Polydimethylsiloxane loaded capsaicin afflicts membrane integrity, metabolic activity and biofilm formation of nosocomial pathogens

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Abstract:

Biofilms constitute 80% of all nosocomial infections associated with invasive medical devices. Polydimethylsiloxane, a silicone polymer widely used as implants, suffers from microbial adhesion. Inhibition of biofilm formation on PDMS surfaces is significant to prevent morbidity, mortality and replacement. The present study investigates the efficacy of capsaicin (0.5% w/v) loaded PDMS as a broad spectrum antimicrobial surface against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Capsaicin exhibited minimum inhibitory concentration (MIC) of 1024 µg mL^{-1} for *S. aureus*, *E. coli* and 256 µg mL^{-1} for *C. albicans*. Capsaicin inhibited biofilms of *S. aureus, E. coli* and *C. albicans* at much lower concentrations of 2, 64 and 8 µg mL^{-1} respectively. The minimum capsaicin concentrations required for total biofilm eradication (MBEC) was found to be 256, 512, 128 µg mL^{-1} for *S. aureus*, *E. coli* and *C. albicans* respectively. Probing sub-lethal concentrations (64 µg mL^{-1}) of capsaicin revealed 38, 32, 30% reduction in metabolic activity of *S. aureus*, *E. coli* & *C. albicans* planktonic cells respectively. Similarly, there was an increase in permeability of cells to propidium iodide compared to control. By reducing the metabolic activity and perturbing membrane integrity, capsaicin could prevent biofilm formation and this was also observed with capsaicin-PDMS surfaces that exhibited 1 log (~90%) reduction of viable bacterial counts.

Keywords: Capsaicin, Polydimethylsiloxane, antibiofilm, Membrane damage, nosocomial pathogens.

1. Introduction

Polydimethylsiloxane (PDMS) is a silicone polymer widely used in various fields such as microfluidics, optics, electronics, biology, medicine, energy, etc. PDMS based devices/surfaces are increasingly used as implants/biomaterial due to their superior biocompatibility, non-immunogenicity, biomechanical behaviour such as flexibility, chemical stability, resistance and inert properties. PDMS has been used as versatile scaffold, prostheses and implants at 20 different points in the human body such as spinal implants, voice prosthesis, cochlear implants, hybrid dental implants, drug delivery devices, breast implant coatings, urinary catheters etc, [1,2]. However due to its hydrophobic nature, PDMS surfaces are prone to microbial adhesion and subsequent biofilm formation [3]. Biofilms are hard to eradicate compared to their planktonic counterparts due to their different phenotype, gene expression, protein synthesis and production of viscous exopolymeric substance [4].

It is also obvious that any implanted object which is prone to biofilm development can compromise patient safety and material functionality [1],[5]. Several control strategies have been developed to impart antimicrobial property to the implant material which can minimise biofilm formation and subsequent infections. Biofilm formation on implant material is attributed to the reduced host immune response, besides implants offer an interface for microbial adhesion and growth [6]. The major dominant microbes on implants and prosthetic devices are *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *S. aureus* is known to cause very serious infections such as ventilator associated pneumonia, orthopaedic and breast implant infections, cardiac device infections and infective endocarditis of prosthetic aortic and mitral valves, etc. [7]. Uropathogenic *E. coli* causes symptomatic urinary tract infections (UTI) as well as asymptomatic bacteriuria. *E coli* occurs on prosthetic joints, shunts, grafts and causes bacteraemia and sepsis [8]. Bacterial contamination of Intravenous (IV) and urethral catheters cost approximately $300 million to $2.3 billion annually [5]. Fungal infections caused by *Candida* spp. account for over 400,000 per year. *C. albicans* can form biofilms on various implants such as cochlear, dental, hip implants, contact lenses, silicone and polyurethane catheters and attributed to healthcare associated infections [9].

Impregnating polymers with antimicrobials and their release over time has gained more importance recently. In this direction, antimycotics incorporated into PDMS were developed to

inhibit *C. albicans* [10]. Recent studies have shown that *Candida* spp. have developed wide range of resistance mechanism to antimycotics and also cross resistance mechanisms between species. *Candida* spp. related biofilm infections have high mortality rate of around 41%. Currently different species of *Candida* have developed resistance to conventional antimycotics viz: polyenes, azoles, echinocandins, 5-flucytocine. This has warranted the search for alternate antimicrobial agents. It is imperative to follow antibiofilm approaches to effectively culminate device associated infections. Antibiofilm approaches for polymeric implants / prosthesis like PDMS, involve incorporation of nitric oxide releasing xerogel coatings; enzymes; antibiotics, nanoparticles, surface modifications, etc to improve their antimicrobial properties [2,11]. However, most of these studies have demonstrated antibacterial activity under *in vitro* conditions and elicit biocompatibility issues for *in situ* applications. Hence there is a search for a less cytotoxic, more biocompatible, antimicrobial agent.

In comparison, capsaicin (8-methyl-N-vanilyl-6-nonenamide) possesses all the required attributes such as biocompatibility, excellent antibacterial properties, approved for topical use, which makes it a viable, safe and low cost alternative antimicrobial for implant material. Capsaicin, a pungent compound of chilli pepper, has topical pharmaceutical application in rheumatoid arthritis, osteoarthritis and diabetic neuropathy. Capsaicin has anticancer activity by targeting signalling pathways that regulates oncogenes and tumour suppressor genes [12]. Capsaicin has been found to possess prolonged release and good bioavailability in topical gel formulations [13]. Several studies have reported the *in vitro* antimicrobial property of capsaicin [14,15]. Capsaicin also inhibited the NorA efflux pumps in *S. aureus* by reducing its intracellular invasion ability [16] and demonstrated synergistic antibacterial activity in combination with antibiotics [17]. Capsaicin has been shown to possess antibiofilm and biofilm dispersal properties [18]. Capsaicin loaded into different polymers as fillers has shown excellent anti-algal and marine antifouling property [19,20]. The present study aims at evaluating the antimicrobial activity of capsaicin on planktonic and biofilms of three (*S. aureus, E. coli* and *C. albicans*) most common nosocomial pathogens and their mechanism of action. This study also evaluates the efficacy of PDMS loaded capsaicin surfaces *in vitro*.

2. Materials & Methods

2.1. Microbial strains and growth conditions

S. aureus (V329) and *E. coli* (ATCC 10536) were grown in Tryptic soy broth (TSB) supplemented with 0.25% glucose and Luria Bertani (LB) broth respectively at 37°C in an orbital shaker at 160 rpm to mid log phase. *C. albicans* (ATCC 90028) was maintained in potato dextrose broth/ agar (PDB/PDA) in an orbital shaker at 37 ± 1°C. Spider broth (0.2% K_2HPO_4, 1% mannitol, 0.005% beef extract, 0.01% peptone and 0.01% NaCl) was used for filamentous growth.

2.2. Evaluation of Minimum inhibitory and microbicidal concentrations of capsaicin

Minimum inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of capsaicin (Sigma-Aldrich) against planktonic cells of *S. aureus* and *E. coli* were determined using 96 well microtitre plates. MIC and Minimum fungicidal concentration (MFC) were also determined against *C. albicans* planktonic cells [21]. Varying concentrations of capsaicin (1024, 512, 256, 128, 64, 32, 16, 8, 4 & 2 µg mL^{-1}) were achieved by microdilution from the stock of 4096 µg mL^{-1} capsaicin in ethanol and appropriate vehicle controls were followed. The overnight grown cultures were adjusted to ~10^5 CFU mL^{-1} using UV-vis spectrophotometer (Shimadzu, uv-1601, Japan) at 600 nm, added as initial inocula and the assay volume was fixed as 200 µL per well. Respective media alone served as blank and media with inoculum served as controls. The plates were incubated for 24 h for growth in a temperature controlled incubator at 37°C ± 1°C. The MIC, MBC and MFC values were determined from optical density measurements at 600 nm using a multi-mode reader (BIOTEK-SYNERGY, India). MBC and MFC were confirmed by plating on respective agar where there was no colony observed. The percentage inhibition of planktonic cell growth was calculated using the formulae

$$\% \text{ Inhibition} = [(\text{Control } OD_{600} - \text{treated } OD_{600}) / \text{Control } OD_{600}] \text{ X } 100$$

2.3. Planktonic cell metabolic activity – XTT Reduction assay

The metabolic status of control planktonic cells and cells exposed to sub-MICs of capsaicin (64, 128 µg mL^{-1}) was evaluated using the XTT [2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide sodium salt] reduction assay. The cell suspensions were prepared by centrifuging 1 mL of control and treated cells and the pellets were adjusted to 0.5 OD using PBS. Briefly 200 µL of XTT (0.2 mg mL^{-1} in 1X PBS) and menadione (0.172 mg mL^{-1} in acetone) were mixed in a ratio of 25:1 and added to 200 µL of planktonic cell suspension and incubated at 37°C for 3 h in dark. The coloured supernatant containing XTT formazan was transferred to new

microtitre plates and measured at 490 nm [22]. Percentage of inhibition in metabolic activity was calculated as described below.

$$\% \text{ Inhibition} = [(\text{Control } OD_{490} - \text{treated } OD_{490}) / \text{Control } OD_{490}] \times 100$$

2. 4. Propidium Iodide uptake and membrane damage of planktonic cells

Propidium iodide (PI) uptake is used as a tool to assess membrane damage. PI is a fluorescent probe binds to nucleic acids producing a red fluorescence in cells with compromised cell membranes. A 10 µM working solution of PI (Molecular probes®, Invitrogen) was prepared in 1X PBS and stored in dark at 4^{o}C. 200 µL of control and sub-lethal concentrations of capsaicin (64, 128 µg mL^{-1}) treated planktonic cell suspensions prepared as mentioned above were stained with 200 µL of PI and incubated for 10 min in dark. The cells were then washed with sterile PBS to remove excess PI and re-suspended in PBS. The relative fluorescence intensity units (RFU) were measured using a Biotek-Synergy Multimode reader with an excitation and emission of 530 nm and 620 nm respectively [23].

2. 5. Evaluation of antibiofilm activity of capsaicin

After evaluation of MIC and MBC of planktonic cultures, the 96 well plates were quantified for biofilms formed in the presence of varying concentrations of capsaicin (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 & 0 µg mL^{-1}). The planktonic cultures were discarded and the wells were washed with sterile 1X PBS (pH=7.4) to remove any loosely adhered cells and stained with 200 µL of 0.1 % crystal violet (CV) for 5 min [23]. Crystal violet solution was then discarded and the plates were washed twice with PBS to remove any unbound CV and the plates were air dried. Subsequently 200 µL of glacial acetic acid (33 %) was added to each of the wells to solubilise the cell bound CV for 10 min and optical density was measured at 570 nm. The minimum concentration of capsaicin required to inhibit maximum biofilm formation was identified as the biofilm eradication concentration (MBEC) from the values. The percentage of biofilm inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = [(\text{Control } OD_{570} - \text{treated } OD_{570}) / \text{Control } OD_{570}] \times 100$$

2. 6. Microscopic Evaluation of effect of capsaicin on biofilms

Effect of various concentrations (64, 128, 256, 512 and 1024 µg mL^{-1}) of capsaicin on biofilm formation of test pathogens was investigated using epifluorescence microscope [23]. The assay

was performed in a polystyrene 24 well plate. The test pathogens were allowed to form biofilm in a 1 X 1 cm glass surface in the presence of capsaicin at 37°C for 24 h. Control biofilms were formed in the absence of capsaicin. After 24 h incubation, the planktonic cells were discarded and glass slides were rinsed with sterile PBS to remove loosely adhered cells. The glass slides containing biofilms were stained with BacLight™ Live/dead staining solution (L13152-Molecular Probes, Invitrogen) and observed under epifluorescence microscope.

2. 7. Preparation & characterization of capsaicin loaded PDMS surfaces

PDMS surfaces were prepared from Sylgard 184 (DOW Corning, Midland, MI) by mixing the base polymer (A) and the curing agent (B) in a ratio of 10:1 [23]. Capsaicin (0.5% w/v) was first added to the curing agent (B) and kept in an ultra sonicator bath (frequency 33 ± 3 kHz), for 15 min for uniform dispersion. Then part B was mixed with part A and vortexed for 15 min for uniform dispersion into the matrix. The mixture was then degassed to remove air bubble in a vacuum desiccator for 30 min. The solutions were poured into glass trays and allowed to vulcanize at room temperature overnight, to prepare free standing PDMS films. Plain PDMS surfaces without capsaicin were also prepared.

2. 8. Evaluation of *in vitro* antibiofilm activity of capsaicin loaded PDMS

Plain PDMS control surfaces as well as capsaicin loaded PDMS surfaces were cut into the size of 2 x 2 cm in triplicates and were UV sterilized for 30 min prior to performing assay. Biofilm inhibition assays were conducted in 6 well polystyrene plates using 5 mL of TSB for *S. aureus*, LB for *E. coli* and spider broth for *C. albicans*. 500 µl of 0.05 OD (~10^5 CFU mL^{-1}) adjusted cultures were inoculated and the plates were incubated at 37°C for 24 h in a temperature controlled incubator. After 24 h of incubation, the polymer films were taken out and rinsed with sterile PBS to remove loosely adhered cells and was stained with Live/Dead BacLight® stain for 2 min. Excess stain was rinsed with sterile PBS. The surfaces were visualized under an epifluorescence microscope (Axioscope-I, Carl Zeiss, Germany), using FITC (SYTO 9) and dsRed (Propidium Iodide) filters for green and red fluorescence respectively. Live cells appear green and dead cells appear red in colour. Parallelly another set of similar surfaces were incubated and after 24 h the PDMS surfaces were collected into centrifuge tubes containing sterile PBS (10 mL). The surfaces were ultra-sonicated (10 min) followed by vigorous vortexing for 2 min. The cell suspensions were appropriately diluted and plated on respective agar plates

for determining the cell density. Total viable cells attached on surfaces were calculated and expressed as CFU cm^{-2} [24].

2. 9. Statistical Analysis

All the assays were carried out using minimum three biological replicates and the results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3. 1. MIC & MBC of planktonic cells

Susceptibility of *S. aureus* and *E. coli* to capsaicin increased significantly ($p<0.05$) in a concentration dependent manner. The MIC of capsaicin inhibiting visual planktonic growth was observed at 4 µg mL^{-1} for *S. aureus* and *E. coli*. A 50% reduction in cell growth was observed at concentrations of 128 µg mL^{-1} [MIC_{50}] for both the bacterial strains (Figure 1a). In comparison, a lower MIC value of 2 µg mL^{-1} was observed for *C. albicans* and 50% inhibition of fungal growth was observed at a lower concentration of 64 µg mL^{-1} [MIC_{50}]. Analysis of minimum fungicidal concentrations also revealed that capsaicin was more effective against the fungus *C. albicans* with 100% growth inhibition achieved at 256 µg mL^{-1} of capsaicin. The next most susceptible organism among tested was *E. coli* in which, 100% inhibition was observed at 1024 µg mL^{-1}. However, *S. aureus* showed only 97.8% growth inhibition at the highest concentration tested (1024 µg mL^{-1}).

3. 2. Metabolic activity of planktonic cells

Reduction of tetrazolium salt is a common method to assess metabolic activity of planktonic cells which was assessed using sub-lethal concentrations of capsaicin (64 and 128 µg mL^{-1}). XTT assay revealed 38% decrease in metabolic activity at 64 µg mL^{-1} for *S. aureus,* 32% for *E. coli* and 30% for *C. albicans*. The next increased sub-lethal concentration of 128 µg mL^{-1} tested revealed 57%, 45% and 57% reductions in *S. aureus*, *E. coli* and *C. albicans* metabolic activity, respectively (Figure 2a). Reduced metabolic activity with increasing concentration of capsaicin on planktonic cells may be one of the reasons for the observed mortality of planktonic cells.

3. 3. Uptake of Propidium Iodide and membrane damage in planktonic cells

Propidium iodide uptake of cells exposed to sub-lethal concentration of capsaicin (64 and 128 µg mL^{-1}) showed increased membrane damage than respective controls (Figure 2b). A 3.6 fold increase in RFU was observed with *S. aureus*, 1.4 fold with *E. coli* and 1.3 fold increases with *C. albicans* at 64 µg mL^{-1} capsaicin treatment compared to untreated controls. In comparison the higher concentration of capsaicin (128 µg mL^{-1}) elicited an even higher response of 5.8, 3.7 and 2 fold increase in membrane permeability to PI with respective to *S. aureus*, *E. coli* and *C. albicans*.

3. 4. MBIC and biofilm eradication concentrations

Compared to planktonic cells, the lowest concentration of capsaicin tested *viz*: 2 µg mL^{-1} elicited 53% reduction in *S. aureus* biofilm formation and the next most susceptible organism was *C. albicans* with 56% reduction at 8 µg mL^{-1}. In comparison higher concentrations of 64 µg mL^{-1} was needed for *E. coli*. The biofilm eradication concentrations for total biofilm were much lower than planktonic cells *viz*: 256 µg mL^{-1} for *S. aureus*, 512 µg mL^{-1} for *E. coli* and 128 µg mL^{-1} for *C. albicans* (Figure 1b). Microscopic analysis revealed the dense and confluent architecture of *S. aureus* and *E. coli* control biofilms. *C. albicans* control biofilm was composed of highly virulent filamentous hyphae, whereas capsaicin (>128 µg mL^{-1}) abrogated hyphal forms and more yeast forms were observed (Figure 3). Steady increase in red cell population was observed right from 64 µg mL^{-1} of capsaicin treatment, which indicates the increase of dead cells or cells with compromised membrane integrity stained by PI and complete inhibition of biofilm was observed at concentrations of 512 and 1024 µg mL^{-1}.

3. 5. Inhibition of Biofilm formation on Capsaicin loaded PDMS surfaces

Incorporation of capsaicin into PDMS (0.5%) inhibited biofilm formation of all three test strains (Figure 4). An 87% reduction in viable counts was observed between (control 5 $X10^6$ CFU cm^{-2}) and capsaicin loaded surfaces (6 X 10^5 CFU cm^{-2}) for *S. aureus*. Similarly 92% reduction was observed between plain PDMS (5 X 10^5 CFU cm^{-2}) and capsaicin loaded PDMS (4 X 10^4 CFU cm^{-2}). Control *C. albicans* colonized PDMS surfaces with a magnitude of 5 X 10^4 CFU cm^{-2}, whereas 95% reduction was observed in capsaicin infused PDMS (2 X 10^3 CFU cm^{-2}).

4. Discussion

Biofilms contribute to 50-70% of device associated infections [25]. Biofilm mode offers 10 to 1000 times more resistance to antimicrobials compared to their planktonic counterparts which make biofilms hard to eradicate, once formed. Biofilm infections associated with implants, serve as a reservoir of pathogens; cause tissue destruction, dysfunction of device resulting in morbidity or fatality of infected patients [26]. Elimination of biofilm formation on implants and biomaterials is a major challenge. Predominantly the common implant associated infections are caused by *S. aureus*, *S. epidermidis*, *E. coli*, *Pseudomonas aeruginosa*, *C. albicans*, *Enterococcus faecalis*, *Proteus mirabilis* [27]. Gram positive cocci, Gram negative rods and *C. albicans* respectively constituted 77%, 15% and 8% of all infections caused due to ureteral stents inserted for a period ranging from 5-128 days [28]. For a surgical implanted device to be successful, tissue integration should occur prior to bacterial adhesion, thereby inhibiting biofilm formation is crucial which usually happens in the first 24 h of implantation. Whereas in the case of non-surgical indwelling medical devices, such as urinary catheters, the source of contamination may originate from the point of insertion around the catheters [29]. Implant associated infections caused by biofilms are hard to eradicate and often results in surgical removal of the implanted devices as the infection persists for long time periods [6]. Hence current research focus has been aimed at prevention of biofilm formation and infection following implantation by developing methods for loading or embedding biomaterial surface with antimicrobial substances. These techniques offer more advantages over systemic drug delivery as the antimicrobials are present within the implant and offer sustained delivery of a low dose antimicrobial for prolonged periods until tissue integration is complete [30].

The microbicidal action of capsaicin on all the three microbes assessed by Live/Dead assay revealed membrane damage at sub lethal concentrations of 64 and 128 µg mL^{-1}. Cell membrane is an important component involved in maintaining cell architecture as well as protection from environmental stressors. Propidium iodide (PI), a fluorescent probe, binds to nucleic acids producing a red fluorescence in cells with compromised cell membranes. Capsaicin treated cells were more permeable to uptake of propidium iodide suggesting membrane damage which is in accordance with earlier observations for the compound [15]. In addition, the XTT assay revealed that capsaicin also reduces the metabolic activity of all the strains tested. At sub-lethal concentration of 128 µg mL^{-1}, XTT assay detected 57% reduction of metabolic activity in *S. aureus* and 45% in *E. coli* and 57% in *C. albicans*. This well explains the fractional

occurrence of planktonic and biofilm growth at sub-lethal concentrations. The fraction of metabolically active cells could grow and repopulate at sub-lethal concentrations, and hence there is a need for bactericidal concentrations which are found to be higher (1024 µg mL^{-1}). Biomass production has also been shown to influence the metabolic activity of the population and hence in the present XTT assays cell density was maintained similar to differentiate effects of capsaicin on reduction of metabolic activity [31]. Similar observations were reported by Peters et al., (2013) for ethanol on mono and polymicrobial biofilms of *S. aureus* and *C. albicans* [32].

A concentration dependent increase in biofilm inhibition by capsaicin was observed for all the three strains investigated. Complete biofilm eradication concentrations varied with different microbial species like *C. albicans* requires less concentration (128 µg mL^{-1}) compared to *S. aureus* (256 µg mL^{-1}) and *E. coli* found to be the most tolerant requiring higher concentrations of capsaicin (512 µg mL^{-1}). Such high concentration of capsaicin (7 mM) was required in earlier report [18] for reducing the adherence of *E. coli*, wherein the present study 1.6 mM capsaicin effectively controlled *E. coli*. This is attributed to the difference in inoculum size. Apart from reducing biofilm formation, capsaicin compromises membrane integrity of attached cells which is evident from the increased proportion of PI stained microcolonies present in capsaicin treated samples. Reduced metabolic activity of cells treated with capsaicin will also significantly contribute to the reduction of biofilm mass. Apart from this, capsaicin is known to inhibit various biofilm components such as protein, carbohydrate and DNA which are key components of EPS [18]. In addition, capsaicin has been found to inhibit the release of DNase enzyme in *S. aureus* [33].

In the present work embedding / loading of capsaicin in PDMS polymer matrix and evaluating its antimicrobial efficacy in inhibiting biofilm formation of the nosocomial pathogens *S. aureus*, *E. coli* and *C. albicans* was evaluated. Plain PDMS surfaces were colonized by 4.8 X 10^6 CFU cm^{-2} for *S. aureus*; 5.1 x 10^5 cfu cm^{-2} for *E. coli* and 5.2 x 10^4 cfu cm^{-2} for *C. albicans* after 24 h incubation. In comparison, nearly a one log (~90%) reduction of viable cells on capsaicin loaded PDMS (0.5%), was observed for all three strains with 87% reduction with *S. aureus,* 92% for *E. coli* and 95% for *C. albicans*. The optimum concentrations of capsaicin required for complete biofilm inhibition of all three strains in the present study was observed to be 512 µg mL^{-1}, however membrane damage occurred at even much lower concentration of 128 µg mL^{-1} for all the three strains. PDMS surfaces were infused with ~10 fold higher concentration

of capsaicin (0.5% w/v) considering the thickness of the film and immiscibility of capsaicin. Capsaicin incorporated PDMS exhibited similar mechanical properties as that of control. Previous study by Al-Juhani and Newby (2014) has extensively revealed the indifference in curing behaviour, bulk and surface properties of PDMS *viz;* water contact angle, surface roughness while incorporating low content of capsaicin (up to 1%) [34]. Considering the fact, in the present study relatively insignificant capsaicin (<1%) was included which may not affect the property of silicone polymer and the manifested antimicrobial activities are attributed to the infused capsaicin. Similarly, inhibition of *S. aureus* on PDMS-PEEK implant surfaces has been demonstrated by incorporating Ag (bioactivity) doped TiO_2 (biocompatibility) nanoparticles into the PDMS matrix where complete biofilm inhibition was dependent on the amount of Ag doped TiO_2 nanoparticles [35]. Nano-patterned PDMS surfaces inhibited biofilm formation of both *E. coli* (57%) and *S. aureus* (79.2%) and showed increased fibroblast and endothelial cell adhesion which reiterates the biocompatibility of PDMS matrix [20]. Capsaicin is known to inhibit NorA efflux pumps of *S. aureus* and potentiate ciprofloxacin that shows the combinatorial efficacy of capsaicin [16]. Incorporation of capsaicin into carbopol gels decreased the MIC by 50% compared to plain capsaicin for *C. albicans*, *S. aureus* and *E. coli* [13]. These show the plausible way of incorporating capsaicin into biomaterials along with other antimicrobials which can help in the fight against antimicrobial resistance.

Besides inhibiting the adhesion of biofilm forming strains on PDMS, capsaicin exhibited effective contact killing of attached microbes. Similar contact killing of attached cells on functionalized PDMS has also been reported [36]. In the present study capsaicin-PDMS matrix exhibited broad spectrum antibiofilm activity which is a prerequisite for any implant material. Such broad spectrum antimicrobial activity of PDMS nanocomposites have been achieved by incorporating nanoparticles [37,38]; polyimidazolium and covalent bonding of poly (sulfo/carboxy betaine) with PDMS [2]. However one drawback of these approaches for *in situ* applications is their biocompatibility issues as most of the nanoparticles and chemical moieties exhibit cytotoxicity. In comparison, capsaicin is a safe, biodegradable and biocompatible compound which can impart antimicrobial property to any surface which requires biocompatibility.

Capsaicin loaded PDMS exhibited complete biofilm inhibition of the all three strains with a loading of 0.5% (w/v) whereas aqueous suspensions of capsaicin as such required a much

lesser concentration. This may be attributed to the slow release of capsaicin incorporated in the porous PDMS polymer matrix which will have sustained release of capsaicin for extended period of time. Studies on leaching of capsaicin from PDMS matrix have revealed around 60% retention of original capsaicin mass incorporated into PDMS coatings after 30 days of exposure [34]. This will render a prolonged liberation of capsaicin to the microenvironment which can offer sustained antimicrobial property to the surface. Despite of excellent antimicrobial and biocompatible properties, capsaicin-PDMS surfaces lack uniformity in capsaicin distribution due to the immiscibility of capsaicin with PDMS. This forms heterogeneity in capsaicin aggregates which affects uniform distribution and release. Future research should be focused to develop silicones incorporated with capsaicin nano-emulsions that can help in uniform distribution as well as effective and controlled release.

5. Conclusion

The present study provides optimal concentrations of capsaicin required for broad spectrum antibiofilm activity against selected Gram positive, negative and fungal pathogens. Capsaicin imparted antimicrobial property to PDMS surfaces by causing membrane damage and reducing metabolic activity of bacterial and fungal pathogens. Inherent antibiofilm property of capsaicin loaded PDMS, will serve as a potential biomaterial that can be helpful in tackling microbial infections.

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Declaration of Competing Interest

None to declare

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Figure legends

Figure 1. (a) Antibacterial and **(b)** antibiofilm activity of capsaicin against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. * indicates $p \leq 0.05$, ** indicates $p \leq 0.005$, *** indicates $p \leq 0.001$.

Figure 2. Effect of sub-MIC of capsaicin on metabolic activity **(a)** and membrane integrity **(b)** of *S. aureus, E. coli* and *C. albicans*. * indicates $p \leq 0.05$, ** indicates $p \leq 0.005$, *** indicates $p \leq 0.001$.

Figure 3. Effect of various concentrations of capsaicin (μg mL^{-1}) on the biofilm formation of *S. aureus, E. coli* and *C. albicans*.

Figure 4. Biofilms of *S. aureus, E. coli* and *C. albicans* formed on plain PDMS and capsaicin (0.5% w/v) incorporated PDMS.