured with the two probe systems were similar for all animals except number 5. Also, for mice 1–4 and 6, no significant difference was observed between the Helzel and OxyLite probes ($P = 0.508$).

Figure 4c shows a scattergram of paired pO_2 readings for comparable depths along parallel tracks measured alternately with the Helzel (abscissa) and OxyLite (ordinate) pO_2 probe systems for all mice except animal 5. The data show no correlation between the two measurement systems. This is to be expected, because the distance between the parallel tracks is 1 mm, which is large compared to the oxygen gradients known to exist in tumors. pO_2 is believed to fall from 140 mm to 0 mmHg over a distance of about 0.2 mm.

Figure 4d shows the distribution density of non-zero pO_2 measurement points for the two probes. This figure shows the similarity in the spectrum of measured values and demonstrates the statistical equivalence of the two measurement devices.

DISCUSSION

The present study was performed in conjunction with our research to validate non-invasive imaging measurements of tumor hypoxia. At the beginning of our studies, only the OxyLite system was available commercially. In previous studies, we evaluated the effects of various anesthetics on OxyLite probe tumor pO_2 measurements (11,14). This study demonstrated the reliability of OxyLite system measurements in animals under sustained isoflurane anesthesia. Whereas the OxyLite probe has certain advantages, including the ability to measure pO_2 at individual locations over prolonged periods, because the device does not consume oxygen (11,28,33), the long stabilization time requiring 1–2 min per reading is a major limitation, rendering its use laborious and time-consuming. The OxyLite probe can also be used in combination with magnetic resonance imaging and positron emission tomography (19,20).

The Eppendorf pO_2 measurement system has been used successfully by many investigators (9,10), but it ceased to be available commercially about 10 years ago. The Helzel polarographic probe system (based on the same principle as the Eppendorf precursor) became available commercially in 2003. It has the advantage of a more rigid metal probe casing and a more efficient data acquisition process with the use of a stepping motor and computer control. However, it cannot be used in steady-state measurements because of oxygen consumption during the read-out process.

In the present study, we performed a systematic and detailed analysis of the similarities and differences between pO_2 measurements obtained with the Helzel and OxyLite systems. We performed a preliminary experiment to validate the similarity of readings within the two systems in our R3327-AT tumor xenograft model as well as to confirm the compatibility of our data with published data obtained by others from different tumor xenograft models. One unique aspect of this study was that we conducted experiments in which pO_2 measurements were performed using both systems in the same tumors. These measurements were done in animals under normal air-breathing conditions and after the pO_2 profiles were perturbed by allowing the animals to breathe pure (100%).

Consistent with the previous findings in this and other laboratories, the present study indicated that tumors >200 mg are extremely hypoxic in air-breathing mice (Fig. 1 and Fig. 2). Significant heterogeneity in oxygenation status in each measurement track was observed with both pO_2 measurement systems (Fig. 2 and Fig. 3). Our findings support the overall equivalence of the two pO_2 probes as reported by Sedden *et al.* (27) but also the differences at the limits of operation leading to unequivalency, as discussed by Braun *et al.* (28). Specifically, the pooled pO_2 data shown in Table 2 and Table 3 indicated close similarities between the pO_2 frequency distributions measured by the two systems. Detailed statistical analysis of (1) the number of zero readings from each of the two probe systems and (2) the distributions of non-zero pO_2 readings exhibited some statistically significant discrepancies. The small number of zero measurements under oxygen-breathing conditions allows the performance of the two probe systems to be compared over a full range of pO_2 values. In oxygen-breathing animals, these two systems yielded very similar mean or median pO_2 readings and percentages of pO_2 with different thresholds.

The polarographic electrode and fiber-optic probe differ in many respects, including the mechanism of pO_2 measurement, and the tissue volume sensed by the probe tip, the speed and process of pO_2 readings. The fiber-optic probe is most accurate near 0 mmHg, and its signal-to-noise ratio becomes progressively lower with increasing pO_2 , especially above 50 mmHg (34). In contrast, the signal of the polarographic electrode is directly proportional to the pO_2 value being measured and is therefore subject to the greatest errors at the lowest pO_2 readings (27). Considering many these differences, it is reassuring that the two systems give comparable distributions of pO_2 values measured in a large number of tumors.

CONCLUSIONS

In this study, we made pO_2 measurements with both systems in the same tumors measurements in nine parental R3327-AT tumors, 19 R3327-AT/tkeGFP tumors, and an additional six R3327-AT tumors in mice breathing 100% oxygen. There was a slight but significant difference observed for the parental R3327-AT prostate cancer cell line, but no difference was observed in the larger group of tumors grown from a genetically modified subclone, R3327-AT/tkeGFP. Data acquired with the two systems in pure oxygen-breathing animals extended the range of the comparison of pO_2 values and provided more evidence of the equivalence of the Helzel and OxyLite pO_2 measuring systems.

In summary, we demonstrated that Helzel and OxyLite systems provide comparable assessments of tumor oxygenation. The differences in their physical and operational characteristics can be translated into advantages or disadvantages depending on the application. Our conclusion is that the two pO_2 probe systems complement each other and can be used interchangeably in the measurement of tumor hypoxia.

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FIG. 1.

Distributions of pO_2 readings in R3327-AT tumors of different sizes as measured with either the (panel a) Helzel system or (panel b) OxyLite system.

FIG. 2.

Dot plots containing all the pO_2 values for each animal number measured with the Helzel (panels a and c) and OxyLite pO_2 (panels b and d) systems. Measurements were performed along parallel tracks for each of the 19 tumors. The Helzel system measurements are noisier around zero than those with the OxyLite system, and thus thresholds of 2.5 and 1, respectively, were used to identify the zeros.

FIG. 3.

The distributions of non-zero pO_2 for the Helzel system (solid line) and OxyLite system (dashed line) are shown for the R3327-AT (left panel) and R3327-AT/tkeGFP tumors (right panel). The pO_2 values for the R3327-AT tumors were significantly lower for the OxyLite system than for the Helzel system ($P < 0.01$). There was no significant difference in the R3327-AT/tkeGFP tumors ($P = 0.138$).

FIG. 4.

Dot plots of measured pO_2 values in the same six R3327-AT tumors using the Helzel (panel a) and OxyLite (panel b) system while the animal breathed 100% oxygen. Note that there is a large disparity between the two systems in mouse 5, whereas the other mice are comparable. Panel c is a scatterplot of the pO_2 data acquired with the OxyLite (x axis) and Helzel (y axis) probes for equivalent-depth measurement points from parallel tracks in the same tumors. The density of pO_2 readings measured with the two systems is shown in panel d. The dotted line represents data measured with the OxyLite system and the solid line represents data measured with the Helzel system. The pO_2 data obtained from mouse 5 have been excluded in panels c and d. The scatterplot of the data shows that the two are not related, which is to be expected

since the measurements are local at micrometer levels while the points compared are 1 mm apart. The lower right panel shows that while pO_2 values were not correlated, the distributions of the values from the two systems are comparable.

TABLE 1

Number of Tumors used in Different Experiments

	R3327-AT	R3327-AT/HRE
Helzel system alone	19	
OxyLite system alone	21	
Both systems, air	9	19
Both systems, 100% O ₂	6	

TABLE 2

Pooled Data for Percentages of pO_2 Readings with Different Cutoffs for R3327-AT Tumors from Experiment 1 Measured Independently in Different Animals with the Helzel or OxyLite System

Tumor data	Mean	SD	Mean	SD
No. of tumors	19		21	
Volume (mm ³)	565	400	560	416
	Helzel		OxyLite	
Percentage of readings (%)				
<2.5 mmHg	71	26	65	25
<5.0 mmHg	74	25	70	21
<10.0 mmHg	81	20	74	
pO_2 readings (mmHg)				
Minimum	0.0		0.0	
Maximum	57		99	
Mean pO_2	5.0	9.7	9.6	17

TABLE 3
Percentages of pO_2 Readings in Experiment 1 within Different pO_2 cutoff Windows Stratified According to Tumor Size as Measured by the Helzel (left columns) or OxyLite (right columns) System for the R3327-AT Tumors

	<3 mmHg	<5 mmHg	<10 mmHg	<3 mmHg	<5 mmHg	<10 mmHg
~100 mm ³	17 ± 5	20 ± 7	39 ± 10	22 ± 5	35 ± 7	42 ± 6
~200 mm ³	65 ± 3	74 ± 5	81 ± 7	60 ± 8	69 ± 5	74 ± 8
~400 mm ³	74 ± 16	77 ± 16	84 ± 13	67 ± 8	70 ± 6	73 ± 6
~800 mm ³	80 ± 11	83 ± 10	86 ± 8	76 ± 11	78 ± 9	83 ± 8
>1000 mm ³	89 ± 6	90 ± 5	93 ± 5	83 ± 6	85 ± 5	88 ± 4