Exploring the Utilization of Curcumin-Loaded Gel Polymers to Minimize the Risk of Prosthetic Joint Infections (PJI) in Ortho-Implants

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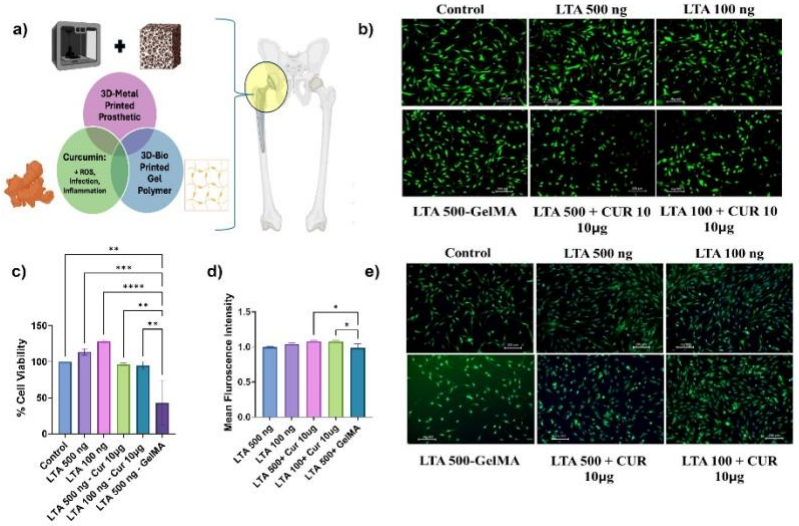
INTRODUCTION: A major concern following joint replacement is prosthetic joint infection (PJI), which may necessitate revision surgery or surgical debridement. Treatment failure with surgical debridement is 30-50% and over 10% following one or two-step revision surgery[1]. Moreover, hospital costs alone (not including provider costs) to treat PJIs in 2020 were estimated to be $1.62 billion[2], with an estimated annual hospital cost for hip and knee PJIs expected to reach $1.85 billion by 2030[3]. Given the high numbers of medical implantations performed and the reported associated infection rates, this study aims to evaluate whether PJIs can be minimized by reducing the processes that drive prosthetic infections? This study aims to explore the innovative approach of preventing inflammatory and oxidative processes driving PJIs by utilizing curcumin-loaded GelMA polymers (CLGP) printed within a porous structure 3D-metal printed prosthetic model. Our long-term goal is to develop Biologically Functionalized 3D Printed Constructs (BF3DC), at the implant interface with a loaded drug to minimize the risk of PJI. In this study, antioxidant compounds such as curcumin act to decrease oxidation by diverting ROS to less harmful products[4]. Curcumin is a bioactive compound derived from Curcuma longa (turmeric) and has been shown to exhibit antioxidant and antibacterial properties, even against bacterial strains that have grown resistant to antibiotics[5]. We hypothesized that by combining the mechanical properties of a metal implant with curcumin-loaded GelMA polymers, the direct delivery of an anti-infectious and antioxidant compound to PJI site would minimize the complications. The study aims to optimize the curcumin concentration and evaluate curcumin's drug release kinetics with lab-scale simulated infectious conditions.

METHODS: Human fibroblast-like synoviocytes (HFLS) were procured from Cell Applications and cultured in DMEM media supplemented with 10% FBS and 1% antibiotics, then maintained at 37°C in a 5% CO2 incubator. Lipoteichoic acid (LTA) from *staphylococcus aureus (s. aureus)* and curcumin powder were both obtained from Sigma Aldrich. LTA was utilized to induce a gram-positive infectious environment in vitro. **A.) Drug and LTA cytocompatibility**: HFLS cells were seeded in 96-well plates and treated with different concentrations of curcumin and LTA. The plates were tested for cellular viability using Alamar Blue assays. **B.) Curcumin-loaded GelMA (CLGP) preparation:** GelMA hydrogel was loaded with 0.1mg/ml of curcumin to create a curcumin-loaded gel polymer (CLGP) and cured using UV light. HFLS cells were seeded in 24-well plates and, after reaching 80% confluency, treated with the CLGP and LTA, then evaluated by various assays. **C.) Cytoxicity assays:** HFLS cells were treated with LTA and curcumin, and a series of experiments were carried out to evaluate its efficacy to minimize PJI while maintaining cytocompatibility with HFLS cells. Cytotoxic evaluation was done using an Alamar Blue assay. Reactive oxygen species (ROS) production was evaluated by DCFDA/H2DCFDA - Cellular ROS assay kit (obtained from Abcam) using a microplate reader. The live-to-dead cell ratio was imaged using live-dead stain and cellular integrity imaging was carried out using FITC-DAPI staining. **D.) Drug release kinetics:** A drug release profile was evaluated with the CLGP over a 24-hour period, wherein samples were taken every hour and evaluated using a microplate reader for curcumin release from CLGP.

RESULTS: Different concentrations of curcumin and LTA showed cytocompatibility with HFLS in the Alamar Blue studies. Using the chosen concentrations of curcumin and LTA from these preliminary studies, 100 ng and 500 ng LTA concentrations were chosen and showed induction of reactive oxygen species. In the release study of CLGP loaded with 0.1mg/ml of curcumin, a burst release was initially present, then followed by a slow release. In ROS assays, a reduction in ROS was noticed in HFLS cells treated with curcumin compared to HFLS cells treated with LTA. The Live/Dead assays showed reduced dead cells with CLGP, despite Alamar Blue showing a reduction in HFLS viability. FITC/DAPI studies indicate little changes to the nuclear integrity of the cells treated with CLGP. Overall, the cytotoxicity studies showed that when HFLS was treated with LTA and curcumin together, there was reduced viability when compared to HFLS cells treated with CLGP.

DISCUSSION: Lipoteichoic acid (LTA), an endotoxin found in gram-positive bacteria, was used to represent *Staphylococcus aureus (S. aureus),* one of the most common bio-forming microbes affecting implants and one of the primary reasons for an implant revision surgery[6]. This study used LTA cultured HFLS cells in-vitro to mimic the microbial organisms that cause prosthetic joint infection sites. Our study on human synoviocytes showed reactive oxygen species in the presence of various LTA concentrations, however it also showed diminished ROS levels when LTA-induced synoviocytes were concurrently cultured with curcumin. Additionally, the CLGP showed increased viability in LTA-induced HFLS compared to cells treated with curcumin particles only. Although there was a reduction of ROS in HFLS cells in the presence of curcumin, at higher concentrations curcumin may also induce toxicity to cells. Our release study of the CLGP illustrates a burst release of curcumin followed by a sustained release, which perhaps is the reason for the reduction in viability in the Alamar Blue studies. Further studies optimizing a sustained release of curcumin from a gel polymer could provide a promising candidate for minimizing cytotoxicity while also reducing infection. Limitations from this study include investigating the effects of only one antioxidant bio-compound as well as being done under a stimulated environment. Therefore, future studies should be explored using direct microbial exposure with *s.aureus* while also using a curcumin concentration that is compatible with human cell lines.



SIGNIFICANCE/CLINICAL RELEVANCE: Our results indicate that CLGPs may be an innovative addition to prosthetics and medical implants given the anti-infectious and anti-oxidative nature of curcumin which can be delivered directly to the surgical site using gel polymers. The initial outcome indicates that BF3DC can be generated for orthopedic and dental implantation or prosthetics to minimize PJI, reduce financial burden, and optimize patient and hospital outcomes.

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REFERENCES: [1] Peng KT et al., (2019) [2] Tubb, CC et al., (2014) [3] Mansour E et al., (2024) [4] Menon et al., (2007) [5] Hussain Y et al., (2022) [6] Inzana et al., (2015)

Figure 1: a) Schematic representation of the proposed study b) Live-dead assay showed an increase in live cells in Curcumin-loaded GelMA (CLGP) **c)** Alamar Blue assay indicates the reduction in cell viability in the treated groups **d)** ROS assay indicates a reduction in ROS in cells treated with CLGP **e)** FITC/DAPI indicates little change to nuclear integrity