**Autologous Blood Clots as a Vehicle for Antibiotic Delivery**

Jennifer Ku, BA1, Haiying Pan, MS2, Jessica Pham, BS1, Genevieve Abd, PhD2, Robert Sawyer, MD1, Yong Li, MD, PhD1

1Medical Student (M2), Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI
2Department of Orthopedic Surgery and Biomedical Engineering, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI
3Department of Surgery, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI

Email of Presenting Author: jennifer.ku@wmdu.edu

**BACKGROUND:** Natural biomaterials designed for drug delivery hold significant potential in tissue healing, targeting precision medicine strategies to address specific patient needs. Nevertheless, existing delivery systems frequently exhibit limitations in adaptability, biocompatibility, and cost-efficiency. Furthermore, current therapies often require oversight of the U.S. Food and Drug Administration, presenting a challenge for widespread clinical adoption. Autologous blood clots (BC) emerge as a compelling contender in the realm of orthobiologics, owing to the intrinsic capacity of BCs to function as natural reservoirs for controlled release of growth factors (GF) in conjunction with the fibrin matrix inherent to the BC structure1. The aim of this study was to assess the duration of sustained antibiotic release from BC and their potential impact on host mesenchymal stem cells (MSC). Additionally, this investigation explored the use of autologous BC composited with antibiotics and mesenchymal stem cells (MSC) as a strategic methodology to treat a comprehensive spectrum of wounds and orthopedic injuries. We hypothesize that the integration of autologous BC with antibiotics and MSC will function as a pro-regenerative delivery vehicle capable of sustained release of therapeutic factors to effectively treat and prevent infections.

**METHODS:** Porcine and murine blood samples were allowed to clot at room temperature to form BC in vitro. BC were composited with vancomycin and gentamicin at concentrations of up to 5.0 mg/mL, composited with antibiotics and bone marrow derived MSC at a concentration of 1 x 10^6 cells/mL, or composited with PBS. Composited BC were evaluated for antibiotic release and antimicrobial activity for a duration of one week. The release of GF, such as vascular endothelial growth factor (VEGF), from composite BC was assessed at daily intervals over a duration of 7 days and quantified by sandwich ELISA assays (DuoSet ELISA for Mouse, R&D Systems).

**RESULTS:** The findings suggest that vancomycin and gentamicin do not exhibit noticeable toxicity on porcine and murine MSC over the span of one week when composited with BC. Notably, composite BC demonstrated a controlled and sustained release of antibiotics over the 7-day period (Figure 1). Moreover, the composited BC with antibiotics indicated the functional capability to inhibit bacterial proliferation for a duration of 7 days (Figure 2). The composite BC combined with MSC also displayed a sustained and prolonged release of GF, such as VEGF, for a period exceeding 7 days in vitro with a concentration diffusion range of 220-350 pg/mL.

**DISCUSSION:** Our results indicate that BC serve as efficient therapeutic carriers for sustained antibiotic release. BC composited with antibiotics effectively inhibited bacterial proliferation for a 7-day period, providing a clinically relevant timeframe for wound healing and infection prevention. Furthermore, when combined with MSC, the composite BC exhibited notable levels of VEGF release, a concentration level sufficient to promote angiogenesis in wounds. The clinical translational potential of this approach holds significant promise. The straightforward methodology, coupled with the inherent capacity for GF release and antibiotic delivery, positions BC as a versatile and natural biomaterial with multifaceted roles in regenerative medicine. Their dual role as a reservoir for therapeutic factors and a biomaterial to treat and prevent infection underscores their efficacy in addressing a wide array of wound and orthopedic injuries.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This simple BC composite system offers several advantages including minimal risk, simple use, and cost-effective approach. Utilizing BC as biomaterials could present a transformative shift in treatments, offering an innovative and clinically impactful solution for widespread clinical implementation.


![Figure 1](image1.png)

**Figure 1:** The release of antibiotics, gentamicin (A) and vancomycin (B), from the composited murine BCs (blue) and pig BCs (red) for up to 7 days.

![Figure 2](image2.png)

**Figure 2:** Murine and pig BC were seen to functionally prevent bacterial growth for up to 7 days. (A) G-gentamicin, D1-Pig BC day1, D2-Pig BC day2, D3-pig BC day3, D5-pig BC day5, D7-pig BC day7; d1-murine BC day1, d2-murine BC day2, d3-murine BC day3, d5-murine BC day5, d7-murine BC day7. (B) Vancomycin, D1-Pig BC day1, D2-Pig BC day2, D3-pig BC day3, D5-pig BC day5, D7-pig BC day7; C1-C7 matched time controls. (C) Both murine and pig BC without antibiotics controls.