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Bio-elimination and Safety Test of Novel Dual-Energy CT Contrast Agent

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Bio-elimination and Safety Test of Novel Dual-Energy CT Contrast Agent

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Bio-elimination and Safety Test of Novel Dual-Energy CT Contrast Agent

Sizhe Wang

ABSTRACT

Dual-energy CT is an FDA approved CT technique introduced into fast clinical scanners 10 years ago. It may be obtained in multiple ways, such as switching the tube voltages of the X-ray source rapidly during the scan (usually between 80 kVp and 140 kVp) to generate images with different energy levels. Different materials including soft tissues, bone, and contrast agent, have different CT number attenuation ratios at low- versus high-energy, where CT number is a quantitative scale for describing radio-density. Based on this property, different materials can be separated. Currently there are very limited numbers of contrast agents designed for dual-energy CT, and the long-term goal of our research is to develop a silicon-based novel enteric contrast agent for dual-energy CT.

In order to get FDA approval for initial human testing, it is important to collect *in vivo* bio-elimination and impurity data for the novel contrast agent. The main purpose of my research is to access the safety of the novel contrast agent, including: 1) Quantification of bio-eliminated contrast agent in mice; 2) Semi-quantification of bio-eliminated contrast agent in phantoms and rats; 3) Heavy metal quantification for fumed silica beads.

Based on our *in vivo* DECT imaging tests, most of the novel contrast agent will be eliminated out of rats in approximately 2 days, but further quantitative verification is still needed. In addition, although the amounts of most heavy metals are below the daily allowable threshold limit, undesirably high amounts of barium and lead are seen in this novel contrast agent. Further tests and modifications are necessary to better quantify and improve the contrast agent before it will be ready for human testing.

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Disclosure: For proprietary reasons, exact details of the materials used, and the numerical values of the experimental formulations of the contrast agent under investigation cannot be disclosed at this time.

1. INTRODUCTION

Dual-energy Computed Tomography (DECT) is a CT technology that uses both a higher energy level X-ray and a lower energy level X-ray spectrum, delivered nearly simultaneously, to make images. The two different photon spectra can be generated either by rapidly switching the potentials of single X-ray tube, or by running two separate X-ray scans at different potentials (Johnson 2012). Typically, the high X-ray energy is at tube voltage of 140 kVp, and the low is 80 kVp. For individual materials such as soft tissues, bone and contrast agent, their ratios of attenuation coefficients between high and low energy X-rays are unique (Mendonca et al. 2010). When an object is imaged by dual-energy CT, objects can be differentiated and quantified based on differences in their X-ray attenuation ratios within voxels. *Figure.1* is a comparison between a conventional CT image and a separated DECT image, and the DECT image clearly distinguishes iodine in arterial phase from tungsten in venous phase. An enteric contrast agent can be digitally separated from an intravascular contrast agent if the agent have sufficiently different attenuation ratios, and this property can be used to improve the evaluation of small bowel.

Current clinical contrast agents are all based on iodine or barium, and the differentiation of their signal from each other is limited due to their similar 80 kVp/140 kVp attenuation ratios (Anderson et al. 2010). The overall goal of this research is to develop a clinically safe novel enteric contrast agent, which can be more efficiently differentiated from iodinated and barium agents on DECT imaging.

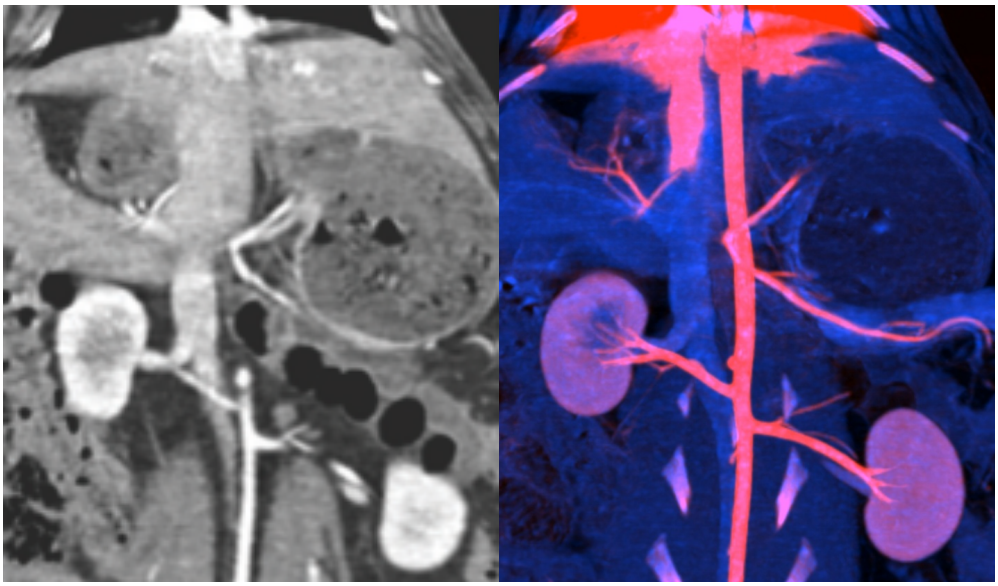

Figure.1 ***Left: conventional CT image of the rabbit with venous tungsten and arterial iodine contrast agents; Right: DECT image of the rabbit with venous tungsten and arterial iodine contrast agents.*** *Arteries (red) and veins (blue) can be readily distinguished in the DECT image.*

The novel contrast agent is primarily made of fumed silica (SiO_2) beads. Two different sized agents are tested; one with a mean diameter of smaller beads around 17 μm, and the larger one around 23 μm. *Figure.2* shows a scanning electron microscopy (SEM) micrograph of the larger bead, taken by Rush University Medical Center. Silica has widespread applications including use as food additive and as excipient in drugs. Small amounts of silica are considered as nontoxic and stable inside the human bodies (Martin et al. 2007). In addition, the silica micro-particles used in the current project exhibit an 80 kVp/140 kVp attenuation ratio of 2.3, differing from that of iodine (1.7), calcium (1.5), and soft tissue (1.0) (Nowark et al. 2011). These properties make silica micro-particles an attractive enteric dual-energy CT contrast agent.

The effectiveness of the novel contrast agent has been confirmed in the rabbit-model research by our lab. In order to get an FDA approval for initial human testing, it is important to

collect *in vivo* bio-elimination and impurity data for the novel contrast agent. The main purpose of this research project was to assess for the bio-elimination properties of the contrast agent in animal models of mice and rats, to verity the quantification of novel contrast agent separation algorithm, and to quantify heavy metal impurities associated with beads.

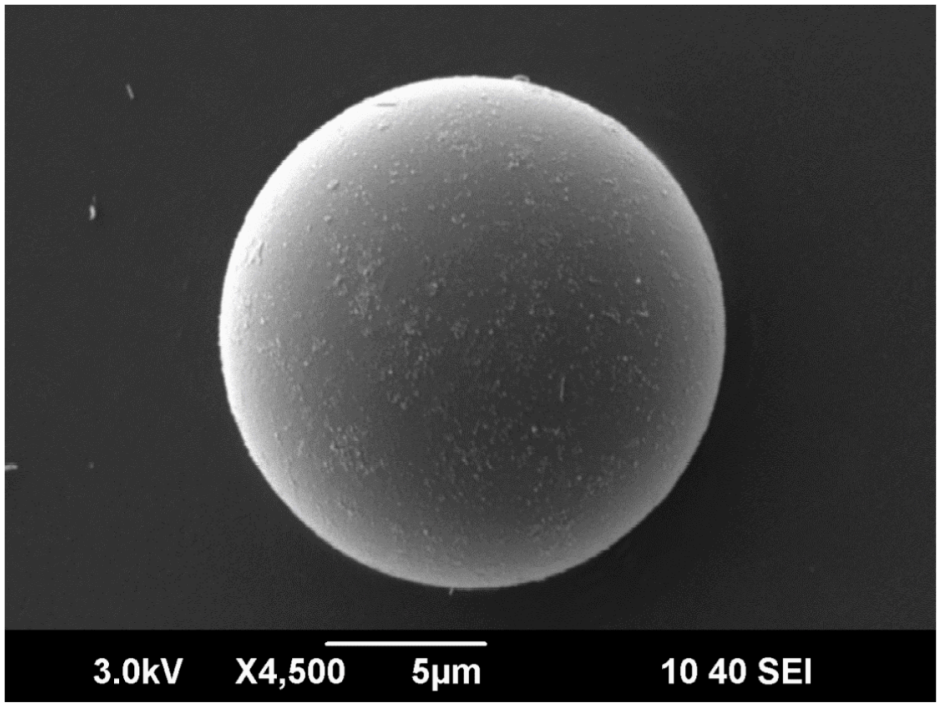

Figure.2 *SEM micrograph of a 15.6µm fumed silica bead (X4500).*

2. MATERIALS AND METHODS

2.1 In vivo bio-elimination of novel contrast agent for mice

The acquisition and housing of mice, and sample collection were handled by Nextrast, Inc in collaboration with MuriGenics, Inc. 10 healthy *CD1* mice with weights between 25-30 g were assigned into two groups. All the mice underwent a 14-day acclimation period on a low silicon diet before they received silica contrast agents by gastric gavage. Group 1 were given 20 mL/kg 30 wt.% novel contrast agent of the smaller beads, and Group 2 were given 20 mL/kg 30 wt.% of the larger beads. After the oral gavage administration, mice were individually housed in

metabolic cages for up to 72 h, and their feces and urine were collected separately and sent for ICP-AES silicon quantification at the UC Davis Analytical Laboratory. The feces and urine from the day before the oral administration was collected and quantified as self-control. Spike tests for linearity of quantification of silicon in the contrast agent by ICP-AES were obtained prior to the fecal / urine silicon quantification, by identifying silicon in different amounts of fumed silica beads (either as dry powder or contrast agent solution).

2.2 Semi-quantitative dual-energy CT scans of phantoms and rats

Novel contrast agent phantoms of larger fumed silica beads with weight percentages varying from 0 % to 40 % were prepared and taken dual-energy CT scans. Contrast agent masks are the images that only contain signals from novel contrast agent, and were generated by the separation algorithm developed by our lab, based on the known unique 80 kVp/140 kVp CT value of the novel contrast agent. Average voxel intensities of contrast agent masks were measured and recorded.

Two *Sprague Dawley* rats with weights around 500 g were given dual-energy CT scans, before and after they received gastric gavage of contrast agents (one with 10 mL 20 wt.% novel contrast agent of larger beads, and the other with 10 mL *Readi-Cat 2* barium sulfate suspension). Rats were under anesthesia during each scan. Between scans, rats were given *ad libitum* access to food and water.

Both phantoms and rats dual-energy CT examinations were performed with a GE Discovery CT750 HD scanner at UCSF Moffitt Hospital. The rats were centrally placed in the scanner to ensure that the entire gastrointestinal tracts were covered by the field of view of tube detector arrays. Scans were taken under protocol GSI-51 with the following settings: tube voltages of 80 kVp and 140 kVp, 0.5 second rotation time, helical thickness of 0.625 mm, helical

pitch of 1.375:1, CTDIvol of 7.4 mGy, 19 cm field of view, and large bow tie filter applied. Green gradient color was applied to the contrast agent mask, in order to better show the distribution of contrast agent inside of the rat.

2.3 Heavy metal quantification with acid washed

About 2g of the large fumed silica beads were incubated in 20 mL acids (either 38 wt.% HCl, or aqua regia containing 28.5 wt.% HCl and 17 wt.% HNO_3), shaking at 200 rpm, 37.6 °C. Beads were washed and dried after the incubation, and then sent for ICP-MS heavy metal quantification by ALS Geochemical, Inc, along with untouched beads as control. A total of 53 metal elements were quantified, as shown in *Figure.3*.

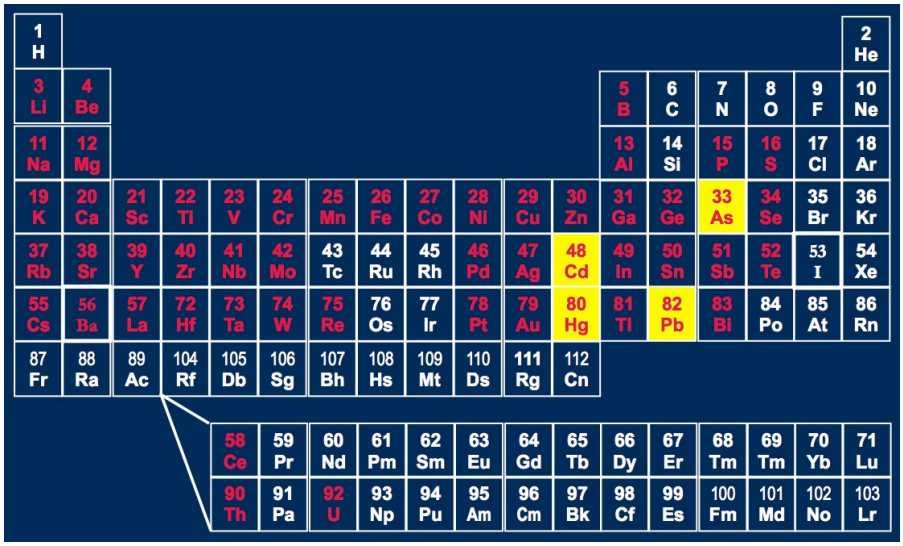

Figure.3 *Quantified metal elements are highlighted as red, and the 4 elements highlighted as yellow are the most toxic elements to the human body.*

3. RESULTS AND DISCUSSION

3.1 In vivo bio-elimination of novel contrast agent for mice

The amounts of silicon in all the feces collected before the gastric gavage are below the detection limit of ICP-AES, which is 0.02 % by weight. In addition, the amounts of silicon in all

the urine samples, including those collected after gastric gavage, are also below the detection limit of ICP-AES. Based on these results, we can consider all the detected silicon to be coming from the gastric gavage, and there was no silicon in any urine sample. Feces samples from 2 mice receiving smaller beads were not well separated from urine during the collection, so their data was not included in the analysis. The in vivo bio-elimination results of the novel contrast agent for mice are shown in *Figure.4*. The unit of values has been converted into percentage, comparing with the amount administered.

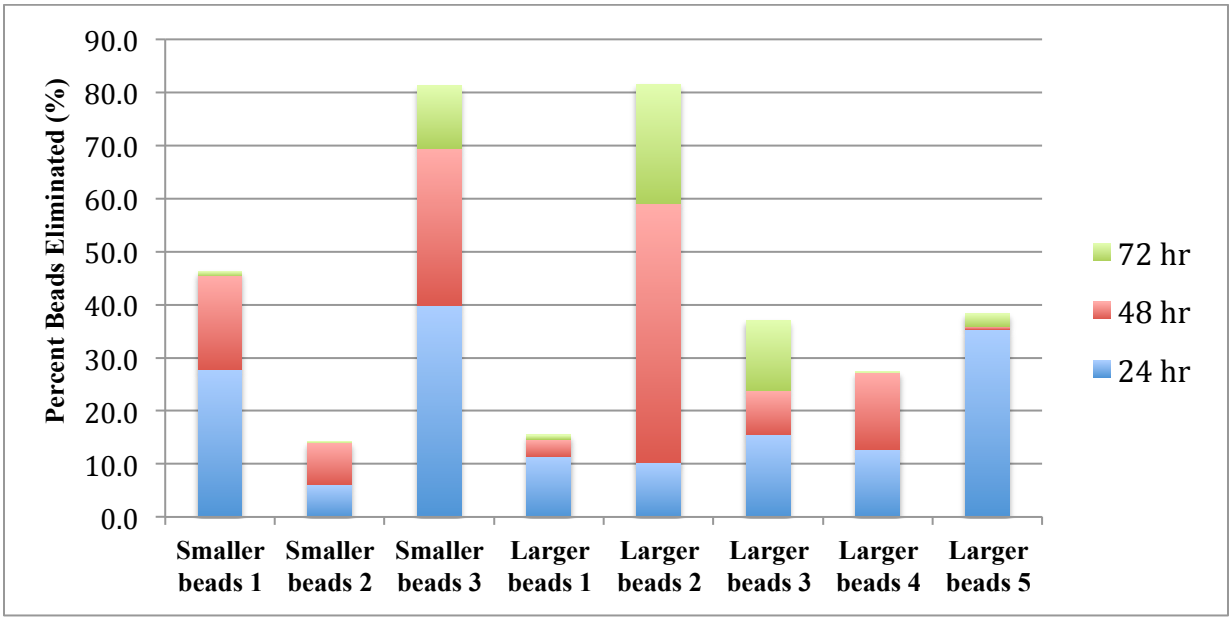

Figure.4 *Percentages of bio-eliminated beads over 72h for mice.*

It can be observed that the eliminated amounts of beads vary largely between different mice, and even for the mice in the same group, the values are highly inconsistent. Some mice eliminated a large fraction of beads on the first day, while some mice eliminated most on the second day. No single conclusion can be generated based on the result. Unfortunately the bowel and carcasses of the mice were not evaluated.

We have identified a flaw in our applied study design. In 1986, Damon et al. reported that when rats were housed in metabolic cages, there was a decrease in food and water intake, urine output and creatinine clearance, and they suggested that a minimum acclimation period of 4 days for rats to recover from the effects of being placed in metabolic cages. Similar effects on mice were reported by Kalliokoski et al. in 2013. Since we did not apply the metabolic cage acclimation period, it is possible that during our test, the mice were in variably stressed statuses, and their elimination systems were therefore inconsistent and/or unstable. We will correct for this possibility when we repeat this test on rats.

2.2 Semi-quantitative dual-energy CT scans of phantoms and rats

Due to the phantom scans, a relationship can be set up between the average voxel intensities of contrast agent masks and the weight percentages of fumed silica beads, as shown in *Figure.5*. The excellent linearity provides strong evidence that our separation algorithm can be used in quantitative analysis.

For the dual-energy CT scans of rats, a baseline scan was acquired prior to administration on the oral contrast agent and follow-up scans were performed at the following time points: right after gastric gavage (0 h), 1 h, 2 h, 4 h, 6 h, 23 h, and 43 h after gavage. *Figure.6* shows the processed images with the novel contrast agent highlighted by green gradient. The current separation algorithm cannot efficiently distinguish novel contrast agent from air gradient, which changes from -1000 HU as pure air to positive HU values in tissue, where HU scale is a quantitative scale for describing radio-density. And our algorithm captures the air gradient signal and treat them as novel contrast agent, appeared as green spots shown in the *pre scan* image. We have not find a proper way to separate the air gradient signal from the contrast agent signal yet, and currently we can only distinguish them based on anatomy structure. Due to this reason, the

quantification analysis of *in vivo* novel contrast agent amount currently cannot be applied to these images.

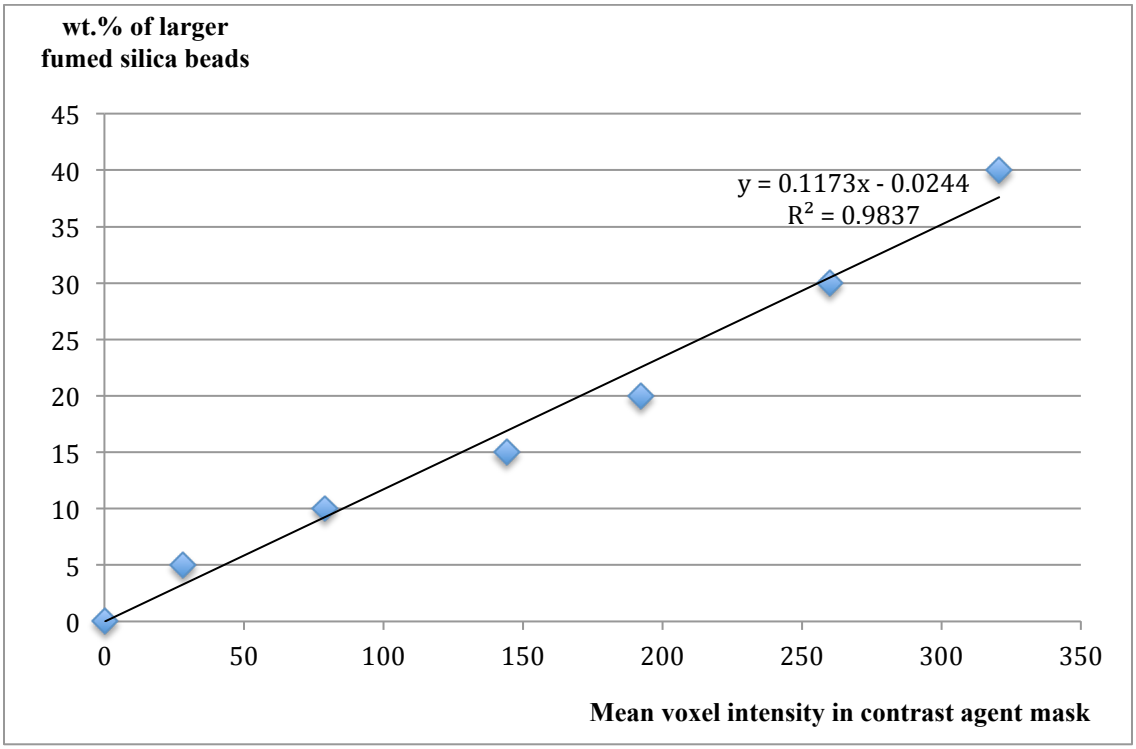

Figure.5 ***Linearity curve of mean voxel intensity in contrast agent mask versus wt.% of larger fumed silica beads.***

The signal of novel contrast agent appeared immediately in the stomach and the small intestine after the oral injection (about 10 min delay between oral injection and the 0 h scan), but no novel contrast agent reached ascending colon until 4 h after the injection, with still plenty of novel contrast agent left in the stomach. From the 23 h scan image, we can see some of the novel contrast agent in the descending colon, and about to be eliminated as feces. And from the 43 h scan image, it can be seen that almost all the novel contrast agent was eliminated, with very little amount remaining in the intestines.

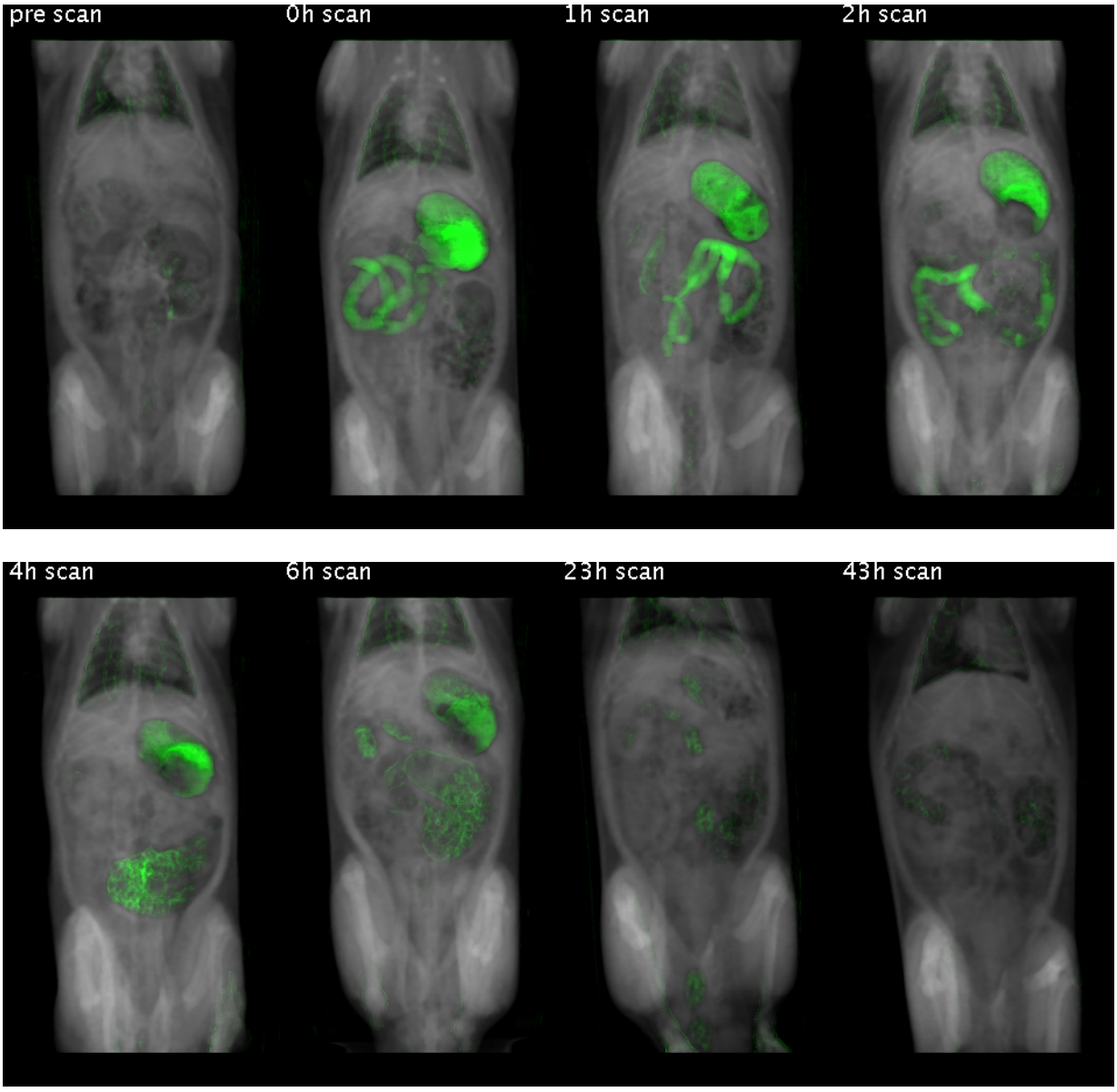

Figure.6 ***Oblique coronal average intensity projection DECT images with novel contrast agent signal highlighted.*** *All the images within one scan are averaged and projected into coronal view. A green gradient is applied to the novel contrast agent, so the brightness of green color is linearly related with the corresponding amount of novel contrast agent there.*

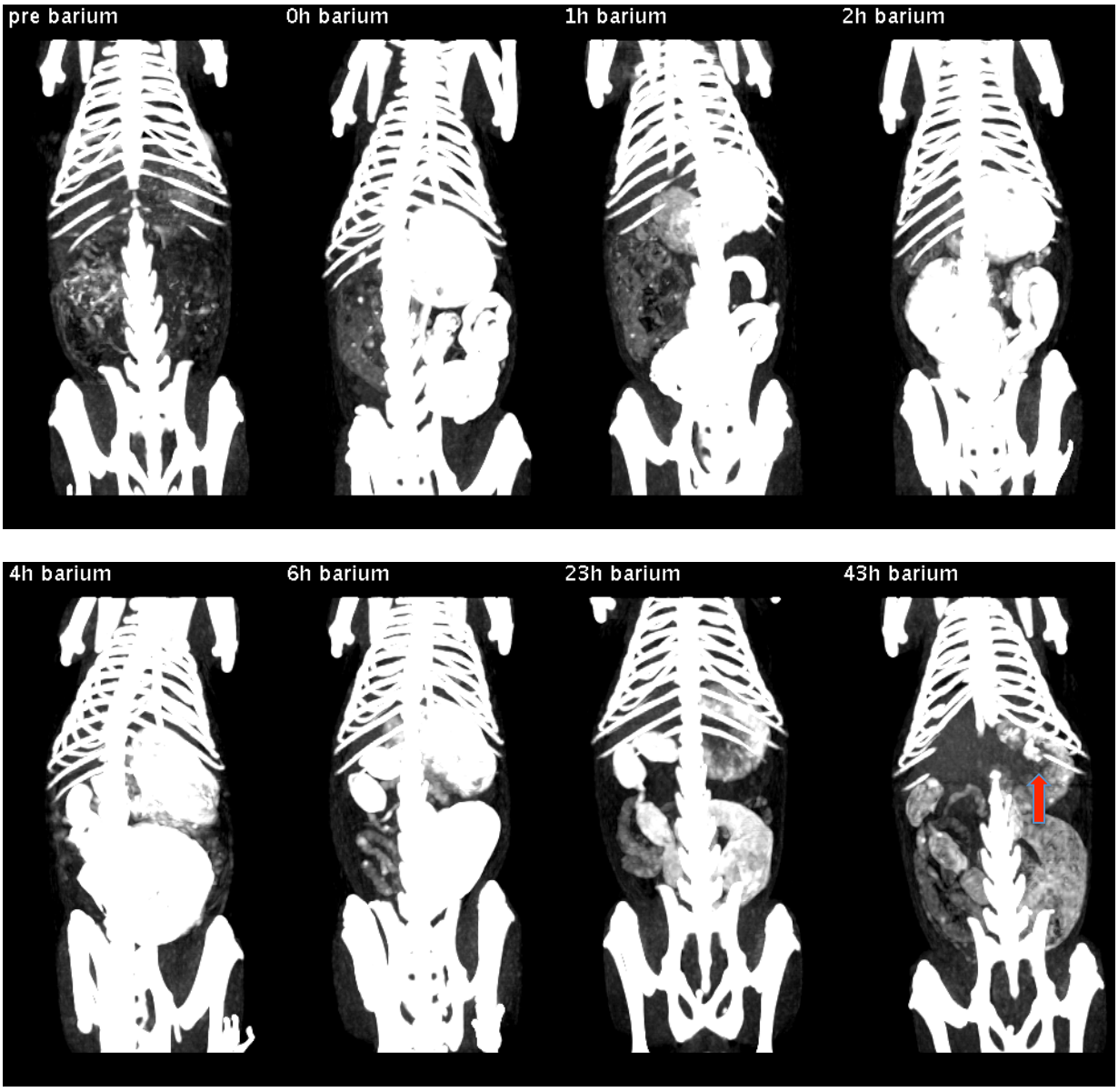

Figure.7 ***Oblique coronal maximum intensity projection CT scan images of barium given by gastric gavage.*** *Barium appears as brightly white in regular CT scan, similar in intensity as bone. In the 43 h scan image, the red arrow points to residual barium in the stomach.*

As the control group, the 60 keV monochromatic images of barium-fed rat are shown in *Figure.7*. It can be clearly observed that in 43 h scan, there was also some barium remaining in the gastrointestinal tract, even in the stomach, as the red arrow highlighted.

Comparing with barium, the novel contrast agent has very similar overall efficiency of bio-elimination, which is around 2 days. However, it takes longer for the contrast agent to get to the colon (around 4 h), while barium takes less than 2 h. Our next goal is to try to reduce the viscosity of the novel contrast agent to potentially improve the bio-elimination efficiency. Also, a larger number of animals will be tested to improve statistical significance.

3.3 Heavy metal quantification with acid washed

The analysis of the 2 g of the larger fumed silica beads indicated that most of the 53 metal elements tested, were under the daily-allowed amount. This was true even in the untouched fumed silica beads. *Table.1* lists the test result of the four most toxic metal elements to human body: As, Cd, Hg, Pb, along with Ba, which is listed due to its high amount in the novel contrast agent.

	As	Ba	Cd	Hg	Pb
Original	0.1ppm	25.7ppm	0.017ppm	0.004ppm	0.74ppm
39% HCl washed	0.03ppm	20.1ppm	0.009ppm	<0.004ppm	0.665ppm
Aqua regia washed (28.5% HCl + 17% HNO_3)	0.02ppm	20.7ppm	0.005ppm	<0.004ppm	0.63ppm
Allowed Daily Amount	0.07ppm	6.5ppm	0.023ppm	0.139ppm	0.02ppm

Table.1 ***Most potentially toxic heavy metals in fumed silica beads, measured by ICP-MS***

All the values are shown in the unit of ppm, and the allowed daily amounts are also converted from mg to ppm, based on ratio over the expected intake of novel contrast agent for a 70 kg adult contains 216 g fumed silica beads.

Acid-washing is very helpful in reducing the amount of heavy metal in fumed silica beads, and most of arsenic can be washed out by either HCl or aqua regia. However, the remaining barium and lead still exceed the daily-allowed limit. Potentially chelators such as EDTA can bind with both barium and lead, and form very stable complexes (Flora and Pachauri, 2010; Chen et al. 2010). Future work will try chelation reactions to reduce the amount of barium and lead in the novel contrast agent.

4. CONCLUSION

We have established preliminary data related to the bio-elimination and impurity levels of a novel enteric contrast agent. DECT data demonstrates the contrast agent has moderate bio-elimination efficiency, similar to that of commercially used barium sulfate, but this needs further verification by feces and urine silicon quantification. Also, although most of the heavy metal elements tested are below the daily-allowed limit thresholds, high amounts of barium and lead remain an unsolved problem.

Future work will involve both evaluations and quantifications of bio-elimination tests *in vivo*, and removal of barium and lead from the fumed silica beads. Developing a new drug is a long-timeline endeavor, and bio-elimination tests and heavy metal quantification are very important safety considerations for a new drug to get FDA approval and eventually be used clinically. The preliminary data acquired as part of this project represent a small step towards this long-term goal.

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