Collagen - Lidocaine Microcapsules with Controlled Release for Tooth Extraction Pain

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The pain management is therefore of paramount importance and the local anesthetic treatment has to be considered. Thus, the purpose of this study was to design and characterize some collagen microcapsules incorporating an anesthetic. The collagen hydrolysate was prepared by spray-drying forming micro sizes spheres (microcapsules) which were loaded with 1% lidocaine (anesthetic) and cross-linked with different cross-linking agent: tannic acid, glutaraldehyde and genipin, in order to obtain solution for tooth pain. The wetting behavior of dried microcapsules powders was determined by contact angle measurement. The microcapsule solutions were characterized by dynamic light scattering (DLS) and in vitro release of lidocaine was investigated. These therapeutically products, based on collagen microcapsules, a local anesthetic and three cross-linking agents, could have potential biomedical application in tooth pain healing treatment.

Keywords: Microcapsules, collagen, lidocaine, pain

Pain is a displeasing sensory and affective experience related with actual or potential tissue damage [1].The tooth extraction is associated with pain at several degrees of severity usually correlated with the amount of tissue damage involved in the procedure [2]. The pain can be treated easily in the immediate recovery period with analgesic drugs. Topical drug administration is a reliable strategy ensuring a good patient compliance and a prolonged drug localized released effect [3, 4].

Lidocaine (2-(diethyl amino)-N-(2,6-dimethyl phenyl)-acetamide) was developed in 1948 as the first amide-type local anesthetic [5]. Recently research has demonstrated that lidocaine is not only able to decrease pain scores, analgesic consumption, and opioid side effects, but also promotes outcomes important for enhanced recovery after intervention. Clinical studies demonstrate analgesic, antihyperalgesic and anti-inflammatory properties of lidocaine [6, 7].

Innovations in biological processes have helped increase the cost efficiency of producing numerous peptides, proteins, and oligo- and polynucleotides doing those attractive candidates for controlled release applications from a clinical and financial point of view. So, research in biomaterials has also been applied to the classic pharmaceutical challenge of designing systems for the extended-release of bioactive substances usable also in different cancers [8, 9]. In an effort to develop these sustained release systems with reproducible and predictable release kinetics, a variety of methods have a risen to address this requirement such as: diffusion controlled water penetration-controlled, chemically controlled, responsive, and particulate systems [10]. An extraordinary amount of synthetic (poly(glycolic acid), poly(l-lactic acid) etc.) and natural materials (collagen, alginate etc.) have been used as biomaterials in controlled release applications [11-13]. From this wide array this study focuses on collagen hydrolysate.

Collagen hydrolysate is a natural polymer that is derived from collagen, and is commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility in physiological environments [14].

A microcapsule is a particle of micrometer size (1-1000 μm) consisting, generally, of a core coated by a shell. The specific morphology may vary and the actual core is sometimes absent. Such a homogenous particle is called microsphere. The active substance is typically residing in the core (or dispersed in the shell matrix) [15, 16]. Microcapsules provide an enormous flexibility regarding the choice of core and shell material. Most gaseous, liquid or solid materials can be encapsulated, regardless of the hydrophlicity/hydrophobicity [17].

Thus, the purpose of this study was to design and characterize some collagen hydrolysate microcapsules, using as an anesthetic model drug the lidocaine hydrochloride, cross-linked with different agents, in order to use them as anti-pain products for dentistry.

Experimental part

Materials

Type I collagen hydrolysate was obtained by acidic hydrolysis as we previously described [18, 19]. Lidocaine was purchased from Sigma-Aldrich, Germany, glutaraldehyde was provided by Merck (Germany). Tannic acid by Sigma-Aldrich, USA.

Microcapsules synthesis

The collagen microcapsules were prepared by spray-drying from liquid collagen hydrolysate. 2% of microcapsules were loaded with 1% lidocaine and cross-linked with tannic acid, glutaraldehyde and genipin in order to obtain solution tooth pain as are presented in table 1.

These solutions were freeze dried in order to obtain solid samples to be characterized by contact angle measurements.

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Samples	Collagen hydrolysate %	Lidocaine %	Tannic acid %	Glutaraldehyde %	Genipin %
M 1	2	0	0	0	0
M 2	2	1	0	0	0
M 3	2	1	0.5	0	0
M 4	2	1	0	0.5	0
M 5	2	1	0	0	0.5

Table 1
COMPOSITION OF MICROCAPSULES SOLUTIONS

Characterization methods

Size and Zeta potential determination for microcapsule solutions

The new obtained microcapsule solutions were characterized by dynamic light scattering (DLS) technique using Zetasizer Nano ZS equipment, Malvern production.

In vitro release of lidocaine from microcapsule solutions

The *in vitro* lidocaine delivery study was performed using the following protocol: the microcapsule solution was placed in a dialysis bag (cut-off 12000 Da) and the bag was then immersed in the release vessel of dissolution equipment (Essa Dissolver). The release medium was phosphate buffer solution, *p*H 7.4, continuousely and constantly stirred at a rotational speed of 50 rpm and kept at the constant temperature of 37°C±0.5°C. At predetermined time intervals, the aliquots of 5mL were collected from the release medium and an equal volume of fresh phosphate buffer solution preheated at 37°C±0.5°C was immediately replaced into the release vessel in order to maintain a constant volume.The absorbance of the withdrawn solutions was spectrophotometrically assessed (Perkin Elmer UV-Vis Spectrophotometer) at the wavelength of 263 nm.

Evaluation of dried microcapsules wettability

The wetting behavior of dried microcapsules powders was determined by contact angle measurement at room temperature, using a KSV Cam 101 Scientific Instrument, as previously described [20]. Briefly, the measurement of the contact angle was performed by placing the tested powder on a glass microscope slide covered by a double sided adhesive tape and the distilled water was dispensed with a Hamilton syringe. The contact angle method was used and the Young-Laplace equation was applied to mathematically describe the drop shape.

Results and discussions

Depending on the type of cross-linking agents, different structures of collagen microcapsules were obtained.

In order to determine samples size distribution and stability using DLS technique, three measurements were made for each sample. From figure 1 it can be seen that all samples have sizes in the micrometer range, sample M5 having the smallest size (0.556 μm). This allows them to penetrate easier the oral mucosa.

Zeta Potential was used as a stability indicator for microcapsule solutions. Therefore, Zeta potential values presented in figure 2 suggest that sample M5, cross-linked with genipin, is the most stable. Also, values sign, in this case being negative, gives information about surface

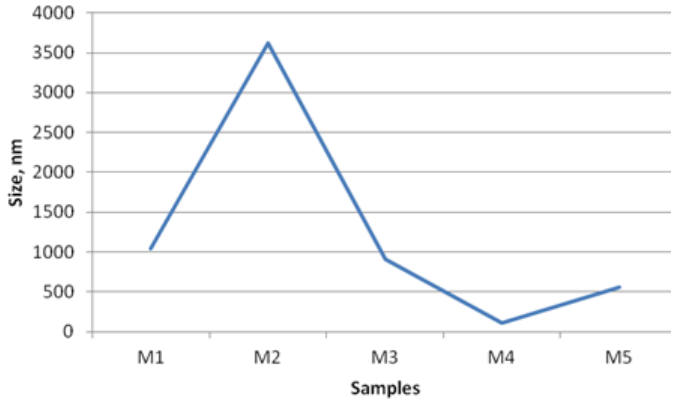

Fig. 1. Size distribution of microcapsule solutions

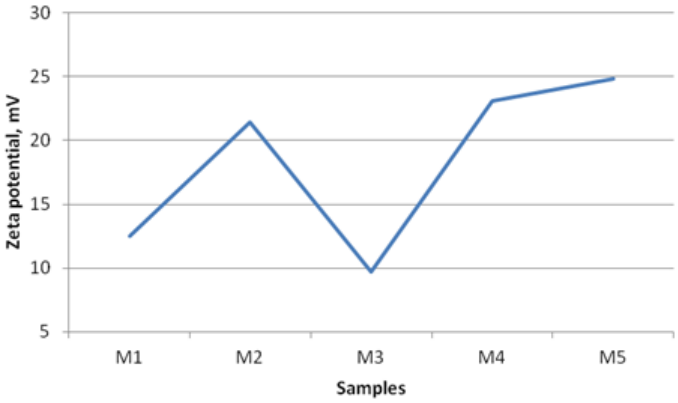

Fig. 2. Zeta potential values of microcapsule solutions

loading. This is a very useful indicator in case of further functionalization.

The values recorded for the contact angles of the dried microcapsules are given in table 2.

Samples	Contact angle (°)
M1	9.25
M2	10.02
M3	22.54
M4	17.95
M5	15.02

Table 2
CONTACT ANGLES OF MICROCAPSULES

The values obtained for the contact angle indicate a high surface hydrophilicity for all the samples un-and cross-linked with different cross-linkers, without or with lidocaine, ranging between 9.25° (M1) and 22.54° (M4), which favors the wetting of the powders by the hydrophilic liquids. The adding of lidocaine induces a slight increase of contact angle, while the presence of different cross-linking agents determines an increase about 1.50-2.25 times compared with the un-cross-linked sample with lidocaine (M2).

The kinetic patterns plotted as cumulative drug released percentage as a function of time is illustrated in figure 3.

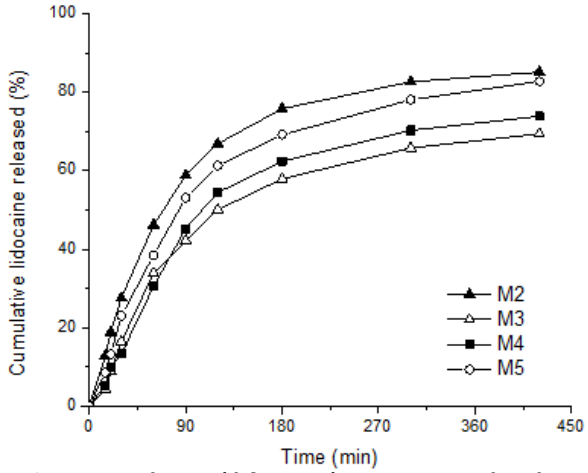

Fig. 3. *In vitro* release of lidocaine from microcapsule solutions

As can be noticed from figure 3, the cross-linking and the type of cross-linking agent influences the release of lidocaine from microcapsule solution. The drug released percent vary from 69.42% (M3), 73.92% (M4), 82.84 (M5) to 85.20% (M2). In the first hour, the percent of the released lidocaine was between 30.53% (M4) and 46.18% (M2), ensuring a rapid pain diminution. It can be seen that the release of the lidocaine was slower in the first hours for sample cross-linked with GA (M4) in comparison with the sample cross-liked with tannic acid (M3), but after 7 h of experiments the drug released percentage for M4 was higher (about 1.06 times). The sample cross-linked with genipin released the highest drug percentage compared to other cross-linked samples (about 1.12-1.19 times).

Conclusions

Different types of microcapsule solutions were obtained from collagen hydrolysate by using three cross-linking agents: tannic acid, genipin and glutaraldehyde and loaded with lidocaine. DLS measurements confirm the obtaining of micro size particles and shows that the solutions are quite stable. The kinetic patterns indicated that the amount of drug released in the first hour could ensure a rapid pain diminution associated with tooth extraction.

As means to manage post intervention pain, the use of the proposed anesthetic-collagen microcapsules with controlled release depending on crosslinking agent has proven to be a promising solution.

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