Introduction:
The majority of long bone fractures heal successfully without complications, however compound and/or comminuted fractures resulting from high impact trauma can result in delayed healing or non-union. Despite currently available treatments to reduce the risk of infection and/or enhance bone healing, such as the use of antibiotic-impregnated beads or bone-morphogenetic proteins, high impact traumatic fractures can take up to a year to fully heal. Some injuries progress to non-union, potentially leading to repetitive surgeries and long-term disability of the injured patient.

Photodynamic therapy (PDT) is a non-surgical, non-ionizing minimally invasive local treatment, which has been successfully applied to treat multiple types of cancer, skin diseases and age related macular degeneration. This treatment involves the local or systemic administration of a photosensitizing drug, which is activated by non-thermal laser light at a photosensitizer specific wavelength. This light activation leads, in the presence of oxygen, to the generation of cytotoxic species (e.g. singlet oxygen) [1], which can induce apoptosis and/or necrosis of targeted cells and tissue and also influence immune responses [2]. Unexpectedly, recent findings from studies aimed at understanding the impact of PDT on skeletal metastases secondary to breast cancer have shown that PDT rapidly improved vertebral bone strength, stiffness and architecture [3]. Based on these observations, the aim of this study is to explore the potential of PDT to enhance healing in traumatic long bone fractures.

Materials and Methods:
A comminuted tibia fracture was generated in 11 adult female Sprague-Dawley (SD) rats. Institutional animal care committee approval was obtained for all procedures (University Health Network, Toronto). General anaesthesia in the rat was induced with 4% isoflurane in oxygen. Under sterile conditions a small lateral skin incision was made at the knee joint. Bending the knee, a 23g needle was used to enter and ream the medullary canal at the tibial plateau. Thereafter, a 0.8 mm Kirschner wire was placed inside the medullary canal. The skin incision was closed and a unilateral fracture was generated in the mid-tibia with a custom drop weight impact apparatus (500g). The tibial and fibular fracture was confirmed using high resolution x-ray imaging (Faxitron X-Ray LLC, Lincolnshire, IL). The rats received antibiotics and analgesics (0.05 mg/kg buprenorphine). The rats were randomly allocated to 3 groups: control (no treatment), PDT applied 1 day (1d) post-fracture or PDT applied 7d post-fracture. Prior to the application of light, a photosensitising drug (Visudyne, Gedeon Richter, Budapest,) was injected intravenously at a concentration of 1mg/kg. Fifteen minutes later, light energy of 75J was delivered at 690 nm using a 1 cm diffuser fibre placed subcutaneously parallel to the fracture under fluoroscopic guidance [4]. Weekly faxitron images and blood samples were acquired. Vascular endothelial growth factor (VEGF) serum concentration was determined using Quantikine Rat/VEGF Immunoassay (R&D Systems, Minneapolis, MN). The rats were euthanized 4 weeks after induction of the fracture and their tibiae were harvested. μCT images at an isotropic 14 μm voxel resolution (SkyScan 1172 High Resolution MicroCT System, Skyscan, Belgium) were acquired of the fracture site and callus for 3D architectural analysis (CTAn, Skyscan, Belgium).

Results:
All rats recovered well from the fracture generation and PDT treatments, with the exception of one animal, which had to be euthanized early due to large displacement of the K-wire. The total bone volume (TV) including callus formation, of the fracture site increased from 148±43mm³ in the control group, to 157±59mm³ in the PDT 1d group (6%) and to 175±25mm³ in the PDT 7d group (18%). Similarly, the bone volume within the callus (BV) increased from 75±8mm³ (control), to 87±19mm³ (1d-PDT) and 85±11mm³ (7d-PDT). The average VEGF serum level concentration was higher in the control group compared to the treatments groups.

Discussion:
PDT treatment of the fractured rat tibiae resulted in an increase of bone formation after 4 weeks as compared to control untreated fractures, despite the high variability in the generation of the comminuted fractures. This observation of increased bone concurs with previous findings of the impact of PDT in the bony spine [3]. Further, the relative increases (compared to control) in both bone and callus volume in the 7d PDT group was found to be ~3 times higher than in the 1d PDT group. This indicates that the tissue response to PDT stimulation is dependent on the fracture healing remodeling stage. As such, PDT may be less effective during inflammation, the first stage of fracture healing at 1d post treatment, vs. during the formation of granulation tissue, the second stage of fracture healing at 7d post-fracture.

Local application of VEGF has been shown to accelerate bone healing [5]. Interestingly, PDT has been reported to locally induce VEGF and enhance angiogenesis in the retina pigment. Yet, a systemic more elevated and prolonged VEGF level has been found in patients after long bone fractures as an indication of impaired bone healing [5]. Histology and immunostaining of the samples will give more information about the local expression of VEGF, angiogenesis and the progress of bone remodeling and fracture healing.

Significance:
If PDT is able to enhance fracture healing in complex fractures it may provide a cost-effective local minimally invasive treatment for long bone fractures at risk for impaired bone healing. Further, if ongoing studies confirm the benefits of PDT are enhanced during the secondary stage of fracture healing, PDT could be applied to patients expected to encounter impaired healing, even without access to immediate medical care.

Acknowledgements:
Funding for this project was provided by the Orthopaedic Research Program (PRORP) of the Office of the Congressionally Directed Medical Research programs (CDMRP), grant # OR090260.

References: