INTRODUCTION

Intravitreal injections remain a common technique for administering ocular drugs to the posterior segment of the eye, especially for treatment of age-related macular degeneration. Aspects of intravitreal injections, such as drug reflux, leakage from the needle tract and needle depth, are known to be of importance for the efficacy of intravitreal drug delivery. Although, several types of symptomatic complications have been shown to occur as a result of these injections. With improved efficacy through injection technique, it is expected there would be a reduction in the number of complications associated with intravitreal injections.

Some complications that are less studied are the introduction of air or silicone into the eye. Air bubbles have been previously documented and are seen clinically following intravitreal injections, but there has been little reporting of the size and fate of these air bubbles. Other clinical reports have found intravitreal silicone oil droplets present within the eye following intravitreal drug injections. These are believed to have originated from the needle as a result of the manufacturing process. Any factors that may affect the therapeutic efficacy of the drug being administered by intravitreal injection, such as the introduction of bubbles in the vitreous, should be addressed.

Pharmacokinetics in the vitreous is influenced by a number of factors. The more well-known and understood include molecular size of the drug, vitreous liquefaction, vitreous volume, intraocular pressure and intraocular inflammation. Some
factors, such as vitreous liquefaction or volume, cannot be changed through treatment options. The differing properties of the vitreous as a result of vitreous heterogeneity\(^6,7\) can significantly alter how a drug travels through the vitreous in terms of time and route. Vitreous liquefaction occurs as a result of patient age or surgical procedures. The distribution of drug in a liquefied vitreous has been shown to be faster than that of a normal vitreous\(^8\) and the effect on drug kinetics is largely influenced by the location of liquefaction.\(^9\)

Many pharmacokinetic studies, and therefore drug dosages, are administered based on assumptions of a specific bolus shape\(^10,11\) and location of injection,\(^12\) but deviations from these assumptions and predications change the flow and dosage window of the drug. Therefore, the most efficient and effective outcomes for the target tissues may not be realized. The purpose of this study is to identify anomalous features found following intravitreal injections using a porcine eye model. With direct spatial and temporal visualization of drug boli from intravitreal injections using micro-computed tomography, we are better able to understand the impact of anomalous features on drug delivery in the eye.

**METHODS**

**Micro-Computed Tomography**

Micro-computed tomography (micro-CT) scanning was performed using a pre-clinical system (eXplore Locus Ultra, General Electric Healthcare Bioscience, London, Ontario, Canada). A flat-panel detector mounted on a slip-ring CT gantry was used to acquire images with an isotropic spatial resolution of 150 µm. Each 3D volume was acquired in 8–16 s and x-ray acquisition parameters (80 kVp and 40–80 mA) were kept constant for each experiment.

**Contrast Agents**

Iodinated (Omnipaque\textsuperscript{TM} 300, GE Healthcare, Oakville, Ontario, Canada) and gold nanoparticle (AuroVist\textsuperscript{TM}, Nanoprobes Inc, Yaphank, New York, USA) contrast agents were used as drug mimics. Iodine based iohexol (Omnipaque\textsuperscript{TM} 300, GE Healthcare, Oakville, Ontario, Canada) was chosen because of its common use as a radiographic contrast agent both clinically and experimentally and is very economical. Omnipaque 300 contains 300 mg of iodine per milliliter and has the molecular weight of 821 g/mol. It has an osmolarity of 465 mOsm/L, absolute viscosity of 6.3 at 37°C and specific gravity of 1.349 at 37°C.

Gold nanoparticles (AuroVist\textsuperscript{TM} 1102, Nanoprobes Inc, Yaphank, New York, USA) were chosen as they have been shown to provide higher contrast at lower concentrations. The molecular weight of gold nanoparticles is much higher than iodine (50 kDa vs. 0.82 kDa) and studies have shown this to attribute to a slower clearing time in biological systems.\(^13\) This provides for a longer period of contrast detection at a lower initial concentration. In addition, the 1.9 nm gold nanoparticles have a similar molecular weight as the most common anti-VEGF drugs used to treat AMD (i.e. pegaptanib (Macugen\textsuperscript{®}) 50 kDa; ranibizumab (Lucentis\textsuperscript{®}) 48 kDa).

**Intravitreal Injections**

Freshly harvested enucleated porcine eyes were obtained from a local abattoir and used within 10 h postmortem. During scanning and for the remainder of the study, they were maintained at 24°C (±2°C). Pre-injection scans confirmed no abnormalities in any eyes. 29-gauge insulin syringe and needles (SS05M2913, Terumo Medical Corporation, Somerset, NJ, USA) were pre-filled with the contrast agent and standard procedures were followed for deairing of the syringe (e.g. vertical positioning of needle, while tapping the syringe and pushing out air). Intravitreal hand injections of a 0.03 mL volume of contrast agent were administered into 24 enucleated eyes. A volume of 0.03 mL was used to compensate for the smaller porcine vitreous volume compared to human, where 0.05 mL is the prescribed intravitreal injection volume for bevacizumab\(^14\) and ranibizumab.\(^14\) The intravitreal injection technique was taught by ophthalmologists and medical residents, with a focus on mimicking a clinical hand injection to humans. Injections were performed 2–3 mm posterior to the superotemporal limbus at a depth of 1 cm. Each injection was completed with a new syringe and needle and lasted for approximately 2–4 s, similar to that of clinical injections.

**Imaging**

Scans were acquired up to 230 min (the anticipated time for the contrast agent to remain at sufficient concentration and/or reach the retina) following the injection to allow for visualization of the contrast agent’s progression through the vitreous. A typical three dimensional (3D) scan is shown in Figure 1, with a central iohexol bolus. Grey scale image intensity variations can be used to differentiate between tissues in the eye and introduced fluids and gases. The temporal and spatial changes in iohexol and gold nanoparticle concentrations can also be observed as the fluid difuses through the vitreous.

**Image Analysis**

The acquired images were analyzed using dedicated 3D computed tomography visualization and analysis
software (MicroView 2.1.2, GE Healthcare, London, Ontario, Canada). Upon examination of the reconstructed scans, anomalous features of air bubbles and irregular bolus shapes were also realized. To better understand the behavior of the air bubbles over time, measures of diameter were taken using signal profiles, plots of grey scale vs. distance. All air bubbles resembled perfect spheres and the volume was calculated using the sphere volume equation with the measured diameter. Plots of bubble volume ($V$) with time ($t$) were produced and assuming first order kinetics, the slope of these lines were found, providing the decay constant ($k$), of the following exponential decay equation:

$$ V = V_0 e^{-kt} \quad (1) $$

where $V_0$ is the assumed initial volume of the bubble.

Analysis of the initial bolus shapes was completed by measuring the length, height and width of a reference rectangular prism manually fit with minimum dimensions into which each bolus fit. It should be noted that due to the irregularity of the shapes, these measures only provide an approximation of the shape of the boli.

### RESULTS

#### Air Bubbles

Despite using typical clinical procedures to eliminate air from entering the vitreous during injection air bubbles were clearly visible within the vitreous (see Figure 2) of 21 eyes following the injections and totaled 36 air bubbles. The initial volume of the air bubbles ranged from 0.01 µL to 1.50 µL and the mean was 0.03 µL. Some movement (~1 mm in any direction) of the bubbles occurred over the scanning period of 230 min. The size of the bubbles was found to decrease over this visualization period (Table 1). Using the first order kinetics assumption, the decay constants of these bubbles ($k$) had a mean of $0.0067 \pm 0.0022 \text{min}^{-1}$. Hence the bubbles had half-lives of $2.16 \pm 0.48 \text{h}$, which is somewhat shorter than in vivo observations of free non-expansile gaseous bubbles have found previously. This may be due to the size of the air bubbles; previous studies have used large bubbles relative to the eye volume and the relationship between surface area and volume is quite different for smaller bubbles. Further investigation of the relative differences in half-life between different sized bubbles is shown in Figure 3, where the decay in bubble volume with time is shown for four typical bubbles with sizes ranging from 0.57 to 0.01 µL are shown. Each decay sequence has been fitted with equation (1) to find the half-life, which is seen to reduce with bubble size.

#### Bolus Shape

Many of the injected boli were found to have abnormal initial shapes (see Figure 4). The assumed shape of a sphere was rarely found; rather characteristics such as two conjoining spheres or tear drop shapes often resulted. For an assumed sphere, the diameter would be approximately 3.86 mm (based on 0.03 mL volume). However, the extent of the initial boli had a larger range and a higher than expected average (Table 2). These asymmetric boli were attributed to movement of the needle tip during the injection and reflux of the drug mimic up the needle path. There was also no true center of the boli (i.e. no single region with a peak contrast concentration) when the initial shape was abnormal.
Although the presence of small air bubbles within the vitreous are not thought to be a significant risk for patients, it is desirable to minimize their size, since they may cause temporary vision impairment. In addition, the transport of the drugs from the injected boli may be hindered due to the increased tortuosity of the diffusion/flow path around the air bubbles. It is important to be aware of the factors influencing pharmacokinetics so that the standard method of drug administration is as effective as possible in disease treatment. Whilst the air bubbles are likely to migrate upwards (due to buoyancy forces), a patient’s position during and following the injection, in addition to pressure gradients or flow in vivo, are also likely to affect air bubble movement. Possible sources for these air bubbles may be inadequate de-airing of the needle barrel system, cavitation or air entrainment during insertion.

The most obvious source of the air bubbles would be transfer from the needle or the barrel of the syringe. Interestingly, the initial volume of the air bubbles was found to be quite large compared to the internal volume of the 29 gauge needles used (up to 5–6 times as great). Thus additional air may have been present in the syringe barrel, lying at the interface of the rubber piston and the sides of the syringe barrel. However, given the care taken during preparation to remove air from the system this source of air is unlikely. Clinical reporting of air bubbles following intravitreal injections did not include sizes of the air bubbles observed,3 providing no means of comparison with those found in this study.

Hydrodynamic cavitation has been observed to cause microscopic air embolisms in other forms of injection,16,17 but it is not thought to occur with standard syringes and injections.18 However, it has been observed through micro-orifices and micro-fluidic devices for low Reynolds number flows.19 Since the barrel-needle interface is defined as a “sharp-edged orifice” in flow terms, the sudden reduction in cross-sectional flow area will induce a static pressure drop at the vena contracta (narrowing of flow) that occurs just beyond the interface. If this drop is sufficiently large and the pressure begins to approach the vapor pressure of the fluid, then cavitation and the growth of micro-bubbles could occur in the flow. Whilst this mechanism is less likely for standard (4–5 s) injection times, this cannot be discounted at this stage for shorter periods of injection. Confirmation of this phenomenon would require further careful study and complex multi-phase computational fluid dynamics approaches.

Another possible source of air could be entrainment during needle penetration of the sclera. A number of examples of physical problems where small objects penetrate liquids and films leading to air entrainment are described in the literature.20 It is therefore conceivable that surface tension forces at the air-liquid-needle
interface are sufficient to pull small volumes of air into the vitreous as the bevel and needle penetrate the sclera. Further study of these injections in a sub-aqueous state would confirm this hypothesis.

Some prefilled syringes are available and useful in reducing or eliminating gas bubbles from the syringe. The techniques in which the syringes are filled vary greatly and not all methods are equally effective in removing air from the syringe barrel.21 More advanced vacuum filling provides bubble-free filling and therefore improves dosage accuracy, product stability, improved sterility and no introduction of air during injection. Recent work has shown foreign materials, such as silicone oil, may still be introduced during intravitreal injections when using prefilled syringes.22 If hydrodynamic cavitation is the source of air in the injection, prefilled syringes would not have any effect.

Not all prefilled syringes are manufactured and filled in the same way, but they do provide potential for improving the efficacy of drug delivery in the eye. Based on the observations of the resulting boli shapes after injection, delivery of the drug cannot be based on predictions where the initial bolus is assumed to resemble a sphere. Further experimental observations by the authors seem to indicate that the initial bolus shape is also related to the fluid velocity exiting the needle and the stability of the syringe. The vitreous is a paucicellular hydrogel with primarily unbranched type II collagen fibrils and hyaluronic acid.23 These fibrils form a tensioned semi-random polymer network, where the hyaluronic acid sustains the tension by osmotic (Donnan) swelling. Investigations of the elastic shear moduli using observations of spherical cavity instability of injected fluids into the vitreous for the content and writing of the paper.

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For potential changes to be made to the delivery method of ocular drugs it is important to understand and address the factors in drug delivery that can be altered. Drug loss as a result of reflux has been shown to be a highly variable factor in ocular drug delivery. It has been estimated that 10% loss of drug could be substantial enough to alter the pharmacokinetics and therefore effectiveness in treating the disease.1 Work has been completed recently that found the use of 30G needles and a deep injection helps to minimize reflux of drug during intravitreal injections.1 The study showed that with minor modifications to injection supplies and technique, significant improvement can be made to administering the drug in the expected manner, thereby maximizing the therapeutic effect.

Our work has broadened and enhanced these observations with direct spatial and temporal visualizations of the drug boli and associated phenomena following injection. It was found that an injected drug boli will be impeded by air bubbles within the vitreous, forcing flow around the air bubble. A greater number or larger air bubbles within the vitreous will decrease symmetrical diffusion, which is the often assumed diffusive pattern in pharmacokinetic studies. In the more diffuse injections of the drug mimic a single region of higher concentration was never attained and is also an indication of greater disruption of the vitreous, potentially leading to prolonged vitreous changes.

These observations have demonstrated the importance of a consistent and accurate injection technique to administer the appropriate concentration of drug to the retinal tissue. They also suggest that the design and length of the needle and the injection fluid velocity must be carefully considered while administering intravitreal drugs, as this can alter the concentration and period of drug reaching the target site. It is only by making these anomalous features known that they can be better addressed to improve the efficacy of intravitreal drug delivery and perhaps influence the design of new drug delivery systems, all of which will improve patient outcomes and safety.

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