ARTICLE IN PRESS

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Advanced Drug Delivery Reviews xx (2003) xxx–xxx

Advanced DRUG DELIVERY Reviews

www.elsevier.com/locate/addr

Collagen as a carrier for on-site delivery of antibacterial drugs

Zbigniew Ruszczak[a,b,*], Wolfgang Friess[c]

[a] *INNOCOLL GmbH, Saal, Germany*
[b] *Dermatology, UMDNJ, New Jersey Medical School, Newark, USA*
[c] *Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, University of Munich, Munich, Germany*

Received 18 August 2003; accepted 26 August 2003

Abstract

Due to its biocompatibility and well-established safety profile, collagen represents a favourable matrix for on-site drug delivery. In this review, we summarize some of the recent developments and applications of collagen as a biomaterial in drug delivery systems for antibiotics, especially gentamicin. The main clinical and experimental applications covered include: treatment and prophylaxis of bone and soft tissue infections, wound healing, as well as ophthalmic and periodontal treatment. Advantages of local drug application and the rationale of use local drug delivery systems for adjuvant (ancillary) therapy are discussed. Recent efforts in the use of collagen and collagen-synthetic polymer composites for controlled drug delivery as well as collagen-based diffusion membranes for prolonged drug release have also been included in this review.

Keywords: Collagen; Collagen sponge; Antibiotic; Gentamicin; Wound healing; Ophthalmology; Periodontology; Collagen membrane; Collagen/PLGA composite

Contents

* Corresponding author. Innocoll Inc., Innocoll GmbH, European Research and Development Center, Donaustr. 24, D-93342 Saal-Donau, Germany. Fax: +49-9441-68600.
E-mail address: ruszczak@innocoll.de (Z. Ruszczak).

0169-409X/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2003.08.007

1. Introduction

Collagen is of particular interest as a natural polymer for drug delivery since it is a major natural constituent of connective tissue and a major structural protein of any organ. Collagen is unique in its different levels of structural order: primary, secondary, tertiary and quaternary. In vivo, collagen molecules form fibers having a specific internal and structural orientation and strengthened together by two types of covalent cross-linking: intramolecular and intermolecular [1]. Intermolecular cross-linking is essential to form macromolecular fibers and, consequently, for its mechanical stability and other physical characteristics [2–4]. Biomaterials made of collagen offer several advantages: they are biocompatible and non-toxic and have well-documented structural, physical, chemical, biological and immunological properties [5–7]. Additionally, drug release kinetics can be influenced by modification of the matrix characteristics (porosity, density) or by different chemical treatment regimes affecting its degradation rate (see [2]).

It is important to define the term collagen before discussing its potential delivery matrix properties. Unfortunately, the term collagen is not consistently used in the literature. In many publications gelatin, a thermally degraded collagen-derived product which has lost many properties of ''real collagen'' is also named ''collagen''. The current main sources of collagen are animal skin, mostly bovine or porcine, or Achilles tendons, mostly bovine or equine. It has to be stressed that collagen properties, i.e. mechanical strength, fluid absorption volume or haemostatic activity differ depending on the animal source and anatomical location of the raw material. In case of Achilles tendons, this organ consists mostly of collagen type-I (approx. 96%) and collagen type-III (approx. 3%) and some other proteins as, e.g. fibronectin or glycosaminoglycans. After extraction and purification, the Achilles tendon-derived collagen consists almost exclusively of collagen type-I. Depending on preparation and purification technology, skin-derived collagen type I material may contain remainders of fibronectin, elastin, glycosaminoglycans, neural and vascular components and different other collagen types (e.g. III, IV, VII). Additionally, collagen-based products are manufactured either from a ''native'', so-called insoluble collagen (not enzymatically digested, degraded or dissoluted collagen consisting of intact fibers in which primary through tertiary structural order has been preserved) or from soluble collagen which has been digested, degraded and dissoluted at extreme pH values or with enzymes leading to partial or complete removal of higher order fibril structures. Consequently, a higher-order in vivo-like structural order may or may not be achieved industrially.

Finally, different in vitro and in vivo behavior including drug release profiles may be obtained if the collagen product has been cross-linked additionally [8–10]. Furthermore, a sterilization process may additionally influence the material properties [10]. The diversity of collagen products described in the literature and used as drug delivery systems, e.g. as powders, liquids, solid compressed masses, membranes or sponges of different porosity and of different collagen content, may lead to misinterpretation of study results, mostly in terms of mechanical, biological and drug delivery properties.

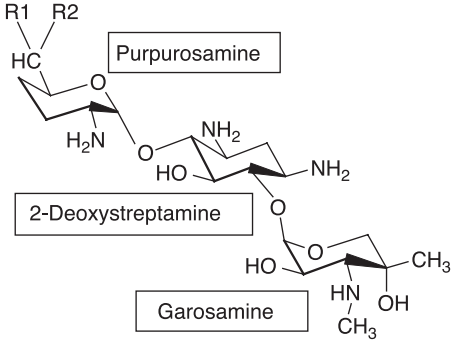
In this review, the current progress in the use of collagen sponges for delivery of antibacterial drugs will be discussed especially from the perspective of marketed products. In parts of this paper in which our own scientific, product development and manufacturing experience will be discussed, the term collagen describes a highly purified, bovine or equine Achilles tendon-derived, native, fibrillar, insoluble type-I collagen without any additional cross-linking.

1.1. Rationale for local antibiotic delivery

Despite a reduction in the risk of contamination due to improved material, implant, and clean room technique as well as preoperative antibiotic prophylaxis, infections still remain a feared complication in orthopedic and traumatic surgery [11]. In many surgical disciplines, topical administration of antibacterial drug is not possible or practicable, and achieving of a sufficient antibacterial dose by systemic delivery may lead to adverse reactions negatively influencing overall patient's conditions. Especially the use of specific antibiotics may be limited by their high cumulative cell and organ toxicity. Moreover, insufficiency in local blood supply due to post-traumatic or post-operative tissue damage as well as inadequate tissue penetration or bacterial resistance increase the local ineffectiveness of systemic antibiotic therapy,

both in terms of preventive or curative drug administration [12]. This dilemma can be resolved by local delivery of antibiotics which ideally show: a low allergization quota, stability at body temperature, tissue compatibility, bactericidal activity, a low rate of bacterial resistance, broad spectrum activity, a low-resorption rate, no interference with wound healing and chemical stability in the presence of biological material such as pus or fibrin [13]. Local delivery has been established successfully in the clinics specifically for aminoglycosides like gentamicin [14–16] and tobramycin [17–20] but has also been studied with minocycline [21], tetracycline [22], teicoplanin [23] or sulbactam-cefoperazone [24]. Especially gentamicin fulfills the abovementioned requirements for potential local application for the most part. Gentamicin exhibits bactericidal activity against a broad spectrum of microorganisms including clinically critical microbes such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* species, and Enterobacteriaceae. Therefore, serious infections such as meningitis, endocarditis, pneumonia, or osteomyelitis caused by gram-negative pathogens are treated by parenteral application of gentamicin, frequently in combination with other antibiotics [25]. Gentamicin represents a chemical mixture with 16 different molecules having been identified [26]. The major components are based on the aminocyclitol 2-deoxystreptamine with two additional amino sugars (Fig. 1). Gentamicin sulfate is characterized by high solubility in water (>1 g/ml) but low solubility in organic solvents (0.678 mg/ml in chloroform, 0.2 mg/ml in methanol, 0.04 mg/ml in acetone) [27]. Because of its polar nature, the oral resorption rate and the tissue penetration of gentamicin are poor and it is largely excluded from most cells [25]. Consequently, tissue concentrations are lower than the corresponding plasma levels [28]. High-tissue concentrations are only found in the renal cortex and in the endolymph and perilymph of the inner ear [29]. The incidence of nephro- and ototoxicity has been attributed to this accumulation [30]. Gentamicin was approved by the FDA in 1966. Against gram-negative aerobic rods, aminoglycosides exhibit "concentration-dependent killing" and a "post-antibiotic effect" (PAE). "Concentration-dependent killing" describes the principle that bactericidal effects increase as the concentration increases. PAE reflects the suppression of bacterial growth continues after the antibiotic concentration falls below the bacterial MIC. The post-antibiotic effect can be bacteria specific, as well as drug specific [31]. The PAE of aminoglycosides is short for most gram-positive organisms (<2 h) and longer for gram-negative organisms (2–7 h), such as *Escherichia coli*, *K. pneumoniae*, and *Ps. aeruginosa*. Both of these phenomena are being exploited in designing dosage regimens that employ higher doses administered at longer intervals [31].

Approximately 30 to 60 min after intramuscular injection, peak concentrations in plasma of approximately 2 to 4 mg/l are reached. The therapeutic level ranges from 4 to 12 mg/l. Although it has not been yet established exactly what plasma concentration is toxic, careful drug monitoring is strongly recommended with an upper limit of 10–12 mg/l and a lower limit of approximately 2 mg/l ($t_{1/2\ \mathrm{plasma}} \sim 2$ h) [29]. Based on an intravenous injection or infusion every 8 h (3 × 1 to 2 mg/kg per day), the serum concentration in the middle of the application interval is used to discrim-



type	R1	R2
C1	-CH_3	-$NHCH_3$
C1a	-H	-NH_2
C2	-CH_3	-NH_2

Fig. 1. Structural formula of the gentamicin complex.

inate microorganisms for their susceptibility to gentamicin [32]. Accordingly, microbes with a minimal inhibitory concentration (MIC) smaller or equal to that concentration at either low dose therapy - called the break point [33] - of 1 mg/l or at maximum dose therapy of 4 mg/l are considered "sensitive" or "moderately sensitive", respectively [13]. Microorganisms with a MIC higher than 4 mg/l are rated resistant. A study testing more than 250,000 isolates from patients showed that the number of resistant microbes was up to 6% for Enterobacteriaceae, 10% for *S. aureus* and *Ps. aeruginosa*, 20% for coagulase-negative Staphylococci and 90% for *Escherichia faecalis* [16]. The corresponding MIC for gentamicin-resistant samples was analyzed, and collectively, 62% of the resistant isolates were inhibited by 16 mg/l gentamicin whereas 5% were resistant to even 64 mg/l. Gentamicin is active against Enterobacteriaceae organisms including *E. coli*, *Proteus*, *Klebsiella*, *Serratia*, *Enterobacter,* and *Citrobacter*. In general, *Ps. aeruginosa* is usually sensitive to gentamicin, although considerable variability in its susceptibility exists. Other pseudomonal species also may be susceptible but are usually less so than *Ps. aeruginosa*. *S. aureus* as well as many strains of *Streptococcus* are sensitive [34]. The main mechanism of resistance to gentamicin is inactivation of the aminoglycoside by bacterial enzymes [29]. As could be shown, there is a rather high number of patients with at least partial resistance [16]. Secluded foci of infection are difficult to treat systemically with gentamicin due to the extremely low concentrations in this tissue of low blood supply and the limitation of the gentamicin plasma levels by its vestibular, cochlear, and renal toxicity.

1.2. Carrier materials for local antibiotic delivery

The consequent need for local drug delivery has been recognized since many years. During the last decades, different forms of local drug delivery have been used. The most common and simple way was to spread the drug in a powder form over the wound area after an extensive debridement and before wound closure [35]. Consequently, high local concentrations for a short period of time are achieved which potentially result in tissue damage. Another approach was to applied drugs in liquid form by injection or irrigation or, to extend the effectiveness by continuous perfusion. However, this method is labor intensive and requires experienced nursing staff to avoid leakage and drain blockage. Furthermore, the use of implantable pumps which can be refilled percutaneously is described [36]. An additional method used was to soak the cotton gauze or linen operative material with the drug and leave it in the wound until the final closure. This procedure is still in use in many countries to minimize the post-operative risk of infection, e.g. in dirty abdominal wounds or in trauma patients.

The first step to standardized commercially available antibiotic delivery systems has been made early 1970s. Buchholz and Engelbrecht [37] reported a technique of local antibacterial therapy in treatment of bone infection. The concept involved the prophylactic placement of antibiotic-impregnated beads made from polymethylmethacrylate (PMMA) bone cement into the bone cavity. Addition of macrolide antibiotics especially gentamicin to acrylic cement allows for sustained postsurgical anti-infectious treatment. However, the compression strength of cement can be affected by added antibiotic [38,39]. Drug release is typically extended for periods of weeks to months and incomplete [40–42]. Bunetel et al. [43] demonstrated a biphasic release with primary half-lives of 2.7 h in drainage fluid and 7.16 h in urine and secondary half-lives of 13.5 and 47.12 h, respectively. The exothermic nature of the curing process of the cement assisted the antimicrobial effect of the antibiotic. Besides the factors mentioned previously (see Section 1.1), the selection of the antibiotic agents suitable for use in PMMA bone cement was restricted by (i) adequate water solubility to permit diffusion of the antibiotic from the cement, (ii) short-term heat stability at temperatures up to 100 °C which occur frequently when the powdered polymer catalyst is mixed with the liquid monomer to form the bone cement and (iii) a bactericidal effect at low doses in order to avoid any adverse effect of higher drug loading on the mechanical properties of the bone cement. This initial use of antibiotic-impregnated bone cement in the treatment of osteomyelitis was very successful with relatively few disadvantages, as follows. First, the use of cement blocks inhibited drainage of secretions from the debridement area. Secondly, the same cement was also very difficult to remove if redebridement was necessary.

To overcome these drawbacks, a system with antibiotic-impregnated cement beads strung on steel surgical wire has been developed. These bead chains were flexible and were impregnated with gentamicin. The antibiotic beads were designed to treat localized infections residing in bone and soft tissues [44–46]. In the following years, gentamicin-impregnated PMMA beads have been commercialized in Europe under the trade name Septopal®. The product has not been approved in the US. Based on this development, many US surgical and orthopedic centers started to prepare their own bone cement beads containing tobramycin, another FDA-approved aminoglycoside, to support systemically given drug by local administration. These homemade beads are now a non-approved standard in local drug delivery in trauma, orthopedics and soft tissue infections [47,48]. The most substantial disadvantage of these bead systems is the need to remove the beads after the infection has been treated. This removal surgery is usually more difficult than the implantation because of local tissue scaring and adhesion and may lead to postoperative infection due to both the patient local and systemic condition. In addition, the second procedure poses the risk of additional pain, anaesthetic complications, and inferring extra costs. Recently, a Dutch group of scientists has found that despite of antibiotic release, beads act as a biomaterial surface at which bacteria preferentially adhere, grow and potentially develop antibiotic resistance [49]. Extensive culture procedures indicated the presence of bacteria on gentamicin-loaded beads in 18 of the 20 patients. Nineteen of twenty-eight bacterial strains isolated were gentamicin resistant and cultures from three patients yielded highly gentamicin-resistant sub-populations. In vitro release tests under sink-conditions revealed that gentamicin liberation from PMMA beads increased steadily for several weeks (Fig. 2) [50]. In vivo, PMMA beads slowly liberate gentamicin resulting in exudate levels of approximately 30 mg/l within the first day postoperatively and provide local concentrations of approximately 5 mg/l for several weeks [51,52]. These levels are well above the concentrations necessary to inhibit sensitive and moderately sensitive ethiologically important pathogens, whereas resistant microbes may not be affected. The effect in tissue approximately 1–2 mm away from the implant, where the concentration will be much lower, is still unclear [13]. Following implantation of PMMA beads, recurrence of infection most likely occurs within the first 2 weeks after surgery [53].

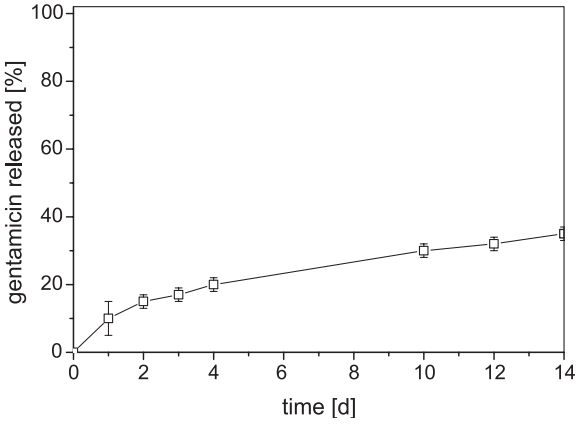


Fig. 2. Gentamicin release in vitro from PMMA beads (Septopal®) (modified from Ref. [50]).

Inorganic materials like calcium sulfate or calcium phosphate both as particles and cements can be utilized to delivery antibiotics at bony sites for a few weeks' periods as well [18,54,55]. The addition of an antibiotic to cement may negatively affect their consistency and porosity [55,56]. Carriers based on biodegradable, synthetic polymers like polylactic acid, polyglycolic–polylactic acid, poly(ortho esters), polyhydroxybutyrate–co-hydroxyvalerate have only been tested in a few studies. The systems studied were either monolithic implants [50,57] or microparticulate [58,59]. One of the most advanced biodegradable synthetic systems is Septacin®, a polyanhydride implant containing gentamicin sulfate for osteomyelitis treatment. The system has recently been reviewed by Li et al. [60]. Polymer-drug pellets are prepared by extrusion, pelletizing and injection molding and γ-irradiation. In vitro and in vivo release sustains for approximately 4 weeks. A similar gentamicin containing local delivery system has been described by Yang et al. [61]. The authors used poly(oleic/linoleic acid dimmer: sebacic acid (PolyOAD/LOAD:SA) loaded with 20% of gentamicin to form implantable beads. In an in vitro dissolution study, the gentamicin concentration peak was found on day 2. The concentrations slowly decreased and considerable amounts of gentamicin were still released on day 50. From day 2 to day 50, the

gentamicin concentration in the releasing fluids was from 59- to 42128-fold and 1.8 to 1314-fold of the MIC for *S. aureus* and *E. coli*, respectively. Furthermore, hydrogel-type systems based on fibrin glue or calcium alginate have been tested in vivo and increased local antibiotic levels and activity could be demonstrated [15,62–64]. Another recently introduced model describes gentamicin coating of metal implants as a way to reduce postoperative complications [65]. In this animal study, 10% gentamicin-loaded poly(D,L-lactide) (PDLLA) coating of orthopedic devices have been tested in preventing implant-related bacterial osteomyelitis. Bacteriological and radiological as well as histological signs of infection were significantly reduced in the gentamicin-coated group compared to controls.

In the early 1980s, another commercial drug delivery system for antibiotics has been developed. This system utilized a collagen sponge matrix as a carrier for gentamicin and has two main advantages: (i) it leads to a very high local drug level without achieving systemic drug concentration, and (ii) is fully biocompatible and biodegradable which eliminates the need of secondary surgery to remove a carrier. In the following sections, this paper will subsequently provide a systematic review of collagen as a drug carrier for anti-infective drug mostly in trauma, orthopedics, soft tissue infection, dirty abdominal surgery and wound healing.

2. Collagen carriers for local delivery of antibacterial drugs

2.1. Drug release characteristics from collagen matrices and clinical experience in wound healing

For local antibiotic delivery, the goal should be able to maintain the highest possible, but not toxic, local drug concentration without achieving systemic effects. This can be achieved by physical and possibly also chemical incorporation of the drug into a collagen matrix in the course of the manufacturing process to assure drug immobilization. Drugs may be complexed to collagen through direct binding of the drug to free amino or carboxylic groups of the collagen molecule [5]. Drug release occurs by diffusion from a collagen matrix implanted or injected as such or polymerized after intra-tissue injection [5,6,16]. For example, a tetracycline solution injected subcutaneously reached a maximum serum concentration after 3 h which slowly decreased within the next 20 h. When the same amount of tetracycline solution was soaked into a collagen sponge and inserted into a natural body cavity, the drug release was detected over a period of 14 days resulting in a relatively constant serum concentration of the drug [5].

This approach forms the principle of a currently marketed collagen-based product designed for on-site delivery of gentamicin. This product has been primarily approved and marketed in Europe and is now available also in many different counties worldwide (manufacturer: Innocoll, Saal/Donau, Germany, distributors: i.e. Sulmycin®-Implant, Garacol®, Gentacoll®, Garamycin®-Schwamm, Collatamp®-G, Collatamp®-EG, etc., Schering-Plough (USA) and its local subsidiaries or Collatamp®-G, Collatamp®-EG, Syntacoll (Switzerland)). The matrix which delivers the drug is a biocompatible and locally re-modelable, partially close-porous sponge in which the drug is incorporated. The design of the sponge and the drug incorporation by colyophilization allow a uniform distribution of the drug within the spongy matrix and assure an equal drug dose applied per square centimeter of the treated surface (Fig. 3). Pore size of collagen sponges can be adapted by the lyophilization process [66]. The collagen used may be of either bovine or equine origin and is isolated from Achilles tendons. Collagen sponges used for the local gentamicin delivery of antibiotics have been designed to assure a specific drug kinetic and prevent potential development of resistance. The unique character of the collagen matrix provides five major advantages: (a) partial open porosity for quick release of the drug after implantation into the tissue, (b) partial close porosity for ‘‘secondary’’ release of the drug enclosed within pores, (c) ‘‘tertiary’’ release of the drug partially immobilized within the fibrillar collagen structure, (d) a three-dimensional structure which works as a ‘‘natural’’ distance barrier between the drug incorporated into the sponge and the surrounding environment and (e) a network which enhances cell penetration and new tissue formation. Consequently, the collagen matrix provides both rapid and prolonged gentamicin release.

Pharmacokinetic data collected from over 1500 patients with either soft tissue-related or bone-related

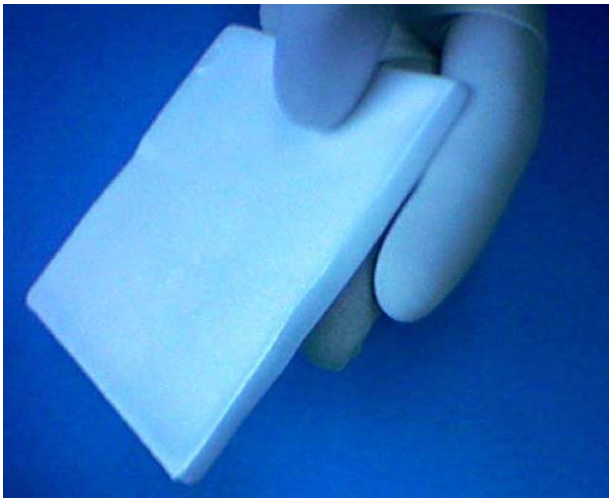


Fig. 3. Commercially available collagen–gentamicin sponge (200 mg gentamicin sulfate and 280 mg highly purified native fibrillar collagen).

infections demonstrate that surgical implantation of 1 to 5 gentamicin–collagen sponges which corresponds to a drug dose (gentamicin sulfate) of 200 to 1000 mg (depending on wound size, by always constant drug amount applied per square centimeter of wound area) which generated very high concentrations of gentamicin (170–9000 μg/ml) in the local tissue (depending of a local tissue vascularization level and anatomical localization). These levels of antibiotic, which are achieved within 24 h following implantation of the sponges into the surgically debrided site, are well above the established MIC for gentamicin-sensitive or low-sensitive organisms (4 and 8 μg/ml, respectively). At the same time, systemic levels of gentamicin remained well below the established toxicity thresholds of 10–12 μg/ml for peak values and fell below 2 μg/ml by 24 h for all patients evaluated. An example of the in vitro and in vivo release profile of the product is shown in Fig. 4a and b. In vitro, the aminoglycoside is rapidly release due to fast dissolution from the porous matrix and liquid exchange with the incubation medium. The sustained in vivo release profile should assure total eradication of pathogenic bacteria from the wound area by wound surface and volume-related manner [67–69]. This release kinetic cannot be achieved using local drug injection or powder spreading or drug loaded polymer beads. Despite the high local drug concentration after in vivo administration of collagen–gentamicin sponges, significant or therapeutic serum gentamicin levels are not reached (Fig. 4c). Consequently, systemic side effects or cumulative effects with collagen–gentamicin implants have not been reported for more than 1 million patients treated. The efficacy of the gentamicin–collagen sponge in the treatment and prevention of soft tissue-related infections is supported by data from 661 patients who received treatment with the gentamicin–collagen sponge primarily for intra-abdominal-related surgeries or wound infections following surgical procedures or traumatic events. Approximately, 40% of these patients had clean-contaminated or contaminated surgical procedures. In the majority of cases, one to three sponges (corresponding to 200 to 600 mg of gentamicin sulfate, 2.0 mg/cm^2 of the sponge) were used. The combined results of the randomized, controlled studies in these patient populations yielded a positive outcome in favor of the gentamicin–collagen sponge: 95.6% compared with 72.5% of patients healed by either primary intention or without evidence of post-operative infection. Additionally, two controlled, parallel-group studies showed a reduction in duration of hospital stay by 15% and 22%, respectively, when collagen–gentamicin sponges ware used to support standard therapy.

Frequently, soaking of collagen sponges with antibacterial drug containing solution is described [70,71]. However, there is a significant difference whether the drug is immobilized in a collagen matrix by immersion before use, by immersion and subsequent drying or by

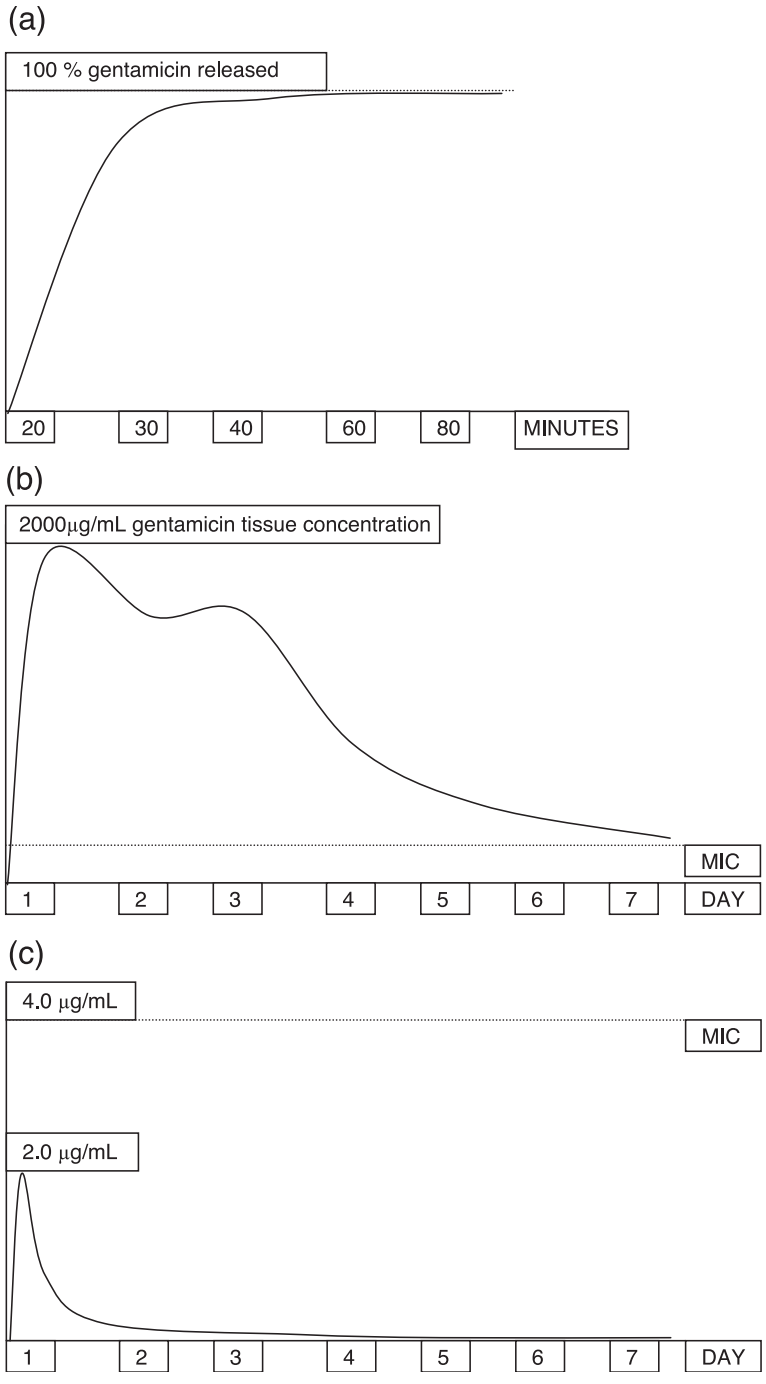


Fig. 4. (a) In vitro drug release profile from one collagen–gentamicin sponge (200 mg gentamicin sulfate and 280 mg collagen) collected by standard EP/USP dissolution method; (b) local concentration of gentamicin in soft tissue (after implantation of 3 units of Sulmycin®-Implant, total dose of 600 mg gentamicin sulfate), and (c) serum concentrations after implantation of three collagen–gentamicin sponges (Sulmycin®—Implant, total dose of 600 mg gentamicin sulfate) into postoperative abdominal wound.

incorporation of the drug into collagen dispersion during the manufacturing process of a sponge or a membrane. A comparison study of five different antibiotics (gentamicin sulfate, cefotaxim, fusidic acid, clindamicin, and vancomycin) ''immobilized'' in a pre-prepared collagen sponge by soaking demonstrated that drug was released completely within mostly 3–4 days if tested in adapted dissolution test [70]. Only in case of gentamicin sulfate that dissolution continued up to day 7 and retained appropriate bacterial grow inhibition, regardless of what preparation was used (immersed in antibiotic solution or lyophilized sponge). This specific release of gentamicin cannot only be attributed to an ionic interaction between the amino groups of the gentamicin and the carboxyl groups of the collagen, since the same interaction occurs also in the case of clindamycin and cefotaxime. Both drugs had, however, much shorter release time of only 1 day. This binding of gentamicin sulfate to collagen fibers requires further investigation [70].

Another way to achieve prolonged release of the drug has been demonstrated by a product utilizing chemically modified antibiotics to decrease its water solubility and—consequently—to prolong its local bioavailability. Recently, a medical device (Septocoll®, Merck Biomet, Berlin, Germany) ancillary utilizing two gentamicin salts, sulfate (high solubility) and crobefat (low solubility) to protect collagen hemostatic sponge from potential infection has been approved in Europe. In this combination, gentamicin sulfate is released over a few hours while gentamicin crobefat remains in place over days [72].

2.2. Clinical experiences in the use of collagen for local antibiotic delivery in ophthalmology

The need to prolong the presence of the drug in the tear pool has been recognized since many years. Diverse systems have been proposed to prevent quick wash out of the drug. Some authors evaluated the therapeutic effect of soft contact lenses on antibiotic delivery to the cornea [73,74]. Later in 1980, collagen shields have been introduced as a drug delivery system for ophthalmic applicable antibiotics. The drugs of choice were gentamicin, widely used for treatment of ocular infections, as well as vancomycin. Collagen corneal shields, dry contact lens-shaped films have been immersed with gentamicin or vancomycin solution, respectively. Additionally, a mixture of gentamicin and vancomycin has been used [75]. Experiments performed both in vitro and in rabbits demonstrated, that presoaked collagen shields released the majority of gentamicin within the first 30 min, while vancomicyn was released gradually over 6 h. In vivo, this release time was longer and the local drug concentration higher securing the drug dose well above the therapeutic range. Negative, in particular, cell toxic side effects of the drug to rabbit eye have not been found. All of these characteristics in combination with the potential for improvement of patient comfort during treatment (i.e. for bacterial keratitis) make the collagen shields an attractive modality for drug delivery. Additionally to drug release, collagen corneal shields have been previously found to speed up epithelial cell differentiation and reduce stromal swelling in corneal wounding studies or in human studies after cataract surgery [76–78].

Untermann et al. [79] demonstrated that tobramycin was released within 72 h. Collagen shields immersed in two drug concentrations produced significantly higher (200 mg/ml) or higher (40 mg/ml) concentration of antibiotic in the cornea than subconjunctivally injected drug. However, drug-related toxic side effects have been observed in the high-concentration group. Local distribution of the drug was found to be more reproducible and uniform. Also in this study, a positive effect of collagen on corneal wound healing has been described. Less promising results have been reported if similarly gentamicin-soaked collagen shields have been used [80]. The authors concluded that only a fortified topical gentamicin application pathway lead to therapeutic drug concentration in the aqueous.

In another study, discussing the role of corneal collagen shields as a drug delivery device for treatment of bacterial keratitis, gentamicin eye drops (13.6 mg/ml) and collagen ocular shields soaked with 13.6 mg/ml gentamicin solution for 5 min have been analyzed [81]. It was found that the single use of gentamicin-soaked collagen shields does not improve the treatment if compared to topical delivery of fortified antibiotic concentrations by drops every 30 min over 24 h. However, if a combination of collagen shield (pre-soaked and not pre-soaked with the drug) with a continuous administration of gentamicin eye drops has been used, bacterial eradication was

significantly more effective than in the group treated with the standard gentamicin drops administration alone. Collagen corneal shields discussed above have been approved by FDA as medical device class-I and listed in the year 2000. Despite many positive impulses coming from different animal studies and from studies combining ophthalmic drug and anti-inflammatory agents [82,83], collagen ocular shields containing drugs were never been further developed and approved as a product for treatment of ophthalmic infections.

2.3. Clinical experiences in the use of collagen for local antibiotic delivery in odontology

At the end of 1980s, collagen-derived matrices containing tetracycline have been proposed for treatment of periodontal disease. The matrices have been prepared by mixing 1% of bovine collagen solution with either 5 or 10 mg/ml of pulverized tetracycline, placing into plastic molds and air-drying or freeze-drying. Subsequently, collagen membranes have been chemically cross-linked by immersion with 2% glutaraldehyde [84]. It was found that the degree of drug release could be partially controlled by the collagen concentration in the matrix and the time of the cross-linking process. If compared to other applications (e.g. intra-pocket drop application), tetracycline immobilized in glutaraldehyde cross-linked collagen matrix released much slower. A therapeutic dose of the drug was present in the pocket for up to 10 days. In patients treated with collagen–tetracycline composites for a 4-week time period in 1-week intervals, microbiological studies demonstrated that bacterial eradication was much more effective and bleeding index improved [85]. The clinical effects of a single application of a 5% metronidazole collagen device in periodontal pockets deeper than 5 mm can be improved in association with debridement and without re-enforcement of home care and hygiene as practiced by the patient at any time [86].

Recently, PerioChip® has been developed in Israel and approved both in US and Europe. PerioChip® is a small chip, rounded at one end, for insertion into the periodontal pocket to support the standard therapy of adult parodontitis. PerioChip® is composed of 2.5 mg of chlorhexidine-D-gluconate in a non-porous biodegradable matrix of hydrolyzed gelatin cross-linked with glutaraldehyde. The product releases chlorhexidine subgingivally before and as the chip degrades over a total period of up to 7–10 days. The mean concentration of chlorhexidine in the gingival crevicular fluid (GCF) was 2007 μg/ml at 2 h and remained in the range of 1400–1900 μg/ml for the next 70 h [87]. Chlorhexidine remained at clinically effective levels (MIC 125 μg/ml) in the GCF of the periodontal pockets for over 1 week without detectable systemic absorption. PerioChip® is completely degraded by enzymes within 7 to 10 days and does not need to be removed. It has been demonstrated that PerioChip® used as an adjuvant to the classical surgical root cleaning (SRP) enhance the reduction of pocket depth by approx. 0.4 mm within 6 months if compare to SRP alone [87,88].

2.4. Diffusion restricted collagen sponge matrices

Many efforts have been made to sustain antibiotic release from collagen systems and to obtain the following theoretical drug release characteristic: quick release in the initial phase following implantation into the tissue followed by a possibly constant and high level over a defined time period (e.g. up to 7 days) and a short end phase in which the drug level rapidly drops (Fig. 5). It is still under discussion if such prolonged "Minivan-like" release model has advantages over the currently used products such as collagen gentamicin sponges containing either gentamicin sulfate or combination of gentamicin salts (see above). Additionally, the activity area of the drug released from the product should be restricted to the place of application and, possibly, to adjacent tissue, but the drug should not be available systemically.

Current known ways to change local kinetics in on-site drug delivery using collagen-based matrices are: (a) increasing collagen content (up to 30%) to make the matrix more dense and less permeable [89], (b) cross-linking the matrix to make it less permeable and longer standing [8,9,84], (c) changing drug molecule to make the drug less (water) soluble [72,90], (d) using diffusion restrictors in order to prolong the diffusion distance or to create a diffusion barrier [91], and (e) combination with other polymers, e.g. coating the collagen sponge containing the antibiotic additionally with resorbable poly(α-hydroxy acid) [92] or addition of anionic polymers such as alginic

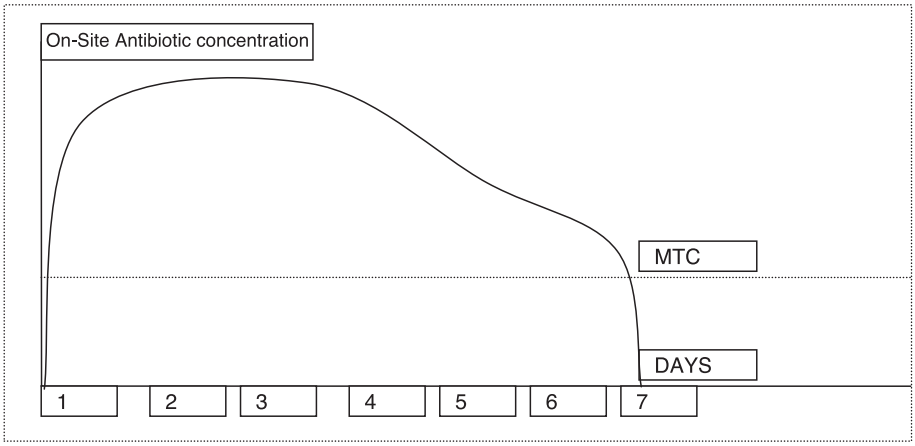


Fig. 5. A model proposed for an optimal in vivo antibiotic delivery profile (“minivan-like” release curve) based on quick initial release assuring high on-site level that remains constant over a period of approximately 7 days and final quick dose reduction.

acid or pectin [93] (see also Section 2.5). The diffusion restriction approach will be discussed further in detail in this section.

Diffusion restrictors (barriers or multilayer design) without changing the basic biological parameters of the collagen carrier appear to be a promising approach since chemical modifications of collagen or the drug can be avoided. Advanced technologies in manufacturing of collagen-based implants allow to create novel collagen-based materials, e.g. leather-like collagen sheets of different strength, collagen “pockets” [94], or “pillows or tortellini-like” and collagen “sandwich”-like structures of different permeability and porosity as well as collagen tubes and channels with or without lumen (Fig. 6) [95]. The technology allows manufacturing of collagen matrices having not only different porosity and permeability, but also different wetting time, fluid absorption and drug release characteristics.

Preliminary data collected with novel collagen matrix preparations demonstrated that depending on the basic collagen content, the way of manufacturing and applied combination of different matrix variants, different drug release characteristic may be achieved [95]. For example, variation of compression pressure at identical temperature and compression time leads to different release profiles (Fig. 7). It is important to stress that the use of thermal compression at high temperature and high pressure does not negatively influence collagen nativity and main biological functions (e.g. platelets docking capacity, cell growth in vitro). As an alternative, a release restriction barrier in form of a collagen pillow cover can be applied to coat a gentamicin–collagen sponge core. The drug release profile is changed significantly and an almost linear release profile could be achieved (Fig. 8). This unique technology has been applied recently for development of a novel family of collagen-based matrices allowing specific drug release kinetic. Another possibility is to prepare collagen membranes which during fluid absorption may expand into a sponge-like, three-dimensional structure [91]. Such matrix can be used to incorporate drug components which are sensitive to final sterilization, since it may be pre-fabricated, loaded and finally manufactured under sterile conditions.

2.5. Collagen/PLGA composite

Gentamicin release from a porous collagen sponge is completed in vitro within 1 h and in vivo at well-perfused sites within 1 to 4 days [10,14,96,97]. Therefore, in accordance with Ipsen and Wachol-Drewek, an implantable, biodegradable drug delivery system based on collagen was developed providing an initial antibiotic dose followed by a sustained drug delivery of gentamicin within 1 week [70,98]. The device was based on biodegradable poly(lac-

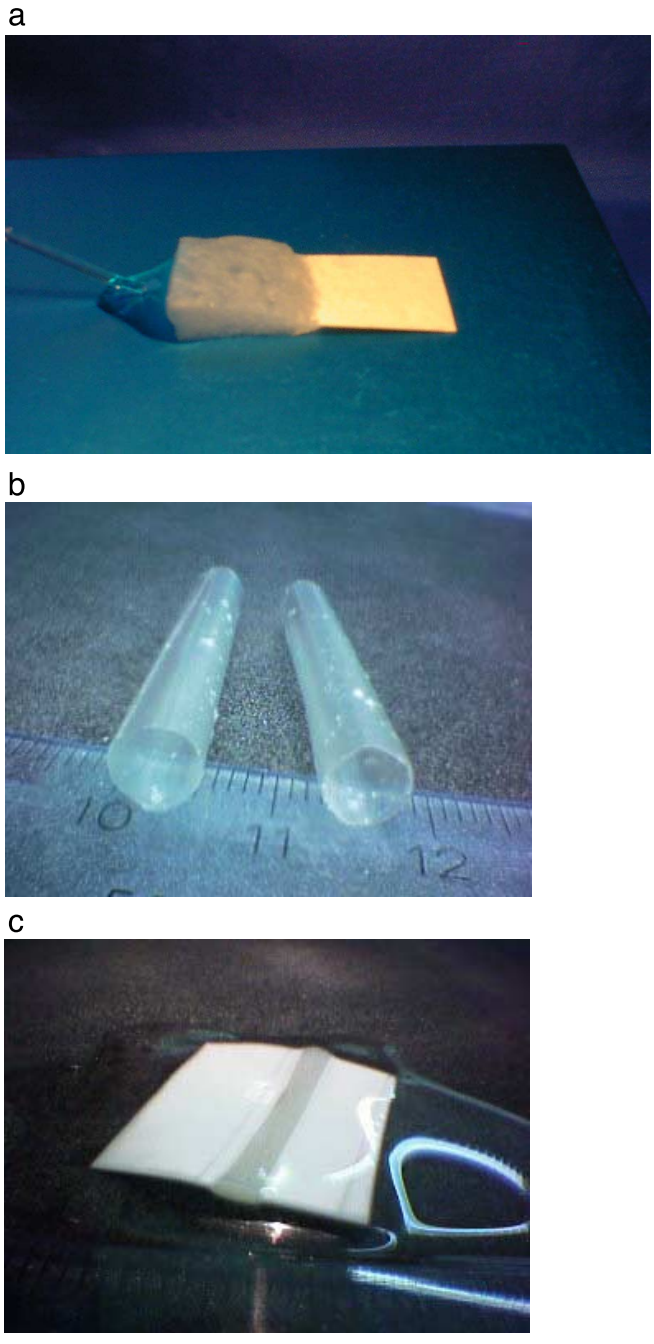


Fig. 6. (a) New highly absorbing collagen matrix developed for drug immobilization. Preparation by thermal compression, initial thickness of 0.03 mm. A "spontaneous" soaking of drug solution within 3 s (5.0 × 8.0 cm); (b) collagen tubes; (c) a collagen-based construct containing both highly absorbing part (core) and low-absorbing part. Each of the parts can contain either in-process immobilized or secondary immobilized drug component (with permission from Refs. [91,95]).

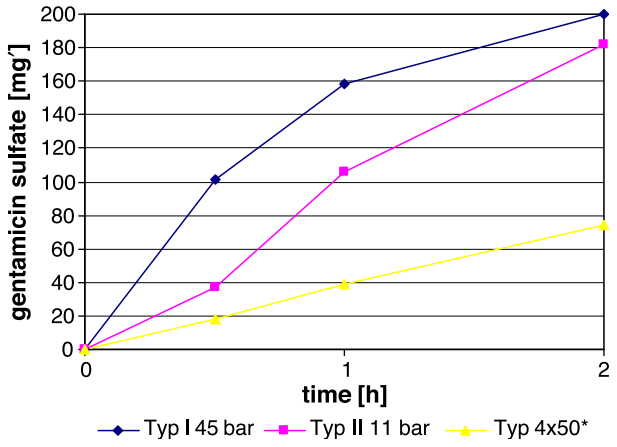


Fig. 7. In vitro drug release kinetic obtained from three different collagen-gentamicin constructs (containing identical drug dose, 200 mg each) manufactured by different compression parameters. Type-I–10.0 kg/cm^2, Type-II–2.5 kg/cm^2, Type-4×50–2.5 kg/cm^2. (according to Ref. [95], modified).

tide–co-glycolide) (PLGA) microparticles which were combined with collagen in order to achieve their fixation in an implantable drug delivery system presenting the favourable effect of collagen on wound healing.

Preparation of the microparticles was carried out applying a W/O/W double emulsion technique [99]. An aqueous solution of the antibiotic was mixed with the organic polymer solution and subsequently transferred into a second aqueous phase. After hardening,

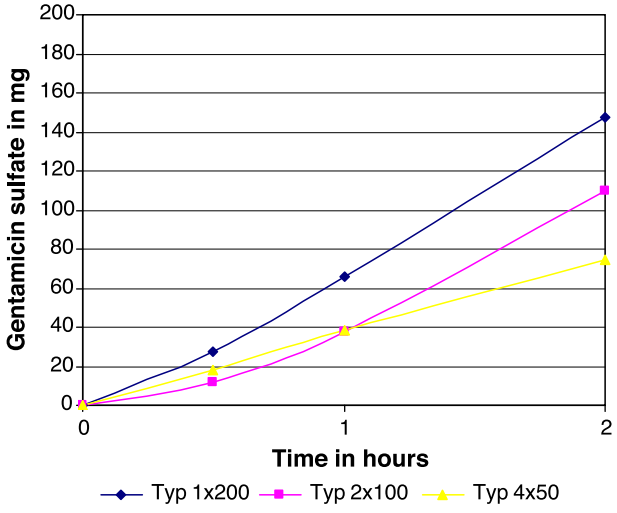


Fig. 8. In vitro drug release kinetic obtained from three different collagen-gentamicin constructs (containing identical drug dose, 200 mg each) manufactured by thermal compression (identical pressure, time and temperature) using additional barrier (release restrictor) made of a plane collagen. Type-1×200–collagen/gentamicin sponge covered by one diffusion barrier; Type-2×100–two collagen/gentamicin sponges (a 100 mg) covered by one diffusion barrier; Type 4×50–four collagen/gentamicin sponges (a 50 mg) covered by one diffusion barrier. (according to Ref. [95], modified)

while stirred, the particles were separated and freeze dried. This process ensured 15% loading of the particles with gentamicin to allow for combination of 5 mg microparticles (containing 0.75 mg gentamicin) with an aqueous preparation containing 1 mg collagen dispersed and 0.75 mg gentamicin dissolved which is lyophilized to yield a homogeneous collagen/PLGA microparticles implant [100]. A 50:50 polymer mixtures of RG 503 and RG 502H yielded the desired release profile over 1 week. The release of gentamicin was connected with a decrease in polymer molecular weight and glass transition temperature until a structural breakdown of the particles reaching a critical molecular weight of 15,000 Da occurred [100–102]. At the same time, the water content increased and reached its maximum after 3 days. Consequently, the release of gentamicin from 50/50 RG 503/RG 502H microparticles was completed after 7 days. Different concentrations, pH values and temperatures of the collagen dispersion were tested rheologically, microscopically and spectroscopically to optimize processing of the composite including avoidance of particle sedimentation (Fig. 9) and particle loss upon incubation in liquid (Fig. 10) [103,104]. The final composite was prepared using a 1% collagen dispersion, pH 4.5 at room temperature (Fig. 11a, b).

The collagen/PLGA microparticles dispersion with additional free gentamicin for an initial burst was lyophilized to obtain an implant with microparticles

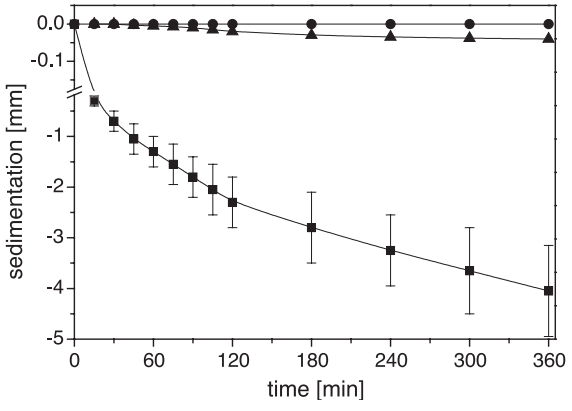


Fig. 9. Sedimentation of PLGA microparticles in collagen dispersions pH 4.5:0.1% at 22 °C (■), 0.56% at 22 °C (●) and 0.56% at 40 °C (▲) (with permission from Ref. [104]).

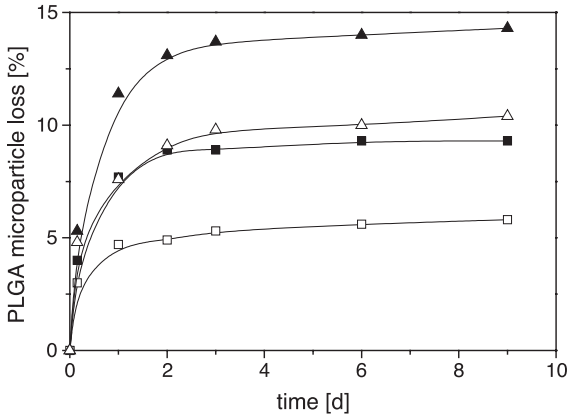


Fig. 10. Microparticle loss from collagen sponge/PLGA microparticles composite in PBS: composites prepared at a freezing rate of 5 °C/h (a) and 20 °C/h (b) at 0.56%/22 °C (▲), 1%/22 °C (△), 0.56%/40 °C (■), 1%/40 °C (□) collagen concentration/suspension temperature (modified from Ref. [103]).

embedded homogeneously. Freezing the dispersion with 5 °C/h increased the amount of initially liberated gentamicin to 35% as compared to 15% to 20% for the non-processed microparticles whereas the original release profile could be preserved by freezing with 20 °C/h (Fig. 12) [103]. The increase in the initial liberation of gentamicin was due to longer exposure of the microparticles to the acidic aqueous environment of the collagen dispersion. Additional investigations demonstrated a marked loss of gentamicin from microparticles dispersed in the collagen gel at 4 and 22 °C (Fig. 13). At 40 °C, this loss was limited to 10% due to closing pores at a temperature above the glass transition temperature as shown microscopically [104]. Ethylene oxide as well as β- and γ-irradiation were tested for sterilization of the collagen/PLGA microparticles composite. All methods resulted in a decrease of molecular weight and glass transition temperature of polymer raw material and microparticles [103,105]. In addition, ethylene oxide treatment yielded aggregation of microparticles leading to a substantial increase in the initially liberated gentamicin dose. Furthermore, chemical changes of gentamicin after ethylene oxide sterilization could be identified using NMR spectroscopy. Despite of a decrease in the molecular weight and glass transition temperature after irradiation, neither morphological changes of the composites nor changes regarding the

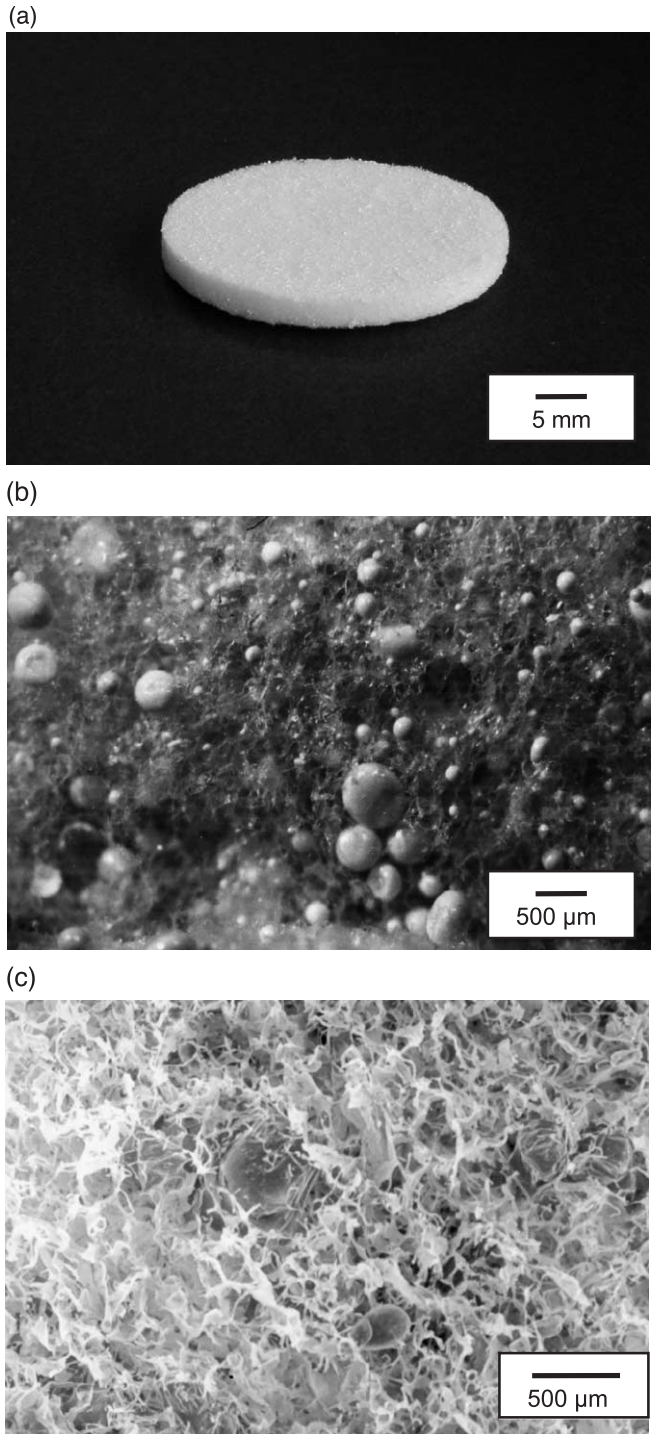


Fig. 11. Overview (a) and SEM of cross section (b) of collagen sponge/PLGA microparticles composite (1% collagen dispersion, 22 °C suspension temperature, 20 °C/h freezing rate) and (c) γ-sterilized collagen sponge/PLGA microparticles composite (with permission from Ref. [103]).

gentamicin release profile from β- and γ-sterilized material were observed. Free radicals, which could only be detected in gentamicin drug substance and at marginal level in gentamicin-loaded microparticles, disappeared within 4 weeks. Additional microbiological testing verified the microbiological activity of gentamicin liberated from β-sterilized collagen/PLGA microparticles composites [104]. Storage at 4 °C/35% r.h. for 3 months did not influence morphology, molecular weight, glass transition temperature and release profiles of microparticles and collagen/PLGA microparticles composites. However, storing at 25 °C/60% r.h. and 40 °C/75% r.h. yielded a marked decrease of molecular weight and glass transition temperature. This effect was due to a higher humidity, water uptake into polymers and subsequent hydrolysis of polymers and microparticles. Thus, the collagen sponge/PLGA microparticles composite presents an advancement in collagen carriers from antibiotics which enables further delay of antibiotic release. The results are encouraging and in vivo testing would be desirable.

Besides these monolithic porous or non-porous systems, collagen gels [106] as well as collagen microparticulates have been described in the literature [107,108]. Additionally, collagen-modified hyaluronan microparticles have been found useful as local antibiotic carrier [109].

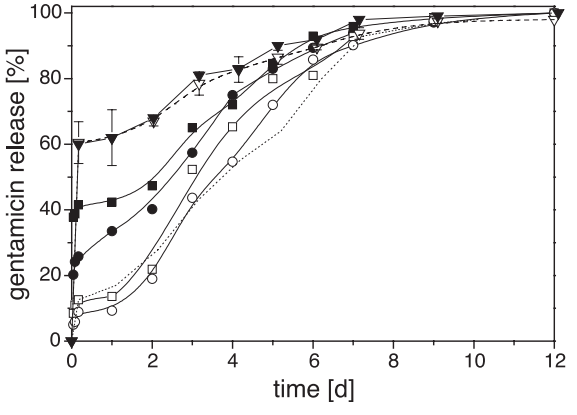


Fig. 12. Gentamicin release from collagen sponge/PLGA microparticles composites prepared from 1% collagen dispersion (22 °C/5 °C/h (■), 22 °C/20 °C (□), 40 °C/5 °C/h (●), 40 °C/20 °C/h (○)); additionally, original PLGA particles (···) and a composite which contains an additional 0.75 mg gentamicin bolus per mg collagen non-sterilized (··· ▽ ···) and γ-sterilized (··· ▼ ···).

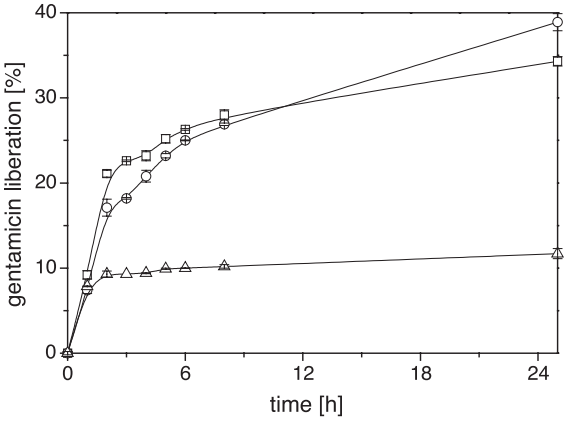


Fig. 13. Gentamicin loss from PLGA microparticles in acidic environment pH 4.5 at 4–8 °C (□), 22 °C (○) and 40 °C (△) during collagen sponge/PLGA microparticles composite preparation (modified from Ref. [104]).

3. Future and perspectives

There is a growing interest in collagen as a drug delivery vehicle. During the last 5 years, new developments enhancing mechanical and saving biological performance of collagen have been made. It seems that the near feature may be a renaissance period for collagen, an excellent natural, fully biocompatible and biodegradable drug carrier material. Depending on its fibrillar and structural nativity, process-controlled degradation time (e.g. by enzymatic pre-treatment before reconstitution into fibers or by chemical cross-linking to stabilize the final construct) collagen-based products can assure a bright spectrum of release kinetics. In products designed to have short diffusion distance or having open porosity, immediate, almost explosive drug release may be achieved. In contrast, product designed to have long or multilayer (multi-barrier) diffusion distance, having predominantly closed pores, or utilizing high amount of collagen which results in long degradation time, can achieve prolonged drug release. These technological possibilities open a new perspective, especially in creation of product utilizing antibacterial drug for either prophylactic or therapeutic use.

Currently, drug delivery systems containing only a single antibiotic drug (gentamicin) are available on the market. The next generation of collagen–drug delivery system will be focused on both drug combination and different release profiles which will lead to better infection control. Moreover, together with this development and better understanding of benefits coming from local drug delivery, some new collagen-based system may, in selected indications, even replace current standard of systemic antibiotic treatment.

It is important that clinical studies demonstrate the optimum antibiotic delivery regime as a target. The technical developments in collagen processing as well as combination of collagen with other materials should allow to specifically tailor the release rate to the desired kinetic for local delivery.

References

[1] J.C. Brown, R. Timple, The collagen superfamily, Int. Arch. Allergy Immunol. 107 (1995) 484–490.
[2] R.J. Todhunter, J.A.M. Wooton, G. Lust, R.R. Minor, Structure of equine type I and type II collagens, Am. J. Vet. Res. 55 (3) (1994) 425–431.
[3] J.Y. Exposito, C. Cluzel, R. Garrone, C. Lethias, Evolution of collagens, Anat. Rec. 268 (2002) 302–316.
[4] S.H. Liu, R.S. Yang, R. Al-Shaikh, J.M. Lane, Collagen in tendon, ligament and bone healing, Clin. Orthop. Res. 318 (1995) 265–278.
[5] M. Chvapil, Industrial uses of collagen, in: D.A.D. Parry, L.K. Creamer (Eds.), Fibrous Proteins: Scientific, Industrial and Medical Aspects, Academic Press, London, 1979, pp. 247–269.
[6] J.A.M. Ramshaw, J.A. Werkmeister, V. Glattauer, Collagen-based biomaterials, Biotechnol. Rev. 13 (1995) 336–382.
[7] K. Panduranga Rao, Recent developments of collagen-based materials for medical applications and drug delivery, J. Biomater. Sci., Polym. Ed. 7 (7) (1995) 623–645.
[8] J. Angele, J. Abke, R. Kujat, H. Faltermeier, D. Schumann, Z. Ruszczak, R. Mehrl, M. Nerlich, R. Kinner, C. Englert, R. Mueller, Influence of different collagen species on physico-chemical properties of crosslinked collagen matrices, Biomaterials (2003) (submitted for publication).
[9] E. Lesiak-Cyganowska, D. Sladowski, J. Komender, Modification of collagen film by certain chemical agents, Arch. Immunol. Ther. Exp. 49 (2001) 247–251.
[10] W. Friess, Drug Delivery Systems Based on Collagen, Shaker Verlag, Aachen, 2000, ISBN, 3-8265-6994-6.
[11] E.W. Taylor, Surgical infection: current concerns, Eur. J. Surg. 163 (S578) (1997) 5–9.
[12] S. Mehta, J.S. Humphrey, D.I. Dchenkmann, A.V. Seaber, T.P. Vail, Gentamicin distribution from collagen carrier, J. Orthop. Res. 14 (1996) 749–754.
[13] H. Grimm, Bakteriologische und pharmakokinetische Aspekte der topischen Antibiotikaanwendung, in: A. Stemberger, et al. (Eds.), Kollagen als Wirkstoffträger-Einsatzmöglichkeiten in der Chirurgie, Verlag Schattauer, Stuttgart, 1989, pp. 33–40.
[14] H.J.T. Rutten, P.H.A. Nijhuis, Prevention of wound infection in elective colorectal surgery by local application of a gentamicin-containing collagen sponge, Eur. J. Surg. 163 (S578) (1997) 31–35.
[15] V. Iannuccelli, G. Coppi, M. Bondi, M. Pinelli, A. Mingione, R. Cameroni, Biodegradable intraoperative system for bone infection treatment: II. In vivo evaluation, Int. J. Pharm. 143 (2) (1996) 187–194.
[16] A. Stemberger, H. Grimm, F. Bader, H.D. Rahn, R. Ascherl, Local treatment of bone and soft tissue infections with the collagen–gentamicin sponge, Eur. J. Surg. 163 (S578) (1997) 17–26.
[17] R.W. Lindsey, R. Probe, T. Miclau, J.W. Alexander, S.M. Perren, The effects of antibiotic-impregnated autogeneic cancellous bone graft on bone healing, Clin. Orthop. Relat. Res. 291 (1993) 303–312.
[18] C.L. Nelson, S.G. McLaren, R.A. Skinner, M.S. Smeltzer, T.J. Roby, K.M. Olsen, The treatment of experimental osteomyelitis by surgical debridement and the implantation of calcium sulfate tobramycin pellets, J. Orthop. Res. 20 (4) (2002) 643–647.
[19] K.J. Hendricks, D. Lane, T.A. Burd, K.J. Lowry, D. Day, J.G. Phaup, J.O. Anglen, Elution characteristics of tobramycin from polycaprolactone in a rabbit model, Clin. Orthop. Relat. Res. 392 (2001) 418–426.
[20] A. Gonzalez Della Valle, M. Bostrom, B. Brause, C. Harney, E.A. Salvati, Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement, Acta Orthop. Scand. 72 (3) (2001) 237–240.
[21] S. Galandiuk, W.R. Wrightson, S. Young, S. Myers, H.C. Polk Jr., Absorbable, delayed-release antibiotic beads reduce surgical wound infection, Am. Surg. 63 (9) (1997) 831–835.
[22] M.P. Singh, R. Brady Jr., W. Drohan, M.J. MacPhee, Sustained release of antibiotics from fibrin sealant, in: D.H. Sierra, R. Saltz (Eds.), Surgical Adhesives and Sealants, Technomic Publ., Lancaster, 1996, pp. 121–133.
[23] I. Yenice, S. Calis, H.S. Kas, M. Ozalp, M. Ekizoglu, A.A. Hincal, Biodegradable implantable teicoplanin beads for the treatment of bone infections, Int. J. Pharm. 242 (1–2) (2002) 271–275.
[24] M.F. Yagmurlu, F. Korkusuz, I. Gursel, P. Korkusuz, U. Ors, V. Hasirci, Sulbactam-cefoperazone polyhydroxybutyrate–co-hydroxyvalerate (PHBV) local antibiotic delivery system: in vivo effectiveness and biocompatibility in the treatment of implant-related experimental osteomyelitis, J. Biomed. Mater. Res. 46 (4) (1999) 494–503.
[25] M.A. Sande, G.L. Mandell, Antimicrobial agents; the aminoglycosides, in: A. Goodman Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), Goodman and Gilman's—The Pharmacologi-

cal Basis of Therapeutics, Pergamon, New York, USA, 1990, pp. 1098–1116.
[26] R. Okachi, T. Nara, The aminoglycosides: properties, biosynthesis, and fermentation, in: E.J. Vandamme (Ed.), Biotechnology of Industrial Antibiotics, Marcel Dekker, New York, USA, 1984, pp. 329–365.
[27] B.E. Rosenkrantz, J.R. Greco, J.G. Hoogerheide, E.M. Oden, Gentamicin, Anal. Profiles Drug Subts. 9 (1980) 295–340.
[28] H. Lorentzen, F. Kallehave, H.J. Kolmos, U. Knigge, J. Bülow, F. Gottrup, Gentamicin concentrations in human subcutaneous tissue, Antimicrob. Agents Chemother. 40 (1996) 1785–1789.
[29] D. Adam, W. Christ, Antibiotika und Chemotherapeutika; antiinfektiöse Therapie, in: W. Forth, D. Hentschler, W. Rummel (Eds.), Pharmakologie und Toxikologie, Wissenschaftsverlag, Mannheim, Germany, 1987, pp. 580–715.
[30] J.M. Aran, J.P. Erre, D. Lima da Costa, I. Debbarh, D. Dulon, Acute and chronic effects of aminoglycosides on cochlear hair cells, Ann. N.Y. Acad. Sci. 884 (1999) 60–68.
[31] http://www.medicine.mcgill.ca/cai/meded/drugdb/gentamicin/gentamicin_db.htm.
[32] DIN-Norm 58940, Medical microbiology—methods for the determination of susceptibility of pathogens (except mycobacteria) to antimicrobial agents (1989).
[33] M.E. Levison, L.M. Bush, Pharmacodynamics of antimicrobial agents, Infect. Dis. Clin. North Am. 3 (1989) 415–421.
[34] B.G. Katzung, Basic and Clinical Pharmacology, 8th ed., McGraw-Hill/Appelton and Lange, New York, 2000, pp. 784–792.
[35] N. Rushton, Applications of local antibiotic therapy, Eur. J. Surg. 163 (S578) (1997) 27–30.
[36] C.R. Perry, R.L. Pearson, Local antibiotic delivery in the treatment of bone and joint infections, Clin. Orthop. Relat. Res. 263 (1991) 215–226.
[37] H.W. Buchholz, E. Engelbrecht, Uber die Depotwirkung einiger Antibiotika bei Vermischung mit dem Kunstharz Palacos, Chirurgie 41 (1970) 511–513.
[38] R.C. Nelson, R.O. Hoffman, T.A. Burton, The effect of antibiotic additions on the mechanical properties of acrylic cement, J. Biomed. Mater. Res. 12 (4) (1978) 473–490.
[39] W.R. Krause, A. Hofmann, Antibiotic impregnated acrylic bone cements: a comparative study of the mechanical properties, J. Bioact. Compat. Polym. 4 (4) (1989) 345–361.
[40] E. Diez-Pena, G. Frutos, P. Frutos, J.M. Barrales-Rienda, Gentamicin sulphate release from a modified commercial acrylic surgical radiopaque bone cement: I. Influence of the gentamicin concentration on the release process mechanism, Chem. Pharm. Bull. 50 (9) (2002) 1201–1208.
[41] H. van de Belt, D. Neut, D.R.A. Uges, W. Schenk, J.R. van der Mei, H.C. van der Mei, H.J. Busscher, Surface roughness, porosity and wettability of gentamicin-loaded bone cements and their antibiotic release, Biomaterials 21 (19) (2000) 1981–1987.
[42] L. Bunetel, A. Segui, F. Langlais, M. Cormier, Osseous concentrations of gentamicin after implantation of acrylic bone cement in sheep femora, Eur. J. Drug Metab. Pharmacokinet. 19 (2) (1994) 99–105.
[43] L. Bunetel, A. Segui, M. Cormier, E. Percheron, F. Langlais, Release of gentamicin from acrylic bone cement, Clin. Pharmacokinet. 17 (4) (1989) 291–297.
[44] K. Klemm, Treatment of chronic bone infection with gentamicin–PMMA chains and beads, Accid. Surg. 4 (1976) 1–20.
[45] I.S. Bailey, N.J. Bundred, H.J. Pearson, P.R.F. Bell, Successful treatment of an infected vascular graft with gentamicin beads, Eur. J. Vasc. Surg. 1 (1987) 143–144.
[46] H. Wahlig, Gentamicin–PMMA: a drug delivered system in treatment of chronic bone soft tissue infections, J. Antimicrob. Chemother. 10 (1982) 463–465.
[47] N.Y. Gunya, Infections of bone and joints. In: E. Sherry (Ed.), Electronic Orthopedic Text Book 2003; http://www.worldortho.com/database/etext/infection.html.
[48] C.E. Wheerlees, Textbook of Orthopedics 2001; http://www.ortho-u.net/med.htm.
[49] D. Neut, H. van de Belt, L. Stokroos, J.R. van Horn, H.C. van der Mei, H.J. Busscher, Biomaterial-associated infection of gentamicin-loaded PMMA beads in orthopaedic revision surgery, J. Antimicrob. Chemother. 47 (2001) 885–891.
[50] C. Schmidt, R. Wenz, B. Nies, F. Moll, Antibiotic in vivo/in vitro release, histocompatibility and biodegradation of gentamicin implants based on lactic acid polymers and copolymers, J. Control. Release 37 (1995) 83–94.
[51] S.Å. Hedstrøm, L. Lidgren, S. Tørholm, R. Önnerfält, Antibiotic containing bone cement beads in the treatment of deep muscle and skeletal infections, Acta Orthop. Scan. 51 (1980) 863–869.
[52] P.L. Becker, R.A. Smith, R.S. Williams, J.P. Dutkowsky, Comparison of antibiotic release from polymethylmatacrylate beads and sponge collagen, J. Orthop. Res. 12 (1994) 737–741.
[53] G.H.I.M. Walenkamp, Gentamicin PMMA beads. A clinical, pharmacokinetic and toxicological study. PhD Thesis, University of Nijmegen, Netherlands, 1983.
[54] H. Winkler, O. Janata, C. Berger, W. Wein, A. Georgopoulos, In vitro release of vancomycin and tobramycin from impregnated human and bovine bone grafts, J. Antimicrob. Chemother. 46 (3) (2000) 423–428.
[55] M. Takechi, Y. Miyamoto, K. Ishikawa, M. Nagayama, M. Kon, K. Asaoka, K. Suzuki, Effects of added antibiotics on the basic properties of anti-washout-type fast-setting calcium phosphate cement, J. Biomed. Mater. Res. 39 (2) (1998) 308–316.
[56] A. Ratier, I. Gibson, S.M. Best, M. Freche, J.L. Lacout, F. Rodriguez, Setting characteristics and mechanical behavior of a calcium phosphate bone cement containing tetracycline, Biomaterials 22 (9) (2001) 897–901.
[57] F. Turesin, I. Gursel, V. Hasirci, Biodegradable polyhydroxyalkanoate implants for osteomyelitis therapy: in vitro antibiotic release, J. Biomater., Sci., Polym. Ed. 12 (2) (2001) 195–207.
[58] S. Prior, C. Gamazo, J.M. Irache, H.P. Merkle, B. Gander, Gentamicin encapsulation in PLA/PLGA microspheres in view of treating *Brucella* infections, Int. J. Pharm. 196 (2000) 115–125.

[59] D. Sendil, I. Gursel, D.L. Wise, V. Hasirci, Antibiotic release from biodegradable PHBV microparticles, J. Control. Release 59 (1999) 207–217.
[60] C.L. Li, J. Deng, D. Stephens, Polyanhydride implant for antibiotic delivery—from the bench to the clinic, Adv. Drug Deliv. Rev. 54 (7) (2002) 963–986.
[61] X.F. Yang, F.D. Zeng, Z.B. Zhou, K.X. Huang, H.B. Xu, In vitro release and antibacterial activity of poly(oleic/linoleic acid dimer: Sebacic acid)-gentamicin, Acta Pharmacol. Sin. 24 (2003) 306–310.
[62] C.J. Woolverton, J.A. Fulton, S.J. Salstrom, J. Hayslip, N.A. Haller, M.L. Wildroudt, M. MacPhee, Tetracycline delivery from fibrin controls peritoneal infection without measurable systemic antibiotic, J. Antimicrob. Chemother. 48 (6) (2001) 861–867.
[63] C.J. Woolverton, K. Huebert, B. Burkhart, M. MacPhee, Subverting bacterial resistance using high dose, low solubility antibiotics in fibrin, Infection (Munich) 27 (1) (1999) 28–33.
[64] P. Marone, V. Monzillo, C. Segu, E. Antoniazzi, Antibiotic-impregnated fibrin glue in ocular surgery: in vitro antibacterial activity, Ophthalmologica 213 (1) (1999) 12–15.
[65] M. Lucke, G. Schmidmaier, S. Sadoni, B. Wildemann, R. Schiller, N.P. Haas, M. Raschke, Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats, Bone 32 (5) (2003) 521–531.
[66] M. Geiger, W. Friess, Collagen sponge implants—applications, characteristics and evaluation: Part I, Pharm. Tech. Europe 14 (2002) 48–56.
[67] W. Attmanspacher, V. Dittrich, A. Schaetzler, H.W. Stedtfeld, Mittelfristige Ergebnisse nach postoperativen Infektionen an der Schulter, Unfallchirurg 103 (2000) 1048–1056.
[68] S.J. Humphrey, S. Mehta, A.V. Seaber, T.P. Vail, Pharmacokinetics of a degenerable drug delivery system in bone, Clin. Orthop. Res. 349 (1998) 218–224.
[69] S. Mehta, J.S. Humphrey, D.I. Dchenkmann, A.V. Seaber, T.P. Vail, Gentamicin distribution from collagen carrier, J. Orthoptera Res. 14 (1996) 749–754.
[70] Z. Wachol-Drewek, M. Pfeiffer, E. Scholl, Comparative investigation of drug delivery of collagen implants saturated in antibiotic solutions and a sponge containing gentamicin, Biomaterials 17 (1996) 1733–1738.
[71] S. Baar, C. Schoerner, M. Roellinghoff, M. Radespiel-Troeger, H.P. Huemmer, R.T. Carbon, Collagen patches impregnated with antimicrobial agents have high local antimicrobial efficacy and achieve effective tissue gluing, Infection 29 (2001) 27–31.
[72] U. Gruessner, M. Clemens, P.V. Pahlplatz, P. Sperling, J. Witte, P. Sperling, H.R. Rosen, Improvement of perineal wound healing by local administration of gentamicin-impregnated collagen fleeces after abdominoperineal excision of rectal cancer, Am. J. Surg. 182 (2001) 502–509.
[73] H.E. Kaufman, M.H. Uotila, A.R. Gasset, T.O. Wood, The medical use of soft contact lenses, Trans. Am. Acad. Ophthalmol. Otolaryngol. 75 (1971) 361–372.
[74] A.Y. Matoba, J.P. McCulley, The effect of therapeutic soft tissue lenses on antibiotic delivery to the cornea, Ophtalmology 92 (1985) 97–99.
[75] R.B. Phienney, S.D. Schwartz, D.A. Lee, B.J. Mondino, Collagen-shields delivery of gentamicin and vancomycin, Arch. Ophtalmol. 106 (1988) 1599–1604.
[76] J.V. Aquavella, M. del Cerro, P.S. Musco, The effect of a collagen bandage lens on corneal wound healing, Ophtalmic Surg. 18 (1987) 570–573.
[77] G. Renard, N. Bennani, P. Lutaj, C. Richard, C. Trinquand, Comparative study of collagen corneal shield and subconjunctival injection at the end of cataract surgery, J. Cataract Refract. Surg. 19 (1933) 48–51.
[78] J.V. Aquavella, J.J. Ruffini, J.A. LaCascio, Use of collagen shields as a surgical adjunct, J. Cataract Refract. Surg. 14 (1988) 492–495.
[79] S.R. Untermann, D.S. Rootman, J.M. Hill, J.J. Parelman, H.W. Thompson, H.E. Kaufman, Collagen shield drug delivery: therapeutic concentrations of tobramycin in the rabbit cornea and aqueous humor, J. Cataract Refract. Surg. 14 (1988) 500–504.
[80] N. Baziuk, C.M. Gremillon Jr., G.A. Peyman, H.K. Cho, Collagen shields and intraocular drug delivery: concentration of gentamicin in the aqueous and vitreous of a rabbit eye after lensectomy and vitrectomy, Int. Ophthalmol. 16 (1992) 101–107.
[81] J. Silbinger, G.A. Stern, Evaluation of corneal collagen shields as a drug delivery device for treatment of experimental *Pseudomonas* keratitis, Ophthalmology 99 (1992) 889–892.
[82] J.K. Milani, I. Verbkuh, U. Pleyer, H. Sumner, S.A. Adamu, H.P. Halabi, H.J. Chou, D.A. Lee, B.J. Mondino, Collagen shields impregned with gentamicin-dexamethasone as a potential drug delivery device, Am. J. Ophthalmol. 116 (1993) 622–627.
[83] C. LeBourlais, L. Acar, H. Zia, P.A. Sado, T. Needham, R. Leverge, Ophthalmic drug delivery systems—recent advantages, Prog. Retin. Eye Res. 17 (1) (1998) 33–58.
[84] M. Minabe, A. Uematsu, K. Nishijima, E. Tomomatsu, T. Tamura, T. Hori, T. Umemoto, T. Hino, Application of local drug delivery system to periodontal therapy. Development of collagen preparations with immobilized tetracycline, J. Periodontol. 60 (2) (1989) 113–117.
[85] M. Minabe, K. Takeuchi, E. Tomomatsu, T. Hori, T. Umemoto, Clinical effects of local application of collagen film-immobilized tertacacline, J. Clin. Periodontol. 16 (1989) 291–294.
[86] C.Y. Hitzig, C. Charbit, T. Bitton, M. Fosse, L. Teboul, L. Hannoun, V. Varonne, Topical metronidazole as an adjunct to subgingival debridement in the treatment of chronic periodontitis, J. Clin. Periodontol. 21 (1994) 146–151.
[87] W.A. Soskolne, P.A. Heasman, A. Stabholz, G.J. Smart, M. Flashner, M. Flashner, H.N. Newman, Sustained local delivery of chlorhexidine in the treatment of periodontitis: a multicenter study, J. Periodontol. 68 (1997) 32–38.
[88] M.K. Jeffcoat, K.S. Bray, S.G. Ciancio, A.R. Dentino, D.H. Fine, J.M. Gordon, J.C. Gunsolley, W.J. Killoy, R.A. Lowenguth, N.I. Magnusson, S. Offenbacher, K.G. Palcanis, H.M. Proskin, R.D. Finkelman, M. Flashner, Adjuvant use of a subgingival controlled release chlorhexidine chip reduces probing depth and improves attachment level com-

pared with scaling and root planning alone, J. Periodontol. 69 (1998) 989–997.
[89] K. Fujioka, Y. Takada, S. Sato, T. Miyata, Novel delivery system for proteins using collagen as a carrier material: the minipellet, J. Control. Release 33 (1995) 307–315.
[90] R. Gericke, H. Wahlig, E. Dingeldein, Flavanone derivatives. US Patent 4938257 (1990).
[91] Z. Ruszczak, R. Mehrl, J. Jeckle, M. Stoltz, Improved natural polymer-based material for use in human and veterinary medicine and method of manufacturing such. (2000). Patent application No. WO-01/66159.
[92] A. Stemberger, M. Unkauf, D.E. Arnold, G. Blümel, Drug carrier systems based on resorbable polyester collagen and/or biomaterial combinations, in: C.G. Gebelein, T.C. Cheng, C.C.M. Yang (Eds.), Cosm. Pharm. Appl. Polym., Plenum, New York, USA, 1990, pp. 263–268.
[93] P. Fleckenstein, H. Wahlig, Dingeldein, E. Wirkstoff-Depot, Dt. Off. 3429038 (1986).
[94] M. Antiszko, J. Grzybowski, A. Tederko, R. Braczko, High-absorbing collagen membranes as carriers for antibacterial drugs, Polim. Med. 26 (3–4) (1996) 21–27.
[95] Z. Ruszczak, R. Mehrl, M. Stoltz, J. Jeckle, Multilayer collagen material for tissue reconstruction (2000). Patent application No. WO-01/66162.
[96] L.G. Jørgensen, T.S. Sørensen, J.E. Lorentzen, Clinical and pharmacokinetic evaluation of gentamicin containing collagen in groin wound infections after vascular reconstruction, Eur. J. Vasc. Surg. 5 (1991) 87–91.
[97] T.S. Soerensen, A.I. Soerensen, S. Merser, Rapid release of gentamicin from collagen sponge. In vitro comparison with plastic beads, Acta Orthop. Scand. 61 (4) (1990) 353–356.
[98] T. Ipsen, P.S. Jørgensen, V. Damholt, C. Tørholm, Gentamicin–collagen sponge for local applications, Acta Orthop. Scand. 62 (1991) 592–594.
[99] Y. Ogawa, M. Yamamoto, H. Okada, T. Yashiki, T. Shimamoto, A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly (lactic/glycolic) acid, Chem. Pharm. Bull. 36 (1988) 1095–1103.
[100] W. Friess, M. Schlapp, Modifying the release of gentamicin from microparticles using a PLGA blend, Pharm. Dev. Technol. 7 (2) (2002) 235–248.
[101] W. Friess, M. Schlapp, Release mechanisms from gentamicin loaded poly(lactic–co-glycolic acid) (PLGA) microparticles, J. Pharm. Sci. 91 (3) (2002) 845–855.
[102] C.G. Pitt, M.M. Gratzl, G.L. Kimmel, J. Surles, A. Schindler, Aliphatic polyesters: II. The degradation of poly(DL-lactide), poly(epsilon-caprolactone), and their copolymers in vivo, Biomaterials 2 (1981) 215–220.
[103] M. Schlapp, Kollagen/PLGA-Mikropartikel Komposite zur gesteuerten Freigabe von Gentamicin, PhD Thesis, Erlangen, Germany, 2001.
[104] M. Schlapp, W. Friess, Collagen/PLGA microparticle composites for local controlled delivery of gentamicin, J Pharm Sci., in print.
[105] B. Bittner, K. Mäder, C. Kroll, H.-H. Borchert, T. Kissel, Tetracycline–HCl loaded poly (D,L-lactide–co-glycolide) microspheres prepared by a spray drying technique: influence of γ-irradiation on radical formation and polymer degradation, J. Control. Release 59 (1999) 23–32.
[106] M.P. Singh, J. Stefko, J.A. Lumpkin, J. Rosenblatt, The effect of electrostatic charge interaction on release rates of gentamicin from collagen matrices, Pharm. Res. 12 (8) (1995) 1205–1210.
[107] M.S. El-Samaligy, P. Rohdewald, Reconstituted collagen nanoparticles, a novel drug carrier delivery system, J. Pharm. Pharmacol. 35 (1983) 537–539.
[108] W. Friess, Verfahren zur Herstellung von Kollagenmikropartikeln. Offenlegungsschrift DE 19934894 (2001).
[109] J.E. Lee, J.C. Park, J.G. Kim, H. Suh, Preparation of collagen modified hyaluronan microparticles an antibiotic carrier, Yonsei Med. J. 42 (3) (2002) 291–298.