

Collagen – vinblastine Delivery Systems as a New Treatment for Kaposi's Sarcoma

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Collagen-vinblastine composites have been prepared by crosslinking with genipin and glutaraldehyde followed by freeze-drying in order to obtain drug delivery systems in spongy form. The resulting composites have been characterized with respect to their swelling capacity, FT-IR spectroscopy, scanning electron microscopy and vinblastine release. It was observed that the composites absorbed a high amount of water depending on the degree of crosslinking and the amount of vinblastine. They have porous structures as SEM images showed and the native collagen structure was kept for all composites. The release of vinblastine was between 67.72% and 84.91%. In the first hour of experiment, vinblastine is released faster inducing the remission of tumor cells, while during the next four hours the drug is released more slowly and gradually to maintain an appropriate concentration for remission consolidation and tumor healing. In conclusion, the obtained drug delivery systems based on collagen and vinblastine are a promising biomaterial for treatment of Kaposi's sarcoma.

Keywords: collagen, genipin, vinblastine, drug delivery systems

Kaposi's sarcoma is a vascular, multicentric lesion, of four clinical types: classic (elderly men from the Mediterranean, Eastern Europe), endemic African variant, iatrogenic (patients receiving immunosuppressive drugs), epidemic AIDS related form [1]. Cutaneous involvement is characteristic, but it can affect visceral sites (lung, stomach, liver) causing significant morbidity. The cutaneous form typically affects the upper body, face, oral mucosa and genitalia. Kaposi's sarcoma has many treatment options that include local or systemic therapy and highly active antiretroviral therapy [2]. Local therapies such as cryotherapy, topical therapy with 9-cis retinoic acid and radiotherapy can be used for KS patients. Intralesional chemotherapy can determine short term regression, but the duration of palliation is of 3 to 4 months [1]. The management of the chemotherapeutic side effects in a Kaposi's sarcoma patient (grade IV neutropenia, neurotoxicity) has been a burden for many oncologists. There are multiple clinical studies that describe intralesional vinblastine administration as a viable solution for KS patients, less toxic and less costly.

Flaitz et al. published in 1995 a 144 patients' study which concluded that intralesional vinblastine administration can lead to complete healing in an impressive number of intraoral KS lesions. The treatment was well tolerated, with minimum adverse effects (pain, ulceration, paresthesia) [3]. A report on 10 patients who were treated with up to three administrations of intralesional vinblastine (0.1 mg/mL), with 2 year follow-up, showed that it was an effective treatment alternative for intraoral KS patients [4].

Vinblastine is indicated in lymphomas, germ cell tumors, Kaposi's sarcoma, breast cancer. Unlabeled uses include

melanoma, non small cell lung cancer, ovarian cancer, bladder cancer and soft tissue sarcoma [1, 2]. The drug may cause severe myelosuppression (nadir occurs on day 7-10, recovery by day 17). Other common side effects are hypertension, alopecia, rash, nausea and vomiting, diarrhea, constipation, peripheral neuropathy [2, 4]. In order to decrease the side effects, local therapy is more useful when there are just a few lesions in a very visible area (such as the face) [5]. Alitretinoin 0.1%, a retinoid drug related to vitamin A, is available as a gel (Panretin®) and is approved for the treatment of cutaneous lesions of AIDS-associated Kaposi's sarcoma, with favourable evolution in spite of an intense local reaction. Side effects of this gel include skin irritation and lightening of the skin color [6]. In order to prevent skin irritation, topical agents are indicated for the treatment of superficial wounds [7]. Collagen is the most used biomaterial for tissue engineering and regenerative medicine, being commonly used in medical and pharmaceutical industries as carrier molecules for drugs, proteins and genes [8, 9]. The microfibrillar collagen sheet is considered by Sato et al. as a promising drug carrier for the treatment of cancer [10]. In recent publications we reported that collagen in the form of hydrogels or matrices exhibited good properties as drug delivery systems [11-14].

The aim of this study is to prepare and describe some drug delivery systems based on collagen as a support, vinblastine - a drug used to treat a number of types of cancer, cross-linked with classical glutaraldehyde on one side and with genipin on the other side. The drug delivery systems obtained will be used for the treatment of Kaposi's sarcoma.

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Sample code	Glutaraldehyde*, %	Genipin*, %	Vinblastine*, %
G1	0	0	0
G2	0.05	0	0
G3	0	0.05	0
G4	0	0	0.05%
G5	0.05	0	0.05%
G6	0	0.05	0.05%
G7	0	0	0.1%
G8	0.05	0	0.1%
G9	0	0.05	0.1%

* Reported to collagen gel volume

Table 1
COMPOSITION OF COLLAGEN-
VINBLASTINE GELS

Experimental part

Materials

Type I collagen of bovine origin was extracted by the currently used technology as previously described [15]. The collagen (Coll) was obtained as a gel in native form with fibrillary structure with an initial concentration of 2.11%, pH 2.5, and free of fat and ash. Glutaraldehyde (GA) was supplied by Sigma-Aldrich (Germany), genipin (GP) by Sigma Aldrich, USA, and sodium hydroxide by Merck (Germany). Vinblastine sulfate was purchased from Sigma-Aldrich, Germany. All the chemicals used in this work were of analytical grade and the water was distilled.

Preparation of collagen delivery systems

The 0.8% collagen gels, different concentrations of crosslinking agents and vinblastine were prepared according to the composition presented in table 1.

Subsequently, gels (G1 – G9) were frozen at -40°C for 12 hours and then freeze-dried according to the previously described method [16] using the Christ Model Delta 2-24 LSC freeze-dryer (Germany). 3D collagen delivery systems in matrix form were obtained, coded M1 – M9 and described as follows.

Water absorption

In order to determine the water absorption we used the previously described method [16]. Briefly, the composites were first immersed in PBS at 37° C. At scheduled time intervals, the samples were withdrawn, wiped (to remove the surface water) and weighed. The water absorption was calculated using the following equation:

$$\text{water absorption} = \left(\frac{W_t - W_d}{W_d} \right) \quad (1)$$

where W_t denotes the weight of the swollen samples at immersion time t , and W_d denotes the weight of the dry samples.

Drug release kinetics study

The release of Vinblastine from 3D collagen spongy composites was determined using the method reported in our previous studies [12]. Briefly, the samples were fixed in the transdermal sandwich devices and then placed in the release vessels of a dissolution equipment in conjunction with paddle stirrers (Essa Dissolver). The release medium used was a phosphate buffer solution (pH 7.4), maintained at 37°C. At predetermined time intervals aliquots of 5 mL were withdrawn from the release vessels and replaced with the same volume of fresh, preheated buffer solution. The absorbance of the extracted solutions was spectrophotometrically assessed (Perkin-Elmer UV-Vis spectrophotometer). The Power law equation [17] was used for the evaluation of vinblastine release kinetics from the designed composites:

$$\frac{m_t}{m_\infty} = k \cdot t^n \quad (2)$$

where, m_t/m_∞ is the fraction of drug released at time t , k – the kinetic constant, and n – the release exponent in relation with the drug transport mechanism.

Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups present in the structure of the prepared samples were identified by FTIR using a Shimadzu 8400 FT-IR Spectrometer. The spectra were recorded in the range of 500 – 4000 cm^{-1} , with a resolution of 2 cm^{-1} .

Scanning Electron Microscopy (SEM)

In order to investigate the structure and morphology of the samples, SEM studies have been performed using a HITACHI S2600N-type scanning electron microscope (SEM). Prior to the analysis, all samples were covered with a layer of silver by plasma sputtering.

Results and discussions

Collagen-vinblastine delivery systems were prepared according to the process described in the experimental section. Two types of cross-linking agents were used to obtain a spongy form with improved stability: genipin and glutaraldehyde which may form intramolecular and intermolecular crosslinking [18, 19].

The swelling capacity of these composites after one hour, cross-linked with genipin or glutaraldehyde, with or without vinblastine is presented in figure 1.

The composites cross-linked with GA and GP showed a higher swelling capacity compared to the corresponding un-cross-linked composites. This can be explained by the porous network formed during crosslinking which is able to keep water as a sponge. Furthermore, it was shown that the addition of vinblastine resulted in a reduced swelling capacity which can be associated with an increased crosslinking degree and formation of a more dense and compact structure.

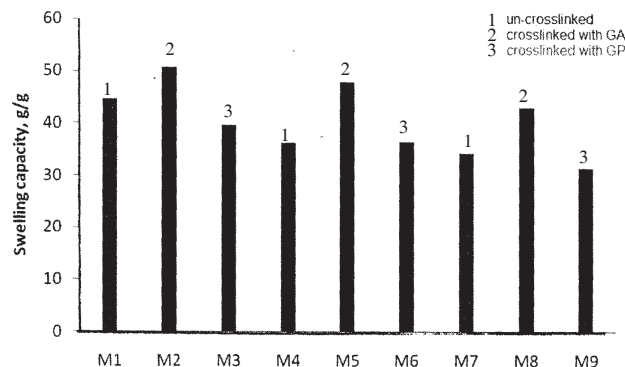


Fig. 1. Swelling capacity of water for M1 ÷ M9 composites

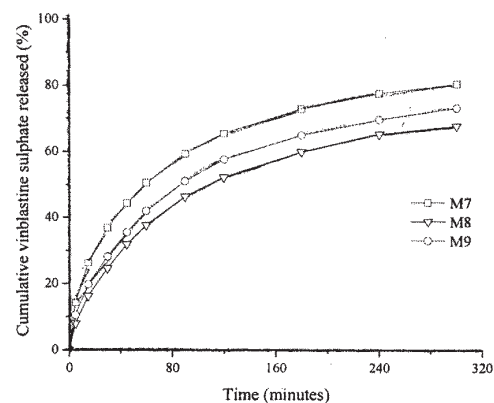
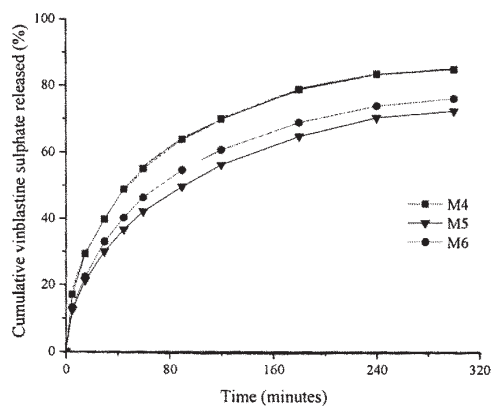


Fig. 2. Cumulative release profiles of drug from M4-M9 collagen spongy composites with: a) 0.05% vinblastine; b) 0.1% vinblastine, over time

Spongy composites	Drug released (%)	Kinetic constant (1/min ⁿ)	Release exponent	Correlation coefficient
M4	84.91	0.128	0.342	0.9924
M5	72.45	0.081	0.392	0.9955
M6	76.17	0.093	0.377	0.9932
M7	80.35	0.112	0.355	0.9922
M8	67.72	0.060	0.436	0.9917
M9	73.18	0.074	0.409	0.9925

Table 2
DRUG RELEASED PERCENTAGE
KINETIC PARAMETERS AND
CORRELATION COEFFICIENT FOR
THE POWER LAW MODEL

The release kinetics patterns of vinblastine from un-cross-linked and cross-linked collagen spongy composites were recorded as percentage of drug released over time, and presented in figure 2 a-b.

The drug released percent varied between 67.72% (M8) and 84.91% (M4) (table 2). In the first hour of experiment, vinblastine is released faster inducing the remission of tumor cells, while during the next four hours the drug is released more slowly and gradually to maintain an appropriate concentration for remission consolidation and tumor healing.

It can be noticed that the released drug percentage for all the samples from series with a smaller vinblastine concentration (M4-M6) is higher (about 4% to 7%) compared to the corresponding ones from series M7-M9

having a double drug concentration. These results are in line with the ones obtained for the water absorption determinations for the above composites (fig. 1).

The presence of the crosslinking agents (GP and GA) induces a decrease of the drug released percent both for the composites with a lower vinblastine concentration (1.14 to 1.17 times) as well as for the ones with maximum drug concentration (1.10 to 1.18 times).

The application of Power law equation to the kinetic data showed a release exponent smaller than 0.5 indicating a non-Fickian drug transport mechanism, specific to the drug spongy composites [12]. The values recorded for the correlation coefficient as well as the values obtained for the kinetic descriptors characteristic for the above model are given in table 2.

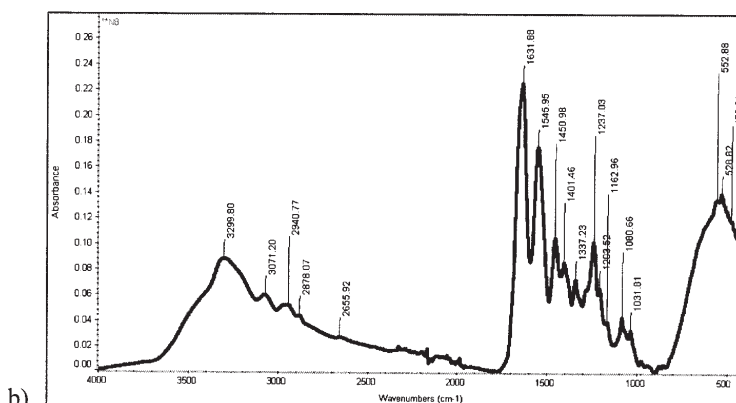
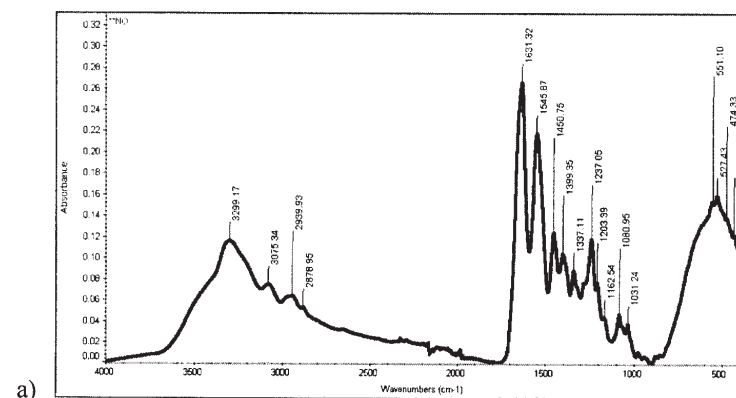


Fig. 3. FT-IR spectra for M1 and M9 composites

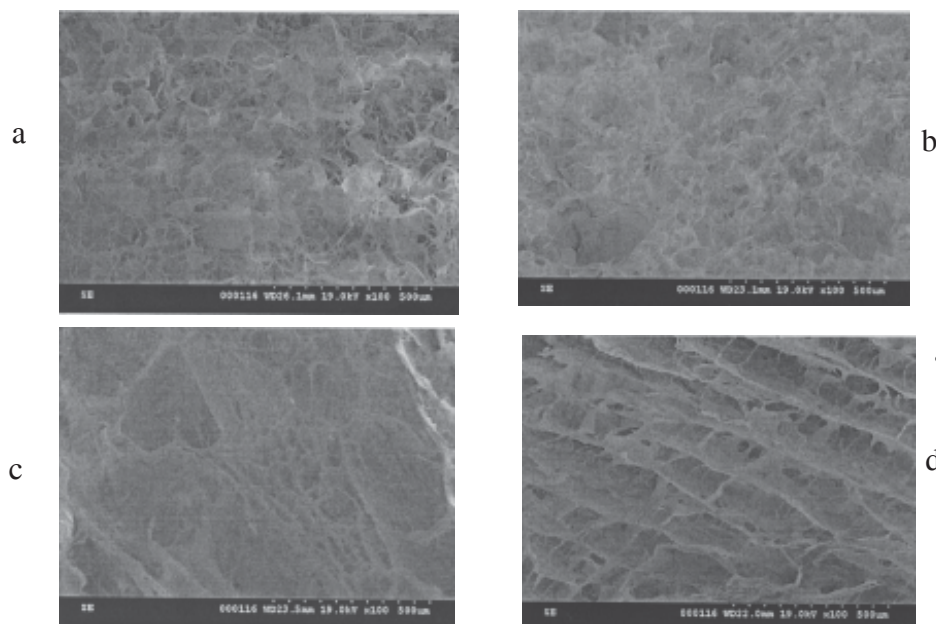


Fig. 4. SEM images for composites: a) M1; b) M2; c) M3 and d) M9 (x 100)

The FT-IR spectra showed the characteristic bands of the amide I, II and III: 1631, 1545 and 1237 cm^{-1} respectively. The peaks of 3299 cm^{-1} correspond to N-H and O-H stretching vibrations. In figure 3 the spectra for control sample (M1) and for M9 (cross-linked with GP and highest amount of vinblastine) are presented for exemplification.

As figure 3 shows, there are no evident changes in collagen structure due to crosslinking or vinblastine adding.

The changes in collagen structure can be seen in SEM microscopy images presented in figure 4a-d.

SEM microscopy showed that pores inside the composites were interconnected for all samples, with irregular patterns for samples without vinblastine and with an ordered pattern and more regular pores for composite M9. The porosity was dependent on cross linking agents and the pore sizes varied between 50 and 500 μm .

Conclusions

Drug delivery systems were obtained by freeze-drying of composite gels based on collagen, vinblastine, cross-linked with glutaraldehyde or genipin. The obtained composites showed a high swelling capacity, up 50 g/g, which decreased while vinblastine was added. Moreover, the vinblastine which is used as drug played also the role of a cross-linker, forming a denser and compact structure. The SEM images showed porous structure with interconnected pores for all composites and regular network for composites containing vinblastine. The vinblastine is released faster in the first hour and slower and gradually in the next ones to maintain an appropriate concentration for remission consolidation and tumor healing. In conclusion, the obtained drug delivery system based on collagen and vinblastine is a promising biomaterial for treatment of Kaposi's sarcoma.

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Manuscript received: 15.07.2014