Hernia-repair prosthetic devices functionalised with chitosan and ciprofloxacin coating: Controlled release and antibacterial activity

Paola Avetta[a,†], Roberto Nisticò[a,b,*,†], Maria Giulia Faga[c], Domenico D'Angelo[d], Elisa Aimo Boot[d], Roberta Lamberti[e], Selanna Martorana[e], Paola Calza[a,b], Debora Fabbri[a], Giuliana Magnacca[a,b]

[a] University of Torino, Department of Chemistry, Via P. Giuria 7, 10125 Torino, Italy
[b] NIS Centre of Excellence, Via P. Giuria 7, 10125 Torino, Italy
[c] CNR-IMAMOTER, Strada delle Cacce 73, 10135 Torino, Italy
[d] Environment Park S.p.A., Plasma Nano Tech, Via Livorno 60, 10144 Torino, Italy
[e] Herniamesh S.r.l., Via F.lli Meliga 1/C, 10034 Chivasso, Italy
* Corresponding author. E-mail: roberto.nistico@unito.it, Ph. +39-011-6707533, Fax: +39-011-6707855
† Both authors contributed equally to this work.

SUPPORTING INFORMATION

Antibacterial activity evaluation tests

Culture media.

i) Suspension medium – 1/500 nutrient broth (1/500 NB). A nutrient broth was prepared by dissolving 3.0 g of meat extract, 10.0 g of peptone and 5.0 g of sodium chloride in 1 L of distilled water and diluting the nutrient broth with distilled water to a 500-fold volume. In this way, pH shall be in the range 6.8 - 7.2. When necessary, an adjustment of the pH by addition of sodium hydroxide or hydrochloric acid was done. Finally, sterilisation was carried out by autoclaving at 121 ± 2 °C for at least 15 minutes. The suspension was stocked at 5 ± 3 °C up to one week after preparation.

ii) Slant culture medium (Tryptic Soy agar, TSA) was obtained from Sigma Aldrich. The TSA was prepared in accordance with the manufacturer instructions by pouring the warm medium into a screw-capped test tube. Sterilisation was carried out by autoclaving at 121 ± 2 °C for at least 15 minutes. After sterilisation, the test tube was placed at a slant angle of about 15° to the horizontal in order to allow the solidification of the content.

iii) Plate count agar was obtained from Nissui Pharma, product "Plate Count Agar PCA", certified for detection of aerobic mesophilic bacteria. The composition of 1 L medium was: 2.5 g of yeast extract, 5.0 g of peptone, 1.0 g of dextrose and 15.0 g of agar powder.

iv) Soybean casein digest broth with lecithin and polyoxyethylene sorbitan mono-oleate (SCDLP broth) was obtained from Sigma Aldrich. The SCDLP broth was prepared in accordance with the manufacturer instructions by addition of 1.0 g/L of lecithin to the medium. Sterilisation was carried out by autoclaving at 121 ± 2 °C for at least 15 minutes.

v) Phosphate buffer solution was prepared by dissolving 34.0 g of potassium dihydrogen phosphate in 1 L of distilled water; the pH shall be in the range 6.8 ÷ 7.2. Sterilisation was carried out by autoclaving.

vi) Phosphate-buffered physiological saline solution was prepared by dissolving 8.5 g of sodium chloride in 1 L of distilled water and diluting the solution with the physiological saline one to an 800-fold volume. Sterilisation was carried out by autoclaving.

vii) Physiological saline solution for incubation of the samples was prepared by dissolving 9.0 g of sodium chloride and 1.00 g of Tween 80 in 1 L of distilled water. Sterilisation was carried out by autoclaving.

Pre-culture of *Staphylococcus aureus*.
A disc of *Staphylococcus aureus* was placed in 1 mL of sterile Tryptic Soy broth. The culture was revitalised by incubating for 1 hour at 35 ± 2 °C and then the softened disc was spread around the plate of a slant culture medium (TSA) by means of a sterile inoculating loop, in order to facilitate the isolation of colonies. Incubation was carried out at 35 ± 2 °C for 48 hours. After incubation, this primary culture was stored in refrigerator at 5 ± 3 °C.
After the first 24 hours of contact, samples for the subsequent testing were kept in physiological solution under the same conditions at 35 ± 2 °C.

Preparation of test specimens and inoculum.
Specimens were individually placed in a sterile Petri dish with a diameter size of 90 mm avoiding any contamination with micro-organisms or extraneous organic debris and, after inoculum with the suspension of bacteria each sample was covered by the PP cover specimen (contact surface between test material and bacteria suspension was 1600 mm²).
Using a sterile inoculating loop, one loop of the pre-incubated test bacteria was transferred into a small amount of 1/500 NB, curing that the bacteria were evenly dispersed. The suspension was diluted with 1/500 NB to obtain a bacterial concentration between 2.5 x 10^5 cells mL^{-1} and 1.0 x 10^6 cells mL^{-1}: this resulting suspension was our test inoculum.
The viable bacteria were enumerated by performing10-fold serial dilutions of the 1/500 NB; 1 mL of each dilution was placed into separate sterile PCA dishes. All plating was performed in duplicate and bacteria were incubated at 35 ± 2 °C for 24 hours. Micro-organisms on each plate were counted and the average value on two plates was calculated in order to obtain the concentration of the bacterial suspension for inoculums.

Equation SI-1
The number of viable bacteria recovered per cm^2 of test specimen (N) can be calculated as follows
$N = (100 \times C \times D \times V)/A$
where C is the average plate count for duplicate plates, D is the dilution factor for the plates counted, V is the volume in mL of SCDLP broth added to the specimen (10 mL) and A is the surface area in mm^2 of the cover film (1600 mm^2).

Inoculation an incubation of test specimens.
Each test specimen was placed into a separate sterile Petri dish by adding 0.4 mL of the test inoculum (bacterial concentration 4.4 x 10^5 cfu/test specimen) onto the specimen surface and a section of PP (4 x 4 cm) was used to cover the test inoculum and gently pressed down on the film. Thus the test inoculum spread to the edges, but not leaked beyond the edges of the film. Incubation of the inoculated test specimens was done at a temperature of 35 ± 2 °C and a relative humidity of 90 % for 24 hours.
Bacteria were recovered from untreated test specimens 1 h after inoculation by processing half of the blank samples by adding 10 mL of SCDLP broth. This value was used to determine the recovery rate of the bacteria from the test specimens under investigation, ensuring that the neutraliser SCDLP broth completely washed the specimens.

Table SI-1. *Staphylococcus aureus* test inoculum concentration: 1.1 x 10^6 cfu mL^{-1}.

Samples	10^{-1} dilution[a]	10^{-2} dilution	10^{-3} dilution	10^{-4} dilution
PCA plate A1	> 300	> 300	> 300	109
PCA plate A2	> 300	> 300	> 300	115

[a]Dilutions are reported in cfu mL^{-1}.

Table SI-2. *Staphylococcus aureus* test inoculum concentration: 9.2 x 10^5 cfu mL^{-1}.

Samples	10^{-1} dilution[a]	10^{-2} dilution	10^{-3} dilution	10^{-4} dilution
PCA plate B1	> 300	> 300	> 300	88
PCA plate B2	> 300	> 300	> 300	95

[a]Dilutions are reported in cfu mL^{-1}.

Table SI-3. Untreated test specimens 1 hour after inoculation.

Samples	Inoculum SCDLP	10^{-1} dilution[a]	10^{-2} dilution	10^{-3} dilution
Blank A1	> 300	> 300	> 300	31
Blank A2	> 300	> 300	> 300	33
Blank A3	> 300	> 300	> 300	35
Blank B1	> 300	> 300	> 300	37
Blank B2	> 300	> 300	> 300	31
Blank B3	> 300	> 300	> 300	38

[a]Dilutions are reported in cfu mL^{-1}.