Gentamicin, Salicylate and Their Combinations as Anti-Infective Coating of Orthopedic Implants

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Abstract

Our objective was to determine the efficacy of gentamicin, salicylate and their combinations- PDLLA-coated orthopedic stainless steel wires in preventing gram positive and gram negative bacterial colonization. Stainless steel wires were coated with gentamicin/salicylate combinations. The zone of inhibition (ZoI) around the wires with and without drugs was determined by a modified Kirby–Bauer method. The adherence of bacteria to coated and uncoated wires and after 3 months shelf life followed by 4 weeks of immersion in serum were determined. Gentamicin/salicylate coated metal segments showed the highest inhibitory effect on bacterial adherence when compared with controls (93.1%–100%). These segments showed zones of inhibition ranged from (20-45 mm) diameter. After 4 weeks immersion in serum, the coated wire segments maintained significant durability and inhibited bacterial adherence when compared with controls by 92.8%–93.7%. Salicylate increases the coating efficacy of gentamicin when used in combination with them. These combinations can be used as coating for the orthopedic implants due to their effect in preventing biofilm formation.

Keywords: Gentamicin, Orthopedic, Salicylate, Biofilm, Implants

1. Introduction

Orthopedic implants are the most widely utilized and researched medical devices. Their applications range from hip and knee replacement to cranial implants (Omri et al, 2007).

The presence of biomaterials in orthopedic surgery involves a high risk of developing deep infections (Rimondini et al, 2005). Infections that occur following implantation of orthopaedic implants may require long-term treatment including replacement of the infected artificial joint, resection arthroplasty, or amputation, depending on the severity of the symptoms. In some cases, fulminating infections may also lead to patient death (Cierny & DiPasquale, 2002 and McPherson et al, 2002). In recent years there have been several advances in anti-infective orthopaedic technology but most of them were concerned with Staphylococcus spp.

One of the main factors is the phenomenon of the adhesion of the bacteria to the biomaterials and the production of a biofilm from the bacterial strains (Olson et al, 2006 and Rohde et al, 2010). It was demonstrated that bacteria have the ability to bind to the surface of biomaterials due to specific physical and chemical properties (Higashi et al, 1998 and Cerca et al, 2005). Orthopedists want to ensure the eradication of postoperative infections. Recently, biofilms produced by bacteria have attracted attention as a cause of chronic and intractable device-related infections (Stewart & Costerton, 2001). It has been suggested that the bacteria in biofilms are 100 to 1,000 fold more resistant to antibacterial agents than planktonic bacteria (Gristina & Costerton, 1985).

The staphylococci species including Staphylococcus aureus and Staphylococcus epidermidis are responsible for...
the majority of biofilms found on explanted orthopedic devices (Bengtson & Knutson, 1991). *Pseudomonas aeruginosa* and other gram-negative species have also been identified in implant infections, but are less frequent (Tattevin et al, 1999).

In the biofilm mode of growth, bacteria embed themselves in an extracellular material (slime), which holds the bacteria together and firmly attaches them to an implant surface (Hall-Stoodley et al, 2004). The ability to form a biofilm is believed to make the organisms more resistant to antibiotic treatment and the host defence system (von Eiff et al, 1999).

Conventional therapy with systemic antibiotics is expensive, prone to complications and often unsuccessful. Major problems in treating osteomyelitis include poor antimicrobial distribution at the site of infection because of limited blood circulation to infected skeletal tissue, and inability to directly address the biofilm pathogen scenario. A high systemic dosage of antibiotics to facilitate sufficient tissue and biofilm penetration is undesirable due to possible grave toxic side effects (Aviv et al, 2007).

Current approaches towards reduction of implant related infection include initiation of initial bacterial adhesion, coating implants with polymers which have inherent antibacterial activity or by loading of polymers with antimicrobial agents (Gabriel et al, 2009).

As reported, poly (D,L)-lactic acid (PDLLA) has excellent features with respect to implant coating including high mechanical stability (Schmidmaier et al, 2001b), good osteoinductive potential, and excellent biocompatibility in vivo (Schmidmaier et al, 2001a). The products of degradation are metabolised in the citric acid cycle (Schmidmaier et al, 2001a). Because of these characteristics, PDLLA can be used in orthopaedic coatings.

Gentamicin is the antibiotic most frequently used in orthopaedic surgical procedures throughout Europe (Malchau et al, 2002). Gentamicin considered being the antibiotic of choice because of its broad spectrum of activity against both Gram-positive and Gram-negative bacteria, excellent water solubility, thermal stability and its low allergenicity (Dunne et al, 2008). Salicylates and other non steroidal anti-inflammatory drugs (NSAID) are known to prevent bacterial adhesion on to medical devices (Arciola et al, 1998). They are antiseptic, anti-infective but not antimicrobial, hence there is little long-term possibility of creating microbial resistance, as shown with some antimicrobial substances (Gilbert et al, 2002 and Prithiviraj et al, 2005) but enhance the activities of certain antibiotics (Price et al, 2000).

Byers et al (2006) reported that poly (anhydride-esters) composed of non-steroidal anti-inflammatory drugs that biodegrade to salicylic acid (SA) and adipic acid inhibited colonization to foreign bodies in vivo and *in vitro* by *Pseudomonas aeruginosa*.

Coating the surface of fracture-fixation devices with effective antimicrobial agents would thus reduce the rate of bacterial colonization and provide significant protection against clinical infection. Objective of this study was to determine the efficacy of gentamicin, salicylate and their combinations- PDLLA-coated orthopedic stainless steel wires in preventing gram positive and gram negative bacterial colonization.

2. Experimental

2.1. Bacterial Strains

5 Clinical isolates of a biofilm-forming strain isolated from patients suffering from orthopedic implant infections at the department of Orthopedics, Minia University Hospital, Egypt were used for the *in vitro* studies. Strains were *Staphylococcus aureus*, *Staphylococcus epidermidis*, Klebsiella sp, *Pseudomonas aeruginosa* and Proteus spp.

2.2. Chemicals

Gentamicin sulphate (Winlab), sodium salicylate (El-Nasr Pharmaceutical Chemical Co. Abu-Zaabal), Poly D, Lactic acid polymer (PDLLA): Resomer R203H (Boehringer-Ingelheim, Ingelheim, Germany) and chloroform (El-Nasr Pharmaceutical Chemical Co. Abu-Zaabal).

2.3. Orthopedic Implants

Commercially available Kirschner-wires (K-wires) cut into 1 cm segments and screws of stainless steel (Suzhou ideal Medical Instrument Co. Ltd., France).

2.4. PDLLA-Drug Coating of Orthopedic Implants (Kirschner Wires and Stainless Steel Screws)

2.4.1. The Polymer Coating

Implants were coated with PDLLA by a solvent casting technique (Schmidmaier et al, 2001b). 100 mg PDLLA were dissolved in 1.5 ml volatile solvent (chloroform) at room temperature and the solution was sterile filtered. 3, 5 and 10 % w/w of gentamicin sulphate and 10, 15 and 20 %w/w sodium salicylate each alone or in combinations were added to the polymer solution. Drugs were dissolved in the PDLLA/chloroform solution to obtain the desired concentration. Sterilized biomaterials were dipped two times into the coating solution and dried under laminar air flow conditions.
2.4.2. Bacterial Adherence Testing of the Coated Implants

These coated implants were placed in 1 ml of donor calf serum and incubated overnight at 37 °C. Coated implants were then immersed in 1 ml of Mueller–Hinton broth inoculated with 5.5×10^5 CFU/ml of a biofilm-forming bacterial isolates and incubated overnight at 37 °C. The broth was then replaced with 1 ml of 0.9 % sterile saline and washed with shaking for 30 minutes to discard any planktonic bacteria. Without disturbing the biofilm, the implants were then transferred to 5 ml of 0.9 % saline, sonicated to dislodge the bacterial biofilm for 15 minutes and vortexed for 30 seconds. An aliquot of 100 µl was spread onto trypticase soy agar with 5 % sheep blood, incubated for 24 hours at 37 °C and then counted. A value of 100 CFU was used for any plate that had at least 100 colonies. Final colony counts were then calculated accounting for the dilution factor (Bahna et al, 2007).

2.4.3. Shelf Life and Durability Testing

The ability of coated implants to maintain efficacy over a period of time was determined by testing coated rods that were stored for 3 months in non-sterile ambient room temperature conditions. The coated rods were then incubated in donor calf serum at 37 °C for 4 weeks and tested for bacterial adherence using the Bahna et al (2007) method.

2.4.4. Zone of Inhibition (ZoI) Testing

The antimicrobial activity of rods was evaluated through ZoI testing using a modified Kirby–Bauer method (Bauer et al, 1966). Rods were vertically embedded, displacing the existing agar, in Mueller–Hinton agar plates inoculated with a 0.5 McFarland (1.5×10^8 CFU/ml) of biofilm-forming bacterial strains. After overnight incubation at 37 °C, the diameter of the ZoI was measured in mm and used to assess and compare the antibacterial efficacy of coated and uncoated rods.

2.5. Scanning Electron Microscope (SEM)

Orthopedic coated implants (controls and that with drugs) were fixed in 2.5 % (v/v) glutaraldehyde in Dulbecco PBS (Phosphate Buffered Saline) (pH 7.2) for 1.5 hours, rinsed with PBS, and then dehydrated through an ethanol series. Samples were critical point dried and gold-palladium coated. SEM examinations were made on JSM-840 SEM (Hudetz et al, 2008).

2.6. Statistical Analysis

One-Way ANOVA was employed to evaluate any significant difference between values obtained without the drug (controls) and those observed in the presence of different drug concentrations. One-Way ANOVA was also employed to evaluate any significant difference between values obtained without the drug (controls) and coated wires after immersion in serum for 4 weeks. Differences were done using graphpad prism 5 software. P values <0.05 were considered significant.

3. Results and Discussion

3.1. Bacterial Adherence Testing of the Coated Implants

Coating of stainless steel wire segments with the tested drugs was observed to decrease the number of adherent cells to wires surfaces. The effect was concentration dependent as the reductive effect of gentamicin at a concentration of 10 %w/w ranged from 94.7%-99.9% while at concentrations of 3 and 5 % w/w, it was in the range of 23.7%-88.8% and 76.3%-96% of the controls, respectively. Sodium salicylate at a concentration of 20% w/w had a significant inhibitory effect on bacterial adherence (79.7%-99.5% of the controls) while the effects of 15% w/w and 10% w/w were in the range of 71.3%-98% and 36.7%-90.6% of the controls, respectively. Gentamicin/salicylate combinations were found to be more effective than the effect of each alone as Gentamicin/salicylate at 10/20 % w/w showed the highest effect on bacterial adherence (99.9%-100%) while at concentrations of 5/15 and 3/10 % w/w reduced the bacterial adhesion by 98.2%–99.9% and 93.1%-97.4% when compared to controls, respectively so salicylate increases the inhibitory effect of gentamicin resulting in a significant decrease (P< 0.05) in the number of adherent cells to wire segments (Table 1).

3.2. Zone of Inhibition (ZoI) Testing

Gentamicin /sodium salicylate-coated wires (1 cm length) produced a ZoI ranged from (20-45 mm) diameter (Table 2).

3.3. Shelf Life and Durability Testing

Gentamicin / sodium salicylate-coated wires (1 cm length) stored at room temperature for 3 months and immersed in serum for 4 weeks showed a significant (P <0.05) reduction in adherence of the tested microorganisms compared with uncoated control rods (Table 3).

3.4. Scanning Electron Microscope (SEM)

Figure 1 demonstrates the morphology of biofilm on uncoated implant. Biofilm of Staphylococcus spp. was shown to be composed of many multilayered bacterial colonies, forming different sized colony masses on the
biomaterial (A) and *Pseudomonas* spp. biofilm completely covered the implant surface (B).

Sheehan et al (2001) reported that K-wires were coated with 10 to 50 nm of silver showed a log 2.95 fold reduction in biofilm formation but significant increase in the silver serum level led us to consider it ethically unacceptable to continue this investigation. Also, in a similar study Silver-coated fracture fixation devices were found to inhibit bacterial adherence in vitro of some but not all bacteria (Darouiche, 1999).

Bahna et al (2007) reported that gendine coated Schanz metal rods showed a net Zol of 16 mm against MRSA. Gendine-coated rods showed no biofilm formation. After 2 weeks, gendine-coated rods maintained significant durability, resulting in 90% reduction in MRSA biofilm adherence compared with uncoated control rods. On the other hand gentamicin/salicylate coated wires produced significant reduction in viable counts after 4 weeks.

### Table 1. Effect of Coated Orthopedic Implants with PDLAA-Gentamicin, Sodium Saicylate and Their Combinations on the Number of Adhering Bacterial Cells

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gentamicin</th>
<th>Sodium Salicylate</th>
<th>Gentamicin/Salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% w/w Conc.</td>
<td>Viable Count (CFU/ml)</td>
<td>Reduction (%)</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>CTR 48×10³</td>
<td>3 54×10² 88.8</td>
<td>10 45×10² 90.6</td>
</tr>
<tr>
<td></td>
<td>5 31×10² 93.5</td>
<td>15 14×10² 97</td>
<td>5/15 4×10 99.9</td>
</tr>
<tr>
<td></td>
<td>10 11×10² 97.7</td>
<td>20 24×10 99.5</td>
<td>10/20 0 100</td>
</tr>
<tr>
<td><em>Coagulase negative staph.</em></td>
<td>CTR 51×10³</td>
<td>3 8×10³ 84.3</td>
<td>10 28×10³ 45</td>
</tr>
<tr>
<td></td>
<td>5 2×10³ 96</td>
<td>15 7×10³ 86.3</td>
<td>5/15 9×10² 98.2</td>
</tr>
<tr>
<td></td>
<td>10 5×10 99.9</td>
<td>20 8×10³ 94.1</td>
<td>10/20 0 100</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>CTR 3×10⁶</td>
<td>3 14×10⁴ 53.3</td>
<td>10 19×10⁴ 36.7</td>
</tr>
<tr>
<td></td>
<td>5 22×10⁴ 92.7</td>
<td>15 86×10³ 71.3</td>
<td>5/15 9×10² 99.7</td>
</tr>
<tr>
<td></td>
<td>10 4×10³ 98.7</td>
<td>20 61×10³ 79.7</td>
<td>10/20 3×10² 99.9</td>
</tr>
<tr>
<td><em>Proteus spp.</em></td>
<td>CTR 25×10⁴</td>
<td>3 84×10³ 66.4</td>
<td>10 73×10³ 70.8</td>
</tr>
<tr>
<td></td>
<td>5 51×10³ 79.6</td>
<td>15 5×10³ 98</td>
<td>5/15 25×10² 99</td>
</tr>
<tr>
<td></td>
<td>10 13×10³ 94.8</td>
<td>20 3×10³ 98.8</td>
<td>10/20 0 100</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>CTR 38×10³</td>
<td>3 29×10³ 23.7</td>
<td>10 7×10³ 81.6</td>
</tr>
<tr>
<td></td>
<td>5 9×10³ 76.3</td>
<td>15 5×10³ 92.1</td>
<td>5/15 7×10² 98.2</td>
</tr>
<tr>
<td></td>
<td>10 2×10³ 94.7</td>
<td>20 8×10² 97.9</td>
<td>10/20 3×10 99.9</td>
</tr>
</tbody>
</table>

*CTR: Control Without Drugs, *P value: < 0.05 (Significant)*

### Table 2. Zol Diameter of Coated Wires in Comparison to Uncoated Control Wires

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zol of Coated Wire (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>45</td>
</tr>
<tr>
<td>Coagulase negative staph.*</td>
<td>40</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>25</td>
</tr>
<tr>
<td><em>Proteus spp.</em></td>
<td>20</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>30</td>
</tr>
</tbody>
</table>

SEM revealed that gentamicin/sodium salicylate coating metallic implant eradicates bacterial biofilms removing both the sessile cells and the extracellular matrix from implant surface (Figure 2).
In the current study, stainless steel wire segments coated with gentamicin/salicylate-PDLLA combinations (10/20 %w/w) showed the highest efficacy and the highest protective effect against microbial adherence, produced an inhibition zone ranged from (20-45 mm) and durability lasted for at least 4 weeks as salicylate was found to increase the therapeutic activity of gentamicin as said before. Similar results were obtained by Polonio et al (2001) who reported that Salicylate made synergistic effect with vancomycin. Gollwitzer et al (2003) reported that Combination of PDLLA with either gentamicin (5% w/w) or teicoplanin (5% w/w) or both antibiotics on the implant together significantly reduced adhesion of viable of Staphylococcus spp. While, in a rabbit model, rabbit tibia fractures were contaminated with Staphylococcus aureus. After plate implantation, it was found that 32% of rabbits implanted with the norvancomycin PDLLA-coated plates were infected but 92% of rabbits implanted with the uncoated plates were infected (P<0.05) (Fei et al, 2010).

Price et al (1996) showed a significant reduction in bacterial growth on 20% PLGA gentamicin coated stainless steel fracture plates over uncoated control plates. On the other hand, Colonisation and initial attachment of Staphylococcus aureus to PDLLA 10% gentamicin-coated

Table 3. Bacterial Adherence to Gentamicin / Sodium Salicylate-Coated Wires after 3 months Shelf Life Followed by 4 Weeks Immersion in Serum

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. of Viable Cells (CFU/ml)</th>
<th>% Reduction</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Coated</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>48×10^3</td>
<td>31×10^2</td>
<td>93.5</td>
</tr>
<tr>
<td>Coagulase Negative staph.</td>
<td>51×10^5</td>
<td>36×10^2</td>
<td>92.9</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>3×10^5</td>
<td>19×10^3</td>
<td>93.7</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>25×10^4</td>
<td>18×10^3</td>
<td>92.8</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>38×10^3</td>
<td>28×10^2</td>
<td>92.6</td>
</tr>
</tbody>
</table>

*P value < 0.05, Significant
K-wires was reduced by 90% when compared to the non-coated control group (McMillan et al, 2011) which was less than obtained in our results.

4. Conclusion

Coating orthopedic stainless steel rods with gentamicin/salicylate proved effective in inhibiting gram positive and negative bacterial activity and adherence to the surface of steel implants. The antibacterial activity of the coated rods remained stable and was not altered by serum or storage. Gentamicin/salicylate combinations showed a promising long-term efficacy and anti-adherence ability. The durability of tested rods and the non-labour-intensive method of coating make this novel combination a viable alternative for coating metallic devices.

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References


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