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## Viral Inactivation of Human Osteochondral Grafts with Methylene Blue and Light

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### Abstract

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#### Objective:

Cartilage injury is one of the most common disorders of synovial joints. Fresh osteochondral allografts are becoming a standard treatment; however, they are supply constrained with a potential risk of disease transmission. There are no known virucidal processes available for osteochondral allografts and most methods presently available are detrimental to cartilage. Methylene blue light treatment has been shown to be successful in the literature for viral inactivation of fresh frozen plasma. The purpose of this study was to determine the capacity of methylene blue light treatment to inactivate a panel of clinically relevant viruses inoculated onto osteochondral allografts.

#### Design:

Osteochondral grafts recovered from human cadaveric knees were inoculated with one of the following viruses: bovine viral diarrhea virus (BVDV), hepatitis A virus (HAV), human immunodeficiency virus type 1 (HIV-1), porcine parvovirus (PPV), and pseudorabies virus (PrV). The samples were processed through a methylene blue light treatment, which consisted of an initial soak in nonilluminated circulating methylene blue at ambient temperature, followed by light exposure with circulating methylene blue at cool temperatures. The final titer was compared with the recovery control for the viral log reduction.

#### Results:

HIV-1, BVDV, and PrV were reduced to nondetectable levels while HAV and PPV were reduced by 3.1 and 5.6 logs, respectively.

#### Conclusions:

The methylene blue light treatment was effective in reducing (a) enveloped DNA and RNA viruses to nondetectable levels and (b) nonenveloped DNA and RNA viruses of inoculated human osteochondral grafts by 3.1 to 5.6 logs. This study demonstrates the first practical method for significantly reducing

viral load in osteochondral implants.

**Keywords:** osteochondral, allograft, viral inactivation, methylene blue

## Introduction

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The use of allografts in musculoskeletal procedures has increased over the decades to address the growing needs in orthopedic and sports medicine applications.<sup>1</sup> Cartilage injury is one of the most common disorders in the knee with about 1.3 million cartilage lesions observed annually.<sup>2</sup> For full thickness cartilage lesions, surgeons use as a replacement autologous osteochondral grafts as a composite of bone and hyaline cartilage surface harvested from a less- or non-weightbearing portion of the knee. These grafts have similar biomechanical properties to the native tissue. If properly sized and placed, the graft bone typically integrates well with the host bone resulting in stability, and the immediate replacement of the cartilage restores the joint function. It has shown good clinical outcomes but is limited to small- to medium-sized cartilage lesions because of the limited amount of autologous tissue available for transplantation, donor site morbidity and topographical mismatch.<sup>3</sup> Transplantation of fresh osteochondral allografts also provides a composite graft of bone and a hyaline cartilage from donor joints. Harvesting the allografts from a size-matched donor at the anatomical site corresponding to the location of the patient's lesion allows for better topographical matching and avoids the donor site morbidity issues associated with autograft transplants.<sup>3</sup> It is becoming a standard procedure for treatment of cartilage defects, particularly for resurfacing large defects and for revision of failed primary cartilage procedures. In addition, allograft usage can be associated with the potential risk of communicable disease transmission, with the current reported risk from human immunodeficiency virus (HIV)-infected donors to be between 1 in a million to 4 in a million,<sup>4</sup> and potential bone cyst formation in the subchondral bone possibly due to a local immune response.<sup>5,6</sup>

Donor screening, including infectious disease testing, is the standard practice for mitigating infectious disease transmission risks. The Food and Drug Administration (FDA) states that although screening is crucial, it alone does not minimize these risks.<sup>7</sup> Incidences of allograft-associated viral infections have occurred,<sup>4,7-9</sup> such as HIV,<sup>4,10-15</sup> hepatitis B virus (HBV),<sup>14</sup> and hepatitis C virus (HCV),<sup>11,4,14,16,17</sup> and the Centers for Disease Control and Prevention warns that the actual incidence rate may have been higher due to a previously ill-defined reporting mechanism.<sup>18</sup> The implementation of requirements for nucleic acid testing<sup>19,20</sup> has further mitigated the risks by reducing the window of infectivity, but is only for specific viruses and may not detect all genetic variants of viruses or donors who may have been infected with an emerging disease.<sup>7,21,22</sup> For an additional level of protection, the tissue banking industry has invested in methods to improve the safety of allografts by including virucidal and bactericidal processing steps.

Therefore, alternative chemical methods have been explored. Cartilage has been considered immunoprivileged, which may be the reason that no viral inactivation method has been applied to osteochondral allografts. However, these composite grafts contain cancellous bone that house bone marrow, a primary location for potential viral particles. According to the best knowledge of the authors, there are no current methods in the industry that inactivate clinically relevant viruses while maintaining the biomechanical functionality of osteochondral allografts. Maintaining relevant mechanical properties and the structural integrity of osteochondral implants is essential to facilitate early load-bearing of the operated joint and diminish the risk of subsequent implant collapse.

Gamma irradiation is the most commonly accepted sterilization procedure for bacteria. However, the higher doses required to inactivate viral DNA (>30 kGy) is detrimental to the biomechanical properties of tissue.<sup>23-25</sup> The purpose of the present study was to determine the potential of methylene blue light treatment (MBLT) as a viral inactivation step for osteochondral allograft. Photosensitizers, such as

methylene blue (MB), in combination with illumination have been shown in the literature to be successful in inactivating HIV and HCV in fresh frozen plasma production while maintaining most of the activity of the proteins.<sup>26,27</sup> Viral nucleic acid is considered a critical target for photosensitized oxidation of viruses.<sup>27-35</sup> It is assumed that MB, in the presence of light and oxygen can produce a highly reactive oxidizing agent, singlet state oxygen ( $^1\text{O}_2$ ),<sup>36-38</sup> which is believed to destroy the viral genome reducing viral viability and prevents its replication. A viral inactivation study was therefore conducted here with a panel of clinically relevant viruses chosen to provide a range of physiochemical resistances and that show the robustness of the virucidal process.<sup>20,39-46</sup> The panel in this study therefore includes HIV type 1 (HIV-1), bovine viral diarrhea virus (BVDV–HCV model), pseudorabies virus (PrV–herpes virus model), hepatitis A virus (HAV), and porcine parvovirus (PPV–parvovirus B19 model).<sup>19,20,44</sup> It was hypothesized that MB with light treatment of inoculated osteochondral allografts would reduce the titers of a comprehensive panel of viruses.

## Materials and Methods

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The design of the study was to inoculate the cancellous portion of the osteochondral grafts, subject the grafts to a MBLT and then determine the remaining virus particles after the treatment (**Fig. 1**). Inoculation of the cancellous portion would allow for better absorption of the virus suspension and is the more likely location of the virus particles. The study evaluated a robust panel of relevant viruses including both DNA and RNA viruses as well as enveloped and nonenveloped viruses (**Table 1**). WuXi-AppTec, Inc. (Philadelphia, PA) provided all of the virus stocks and reagents used in the titration of the samples. The MB solutions used in processing were supplied sterile by KSE Scientific (Durham, NC). Cylindrical osteochondral allografts 15 mm in diameter and 10 mm in length were recovered from femoral condyles and tibial plateaus of human donors (tested negative for infectious disease) supplied by LifeNet Health (LNH, Virginia Beach, VA). The knee joints were from 3 seronegative, research-consented male donors, ages 46, 61, and 63 years. Each was inspected for trauma and was evaluated for degeneration such as fibrillation. The samples were similar to those of implant quality. The grafts were pretreated with a proprietary decellularization, cleaning and delipidation process through serial alcohol processing and processing with an organic solvent. Removal of tissue debris is vital to allow penetration of MB and light for the viral inactivation step. The samples were then stored in phosphate-buffered saline (Gibco, Grand Island, NY) and shipped on wet ice to WuXi-AppTec for a viral inactivation study. Ten samples were used to determine toxicity of the processing reagents to the associated indicator cells for each virus and 15 were used in the viral inactivation experiment.

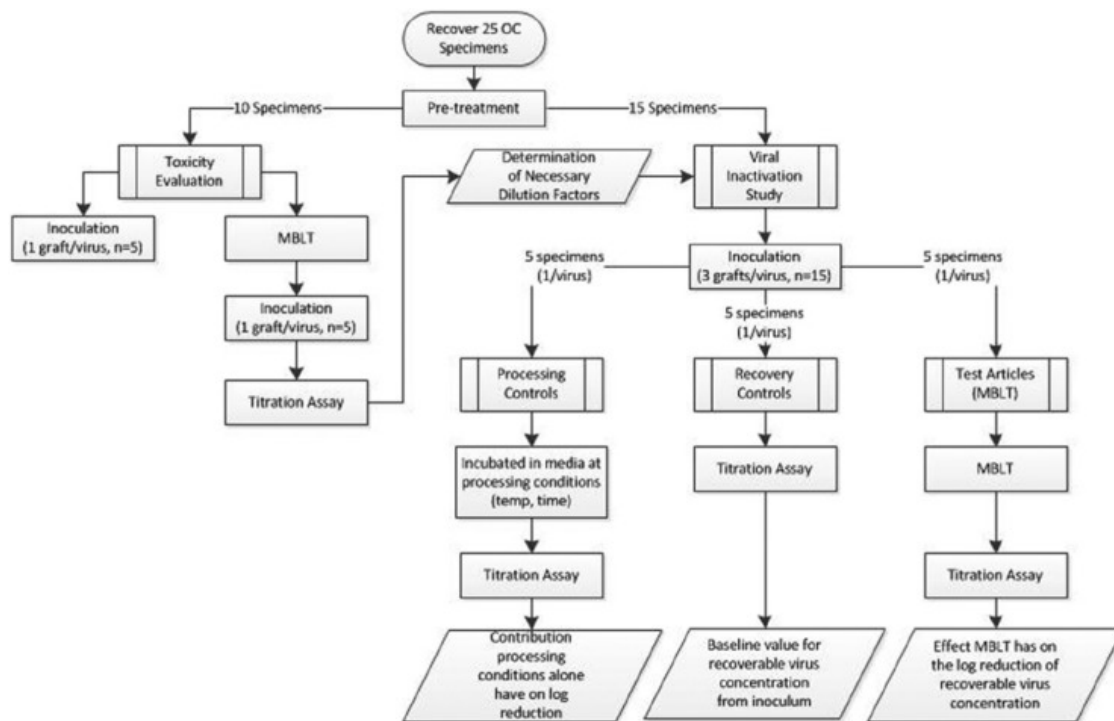


Figure 1.

Flowchart of the experimental design for executing the viral inactivation study.

Table 1.

Characteristics of the Panel of Viruses.

Virus	Indicator Cell	Envelope	Genome	Approximate Size (nm)
BVDV	BT	Yes	RNA	50-70
HAV	FRhK-4	No	RNA	28-30
HIV-1	CEM-A	Yes	RNA	80-130
PPV	ST	No	DNA	18-26
PrV	CV-1	Yes	DNA	150-200

Note: BVDV = bovine viral diarrhea virus (Singer strain); HAV = hepatitis A virus (HM175 strain, 18f); HIV-1 = human immunodeficiency virus type 1 (HTLV-IIIIB strain); PPV = porcine parvovirus (NADL-2 strain); PrV = pseudorabies virus (SHOPE strain).

In the viral inactivation experiment, the capacity of the MBLT to inactivate viruses was measured as the log reduction in the virus titer of the test samples determined from a baseline value. The baseline value, deemed recovery control, was determined to be the amount of inoculum that could be recovered

from the tissue specimen (**Table 2**). The recovery controls were inoculated with virus stock and then titrated to determine a baseline for the reduction calculations. A processing control was used to test interference of the test article. Therefore, the specimens were inoculated with virus stock but incubated in media only at the same temperature and times as the MBLT group. Interference controls were prepared with inoculated serum-free media incubated at processing conditions. Interference was defined as a  $>0.5 \log_{10}$  reduction in the virus titer.

**Table 2.**

Virus Log<sub>10</sub> Reduction Summary With 95% Confidence Limits.

	<b>BVDV</b>	<b>HAV</b>	<b>HIV-1</b>	<b>PPV</b>	<b>PrV</b>
Inoculum titer (PFU)	7.64	7.96	7.25	7.62	7.39
Recovery control titer	7.35	7.38	6.47	7.39	7.06
MB/light titer (PFU)	<2.28 <sup>a</sup>	<4.30	<3.15 <sup>a</sup>	1.81	<1.58 <sup>a</sup>
Log reduction	>5.07 <sup>a</sup>	>3.08	>3.32 <sup>a</sup>	5.58	>5.48 <sup>a</sup>
	±0.21	±0.15	±0.29	±0.37	±0.07

Note: BVDV = bovine viral diarrhea virus (Singer strain); HAV = hepatitis A virus (HM175 strain, 18f); HIV-1 = human immunodeficiency virus type 1 (HTLV-IIIIB strain); PPV = porcine parvovirus (NADL-2 strain); PrV = pseudorabies virus (SHOPE strain); PFU = plaque-forming units; MB, methylene blue.

<sup>a</sup>Virus reduced to nondetectable levels.

## Virus Quantitation

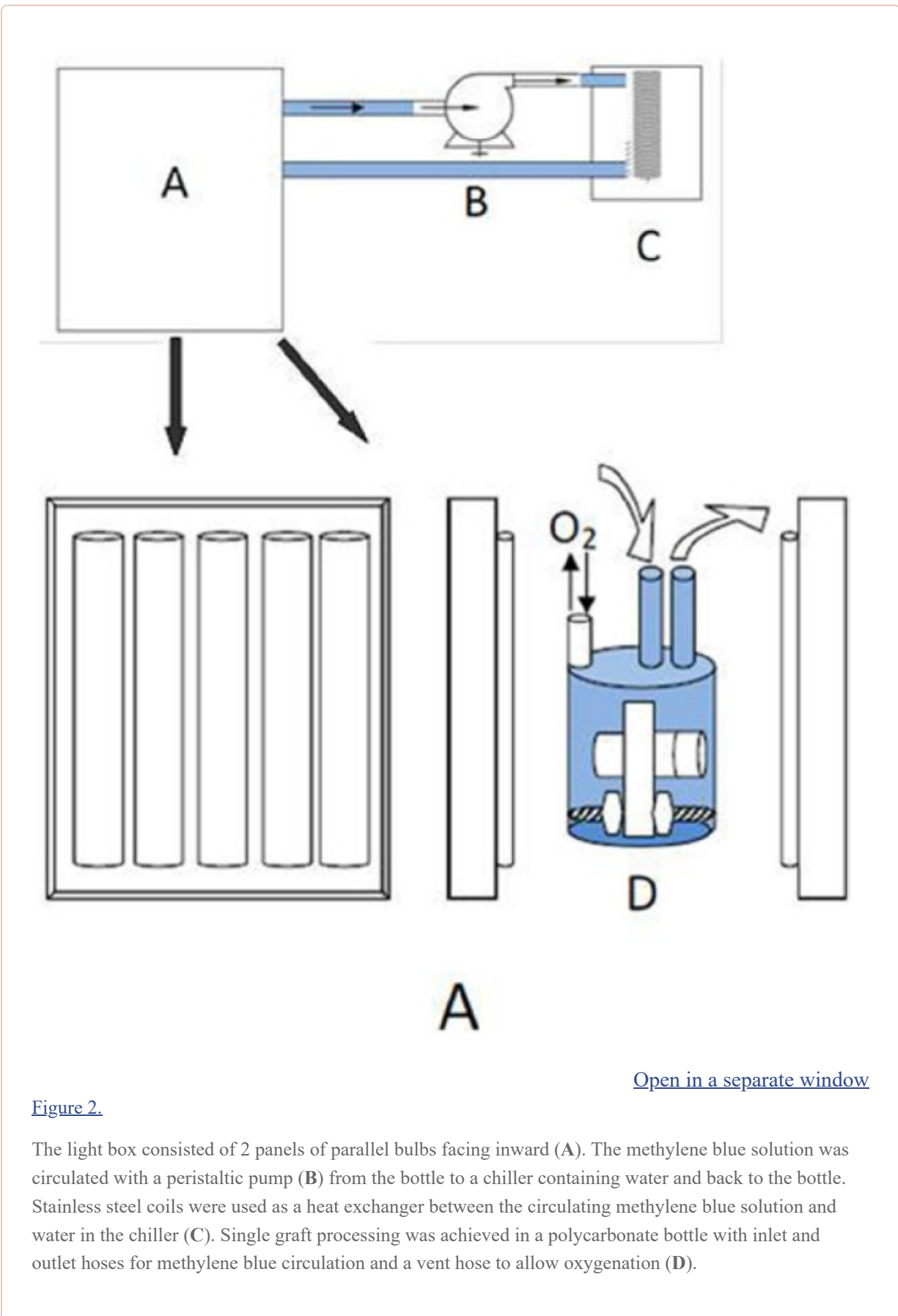
Two methods were used to measure the concentration of a virus in a sample. The plaque assay is a quantitative method in which each plaque corresponds to a single infectious unit. Only viruses that cause visible damage to cells, thus an active virus, can be assayed this way. Ten-fold dilutions of virus stock are inoculated onto susceptible cell monolayers. Cells release viral progeny after infected, and the new viruses are spread to neighboring cells forming plaques or the cells fuse forming syncytium. For plaque and syncytia forming assays, viral titers were determined by multiplying the mean units (plaque forming units [PFU] or syncytium forming units [SFU]) of 3 wells or dishes by the dilution and dividing by the volume per well or dish. Tissue culture infectious dose (TCID<sub>50</sub>) is a quantal method that is scored as infected or not, and quantifies the amount of virus required to produce a cytopathic effect in 50% of inoculated tissue culture cells. This assay can be used where a virus does not form plaques.

An aliquot of each test and control sample was diluted in medium to the end point ( $10^0$ - $10^{-8}$ ). Each appropriate dilution was assayed by the respective standard virus titration procedure at WuXi-AppTec. Indicator cells are required since viruses need a host to infect. BVDV was used as a model virus for HCV because it is of the same virus family (Flaviviridae). The titer of BVDV stock solution (Singer strain) was assayed in multiple wells and/or dishes for infectious viral particles by the BVDV plaque assay using Bovine turbinate (BT) indicator cells. The titer of the HAV stock solution (HM175 strain, 18f) was assayed in 24 wells (using 3-fold dilutions) for infectious viral particles by the HAV TCID<sub>50</sub> assay using fetal Rhesus kidney (FRhk-4) indicator cells. The viral titers for the assay for HAV were

determined by adding the  $\log_{10}$  of TCID<sub>50</sub> and 1.602 (which is the  $\log_{10}$  of the adjustment made to express the titer on a per milliliter basis). These values were adjusted by the dilution of the virus stock used in the inoculum. The  $\log_{10}$  reduction value was calculated by subtracting the  $\log_{10}$  of the adjusted titer for the MB/light treatment from that of the stock virus control. The titer of the HIV-1 stock virus solution (HTLV-III<sub>B</sub> strain) was assayed in multiple wells for infectious viral particles using CEM-A indicator cells. HIV-1 induces syncytium formation in CEM-A. CEM cells are adherent T-lymphoid cells that are permissive for HTLV-1 replication. Porcine parvovirus served as a model for human parvovirus B19. The titer of the PPV stock virus solution (NADL-2 strain) used was assayed in multiple wells and/or dishes for infectious viral particles by the PPV plaque assay using swine testis (ST) indicator cells. PrV was used as a model virus for other herpes viruses such as Cytomegalovirus. The titer of PrV stock virus solution (SHOPE strain) used was assayed in multiple wells and/or dishes for infectious viral particles by the PrV plaque assay using CV-1 (African green monkey kidney cell line) indicator cells.

### Sample Preparation and Processing

To determine the remaining virus titer after MBLT without cross-contamination, the samples underwent MBLT in isolation ([Figure 2](#)). Each individual graft was contained in a sterile polycarbonate bottle (125 mL) with circulating MB solution (62  $\mu$ M) and ventilation for singlet oxygen production. Approximately 216 mL of MB solution were circulated with a peristaltic pump at a flow rate of approximately 195 mL/min, confirmed to be sufficient to maintain the solution temperature within the bottle at the specified treatment temperatures.



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**Figure 2.**

The light box consisted of 2 panels of parallel bulbs facing inward (A). The methylene blue solution was circulated with a peristaltic pump (B) from the bottle to a chiller containing water and back to the bottle. Stainless steel coils were used as a heat exchanger between the circulating methylene blue solution and water in the chiller (C). Single graft processing was achieved in a polycarbonate bottle with inlet and outlet hoses for methylene blue circulation and a vent hose to allow oxygenation (D).

In a biosafety hood, the specimen was secured in the bottle and inoculated after the pretreatment in order to assess the MBLT alone as a viral inactivation step. A pipette was used to apply 0.5 mL of virus stock solution to the circumferential and bottom surface of the bone portion of the graft and then placed

at 2 to 8 °C for 15 minutes to allow absorption. Dispersion of the suspension was uniform to ensure consistent coverage. The high concentration of the initial inoculum, seen in [Table 2](#), was chosen in order to have a measurable quantity for log reduction calculations as well as represent a high potential dose in an infected donor. This method is standard practice in the industry for viral inactivation and sterilization evaluations. The light box was assembled with 2 light panels facing inward, allowing the specimen to receive exposure to both direct and incidental light from all angles. The light intensity within the light box was mapped with a light meter (ExTech, Model 407026) to provide the locations to achieve the target value range of 23,000 to 24,000 lux. Each bottle was secured within the light box at these predetermined locations. The HIV test was conducted separately in a BSL-3 laboratory. For MBLT, the samples were then soaked to a point of saturation in circulating MB solution for 24 hours at 22 to 23 °C. Saturation was determined as the time the graft achieves maximum MB content. The solution was shielded from light to maintain MB activity. After 23 hours, the bath was set to 8 °C. At 24 hours, the light box was turned on for a total of 72 hours at 8 to 12 °C.

### Toxicity

To ensure the process intermediates did not influence the limits of detection for the assays, they were tested for toxicity to the indicator cells used for titration of the respective virus, shown in [Table 1](#). The toxicity was evaluated prior to and following MBLT. Two grafts per indicator cell were mock-spiked with 0.5 mL of virus resuspension media sans viruses. The samples were then incubated at 2 to 8 °C for 15 minutes to allow absorption. One sample was tested immediately. The other sample underwent MBLT. The solution was removed and saved.

The specimens were resuspended in a small amount of virus resuspension media and homogenized. The samples not treated with the MBLT were brought to a final volume of 216 to 219 mL in the virus resuspension media. The treated samples were brought to a final volume of 5 mL, combined with the saved MB processing solution, confirmed to be pH 6.5 to 7.5 and filtered (0.45 µm).

The aliquots were serially diluted (undiluted, 3-fold, 10-fold, 30-fold, 100-fold, 300-fold, 1,000-fold, and 3,000-fold diluted) and tested in duplicate via standard toxicity procedures for the BT, CEM-A, ST, and CV-1 indicator cell lines at WuXi-AppTec. For each of these indicator cell lines, samples that reduced the monolayers to less than 80% of the controls were considered cytotoxic. For the FRhK-4 indicator cells, the samples were serially diluted (full strength, 3-fold, 9-fold, 27-fold, 81-fold, 243-fold, 729-fold, 729-fold, and 2,187-fold dilutions) and tested in 8 wells for toxicity in culture medium via standard toxicity procedure at WuXi-AppTec. The dilution was considered toxic if any of the wells at any dilution was negative for cell growth. The results determined what dilution was necessary, if any, to quantify the concentration of virus.

### Viral Inactivation

Fifteen osteochondral grafts were divided evenly among the test group and the 2 control groups. Three grafts per virus were spiked with 0.5 mL of the stock virus solution. The grafts were incubated at 2 to 8 °C for 15 minutes to allow for absorption. One graft per virus was prepared for titration for the recovery control ( $n = 5$ ) or the process control ( $n = 5$ ). The remaining inoculated grafts ( $n = 5$ ) underwent MBLT. Following treatment, the solution was removed and saved. All samples were resuspended in a small amount of virus resuspension media and homogenized. The processing controls and recovery controls were brought to a final volume of 216 to 219 mL in virus resuspension media. The MBLT samples were brought to a final volume of 5 mL in virus resuspension media and then mixed with the saved MB solution. These solutions were prepared for virus quantitation by diluting to the predetermined non-toxic dose with Eagle's minimum essential medium (EMEM), adjusting to pH 6.5 to 7.5 and filtering (0.45 µm).



## Statistical Analysis

The objective of the study is to be carried out to an acceptable level of virological competence. Both quantal and quantitative methods are being used in the TCID<sub>50</sub> assay and plaque assay, respectively. For within assay variation, a 95% confidence limit determined that the variation should be of the order of  $\pm 0.5 \log$  or better. The 95% confidence limit of reduction factors was approximated by  $\pm(s^2 + a^2)$  where  $\pm s$  is the 95% confidence limit for viral assays of recovery controls and  $\pm a$  is the 95% confidence limit for viral assays of the test material.

## Results

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### Toxicity

The pretreated samples prior to MBLT were nontoxic undiluted for all indicator cells. The MBLT samples were nontoxic undiluted for all but one of the indicator cells, which includes BT cells (BVDV), FRhK-4 cells (HAV), ST cells (PPV), and CV-1 cells (PrV). The MBLT samples were not toxic to the CEM-A cells (HIV-1) at a 10-fold dilution. The study evaluated the viral inactivation capacity of the MB step without the influence of the subsequent wash steps used to remove the excess reagent from the grafts. Therefore, these samples contained MB at concentrations more than 13-fold greater than the levels seen in the final process. A 10-fold dilution, while still more concentrated, was sufficient to prevent toxicity to the CEM-A cells.

### Viral Inactivation

The assay variation for all viruses was within the 95% confidence limits ( $\pm 0.5 \log_{10}$ ) recommended by the FDA.<sup>41</sup> The recovery efficiency ranged from 89% to 97% indicating successful processes for absorption of the virus particles into the graft and subsequent recovery. [Table 2](#) shows the initial titer for the inoculum, the titer recovered in the untreated control samples and the resultant titer following MBLT. HAV and PPV were reduced by 3.08 and 5.58 logs, respectively. BVDV, HIV-1, and PrV were reduced to nondetectable levels. For BVDV and PrV, the volume was increased to 7 mL and additional dishes were plated, still with no virus detection. The number of wells was increased to 48 for HIV-1 at undiluted, still with no virus detection resulting.

The media controls and the processing control titers for PPV, PrV, and HAV showed no effect and an insignificant  $\log_{10}$  reduction (0.94) was seen in the BVDV titer in the processing control group. This indicates that the effect comes from the MBLT mechanism. The HIV-1 titer was reduced by 2.46  $\log_{10}$  in the processing control group, showing that this virus was susceptible to the processing conditions.

## Discussion

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The results of this study show that MBLT reduced all enveloped viruses (HIV-1, BVDV [HCV model], and PrV) to nondetectable levels, 2 of which have been reported in allograft-associated infections. The more resilient nonenveloped viruses, HAV and PPV, were reduced to 3.08 and 5.58  $\log_{10}$ , respectively. Similar reductions were only achieved in the literature for HIV, BVDV, and HAV with the addition of heat (above temperatures at which collagen begins to degrade,  $>60^\circ\text{C}$ ), high-dose gamma irradiation ( $>30 \text{ kGy}$ ), a combination of heat and gamma irradiation, or extensive washes in reagents known to be damaging to collagen.<sup>24,25,48,52,53</sup> Additionally, MB and light have been shown to have an effect on viruses that are not part of the screening process yet, such as West Nile virus.<sup>54,55</sup>

Limitations to this study include modeling infected donors and that each virus in each group was tested on a single sample making it difficult to show statistical significance. Unlike those for bacterial contamination, there are no standards for viral inactivation. The present study followed the FDA and

EMA (European Medicine Agency) guidelines.<sup>39-44,46,47</sup> The measurements were done in triplicate and compared to the series of controls (recovery, interference, and toxicity). The test methods were validated to be well controlled (within-assay 95% confidence interval of  $\pm 0.5 \log_{10}$ ). Additionally, the viral loads in the inoculums were similar to those used in the literature and above what would be expected clinically.<sup>24,32,48,49</sup> For HIV, the levels measured in the peripheral blood during the chronic (asymptomatic) period range between 1,000 and 1 million copies/mL. The average set point load of 33,000 was determined to be optimal for HIV transmission, not the periods of the disease with the highest loads.<sup>50</sup> Additionally, death occurs before the HIV load reaches  $7 \log_{10}$ .<sup>51</sup>

An improvement would be to test infected donors. However, this type of analysis comes with its own limitations. It is a difficult study to control as the viral load would be unknown, making it challenging to obtain meaningful data that can be applied to all potentially infected donors. The viral load in the tissue may be measured experimentally, but would vary greatly depending on the phase of the infection. Therefore, the robustness of the process could not be determined by this method. Viral inactivation studies involve a deliberate addition of a known concentration of virus high enough to be detected after processing in order to calculate an effect. Therefore, this study is considered a characterization of the potential effect of MBLT on an extensive virus panel inoculated within the interstices of the cancellous bone.

Currently, there are no known viral inactivation methods that retain cell viability. As such, preservation of relevant mechanical properties must be considered in selecting an appropriate viral inactivation method for decellularized osteochondral allografts. Photosensitizers, such as MB, have been shown to be successful in inactivating HIV and HCV with small concentrations (1  $\mu\text{M}$ ) and short processing times in fresh frozen plasma while maintaining protein activity.<sup>26,27</sup> Therefore, MB and light were used in this study as a tissue-sensitive viral inactivation process for osteochondral allografts. Because of the dense nature of the cartilage tissue, MB solution concentrations and processing times used were at least 60-fold greater than those typically used for plasma processing to enhance the probability of viral inactivation. While the mechanism of MBLT is unclear, it is proposed that singlet oxygen production occurs both within the saturated tissue and in the circulating MB. Therefore, the circulating MB contributes to the effect through diffusion of the singlet oxygen within the extracellular matrix.

According to the best knowledge of the authors, there are no other methods in the industry that inactivate viruses while maintaining the biomechanical functionality of osteochondral allografts. For high-dose gamma irradiation, while effective against viruses and able to penetrate the dense cartilage matrix, the minimum dose required to inactivate viral DNA ( $>30 \text{ kGy}$ ) is detrimental to the biomechanical integrity of the tissue.<sup>23-25</sup> Low-dose gamma irradiation is only adequate for killing surface bacteria, not viruses. LifeNet Health (Virginia Beach, VA) uses low-temperature, low-dose gamma irradiation with its Allowash XG method. Together, these processes have been shown to inactivate a panel of model viruses from inoculated human tendon and bone.<sup>56</sup> RTI Biologics (Alachua, FL) has three sterilization processes for soft tissue and bone that have been validated to inactivate a similar panel of viruses.<sup>57-59</sup> However, there have been no reports of the efficacy of these processes on viral inactivation in human osteochondral grafts.

Ethylene oxide was once widely used because of its efficacy in penetrating bone, but has been abandoned because of evidence that its by-products are carcinogenic and that it has caused significant inflammatory responses in anterior cruciate ligament reconstruction procedures where ethylene oxide-sterilized anterior cruciate ligament allografts were transplanted.<sup>18,60-62</sup> Several companies are employing new methods for the sterilization of musculoskeletal tissues. These processes, however, require the use of chemicals that could potentially denature collagen matrices and are not applicable to cartilage tissue.<sup>4</sup>

Although osteochondral allografts are becoming standard care, fresh tissue can be difficult to obtain because of a shortage of qualified donor tissue and challenging storage requirements. The grafts are a composite of overlying cartilage and underlying bone. Bone has been implicated in reported cases of viral transmission and cartilage matrix has been shown to harbor infectious retrovirus.<sup>11,13-16,63</sup>

Although the perceived risk of viral infection associated with allografts is low, the consequences can be serious. The estimated risk of HIV transmission from a bone allograft is 1 in 1.6 million.<sup>64,65</sup> This risk increases to 1 in 161 when screening procedures are compromised.<sup>66</sup> Donor evaluation and abnormal serological tests were responsible for a majority of musculoskeletal tissue specimens recalled from 1994 to 1997.<sup>10,12</sup>

Decellularized osteochondral grafts of the type modeled here serve as an alternative to fresh allograft tissue as a replacement graft. Removal of endogenous cells and cellular debris and fatty tissue reduce the immune reaction and provide a porous scaffold for bone ingrowth during remodeling. The function of such grafts is not based on cellular activity but on providing articular cartilage and subchondral bone to restore structural integrity to the joint surface. This allows for the grafts to be treated with virucidal processes, making them potentially safer than fresh allografts. Restoring the joint surface facilitates early load-bearing of the operated joint, potentially shortening rehabilitation time and diminishing complications resulting from joint immobilization.

The results of this study show that MBLT was effective in reducing (a) enveloped DNA and RNA viruses to nondetectable levels and (b) nonenveloped DNA and RNA viruses of inoculated human osteochondral grafts by 3.1 to 5.6 logs. This study represents the first practical method for significantly reducing viral load in osteochondral implants.

## Footnotes

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**Ethical Approval:** This study was approved by our institutional review board.

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