Nouveau système résorbable pour la libération locale d'agents antimicrobiens dans les poches parodontales

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NOUVEAU SYSTEME RESORBABLE POUR LA LIBERATION LOCALE D'AGENTS ANTIMICROBIENS DANS LES POCHES PARODONTALES

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La Faculté de médecine, sur le préavis de Monsieur Andrea MOMBELLI, professeur ordinaire à la Section de médecine dentaire, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

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Table of contents

1 Summaries

1.1 Abstracts

NEW BIOERODIBLE SYSTEM FOR THE LOCAL DELIVERY OF ANTIMICROBIALS TO PERIODONTAL POCKETS

Clinical application of poly(ortho ester) as vehicle of antimicrobial agents for the treatment of periodontal pockets has been tested for the first time. Retention of the active agent tetracycline was measured and tolerance to the polymer was examined. The compound has the potential to remain in the periodontal pockets for up to 11 days. Retention was, however, variable and depended on clinical conditions at injection. Tetracycline concentrations in positive sites were up to 100 times higher than the MIC. Tolerance was excellent. Further studies are needed to improve the adhesive properties of the polymer.

NOUVEAU SYSTEME RESORBABLE POUR LA LIBERATION LOCALE D'AGENTS ANTIMICROBIENS DANS LES POCHES PARODONTALES

L'application clinique de poly(ortho ester) comme véhicule d'agents antimicrobiens pour le traitement local des maladies parodontales a été testée pour la première fois. La tolérance au polymère a été examinée et la rétention de son principe actif, la tétracycline, a été mesurée. Le produit a été retrouvé jusqu'à 11 jours après son administration, mais la rétention dans les poches parodontales n'est pas constante et dépend des conditions cliniques lors de l'injection. Dans les sites positifs, la concentration de tétracycline dépasse de 100 fois la CMI. La tolérance s'est révélée excellente. Des développements sont nécessaires pour améliorer les propriétés adhésives du polymère.

1.2 Résumé en français

1.2.1 Introduction

Les maladies parodontales sont reconnues comme des processus infectieux ayant une étiologie bactérienne. L'emploi d'agents antimicrobiens, par voie systémique ou locale, a été proposé de manière à potentialiser l'effet des traitements mécaniques classiques. Comme la destruction des tissus parodontaux n'est généralement pas uniforme, la thérapie médicamenteuse peut se limiter, localement, aux poches parodontales. Des systèmes à libération locale d'agents antimicrobiens ont été développés dans ce but, visant plus particulièrement une longue durée d'action et des concentrations élevées de principe actif directement au site d'action.

Les poly(ortho esters) forment une famille de polymères biodégradables. Quatre générations ont été développées successivement. Les expérimentations chez l'animal ont montré des résultats prometteurs pour des applications en ophtalmologie. Les polymères les plus récents possèdent plusieurs caractéristiques qui les rendent intéressants en tant que véhicules d'agents antimicrobiens pour la thérapie parodontale. Ils sont biocompatibles, biodégradables, injectables, et présenteraient des propriétés adhésives qui pourraient augmenter leur rétention au contact des surfaces radiculaires.

Les tétracyclines, un groupe d'agents antimicrobiens largement utilisés en parodontie, sont bactériostatiques contre un grand nombre de pathogènes parodontaux. Leur action inhibitrice vis-à-vis de la collagénase et leur affinité pour la surface radiculaire ont contribué à les rendre populaires depuis plusieurs décennies. L'hydrochlorure de tétracycline, la doxycycline et la minocycline, toutes des tétracyclines semi-synthétiques, ont été étudiées et utilisées aussi bien en administration systémique que locale. La tétracycline base, qui possède les mêmes propriétés que l'hydrochlorure de tétracycline quant à son mode d'action et son utilisation, peut être incorporée au poly(ortho ester) par simple mélange. *In vitro*, le principe actif est relâché graduellement selon une cinétique d'ordre zéro, car sa libération s'opère par érosion de surface, et la dégradation complète du polymère s'effectue en deux semaines.

But

Le but de cette étude *in vivo* de phase I était de tester l'application clinique de poly(ortho ester) comme véhicule d'agents antimicrobiens pour le traitement local des maladies parodontales. Les observations ont porté sur la rétention du matériel dans les poches et sur la tolérance au produit. De plus, la quantité de la tétracycline libérée par le polymère a été mesurée. L'étude était divisée en trois parties. Le but spécifique de la partie I était de déterminer de manière préliminaire la rétention possible du polymère et la biodisponibilité de la tétracycline suite au traitement de dents monoradiculées ou de molaires atteintes de furcations de degré II. Le but de la partie II était d'explorer si la rétention pouvait être améliorée en modifiant les conditions cliniques lors de l'application, en

minimisant la présence de fluides sur la surface radiculaire. La partie III avait pour but de déterminer si une quantité faible et contrôlée de polymère montrait une meilleure rétention dans la poche, comparé à une quantité non-contrôlée, débordant de la poche.

1.2.2 Matériel et méthodes

Sujets

Dix-huit volontaires ont participé à cette étude, six dans chaque partie. Les sujets étaient en bonne santé générale, mais étaient affectés d'une parodontite chronique avec des poches parodontales $\geq$ 6 mm.

Formulations

Un poly(ortho ester) (POE), dit auto-catalysé, de bas poids moléculaire et contenant 30 mol% d'acide lactique (LA) a servi de véhicule ($POE_{70}LA_{30}$). Les deux formulations testées étaient chargées de 10%, respectivement 20%, de tétracycline base (TB_{10}, TB_{20}). Après mélange, les produits avaient la consistance d'une pâte de viscosité élevée.

Protocole clinique

Les patients ont été instruits à un contrôle de plaque adéquat afin d'obtenir une bonne hygiène. Les dépôts supragingivaux ont été éliminés par détartrage et polissage. Au jour 0, la profondeur de poche (PPD), le saignement au sondage (BoP) et la récession gingivale (REC) ont été mesurés dans tous les sites étudiés. Le surfaçage radiculaire des dents sélectionnées a été effectué au moyen d'ultrasons et de curettes sous anesthésie locale. Les sites tests ont reçu la formulation et ont été suivis selon le protocole décrit ci-dessous. Le polymère était introduit dans la poche au moyen d'une seringue conçue pour les anesthésies intraligamentaires sur laquelle était montée une aiguille de 0.8 mm de diamètre. A chaque séance de suivi, le fluide gingival (GCF) était collecté.

Partie I

Les deux formulations décrites plus haut ont été testées chacune dans la moitié des 22 sites, chez les six patients. 12 sites interproximaux ont été traités sur des dents monoradiculées (sujets 1.1, 1.2, 1.3). Les autres individus présentaient des atteintes de furcations (1.4, 1.5, 1.6). Les poches ont été remplies en excès, jusqu'à ce que les formulations apparaissent à la marge gingivale. Les sujets ont été examinés aux jours 0, 3, 5, 7 et 10.

Partie II

Le protocole a été modifié dans le but d'éviter, si possible, tout saignement lors de l'application du polymère. Chez six sujets (2.1 à 2.6), quatre sites interproximaux sur des dents monoradiculées ont été traités par surfaçage radiculaire. Une fois l'instrumentation terminée, un fil de rétraction a été inséré dans tout les sites pour 15 minutes. Après avoir retiré le fil et observé la présence d'un éventuel

saignement, les poches ont été remplies avec le mélange contenant 20% de tétracycline base ($POE_{70}LA_{30}TB_{20}$). Les patients ont été suivis aux jours 0, 4, 7, et 11.

Partie III

Afin de faciliter le recrutement des patients, les critères d'admission ont été simplifiés (minimum 2 sites par individu), et la durée d'observation réduite à 7 jours. Cinq à 10 mg de la formulation contenant 20% de tétracycline ($POE_{70}LA_{30}TB_{20}$) ont été injectés dans 21 sites chez six sujets (3.1 à 3.6). Dans 7 sites additionnels, chez deux individus (3.4 et 3.6), l'entrée de la poche a été scellée au moyen d'une colle de type cyanoacrylate après l'injection du polymère. Les sujets ont été examinés à deux reprises, aux jours 0 et 7.

Procédures de laboratoire

La rétention des formulations a été déterminée de manière indirecte en mesurant la concentration de tétracycline dans le fluide gingival créviculaire. Pour chaque prélèvement, le volume de fluide était mesuré au moyen du Periotron®8000 et la quantité de tétracycline au moyen d'un bioessai. Pour ce dernier, les prélèvements ont été déposés sur l'agar de boites de Pétri ensemencées par du *Bacillus cereus*. Après 24 h d'incubation, les diamètres d'inhibition ont été mesurés et comparés à une courbe standard. La concentration de tétracycline a été calculée en divisant la quantité d'antibiotique par le volume de fluide mesuré.

1.2.3 Résultats

Tolérance

Aucun patient ne s'est plaint de mauvais goût, d'inconfort ou de douleurs. Un sujet (1.2) a décrit une sensibilité généralement augmentée au froid. Dans un site, chez un individu (1.1), la gencive était localement rouge et gonflée 4 mm apicalement au rebord gingival. Aucun autre signe d'inflammation n'a été détecté dans l'ensemble de l'étude.

Rétention

Partie I

De manière générale, la rétention des formulations a été faible. Au jour 3, la tétracycline a été détectée dans seulement deux sites, au jour 5 également. Un site était positif au jour 7, et aucun au jour 10. La concentration de tétracycline s'élevait de 70 à 330 µg/ml dans les sites positifs. Les valeurs les plus élevées sont apparues au jour 5. Ces résultats ont été obtenus chez deux sujets, un dans chaque sous-groupe (monoradiculées *vs.* furcations).

Partie II

Ici, la rétention du système antibiotique s'est révélée meilleure, cinq sujets sur six présentant au moins un site positif, au moins une fois. Sept surfaces traitées sur 24 étaient positives au jour 4, huit au jour

7, et deux au jour 11. La concentration maximale de tétracycline s'élevait à 280 µg/ml, la plus faible étant de 0.0004 µg/ml.
Trois patients sur six montraient des poches positives au jour 4, et quatre au jour 7. Chez deux sujets, la tétracycline a pu être détectée aussi au jour 11.

Partie III

Seuls trois sites sur 28, chez trois sujets différents, ont montré un petit diamètre d'inhibition lors du bioessai (jour 7). La concentration de tétracycline dans ces sites «positifs» variait de 0.002 à 0.006 µg/ml. Deux de ces sites avaient reçu la colle cyanoacrylate après injection du polymère.

Libération de tétracycline

La tétracycline mesurée avait la tendance générale de diminuer au cours du temps. En effet, la moyenne des concentrations aux jours 4, 7 et 11 décroît exponentiellement.

1.2.4 Discussion

Les résultats obtenus dans cette série d'études préliminaires confirment que les poly(ortho esters) sont des agents prometteurs pour la libération locale d'antimicrobiens lors de la thérapie parodontale. Ces polymères sont biocompatibles, biodégradables, injectables, et, dans une certaine mesure, adhésifs.

Pour des raisons évidentes, un produit destiné à être placé sous la gencive doit être biocompatible. Chez l'animal déjà, les poly(ortho esters) ont été remarquablement bien tolérés. Chez nos patients, aucun effet indésirable n'est apparu au cours de la période d'observation. A l'exception d'un individu, qui a décrit des sensibilités au froid, aucun patient ne s'est plaint de mauvais goût, d'inconfort ou de douleurs. Une sensibilité augmentée aux stimuli thermiques est un phénomène communément observé après traitement parodontal, et peut être attribuée au traitement mécanique. Dans ce cas particulier, en effet, toutes les dents traitées mécaniquement se sont révélées sensibles, indépendamment de l'application ou non du polymère. Toutefois, des sensibilités locales dues au mordançage par la tétracycline (déminéralisation et ablation de la boue dentinaire) ne peuvent être exclues.

La dégradation par érosion de surface est une caractéristique des poly(ortho esters). Cette particularité mène à une libération de principe actif contrôlée par la dégradation même du polymère. Si le principe actif ne peut plus être détecté, cela signifie que le polymère s'est entièrement dégradé. Au contraire, d'autres systèmes relâchent le médicament par diffusion. Dans ce cas, des résidus polymériques peuvent être présents dans la poche même s'il n'y a plus de principe actif disponible.

Les systèmes à libération locale non-injectables, comme les fibres de tétracycline, sont relativement difficiles à manipuler et leur application requiert du temps. Ces produits n'ont pas obtenu le succès escompté auprès des praticiens. Un système injectable, quant à lui, possède des avantages comme la

facilité et la rapidité d'application, dans un nombre élevé de sites. Les formulations utilisées ici ($POE_{70}LA_{30}TB_{10/20}$) étaient injectables au moyen d'une aiguille de 0.8 mm de diamètre.

Des tests en laboratoire pouvaient faire croire à une bonne adhésion du poly(ortho ester) à la dentine, mais la rétention limitée observée *in vivo* dans la présente étude n'a pas, jusqu'ici, confirmé ces observations. La première série de traitements n'a rapporté que de faibles résultats quant à la rétention du polymère. Aucune différence n'a pu être décelée entre le sous-groupe des dents monoradiculées et celui des dents avec atteintes de furcation. Néanmoins, il était encourageant d'observer que, dans les sites positifs, le produit était présent pendant plusieurs jours à des concentrations élevées. Dans la deuxième partie, un nombre plus élevé de poches était positif. La tétracycline a été détectée jusqu'au jour 11. Ainsi, un changement dans les conditions cliniques (92% des sites 'secs' dans la partie II) a contribué à augmenter le nombre de sites positifs, ainsi que la durée de rétention du polymère. La troisième partie, quant à elle, a montré qu'une petite quantité de polymère était insuffisante pour obtenir des résultats positifs après une semaine.

L'absence de résultats positifs dans plusieurs sites peut être expliquée par la pression exercée par les tissus parodontaux. Dans la phase de guérison, après surfaçage radiculaire, la rétraction de la gencive pourrait avoir expulsé le produit hors de la poche. A cela, il faut ajouter l'action du fluide gingival qui contribue, par un effet de lavage, à diluer toute substance placée sous la gencive.

Après administration systémique, la concentration de tétracycline dans le fluide gingival est de 3 à 8 µg/ml. Or, l'administration locale d'antibiotiques conduit à des niveaux beaucoup plus élevés. La fibre de tétracycline peut produire 1500 µg/ml pendant 10 jours. Un autre système, à base de doxycycline, a montré des concentrations supérieures à 150 µg/ml pendant 7 jours. Nos résultats soutiennent la comparaison, avec des niveaux de tétracycline de 70 à 330 µg/ml pour les sites positifs de la partie I. C'est environ 100 fois plus que la concentration minimale inhibitrice (CMI) qui est de 1-2 µg/ml.

Dans la partie II, les concentrations maximales ont été comparables à la première partie, mais plusieurs sites montraient des concentrations basses. Deux tiers des résultats positifs étaient en dessus de la CMI. Dans 1/3 des sites, le bioessai a montré de petits diamètres d'inhibition, les quantités correspondantes de tétracycline atteignant la limite de détection. Dans ces sites, les mesures n'ont donc pas pu être obtenues de manière précise. Ce phénomène a été également observé dans la partie III, où les concentrations de tétracycline étaient basses. De plus, les résultats décevants de la partie III sont certainement dus à la faible quantité de formulation injectée au départ.

Conclusion

Le système à libération locale d'agents antimicrobiens basé sur du poly(ortho ester) peut rester dans la poche parodontale jusqu'à 11 jours. La tétracycline incorporée dans le polymère peut être relâchée à des concentrations thérapeutiques durant la même période. Néanmoins, la rétention du système est variable et dépend, notamment, des conditions cliniques. La reproductibilité des résultats n'a pas pu

être obtenue. De futures études sont nécessaires afin d'obtenir une rétention qui soit constante, soit en améliorant encore les conditions cliniques lors de l'application, soit en développant les propriétés adhésives du polymère.

2 Introduction

Periodontal diseases are considered as infectious processes with bacterial etiology (Socransky 1977, Socransky & Haffajee 1994). Thus the adjunctive use of antimicrobial agents, either systemically or locally, has been advocated in order to enhance the effect of classical mechanical therapy (Listgarten & Hellden 1978, Goodson et al. 1979, Loesche et al. 1981). In subjects with periodontitis, all parts of the dentition are usually not affected with equal severity. It has therefore been suggested to focus therapy on the affected areas and to limit the delivery of drugs to the periodontal pockets. Local drug delivery systems have been developed aiming at longer duration and higher concentration of active agents directly at the site of action. For more details on local delivery systems, see section 2.1.

Recently developed biodegradable polymers, poly(ortho esters), have a number of interesting characteristics, such as biocompatibility, bioerodibility and injectability, which make them promising candidates as carriers of antimicrobial agents for local subgingival therapy. Some types of these polymers also have adhesive properties that may improve their retention on dental surfaces (Schwach-Abdellaoui et al. 2001). For more details on poly(ortho esters), see 2.2.

Tetracyclines comprise a group of antimicrobial agents widely used in the field of periodontology. Their bacteriostatic activity against a large panel of periodontal pathogens (Walker 1996), as well as their ability to inhibit collagenase (Golub et al. 1991) and to bind to the tooth surface (Baker et al. 1983), have made them popular for several decades. Tetracycline hydrochloride (TH), doxycycline and minocycline, all semi-synthetic tetracyclines, have been studied and used for the systemic route as well as for local applications (for review, see Seymour & Heasman 1995). Tetracycline base, having the same actions and uses as TH can easily be incorporated into poly(ortho ester) by mixing the two components. *In vitro*, the drug is released according to a zero order process, confirming that delivery occurs by surface erosion of the polymer (Schwach-Abdellaoui et al. 1998, Schwach-Abdellaoui et al. 2001). Previous *in vitro* results are presented in section 2.3.

2.1 Local Delivery Devices

As irrigation of pockets can achieve therapeutic drug levels for only short periods of time (Pitcher et al. 1980, Eakle et al. 1986, Oosterwaal et al. 1990), controlled delivery systems have been proposed to extend the time of action of locally applied antimicrobial agents. The use of such systems has been advocated particularly in the therapy of sites difficult to reach by mechanical means, for example furcation defects (Nordland et al. 1987), and sites not responding to conventional mechanical therapy (see Greenstein & Polson 1998 for review). Although numerous local controlled delivery systems have been designed in the past two decades, only few have been tested extensively, and even fewer have become commercially available for broad use by dentists (for review see Mombelli & Tonetti 2001).

Two multi-center controlled clinical trials have shown the efficacy a 2% minocycline ointment (Dentomycin, Lederle, Wayne, USA) to improve clinical parameters, if applied repeatedly. In the first study, subjects were treated 4 times in addition to scaling and root planing with minocycline or placebo ointment (van Steenberghe et al. 1993). The second study evaluated a seven-time repeated application of the drug over a 15-months period (van Steenberghe et al. 1999). The repeated subgingival administration of minocycline ointment yielded an adjunctive improvement after subgingival instrumentation in both clinical and microbiologic variables.

Another product contains metronidazole as active principle (25%) in glyceryl-mono-oleate and sesame oil as carriers (Elyzol®, Dumex, Copenhagen, Denmark). This degradable compound can be injected in the form of a gel with a syringe directly into the pocket. Although the gel increases its viscosity in the pocket due to the presence of water, about half of the product is lost immediately following placement, and in the hours thereafter the concentration of metronidazole decreases exponentially (Stoltze 1992, Stoltze 1995). The manufacturer therefore recommends two applications of the gel within one week. In clinical studies no significant difference in periodontal parameters was demonstrated between Elyzol® and conventional SRP (Ainamo et al. 1992, Grossi et al. 1995, Pihlstrom et al. 1995); these results were interpreted to suggest equivalence of the two treatments.

In one of the first clinical trials testing the controlled local administration of a drug into a periodontal pocket, delivery of tetracycline was attempted by placing hollow dialysis tubes filled with the active

agent into the diseased sites (Goodson et al. 1979). Subsequent developments resulted in the Actisite® tetracycline fiber (Alza, Palo Alto, USA), consisting of a monolithic ethylene-vinyl-acetate copolymer loaded with tetracycline hydrochloride crystals (Goodson et al. 1983). The nondegradable carrier assumes the form of a fiber with a diameter of 0.5 mm and a length of 23 cm, and contains 12.7 mg of the antimicrobial agent. The device is densely packed into the periodontal pocket and left *in situ* during 7 to 10 days. Measurements in humans have shown very high and constant concentrations of drug at the treated site during the entire time of application (Tonetti et al. 1990). In several multi-center studies, the tetracycline fiber has proved to be effective. Used as an adjunct to scaling and root planing (SRP), it improved probing pocket depth (PPD) and clinical attachment level (CAL) significantly better than mechanical treatment alone (Newman et al. 1994, Michalowicz et al. 1995). Despite of its proven efficacy, the device has been withdrawn from the market in early 2002, and is presently no longer available commercially.

A more recently developed system contains 8.5% doxycycline hyclate as active agent. Polylactic acid is the biodegradable vehicle and N-methyl-2-pyrrolidone (NMP) the solvent (Atridox™, Atrix Laboratories, Ft.Collins, USA). Two separate syringes are coupled and mixed just prior to use. As soon as the compound is injected into the pocket, the NMP is replaced by water, which causes the polymer to return to its solid state (Stoller et al. 1998). On the short term, clinical studies have demonstrated that chemical therapy alone is at least as effective as SRP in improving clinical parameters (Garrett et al. 1999, Garrett et al. 2000). In one study, even better results were obtained in a group treated with the drug during a single session of scaling, compared to those obtained in subjects treated in four appointments with subgingival mechanical therapy (Wennstrom et al. 2001).

The potential usefulness of microcapsules as local delivery device for periodontal therapy has also been investigated. In 1994, Jones et al. described the use of minocycline HCl microencapsulated in poly(lactic acid-co-glycolic acid) (PLGA) for the treatment of chronic periodontitis. The biodegradable polymer containing the active agent had the form of a powder and was administered into the periodontal pocket with a syringe. Maybe due to the small number of subjects and/or insufficient plaque control, clinical and microbiological parameters failed to demonstrate superiority in comparison to control or mechanical treatment alone (Jones et al. 1994). A few years later, Yeom (1997) presented a new bioabsorbable polymeric system consisting of chitosan-encapsulated alginate

microspheres loaded with 10% minocycline. The injectable polymeric compound was tested in 15 patients as adjunct to supragingival scaling compared to SRP alone. The six-week observation period permitted to conclude that BoP was better reduced by antimicrobial therapy than by SRP. Microbiological indicators of periodontal health tended to be better in minocycline sites than SRP sites (Yeom et al. 1997). A multicentric study, including nearly 750 subjects in 18 centers, demonstrated the superiority of locally delivered minocycline (in PLGA) over nine months upon SRP alone, or SRP plus vehicle (Williams et al. 2001).

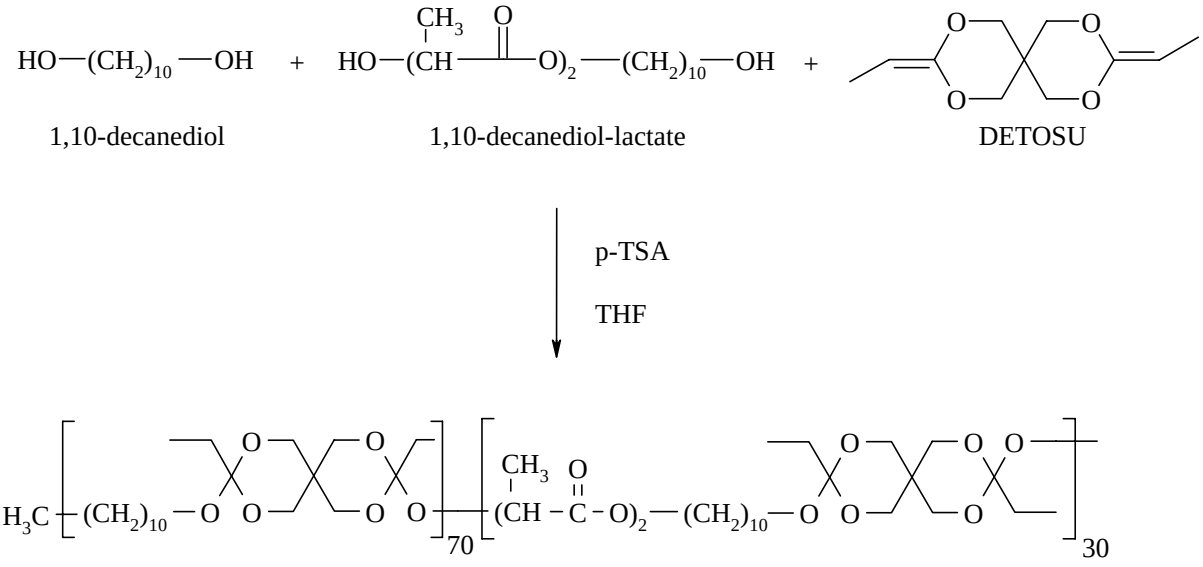
2.2 Poly (ortho esters)

Because their physico-chemical properties can be varied to a large extent, polymers attract much attention for their potential use as biomaterials in various fields of medicine. A wide range of applications are currently explored in orthopedics, ophthalmology, or dentistry, including their potential use as carriers of active agents for controlled local drug delivery (Sintzel et al. 1996, Peter et al. 1998).

Poly(ortho esters) (POE) are synthetic biodegradable hydrophobic polymers that allow the liberation of incorporated agents by surface erosion. This mechanism has been validated in animal models for applications in ophthalmology (Einmahl et al. 2000, Einmahl et al. 2001a, Merkli et al. 1994, Zignani et al. 1998, Zignani et al. 2000b). The use of semi-solid POEs is expected to lead to significant advances in glaucoma filtering surgery, and may make drug delivery to various parts of the human eye possible (Zignani et al. 2000a, Einmahl et al. 2002). Investigations are currently in progress in several other fields of veterinary and human medicine (for review see Einmahl et al. 2001b). POEs may also become suitable candidates for local controlled drug delivery in periodontal pockets. Preliminary laboratory investigations have been presented (Roskos et al. 1995).

The polymer described in the present work is a low molecular weight auto-catalyzed POE containing 30 mol% of lactic acid units ($POE_{70}LA_{30}$). It was recently synthesized, characterized and optimized (Schwach-Abdellaoui et al. 1999, Schwach-Abdellaoui et al. 2001). Synthesis reaction is an acid-catalyzed condensation of 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5,5]undecane (DETOSU) with 1,10-decanediol and 1,10-decanediol-dilactate. *p*-toluenesufonic acid (p-TSA) is added as catalyst and tetrahydrofuran (THF) is the solvent (Figure 1).

Figure 1 : Synthesis of auto-catalyzed POE is a condensation reaction of DETOSU with 1,10-decanediol and 1,10-decanediol-dilactate. p-TSA is the catalyst and THF the solvent.

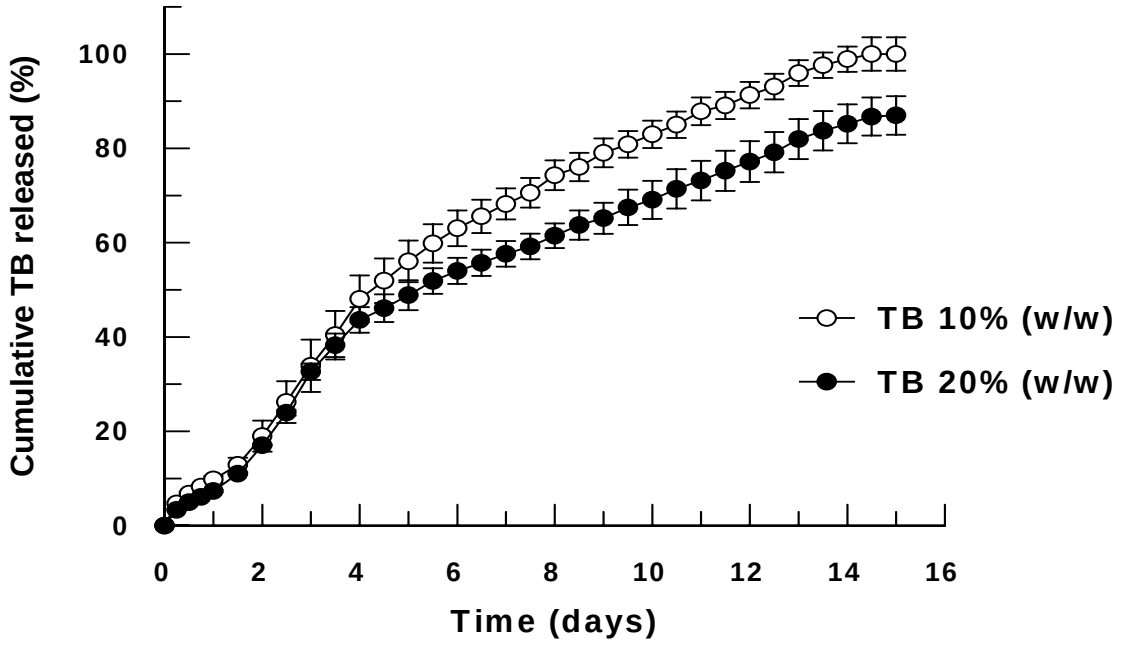


By modifying the proportion of lactic acid in the polymer, different degradation rates can be obtained (see 2.3) and the physicochemical properties of the compound (POE_xLA_y) can be varied. For example, $POE_{70}LA_{30}$ shows a low viscosity and, therefore, is suitable for injectable preparations (Schwach-Abdellaoui et al. 2001).

2.3 Previous experiments

Degradation of auto-catalyzed POE occurs mostly by surface erosion. An incorporated drug could therefore be released for days to weeks (Ng et al. 1997, Schwach-Abdellaoui et al. 1998, Sintzel et al. 1998a, Sintzel et al. 1998b, Schwach-Abdellaoui et al. 1999). Delivery of tetracycline incorporated in $POE_{70}LA_{30}$ has been previously studied *in vitro* in thermostated cells at 37°C, circulated with phosphate buffer (Schwach-Abdellaoui et al. 2001). The released amount of drug was measured by high-performance liquid chromatography (HPLC). Figure 2 shows, for 10% or 20% tetracycline load, that the delivery lasted two weeks at an almost constant rate. The complete degradation of the polymeric matrix was simultaneous.

Figure 2 : *In vitro* tetracycline release profiles from $POE_{70}LA_{30}$ loaded with 10% and 20% tetracycline base (TB) (Schwach-Abdellaoui et al. 2001).



2.4 Purpose

The aim of this phase I *in vivo* study was to explore the clinical potential of poly(ortho esters) as carriers of antimicrobial agents for the local treatment of periodontal disease. Observations focused on the retention of the material in periodontal pockets and the tolerance to the product. Additionally, the release of tetracycline incorporated in the polymer was measured. The study was subdivided in three parts. The specific purpose of part I was to preliminarily determine the potential for retention and the possible bioavailability of tetracycline after treatment of single rooted teeth and molars with class two furcation (Hamp et al. 1975) defects. The purpose of part II was to explore if retention could be improved by modifying the clinical conditions at application time to minimize the presence of fluid on the root surface. The purpose of part III was to determine whether a small and controlled amount of polymer could be better retained within the pocket than an uncontrolled quantity overfilling the pocket.

3 Material and methods

3.1 Subjects

Eighteen systemically healthy volunteers with chronic periodontitis (4 females and 14 males, mean age 54, range 36-77), with periodontal pockets ≥ 6 mm, participated in this three-part study. Each part was carried out in six persons. Subjects had not received any periodontal treatment during the year preceding the study and had neither taken antibiotics nor long-term anti-inflammatory medication during the previous six months. The general health history and oral health status were registered for each patient. Subjects with a medical condition (diabetes, rheumatic fever, blood dyscrasia, immunologic anomalies) or allergic to tetracycline or poly(ortho esters) were not included. Pregnant or lactating females were also not considered (see Patient Record Form in Appendix). The study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethical board of the School of Dental Medicine, University of Geneva. The participants were informed on the aim of the study, the possible side effects, and gave their consent in written form.

3.2 Formulations

Self-catalyzed poly(ortho ester) (POE) of low molecular weight, containing 30 mol% of lactic acid (LA) units in the polymeric backbone, was used as vehicle ($POE_{70}LA_{30}$). The compound was manufactured by the laboratory of Pharmaceutics and Biopharmaceutics of the University of Geneva. Drug loading was of 10% (w/w) or 20% (w/w) tetracycline base (TB_{10}, TB_{20} ; Sigma, Steinheim, Germany). Synthesis and degradation of these polymers have been presented in sections 2.2 and 2.3. Both formulations were prepared by mixing the polymer with the corresponding quantity of tetracycline. All samples were γ-irradiated under argon at -70°C with a total of 2 Mrad, a dose validated in previous studies (Sintzel et al. 1998c). The final products had the consistency of a highly viscous paste.

3.3 Clinical protocol

Patients were instructed in proper plaque control methods to reach a good level of oral hygiene before the experiments were carried out. Hard and soft deposits were removed mechanically from all supragingival tooth surfaces by scaling and polishing.

At day 0, PPD, BoP and gingival recession (REC) were recorded from all study sites. The selected teeth were then scaled and planed subgingivally under local anesthesia, using ultrasonic scalers and hand curettes. Subgingival treatment was not limited in time and lasted approximately 5 to 10 minutes *per* tooth. Prior to the placement of the compound into the pocket, each site was isolated from saliva with cotton rolls and dried with gauze. The prepared teeth then received the trial medication and were followed up according to the protocols outlined below (see 3.3.1.-3.3.3, parts I-III). In all subjects the polymer was placed into the periodontal sites with a syringe designed for intraligamentar anesthesia (Ligmaject®, Henke-Sass Wolf, Tuttlingen, Germany), fitted with a canula 0.8 mm in diameter. At each follow-up session the GCF was assayed. The tolerance to the product was evaluated by visual inspection of the gingiva at each site. Changes in texture and color as well as presence or absence of suppuration were recorded. Bad taste, discomfort, or pain were also assessed. For the collection of GCF, teeth were first isolated and dried by a gentle stream of compressed air. Three minutes later, paper strips (Periopaper®, Ora*flow*, Plainview, NY, USA) were placed for 30 seconds at the gingival margin of the test sites without entering the pocket. As a general rule, if no active agent could be detected at the first and second visits following placement in any given site of a subject, no further GCF samples were taken thereafter in this individual.

At the completion of the observation period, PPD, BoP and REC were recorded again from all selected study sites. Patients received additional periodontal therapy according to their needs.

3.3.1 Part I

The polymeric system was tested at concentrations of 10% and 20% of active agent in six patients (# 1.1 - 1.6). In each subject, half of the sites were treated with the 10% concentration and half with the 20% tetracycline concentration. In total, 22 pockets were treated. Four single rooted teeth with mesial or distal pockets of at least 6 mm were treated in each of three subjects (subjects # 1.1, 1.2

and 1.3). On each of these twelve teeth, one proximal site was chosen as experimental unit. Two to four molar sites with a furcation involvement were treated in the other three patients (# 1.4, 1.5 and 1.6). Nine study sites showed a degree-two furcation involvement. One site in one subject (# 1.4) had only a degree-one involvement. The pockets were filled until the preparation re-appeared at the gingival margin. The injected amount was estimated to the nearest 10 mg.

Subjects were examined at day 0 and then at days 3, 5, 7 and 10.

3.3.2 Part II

In the second part of the study, the protocol was slightly modified in order to optimize the conditions for the application of the polymer. Special care was taken to stop bleeding before placing the preparation into the periodontal pockets.

Four single rooted teeth with mesial or distal pockets of at least 6 mm were treated in each of six subjects. After subgingival treatment by scaling/root planing and isolation from saliva, each site was packed with a retraction cord (GingiBraid®n°2a, Van R, Oxnard, CA, USA), inserted into the pocket by using a spatula. The cord was left *in situ* for 15 minutes in order to achieve hemostasis. Immediately after the removal of the cord and visual evaluation of any possible bleeding, the pocket was filled with the 20% (w/w) tetracycline base preparation ($POE_{70}LA_{30}TB_{20}$), and the excess was eliminated.

Patients were examined at baseline and at days 4, 7 and 11.

3.3.3 Part III

The aim of this third part was to determine whether a small and controlled amount of polymer could be better retained within the pocket, as compared to an overfilling, uncontrolled quantity.

In order to facilitate the recruitment of patients, the admission criteria were simplified (minimum 2 sites per patient) and the duration of the study reduced to 7 days with two examinations, at day 0 and day 7. Five to 10 mg of the 20% (w/w) tetracycline base preparation ($POE_{70}LA_{30}TB_{20}$) were injected into 21 sites in six patients (3.1 to 3.6). To explore the possibility of securing the material from premature loss, 7 additional sites, in two subjects (3.4 and 3.6), were treated as follows: after the placement of 7 mg of

the preparation, a small amount of cyanoacrylate (Histoacryl®, B. Braun Surgical, Melsungen, Germany) was applied at the gingival margin with a disposable pipette.

3.4 Laboratory procedures

The retention time of the formulations was determined indirectly by measuring the concentration of tetracycline in gingival crevicular fluid (GCF) with a bioassay (Gordon et al. 1980, Goodson et al. 1983, Tonetti et al. 1990). The volume of GCF was determined by a fluid analyzer (Periotron®8000, Ora*flow*, Plainview, NY, USA). A standard curve was obtained for each of the three parts by recording fixed volumes of serum with the fluid analyzer (see Appendix), and by fitting a 4^{th} order polynomial plot to the data points as recommended by the manufacturer (www.oraflow.com). To determine the amount of tetracycline, strips were placed onto the surface of Müller-Hinton 2 agar (OXOID, Basingstoke, UK) previously inoculated with a strain of *Bacillus cereus* (ATCC 11778). After 24h incubation at 37°C, the inhibition diameter around the strip was measured and the quantity of tetracycline determined according to a standard curve (Goodson et al. 1983, Tonetti et al. 1990). The concentration of tetracycline was computed by dividing the quantity of antibiotic by the volume of GCF on the strip.

4 Results

4.1 Part I

4.1.1 Tolerance

No complaints were recorded with regard to bad taste, discomfort, or pain. One patient (subject 1.2) reported sensitivity to cold after treatment. The gingiva of one treated site showed light redness and swelling 4 mm below the margin during one week (subject 1.1.). No redness or swelling was noted in any of the other treated sites.

4.1.2 Retention

In general, a poor retention of the system was observed. In only two out of the 22 monitored sites tetracycline could be detected on day 3, two were positive at day 5, and one at day 7. No tetracycline was detected at day 10 in any site. In positive samples, however, high tetracycline concentrations, ranging from 69 to 327 μg/ml, of GCF were measured.

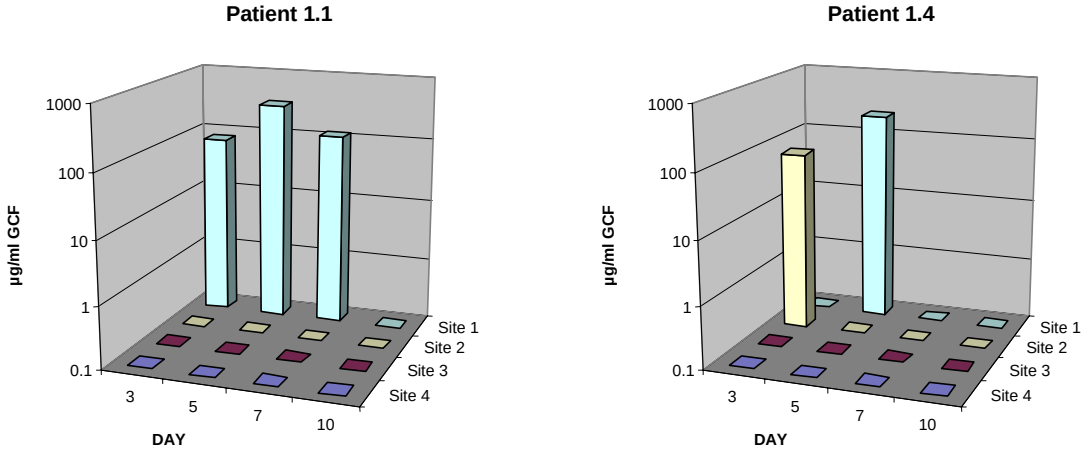
Positive results were obtained from subjects 1.1 and 1.4. The results of these two individuals are shown in Figure 3. In subject 1.1, one site on a single-rooted tooth was positive at days 3, 5 and 7. In subject 1.4, one furcation site was positive at day 3 and another one at day 5. The highest concentrations were found at day 5 in both patients.

Clinical parameters (PPD, BoP, and REC) did not show significant changes between baseline and the end of the observation period (data not shown).

4.2 Part II

From the six patients enrolled in this second part (subjects 2.1 to 2.6), five completed the study; subject 2.5 missed the last appointment (day 11) because of sickness. Placement of the retraction chords took 2 to 5 minutes. Four to 12 cm of chord were used. Clinical inspection of the sites after the removal of the chords indicated that 22 out of 24 sites (92%) were dry.

Figure 3 : Part I : Tetracycline concentrations in positive patients. Rows represent sites at different days and columns give the concentrations of tetracycline in the GCF (vertical axis : logarithmic scale).



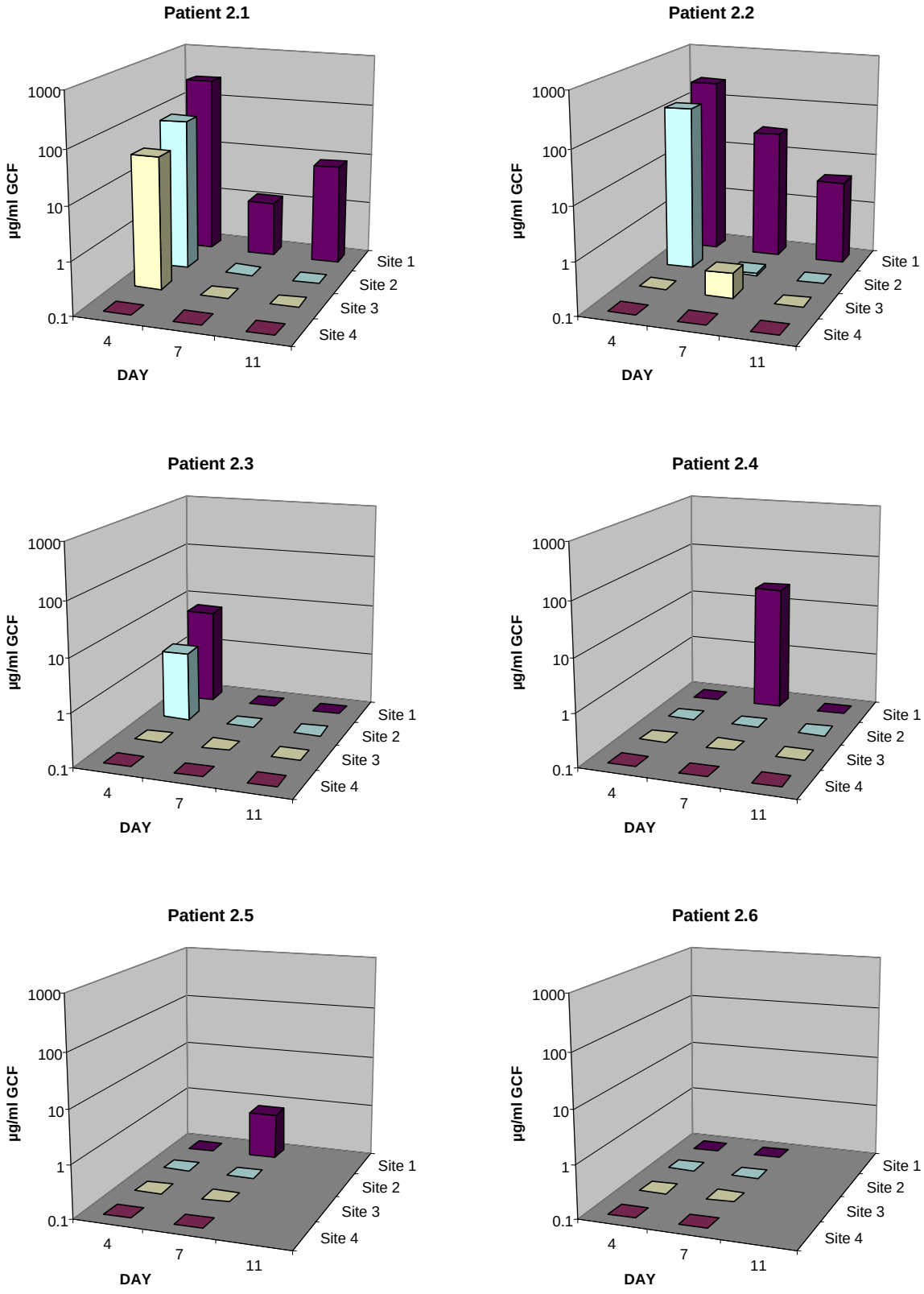
4.2.1 Tolerance

No complaints were recorded with regard to discomfort, pain, or bad taste. No signs of irritation or inflammation were noted.

4.2.2 Retention

In this second part of the study, a better retention of the polymer was observed: as shown in Figure 4, 5 out of 6 subjects showed evidence for tetracycline release in at least one site, at least once. Seven out of 24 treated surfaces (29%) were positive on day 4, eight on day 7 (33%), and two on day 11 (8%). The tetracycline concentration in positive sites ranged from 0.0004 µg/ml to 281 µg/ml of GCF. Three out of the six patients showed positive pockets on day 4 (subjects 2.1, 2.2, 2.3), and four on day 7 (subjects 2.1, 2.2, 2.4, 2.5). In two patients (2.1, 2.2), tetracycline was also detected on day 11 (Figure 4).

Figure 4 : Tetracycline concentrations in the six patients of the part II.



4.3 Part III

4.3.1 Tolerance

Once again, no complaints were recorded with regard to discomfort, pain, or bad taste, and no signs of irritation or inflammation were noted.

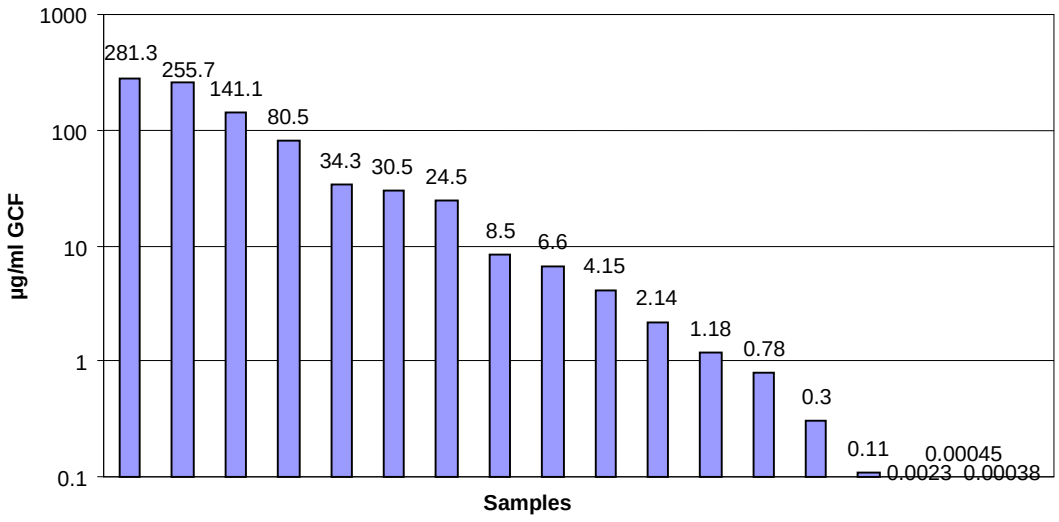
4.3.2 Retention

Out of 28 treated sites, only three, in three different subjects (3.4, 3.5, 3.6) showed a small inhibition diameter in the bioassay at day 7 (11% of the samples). Two of them had been treated additionally with the cyanoacrylate. No residues of the glue were detected at follow up. The tetracycline concentration in positive sites ranged from 0.002 μg/ml to 0.006 μg/ml of GCF.

4.4 Release of tetracycline

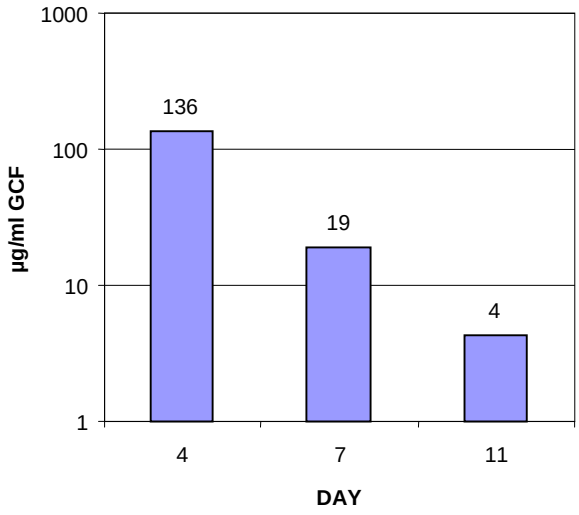
As mentioned above, retention of POE could be demonstrated only in a few samples taken in part one, but in positive specimens high tetracycline concentrations were recorded (Fig. 3). The positive samples obtained in part II are enumerated in Figure 5. Here, low concentrations were detected in many specimens. In 1/3 of the positive samples the concentration did not reach 1 μg/ml.

Figure 5 : Part II : Tetracycline concentrations in positive samples.



An overall tendency for concentrations to decrease with time was noted. Figure 6 shows the average concentrations found in positive sites at day 4, day 7 and day 11. An exponential decrease can be observed.

Figure 6 : Overall mean tetracycline concentrations in positive samples at days 4, 7 and 11.



In Part III, tetracycline concentrations were extremely low in the three positive sites, ranging from 0.001 μg/ml to 0.003 μg/ml.

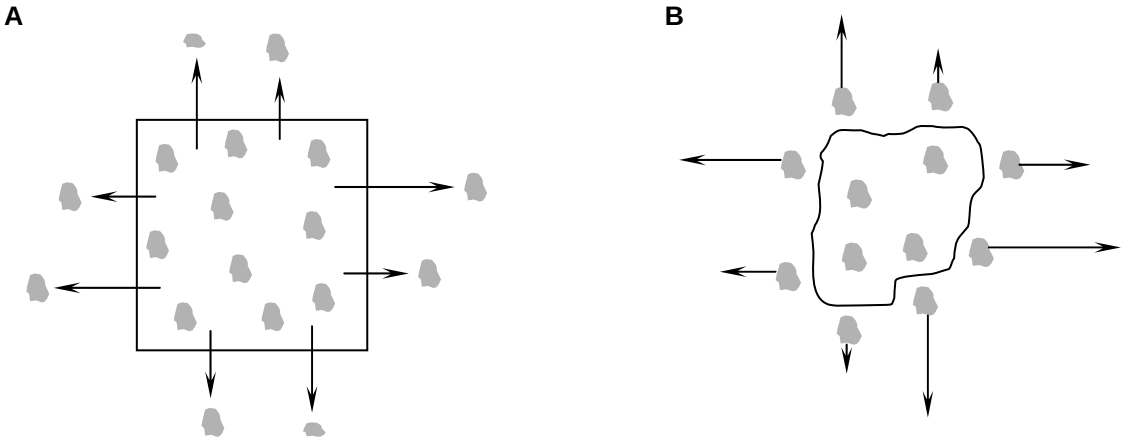
5 Discussion

The aim of this study was to explore the clinical potential of poly(ortho esters) as carriers of antimicrobial agents for the local treatment of periodontal disease. The results obtained in the present series of preliminary experiments indicate that this material has promising characteristics to be used as a carrier of antimicrobial agents for local delivery in periodontal therapy. The polymeric matrix of the present system, poly(ortho ester) and lactic acid, is biocompatible, biodegradable, injectable and shows adhesive properties (Schwach-Abdellaoui et al. 2001), all characteristics which are suitable for the local delivery of drugs to the periodontal pocket.

Biocompatibility is essential to minimize risks of allergic reactions, intolerance or sensitivity to the product. Poly(ortho esters) have shown excellent biotolerance in animal studies (Bernatchez et al. 1993, Zignani et al. 1998, Zignani et al. 2000b). In our patients, no adverse events occurred during the observation period. No signs of irritation or inflammation were noted, suggesting that the compound was well tolerated. With the exception of one participant, who experienced sensitivity to cold after treatment, the placement of the material was not associated with discomfort, pain or bad taste in any of the experiments performed. Increased sensitivity to thermal stimuli is a common phenomenon observed after all types of periodontal treatment, and can be attributed to the mechanical treatment (SRP) performed before the application of the compound. In fact, in the individual showing this reaction, all mechanically treated teeth were sensitive, irrespective of the additional application of the product. Theoretically, a local sensitivity reaction could also be due to root surface demineralization caused by the low pH of the tetracycline, or could be associated with the removal of the smear layer from the root surface (Wikesjo et al. 1986).

Biodegradability of a delivery system may be an advantage in clinical practice, because an additional visit to remove the compound can be avoided. It is characteristic for poly(ortho esters) to degrade by surface erosion (Schwach-Abdellaoui et al. 2001), whereby the liberation of the active agent is coupled with the degradation of the polymeric matrix. This mechanism implies that the polymer is no longer present if the active agent can not be recovered and *vice versa*. In other polymeric systems leading to diffusion of the drug out of the carrier, the latter can still be present in the pocket even if no drug is available anymore. Figure 7 illustrates the difference between diffusion and surface erosion.

Figure 7 : Schematic representation of two different release kinetics. A: Active agent diffuses through the matrix, which remains *in situ*. B: During surface erosion the active molecules are liberated through the progressive breakdown of the matrix.



Non-injectable LDDs, such as tetracycline fibers, are relatively difficult to handle and require a considerable time for application. As only a small number of teeth can be treated in a given time, these products have not obtained the general acceptance by the dental practitioners initially expected by the developers. In comparison, the application of an injectable device is quick and easy, and many sites may be treated in a short period of time. Injectability of poly(ortho esters) depends on their chemical characteristics (molecular weight, percentage of lactic acid, *etc.*) and other factors such as γ-sterilization (Sintzel et al. 1998c). The compounds used in the present trial ($POE_{70}LA_{30}TB_{10/20}$) were injectable through a needle of 0.8 mm in diameter.

Adhesion of poly(ortho ester) to dry or wet bovine dentin slabs was evaluated by Roskos (1995). Tensile strength tests showed consistently a cohesive failure within the viscous polymer. The required "forces of detachment" were 0.4 and 0.1 N/cm^2 for dry and wet surfaces respectively. These weak forces were estimated by the authors to be well under the polymer-to-substrate bond. Although not measurable directly, it was assumed that adhesive forces were sufficiently strong to retain the polymer within a periodontal pocket (Roskos et al. 1995). The limited retention recorded *in vivo* in our trials does not confirm these claims so far.

5.1 Retention

The initial series of treatments (part I) yielded disappointing results with regard to the retention of poly(ortho ester). No differences could be observed between the single- and multi-rooted teeth subgroups. It was, however, encouraging to note that in those sites where the material was retained, drug was available during several days at considerable concentrations, suggesting the establishment of a stable subgingival drug reservoir, fulfilling the requirements for successful local chemotherapy (Mombelli 1997). In fact, the tooth with a swelling of the gingiva a few millimeters apically to the site of injection (see 4.1.1) also showed the highest sustained levels of tetracycline in GCF, indicating that a physically stable reservoir had been formed.

In part two, a somewhat higher number of pockets were found to harbor the antibiotic system. Some of these positive sites demonstrated bacterial inhibition until day 11. Thus, changing the clinical conditions by “drying” the pocket at application time improved the number of positive sites and the retention time of the product as well. Shen et al. (1997) used a retraction cord to improve the effectiveness of SRP. Packed periodontal pockets gave enhanced access and visibility. Significantly more calculus was removed from the root surfaces of the test group compared to pockets where no cord was used (Shen et al. 1997). In both cases, the retraction cord gave a benefit for the treatment outcome but the clinical relevance of using such a device is questionable. Insertion of the cord into periodontal pockets and hemostasis requires attention and is time consuming.

The loss of the local delivery device in several sites could be explained by an increase of tissue tonus resulting from a favorable response to treatment. Shrinkage of the gingiva could have pushed the compound out of the pocket. The retention of the device could also be compromised by the flushing action of the GCF. It has been estimated that in a periodontal pocket the GCF is replaced as much as 40 times *per* hour (Goodson et al. 1979). A relatively large reservoir of the drug seems to be necessary to maintain sufficiently high levels of an active agent in GCF over a prolonged period. Part III demonstrated that a small quantity of compound was insufficient to obtain positive results after one week.

5.2 Bioavailability

Tetracyclines are broad-spectrum antimicrobials affecting anaerobes and facultative microorganisms. After systemic administration, such antibiotics are found in the periodontium at bacteriostatic concentration; 3 to 8 μg/ml were reported in GCF (Gordon et al. 1981, Sutter et al. 1983). Local delivery to periodontal pockets produces much higher, bactericidal, concentrations. By using tetracycline fibers, Tonetti et al. (1990) were able to demonstrate sustained concentrations of 1500 μg/ml in the GCF for ten days. Stoller et al. (1998) showed levels of doxycycline in excess of 150 μg/ml for 7 days with a degradable injectable system. Our results compare favorably with the latter, showing GCF levels of tetracycline base ranging from 70 to 325 μg/ml for positive sites in part 1 (Fig. 3). This is about 2 log over the MIC, which is 1-2 μg/ml for most of the periodontal pathogens (Seymour & Heasman 1995, Kleinfelder et al. 1999) but may be as high as 8 μg/ml for some organisms (Walker et al. 1981).

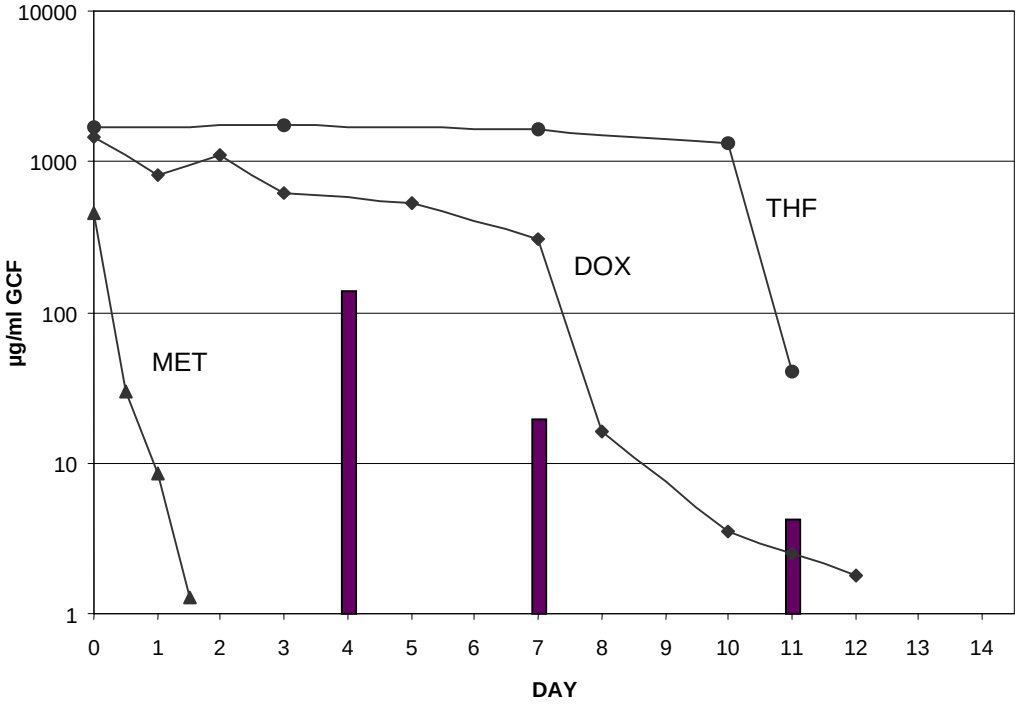
In Part II, maximal concentrations were comparable to those found in the first part, but a higher proportion of sites showed low concentrations (Fig. 5). 2/3 of the antibiotic concentrations recorded in positive samples were above the MIC. In the other positive sites, inhibition zones on the bioassays were very small, reaching the detection limit of the assay, and indicating minimal amounts of retained tetracycline. This phenomenon was observed again in part III, where tetracycline quantities were extremely low in all three positive sites. It should be noted that close to the detection limit of the assay tetracycline concentration could not be determined precisely.

5.3 Release kinetics

An overall tendency for concentrations to decrease with time was noted, suggesting that the compound was eliminated exponentially. Figure 8 compares the mean concentrations found with the poly(ortho ester)/tetracycline device as reported in section 4.4, to the pharmacokinetics of three commercially available drug delivery systems. Metronidazole gel (MET) showed rapidly decreasing concentrations, resulting in low values after the first day (Stoltze 1992). The release profile of tetracycline from monolithic, non-resorbable ethylene-vinyl-acetate fibers (THF) has been

characterized as zero-order for ten days by Tonetti et al. (1990). The release profile of doxycycline from biodegradable (DOX) was characterized by Stoller et al. (1998). Doxycycline level decreased with time but concentrations were above 150 μg/ml for 7 days. The release kinetics of the poly(ortho ester)/tetracycline compound can be compared to the doxycycline system. Both have similar profiles, which differ from the solid device on one hand and the gel on the other.

Figure 8 : Pharmacokinetics of different controlled drug delivery systems. Rapidly decreasing concentrations for metronidazole gel (MET), constant release for tetracycline fiber (THF) and decreasing levels for doxycycline system (DOX) and POE.



Conclusion

- Retention of POE was observed for up to 7 days in part I. However, only a limited number of sites were positive. No differences could be found between pockets on single rooted teeth and furcation defects. If positive, experimental sites showed high levels of tetracycline.

- In part II, retention was improved by modified clinical conditions minimizing the presence of fluid on the root surface. About 40% of the sites showed evidence for retention of POE. The compound had the potential to remain in periodontal pockets for up to 11 days. Tetracycline was released at therapeutic levels during the same period.

- The controlled doses of polymer applied in part III were too small to produce positive results.

- As a general rule tolerance to the compound was excellent throughout the study.

Further studies on POE are needed to obtain sustained delivery of active agent at sufficient concentration in a predictable way. Variations in the retention of POE could be minimized by improving the adhesive properties of the polymer or modifying further the clinical conditions at application time.

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8 Appendix

A Patient Record Form

B Clinical Record Form

C Standard curve - PERIOTRON®

Subject Number

Initials

PATIENT RECORD FORM

Name _____ First Name _____

Address _____

City code _____City _____ Country _____

Telephone home nr _____ Telephone work nr _____

Age _____ Sex _____ Weight _____ Size _____

Baseline admission criteria

Yes	No	
☐	☐	Systemic health : no history of diabetes, rheumatic fever, blood dyscrasia, immunology anomalies
☐	☐	Medication : no exposure to antibiotics within previous 6 months, no long-term exposure to anti-inflammatory medications
☐	☐	No hypersensitivity or allergy to tetracycline or poly (ortho esters)
☐	☐	Female : No pregnancy.
☐	☐	No periodontal treatment within one year.
☐	☐	Presence of four or more residual periodontal pockets > 5 mm at proximal location
☐	☐	Informed consent signed by patient
☐	☐	Patient admitted to study.

Examiner _____

Visit _____

Date _____

Subject Number
Initials
Examiner

CLINICAL RECORD FORM

(Sample # POE15TB20)

		SITE	1	2	3	4	REMARKS
		Tooth					
		Location					
DAY 0		Date					
		PPD					
		REC					
		Suppuration					
		BOP					
		Sample n^{r}					
		Retract. cord					
		Visibility?					
		Pocket dry ?					
		Amount POE					
		Periotron					
DAY 4		Date					
		Sensitivity					
		Suppuration					
		Sample n^{r}					
		Periotron					
DAY 7		Date					
		Sensitivity					
		Suppuration					
		Sample n^{r}					
		Periotron					
DAY 11		Date					
		Sensitivity					
		PPD					
		REC					
		Suppuration					
		BOP					
		Sample n^{r}					
		Periotron					

Standard curve - PERIOTRON®

Standard curve : Fixed volumes of serum are recorded. A 4th order polynomial plot is fitted to the data points