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Cyanoacrylate Adhesive Provides Efficient Local Drug Delivery

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Biodegradable drug delivery systems have advanced treatment of a wide spectrum of musculoskeletal problems. However, their lack of availability and cost can restrict use. To find an easily available and inexpensive biodegradable implant, we tested a widely used tissue adhesive, n-butyl-2-cyanoacrylate, as a drug-trapping material. We tested vancomycin with commercially available absorbable gelatin-sponge pieces as the scaffold. We evaluated the in vitro and in vivo drug release profiles and in vivo inflammatory response. A mouse muscle pouch model was used for in vivo evaluations. The released vancomycin level was measured by fluorescence polarization immunoassay technique, and a leukocyte count-based grading system was used to evaluate inflammatory response. Our findings suggest the proposed implant provides effective drug release for as much as 42 days in vitro and 14 days in vivo. The presence of n-butyl-2-cyanoacrylate led to a local inflammatory response which decreased after 3 weeks in the group with less adhesive. These results showed that n-butyl-2-cyanoacrylate could efficiently trap and slowly release a drug when used in the structure of a biodegradable local drug delivery device.

Emerging drug delivery systems promise advances for local treatment of a wide spectrum of musculoskeletal system problems such as nonunions, bone or chondral defects, tumors, and infection prophylaxis in high-risk patients.[5,19–21,25,29] However, their main use is for treating musculoskeletal infections. In such infections, only high parenteral doses of antibiotics can overcome local ischemia and bacteria-produced mucus barriers (biofilms) to achieve effective local concentrations.[4,11,14,16,23] Local drug delivery systems have been developed to eliminate the disadvantages of high-dose parenteral drug administration.[3,6,11,16,22,23,25] Ideally, a local drug delivery system should: (1) provide high local drug concentrations (for antibiotics the level must be greater than the minimum inhibitory concentration [MIC] value and should not promote development of drug-resistant bacterial strains by an additional low drug release period); (2) provide drug release over a long time with a linear curve; (3) be biodegradable without requiring a second operation for removal; (4) avoid adverse effects on healing processes and encourage new tissue formation; (5) provide easy handling and production to allow a wide range of different drug dosages; (6) not lead to allergic reactions or excessive local inflammatory responses; and (7) be inexpensive and widely available.[3,6,11,16,23,25,28]

The most widely used implant in orthopaedic surgery is antibiotic-impregnated polymethylmethacrylate (PMMA), but biodegradable systems also are used.[3,4,6,11,14,16,23,28,34,41] Polymethylmethacrylate has advantages such as commercial availability, possibility of handmade production in the operating room, and structural solidity to support bone defects or joint spaces. Disadvantages include being restricted to thermoresistant drugs, poor drug release, and requiring a secondary operation for removal because it is not biodegradable.[3,4,6,11,14,16,23,28,42] Biodegradable implants such as polymers of lactic or glycolic acid and calcium sulfate, which are commercially available and provide better characteristics than PMMA,[28,41,42] still have important disadvantages such as lack of availability for urgent conditions, lack of an alternative drug option other than the loaded one, and expense. Other biodegradable systems such as antibiotic-impregnated bone grafts or

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collagens, which allow intraoperative drug impregnation, provide only short periods of drug release.[4,14,34,42]

We hypothesized that n-butyl-2-cyanoacrylate (NBCA) (0.5 mL) (Histoacryl, Aesculap, Tuttlingen, Germany), a biodegradable tissue adhesive, could efficiently trap and release a drug when used in the structure of a local delivery implant. We also tried to determine the changes in the severity of the tissue inflammatory response in accordance to the volume of used NBCA.

MATERIALS AND METHODS

Different combinations of NBCA and vancomycin (1 g) (Vankomisin HCL Abbott, USP, Abbott, Rungis Cedex, France) mixtures were loaded to constant volumes of absorbable gelatin-sponge (Spongostan Standard, Johnson & Johnson, Skipton, UK) pieces as the carrier scaffold of the implant. In vitro tests were performed to determine the elution characteristics of five different combinations. For this reason, a buffer system was used to mimic the seroma that forms around the implants in living tissue. A mouse muscle pouch model[4] was used to evaluate in vivo local and systemic drug release profiles and the severity of the tissue inflammatory response to three different combinations. The released vancomycin levels in buffer and tissue samples were measured quantitatively by the fluorescence polarization immunoassay (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany) technique. A leukocyte count-based grading system[8,12] was used to evaluate muscle tissue inflammatory response. Vancomycin was selected because of its wide use in orthopaedic drug delivery systems and the availability of biochemical level measurement tests. Because of the stickiness of the NBCA, a carrier scaffold was necessary to provide a shape for the implant and to make application easier. For this reason, we used an absorbable gelatin sponge. It is a widely used local hemostatic agent that is well tolerated in vivo and does not impair wound healing.[1]

Cyanoacrylates are biodegradable, hemostatic, and nonallergic tissue adhesives with local antibacterial properties.[2,9,13,36,38] Surgical and clinical use of these adhesives are expanding. They currently are used in the treatment of arteriovenous malformations, cerebrovascular problems such as aneurysms, retinal ruptures, corneal perforations, skin wounds, and skin graft placements.[36,38] They also have been used as the surrounding thin wall of the nanoparticle form of colloidal drug carriers, particularly for transdermal, oral, or ocular administrations.[30] Cyanoacrylates stiffen by a slightly exothermic polymerization process that takes 1 to 5 minutes depending on the moisture of the environment.[2,9,13] In a nonbiologic environment these adhesives hydrolyze to cyanoacetate and formaldehyde,[9,36] while in a biologic environment the degradation end products are CO_2 and small amounts of cyanoacetate.[9] The end products of the adhesive are excreted through urine and feces for approximately 1 year.[2,13] The main adverse effect of cyanoacrylates is a local inflammatory response at the implant site.[36,38] This effect is directly related to the degradation rate of the adhesive.[9,36,38] N-butyl-2-cyanoacrylate, which is a newer formulation with a longer alkyl chain and longer half-life (2–4 months), leads to less inflammatory response than the older formulation shorter chain forms of cyanoacrylates.[9,36,38] N-butyl-2-cyanoacrylate is commercially available as sterilized 0.5-mL vials.

We tested one control group and four study groups with drug delivery implant combinations differing in vancomycin dose, NBCA volume, and vancomycin/ NBCA proportion, which were prepared under aseptic conditions. The volume of the gelatin-sponge scaffold was arranged to remain constant (pieces with original shapes of 2 × 4 × 5 mm). The liquid component (NBCA) of the implant was used in two different volumes in the four study groups. In the control group, we used sterilized bidistilled water rather than NBCA. Vancomycin was added for delivery in all five groups in two different amounts. The prepared implant combinations were: (1) V10 or control combination: 20 μL sterilized bidistilled water + 10 mg vancomycin; (2) N5V5 combination: 5 μL NBCA + 5 mg vancomycin; (3) N5V10 combination: 5 μL NBCA + 10 mg vancomycin; (4) N10V5 combination: 10 μL NBCA + 5 mg vancomycin; and (5) N10V10 combination: 10 μL NBCA + 10 mg vancomycin. The combinations were prepared based on three factors. First, The loaded amounts of vancomycin were aimed to be 1250- and 2500-fold of its minimum inhibitory concentration value (4 μg/mL)[32] for standard Staphylococcus aureus strains. These measures were used before,[3,4] and also are the lowest amounts of carried antibiotics in previously examined delivery systems.[23,28,42] Second, the volume of NBCA and the amount of vancomycin were adjusted to use the quantities in commercially available forms. This could ease the future preparation of an implant in the operating room (eg, in one of the combinations, 10 μL NBCA was used with 5 mg vancomycin in which the proportion corresponded to 1 cc or two vials of NBCA and 500 mg or one flacon vancomycin). Third, the volume of the implants was adjusted to fit into a mouse muscle pouch model. We prepared the implants using sterile commercial surgical forms of NBCA and absorbable gelatin sponge and under aseptic conditions to eliminate the necessity for resterilization before the in vivo study. We first prepared several pieces of absorbable gelatin sponge (2 × 8 × 50 mm). Depending on the combination, 100 or 200 mg vancomycin were mixed by 100 or 200 μL of NBCA and applied to the absorbable gelatin sponge sheets. Vancomycin powder was weighed and mixed with NBCA in sterile Petri dishes. The mixture then was applied to both surfaces of the absorbable gelatin sponge sheet using a mini-spatula to provide a relatively homogenous distribution. The mixture was applied using a gloved fingertip until it was completely adsorbed by the gelatin sponge sheet. The dryness of the vancomycin powder and the gelatin sponge sheets prolonged the setting time of the NBCA to approximately 5 minutes,[2,9,13] which allowed enough time for mixing and applying the mixture to the gelatin sponge sheets. The control group was prepared similarly with 200 mg vancomycin and 400 μL sterilized bidistilled water. The prepared sheets were left to dry and cut into 20 equal pieces each. After drying, the control group implants showed retraction to a smaller volume, whereas the other groups showed only flattening to approximately 1 mm thick because of compression from manual drug application (Fig 1).

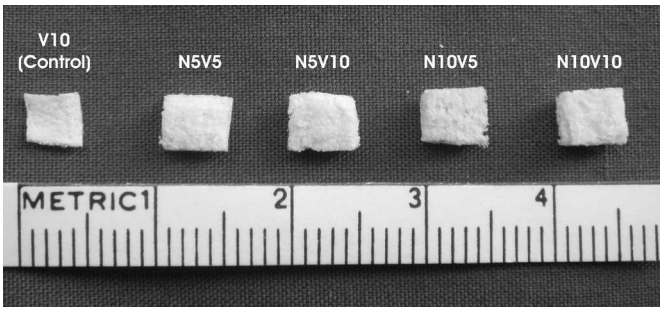

Fig 1. A photograph shows the five different combinations of the prepared drug delivery implants. The implants had a final shape of approximately 1 × 4 × 5 mm, except the V10 combination which was smaller.

The in vitro elution of the drug from the implants were assessed using fluorescence polarization immunoassay, after daily incubation of the implants in 0.5 mL phosphate-buffered saline (PBS) (0.15 mol/L $NaPO_4$, 0.15 mol/L NaCl; pH, 7.2) and a sample mixing technique to reduce the number of samples and also to minimize the effect of unequal NBCA and vancomycin mixture distribution on the gelatin-sponge scaffolds. The PBS volume was determined taking into consideration the volume of seroma formed in vivo conditions. Seroma in the space around a drug delivery implant is composed of serum, inflammatory fluid, and the drug collects.[28] It has been estimated to collect 1 mL seroma per day for beads with a diameter of 8 mm (volume, ~151 mm^3).[28] Because our implants had a maximum volume of 40 mm^3, the possible surrounding seroma volume was calculated as 0.27 mL. We selected 0.5 mL PBS for daily changes because the adequate volume of PBS for each implant of our study should be greater than 0.27 mL to avoid drug hyperconcentration. We randomly selected nine implants from each of the five combinations which were inserted into micro test tubes containing 0.5 mL PBS. The tubes were incubated at 37°C for 24 hours. At the end of each period, drug delivery implants were transferred to a new micro test tube using surgical microforceps. This procedure was repeated daily until the implants lost their integrity and transfer to a new micro test tube was impossible, or for 42 days in combinations without loss of integrity. Micro test tubes were stored at −20°C until used for determining the vancomycin level. Before measuring the vancomycin levels, the tubes' contents were left to thaw at room temperature. The vancomycin levels of the PBS solutions were measured using the fluorescence polarization immunoassay technique on Days 1, 4, 7, 14, 20, 28, 35, and 42. We also recorded the measurements from the day before the last transfer. The nine daily samples of each combination were divided randomly into three subgroups. Equal volumes (100 μL) of PBS from each of the micro test tubes of the subgroups were obtained and collected in another tube. Therefore, three tubes of three samples mixed inside were obtained for each combination daily. The analytic sensitivity of the vancomycin assay was 1.39 μg/mL, defined as the lowest measurable concentration. The within and between-run coefficient of variations of the assay were 3.1% and 3.3%, respectively.

In vivo drug release characteristics and tissue inflammatory response to the NBCA content of the implants were evaluated by inserting the implants in a mouse muscle pouch model.[4] Our primary aim was to evaluate the drug-trapping property of NBCA, therefore we chose a previously described[4] muscle pouch model for in vivo testing. This model has advantages such as ease of performance and the possibility of objective biochemical drug level measurements in muscle tissue.[4] Diseased bone-defect models used to evaluate the therapeutic effects of the drug delivery systems have disadvantages such as difficulty of establishment[27,31,33] and unsuitability for testing tissue drug levels.[37] Such a model could overload the study with specific burdens and make objectively determining characteristics of the implant impossible. Thirty-six adult male Balb-C mice (weight, 26–35 g) were obtained from the experimental animal producing center of our institution. They were cared for under normal laboratory conditions with normal nutrition in 20° to 22°C room temperature and humidity, with 12 hours dark and l2 hours light periods. Experiments were performed in accordance with the guidelines of the National Animal Welfare Law and the Helsinki Declaration of Animal Rights. The animals were divided into three groups of 12. Randomly selected delivery implants of the combinations V10, N5V10, and N10V10 were inserted into mouse muscle pouches. Although the quadriceps muscle was used for testing previously,[4] we used the hamstring muscles as the drug delivery implant site because of the flatness and inappropriate volume of the quadriceps muscle. The animals were anesthetized by intraperitoneal injection of 150 mg/kg ketamine (Ketalar 10 mL, Eczacibasi, Luleburgaz, Turkey) and 6 mg/kg xylazine (Basilazin 2%, Bavet, Istanbul, Turkey). After a posterior incision, the right hamstring muscle belly was explored to prepare a pouch wide enough to accept a drug delivery implant. After inserting the implant, the muscle and skin were closed by 5.0 absorbable chromized catgut. Animals were kept under control until after the effects of anesthesia completely wore off. No postoperative pain medication was given because of the simplicity of the surgical procedure, and to prevent interaction with the inflammatory response to the implants. The mice were observed daily for any local signs of infection, inflammation, or reduced ambulation. No signs of these problems were seen in any of the mice. Two animals from each group were randomly selected on Days 1, 4, 7, 14, 21, and 35. Under high-dose anesthesia (250 mg/kg ketamine and 10 mg/kg xylazine), the thorax was opened to sacrifice the animals by cardiac blood aspiration. The blood samples were left at room temperature to clot and then were centrifuged at 4000 rpm for 5 minutes. The sera of the samples were collected and stored at −20°C until measurement. The skin and the surgically treated extremities were examined for signs of infection or inflammation, and the drug delivery implants were excised with the surrounding muscle tissue cuff. The excised muscle was approximately 1 to 2 mm thick. One-half of the muscle tissue then was dissected from the implant and stored in micro test tubes at −20°C for later measurement of the vancomycin level. The other ½ of the muscle tissue with its drug delivery implant was fixed in 10% formalin solution for histologic examination.

Biochemical measurement of the vancomycin level in the mouse serum and muscle tissue samples was performed using

fluorescence polarization immunoassay. The sera were tested after thawing at the room temperature. Measurement of the tissue vancomycin level was performed three times in each of the two muscle samples to provide a better sampling distribution. For this purpose, the samples were left at room temperature to thaw. The muscle tissue of each animal then was divided into the equal parts. Randomly selected parts of each muscle tissue were gathered with another randomly selected part from the other mouse's muscle. The samples were weighed (approximately 100 mg total weight), divided into small pieces, and transferred to a micro test tube with 0.5 mL PBS and left to be macerated at 37°C for 12 hours. After filtration, the vancomycin level was measured using fluorescence polarization immunoassay. The vancomycin level then was calculated per milligram of muscle tissue.

The inflammatory response to the implants was evaluated using a histologic leukocyte count-based grading system.[8,12] The muscle tissue samples were embedded in paraffin, and sections at 3-μm intervals were obtained and stained by hematoxylin and eosin. Sections that showed implant and muscle tissue were selected for microscopic study. In each section, 10 separate high-power magnification (×400) fields from the locations close to the junction of the muscle tissue with the drug delivery implant were studied. The degree of leukocyte infiltration (polymorphonuclear neutrophils and mononuclear cells) in each high-power field was determined by two histologists (EB and IO) who were blinded to the study. The amounts were graded using the following scale: Grade 0: no extravascular leukocytes; Grade 1: less than 20 leukocytes per high-power field; Grade 2: 20–45 leukocytes per high-power field; Grade 3: greater than 45 leukocytes per high-power field.[8,12] Therefore, scales of 20 high-power fields for each day of study in each study group were available for additional statistical analysis. Analyses were performed using Mann–Whitney U tests to compare the results between different groups and Kruskal–Wallis H tests to evaluate results within a single group. A linear regression test was performed to compare in vivo durability and drug release times of different groups. Probability values less than 0.05 were considered significant. Commercial software (SPSS for Windows, V 9.05, SPSS Inc, Chicago, IL) was used for the statistical analyses.

RESULTS

Drug release profiles from in vitro and in vivo parts of the study revealed that NBCA could efficiently trap and release vancomycin (Tables 1 and 2; Figs 2 and 3). In vitro tests revealed that adding NBCA resulted in increased structural durability of the implant and prolonged elution rate of the vancomycin (Table 1; Fig 2). Daily transfer of the drug delivery implants to new micro test tubes was possible until Day 20 in the V10 group, and until Day 39 in the combination N5V5 and N5V10 groups. The study ended at Day 42 for combinations N10V5 and N10V10, although the implants in these groups were still durable and transferable. The V10 combinations had a high vancomycin elution during the early period and then a rapid decrease in elution until Day 20. In the other four combi-

TABLE 1. Levels of In Vitro Eluted Vancomycin*

Combination	Day 1	Day 4	Day 7	Day 14	Day 19	Day 20	Day 28	Day 35	Day 38	Day 39	Day 41	Day 42
V10 (Control)	1410.2 ± 98.8	45.6 ± 10	16.3 ± 6.3	12.1 ± 3.4	6.1 ± 0.7	3.8 ± 0.4	—	—	—	—	—	—
N5V5	147 ± 73.2	190.8 ± 9.8	195.7 ± 32.6	148.4 ± 4	—	143.9 ± 4.3	109.4 ± 8.5	97.5 ± 5.8	44.9 ± 4.7	29.5 ± 13.7	—	—
N5V10	219.9 ± 60.4	436.4 ± 72.3	567 ± 86.2	316.1 ± 90	—	146.4 ± 3.1	96.2 ± 2.8	61.4 ± 6.1	40.5 ± 3.9	20.6 ± 1.5	—	—
N10V5	62.7 ± 16.6	222.9 ± 25.6	176.7 ± 1.5	155.9 ± 14.9	—	142.8 ± 4.9	110 ± 5.1	70 ± 5.1	—	—	22.5 ± 3.8	16.5 ± 5.3
N10V10	109.2 ± 15.3	162.3 ± 8.3	188.5 ± 10.9	182.1 ± 0.9	—	146.2 ± 5.4	98.8 ± 3	58 ± 3.1	—	—	35 ± 9	33.8 ± 4.5

*μg vancomycin per mL of phosphate buffer system; mean ± standard deviation

TABLE 2. Levels of In Vivo Released Vancomycin*

Combinations	Day 1	Day 4	Day 7	Day 14	Day 21	Day 28	Day 35
V10 (Control)	22.4 ± 10.1	UD	UD	UD	UD	UD	UD
N5V10	25.3 ± 25.3	3.1 ± 1.8	3.3 ± 0.8	UD	UD	UD	UD
N10V10	10.1 ± 1.4	6 ± 1.4	7.3 ± 6.2	5.6 ± 0.4	UD	UD	UD

UD = undetectable level of vancomycin; *μg/mg of muscle tissue; mean ± standard deviation

nations, the elution rate of the vancomycin was slower and more linear (Table 1; Fig 2). The decreasing standard deviation and mean rates of the eluted vancomycin levels revealed a more homogeneous drug elution during the advancing days of the study (Table 1). In vivo tests revealed that a higher volume of NBCA content resulted in longer durability ($p < 0.05$) and drug release times ($p < 0.05$) (Table 2; Fig 3). However, local placement of the implant components led to an immeasurable serum vancomycin level. During the muscle sampling procedure, N10V10 implants were mostly intact even at Day 35. The N5V10 implants preserved their integrity until Day 21. They were fragmented but still visible at Day 35. The V10 implants appeared fragmented at Days 14 and 21, and were almost invisible at Day 35. The level of vancomycin in the mouse muscle tissue with V10 combination was only detectable the first day after insertion. In the N5V10 group, Day 7 was the last day of detectable vancomycin levels. In the N10V10 group, tissue vancomycin levels could be detected until Day 14. On Days 21 and 35, the level of tissue vancomycin was not detectable in any group (Table 2; Fig 3). The level of vancomycin was not detectable (< 1.39 μg/mL) in any of the mouse serum samples.

Our histologic study results showed that adding NBCA led to an inflammatory response of the host tissue which did not change in severity based on the volume of NBCA, but in the group with less NBCA this response decreased after 21 days (Fig 4). Leukocyte infiltration was more evident at the site of the loose connective tissue formed around the drug delivery implants or their remnants (Fig 5). We also observed leukocyte infiltration inside the pores of the implants (Fig 5). The severity of the inflammatory response was less ($p < 0.05$) in V10 group than in the N5V10 group on Days 7, 14, 21, and 35. Also, the severity of the inflammatory response was less ($p < 0.05$) in the V10 group than in the N10V10 group at Days 14, 21, and 35. There was no difference between the results of groups N5V10 and N10V10. The V10 group's inflammatory response decreased ($p < 0.05$) on Day 14 compared with on Day 7, and on Day 21 compared with on Day 14. In the N5V10 group, the inflammatory response on Day 35 day was less ($p < 0.05$) than on Day 21. There were no differences in the results of the N10V10 group (Fig 4).

DISCUSSION

The desirability of a biodegradable, inexpensive, and easily handled drug delivery system that can be prepared in the operating room led us to test a combination of com-

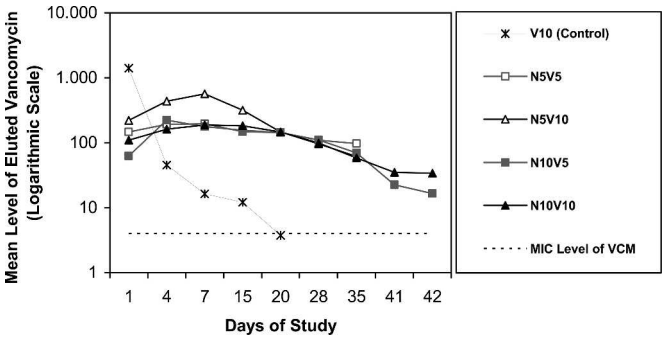

Fig 2. A graph shows the five in vitro vancomycin elution curves from each drug delivery implant. The minimum inhibitory concentration (MIC) of vancomycin for standard Staphylococcus aureus strains (4 μg/mL) is shown as a straight shaded horizontal line. Combinations containing NBCA show a more linear elution than V10, and more content NBCA leads to longer elution times in N10V5 and N10V10 combinations.

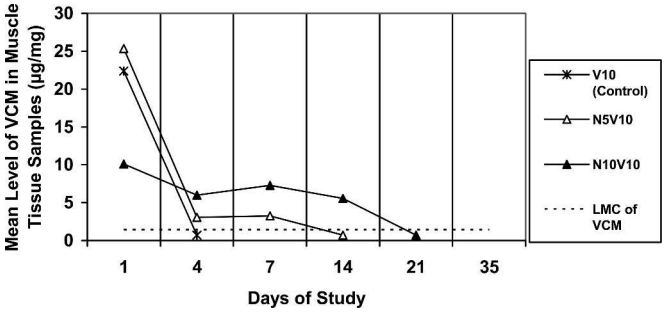

Fig 3. A graph shows the three in vivo vancomycin release curves from drug delivery implants with different NBCA volumes. The lowest measurable concentration of vancomycin by the fluorescence polarization immunoassay technique (1.39 μg/mL) is shown as a straight shaded horizontal line. Undetectable vancomycin levels developed after Day 1 for the V10 combination and after Days 7 and 14 for N5V10 and N10V10 combinations, respectively.

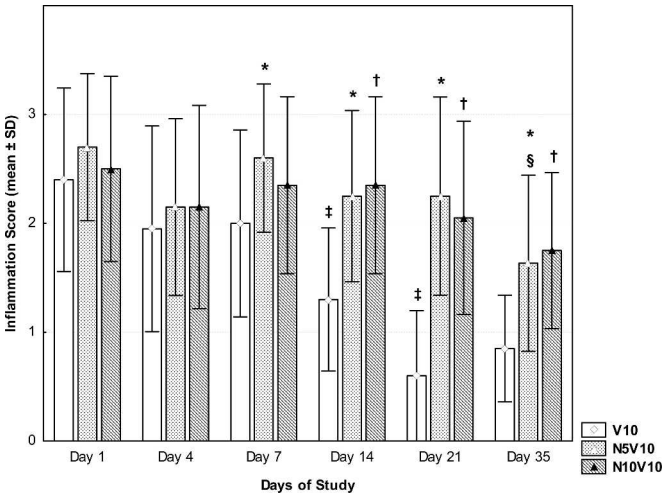

Fig 4. A graph shows the histologic results based on of the mean values of the inflammation scores (*significant difference between the results of V10 and N5V10 groups; †significant difference between the results of V10 and N10V10 groups; ‡significant difference between V10 group results and results on an earlier study day; §significant difference between N5V10 group results and results on an earlier study day). The N5V10 and N10V10 groups showed more evident inflammatory responses than the control group on advanced days of study. There was a decrease in inflammatory response by Days 14 and 21 in the control group and by Day 35 in the N5V10 group.

mercially available sterile forms of NBCA, vancomycin, and absorbable gelatin sponge materials. The first step was evaluating the in vitro elution characteristics of the various combinations, and the second step was evaluating the in vivo drug release and inflammatory response to the combinations based on their volume of NBCA.

Our study has several limitations including the unpredictable amount and distribution of NBCA and vancomycin mixture over the absorbable gelatin-sponge scaffold, the limited number of fluorescence polarization immunoassay tests of the vancomycin, and the limited number of animals used in the vivo test. We tried to overcome the nonhomogeneous distribution of the mixture over the scaffolds by randomized sampling of implants, PBS, and the muscle tissues. Mixing and reducing the number of in vitro samples helped to limit the number of fluorescence polarization immunoassay tests of the vancomycin. Despite carefully transferring the NBCA and vancomycin mixture onto the gelatin sponge sheets, some mixture remained on the Petri dishes. These factors prevented us from determining the exact amount of NBCA or vancomycin in each implant group. For this reason, we reported the amounts of daily vancomycin release instead of the percentage of released vancomycin from the total amount of vancomycin. We think this restriction does not decrease the practical convenience of the proportional amounts. Limited numbers of animals and distribution through the days of the study prevented testing the muscle tissue vancomycin level changes at shorter intervals and establishing the fate of the inflammatory response on a longer time scale. Similar to other studies which require multiple assessments on several times, a statistical analysis was not performed for evaluation of biochemical vancomycin levels. However, the standard deviation and mean rates of the vancomycin levels in almost all of the study groups were within acceptable (< 50%) or ideal (< 30%) limits of variability (Tables 1 and 2).

The in vitro and in vivo objective biochemical measurements provide evidence for the ability of NBCA to trap the vancomycin powder and then release it slowly during degradation. The effective drug release periods were 42 days in vitro and 14 days in vivo. These times occur within the range of the release periods of other delivery systems (Table 3).[3,7,11,18,26,28,39–42] The in vivo drug release time of the proposed implant, although shorter than its in vitro elution time, was twice the reported effective release period of PMMA beads (maximum, 7 days)[14] and 3.5 times that of collagen-based carriers (maximum, 4 days).[14,34] A reason for the difference between the in vivo and in vitro vancomycin release times was the high metabolic rate of the muscle tissue leading to rapid biodegradation of the drug delivery system. The implant could provide a longer release time when inserted in spaces like cavitary bone defects. In these cavities, the implant would be coated by seroma, which would act on the implant in a manner similar to that of the PBS solution, rather than in a manner of adjacent healthy muscle tissue with a high metabolic rate. When compared with the original article on the muscle pouch model,[4] intentionally isolating the muscle tissue from the drug delivery system might have had a role in the lower levels of drug measurement and shorter period of drug release. In that article, a microsphere form of delivery system was used and the measurements also included the intramuscular unreleased drug particles.[4]

Histologic analysis of muscle tissue samples suggested NBCA led to a local inflammatory response and provided evidence for a decrease in the severity of response after 3 weeks of insertion. NBCA causes a lower inflammatory response than short-chain cyanoacrylates.[38] The response is more severe in well-vascularized soft tissues like muscle compared with tissues like cartilage or bone.[38] The mild histotoxicity of NBCA attributable to the inflammatory response is clinically negligible.[9,36,38] Although a local inflammation period longer than 3 weeks is considered to indicate the inserted implant is not biocompatible,[4] long-standing responses are reported against frequently used orthopaedic biodegradable materials like poly-L-lysine–coated alginate (28 days),[40] calcium phosphate (6

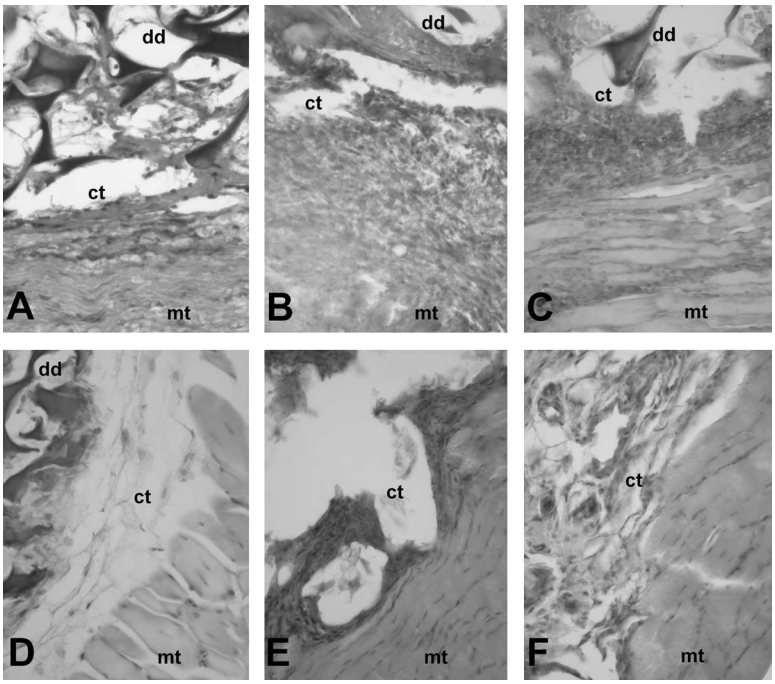

Fig 5A–F. Histologic samples show the drug delivery implant and muscle tissue samples from three mouse groups (Stain, hematoxylin and eosin; original magnification, ×400). The upper row is from Day 7 and the lower row is from Day 35. Leukocyte infiltration is more evident at the site of the loose connective tissue that formed around the implants or their remnants. A decrease in leukocyte infiltration with time was more evident in groups V10 (A and D) and N5V10 (B and E) than in group N10V10 (C and F) (dd = drug delivery implant; mt = mouse muscle tissue; ct = loose connective tissue).

weeks),[23] polyglycolide (6 months),[10] and poly-L-lactide (4–5 years).[10] However, inflammatory response is the cost paid for the advantages of the biodegradable implants.[10] We did not identify the end of the inflammatory response, but we think complete degradation of the proposed implant would end the stimulus to inflammation. Less inflammatory response in a diseased bone cavity than in healthy muscle tissue is also a fact that should be considered. It is not known whether a limited inflammatory effect is useful in the treatment of chronic conditions like osteomyelitis.[17,24,35]

The advantages of our proposed drug delivery system outweighed its disadvantages. The implant: (1) offers slow and linear local drug delivery, (2) is biodegradable and does not require secondary operations for removal or act as a physical barrier to tissue growth and regeneration; (3) can be made from sterile surgical products; (4) can be prepared practically and easily in operating rooms for urgent conditions such as highly contaminated open fractures, without the need for secondary sterilization; (5) slight exothermic polymerization and local antibacterial properties of NBCA[2,9,13] may provide preference in carrying a wide spectrum of drugs for local applications; and (6) provides an inexpensive local delivery system especially for research purposes. Its disadvantages are biocompatibility issues of the NBCA component and its structural

TABLE 3. Results of Drug Delivery Devices

Authors	Device Composition	Effectivity or Release Times (Day)	
		In Vitro	*In Vivo*
Ambrose et al[3]	Poly (DL-lactic-co-glycolic acid) + poly (ethylene glycol) + tobramycin	28	28
Benoit et al[7]	Plaster of Paris coated with poly lactide-co-glycolide + vancomycin	35	28
Buranapanitkit et al[11]	Hydroxyapatite + plaster of Paris + Chitosan + vancomycin	90	ND
Joosten et al[18]	Hydroxyapatite + vancomycin	20	ND
Magnan et al[26]	Collagen-sealed dacron + vancomycin	No adequate effectivity	ND
Mader et al[28]	Polymethylmethacrylate + vancomycin	12	ND
Ueng et al[39,40]	Poly-L-lysine-coated alginate + vancomycin	17	21
Wernet et al[36]	Collagen sponge + gentamicin	ND	5
Wichelhaus et al[42]	Plaster of Paris + vancomycin	10	ND
Current authors	N-butyl-2-cyanoacrylate + absorbable gelatin sponge + vancomycin	42	14

ND = not detected

weakness limiting use at sites requiring a strut-interposed implant. Regarding the above mentioned advantages and disadvantages, the vancomycin-carrying implant developed in the present study could serve as a local management option for Types 1 to 3 osteomyelitis in Cierny-Mader anatomic classification.[15] These types of osteomyelitis do not lead to instability before or after surgical intervention.[15] The vancomycin implants also might be used for treatment of highly contaminated open fractures, infected bone defects stabilized by rigid external fixators, and spondylodiscitis. Our results provide evidence of the efficacy of a combination of NBCA with an absorbable gelatin sponge when used as a biodegradable drug delivery system without systemic increases in the level of the delivered drug. It probably could be used to provide numerous drugs. This implant is the first biodegradable implant with a sustained drug release longer than PMMA which, similar to PMMA, can be prepared in the operating room using commercially available sterilized surgical materials.

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