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Controlled release of vancomycin from cross-linked gelatine

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Abstract This paper explores the possibility of using biodegradable cross-linked gelatines as antibiotic devices for a long-term elution (80 days). Capillary electrophoresis (CE) has been utilized to evaluate the mass percentage of vancomycin and gelatine contemporary released from differently cross-linked vancomycin loaded gelatine samples in an elution time ranging from 24 to 1920 h. While the solubilization kinetic of gelatine samples differently cross-linked can be very close described by the simplified Higuchi model, the vancomycin release kinetic is contemporary governed by both the Fickian diffusion process trough the gelatine matrix network and the dissolution process of the matrix due to its degradation. Comparing the antibiotic eluting kinetics from gelatine at diverse cross-linking degree we observed that the degradation of the proteic matrix appears to have a minor influence in the drug release control. Vancomycin released from all the gelatine partially cross-linked samples results active against *Staphylococcus aureus* and *Streptococcus faecalis* which represent the most pathogens commonly isolated in orthopaedic infections. Vancomycin overcomes the minimum inhibitory concentration for both the bacteria in the whole range of elution time. Cross-linked gelatine devices appear to represent a useful biodegradable delivery system for local anti-infective therapy in arthoplasty.

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1 Introduction

Controlled delivery systems are designed to release definite amounts of therapeutic agents to specific target over extended duration time and with a definite kinetic. The local delivery eliminates the risks at side effects associated with oral or parenteral therapies such as systemic toxicity. It also improves the efficacy of the treatment by achieving higher drug concentrations at target site than those reachable with systemic administration. The localized delivery of antibiotics from an implantable bone replacement material offers considerable advantages over the traditional methods. Polymethylmethacrylate (PMMA) beads and cements are today's antibiotic delivery carrier clinically used [1, 2, 3]. Antibiotic is released as it desorbs from the PMMA surface or diffuses through the polymer matrix or capillaries as fluid penetrates into the PMMA carrier [4]. However this kind of carrier provides an amount of therapeutic agent which can be not enough to cover all the prolonged period of time needful to treat bone infection. Moreover exothermic polymerization of this carrier could induce thermal degradation of antibiotic. Furthermore PMMA is non-bioresorbable and unfortunately non bone-bioactive. The modern drug delivery biomaterials for topical applications during surgery in order to avoid following explantation, have to be not only biocompatible, but also bioresorbable and osteoconductive. These desirable properties have been obtained in inorganic drug delivery systems using calcium phosphate ceramics [5, 6], apatite [7], porous hydroxyapatite [8], biphasic calcium

phosphates [9], biomimetic apatitic nano-crystals [10, 11] and silica xerogels [12].

In the same way as the PMMA is the most studied among non-bioresorbable synthetic polymers poly(glycolic acid), poly(L-lactic acid) are the most considered among the biodegradable ones [13, 14]. Among natural polymers studied such as biomaterials in controlled-release applications (i.e. collagen, alginate, fibrin, etc) certainly collagen is the most considered for its unique possibilities of allowing the synthesis of real biomimetic materials [15, 16, 17]. Gelatine which is obtained by physicochemical degradation of collagen does not express antigenicity like all the common proteins and it is completely bioresorbable in biological environments. Gelatine is commonly used for pharmaceutical, biomedical and surgical applications because of its biodegradability [18, 19, 20] and biocompatibility in physiological environments [21, 22]. The extensively investigated gelatine sol-gel transformation is due to a conformational disorder-order transition of the protein chains forming a thermoreversible hydrogels [23, 24]. Different physical and chemical cross-linking methods have been described for gelatine in order to decrease its solubility in aqueous medium and improve its thermal and mechanical behaviour in physiological conditions, as well as control its rate of biodegradability [25, 26]. Physical cross-linking methods are just efficient and their cross-linking effect on gelatine can be hardly controlled [27]. On the other hand control over the cross-linking density of gelatine is possible using aldehydes, isocyanates, polyepoxides, carbodiimides and acyl-azides to chemically bridge the gelatine functional side groups such as free carboxylic acid residues or amine groups between adjacent protein fragments [28]. Glutaraldehyde (GA) is by far the most widely used bifunctional agent to prepare gelatine hydrogel with specific extents of cross-linking, which can be increased by either prolonging the cross-linking reaction period or increasing the GA concentrations [29].

The availability of gelatine cross-linked by GA at different extent can contribute to obtain a biomaterial particularly suitable for medical applications because of the good biocompatibility of the hydrogels and the possibility of adapting the degradation rate of gelatine to a specific application [30]. Gelatine hydrogels cross-linked by GA have been successfully proposed as biomolecules release [31, 32] and drug delivery [33] systems with controlled diffusion and can represent a good carrier for antibiotics during the implantable bone replacements in arthoplasty.

Vancomycin, one the most studied antibiotic, has a broad spectrum and efficiency on staphylococci and is of particular interest in orthopaedic surgery where it can be associated to bone fillers commonly used to repair bone defects [34]. In this way systemic toxicity and side effects following parenteral administration of antibiotics can be avoided [35].

The aim of this study is to control the kinetic release and the antibacterial efficacy of vancomycin for a period up to 80 days by using a bioresorbable gelatine matrix at different cross-linking extent in order to establish a new delivery system for local anti-infective therapy in arthoplasty. The use of antibiotic loaded gelatine is based on the principle that the antibiotic is totally released during the gelatine degradation. In fact the antibiotic will gradually be released from the gelatine during the contemporary gelatine biodegradation in such a way that the vancomycin local level vastly exceed the minimal inhibitory concentration of most susceptible pathogens. The determination of vancomycin released which is strongly hindered by the contemporary gelatine degradation was performed by capillary electrophoresis. High efficiency, fast analysis, and low cost operation of capillary electrophoresis (CE) have been viewed as promising features making CE an alternative technique to liquid chromatography for therapeutic drug monitoring in recent years.

2 Materials and methods

2.1 Chemicals reagents

Glutaraldehyde (water solution 25%) and glycine were from Sigma-Aldrich S. r. l. Physiological solution (sodium chloride 0,9 g/100 ml) was from Fresenius Kabi. Vancomycin was from Abbott. Gelatine was from Sanofi Bio-Industrie. Sodium dodecyl sulphate (SDS), Dimethyl Phthalate (DEP) were from Fluka (Buchs, Switzerland). Phosphoric acid, sodium hydroxide, were purchased from Carlo Erba Reagenti (Milan, Italy). Water used for the preparation of solutions and running buffers, was purified by a Milli-RX apparatus (Millipore, Milford, MA, USA).

2.2 Vancomycin loaded gelatine

Preparation of vancomycin loaded gelatine at different cross-linked degrees was performed laying 1 g of lyophilized gelatine in a Petri capsule and successively pouring 10 ml of a vancomycin water solution (0.2 mg/ml) at the edge of the gelatine. The capsules were maintained in an oven at 45 °C until to obtain a totally liquid mixture. After this, the capsules were put at room temperature for 15–20 min until the gelification was completed. About 12 ml of GA water solution ranging from 0.035% to 1% (w/w) for each sample were added onto the gelatine-antibiotic composites in order to obtain different cross-linking degrees. Then the capsules were closed and put in a laminar flow

hood for 24 h. The cross-linked samples were washed with 0.1 M glycine aqueous solution and then immersed in an aqueous 0.1 M glycine solution at 37 °C for 1 h to block residual GA aldehyde groups and then rinsed with bidistilled water for 5 times to eliminate GA excess. The composites were kept in a fume hood until obtaining thin homogeneous samples.

2.3 Determination of the gelatine cross-linking extent

The extent of cross-linking of gelatine samples was determined by a UV assay of uncross-linked ε-amino groups before and after cross-linking [30]. Following reaction with 0.5% TNBS (Trinitrobenzene sulfonate), gelatine was hydrolyzed with 6 M HCl, and extracted with ethyl ether. The absorbance of the diluted solution was measured at 346 nm using a Cary 5 UV-Vis-NIR spectrophotometer (Varian, Palo Alto, CA) against blank. The relationship between absorbance and moles of ε-amino groups per gram of gelatine is:

$$\frac{\text{moles of } \varepsilon \text{ - amino groups}}{\text{g gelatin}} = \frac{2(\text{absorbance})(0.020\,\text{L})}{(1.46 \times 10^4\,\text{L/mol cm})(b)(x)}$$

where 1.46×10^4 L/mol cm is the molar absorptive factor of TNP-lys, (b) is the cell path length in cm, and (x) is the sample weight in grams and 0.020 L represents a dilution factor.

2.4 Vancomycin release from gelatine

The samples of Vancomycin loaded gelatine were immersed in 20 ml of physiological solution inside sterile containers in a bascule bath at 37 °C for period of time ranging from 1 to 80 days. At scheduled times sampling was performed: each container was placed in a vortex for 1 min and an aliquot of 2 ml of physiological solution from each container was transferred into conical polyethylene eppendorf tube. The tubes were kept in a freezer at –20 °C until analyses were performed. In order to keep the same volume during the release, the initial volume of the physiological solution with the immersed gelatin sample was replaced with 2 ml of fresh solution.

2.5 Capillary electrophoresis analysis

The release of vancomycin and gelatine was monitored by capillary electrophoresis capillary (Bio-Rad Biofocus 3,000 Capillary Electrophoresis System; Hercules, CA, USA) was equipped with a multiwavelength detector; the data were collected on a PC using the Integration Software by Bio-Rad. An untreated fused silica capillary tube (BioCAP^{TM} Bare Silica Capillary, Bio-Rad) of 50 μm internal diameter with an effective length of 195 mm (total length of 240 mm) was used. The experimental temperature was constant at 28 °C, the detection wavelength was at 210 nm and the separation voltage was at 8 kV. Injection of the samples were performed hydrodynamically at 4 psi × s. The running buffer was a borate buffer (pH 12.0) (25 mM borate, 75 mM SDS). The buffer was filtered by 0.45 μm filter (Millipore, Bedford, MA) and ultrasonically degassed before use. Capillaries were conditioned by flushing sodium hydroxide 1.0 M, sodium hydroxide 0.1 M and finally water for 10 min. Between the runs the capillary was simply rinsed by flushing electrophoretic buffer for 3 min. Dimethyl Phthalate (DEP) was used as internal standard. Triplicate injections were made for each solution.

2.6 Vancomycin release data analysis

The percentage of vancomycin released was plotted as a function of $t^{1/2}$ according to the simplified Higuchi model following the expression:

$$f_t = K_H t^{1/2}$$

where K_H is the Higuchi dissolution constant of which differently defined by different author and theories. Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent [36].

2.7 Calculation of rate constant k and Half-Life $t_{1/2}$

To prove the assumption, that all our release experiments obey a first-order release rate, $\ln([A]/[A]_0)$ was plotted against time, giving a straight line function with slope –k, where A is the concentration of the vancomycin and k is the rate constant. The equation of first-order release can be written as:

$$\frac{d[A]}{[A]} = -kdt$$

which can be integrated directly, because k is a constant independent of t, giving:

$$[A] = [A]_0 e^{-kt}$$

where $[A]_0$ is the initial concentration of A.

This means that the $([A]/[A]_0)$ ratio decays exponentially as a function of time. The rate constant k in a first order reaction can also be determined from the half-life $t_{1/2}$.

To calculate the half-life $t_{1/2}$, the equation of first-order release can also be written as:

$$kt_{1/2} = In\frac{[A]_0}{[A]_0/2}$$

hence

$$t_{1/2} = \frac{In2}{k}$$

The rate constant k was determined for each experiment by calculating the slope of the function for ln[A] plotted against time.

2.8 Vancomycin antimicrobial bioactivity

The antibiotic activity of released vancomycin was checked by an in vitro experiment using strains of *Staphylococcus aureus* (ATCC 2921) and *Streptococcus faecalis.* Culture mediums were made for each strain. These mediums were seeded respectively, in Mueller Hinton (Oxoid) agar plates for *S. aureus* and in KF Streptococcus for *S. faecalis*, with a 10 µl calibrated loop as control plates, 100% bacterial growth.

We tested the bioactivity of the released antibiotic after 1920 h (80 days), for each degree of cross-linking. Bacterial colonies were transferred into tubes of sterile water and vortexed, and the turbidity was adjusted to 0.5 McFarland Standard (10^8 CFU/ml) and to <<0.5 McFarland Standard (10^5 CFU/ml). 760 µl of physiological solution with the released antibiotic (sample 0.035%, 0.070%, 0.140% and 1%) were combined with 2.5 mL of 0.5 and <0.5 McFarland Standard *S. aureus* suspension and incubated at 37 °C for 48 h. Likewise 760 µl of physiological solution with released vancomycin were mixed with 1 mL of 0.5 and <0.5 McFarland Standard *S. faecalis* suspension and incubated at 37 °C for 48 h.

In order to understand if the vancomycin preserves its antibiotic activity, we have evaluated changes in the turbidity of the growth medium. We also tested a control sample made of physiological solution corresponding to 0.2 McF, in fact under this threshold value the inhibition of bacterial growth is effective. For values higher than 0.2 McF bacteria were seeded again to understand if they were still alive or dead.

2.9 Statistical analysis

The degradation and drug-release tests were performed in triplicate, and data were represented as means ± one standard deviation (1 SD). One-way analysis of variance (ANOVA) was carried out to compare the data on each group, and statistical significance was considered at $P < 0.05$.

3 Results

The gelatine-vancomycin samples were treated with GA water solution ranging from 0,035% to 1,000% (w/w) and the relative cross-linking degree (%) has been determined.

The extent of cross-linking of gelatine has been calculated from the moles of free ε-amino groups per gram of gelatine. The results shown in Table 1 indicate that a treatment with 0.035% (w/w) GA is enough to cross-link about 55% of the ε-amino groups. The cross-linking extent raises increasing GA concentration up to the maximum value of about 98% which has been obtained after treatment with 1% wt GA solution.

The choice of the GA concentration (% wt) values has been driven by the correspondent cross-linking extent obtained. The large gap between 0.140 (% wt) GA and 1.000 (% wt) GA is explained with the slightly difference of cross-linking degree obtained with these two GA concentrations.

The total amount of vancomycin and gelatine released as a function of time was measured by capillary electrophoresis. This technique is able to separate gelatine from vancomycin with high selectivity (Fig. 1).

3.1 Gelatine release profiles

We have determined the mass percentage of gelatine released from differently cross-linked vancomycin loaded gelatine samples in the range from 24 to 1920 hours in order to consider the whole period of time needed to completely treat the particular orthopaedic infection (Fig. 2). Any released gelatine has been observed in the whole experimental time (80 days) in physiological solution for the 1% GA treated samples coherently with their complete cross-linking. On the other hand mass percentage of gelatine released from 73%, 64% and 55% cross-linked proteins samples were 46%, 48% and 63% respectively and

Table 1 Cross-linking degree (%) of vancomycin loaded gelatine as a function of GA (% wt)

GA (%wt)	Cross-linking degree (%)
0.035	55 ± 3
0.070	64 ± 2
0.140	73 ± 4
1.000	98 ± 2

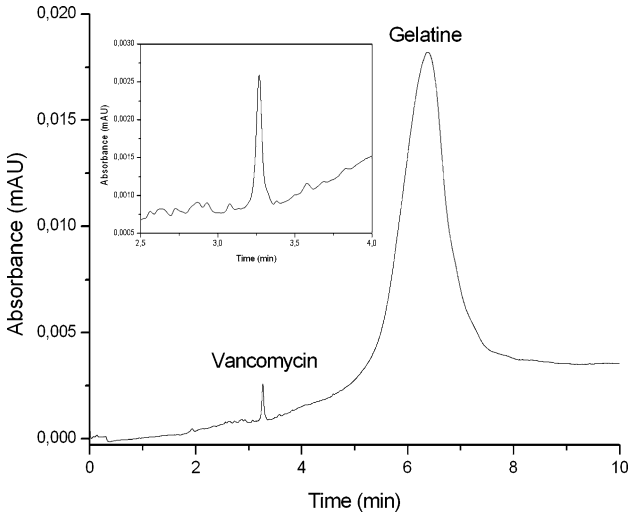

Fig. 1 Representative electropherogram of vancomycin and gelatine from vancomycin loaded gelatine cross-linked sample

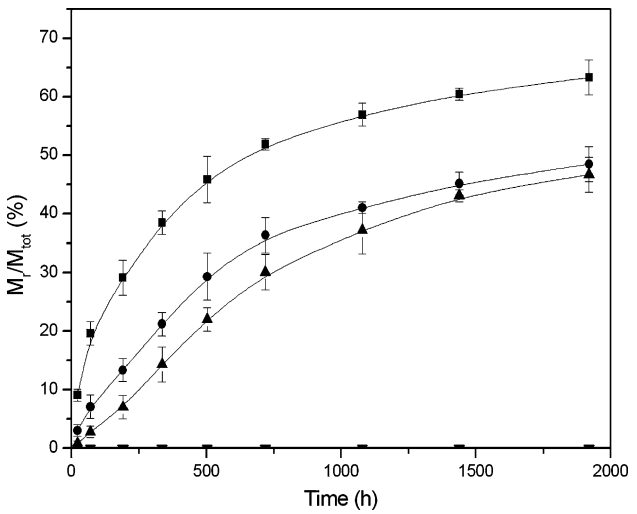

Fig. 2 Mass percentage of gelatine released as a function of time (hours) in physiological solution from differently cross-linked vancomycin loaded gelatine samples. Gelatine samples with 55% (■), 64% (●), 73% (▲) and 98% (▼) cross-linking degree. Each point represents the mean ± SD of three experiments

inversely proportional to their cross-linking degree. The 64% and 73% GA cross-linked samples show a different initial burst release, even if the mass percentage of released gelatine is very similar after 1920 hours.

A curve fitting analysis according to the simplified Higuchi model has been performed on the gelatine solubilization data previously reported and the calculated fitting plots are presented in Fig. 3. It is possible to observe as the simplified Higuchi model can describe very close the solubilization kinetics of gelatine samples cross-linked at 55%, 64% and 73%. A linear relationship of gelatine solubilized amount with the square root of time can be

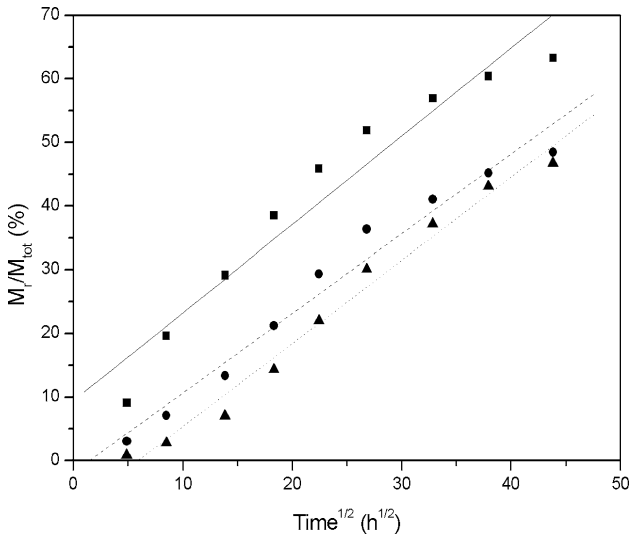

Fig. 3 Fitting of mass percentage of gelatine released in physiological solution from vancomycin loaded gelatine samples with 55% (■), 64% (●), 73% (▲) and 98% (▼) cross-linking degree vs. square root of time

Table 2 Correlation coefficient (R) and standard deviation of the residuals (SD) relative to the linear fitting of gelatine released reported in Fig. 3

Cross-linking degree (%)	R	SD
55	0.969	5.02
64	0.985	3.02
73	0.992	2.42

appreciated, putting in evidence as gelatine release is completely governed by Fickian diffusion. The linearity of the trend increases with gelatine cross-linked degree (Table 2). It means that the value of the correlation coefficient (R) increases and the value of standard deviation of the residuals (SD) decreases.

3.2 Vancomycin release profiles

Mass percentage of vancomycin released as a function of time (hours) determined by CE from differently cross-linked vancomycin loaded gelatine samples are plotted in Fig. 4. Mass percentage of vancomycin up to about 54%, 37%, 38% and 30% have been released after 1920 hours from vancomycin loaded gelatine samples with 55%, 64%, 73% and 98% cross-linking degree respectively.

The samples with 64% (●) and 73% (▲) cross-linking degree show a slightly different initial burst release, even if the mass percentage of released vancomycin is similar after 1920 hours resembling the gelatine released trend previously observed for the same samples in Fig. 2. The mass

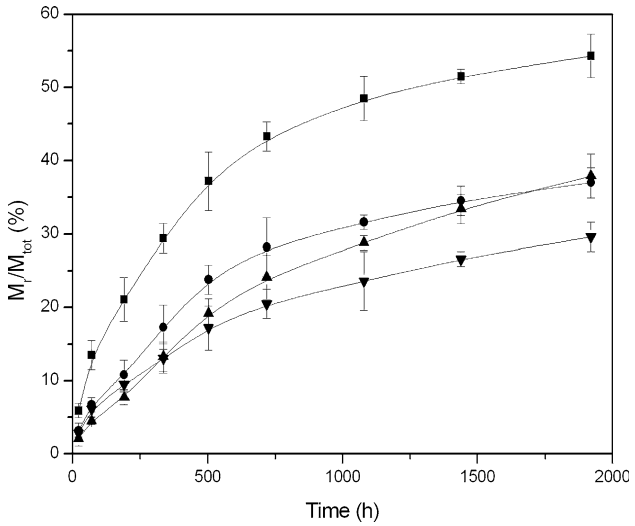

Fig. 4 Mass percentage of vancomycin released as a function of time (hours) from vancomycin loaded gelatine samples with 55% (■), 64% (●), 73% (▲) and 98% (▼) cross-linking degree. Each point represents the mean ± SD of three experiments

percentage of vancomycin released from sample with 98% (▼) cross-linking degree reveals a strong different behaviour respect to the inappreciable mass of gelatine released observed from the same sample (Fig. 2).

A curve fitting analysis according to the simplified Higuchi model has been performed on the vancomycin released data previously reported and the calculated fitting plots are presented in Fig. 5.

The simplified Higuchi model can be successfully applied to the vancomycin release kinetics from samples with 98% cross-linking degree (Fig. 5). The linear relationship of vancomycin released amount with the square root of time

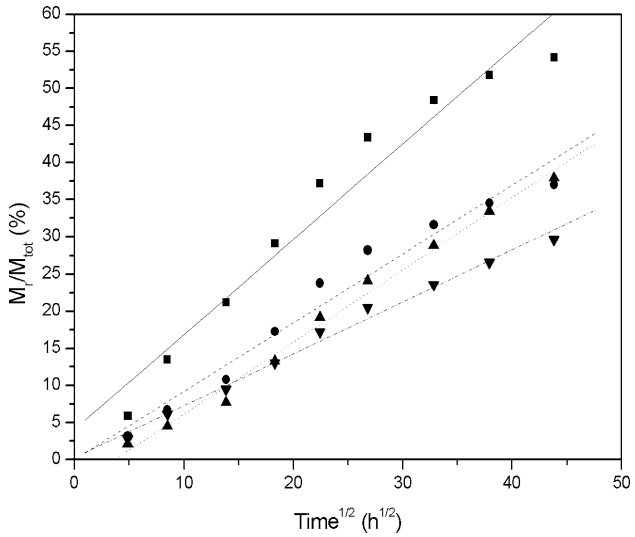

Fig. 5 Fitting of mass percentage of vancomicyn released from vancomycin loaded gelatine samples with 55% (■), 64% (●), 73% (▲) and 98% (▼) cross-linking degree vs. square root of time

Table 3 Correlation coefficient (R) and standard deviation of the residuals (SD) relative to the linear fitting of mass percentage of vancomycin released reported in Fig. 5

Cross-linking degree (%)	R	SD
55	0.977	3.96
64	0.983	2.45
73	0.996	1.27
98	0.995	0.96

puts in evidence that from this sample the vancomycin released is completely governed by Fickian diffusion.

The goodness of the linear fitting decreases in the case of vancomycin release from gelatine sample cross-linked at 73% revealing that a dissolution process of the matrix is becoming important in the release event. On the contrary a linear relationship of vancomycin released amount with the square root of time is not observed for vancomycin loaded gelatine sample with 55% and 64% cross-linking degree. The linearity of the trend increases with gelatine cross-linked degree (Table 3).

The histogram showing the time necessary for vancomycin loaded gelatine samples at different cross-linking degrees to release 50% of total antibiotic loaded ($t_{1/2}$: half-life) is reported in Fig. 6. Vancomycin loaded gelatine samples cross-linked at 55%, 64%, 73% and 98% take 1858, 3057, 2851, and 4216 h respectively to release half of total vancomycin loaded.

3.3 Activity test of eluted vancomycin

Any possible degradation or biological activity loss of vancomycin eluted from antibiotic loaded cross-linked

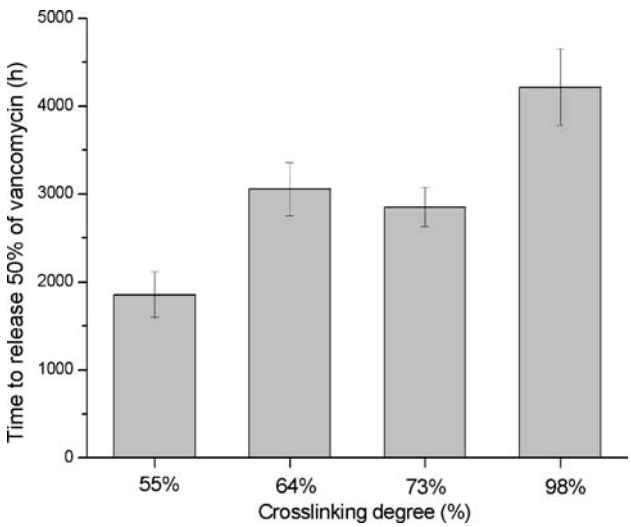

Fig. 6 Time (hours) after which gelatine samples at different cross-linking degree, release 50% of total vancomycin loaded ($t_{1/2}$: half-life)

gelatine samples has been tested as a function of elution time up to 1920 h (80 days). The relative activity of eluted vancomycin from each gelatine samples was tested evaluating the changes in the turbidity of the growth media containing 10^5 CFU/ml and 10^8 CFU/ml *Staphylococcus aureus* and *Streptococcus faecalis*. All the growth media tested with the vancomycin released from gelatine samples cross-linked up to 73%, in the whole elution time ranging up to 1920 h, show the same turbidity value of 0.2 McF. This value is the same as that obtained for the physiological solution sample used as a control, which reveals vancomycin activity against both the bacteria. On the contrary the *Staphylococcus aureus* and *Streptococcus faecalis* (10^5 CFU/ml and 10^8 CFU/ml) growth media tested with vancomycin released from completely cross-linked gelatine samples at 1920 hours of elution time, show a turbidity value of 0.4 McF revealing a vancomycin activity reduction against both the bacteria. The concentration of vancomycin released after 1920 hours is 18 μg/mL, 19 μg/mL, 34 μg/mL and 30 μg/mL for gelatine samples 55%, 64%, 73% and 98% cross-linked respectively. These vancomycin concentration values are higher than the minimum inhibitory concentration (MIC) of vancomycin for *Staphylococcus aureus* and *Streptococcus faecalis* media, which are 0.5–1 μg/mL and 0.5–2 μg/mL respectively [34]. These results show that vancomycin released from all the partially cross-linked gelatine samples is active against both the bacteria, which represent the most commonly isolated pathogens in orthopaedic infections. In fact the local eluted vancomycin concentrations are so much greater than the minimum inhibitory concentration (MIC) for *Staphylococcus aureus* and *Streptococcus faecalis* to overcome any possible degradation or reduction of activity, which the antibiotic could undergo during elution. The strong reduction of activity observed for vancomycin released from totally cross-linked gelatine sample makes us suppose that drug molecule undergo a degradation due to the high cross-linking degree. This hypothesis is supported by the evidence that the antibiotic concentrations released from totally and partially cross-linked gelatine sample are comparable and they are an order of magnitude higher than MIC.

4 Discussion

Management of bone infection is one of the major issues in orthopaedic surgery. In fact systemic antibiotic therapy, which are often not possible for adverse effects, usually fail because of the poor penetration into bone.

An ideal drug delivery system should provide an adequate drug amount at the target site, a drug constant release for long-term elution and be biodegradable into a suitable time period in order to avoid successive surgical removal. This paper explores the possibility of using biodegradable cross-linked gelatine samples as antibiotic device for a long-term drug release. Gelatine can be considered either a non-biodegradable or a bioresorbable material as a function of its cross-linking degree. The results show that the treatment of gelatine samples with amount of 1% wt GA allows to obtain proteic gels with about 100% cross-linking degree and to make them completely insoluble in physiological fluids. Gelatine samples treated with GA solution lower than 1% wt form hydrogels only partially cross-linked which are biodegradable. The lower is the gelatine cross-linking degree, the higher is its biodegradability. The possibility to modulate the rate of bioresorbility through the cross-linking degree, lets gelatine to dissolve opportunely slowly. This process allows to the soft tissue or bone defect to gradually fill with tissue [37].

The use of CE in this work allowed us to overcome the difficulty both to obtain a good separation between a proteic macromolecule and an organic drug and the overlapping of the gelatine signal due to its much larger amount. Moreover the analyses have been performed with a good resolution of signals of antibiotic and gelatine, high sensibility, fast analysis, and low cost operation.

By this technique we have determined the mass percentage of vancomycin and gelatine contemporary released from differently cross-linked vancomycin loaded gelatine samples in the range from 24 to 1920 h in order to characterize the antibiotic release kinetics as a function of gelatine delivery biodegradation.

When the gelatine matrix is completely cross-linked and consequently not biodegradable, vancomycin is eluted according to a kinetic completely governed by Fickian diffusion (Fig. 5). Half of total vancomycin amount loaded is eluted only after 4216 h. The antibiotic concentration after 1920 h of elution is greater than the minimum inhibitory concentration for *Staphylococcus aureus* and *Streptococcus faecalis*. The bacteria inhibition test carried out to determine the relative activity of the released vancomycin reveals a strong reduction of activity and make us suppose that drug molecule undergo a degradation due to the high cross-linking degree.

When the gelatine device is only partially cross-linked and consequently biodegradable, vancomycin is eluted according to a kinetic which is contemporary governed by the Fickian diffusion process trough the gelatine matrix network and the dissolution process of the matrix due to its degradation. In this way, higher antibiotic amount than the completely cross-linked gelatine are eluted and half of total vancomycin amount loaded is released just after 1858, 3057, 2851 h from gelatine samples with 55%, 64% and 73% cross-linking degree respectively (Fig. 6). The amount of antibiotic after 1920 h of elution is greater than

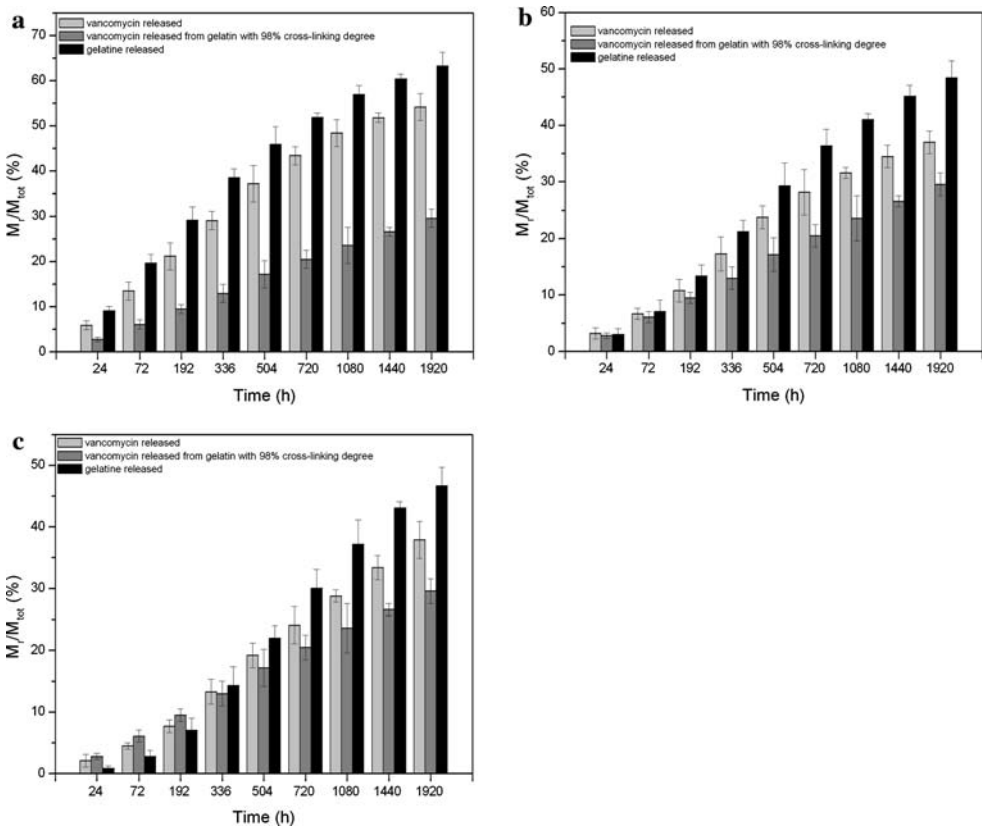

Fig. 7 Mass percentage of vancomicyn and gelatine released from vancomycin loaded gelatine sample with 55% (**a**), 64% (**b**) and 73% (**c**) cross-linking degree vs. time. Mass percentage of vancomicyn from gelatine sample cross-linked with 98% cross-linking degree also reported

the *Staphylococcus aureus* and *Streptococcus faecalis* MIC. The bacteria inhibition test carried out to determine the relative activity of the released vancomycin, reveals any loss of activity, which could take place during elution.

The simplified Higuchi model can be successfully applied to the vancomycin release kinetics from samples with 98% cross-linking degree. This agreement reduces with the decrease of the cross-linking degree as we observed with the standard deviation of the residuals relative to the linear fit reported in Table 3. The release kinetic of vancomycin eluted from the 64%, 73% and 98% cross-linked samples appear similar and not strongly affected by the different dissolution behaviour of the matrices. This result makes suppose that the driving force of the antibiotic elution is its diffusion trough the matrix networks. The degradation of gelatine becomes influent in terms of percentage of released drug when the cross-linking degree is lower than 64%.

In order to elucidate the effect of gelatine cross-linking degree on the relative vancomycin amount eluted, in Fig 7 is reported a graphical comparison between vancomycin released from differently cross-linked gelatine samples and vancomycin released by diffusion from completely cross-linked gelatine sample.

Increasing gelatine cross-linking degree the vancomycin amount eluted decreases getting more and more near the values determined for completely cross-linked gelatine samples. This finding is evident in the range of elution time from 720 (h) to 1920 (h), but less clear in a period of elution time lower than 720 h for high cross-linked gelatine. Probably at the beginning of the vancomycin release the diffusion from cross-linked gelatine matrix is prevalent onto the vancomycin release due to gelatine biodegradation. In fact for highly cross-linked gelatine samples the gelatine released amount due to biodegradation process, became higher in the range of elution time from 720 (h) to 1920 (h).

The results of this work can make us establish that 64% is the minimum cross-linking degree necessary to allow a faster sustained release of antibiotic from a biodegradable gelatine matrix. A lower value of cross-linking degree consents to obtain a quickly biodegradable device with a faster drug release rate. On the other hand a higher value of cross-linking respect to 64% is much more influent on to the matrix degradation than the antibiotic release trend and amount.

The results put in evidence the possibility to use cross-linked gelatine as drug delivery device for a prolonged time (80 days), which is close to the time needed to treat the specific orthopaedic infections. The vancomycin released after 1920 h results active against the common bacterial infections only in the case of partially cross-linked gelatines. The results demonstrated that the device degradation rate can be selected without loss of antibiotic activity in

such a way that its resorbibility could be tailored for specific therapeutic applications.

5 Conclusions

The kinetic release and the efficacy of sustained release of vancomycin from a bioresorbable gelatine matrix at different cross-linking extent has been investigated in order to establish a delivery system for local anti-infective therapy in arthoplasty. The results put in evidence the important role of the cross-linking degree in both controlling matrix biodegradation and antibiotic elution. A similar vancomycin elution rate can be observed in the range 60–100% cross-linking degree, differently of what happens for the matrix degradation, which appears appreciably diverse as a function of cross-linking degree. When the gelatine device is only partially cross-linked and consequently biodegradable, vancomycin is eluted according to a kinetic which is contemporary governed by the Fickian diffusion process trough the gelatine matrix network and the dissolution process of the matrix due to its degradation. However the degradation of the proteic matrix appears to have a minor influence in the drug release control. Vancomycin concentration released from all the partially cross-linked gelatine samples, from 24 h to 1920 h of elution time, is always greater than the minimum inhibitory concentration for *Staphylococcus aureus* and *Streptococcus faecalis* resulting active against both the bacteria which represent the most pathogens commonly isolated in orthopaedic infections. This study could be able to set a drug delivery device in which the biodegradation of the matrix and the antibiotic release kinetic parameters can be controlled in dependence on specific therapeutic needs.

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