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Modulation of bacterial infection and inflammation by release of lactide from a silicone elastomer device

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SUMMARY

The aim of this project is to assess the anti-bacterial and anti-inflammation properties of a matrix-type silicone elastomer device providing sustained release of DL-lactide (a cyclic ester dimer of lactic acid). Lactide-loaded matrices were prepared by injection molding, and the devices were assessed for of in vitro release, anti-bacterial activity, and anti-inflammatory activity. The devices provided sustained release of lactide/lactic acid over five days and were effective against *E. coli* and *S. aureus*. Further, the devices reduced inflammation through activation of the GPR81 receptor via the NF-κB and JAK-STAT signalling pathway. These lactide-releasing silicone elastomer devices could improve clinical outcomes with certain short-term medical devices.

INTRODUCTION

Silicone elastomers are widely used in the manufacture of implantable medical devices. However, bacterial infection is common with such implantable devices (Zare et al., 2021), due to the hydrophobic nature of silicone elastomers resulting in surface attachment of bacteria and biofilm formation (Steffensen et al., 2015). Lactic acid – and lactate, its conjugate base form – is known to have a disruptive action on cytoplasmic membrane by inducing physiological and morphological changes in bacterial cells (Wang et al., 2015). In addition, it possesses anti-inflammatory properties through activation of the GPR81 receptor implicated with NF-κB signalling pathway process, which can subsequently reduce inflammation (Sun et al., 2017). DL-lactide is a cyclic ester dimer of lactic acid. It readily undergoes hydrolysis in aqueous media to form lactic acid. In this study, DL-lactide was loaded into silicone elastomer devices and their anti-bacterial and anti-inflammatory properties were evaluated.

MATERIALS AND METHODS

DL-lactide was mixed with DDU-4320 silicone elastomer and injection molded to form disc-shaped matrices having three different lactide loadings (5, 10 and 15% w/w).

In vitro release experiments were conducted. DL-lactide and lactic acid in the release medium phosphate-buffered saline were quantified by reverse-phase HPLC with UV detection. Fibroblast cells L929 and macrophage RAW246.7 were used to assess cytotoxicity. The anti-bacterial efficacy of lactide/lactic acid in the release medium was assessed against *E. coli* and *S. aureus*. A mouse cytokine antibody array was used to assess the anti-inflammatory activity.

RESULTS AND DISCUSSION

The lactide-loaded silicone elastomer devices showed concentration-dependent activity against *E. coli* and *S. aureus* (Table 1). After 24 h, the 15% w/w-lactide

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device demonstrated a reduction of viable bacterial adhesion to <5% against both species.

Table 1. *Quantitative counts of viable E. coli and S. aureus cells adhering to different lactide surfaces after 24 h of incubation at 37°C.*

Material	*Escherichia coli (25922)*		*Staphylococcus aureus (29213)*	
	% Reduction	Log reduction	% Reduction	Log reduction
Lactide (5% w/w)	16.47±2.71	0.08±0.01	82.82±2.62	0.77±0.07
Lactide (10% w/w)	68.93±3.14	0.51±0.04	97.79±1.30	1.71±0.21
Lactide (15% w/w)	94.72±3.63	1.37±0.33	99.26±0.81	1.84±0.01

Significant bacterial cell attachment was observed on the surface, despite the 15% w/w lactide loaded silicone elastomer reducing *E. coli* and *S. aureus* adhesion by >90%. Subsequent degradation of the lactide released from the device to lactic acid/lactate exerts a broad-spectrum contact-killing effect against bacterial cells.

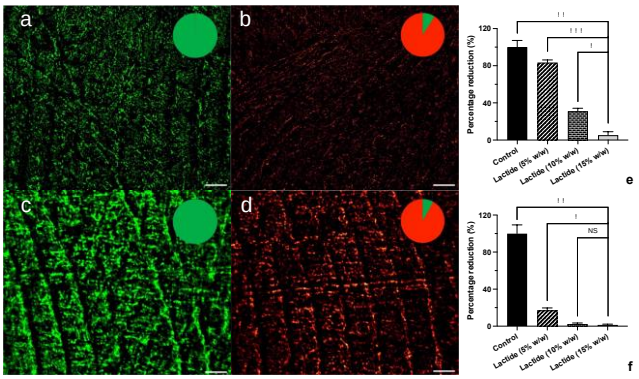

Figure 1. *Live/dead assay: fluorescence microscope image of E. coli (a, b) and S. aureus (c, d) on blank silicone elastomer (a, c) and 15% w/w lactide loaded silicone elastomer (b, d) in 24h incubation. Representative images are shown from one of several examinations (n=6, pie chart represents the percentage viable of live (green) dead (red) cells, scale bars correspond to 100 μm). Bacterial adhesion of E. coli (e) and S. aureus (f) in 24h.*

Multiple expressive pro-inflammatory cytokines were reduced in L929 and RAW246.7 cells. For fibroblasts, ten inflammatory cytokines were expressed. Among them, the pro-inflammatory cytokines monocyte chemoattractant protein-1 (MCP-1), tissue inhibitor of metalloproteinases (TIMP-1), and macrophage colony-stimulating factor (M-SCF) were found highly expressed in cell metabolites and a pronounced reduction were shown after treated by 15% w/w-lactide system. For macrophages, nine inflammatory cytokines were detected. Three highly expressed pro-inflammatory cytokines including macrophages inflammatory protein-1 α (MIP-1α), MIP-1β, stromal cell-derived factor (SDF-1) were found >50% downregulation after treated. Remarkably, Interleukin-1 receptor antagonist (IL-1ra) was increased and IL-1α was not detected, which is known to activate NF-κB signalling pathway.

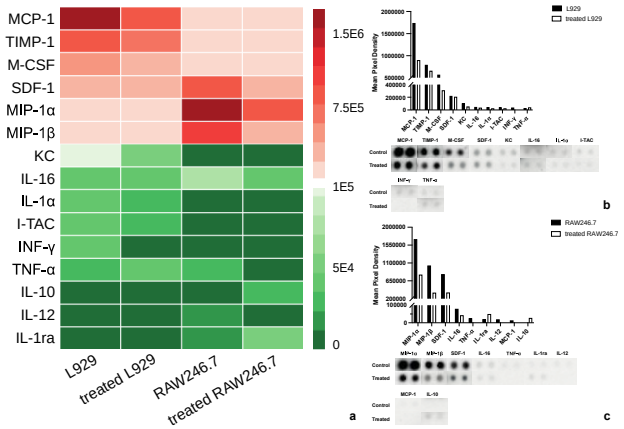

Figure 2. *Heatmap(a) of high expressive inflammatory cytokines(red) and low expressive inflammatory cytokines (green), scale bar represents mean pixel density. Mouse cytokine antibody array of L929 and RAW246.7 cells (b, c), respectively.*

CONCLUSIONS

This study reports the incorporation of lactide into silicone elastomer devices and, upon release, a reduction in bacterial adhesion and inflammation compared to a control. This lactide-releasing system potentially offers a novel alternative non-antibiotic strategy to prevent implant-associated infections and inflammation. In future studies, fluorescent labelled antibody will be applied to determine the activation for GPR81. In addition, western blot assay will be performed to assess GPR81 expression level detection in treated L929 and RAW246.7 cells.

ACKNOWLEDGEMENTS

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