OFLOXACIN LOADED, *IN-SITU* – GELLING, CALCIUM ALGINATE HYDROGEL IN THE LOCAL TREATMENT OF BONE AND SOFT TISSUE INFECTIONS IN ORTHOPAEDIC SURGERY

KÁROLY WELLINGER[1], HRISTIFOR GÁLITY[1], KÁLMÁN TÓTH[1], PIROSKA SZABÓ – RÉVÉSZ[2], EDIT URBÁN[3] , ERZSÉBET CSÁNYI[2*]

[1]*Department of Orthopaedics, Faculty of Medicine, University of Szeged, Semmelweis u. 6, H-6725 Szeged, Hungary*
[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary*
[3]*Central Laboratory for Clinical Microbiology, University of Szeged, Somogyi u. 1, H-6725 Szeged, Hungary*
**corresponding author: csanyi@pharm.u-szeged.hu*

Abstract

Nowadays in orthopedic surgery -in the treatment of bone and soft tissue infections- there is an increasing claim for improving biodegradable drug delivery systems that can be easily placed into the site of application instead of the currently applied polymethylmethacrylate (PMMA) bone cements, since PMMA has some undesirable side effects.

Therefore the aim of the study was to develop an injectable, Ofloxacin-loaded, calcium-crosslinked sodium-alginate based drug delivery system, for local administration of antibiotics. It forms an elastic gel within a short time after injection, while its sustained drug release is controlled by a biodegradable polymer network. *In vitro* Franz vertical cell diffusion test and *in vitro* microbiological evaluation of the drug release suggested, that calcium-crosslinked sodium-alginate hydrogels are good candidates for replacement of the currently used non-biodegradable bone cements in certain circumstances.

Rezumat

In chirurgia ortopedică se impune din ce în ce mai mult necesitatea utilizării unor sisteme biodegradabile de cedare a substanţelor active, acestea fiind extrem de utile mai ales în cazul infecţiilor osoase şi ale ţesuturilor moi. În prezent se utilizează cel mai frecvent sisteme cu polimetilacrilat (PMMA), însă acestea dezvoltă frecvent reacţii adverse.

Scopul acestui studiu a fost acela de a dezvolta şi de a analiza un nou sistem de cedare cu ofloxacina, utilizând un hidrogel cu alginat de calciu.

Keywords: *in-situ* forming hydrogels; drug release; bone cement replacement.

Introduction

Infection comprises a potentially serious complication following joint replacement surgery and considered to be major complications in

traumatic and orthopedic surgery that poses an even greater challenge to the health care. A few decades ago the most common type of bone infection was hematogenic osteomyelitis, but the numbers of cases of exogenous osteitis and osteomyelitis are currently exhibiting a steadily increasing tendency, with pronounced consequences for the patient and substantial medical costs. The treatment of these infections is based on appropriate radical surgical debridement and long - lasting specified parenteral antibiotic therapy [30]. Techniques of local antibiotic defense other than bone cement, e.g. the use of PMMA (polymethylmethacrylate) bead chains or cement spacers have been recommended in orthopedic surgery. However, systemic antibiotic treatment can give rise to side effects, while the necessary high dose may cause hypersensitivity reactions and gastrointestinal intolerance, and may also facilitate the development of bacterial resistance. Moreover, in spite of being administered in such a dose, the drug can not provide the therapeutic concentration in the pocket for the necessary duration. These disadvantages can be decreased substantially by local delivery of the antimicrobial material [13, 23, 27]. The local concentration of the drug can be increased if it is incorporated into a therapeutic system which can be injected directly into the site where it is required and the viscosity of which is increased extensively in response to body temperature or water [1, 2, 4, 7, 8, 14, 20, 31, 32]. The local use of antibiotics is therefore favored when bone infections are treated. In this manner, the active agents act directly in high concentration at the affected area and only a small amount of the applied dosage passes into the circulation, which moderates its toxic systemic effect. The material most commonly used for local antibiotic therapy is bone cement, compounded from a liquid monomer and a mixture of solid methyl and methacrylate PMMA components. As polymerization proceeds in the patient as an endothermic reaction at a temperature sometimes exceeding 100 °C, the added antibiotics should be heat resistant and must not influence the mechanical characteristics of the PMMA. Moreover, in consequence of the increasing bacterial resistance to antibiotics, the currently commercially available gentamicin-loaded bone cement has had to be enhanced by the incorporation of a second antibiotic [30]. After releasing their antibiotic content, these PMMA materials should be removed from the body in a second operation, because PMMA is not biodegradable. Each and every operation carries the risk of sepsis and complication therefore imposing not only physical, but also mental stress on the patient. The elimination of these unwilling procedures with the use of biodegradable materials has great impact on patients' general condition

The possibility of applying an injectable *in-situ* gelling drug delivery system to a localized site, forming a semisolid drug depot, has a number of advantages, e.g. easy application and localized delivery for site-specific action, prolonged drug delivery periods, a decreased body drug dosage with an accompanying reduction in the possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort, as the utilization of biodegradable drug delivery systems eliminates the need for a second operation [15]. In our research work, sodium-alginate (SA) was chosen as a natural polymer which has been widely investigated for drug delivery [11, 17, 18, 22, 26]. Alginates are a family of linear unbranched poly-saccharides which contain varying amounts of 1,4'-linked β-D-mannuronic acid and α-L-guluronic acid residues. The residues may vary widely in size distribution composition and sequence and are arranged in a pattern of blocks along the chain [12]. SA also has the potential for use as a scaffolding material for tissue engineering because of its structural similarity to the natural extracellular matrix, its gentle gelling kinetics, and its low toxicity when purified [19]. SA (in the presence of water and multivalent cations, usually Ca^{2+}, Ba^{2+} or Sr^{2+}) can behave as a hydrogel with controlled swelling characteristics. In orthopedic practice, we can take advantage of its long-lasting *in vivo* degradation characteristics [21, 25, 29].

Ofloxacin, selected as active agent, is a synthetic broad-spectrum antimicrobial agent for oral and intravenous administration, with an *in vitro* activity against a broad spectrum of Gram-positive and Gram-negative aerobic and anaerobic bacteria. It exerts a bactericidal effect on susceptible microorganisms by inhibiting DNA gyrase, an essential enzyme that is a critical catalyst in the duplication, transcription and repair of the bacterial DNA [24]. The choice of the active agent fell on Ofloxacin, as our meticulous survey based on pathogens emerging in our department in the last two years revealed, that this antibiotic is active against the most common strains that had occurred in our clinical practice: *Staphylococcus aureus (32%)*, *Staphylococcus epidiermidis (22%)*, *Pseudomonas aeruginosa (6%)*, *Escherichia coli (6%)*, *Enterococcus faecalis (3%)*. The frequency and distribution of the pathogenes are in accordance with the literature [6, 10, 16, 33].

The aim of our research work was to develop an injectable drug delivery system that forms an elastic gel within a short time after its injection into joints, bone cavities or subcutaneous tissues, prevented from flowing out by virtue of its high viscosity, while the drug release is controlled by the biodegradable polymer network.

Materials and methods

Materials

Sodium alginate (SA) at concentrations of 2% and 3%, Ofloxacin at a concentration of 3% and ethylene–diamine-tetraacetate (EDTA) solution of 5% were used and the materials were donated by Sigma Aldrich. Calcium-sulphate suspension of 0,9% was used for the crosslinking procedure, whereas distilled water was surved for the preparation of hydrogels. All chemicals were used without further purification.

Methods

Preparation of the hydrogel

SA were dispersed in distilled water at room temperature, forming a mucilage. The gelation and crosslinking of the polymers, shown in Figure 1 were achieved by the exchange of sodium ions from the guluronic acids with the divalent cation (Ca^{2+}), and the stacking of these guluronic groups.

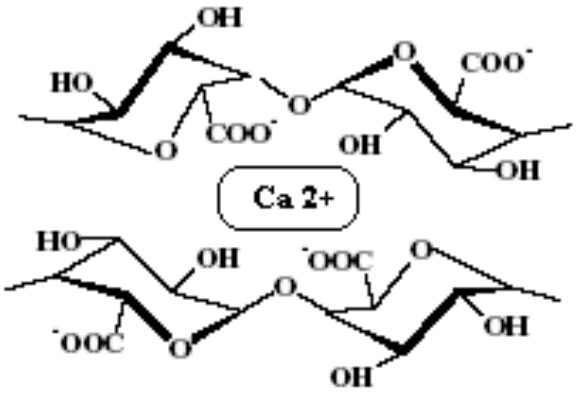

Figure 1
Schematic representation of crosslinking of SA by addition of Ca^{2+}-ions.

We tried to use such procedure for crosslinking that can be performed among conditions of the operating theatre, as well. Crosslinking of the viscous - flowing SA mucilage of 2% and 3% was carried out *ex tempore* with calcium-sulphate suspension of 0.9% in the presence of EDTA solution of 5%, using two injection syringes joined by a thin tube. SA mucilage was present in the first syringe, whereas the other contained a suspension of calcium-sulphate, EDTA solution and Ofloxacin. Content of

the syringes were thoroughly mixed by pushing the pistons for 10 times. After gelling, plastic syringes were removed and the remaining cylinder-like hydrogel was cut into 2 mm thick discs.

Rheological determination

Rheological measurements to find the suitable hydrogel delivery systems were carried out with a Paar Physica MCR 101 rheometer. These periodical measurements comprised of a shear rate of D = 0.1 1/s for 10 seconds, followed by a 300-second-long time interval without shearing. A plate-plate (diameter: 49.953 mm, gap: 1 mm) measuring device with a special rugged surface was used, so that slipping of the probe on the crosslinked hydrogel was avoided thorough the measuring process.

In vitro diffusion studies

In the *in vitro* Franz vertical diffusion cell method (Hanson Microette System, Hanson Research Corporation), the sample was placed as donor phase on the Porafil membrane filter, the pore diameter of which was 0.45 μm. The effective diffusion surface area was 1.767 cm^2. Phosphate buffer (pH = 7.4) was used as acceptor phase to ensure sink conditions. Measurements were performed at 37 °C for 8 h. Ofloxacin was quantitatied with a UV spectrophotometer (Unicam Helios α) at 287 nm.

Microbiological determinations

In vitro microbiological evaluation of the drug release via the Kirby-Bauer disk diffusion method [3] were carried out with cultures of the *Staphylococcus aureus* ATCC 25923 test strain. Ofloxacin - loaded calcium-crosslinked SA discs were prepared and placed on each Petri dish filled with blood agar (Biomerieux, France) and Mueller-Hinton agar (Oxoid Limited, Germany). The Petri dishes were kept under aerobic conditions at 37 °C for 24 h. After incubation, the zones of inhibition around the samples were measured with a metric ruler to the nearest millimeter. Qualitative signs of antibiotic release from alginate discs were the presence of inhibiting zones. Standardized susceptibility test procedures require the use of laboratory control organisms. We used reference filter discs (Macherey-Nagel Limited, U. K.) 6 mm in diameter, containing 5 μg of ofloxacin, that should give a zone 24-28 mm in diameter according to the literature [24]. The inhibitory zones of blank alginate discs, without added ofloxacin were equal to their diameter (d_{disc} = 13 mm), meaning that no substance influencing bacterial growth was released from the control blank alginate discs. Each experiment was performed in triplicate; results are reported as means ± standard error.

Results and Discussion

As a result of crosslinking, a marked increase in viscosity, more than 100 times was observed (Figure 2), which proved suitable for our application purposes. By virtue of the growing viscosity the injected hydrogel is supposed to remain in the application site, filling in bone defects or cavities. Macroscopically, the developed calcium-crosslinked SA hydrogel showed elastic gum-like structure, necessitating further examinations to assess its in vivo behaviour, as well. Evaluation of the viscosity changes was started 60 seconds after the beginning of the crosslinking procedure.

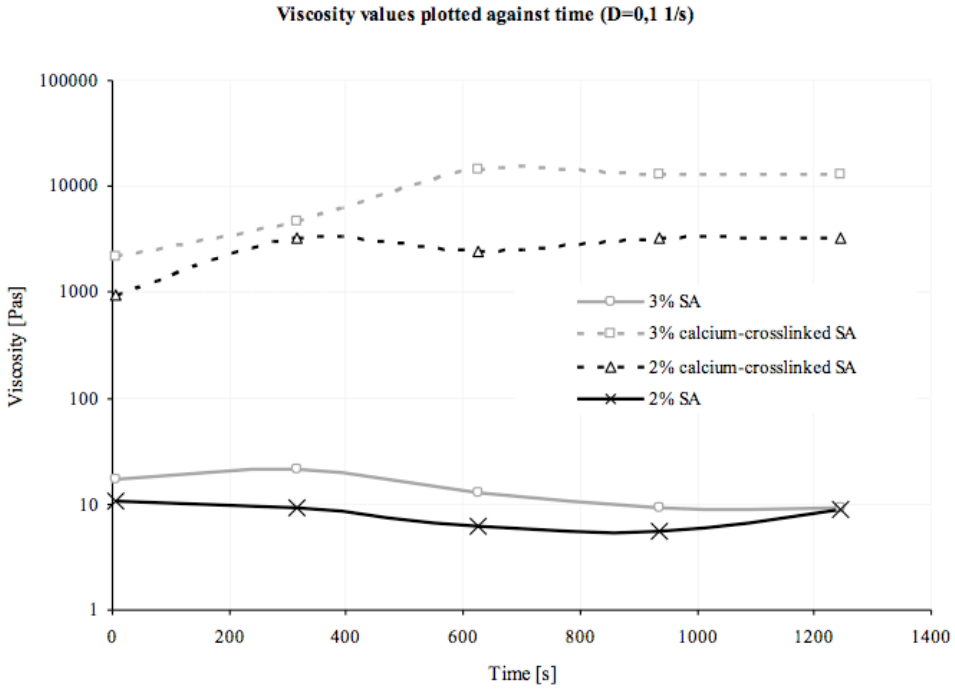

Figure 2
Viscosity of SA mucilages and calcium-crosslinked SA hydrogels

Drug release examinations were performed in vitro, with the use of a Franz-vertical diffusion cell. Figure 3 depicts the *in vitro* liberation of the drug. The results revealed substantial drug release from both systems. The release from the more viscous hydrogel containing 3% SA was more delayed than that from the 2% SA - containing gel.

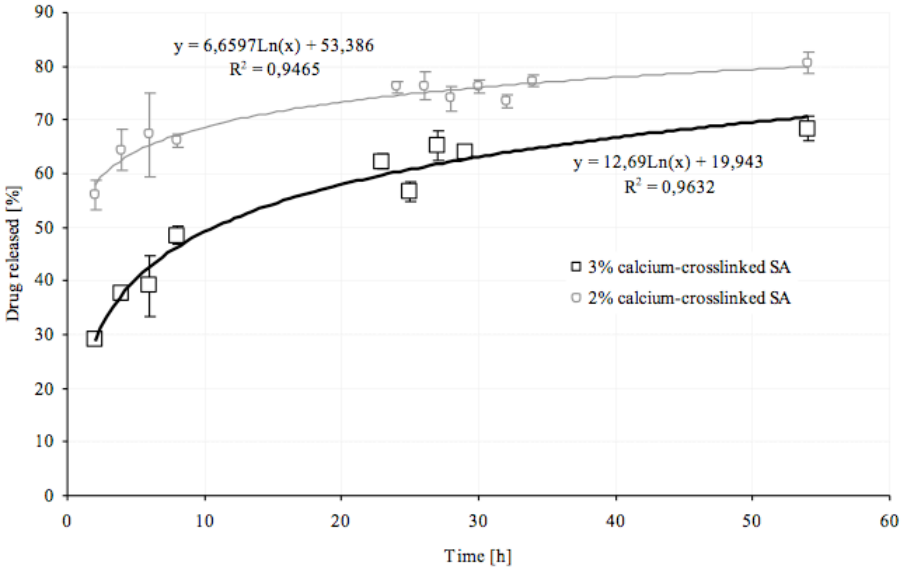

Figure 3

Dissolution of Ofloxacin from calcium-crosslinked SA hydrogels

Microbiological evaluation of the antibiotic release from hydrogel discs in two different types of culture medium disclosed that apart from the blank samples the antibiotic was released from the discs in all cases. The inhibitory zones, presented in Table 1, always exceeded the diameter found for reference discs, irrespective of the culture medium or the alginate concentration, though the total antibiotic content was 1000 times higher in the samples. These findings conform with the *in vitro* Franz vertical cell test results, demonstrating more sustained drug release from the more viscous hydrogel containing 3% calcium-crosslinked SA, than from the 2% calcium-crosslinked SA containing gel. However, considering the effect of water content on the diameter of inhibition zones it can be concluded, that the higher viscosity led to the formation of wider inhibition zones [9].

Table I

Inhibitory zones of calcium-crosslinked SA polymer discs containing 3% Ofloxacin

3% SA with 3% of Ofloxacin				**2% SA with 3% of Ofloxacin**				**Reference disc**			
Blood agar	antibiotic contant (mg)	Disc diameter (mm)	Inhibitory zone (mm)	Blood agar	antibiotic contant (mg)	Disc diameter (mm)	Inhibitory zone (mm)	Blood agar	Antibiotic contant (mg)	Disc diameter (mm)	Inhibitory zone (mm)
Mean	10.6	13	48	Mean	9.9	13	52	Mean	0.005	6	30
SD	0.4	0	1	SD	0.1	0	1	SD	0	0	1
Mueller Hinton agar	antibiotic contant (mg)	Disc diameter (mm)	Inhibitory zone (mm)	Mueller Hinton agar	antibiotic contant (mg)	Disc diameter (mm)	Inhibitory zone (mm)	Mueller Hinton agar	antibiotic contant (mg)	Disc diameter (mm)	Inhibitory zone (mm)
Mean	10.6	13	49	Mean	9.9	13	56	Mean	0.005	6	27
SD	0.8	0	2	SD	0.3	0	1	SD	0	0	1

All the discs remained intact *in vitro:* no damage, perforation or microfractures were observed on their macroscopic examination. The inhibitory zones did not vary in diameter. The inhibitory zones on blood agar and Mueller-Hinton agar did not indicate any significant difference between these culture media.

Conclusions

Ofloxacin - that we had chosen on the bases of our former clinical assessment on pathogens - released effectively by synthetic membrane diffusion from the calcium-crosslinked SA, and increased the inhibitory zones around the hydrogel discs. Finally, it seemed to be effective against most pathogens with a resistance to other antibiotics, emerging in our orthopaedic clinical practice in recent years. The present results suggest that such calcium-crosslinked SA hydrogels are good candidates for replacement of the currently used non-biodegradable bone cements in certain orthopedic surgical interventions, when the bone cement is used only as a drug delivery system, and its mechanical strength is not required. In these selected situations the use of a biodegradable hydrogel system should be taken into consideration not to mention the physiological, emotional, psychological and financial impacts of a second operation, when using traditional PMMA bone cement.

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