Release of Vancomycin and Gentamicin from a Contact Lens Versus a Fibrin Coating Applied to a Contact Lens

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PURPOSE. To develop a contact lens capable of releasing antibiotics for a minimum of 8 hours for the treatment of bacterial keratitis.

METHODS. Fibrin gel was loaded with vancomycin or gentamicin and then shaped into a curved disc. The disc was then used to coat the surface of a commercial contact lens or was sealed between two lenses. Separate contact lenses were soaked in solutions of vancomycin or gentamicin. The in vitro release kinetics for each system was determined using PBS at 37°C and a particle-enhanced turbidimetric inhibition immunoassay. The bioactivity of the antibiotics released from the fibrin was confirmed by using a microbiological assay.

RESULTS. Vancomycin and gentamicin were released at similar rates from soaked contact lenses and a coating of fibrin gel; however, the amounts of antibiotic delivered by the two systems differed considerably. The fibrin coating released over three times more gentamicin but less than one-fifth that of the lenses soaked in vancomycin. When fibrin was encapsulated between two contact lenses, significantly more controlled release was observed. For all systems, bactericidal amounts of vancomycin and gentamicin were released throughout the three-day testing period.

CONCLUSIONS. As a delivery system, fibrin gel loaded with gentamicin performs better than contact lenses soaked in gentamicin. The opposite is true for vancomycin, where soaked lenses outperform fibrin gel. These systems could potentially be used as a treatment for bacterial keratitis. (Invest Ophthalmol Vis Sci. 2012;53:1946–1952) DOI:10.1167/iovs.11-8607

Under normal circumstances, the human eye is highly resistant to bacterial infection. The tear film acts as a potent defense mechanism against pathogens, as tears contain a variety of antimicrobial agents including antibodies, lysozyme, lactoferrin, and beta-lactamase. Antibiotic delivery to the eye is complicated because there is a limited number of antibiotics that are effective against many Gram-positive and Gram-negative bacteria, including important eye pathogens such as Staphylococcus aureus and Streptococcus pneumoniae. Vancomycin is particularly effective against methicillin-resistant Staphylococcus aureus. Both antibiotic agents are remarkably stable, losing very little activity even after heat treatment. Vancomycin and gentamicin were selected for initial investigation because both antibiotics are used in the treatment of severe microbial keratitis, and they are among the few antibiotics for which a robust concentration assay is readily available, facilitating assessment of drug release kinetics from our delivery systems.

Many slow-release drug delivery systems are currently under development for the eye, but presently there is no commercially available system to treat bacterial keratitis. Previous research has shown that soaking soft contact lenses in a solution of gentamicin prior to insertion into the eye can produce a bactericidal concentration in the aqueous humor of patients that lasts for several hours; however, a means of slowing the rate of release from the lens would likely increase the effectiveness of the approach. Ophthalmic drugs are often released very quickly from an unmodified contact lens that has been simply soaked in a solution of the drug. Modifying the lens through the use of nanoparticles loaded with drug or through molecular imprinting yields more controlled release of drugs, but these techniques have not yet been used for gentamicin or vancomycin delivery.

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In this study, we examined the release kinetics of gentamicin and vancomycin from three different delivery systems: contact lenses soaked in solutions of the antibiotics, a fibrin coating loaded with the antibiotics and applied to the inner surface of a contact lens, and a fibrin coating loaded with the antibiotics and sealed between two lenses. The two-lens system was designed to simulate the release profile from fibrin encapsulated within a single contact lens. Golino et al.\textsuperscript{19} recently showed that encapsulation of a polymer-based drug depot within a single, custom-engineered contact lens yielded long-term controlled release of an antifungal drug.

**Materials and Methods**

**Preparation of Fibrin Depot**

Solutions containing 312.5 μg of vancomycin hydrochloride (Sigma-Aldrich, Dorset, England) or 500 μg of gentamicin sulfate (Sanofi-Aventis, Guildford, UK) and 106 KIU of aprotinin (Baxter Healthcare Ltd.) were mixed together to make a 47.8-μL mixture. This solution was then added to fibrinogen powder (TISSEEL Baxter Healthcare Ltd.), containing 8.1 mg of human fibrinogen and placed at 37°C. Once the fibrinogen had completely dissolved, 3.0 IU of thrombin (Baxter Healthcare Ltd., Thetford, UK), which was dissolved in 2.2 μL of calcium chloride (40 μmol CaCl\textsubscript{2} in 1 mL of water; Baxter Healthcare Ltd.) was added to yield a total fibrin gel volume of approximately 62.5 μL. The fibrin was shaped into a curved disk by pressing it against a glass sphere with the approximate curvature of a contact lens.

**Manufacture of Encapsulated Fibrin Device**

A fibrin gel was prepared (as described in Preparation of Fibrin Depot) and placed on top of the concave surface of one contact lens (catalog no. 06/10–14,804; Acuity Contact Lenses, Hoddesdon, UK); 40 μL of fibrin glue was then added, and a second contact lens was placed over the top. The glue was allowed to set for 20 minutes at room temperature.

**Preparation of Vancomycin- and Gentamicin-Soaked Contact Lenses**

The concave surface of a contact lens was filled with vancomycin hydrochloride solution (25 mg/mL in PBS; Sigma-Aldrich) or gentamicin sulfate solution (40 mg/mL; Sanofi-Aventis). Lenses were soaked for two hours at room temperature and then dipped briefly in distilled water to remove droplets of unabsorbed antibiotic.

**Evaluation of Vancomycin and Gentamicin Release**

A fibrin gel was prepared (as described in Preparation of Fibrin Depot) and then placed either onto the concave surface of a contact lens (Acuity Contact Lenses) or glued between two lenses (as described in Manufacture of Encapsulated Fibrin Device). Soaked contact lenses were also prepared (as described in Preparation of Vancomycin and Gentamicin-Soaked Contact Lenses). A 200-μL volume of PBS was added, mostly filling the concave surface of the lens and completely covering the fibrin. At each time point sampled, the entire 200-μL volume was removed and replaced with 200 μL of fresh PBS. After 72 hours (following collection of the final PBS sample) the fibrin gel was placed into 100 μL of 0.5% trypsin-EDTA (Invitrogen, Paisley, UK) and incubated at 37°C until completely degraded. The amount of antibiotic remaining in the fibrin after 72 hours was determined, and this value was used to calculate the total percentage of antibiotic released from the fibrin systems within the 72 hour period; this information was used in the construction of the cumulative release curves presented in Figure 3. The amount of vancomycin or gentamicin present in the samples was determined using a homogenous particle-enhanced turbidimetric inhibition immunoassay (Siemens Healthcare, Camberley, UK) on a Dimension RXL autoanalyzer (Siemens Healthcare). The assay uses a vancomycin-synthetic particle conjugate (PC) or a gentamicin-synthetic PC and a vancomycin-specific monoclonal antibody (AB) or gentamicin-specific monoclonal AB. The antibody in the sample competes with the PC for binding sites on the monoclonal antibody. The PC-AB complex scatters light at 340 nm, whereas the vancomycin-AB complex and the gentamicin-AB complex do not; the amount of light scattered is inversely proportional to the antibiotic concentration in the sample. Higher concentrations of antibiotic in the sample lead to less PC-AB complex being formed and therefore less light scatter. The amount of light being scattered was measured, and the corresponding antibiotic concentration was determined.

**Analysis of Vancomycin and Gentamicin Bioactivity**

A 0.5 McFarland suspension (1.5 × 10^6 CFU/mL) of S. aureus (strain NCTC 6571)\textsuperscript{20} was prepared according to published susceptibility testing protocols.\textsuperscript{20} A cotton swab was then dipped into the suspension and swabbed over the surface of six Iso-Sensitest agar plates (Oxoid Ltd., Basingstoke, UK) and allowed to dry for 30 minutes at room temperature. Three plates were used for the vancomycin assay, and three plates were used for the gentamicin assay. Four wells (in the case of the gentamicin assay) or five wells (in the case of the vancomycin assay) were then cut out of the agar by using a sterile borer (8-mm diameter). For the vancomycin assay, each well was inoculated with 100 μL of PBS solution containing one of the following: no vancomycin, 1 μg of vancomycin, 10 μg of vancomycin, 100 μg of vancomycin, or the supernatant collected from the encapsulated fibrin device. For the gentamicin assay, each well was inoculated with 100 μL of PBS solution containing one of the following: no gentamicin, 0.5 μg of gentamicin, 10 μg of gentamicin, or with the supernatant collected from the encapsulated fibrin device. Supernatant samples from the encapsulated fibrin device contained the entire amount of vancomycin or gentamicin released between 48 and 72 hours. The plates were incubated at 37°C for 24 hours and then photographed. The diameter of the bacteria-free area surrounding each well was measured. Each condition was tested on three separate
plates (N = 3). This assay was performed under the Advisory Committee on Dangerous Pathogens level 2 conditions in the Health Protection Agency microbiology services laboratory at Addenbrooke’s Hospital.

**RESULTS**

**In Vitro Release Kinetics of Vancomycin and Gentamicin from Soaked Contact Lenses and the Contact Lens-Fibrin Systems**

Release of vancomycin and gentamicin was evaluated from soaked contact lenses and fibrin gel, either coating a contact lens or encapsulated between two lenses (Fig. 1). Soaked lenses and fibrin coating released the antibiotics at similar rates (Fig. 2A); however, the concentrations and total amounts of antibiotic released by each system were substantially different (Table 1 and Table 2). For gentamicin, the fibrin coating released an average of 3.3 times more antibiotic than the soaked lens, whereas for vancomycin, the soaked lens released an average of 5.9 times more antibiotic than the fibrin coating. Release of both antibiotics from both systems was more controlled following the initial 8 hours of release (Fig. 2B).

Considerable differences between the release rates were observed between the two contact lens-fibrin systems (Fig. 3A). Within the first 8 hours, a mean percent ± SD of 65.6% ± 2.0% of the vancomycin and 95.1% ± 0.4% of the gentamicin was released from the fibrin coating, whereas only 17.7% ± 1.5% of the vancomycin and 68.0% ± 2.4% of the gentamicin was released from the fibrin sealed between two contact lenses. The total percentage of vancomycin released within three days was 87.7% ± 1.0% for the fibrin coating and 56.0% ± 4.8% for the fibrin encapsulated between two lenses. The total percentage of gentamicin released within three days was 99.4% ± 0.2% for the fibrin coating and 94.8% ± 0.9% for the fibrin encapsulated between two lenses. Release of both antibiotics

![Graph A](image1.png)

**Figure 2.** Vancomycin (black symbols) and gentamicin (white symbols) released from contact lenses soaked in solutions of the antibiotics (triangles) and fibrin gel coating a single contact lens (diamonds). It was not possible to determine the percentage of antibiotics remaining within the soaked contact lenses after sampling was complete; therefore, to compare the soaked lenses with the fibrin coating, we considered only what was released from each system within 72 hours. (A) Percentage of vancomycin and gentamicin released over time. (B) Percentage of vancomycin and gentamicin released from the systems assuming each device was preincubated in PBS for 8 hours prior to sampling. N = 3 for all groups. Error bars represent standard deviations. Lines were added only to guide the eye.

![Graph B](image2.png)

**Figure 3.** Vancomycin (black symbols) and gentamicin (white symbols) released from fibrin gel either coating a single contact lens (diamonds) or encased between two contact lenses (squares). (A) Percentage of vancomycin and gentamicin released over time. (B) Percentage of vancomycin and gentamicin released from the fibrin systems assuming each device was preincubated in PBS for 8 hours prior to sampling. N = 3 for all groups. Error bars represent the standard deviation. Lines were added only to guide the eye.
from both fibrin systems was more controlled following the initial 8 hours of release (Fig. 3B).

Bioactivity of Vancomycin and Gentamicin Released from Fibrin Encapsulated Between Two Contact Lenses

The bioactivity of the vancomycin and gentamicin released from fibrin gel encased between two contact lenses was evaluated using a microbiological assay. Supernatant samples collected from six separate devices (three containing vancomycin and three containing gentamicin) were tested for the presence of active antibiotic. The bioactivity of vancomycin and gentamicin released between 48 and 72 hours in vitro was compared to the bioactivity of known amounts of active vancomycin and gentamicin. Wells cut into the agar plates were filled with PBS containing either vancomycin or gentamicin. For the vancomycin assay, the wells contained the following amounts of vancomycin: 0 μg, 1 μg, 10 μg, and 100 μg, and an unknown amount present in the supernatant from the device. Diameters of the colony-free zones surrounding each of the wells (excluding the diameter of the well itself) were equal to 0 ± 0 mm, 15 ± 0 mm, 26.3 ± 0.6 mm, and 25.7 ± 0.6 mm, respectively (Fig. 5).

DISCUSSION

Ninety percent of ophthalmic drugs are currently delivered via eye drops. Although this approach is often effective, it suffers from several drawbacks, not least is the difficulty of applying drops very frequently for a prolonged period. The safety and comfort of soft contact lenses make them a potentially useful platform for a noninvasive drug delivery system; this type of system could potentially address many of the problems associated with eye drops. The contact lens we chose for this study is used in routine clinical practice as a bandage contact lens on eyes with corneal pathology and has good oxygen permeability with extended wear. We found that it was possible to load significant amounts of vancomycin and gentamicin into a small volume of fibrin gel and then mould the fibrin into a thin layer, which could either be placed over the surface of a single contact lens or sealed between two lenses. The purpose of this study was to evaluate the usefulness of these systems in delivering antibiotics and to determine how they compare with contact lenses soaked in solutions of antibiotic.

Concentrations of vancomycin and gentamicin in all samples collected throughout the three-day testing period (from both fibrin systems) exceeded the minimum bactericidal concentration (MBC) for many bacteria that cause keratitis.

Table 1. Concentration of Vancomycin and Gentamicin Present in PBS Samples

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Vancomycin</th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Encased Fibrin (μg/mL)</td>
<td>Fibrin Coating (μg/mL)</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>242</td>
</tr>
<tr>
<td>3</td>
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<td>212</td>
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<td>8</td>
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<td>81</td>
<td>61</td>
</tr>
<tr>
<td>72</td>
<td>66</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 2. Amounts of Vancomycin and Gentamicin Released

<table>
<thead>
<tr>
<th></th>
<th>Vancomycin</th>
<th>Gentamicin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Encased Fibrin (μg)</td>
<td>Fibrin Coating (μg)</td>
</tr>
<tr>
<td></td>
<td>76 ± 5</td>
<td>171 ± 10</td>
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Table shows average amounts of vancomycin and gentamicin released (± standard deviation) within 72 hours from contact lenses soaked in solutions of antibiotic, fibrin gel coating a contact lens, or fibrin gel encased between two contact lenses. N = 3 for all groups.
A list of relevant bacteria can be found in the article by Sueke et al. The bactericidal range for vancomycin is 0.25 to 6.25 μg/mL and 0.4 to 12.5 μg/mL for gentamicin. The concentration of vancomycin in samples collected from soaked contact lenses also exceeded the MBC throughout the three-day sampling period. However, the concentration of gentamicin in samples collected from soaked contact lenses fell below the MBC for some bacteria after 24 hours.

The amount of gentamicin released from a fibrin coating was found to be greater than triple the amount that was released from a contact lens soaked in a concentrated solution of gentamicin. The concentration of gentamicin in the samples collected from a fibrin coating ranged from 16 to 1845 μg/mL; samples from a soaked lens ranged from 5 to 650 μg/mL. Although both systems delivered bactericidal concentrations of...
gentamicin that were significantly below a toxic level, it is likely beneficial to maintain a higher concentration of gentamicin in an infected eye. Therefore, considering that the release kinetics of gentamicin from a soaked contact lens and a coating of fibrin gel are similar, a coating of fibrin gel is likely a more optimal delivery system for gentamicin.

The concentration of vancomycin in the samples collected from a fibrin coating of a single contact lens ranged from 53 to 242 μg/mL. This is well above the MBC range of 0.25 to 6.25 μg/mL and well below a toxic level. However, in contrast to results with gentamicin, the amount of vancomycin that was released from a fibrin coating was found to be less than one-fifth that of the amount released from a contact lens soaked in a concentrated solution of vancomycin. The concentration of vancomycin in the samples collected from a soaked contact lens ranged from 219 to 2042 μg/mL. Again, it is likely beneficial to maintain a higher concentration of the antibiotic in an infected eye. Therefore, considering that the release kinetics of vancomycin from a soaked contact lens and a coating of fibrin gel are similar, a soaked contact lens is likely a more optimal delivery system for vancomycin.

It was shown previously that concentrated fibrin gel can control the release of therapeutic proteins for a number of weeks. In this study, we found that concentrated fibrin alone did not control the release of small antibiotics as well as it does for larger therapeutic proteins; however, the amounts of vancomycin and gentamicin released throughout a three-day period were consistently above the MBC for relevant bacteria. It is not surprising that the antibiotics released more quickly from the fibrin than larger protein therapeutics, as the mechanism of drug release from fibrin is believed to occur mainly by simple diffusion out of the fibrin matrix. Increasing the amount of cross-linking within fibrin has been shown to dramatically slow the rate of drug release, presumably by reducing the pore size within the matrix and thus making diffusion more difficult.

When a fibrin coating was applied to a single contact lens, antibiotic release occurred in two stages: during the first 8 hours, 64% of the vancomycin was released, the rate of release then slowed and 24% of the remaining drug was released over the following 64 hours. The rate of gentamicin release from a fibrin coating was significantly more rapid: 95% of the gentamicin was released within the first 8 hours, followed by release of 4% of the remaining antibiotic over the subsequent 64 hours. The dramatic difference between the release rates may be related to the size difference between the antibiotics. Vancomycin has a molecular weight more than three times greater than that of gentamicin and therefore it likely diffuses out of the fibrin matrix more slowly. Previous research with collagen shields loaded with vancomycin and gentamicin yielded a similar result; however, the rate of release was much more gradual from our fibrin system and the soaked lenses; most of the gentamicin was released from a collagen shield within 30 minutes, and most of the vancomycin was released within six hours.

Eight hours of controlled release should be sufficient to make a noninvasive antibiotic delivery system clinically viable for the eye, as this amount of time would allow for an uninterrupted sleep cycle, after which the device could be replaced. However, it is also possible that a longer duration of controlled release could have added benefits. If desired, the faster stage of release could be eliminated by simply preincubating the fibrin or soaked lens in solution for 8 hours prior to use. This would result in more consistent amounts of antibiotic being released throughout a three day period. Considering that release of only a small percentage of the total entrapped antibiotic resulted in a bactericidal concentration, even the 5% of gentamicin released from the soaked lens between 8 and 72 hours could be clinically useful. Fluctuations in the amount of antibiotic delivered to the eye over time, using either the fibrin system or soaked lens, would be substantially less than the fluctuations that occur when using eye drops.

In an effort to further control the release of the antibiotics, we sealed the fibrin between two contact lenses. This significantly slowed the rate of release for both vancomycin and gentamicin. Similar to the fibrin coating, bactericidal amounts of antibiotic were released throughout a three day period. However, the thickness of the double contact lens system makes its use impractical. This trial was meant simply to explore the potential of this type of approach. The two-lens system allowed us to determine that both antibiotics are able to diffuse through the contact lens material and that passing through the lens material will slow the diffusion of the antibiotics. These findings establish a basis for engineering a single contact lens containing an antibiotic-loaded fibrin depot.

In order for it to fit comfortably into the eye and to allow sufficient oxygen permeability, the fibrin would need to be sealed within a significantly thinner contact lens. This should be possible with a custom-designed contact lens such as the lens developed by Ciolino et al. A thinner barrier between the fibrin and the eye will result in an increased rate of antibiotic release; however, the potential benefit of more controlled release is worth investigating further.

Using a contact lens completely coated with fibrin will undoubtedly affect the optical clarity of the lens; however, this issue could be addressed by leaving the central part of the lens uncovered (i.e., coating only the outer circumference of the lens with fibrin). This approach has been used by other researchers in the design of their drug delivery lens. For antibiotic delivery, the optical clarity of the delivery system is also not critical, as vision through an infected eye is likely to be significantly impaired already. Antibiotic ointments commonly used to treat ocular infections also reduce vision in the treated eye.

The idea of using a contact lens to deliver drugs dates back to 1960. Since then, there have been many different systems developed that use contact lenses; however, there are still no Food and Drug Administration-approved products available. The results presented in this study suggest that contact lenses soaked in solutions of antibiotics and contact lenses coated with antibiotic-loaded fibrin could be useful for delivering antibiotics to treat bacterial keratitis. Comparing these approaches, we found that a fibrin coating is likely a more optimal delivery system for gentamicin and a soaked lens is likely a more optimal delivery system for vancomycin. These systems are simple, noninvasive, use either the lens itself or fibrin as the drug depot (fibrin was approved for clinical use by the FDA in 1998), and can deliver bactericidal amounts of gentamicin and vancomycin for clinically relevant periods of time.

References


