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IMPLANTS SE FORMANT *IN-SITU* POUR LE TRAITEMENT DE LA PARODONTITE

IN-SITU FORMING IMPLANTS FOR PERIODONTITIS TREATMENT

THESE

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« La connaissance s'acquiert par l'expérience, tout le reste n'est que de l'information

Albert Einstein

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GLOSSARY

2-Pyr : 2-pyrrolidone

ANSM : Agence Nationale de Sécurité du Médicament et des produits de santé/ French National Agency for Medicines and Health Products Safety

API: Active pharmaceutical ingredient

ATB: Antibiotic

ATBC: Acetyl-tributyl-citrate

CAL: Clinical attachment loss

Chx: Chlorhexidine

COX: Cyclooxygenase

CK: Creatine kinase

DDS: Drug delivery system

DMSO: Dimethylsulfoxyde

Eth-Lact: Ethyl lactate

EVA: Ethyl vinyl acetate

GHS: Globally Harmonised System of Classification and Labelling of Chemicals

GPC/SEC: Gel permeation chromatography / size exclusion chromatography

HPLC: High performance liquid chromatography

HPMC: Hydroxypropyl-methyl-cellulose

Ibu: Ibuprofen

ISFI: *In-situ* forming implant

MMP: Matrix metallopeptidase

Mw: Molecular weight

NIH: National Health Institute

NMP: N-methyl-pyrrolidone

NSAID: Non-steroidal anti-inflammatory drug

PCL: Poly-caprolactone

PLGA: Poly (Lactic-co-Glycolic) Acid

PTFE: Polytetrafluoroethylene

PVDF: Polyvinylidene fluoride

RANK: Receptor Activator of NF-kB

RANKL: RANK-Ligand

SRP: Scaling and root planning

Tg: Glass transition temperature

1. INTRODUCTION

1.1. Periodontal diseases

1.1.1. Periodontium anatomy

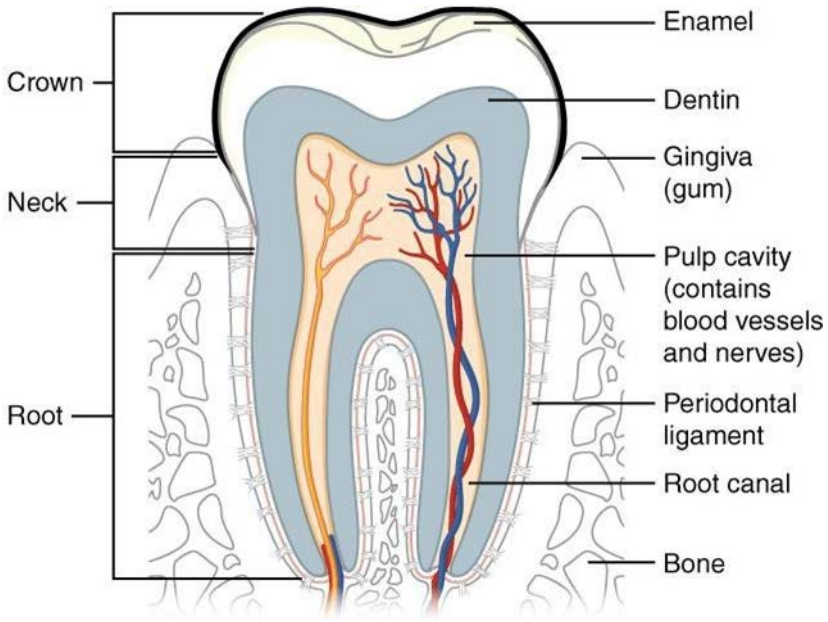


Figure 1: Cross section of a molar (1)

Periodontium comprises the surrounding tissues of the tooth. It is composed of the alveolar bone, the gum, the periodontal ligament and the cement which covers the surface of the tooth's root (1).

The alveolar bone surrounds the tooth and remains on a denser bone structure (maxillary for the upper jaw and mandibular for the downer jaw). Its trabecular internal structure allows for better vascularisation and cell's circulation.

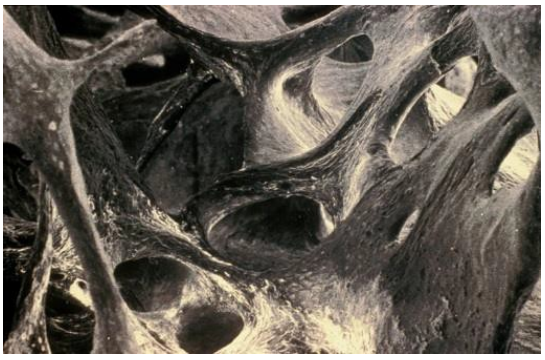


Figure 2: Internal structure of an alveolar bone (© Inserm, George Boivin)

The gum is an epithelium recovering the root and the neck of the teeth. Its histological nature depends on its location (vestibular or lingual face, sulcular or junctional epithelium). It is pluri-stratified and can be keratinized or not.

The periodontal ligament is a soft conjunctive tissue, not mineralized, that links the cement to the alveolar bone. It is composed of many fibres that allows for the teeth integration as well as many nerves that enable for chewing proprioception. The following Figure details the junction between the gum and the teeth (2).

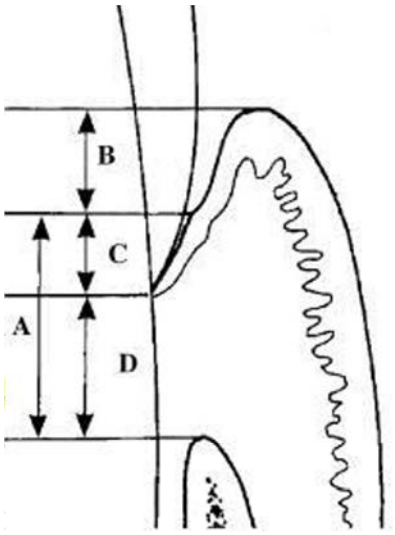


Figure 3: Detail of the tooth's neck (2)
B = 0.69mm: histological sulcus.
C = 0.97mm: epithelial attachment.
D = 1.07mm: conjunctive attachment.
A = C+D: attachment system.

Periodontitis or periodontal diseases regroup a vast domain of pathologies. Those affect the surrounding tissues of the tooth, that include the gum, the periodontal ligament (or periodontal fibre) and the alveolar bone (mandibular or maxillary). Therefore, facing the great diversity of the affections represented by the term "periodontitis" (and the discussions around the subject that it arises (3)), this thesis will be focused particularly on chronic periodontitis with emphasis on local controlled drug delivery systems for its treatment. Those represent 95% of the encountered cases and show a high prevalence in occidental countries, that makes the condition a major public health concern with an important economic impact associated to it (4).

Periodontitis generates an inflammation that is responsible of the tissues destruction, thus leading to other troubles like tooth migrations, tooth loss and are associated with many other systemic diseases (5–7). It represents the first cause of tooth loss in adult and is the sixth more frequent human disease in the world

Chronic periodontitis is progressive and irreversible once the alveolar bone is impacted. The standard treatment is mechanical, consisting in root scaling and surfacing to which is often added antibiotics (ATB) *per os*. Yet, this leads to unwanted

bacterial resistances while acting with a moderate efficacy and many side effects. The need to develop new alternative treatments for a better care management appears now clearly.

1.1.2. Gingivitis

Gingivitis is an inflammation of the gingiva that can be localized or spread. It causes the inflammation and swelling of the gum as well as bleeding. Several gingivitis types are defined whether it is induced or not by the dental plaque (3). If it is, the dental plaque can grow under the gum (on the root surface) and promote a dysbiosis that will lead to periodontitis. Yet, gingivitis is reversible and can be managed with oral hygiene and dental care.

1.1.3. Periodontitis

According to the NIH, periodontitis is the advanced form of gingivitis when it is no longer reversible, that occurs when *"bacterial toxins and the body's natural response to infection start to break down the bone and connective tissue that hold teeth in place*"(8). It refers to the lesion of the deep supporting tissues of the teeth, with an attachment loss coupled with a bone lysis. More precisely, a periodontal pocket is defined as a pathological rupture of the link between the tooth and the crevicular epithelium, limited at its apical extension by a junctional epithelium. It is an abnormal apical extension of the gingival sulcus caused by the apical migration along the radical wall as a consequence of the attachment system's destruction induced by the periodontal disease (2). It is a chronic condition, irreversible and evolving by an alternation of latencies and exacerbations (9).

Periodontal diseases regroup a vast and complex field of pathologies for which numerous classifications have been done. From 1999 to 2017, the one used was made by the AAP (American Academy of Periodontology) in 1999 by Armitage et al. with an international work group. This classification has now evolved to be more clinically relevant (3). Previously, periodontitis was roughly divided into two major groups (*i.e.* "aggressive" and "chronic"). Aggressive periodontitis was of fast development, marked genetic susceptibility, affecting mostly young adults (20 to 30 years old) and concerning a minority of patients whereas chronic periodontitis was of slow development without any particular genetic susceptibility, affecting mostly adults over 30 years old and concerning a majority of patients (10). Though, this classification lacked of precision, especially regarding the clinical outcomes of the disease. Indeed, the clinical attachment loss (CAL) is also a physiological phenomenon with ageing. The previous classification system missed some biological features to better characterise the disease progression. According to the 2017 world workshop, *"the data show that mean annual attachment level change varies considerably both within and between populations. Overall, the evidence does not support or refute the*

differentiation between forms of periodontal diseases based upon progression of attachment level change." (11).

The new classification system is divided in a more dynamic scheme including *stages* and *grades*. It allows a better comprehension of the history and the development of the disease in a particular patient, thus a better anticipation and management of its progression. The following chart resumes roughly the classification scheme (3) (cf. annex 1 & 2 for detailed classification system).

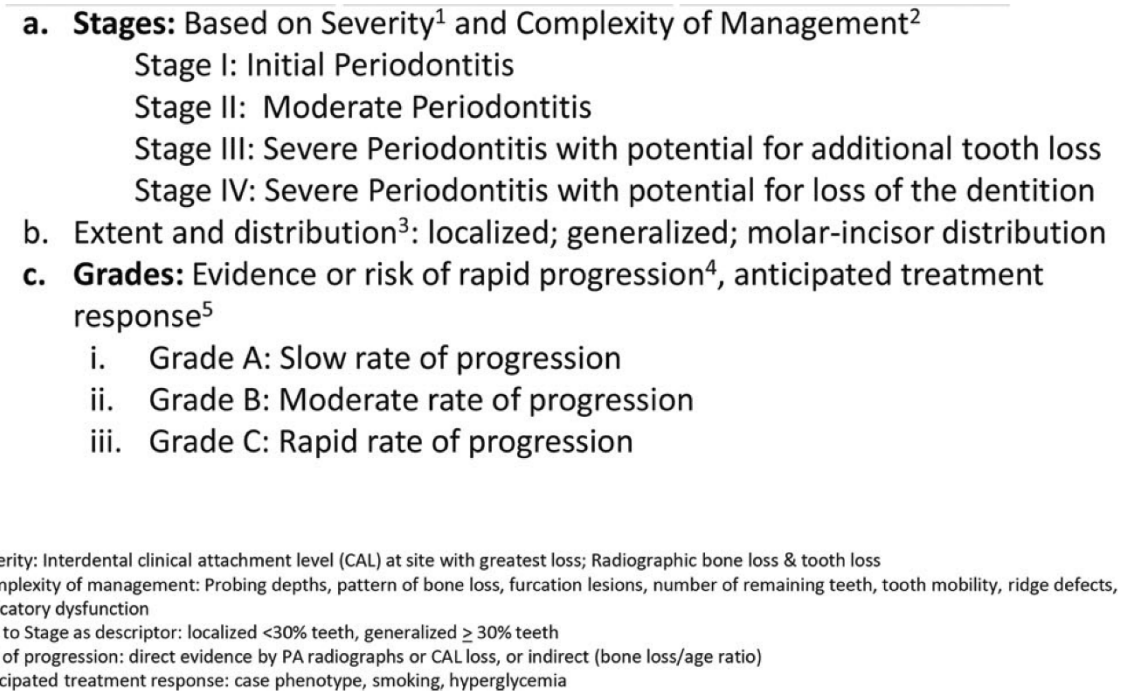


Figure 4: Current classification scheme of periodontitis (3)

1.2. Aetiology

1.2.1. Risk factors and associated diseases

The aetiology and pathogenesis of the disease is multifactorial. It is triggered by the meeting of pathogenic opportunistic bacteria and a site (the gingival sulcus) favourable towards the development of the disease. This site can be favoured as well by genetic factors as by an already existing pathologic condition. This meeting between the pathogens and the host is necessary but not sufficient for the progression of the disease. The complexity of the disease is that the immune response of the host against the bacterial infection is the main component of the tissues destruction's process.

Many other causes have an influence on the prognostic of periodontitis. Thus, oral hygiene is the key and the first component in the disease progression. A lack of hygiene leads to gingivitis, to the accumulation of dental plaque which forms

tartar with time. This is directly linked to the presence of periodontal lesions and the development of the disease (12).

Tobacco consumption exposes the smoker to more periodontal events compared to the non-smoker. The risk increases with the number of cigarettes smoked and with the length of consumption. It decreases slowly when stopped. Smokers heal slower and respond less than non-smokers to medicines. Finally, tobacco consumption impacts numerous immune pathways linked to the disease (13,14)

While some factors are constitutional and can't be changed (age, sex, genetic, morphology), other aspects like environmental factors may be more or less controllable. Those linked to the lifestyle and oral hygiene (smoking, alcohol, drugs abuse, etc.) affect the prognostic of the disease and are the more likely to be changed. Some other factors are on the edge, like nutritional factors or stress, that cannot be always easy to manage. Finally, some factors represent a real burden as they are completely independent from the patient's willingness like iatrogenic effects induced by local treatments (prosthesis, orthodontics treatments) as well as those induced by the medications (e.g. immune suppressants) (15–17).

1.2.2. The bacterial factor

Periodontitis are known to be induced by a dysbiosis of the mouth's commensal flora. Indeed, the oral cavity shelters more than 700 species of bacteria and up to 150 can be found in one subject (18,19). In a healthy status, those are mostly *Streptococcus spp* that represent more than 50% of the buccal flora (9,20).

Since the sixties, several bacteria have been associated with periodontal lesions (21). A correlation was made between the deepness of the pockets and the types of bacteria that were found in these. They were classified into "complex" in function of the clinical state they were associated with (21–23).

Among these, the red complex encompass the three most pathogenic bacteria found in periodontitis (24). It comprises *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* (25). *P. gingivalis* is of particular interest as it exhibits many virulence factors , some of which are really uncommon among bacteria's arsenal (26,27). Indeed, *P. gingivalis* is an asaccharolytic anaerobic Gram-negative bacterium that expresses a broad range of virulence factors (28). In addition to adherence factors, the production of enzymes (e.g. collagenase which is very specific of *P. gingivalis* (29)) allows for the destruction of the soft tissues of the periodontium. Even the metabolites resulting from this destruction exhibits detrimental effects toward the tissues (30). *P. gingivalis* is such a singular bacterium that it is the most studied in periodontitis. It is used to induce periodontal lesion in animal model of periodontitis upon injection into the gum (31). Therefore, it is believed that the presence of *P. Gingivalis* is of major importance for the development and evolution of the disease and is considered as "a keystone pathogen" (32).

Despite the importance of *P. gingivalis*, it has to be pointed out that a lot of other bacteria are involved in the pathogenesis (33–35). It is the result of the interactions between an intricate mix of bacteria that exhibit many virulence factors and the host response that involves complex immune mechanisms (36–38).

1.2.3. The host immune response

If the bacterial factor is needed to induce the disease, it is not sufficient though. The way that immune system will respond to the bacterial aggression constitute the major part of the pathogenesis. Indeed, many people are healthy carriers of bacteria such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* without showing any sign of disease progression (39). The destruction of the tissues is the result of the inflammation caused by the bacterial dysbiosis (40,41). As a response to an infection, all cellular pathways are triggered in periodontitis. Therefore, innate immunity is the first actor with the recruitment of neutrophils via the T-cell line. Then, B-cells intervene also, despite their function remains quite unclear (42).

However, several destruction mechanisms have been elucidated. The destruction of soft conjunctive tissues is mostly the result of direct bacterial aggression, as they secrete a lot of proteolytic enzymes. In this way, *P. gingivalis* secrete at least thirteen enzymes and *Treponema denticola* secretes at least seven of them (29). Moreover, the bacterial lipopolysaccharide induces the secretion of metalloproteinase (MMP) by the neutrophils, responsible of the soft tissues lysis (24).

When the soft tissues are deeply affected, the bacterial products will reach the bone. Bone homeostasis is a finely tuned mechanism orchestrated by the differentiation of osteoblasts. These cells contain RANK receptor (Receptor activator of nuclear factor kappa B) that leads to the formation of osteoclasts, responsible of bone resorption, when activated by its ligand RANKL. In an inflammatory state, many cytokines are produced that favour the equilibrium toward the stimulation and production of RANKL, therefore directly promoting bone resorption (43).

The most complex part is the presence of multiple interactions between bacteria and host response. Indeed, in periodontitis, bacteria have developed many systems to overcome their destruction by the immune system, either innate as adaptive. For example, *P. gingivalis* is capable to manipulate the complement to prevent itself from lysis (40,44,45). It can also avoid leucocyte adhesion, decreasing their efficacy in fighting infection (46). In addition, this manipulation leads to the destruction of periodontal tissues by auto-immune mechanisms (47,48).

Even if many mechanisms remain unclear, it might be worth considering some of these as possible treatment targets. This can be achieved either by inducing immune response as by breaking down the escape or subversion mechanisms triggered by bacteria. Indeed, the systemic inflammation resulting from periodontitis is believed to be associated with many systemic diseases (49), and some like rheumatoid arthritis,

cardiovascular events and particularly diabetes are highly correlated with the periodontal status (17,50–54).

Finally, the immune status represents an interest in the management of periodontal care as a prognostic tool. Indeed, some cytokines or other markers of inflammation are dosed in some studies and finding one specific of periodontal disease would be interesting to be associated with particular clinical outcome.

1.2.4. Treatments

Once the disease is diagnosed, the treatment is essential to stop its evolution as the lesions are irreversible. The medical management of the disease consists first in stopping the progression of the disease by controlling the bacterial biofilm and the inflammation then to prevent the risk of relapse (55). The mechanical disruption of the dental plaque is the main goal of the treatment scheme. Then, it can be added chemical adjuvants to further eliminate residual pathogenic bacteria and limit inflammation for a better recovery and to delay the risk of relapse. Afterward, the patient will need to maintain a healthy periodontal status by oral hygiene and dental care follow up.

1.2.5. Non-surgical treatment

The gold standard treatment is scaling and root planning (SRP). As periodontitis is associated with subgingival tartar, it represents the first step of the medical management. The dentist removes the subgingival tartar with a curette, allowing for the breaking of the bacterial biofilm and a further healing of the wound. This treatment is essential but is not always sufficient (37–40). Depending on the extent and the stage of the disease, this procedure can be very long. The number of sessions and way to manage the procedure is discussed. Some advises to treat the full mouth at once to avoid contamination from the use of instruments whereas others will treat by quadrants (56).

1.2.6. Surgical treatments

When the lesions are profound and the bone is deeply attacked (e.g. furcations) it may be necessary to perform dental surgery in order to remove the tartar and clean up the periodontal pocket. For this, a flap surgery is needed. It consists of an incision into the gum allowing to access correctly into the periodontal pocket. Flap surgery is effective to remove more calculus than SRP alone (57). There are numerous ways to proceed and it depends of the lesion itself as there is no standard flap surgery (58).

For periodontal regeneration, some techniques exist that are complex surgery interventions. Amongst all, the most common is the guided tissue regeneration. It consists of placing a barrier like a membrane between the periodontal ligament and the bone in order to prevent epithelial cells migration into the wound and to allow periodontal ligament cells to get back on the tooth’s root and increase attachment level

(59). When the wound is substantial, it may require other techniques and the need for biomaterials. These comprise autogenous grafts, allogenic grafts, xenogenic grafts or the use of alloplastic materials to fill the bone defect (59).

1.2.7. Medicinal treatment

In addition to the mechanical treatment, drugs are often used as adjunct therapy. In periodontal care, three main classes are used, *i.e.* antibiotics, antiseptics and anti-inflammatory agents. The efficacy of such treatments is still limited due on the one hand to their mechanism of action and on the other hand to their galenic form. Indeed, as antibiotics act against bacterial growth and since periodontal bacteria are organised in biofilms (in which bacteria have a lower metabolic rate), their efficacy is therefore lower than against planktonic bacteria. This is even more noticeable as dental plaque is a complex multi-species biofilm were bacteria often exhibit mutualistic and synergistic interactions (35,37,60–63). On top of that, a systemic treatment involves the patient to experience many side effects even though the concentration at the site of action (*i.e.* the gingival sulcus) remains low.

1.2.7.1. Systemic

The use of antibiotics in periodontics remains a very controversial topic. The trends have been evolving and the subject remains on top of current topics, notably with the emergence of bacterial resistances (64,65). Moreover, every country has a different approach regarding antibiotics use and even the ESE (European Society of Endodontics) for example states its position about their uses but claims that local recommendations may differ (66).

The ANSM (Agence Nationale de Sécurité du Médicament et des produits de santé/ French National Agency for Medicines and Health Products Safety) established guidelines about the use of antibiotics in periodontitis treatment. They are recommended only in a few cases. This is due to the lack of data regarding the clinical efficacy and relevance of such treatment (too few clinical trials or scales not large enough). Antibiotics are not innocuous therapies and side effects can be serious. Mostimportantly, the determination and willingness of the authorities in reducing their use is obviously in order to limit bacterial resistances to this class of compounds (67).

However, their use is common in clinical practice since the existing clinical data tend to exhibit benefits from it (68–71). It must be noted that drugs are only adjuvants of a mechanical and/or surgical intervention, and their use alone may not replace this treatment. The benefit off adding antibiotics to scaling and root planning remains disputable and "*their use can sometimes be justified*" (72).

In prophylaxis, antibiotics are intended for patients with a high infectious risk (immune depressed, patient with high risk of infective endocarditis). Systemic administration will be used in these cases.

In a curative way, antibiotics are addressed to particular cases (traumatisms, necrosis, etc…) as well as for aggressive and severe periodontitis. In these cases, local administration can be used.

It has to be pointed out that the use of antibiotics *per os* depends strongly on the type of patients. Indeed, people will not be considered the same between general population and immune depressed patients or patients with high risk of infective endocarditis. For patients under immune suppressant medication, the use of anti-infectious prophylaxis is recommended, using a bactericidal agent and not a bacteriostatic one (73,74).

Antibiotics used in periodontal care depend on what type of treatment is required (*i.e.* prophylaxis or curative). In the first case, their indication is to avoid possible infective endocarditis or infection of any implantable medical device like prosthesis. The type of bacteria mostly found in these infections are Gram positive bacteria like *Staphyloccocus spp*, *Streptococcus spp* and *Enterococcus spp*. Therefore, prophylaxis is recommended before every intervention that would cause gingival breakthrough, with one unique dose of 2g amoxicillin 1h before intervention. In case of beta-lactamin intolerance, 600mg of clindamycin can be used.

In France, antibiotics are not indicated in curative treatment of chronic periodontitis as an adjunct of scaling and root planning whatever is the infectious risk of the patient (professional agreement). However, these are the official recommendations and the real use of antibiotics in clinical practice cannot be denied.

According to Feres M. *et al.* (Systemic antibiotics in the treatment of periodontitis. Periodontol 2000. 2015), *"the recommendation of antibiotics to treat periodontal infection should follow the same principle used for the treatment of any other infection, that is: the risks need to be clearly offset by benefits to the patient – benefits that could not be otherwise achieved or which would be achieved with much greater difficulty or risk by other means. In this regard, both the reduced need for periodontal surgery and the lower levels of pathogens associated with the administration of systemic antibiotics, especially metronidazole+amoxicillin, need to be considered. In other words, the risks associated with performing additional surgical procedures and with the presence of higher levels of pathogenic bacteria in the subgingival environment (for the patient`s local and systemic health) should be included in the risk–benefit evaluation* (74)."

Regarding this fact, antibiotics should be used as follow [adapted from (67)]:

	First intention	Second intention
General case	• amoxicillin: 2 x 1g/d • azithromycin: 1 x 500 mg/d* • clarithromycin: 2 x 500 mg/d • spiramycin: 3 x 3 MUI/d • clindamycin: 2 x 600 mg/d	• amoxicillin-clavulanic acid (ratio 8/1): 2 x 1g/d to 3x 1g/d (expressed dose in amoxicillin) • amoxicillin: 2 x 1g/d **and** metronidazole: 3 x 500 mg/d • metronidazole: 3 x 500 mg/d **and** azithromycin: 1 x 500 mg/d* **or** clarithromycin: 2x 500 mg/d **or** spiramycin : 3 x 3MUI/d
Necrotic periodontitis	• metronidazole : 3 x 500 mg/d	
Localized aggressive periodontitis	• doxycycline: 1 x 200 mg/d†	
localized or generalized aggressive periodontitis	• amoxicillin: 3 x 500mg/d or 2 x 1g/d **and** metronidazole: 3 x 500 mg/d *In case of penicillin's allergy:* • metronidazole : 3 x 500 mg/d	

Table 1 : Recommended antibiotherapy's schedule in adult with normal kidney function.
*Duration of treatments: 7 days, except * and †.*
** Treatment time of 3 days.*
† In one dose, during lunch or dinner, at the latest one hour before sleeping; under 60kg: 200mg the first day then 100mg/d. Treatment time of 14 days.

These doses are "general guidelines" which aim is to cover the majority of patients, though many particular cases can occur and this will be the physician's work to evaluate the risk-benefit ratio.

First intention is amoxicillin, which spectrum is large and covers well the bacteria involved in the disease. Even the others ATBs used in first intention should be an alternative to amoxicillin (in case of penicillin's allergy or ineffectiveness).

In second intention, amoxicillin should be used firstly with the synergistic clavulanic acid. If not, metronidazole can be used only in association with another ATB. This is due to the particular spectra of metronidazole that targets only the anaerobic bacteria. This is also the reason why it is used alone only in necrotic periodontitis as almost only strictly anaerobic bacteria are found in this type of deep tissue lesions.

Finally, we can cite doxycycline at very low doses (*per os*, 20mg a day) which does not exhibit any antimicrobial properties but seems to provide a better gum healing by inhibiting host-derived enzymes known as the matrix metalloproteinases (MMPs), thus preventing further destruction of the tissues (75,76). The only marketed product is Periostat©: doxycycline 20mg.

1.2.7.2. Local

Some specific delivery systems have been developed to treat periodontitis. Their aim is to be placed into the periodontal pocket after scaling and root planning in order to prevent bacterial recolonization thus enhancing clinical attachment gain.

As previously said, the use of ATB is not welcomed as it represents a threat for the emergence of bacterial resistances. It is clear that exposing pathogenic bacteria to low doses of ATB (*i.e.* sub-MIC doses) for a long time is a major risk of creating new mutants resisting to the ATB. In this way, we can see that authorities limit drastically their use. For example, in France, several specialties have been recently suppressed from the market:

→ METROCOL 4,5 mg sponge for dental use

→ METROGENE 4,5 mg sponge for dental use

These medicines were for hospital use only and consisted of a biodegradable collagen fibres sponge loaded with metronidazole, intended to clean the lesion and eventually let in place. They were suppressed from the market on November 11th 2016.

The only local treatment loaded with antibiotics available in France is Parocline® 2% Gel dent (Minocycline). It is a preformed gel for periodontal pocket injection. Excipients are: Hydroxyethylcellullose (HEC), Magnesium Chloride (MgCl), Acrylates & Methacrylates copolymer (Eudragit RS®) triacetin and glycerol.
Please note that Parocline® is the French proprietary drug name equivalent to Dentomycin®, the UK proprietary drug name, mentioned in the following table 2.

However, other treatments exist that are commercialised in the world, mainly in the US. The following chart resumes the existing specific local delivery formulations currently available on the market [adapted from (73)]:

Brand	Manufactured by companies	Drug	Delivery	Polymer matrix	Biodegrability	Approval
Arestin®	OraPharma, US	Minocycline Hydrochloride	Micro-spheres	Poly (lactide-co-glycolide)	Biodegradable (14-21 days)	USFDA
Atridox®	Atrix Laboratories	Doxycycline	Gel	Poly(dl-lactide) and N methyl pyridine	Bioabsorbable (21 days)	USFDA
Periochip®	Perio Products Ltd., Israel	Chlorhexidine gluconate	Chip	Gelatin crosslinked with glu-taraldehyde	Biodegradable (7 to 10 days)	USFDA
Dentomycin®	Wyeth, United Kingdom	Minocycline HCl	Ointment	Hydroxyethyl-cellulose, aminoal-kyl-methacrylate, triacetine and Glycerine	Biodegradable	European Union but non-FDA cleared
Elyzol®	Dumex-Alpharma	Metronidazole benzoate	Gel	Glyceryl mono-oleate and sesame oil	Biodegradable	Non-FDA
Chlo-Site®	Ghimas Company, Italy	Chlorhexidine	Gel	Xanthan gel	Biodegradable	Non-FDA

Table 2: Currently marketed local delivery systems for periodontitis treatment [adapted from (73)].

Among these treatments, Atridox® and Elyzol® are also approved in France but are not distributed anymore.

Interestingly, most of these systems are loaded with antibiotics and only two of them uses chlorhexidine. Although not detailed in table 2, Chlo-site is loaded with both chlorhexidine salts dihydrochloride and digluconate.

However, many researches focus on using alternative active pharmaceutical ingredients to antibiotics that would present an antibacterial effect but also possibly an anti-inflammatory effect. To name but a few: genistein (77), epigallocatechin-3-gallate (78), taurolidine (79) and curcumin (80–86).

1.2.7.3. Anti-inflammatory agents

Agents used to modulate the inflammatory response have been proposed to treat the disease and slow down its progression. Glucocorticoids have not proven any benefit on the clinical outcomes when the periodontal status of patients with multiple sclerosis taking prednisone was followed (87). On the contrary, corticoids are harmful in animal model of periodontitis as they induce bone resorption (88).

Alternatively, non-steroidal anti-inflammatory agents (NSAIDs) have been proposed in periodontitis treatment as an adjuvant of scaling and root planning. This class of compounds has an interest in reducing alveolar bone resorption. Their mechanism of action relies on the inhibition of cyclo-oxygenase enzymes (COX), responsible of arachidonic acid metabolism and formation of pro-inflammatory agents. Two isoforms of these enzymes exist: COX-1 that is constitutive and physiological, and COX-2 that is induced by autacoids (IL1, TNF-α). COX-1 activity induces the synthesis of prostaglandins that takes place in the stomach (cytoprotection) and the kidneys (maintaining a normal blood flow and pressure). It also induces the synthesis of thromboxane A2 (vasoconstriction and platelet aggregation). Its inhibition leads to unwanted side effects of NSAIDs. In contrast, COX-2 activity is inducible and responsible of specific prostaglandins synthesis intervening in inflammatory response.

That is said, many sides effects preclude their use on a long term schedule, at least via systemic administration. Indeed, COX-2 specific inhibitors are known to have a specific cardiotoxicity upon systemic and chronic use (cf. Vioxx' case). They are represented by the Coxibs.

On the other hand, non-selective COX inhibitors present also a non-negligible toxicity, with an increased risk of gastric ulceration, renal impairment and haemorrhage. Their most common representative molecules are from the class of arylcarboxylic acids that includes Ibuprofen, Ketoprofen, Flurbiprofen, etc…

Because NSAIDs have been proven to be effective in slowing periodontitis evolution and reducing clinical attachment loss and alveolar bone regression both in human and animal, they still are of interest. Yet, their efficacy is molecule dependant and the side effects upon systemic administration and/or chronic use are important.

Thus, adjunction of NSAIDs to scaling and root planning alone should be considered regarding the benefit-risk ratio for the patient. The development of new topical treatments or local delivery systems (such as implants, semi-solid dosage forms, gels, ointments, etc…) may represent a promising tool to improve periodontal care (89–95).

An interesting fact has to be noted is that NSAIDs benefits in wound healing are still discussed. Indeed, some studies have shown that COX inhibition during the first stages of wound healing would impair it (96–98). Though, these studies are mainly conducted to observe skin wounds repair.

In contrast, systemic administration of ibuprofen is widely prescribed in periodontal treatment and has shown to improve the clinical outcomes of patients (90). Finally, very recent studies have shown an improved wound healing when using local drug delivery systems loaded with ibuprofen (99–101).

1.3. Local controlled delivery systems

1.3.1. General

The main success factor in a pharmacotherapy is to reach a desirable drug time-concentration profile at a specific site of action into the body. This must be into a therapeutic window comprised between a minimal efficacy concentration and a minimal toxic concentration (or maximal tolerated concentration) above which the risks will overcome the benefits of the drug used. This is particularly crucial for highly potent drugs with a narrow therapeutic window or with a short half-life compared to the time of treatment needed (e.g. hormones, cytotoxic drugs, psychiatric drugs).

In order to reduce side effects of drugs orally taken, a range of different systems to administer the right dose, at the right place with the correct concentration over time has been developed. Most of those require the use of polymers allowing to reach this purpose (e.g. polymeric coating for intestinal delivery, matrix polymeric core for extended release, etc). A lot of oral dosage forms are now marketed with specific drug release properties.

Despite these improvements, the local delivery issue remains. Even with some controlled release devices (e.g. suspension or solution depot formulations, matrix formulation like pre-formed implants, reservoirs formulations), the treatment appears to be systemic. Among many systems that allow for controlled drug delivery, there are some that are specially designed to treat *locally* specific diseases, or at least particular symptoms of diseases and many researches are made in order to design new ones. As an example, Eligard® (Leuprolide acetate, Atrix laboratories) indicated in the palliative treatment of advanced prostate cancer, is an *in-situ* forming implant based on Poly (Lactic-co-Glycolic) Acid (PLGA) and N-methyl-pyrrolidone (NMP). The same type of technology has been used to develop Atridox® for periodontitis treatment. As every disease represents a specific challenge to overcome, various drug delivery

modalities have been studied. Among different local drug delivery systems (DDSs), the following intrapocket DDSs are already the object of substantial researches: films, chips, strips, fibers, nanofibers, microparticles, nanoparticles, liposomes, gels (102).

Therefore, it paves the way for the development of formulation processes and analytical tools to characterise the resulting systems. A particular emphasis has to be pointed on this very specific notion. Indeed, long term releasing systems generates this challenge of drug release kinetics assessment. A system designed to last for several months or even years cannot be tested such a long time. Thus, there is an obvious need for accelerated methods of drug release tests or innovative characterisation tools. It may allow in the future, via an extensive amount of collected data from those systems, to consider mathematical modelling and *in-silico* prediction of the drug release kinetics.

1.3.2. *In-situ* forming implants

In-situ forming implants (ISFI) encompass a vast domain of different parenteral depots systems. Their interest is growing for several reasons, as they are very promising to overcome some issues with particular conditions. One of the most interesting benefits is that it allows for a better treatment observance, which is a major condition for a successful therapy. As an example, it is particularly true for psychiatric diseases. Indeed, some mental disorders may lead to a lack of awareness responsible of missing doses, even despite the good willingness of the patient. But it can reveal other interests as their nature allow for multiple targeted treatments: intra-muscular, sub-cutaneous, intra-ocular, intra-articular, intra-spinal and even intra-cerebral injections are conceivable.

Several mechanisms of implants formation exist. The following chart resumes the main mechanisms of ISFI formation (103).

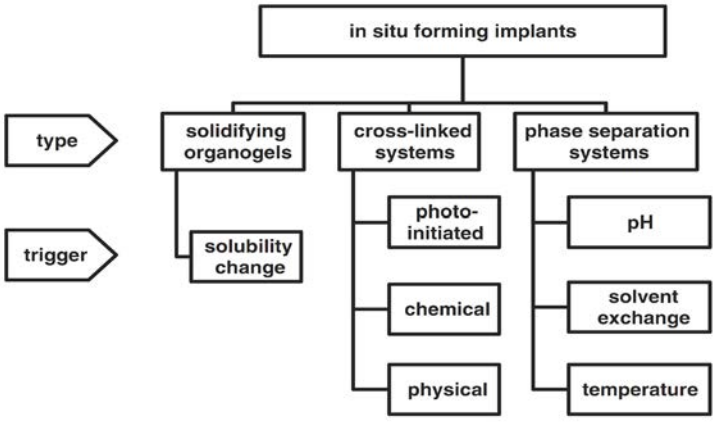


Figure 5: Types and formation's mechanisms of in-situ forming implants (103).

The object of this study was an ISFI based upon solvent exchange mechanism. It consists of a liquid formulation comprising a main polymer solubilised into a solvent, one or more API and additives. The main polymer must be insoluble into water,

allowing it to harden after injection, leading to implant's formation and drug(s) entrapment into the polymeric matrix. Thus, the drug(s) will be released with controlled kinetics. This mechanism is illustrated in Figure 6.

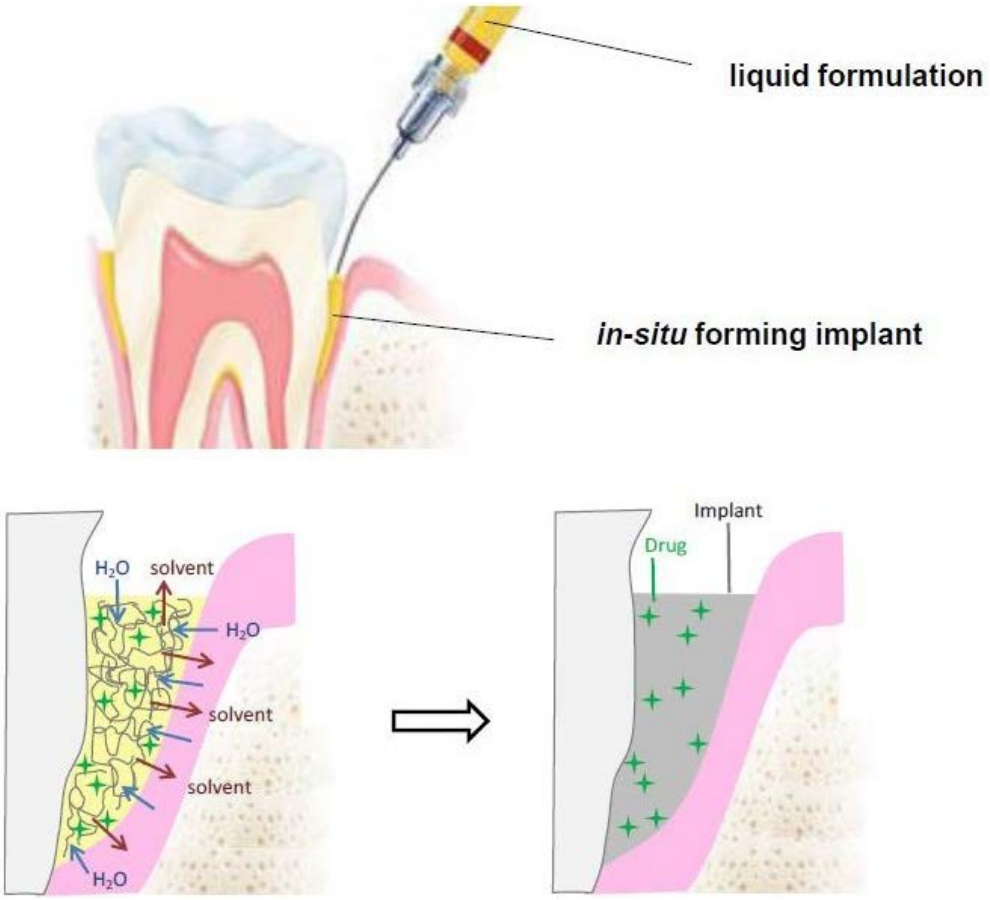


Figure 6: ISFI based upon solvent exchange mechanism. The liquid formulation is injected into the periodontal pocket after SRP. Then, the solvent contained in the formulation goes out as water takes its place, leading to polymer precipitation and implant formation. Once the implant is formed, the drug is entrapped in the polymeric matrix structure of the implant and is slowly released over time.

In-situ forming implants exhibit unique features that are unmet with any other system, but it creates new challenges. While some can be overcome, others are intrinsic to the system. The liquid formulation allows it to take any shape and geometry. It may in some cases be a difficulty to overcome as the shape and geometry will depend on a lot of factors, as seen in Figure 7 (103). However, it can be particularly interesting for periodontitis treatment. Indeed, whatever the size of the patient's periodontal pocket, the liquid formulation will fill the entire space available before hardening and stay in place, making this drug form a personalized medicine adaptable to the periodontal pocket of any patient.

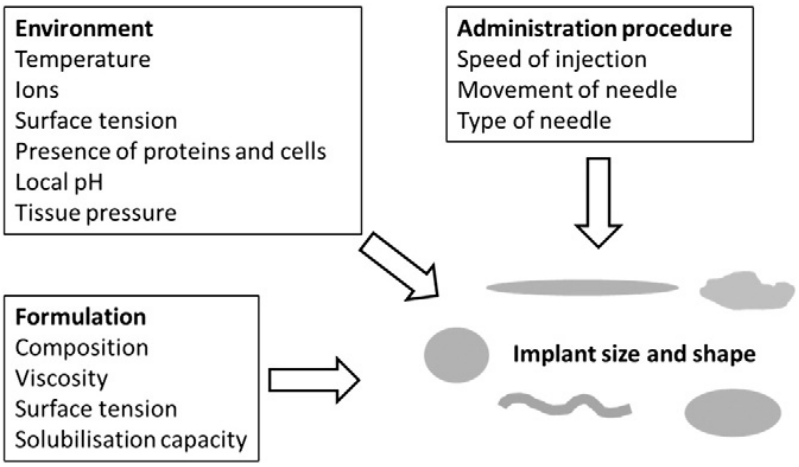


Figure 7: Factors affecting implant's size and shape (103).

It has to be noted that ISFI represents a very interesting and promising way to develop new dosage forms as it relies on already proven to be safe APIs. Indeed, since clinical testing of new products represents the higher cost in a medicine development and the higher risk of failure due to toxicity issues, ISFI are not deprived of interest. It can improve the added medical benefit and participate to the life cycle management of drugs.

1.3.2.1. Composition

1.3.2.1.1. Polymers

Polymers are particular chemical substances. Any molecule, whether natural or synthetic, that is made of repeated subunits (*i.e.*. monomers) is a polymer. They can be organic or not, composed of the same monomers or different ones (copolymers), of various lengths from very short to extremely long. With this definition, it is easy to understand that almost any properties can be found within these molecules. This versatility of applications is of major interest for biopolymers.

Though, polymers used in the pharmaceutical formulation field have to meet common characteristics, which is to be at least biocompatible, whether they are synthetics, natural or hemi-synthetics.

Indeed, a biomaterial is different than any other product even if it is meant to be ingested. This involves a lot of requirements to meet, like cytotoxicity assays (*in-vitro*) and tissue response assessment (*in-vivo*) in animal before human use. Indeed, cellular response might be different than tissue response. The polymer's surface interaction with one cell line doesn't show numerous other factors like the way proteins and saccharides may interact with the polymer's surface, particularly the spatial configuration these elements will take, thus affecting directly the immune response (104,105).

The following concept of biocompatibility proposed in 2008 by David F Williams might be the most suitable definition of the term:

"Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy" (104).

So far, several polymers have been well described and are currently largely used in pharmaceutical production. Some are not biodegradable, like EVA which is the polymer of Actisite®. This is partially the reason why the product failed a few years after its commercialisation in 1994 as the fibre was difficult to put in place and needed a second intervention to be removed.

Other polymers or copolymers are biodegradable. To mention only a few, polycaprolactone (PCL) and poly-(lactide-co-glycolide) (PLGA) might be the most extensively characterised and used in formulation for sustainable/controlled drug delivery systems. Their degradation products after hydrolysis are naturally occurring acids that are non-toxic and easily excreted via the urines.

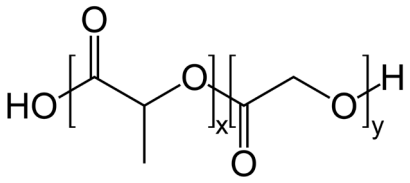


Figure 8: Chemical structure of PLGA (x : lactic acid; y: glycolic acid)

Various properties can be reached depending on the type of polymer used but also of its grade. For PLGA, three main parameters are important:

➔ The ratio between lactic and glycolic acid. As the lactic acid comprises one supplementary methyl group, it is more hydrophobic. It is also less sensitive to hydrolysis due to steric obstruction. Thus, the more lactic acid there is, the longer the polymer takes time to be degraded.

➔ The end capping. It is defined by the "grade" of PLGA by the manufacturing companies. In function of the ending groups of the chains (–H or –OH), the number of hydrogen bonds between the chains are different.

➔ The overall length of the chains, also called average molecular weight (Mw). This parameter is probably the most important (the longer chains there are, the denser the polymer is), but it is also directly linked to the two previous ones.

Other polymers can be way more complex, comprising different side chains or terminal residues. As an example, triblock copolymers can be randomly synthetized as they can be finely designed to achieve specific properties (AAA, BBB, CCC, AAB, BBC, ABC, etc…) thus leading to “infinity” of variations.

The major interest with synthetic polymers unlike natural ones is, beside their tuneable properties, the reproducibility of their fabrication as their chemistry is now well known and established (106). The following table gives us a few characteristics of PLGA and PCL that are commonly found in pharmaceutical formulations (107)

Polymers	Composition	Crystallinity	Glass transition temperature (°C)
Polyglycolide	100	Crystalline	35–40
Poly(L-lactide)	100	Crystalline	56–60
Poly(DL-lactide)	100	Amorphous	50–55
Poly(DL-lactide-*co*-glycolide)	85:15	Amorphous	50–55
	75:25		48–53
	65:35		45–50
	50:50		43–48
Poly(ϵ-caprolactone)	100	Crystalline	−65–(−60)
Poly(L-lactide-*co*-caprolactone)	85:15	Amorphous	20–25
	75:25		20–25

Table 3 : Properties of principal polymers used in ISFI formulation (107).

1.3.2.1.2. Solvents

Solvents used in ISFI must be able to solubilise the polymer and be biocompatible. This last point is the major concern arisen with these systems. Nevertheless, some are tolerated at certain doses given their low toxicity profile and good acceptance. The solvent choice is crucial as it determines the implant’s formation in the early phase just after injection. Indeed, a highly water-miscible solvent will increase the solvent exchange’s rate and lead to a faster implant formation. Thus, the internal structure may exhibit pores, affecting substantially the resulting drug release kinetics from the implant. By contrast, a hydrophobic solvent will slower the implant’s formation, leading to a denser matrix and a reduction of the potential pore formation. The following table list the most common solvents used for ISFI formulation based upon solvent exchange mechanism as well as their main characteristics (107).

Solvents	Water miscibility (mg/mL)	Viscosity (cP) at 20 °C	Classification	LD50 oral rat (mg/kg)
Glycofurol	miscible in all proportions[a]	8–18[a]	/	980[c]
DMSO	miscible[a]	2.19[d]	ICH class III[e]	14,500[c]
NMP	1000[b]	1.89[d]	ICH class II[e]	3914[c]
2P	1000[b]	14.66[d]	/	328[c]
TA	64[b]	19.7[d]	FDA GRAS[a]	3000[c]
BA	35[c]	5.81[d]	FDA IIG[a]	1 230[c]
BB	Insoluble[b]	8.67[d]	FDA IIG[a]	1 680[c]

NMP: *N*-methyl-2-pyrrolidone; DMSO: dimethyl sulfoxide; 2P: 2-pyrrolidone; TA: triacetin; BB: benzyl benzoate; BA: benzyl alcohol; GRAS: generally recognized as safe; IIG: inactive ingredients. LD50: lethal dose 50.

Table 4 : Main characteristics of mains polymers used in ISFI formulations (107).
Please note that exponent letters a,b,c and d in the table refer to specific references that can be found in the original article by Parent et al, PLGA in-situ implants formed by phase inversion: Critical physicochemical parameters to modulate drug release. J Controlled Release. 2013 (107).

The toxicity of the solvent is one of the major drawbacks for the use of ISFI. Indeed, even if the LD50 oral is low, it doesn't mean that an injection of the product will be harmless. Moreover, an ISFI is a matrix comprising a polymer solubilized in this particular solvent. Considering this, some studies have been conducted and the results are contradictory. As an example, one evaluates a good biocompatibility without necrosis or tissue damage when injecting NMP/PLGA and DMSO/PLGA solutions in rhesus monkey (108), as another exhibits no damage in rabbit (109). On the opposite, some studies shows an acute myotoxicity both *ex-vivo* and *in-vivo* by monitoring the creatine kinase (CK) efflux after injection of the ISFI (110,111). Thus, it is still a controversial subject and every study can be discussed. The first ones showing no acute toxicity did not monitor CK efflux but was based on histological studies, showing a very modest fibrosis at the injection's site with a few macrophages and neutrophils. The second ones, while monitoring finely the CK efflux, did not investigate the histological tissue response of the injection, thus it might be difficult to fully conclude. Indeed, an elevation of serum CK can be observed under physiological conditions (112).

However, some products are already on the market and it has to be pointed out that, like any other medicine, the benefit-risk ratio prevails over slight toxicity issues. It is important to keep in mind the detrimental effects of the disease when evaluating a medicine to treat it.

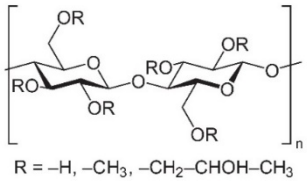
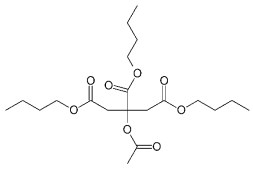
1.3.2.1.3. Additives

Given the desired properties of the implant, many additives can be added into the formulation. Their use can affect tremendously the resulting behaviour of the system. Their properties can be used for a fine tuning of the implant's resulting behaviour, especially concerning the initial burst effect (113). Like the solvents, toxicity of additives must be evaluated before use and their potential interaction with other components of the formulation anticipated.

Adding an additive must be evaluated on a case by case basis, considering the physicochemical properties of the other constituents of the formulation and the desired properties required. It has to be noted that the less ingredients there is in a formulation, the better it is: simplicity of formulation, cost efficiency, avoidance of potential interactions, etc… Nevertheless, this goal cannot be reached all the times and the use of additives might be necessary.

The following table shows an example of two additives that were used for a different purpose of just modifying the initial burst release.

Table 5: Examples of additives used in ISFI formulations

Additive used	IUPAC Name (Abbreviated name)	Properties
	HydroxyPropyl MethylCellulose (HPMC)	Hydrophilic polymer. Loading the implant with HPMC leads to a more porous internal structure and faster implant formation (114,115).
	AcetylTriButyl Citrate (ATBC)	Plasticizer. ATBC lowers the glass transition temperature (Tg) of the polymer and allows to have a softer, "chewing-gum" like texture.

Their choice is not due to hazard; they were investigated in previous studies for their effect on a particular formulation, hence the observed properties.

Finally, there are so many combinations possible between the polymer matrix, the solvent, the additives and the drug that almost every release rate modulation can be considered. The following table illustrates well that diversity of systems and possible adjustments of release rate (113).

Additive	Polymer/Solvent system	Drug	Amount of additive	Mean burst release (no additive)	Mean burst release (with additive)
Triacetin	PLGA/NMP	Aspirin	ND	36.9%	65%
PVP	PLGA/NMP	Chicken egg lysozyme	3%	ND	8 fold increase
Ethyl benzoate	PLGA/NMP	Leuprolide acetate	12.8%	14.50%	5.53%
PEG 400	PLGA/NMP	Aspirin	20%	36.9%	30%
Glycerol	PLGA/NMP	Naltrexone HCl	1%	67%	62%
			3%		61%
			5%		60%
Ethyl heptanoate	PLGA/NMP	Naltrexone HCl	1%	67%	62%
			3%		50%
			5%		44%
PEG 4000	PLLA/NMP	Heparin	5%	40%	5%
	PLLGA/NMP			20%	ND
Glyceryl monosterate	PLGA/BB and BA	Risperidone	2%	32.2%	4.7%
Steric acid	PLGA/BB and BA	Risperidone	2%	32.2%	23.4%
Zinc complexation	PLGA/NMP, triacetin, ethyl benzoate, BB	Human growth hormone	30 mM	ND	Reduction in all cases
Pluronic L101	PDLA/NMP	Lysozyme	5.4%	25%	18%
Pluronic L121	PDLA/NMP	Lysozyme	5.4%	25%	10%

ND—no details.

Table 6: Impact of several additives on the burst release from ISFI (113).

1.3.2.1.4. Active Pharmaceutical Ingredients

The properties of the APIs used are of great importance, as it will interact with the other components of the system and so affect the global characteristics of the implant. If the drug load is determined by the clinical application and the drug concentration needed, it is still however possible to choose between different drug salts. Therefore, given the high solubility differences between these, the resulting release kinetics can be modified.

1.3.2.1.4.1. *Chlorhexidine*

Chlorhexidine (Chx) is an antiseptic drug with a biguanide structure. Its mechanism of action is determined by the presence of two cationic charges, allowing the molecule to bind the negatively charged phospholipids of the bacterial membrane thus leading to its disruption. Different chemical salts exist showing different solubility in water. These include free base, Chx diacetate, Chx digluconate, and Chx diHCl. Chlorhexidine is a widely used broad-spectrum antimicrobial agent that inhibits bacterial growth and is commonly used in patients during periodontal treatment. In addition to its antibacterial activity, Chx is also effective against *Candida*.

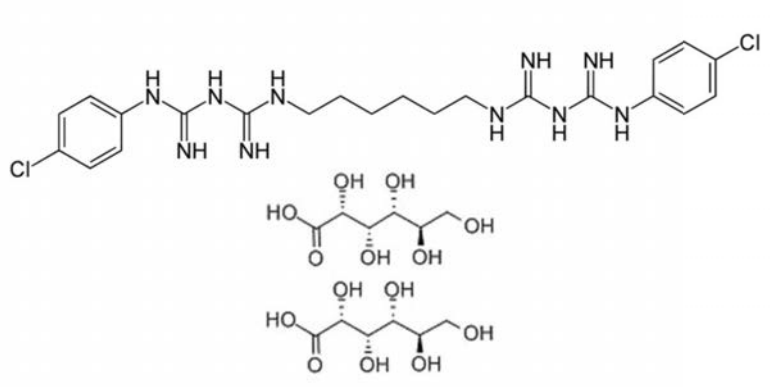


Figure 9: Chemical structure of Chlorhexidine digluconate (Top: Chx; Bottom: Gluconic acid)

Its effectiveness is also attributed by its remanent and cumulative activity: Chx is adsorbed on biological surfaces (teeth and oral mucosa). Moreover, due to its mechanism of action, no resistance mechanism is known for its activity so far. The presence of these two cationic charges is very important as PLGA is a negatively charged polymer. In addition, the hydrolysis of PLGA leads to the release of acids that exhibits negatives charges too. This is of particular importance as these charges will lead to interactions between the different components.

1.3.2.1.4.2. *Ibuprofen*

Ibuprofen (Ibu) is a non-steroidal anti-inflammatory drug (NSAID) that exposes an aryl carboxylic structure. Its mechanism of action rests on the inhibition of cyclo-oxygenase enzyme activity. As for Chx, Ibu exists in free form as in salt form, like Ibu Lysinate (Lys), showing different properties, particularly for their solubility (Ibuprofen is a week acid with a pKa of 4.4).

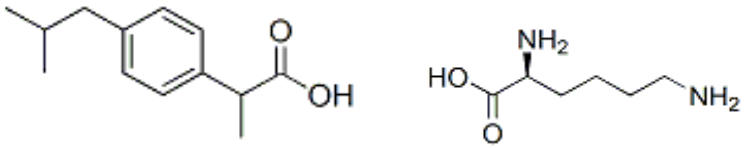


Figure 10: Chemical structure of Ibuprofen Lysinate (Left: Ibu; Right: Lysinic acid)

1.3.2.1.4.3. *CIN-102*

Cin-102 is *"a chemically well-defined, synergistic blend developed by Septeos. The composition of CIN-102 resembles the composition of cinnamon essential oils (Table 4). Its major component is cinnamaldehyde (86.7% w/w). CIN-102 has a broad-spectrum antibacterial activity and no resistance has been observed so far"* (116). At ambient temperature (approx. 20 to 25°C), it is an oily, viscous liquid.

In addition to the antibacterial activity of the blend, cinnamaldehyde has been shown to exhibits anti-inflammatory properties on LPS stimulated cells by increasing anti-inflammatory mediators and decreasing inflammatory mediators (117).

Table 7: Composition of CIN-102 (117).

Component	% (w/w)
Trans-cinnamaldehyde	86.7
Trans-2-methoxycinnamaldehyde	5.35
Cinnamyl acetate	2.5
Linalool	2.4
β-Caryophyllene	1.7
Cineol	1
Benzyl benzoate	0.35

1.3.2.2. Release mechanisms of API from ISFI

In-situ forming implants exhibit various and specific release profiles. Many parameters affect the drug release kinetics of these systems. If some can be controlled (e.g. polymer type and concentration, additives, etc…), the resulting kinetics will be considerably affected by the *in-vivo* environment of the implant. Various drug release profiles have been described and five trends emerge: biphasic release with a burst followed either by a fast release or a zero order kinetic; biphasic release without burst effect and triphasic release with a short or a long phase 2 release time (Figure 11). Depending on the type of application needed, every type of release can be interesting (118).

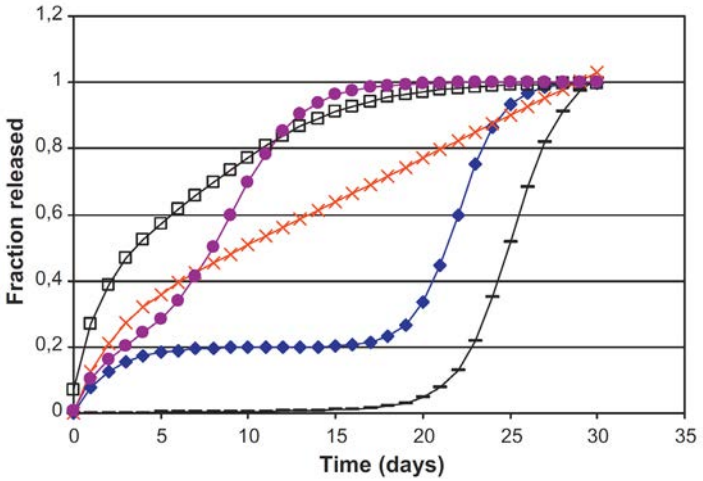


Figure 11: Release profiles consisting of different phases. Open squares: burst and a rapid phase II. Filled circles: tri-phasic release with a short phase II. Crosses: burst and zero-order release. Filled diamonds: tri-phasic release. Dashes: bi-phasic release, similar to tri-phasic but without the burst release (118).

Even if a zero order kinetic is preferred, it is rare and PLGA matrices are well known to mostly exhibit a triphasic release profile. The first burst effect can be explained by the drug's diffusion out of the matrix during the implant's formation. If the API is highly soluble in the organic solvent, a non-negligible fraction of it will diffuse as the solvent exchange mechanism occurs. Then, once the implant is formed, API will diffuse both by diffusion through the matrix and by erosion. Here, two mechanisms appear: bulk erosion and surface erosion. Depending on the internal structure of the implant once formed, one may be more pronounced than the other. However, this linear phase will finally lead to a final burst effect, were the average molecular weight of the polymer will be enough degraded so that cohesion will not be longer assured.

One of the biggest concerns with this kind of system is the obstacles faced to predict accurately long term kinetics. Due to the variability of the implant's shape and geometry that relies on various parameters like the speed of injection, the site of injection, the volume injected, etc… Indeed, models can be established only with numerous presuppositions (e.g. particular shape, known size, constant surface erosion without swelling…(119)). These ideal conditions are very unlikely to be met with ISFI.

If some of these variables can be overcome by designing well the drug release study, it may be quite distant from the *in-vivo* conditions and thus be unhelpful. Thus, once the *in-vitro* release study is designed to be relatively close to therapeutic conditions, its limits must be kept in mind when interpreting the results. It is indeed well known that many *in-vivo* factors affect significantly the polymer's degradation (e.g. macrophages, enzymes, pH…).

Also, with a solvent exchange based ISFI, an initial burst release is often observed, particularly with water miscible organic solvents (107). This phenomenon can lead to an increase in the drug release rate's variability, especially in the first few days of release. This is why obtaining a consistent formulation is a main goal in order to have a good reproducibility. The formulation must lead to a sort of "quality by device" resulting DDS. It is particularly true for long lasting devices (up to several months or even years) for which it is absolutely impossible to make exhaustive pre-clinical and clinical tests. In addition, this kind of device may not be removable depending of its application (e.g. intracerebral implant) in case of issues (allergy, side effects…) which can be a concern. Though, biocompatibility has been shown for these DDS.

Finally, it can be seen both as a limitation and as an opportunity: this type of device may be used only with already known and used APIs, therefore already "clinically fail-proof". It may represent both an opportunity in the life-cycle management of a drug as well as an improvement for the patients.

The following Figure illustrates well the complexity of an ISFI (118).

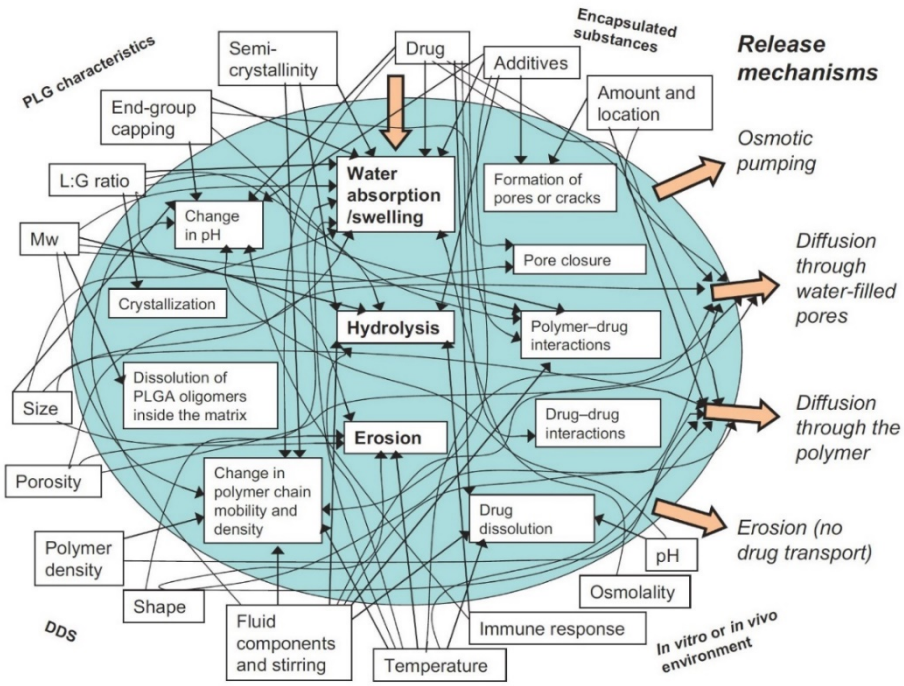


Figure 12 : The complex picture of the different factors that influence drug release from PLGA matrices. The effects of the properties of the DDS and the surrounding environment on the processes that, in turn, influence drug release are illustrated by arrows (118).

2. PURPOSE OF THIS WORK

Different types of local drug delivery systems have been proposed for the treatment of periodontitis; however most of them contain antibiotic drugs and/or exhibit insufficient adhesion to the walls of the periodontal pockets, combined with inappropriate mechanical properties. This leads to possible uncontrolled expulsion of the systems during the treatment and, thus, unreliable drug exposure to the target site. The main objective of this thesis was to develop innovative *in-situ* forming implants, for the treatment of periodontitis. This work was part of an interdisciplinary project IMPERIO (In-situ forming implants for periodontitis treatment) funded by the French National Research Agency (ANR) in collaboration with pharmacists, dentists, microbiologists, immunologists and physicists. The aim of my work was to develop innovative *in-situ* forming implants and to better understand the underlying drug release mechanisms in order to facilitate the optimization of such systems.

The *in-situ* forming implants to develop should ideally:

- ➔ be easy to inject (liquid formulations)
- ➔ readily spread within the patients' pockets and adapt their geometry and size to the individuals' needs
- ➔ exhibit reliable residence times
- ➔ exhibit dual controlled release of an antiseptic and an anti-inflammatory during optimized periods of time
- ➔ be biocompatible and biodegradable
- ➔ present appropriate mechanical properties "chewing-gum like" texture

Based on preliminary experiments in the laboratory and the literature, the following composition was selected for the *in-situ* forming implant (114,115,119–124) and previous knowledge from the literature a formulation of an *in-situ* forming implant has been developed comprising the following elements:

- ❖ **PLGA RG 502 H**: main polymer, composes the matrix structure of the implant.
- ❖ **HPMC:** additive, second polymer, hydrophilic, enhance the ISFI's adhesion.
- ❖ **ATBC**: plasticizer
- ❖ **Solvent**
- ❖ **APIs**

The innovation consists in the association of two APIs into the local controlled drug delivery system. Indeed, as far as we know, there is no existing DDS for periodontitis releasing simultaneously and in a controlled manner an antiseptic agent and an anti-inflammatory drug to treat the two component of periodontitis: the infection and the inflammation. Moreover, the use of an antiseptic instead of currently used antibiotics will help to fight bacterial resistances.

This work will focus on the impact of the drug salt used and the type of solvent used in the formulation as well as the type of release medium on the resulting drug release kinetics of the drug delivery system. To better understand the underlying drug release mechanisms, different parameters were monitored upon drug release: water uptake (gravimetrically), PLGA degradation (by GPC/SEC), change in the pH of the surrounding medium and macroscopic appearance of the implants

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Formulation of the ISFI.

Poly(D,L-lactic-co-glycolic acid) (PLGA, Resomer® RG 502 H; Evonik, Darmstadt, Germany); acetyltributyl citrate (ATBC) (Morflex®, Greensboro, NC, USA); hydroxypropyl methylcellulose (HPMC, Methocel® E50; Colorcon, Dartford, UK); N-methyl pyrrolidone (NMP, Fisher Scientific, Loughborough, UK); Chlorhexidine dihydrochloride and Chlorhexidine digluconate (Evonik, Hanau, Germany); Ibuprofen (free acid) and racemic ibuprofen lysinate (BASF, Ludwigshafen, Germany); CIN-102 (Septeos, Paris, France) (cf.. composition in table 4., components providing from Sigma Aldricht); dimethyl sulfoxide (reag. Ph. Eur. for analysis) (DMSO, PanReac Applichem, Spain); ethyl lactate (Alfa Aesar GmBH & Co KG, Karlsruhe, Germany); 2-pyrrolidone (Alfa Aesar, ThermoFisher (Kandel) GmbH, Germany).

3.1.2. Formulation of the artificial saliva.

Calcium chloride dehydrate p.a. (Acros Organics, Geel, Belgium); Yest extract (Oxoid, Basingtoke, UK); Bacto™ Beef extract desiccated (BD, Sparks, USA); BBL™ Gelysate™ peptone (BD, Sparks, USA), pancreatic digest of gelatin (BD, Sparks, USA), Potassium chloride RECTAPUR® crystallized (Prolabo, Paris, France), sodium chloride (J.T.Baker, Deventer, Netherland); Mucin from porcin stomach, Type III, bound sialic acid 0.5-1.5 %, partially purified powder and sterile urea solution (40% w/v in water) provide from Sigma Aldrich.

3.1.3. Analytical chemicals.

Tetrahydrofuran (THF, 99.99%, analytical reagent grade, stabilized with 0.025% butylhydroxytoluene); acetonitrile (HPLC grade) and orthophosphoric acid provide from Fisher Scientific, Loughborough, UK; sodium dihydrogen phosphate dihydrate (Emsure®, Reag. Ph. Eur. for analysis, Merck KGaA, Darmstadt, Germany); hydrochloric acid 32% solution in water (Acros Organics, Geel, Belgium); sodium hydroxide aqueous solution min. 30% (Rectapur®, Prolabo, Rhône-Poulenc LTD, Manchester, UK), triethylamine (for analysis) (Acros Organics, Geel, Belgium).

3.2. Methods

3.2.1. Preparation of the liquid formulations

PLGA (28% w/w, based on the total liquid formulation without drug) was dissolved in either NMP, DMSO, ethyl lactate or 2-pyrrolidone at 25 °C for 30 min under stirring in a glass vial. ATBC (10% w/w, based on the PLGA mass) and HPMC (10% w/w, based on the PLGA mass) were added and the mixture was vortexed for 3 min,

followed by standing for 3 h at 25 °C. Subsequently, the drugs were added (as indicated), and the mixtures were vortexed for 3 min.

The formulations were always loaded with two drugs: one antiseptic agent, either chlorhexidine or CIN-102, and one anti-inflammatory agent that was always ibuprofen. Drug salts were varied using chlorhexidine dihydrochloride or digluconate, and ibuprofen “free acid” or lysinate. The drugs loadings were always kept at the same ratio between antiseptic and anti-inflammatory of 1:1. Drug loadings were 1.5%, 5.3% or 16.1% for both active pharmaceutical ingredients (w/w, based on the *in-situ* implant formulation without solvent = “theoretical” implant composition), as indicated. The formulations were stored at 2-6 °C and protected from light to avoid drug degradation prior to use.

3.2.2. Preparation of the artificial saliva

One liter of reverse osmosis water was heated at approximately 90°C to facilitate the dissolution of components. 2,5g of porcine mucin, 1g of beef extract, 2g of yeast extract, 5g of peptone gelysate, 0.2g of sodium chloride, 0.3g of calcium chloride and 0.2g of potassium chloride were added to the water and stirred manually to complete dissolution. The resulting solution was put in an autoclave for sterilization for 15min at 121°C. Finally, 1.25mL of urea (sterile 40% solution in water) was added and the resulting solution was kept at <6°C.

3.2.3. In situ implant formation

One hundred microliter of the respective formulation were injected at the bottom of a two mL Eppendorf vial using a standard syringe. One milliliter preheated (37 °C) deionized water was carefully added using a pipette, initiating solvent exchange and implant formation. The vials were horizontally shaken at 37 °C at 80 rpm for 40 days (section *4.1*. Impact of the drug form) or 30 days (sections *4.2*. Impact of the solvent and *4.3*. Impact of the release medium) (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel Germany).

3.2.4. Drug release measurements

At determined time points, the release medium was completely renewed. The drug amounts in the withdrawn bulk fluids were determined by HPLC-UV (Waters Alliance 2695 separation module, Waters 2489 UV/vis detector) as follows: 20 µL samples were injected into an A C18 RP column (Gemini 5 µm C18 110 Å, 100mm x 4.6 mm; Phenomenex, Le Pecq, France). The flow rate was 1.5 mL/min. The following gradient method was applied: Eluent A was a 70:30 mixture of an aqueous solution of 115 mM Na_2HPO_4 containing 0.5% triethylamine (adjusted to pH 2.5 with H_3PO_4) and 30% acetonitrile. Eluent B was acetonitrile. The composition of the mobile phase was as follows: 4 min Eluent A, followed by a blend A:B (55:45) for

6 min. The retention times for chlorhexidine and ibuprofen were approximately 2.5 and 6.9 min, respectively. The column was kept at room temperature and the detection wavelength was λ = 239 nm. The calibration curve was linear (R2 > 0.999) in the range of 1 to 100 µg/mL for chlorhexidine and in the range of 1 to 50 µg/mL for ibuprofen.

Each experiment was conducted in triplicate, the results are presented as mean values ± standard deviation. In the case of ibuprofen (free acid and lysinate), the theoretical drug loadings were considered as “100% reference” values. In the case of chlorhexidine (dihydrochloride and digluconate), the amounts of drug remaining within the implant remnants at the end of the observation period were determined as follows: the remnants were freeze-dried for 3 d (Christ Epsilon 2–4 LSC; Martin Christ, Osterode, Germany) and the lyophilisates were subsequently dissolved in acetonitrile. The solutions were filtered using 0.45 µm PVDF filter syringes (GE Healthcare UK limited, Buckinghamshire, UK), and analyzed for their drug content by HPLC-UV (as described above). The sum of released and remaining chlorhexidine at the end of the observation period was about 100% in all cases. In the case of CIN-102, HPLC measurements were conducted on cinnamaldehyde content, as the major component of the blend (86.7% w/w). The calibration curves were obtained using trans-cinnamaldehyde. All experiments were conducted in triplicate.

In addition, the pH of the release medium was measured at pre-determined time points using a pH meter (InoLab pH Level 1; WTW, Weilheim, Germany) (n=3). Mean values ± standard deviation are reported.

3.2.5. Dynamic mass change monitoring

Implants were prepared and treated as described in Sections *2.2.* and *2.*4. At pre-determined time points, implant samples were withdrawn, carefully blotted with precision wipes (Kimberly-Clark, Rouen, France) to remove liquid at the surface, and accurately weighed [mass (t)]. The mass change % at time *t* was calculated as follows:

$$Mass\ change\ (\%)(t) = 100 \cdot \frac{mass\ (t) - mass\ (t = 0)}{mass\ (t = 0)} \qquad (1)$$

where mass (*t* = 0) is the weight of the liquid formulation used for implant preparation. Each experiment was conducted in triplicate. Mean values +/- standard deviations are reported.

3.2.6. Drugs solubility measurements

3.2.6.1. <u>In water</u>

Solubility measurement of Ibuprofen (free drug and Lysinate) were conducted in the pH-range of pH 2.0 to 7.0 at 37°C (n=3). A large excess of solid drug was added to 1.5mL of deionized water into 2mL Eppendorf tubes. The pH was modified with 0.1N

or 1N HCl or 0.1N, 1N or 3N NaOH aqueous solution and the samples were horizontally shaken for 1 week at 37°C (80rpm). After equilibrium was reached, the samples were filtered (0.2 µm PVDF filter, GE Healthcare UK limited, Buckinghamshire, UK).

Solubility measurement of chlorhexidine (diHCl and digluconate) were conducted in the pH range of 2.0 to 8.0 at 37°C (n=3). A large excess of solid drug was added to 1.5mL of deionized water into 2mL Eppendorf tubes. The pH was modified with 0.1N or 1N HCl or 0.1N, 1N or 3N NaOH aqueous solution and the samples were horizontally shaken for 72h at 37°C (80rpm). After equilibrium was reached, the samples were filtered (0.2 µm PVDF filter, GE Healthcare UK limited, Buckinghamshire, UK).

The drugs concentrations were determined using HPLC-UV and the final pH value was measured. Each experiment was conducted in triplicate.

3.2.6.2. In NMP

Solubility measurement of Ibuprofen (free drug and Lysinate) were conducted at 37°C (n=3). A large excess of solid drug was added to 1.5mL of NMP and the samples were horizontally shaken for 1 week at 37°C (80rpm). After equilibrium was reached, the samples were centrifuged at 10 000 rpm for 2min (Centrifuge Universal 320; Hettich, Tuttlingen, Germany) and 100 µL of the supernatant were carefully removed and diluted.

Solubility measurement of Chlorhexidine (diHCl and digluconate) were conducted at 37°C (n=3). A large excess of solid drug was added to 1.5mL of NMP and the samples were horizontally shaken for 72h at 37°C (80rpm). After equilibrium was reached, the samples were filtered using 0.45µm PTFE filters.

The drugs concentrations were determined using HPLC-UV. Each experiment was conducted in triplicate.

3.2.7. Determination of polymer molecular weight

Implants were prepared and treated as described in Sections *2.2.1* and *2.2.4*. At pre-determined time points, implant samples were withdrawn, freeze-dried for 3 days (Christ Epsilon 2–4 LSC) and the lyophilisates were dissolved in tetrahydrofuran (at a concentration of 3 mg/mL). The average polymer molecular weight (Mw) of the PLGA was determined by Gel Permeation Chromatography (GPC, Separation Modules e2695 and e2695D, 2419 RI Detector, Empower GPC software; Waters, Guyancourt, France) using a PLGel 5 µm MIXED-D column kept at 40 °C (7.5×300 mm; Agilent Technologies, Interchim, Montluçon, France). The injection volume was 50 µL. Tetrahydrofuran was the mobile phase (flow rate: 1 mL/min). Polystyrene standards with molecular weights between 1,090 and 70,950 Da (Polymer Labaratories, Varian, Les Ulis, France) were used to prepare the calibration curve.

All experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

3.2.8. DSC measurements

Differential Scanning Calorimetry (DSC) thermograms of PLGA: ibuprofen blends were recorded with a DSC1 Star System (Mettler Toledo, Greifensee, Switzerland). The blends were obtained upon dissolution of appropriate amounts of PLGA and ibuprofen in acetone (12.5 w/v total solids content), followed by evaporation of the solvent. The ibuprofen content of the samples was varied from 0 to 25 % (w/w). Approximately 5 mg samples were heated in pierced aluminum pans from -70 °C to 120°C, cooled to -70 °C and reheated to 120 °C at a rate of 10 °C/min. The indicated glass temperatures (Tgs) were obtained from the second heating cycles. Each experiment was conducted in triplicate. Mean values +/- standard deviations are reported.

4. RESULTS AND DISCUSSION

4.1. Investigation of the drug form impact

4.1.1. Global observations

Figure 14 shows the simultaneous release of the antiseptic drug chlorhexidine and the anti-inflammatory drug ibuprofen from the investigated *in-situ* forming PLGA implants upon exposure to water. The initial drug loadings were varied as follows: 1.5% / 1.5% (squares), 5.3% / 5.3% (crosses) and 16.1% / 16.1% (circles) (referring to the *in-situ* forming implant formulations without NMP = “theoretical” implant masses). Different drug forms were combined: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate. Importantly, these drug forms exhibit substantially different solubilities in water and NMP (Table 8: chlorhexidine dihydrochloride is much less soluble in water and NMP than chlorhexidine digluconate, whereas ibuprofen free acid is much more soluble than ibuprofen lysinate in NMP, while the opposite is true in water).

Table 8:Solubility of the investigated drugs (in mg/mL) in water or NMP at 37 °C (mean values ± standard deviations, n = 3). The indicated pH values were measured at equilibrium.

Drug	Water	NMP
chlorhexidine dihydrochloride	0.91 (± 0.07) (pH=6.8)	7.02 (± 0.35)
chlorhexidine digluconate	> 200 (pH=6.0)	> 80
ibuprofen free acid	0.093 (± 0.002) (pH=3.9)	> 450
ibuprofen lysinate	> 500 (pH=7.9)	1.55 (± 0.04)

The blue curves in Figure 14 illustrate chlorhexidine release, the black curves ibuprofen release.

Some observations can be made:

- The investigated *in-situ* forming implants are able to control the release of both drugs simultaneously over several weeks.
- Increasing initial drug contents generally lead to decreasing relative ibuprofen release rates, and increasing chlorhexidine release rates, irrespective of the drug form and drug combination (arrows in Figure 14).
- Ibuprofen release is generally slower than chlorhexidine release.

- The release of ibuprofen is not fundamentally affected by the drug form and drug combination, despite the substantial differences in solubility (e.g. factor > 5,000 in water, Table 8).
- The impact of the drug form and drug combination on chlorhexidine release is overall moderate, despite the substantial differences in drug solubility (e.g. factor > 10 in NMP, factor > 200 in water, Table 8).

To better understand the – at a first glance – rather surprising observations in the light of the tremendous differences in drug form solubility, the systems were characterized in more detail. First, ibuprofen release is discussed in more detail, followed by chlorhexidine release.

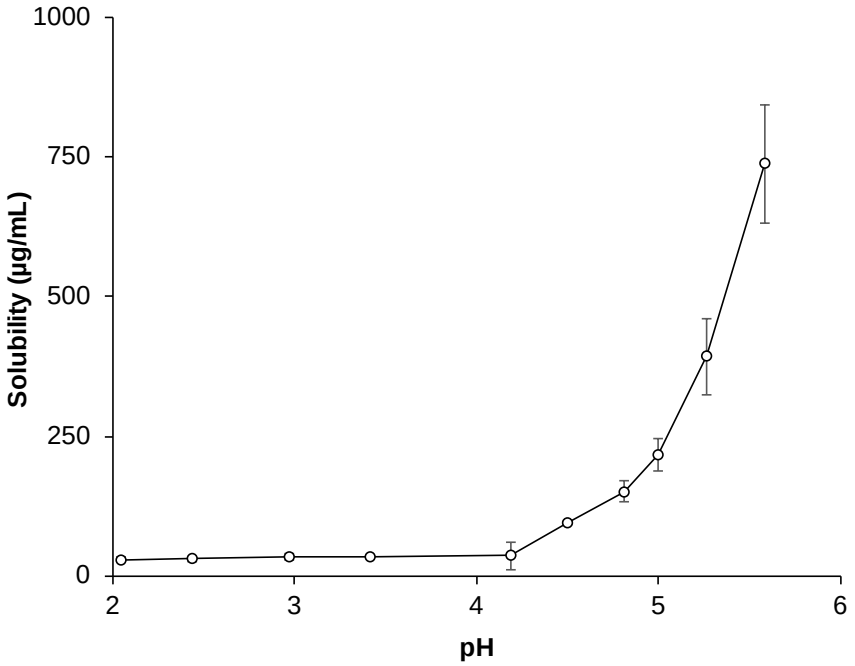


Figure 13 : pH dependence of the solubility of ibuprofen (free acid) in water at 37°C (the pH was adjusted with HCl or NaOH).

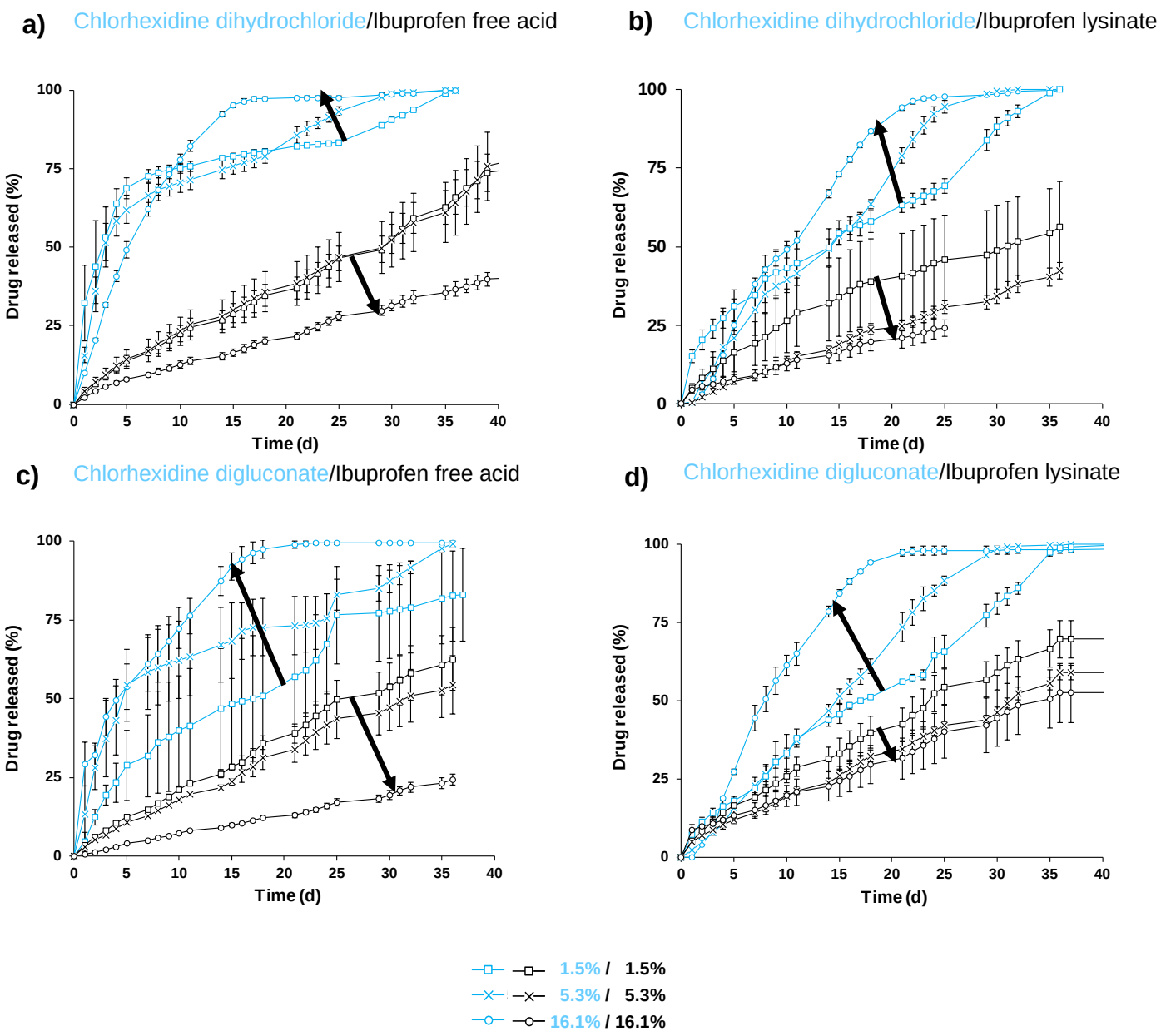


Figure 14: Dual drug release from in-situ forming implants loaded with: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. The blue curves show chlorhexidine release, the black curves ibuprofen release.

4.1.2. Ibuprofen release

Figure 16 illustrates the dynamic changes in the pH of the surrounding bulk fluid during drug release from *in-situ* forming implants, loaded with: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate, respectively. Again, the initial drug content was varied: 1.5% / 1.5%, 5.3% / 5.3% and 16.1% / 16.1%. One hundred µL formulation were exposed to 1 mL water (37 °C). At each measurement time point, the bulk fluid was completely renewed. As it can be seen, in all cases the bulk fluid became rapidly acidic and remained acidic throughout the observation period (roughly in the range of pH 2.5 - 4). This is due to the acidic degradation products of PLGA, which leach out into the surrounding release medium, and the absence of a buffer in the bulk fluid. In vivo, gingival fluid contains bicarbonates (125) and also urea, even if the reason for its which exhibits a pKa of 4.4 (126): below pH 4, ibuprofen's solubility is extremely poor, whereas it is much more soluble at higher pH values. Therefore, the first hypothesis would be that saturation effects in the surrounding fluid play an important role on the release rate of implants loaded with ibuprofen free acid. Importantly, ibuprofen lysinate is freely soluble in water (Table 8) and the resulting release rates from implants containing either ibuprofen free acid or lysinate were not radically different (Figure 14: diagrams on the right vs diagrams on the left). Moreover, when acidifying a highly concentrated ibuprofen lysinate solution with lactic acid to pH approx. 2-3, no precipitation of the drug occurs. This suggests that saturation effects in the surrounding bulk fluid does not play a dominant role.

As illustrated in Figure 13, the pH of the release medium is decisive for the solubility of ibuprofen: below pH 4, the solubility is very limited, whereas at higher pH values, ibuprofen becomes ionized and much more soluble (the pKa of ibuprofen is 4.4). Thus, it might be hypothesized that in the case of ibuprofen free acid containing implants, saturation effects within the bulk fluid play an important role (Figures 16 and 13). However, ibuprofen lysinate is freely water-soluble and the resulting ibuprofen release kinetics from ibuprofen free acid and ibuprofen lysinate containing implants were not fundamentally different (diagrams on the right hand side of Figure 14 vs. diagrams on the left hand side). Please note that even if a highly concentrated ibuprofen lysinate solution is acidified with lactic acid to pH 2-3, the drug does not precipitate. So, limited drug solubility effects in the surrounding bulk fluid cannot be dominant in all cases. In contrast, limited ibuprofen solubility within the implants might be of importance, since the relative ibuprofen release rate monotonically decreased with increasing drug loading, irrespective of the type of system (Figure 14, black curves). This might indicate that at least at higher initial drug loadings not all of the drug is dissolved and dissolved and non-dissolved drug co-exist. Importantly, only dissolved drug is available for diffusion. Thus, increasing the initial drug loading does not increase the concentration gradient of dissolved drug (being the driving force for

diffusion), but increases the 100% reference value, resulting in decreasing relative drug release rates.

The fact that ibuprofen release was rather similar from implants loaded with ibuprofen free acid and ibuprofen lysinate, despite the tremendous differences in drug solubility in water and NMP (Table 8), can serve as an indication for the fact that also other phenomena likely play a major role for the control of the release of this drug (in addition to limited solubility effects within the implants at high drug loading). Certain drugs have been reported to be efficient plasticizers for PLGA. As an example, it has been reported that that the addition of about 25% ketoprofen decreased the glass transition temperature (Tg) of PLGA-based microparticles from about 48 °C to only about 25 °C. At even higher drug loadings, the Tg remained unchanged, indicating that the polymer matrix was saturated with the drug (127). As it can be seen in Figure 15, also ibuprofen acts as a plasticizer for PLGA: The glass transition temperature of the polymer decreases from about 42 °C to about 30 °C upon addition of 10 % ibuprofen. At higher drug contents, a plateau is observed. This suggests that in implants loaded with 1.5 or 5.3 % ibuprofen, the drug might be completely dissolved in the PLGA, but in implants loaded with 16.1 % drug, one portion of the ibuprofen is dissolved in the polymer, while the other portion is dispersed in the form of solid ibuprofen particles. The decrease in the glass transition temperature of the PLGA upon addition of ibuprofen indicates significant drug-polymer interactions. These interactions might (at least partially) explain the rather similar ibuprofen release kinetics from the investigated *in-situ* forming implants for the different drug forms exhibiting substantially different solubilities: The affinity of the ibuprofen to the PLGA might dominate and - to a large extent - control drug release. Above 10 % drug loading the PLGA is likely saturated and limited drug solubility effects *within* the polymeric matrix can also be expected to be of importance.

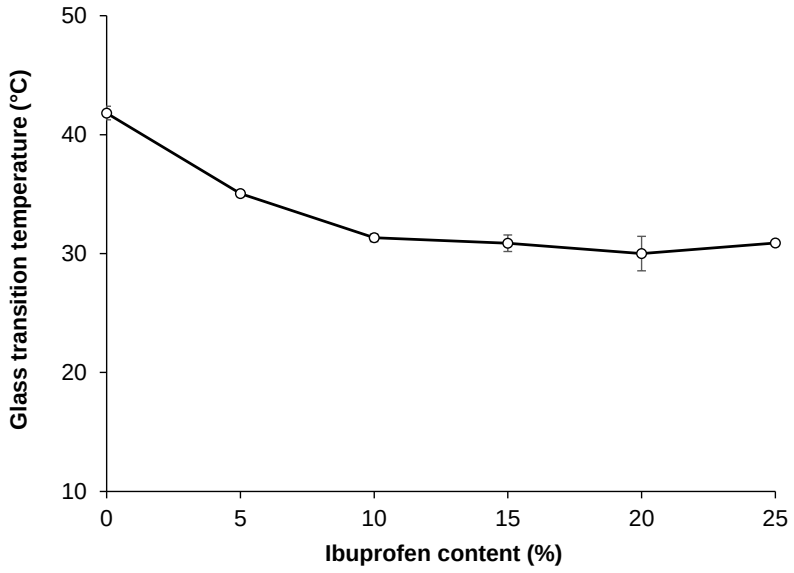


Figure 15: Dependence of the glass transition temperature (Tg) of PLGA:ibuprofen blends (prepared by solvent evaporation) on the drug content.

Importantly, there was no clear correlation between the dynamic changes in the implants' wet mass as a function of time and ibuprofen release (Figure 17 vs. Figure 14). For example, Figure 17a shows that the wet mass of *in-situ* formed implants loaded with 1.5% / 1.5% chlorhexidine dihydrochloride / ibuprofen free acid substantially increased during the first few days, whereas systems with an initial drug loading of 5.3% / 5.3% and 16.1% / 16.1% lost wet mass in this period (and this to a rather similar extend and at a rather similar rate). In contrast, Figure 14 shows rather similar release rates for ibuprofen from these systems at 1.5% / 1.5% and 5.3% / 5.3% during the first few days, and somewhat slower drug release from implants with an initial drug loading of 16.1% / 16.1%. Thus, major differences in wet mass changes do not correlate with ibuprofen release. Also, in the case of chlorhexidine digluconate / ibuprofen lysinate loaded implants, the wet mass changes were rather similar at 1.5% / 1.5% and 5.3% / 5.3% initial drug loading, whereas 16.1% / 16.1% drug loaded systems showed different wet mass changes. This does not correspond to the observed rather similar ibuprofen release kinetics observed at 5.3% / 5.3% and 16.1% / 16.1% initial drug loading, and the somewhat faster drug release at 1.5% / 1.5% drug loading.

The dynamic changes in the wet mass of the *in-situ* forming implants (Figure 17) are reflected in the macroscopic pictures taken at different time points upon exposure to the release medium (Figure 19) (please note that the sampling time points are different in Figures 17 and 19).

Thus, for ibuprofen release from the investigated *in-situ* forming PLGA implants ibuprofen-PLGA interactions seem to play an important role, and – at high drug loadings – also limited drug solubility effects *within* the implants.

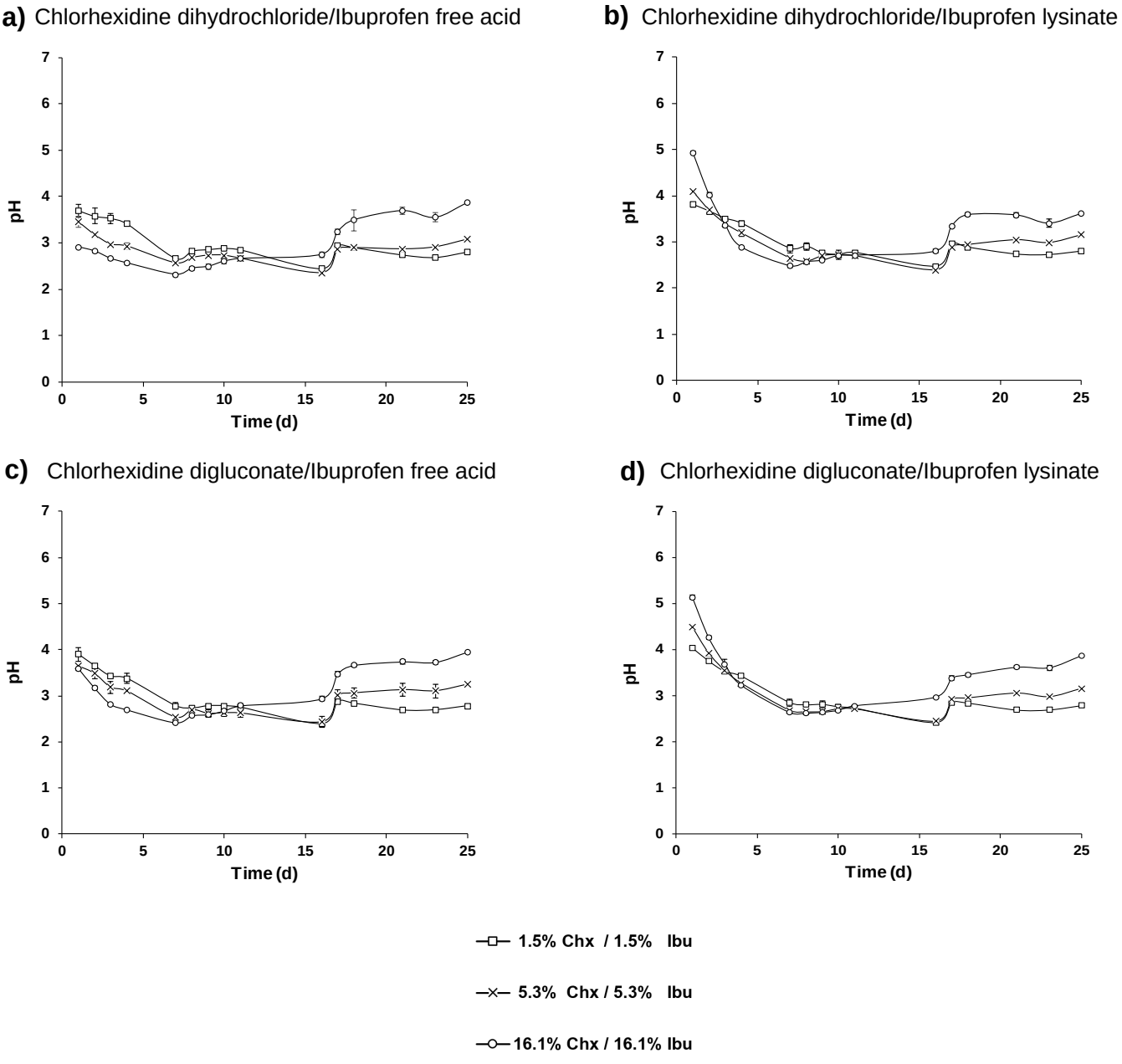


Figure 16: Dynamic changes in the pH of the bulk fluid (water) during drug release from in-situ forming implants loaded with: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated.

Figure 18 shows the decrease in the average molecular weight of PLGA from ISFI upon exposure to the release medium (water, 37°C) in function of time. It is clear that the polymer degradation is faster at higher drug loading, for every combination of drug used. Moreover, the presence of ibuprofen free acid is associated with a faster PLGA degradation than ibuprofen lysinate (Figure 18, left hand vs right hand). This is in good agreement with previous literature reports on other acidic drugs, like ketoprofen and aspirin. Indeed, ester bonds of PLGA are well described to be hydrolyzed by protons. This reaction is even auto-catalyzed (124).

Figure 17 shows the change in the implant's wet mas upon exposure to the release medium over time. Importantly, no correlation could be made between ibuprofen release and variation of ISFI's wet mass (Figure 17 vs Figure 14). For example, Figure 17 a shows that ISFI's wet mass loaded with 1.5% Chx diHCl / 1.5% Ibu increased during the first days, whereas systems loaded with 5.3% and 16.1% lost mass during the same period. Comparing these differences in mass change with the ibuprofen release, which is similar for 1.5% and 5.3% drug loading whereas slower with 16.1% indicates that substantial differences of wet mass changes are not correlated with variations of ibuprofen release. Also, Figure 14 d shows that ibuprofen release from ISFI loaded with 1.5% Chx diglu / 1.5% Ibu Lys is somewhat faster that the higher drug loadings. This does not correlate with Figure 17 d, where ISFI's wet mass of 1.5% and 5.3% are not substantially different compared to 16.1%.

Finally, the ISFI's wet masses shown in Figure 17 are all in good agreement with the macroscopic pictures shown in Figure 19 (please note that the sampling times are different between Figures 17 and 19). As it can be seen (Figure 19 a), ISFI loaded with both hydrophobic drug salts are less prone to swell. However, the swelling occurs during the first initial days. After one week, almost every implant exhibits a negative mass comparing to the initial implant's mass. This limited swelling is of major importance given the application. Too much swelling of the implant is likely to cause its expulsion out of the periodontal pocket.

Therefore, two phenomena are likely to play a major role on the ibuprofen release from the *in-situ* forming PLGA implants. The first one seems to be polymer-drug interactions. This one is very likely to be predominant, as ibuprofen is very hydrophobic, as well as PLGA. The second phenomenon is the solubility of ibuprofen salts, both in water and ISFI's solvents, this one being even more important at higher drug loadings. It is also important to note that these two phenomena are occurring both at the same time. Thus, it is particularly difficult to predict which one might be predominant over the other, at a certain release time for a given concentration and for a particular API. As an example, it can be supposed that if there were zero drug-polymer interaction, Ibu Lys would be released faster than Ibu free acid. But this statement is still a hypothesis, as lysine-PLGA interactions are not elucidated.

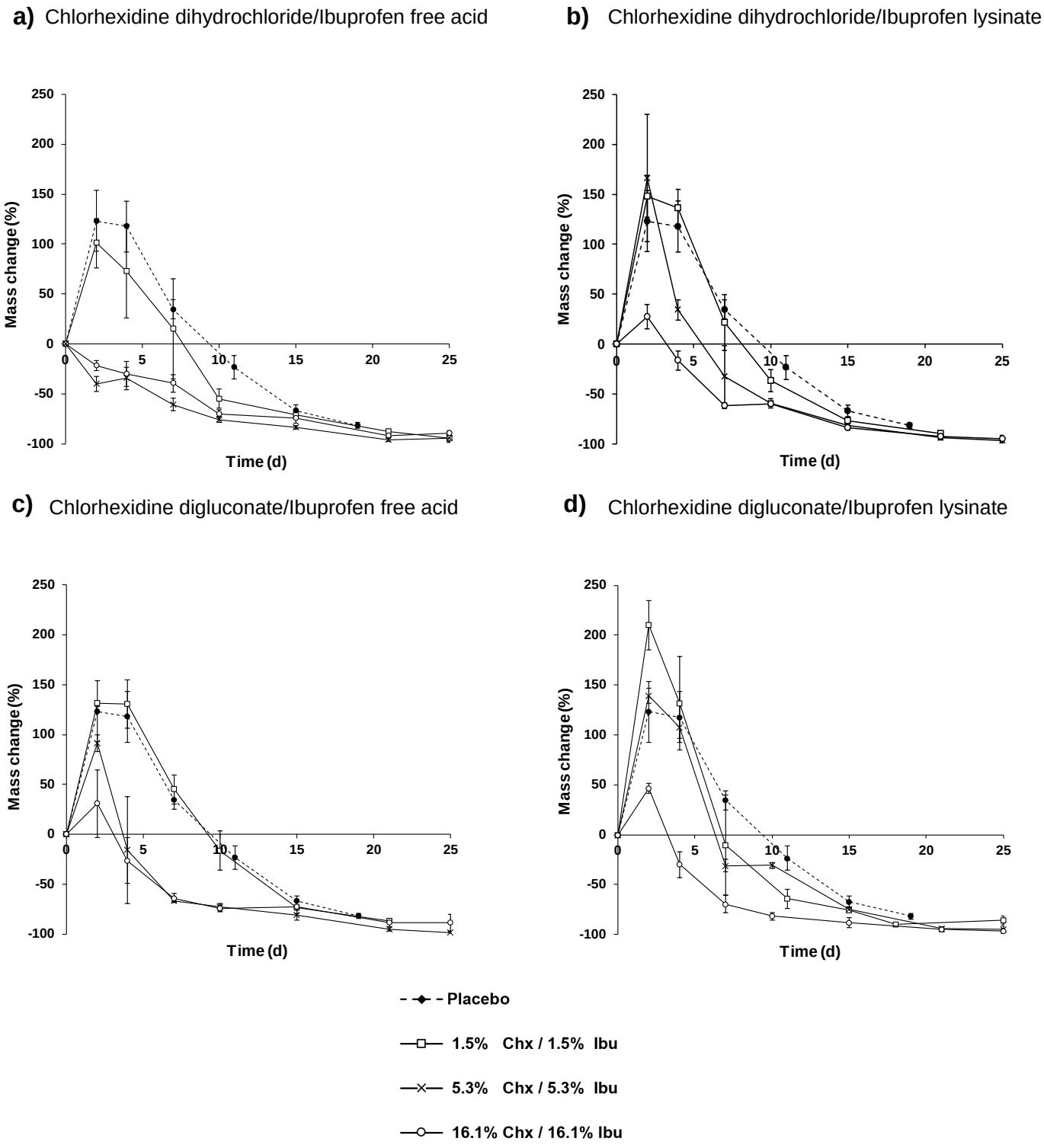


Figure 17: Dynamic changes in the (wet) mass of the in-situ forming implants during drug release. The systems were loaded with: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug-free implants were studied.

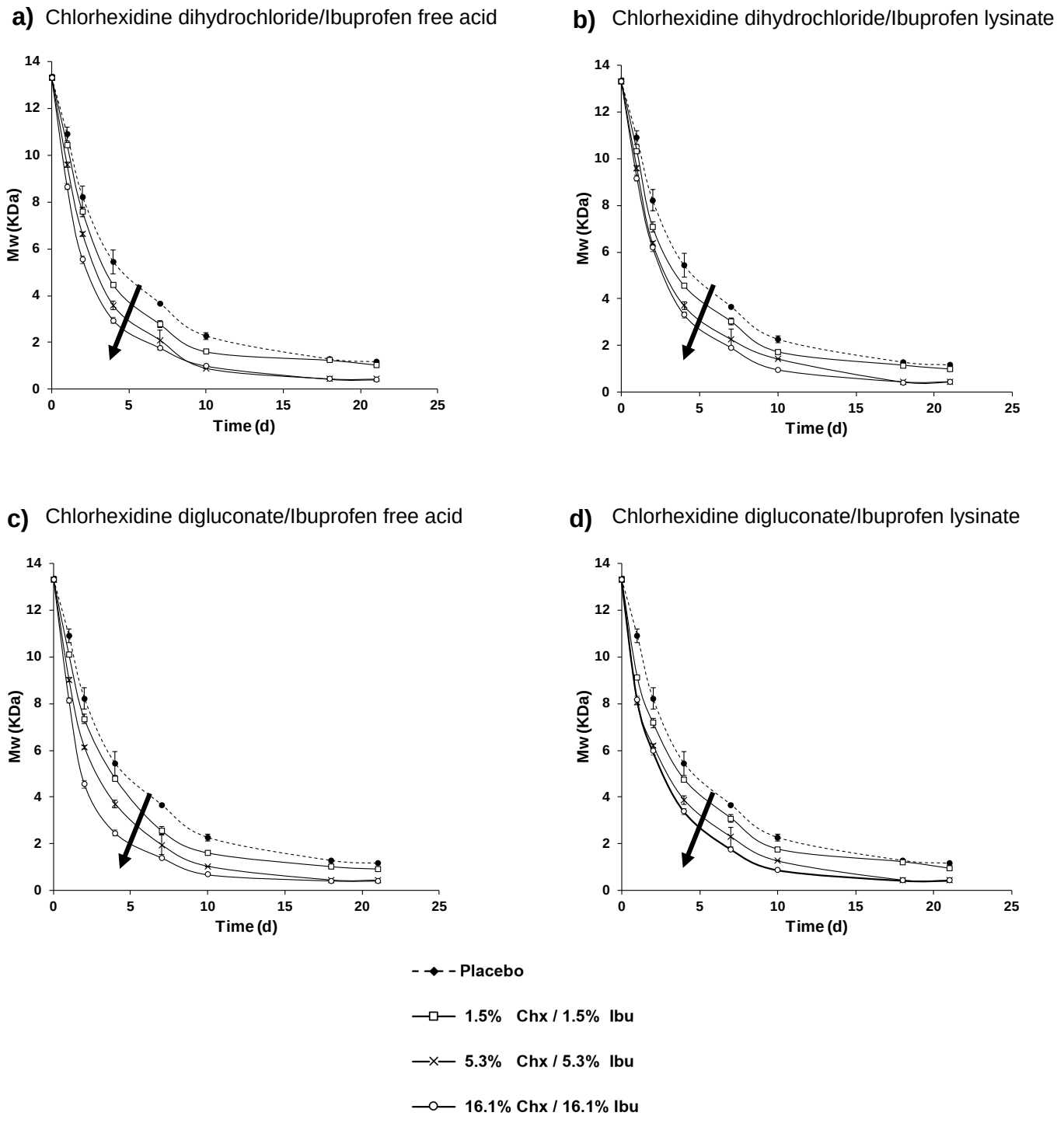


Figure 18 : Dynamic changes in the average polymer molecular weight (Mw) of the PLGA in the in-situ formed implants during drug release. The systems were loaded with: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug-free implants were studied.

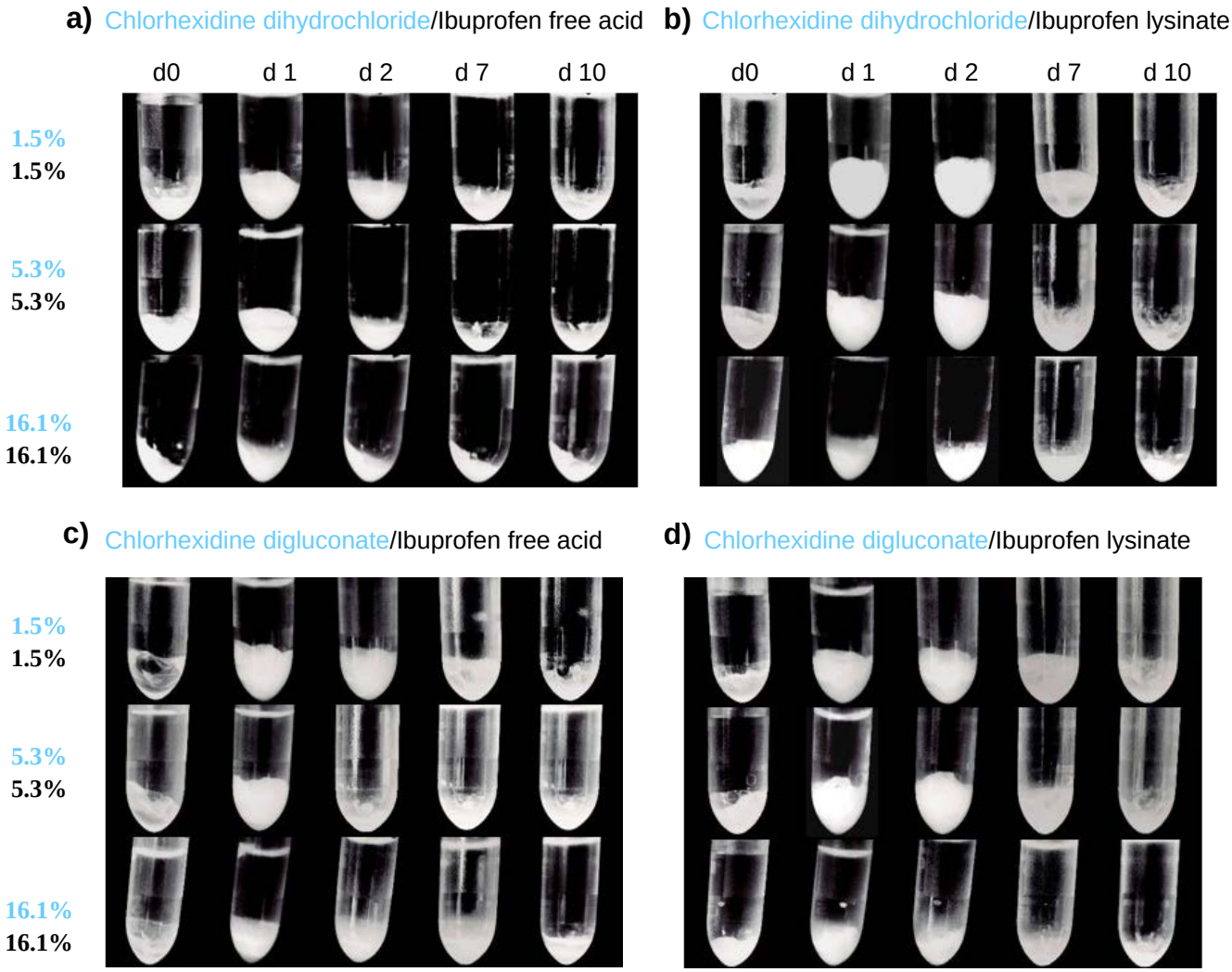


Figure 19 : Macroscopic pictures of in-situ forming implants loaded with: a) chlorhexidine dihydrochloride and ibuprofen free acid, b) chlorhexidine dihydrochloride and ibuprofen lysinate, c) chlorhexidine digluconate and ibuprofen free acid, and d) chlorhexidine digluconate and ibuprofen lysinate, after different time points upon exposure to water (37 °). The initial drug concentrations were varied as indicated.

4.1.3. Chlorhexidine release

In the case of chlorhexidine, drug release was overall somewhat faster from implants loaded with chlorhexidine dihydrochloride and ibuprofen free acid compared to the other systems (Figure 14a vs. Figures 14b, 14c and 14d). This is quite surprising, since the solubility of chlorhexidine dihydrochloride in water and NMP was much lower than the solubility of chlorhexidine digluconate (factor > 200 and > 10, Table 8). This cannot be explained by differences in the dynamic changes in the systems' wet mass over time (Figure 17a vs. Figures 17b, 17c and 17d). Also the degradation kinetics of PLGA in the different implants do not offer an explanation for this observation (Figure 18).

Comparing ibuprofen release and chlorhexidine release from the different types of *in-situ* forming PLGA implants, it becomes obvious that the effect of increasing initial drug loadings on the resulting relative drug release rates is inverse: In the case of chlorhexidine, the release rate increases when increasing the initial drug loading from 1.5% / 1.5% to 16.1% / 16.1% (Figure 14). Thus, limited drug solubility effects within the implants are unlikely in the case of chlorhexidine. In contrast to ibuprofen an increased porosity effect upon drug exhaust seems to dominate. With increasing initial drug loading, the remaining polymer network becomes less dense with time (released drug is essentially replaced by water), facilitating the release of the remaining drug. In all cases, the pH of the surrounding bulk fluid became rapidly acidic and remained acidic throughout the observation period (Figure 16), and PLGA degradation was accelerated at higher drug loadings (Figure 18, due to the acidity of the drugs, as explained above). As in the case of ibuprofen, the dynamic changes in the implants' wet mass do not correlate with the observed drug release kinetics (Figure 17). For instance, during the first 5 days, substantial differences in the wet mass profiles of implants differing in the initial drug loading were observed in the case of implants loaded with chlorhexidine digluconate and ibuprofen lysinate, whereas the respective drug release profiles in this time period were rather similar (Figure 17d vs. Figure 14d).

Thus, also in the case of chlorhexidine the tremendous differences in the solubilities of the digluconate and dihydrochloride did not translate into substantial differences in drug release (even in contrast: higher drug release rates were observed with the much less soluble dihydrochloride compared to the more soluble digluconate, when combined with ibuprofen free acid). The exact reasons for this behavior are not clear. However, in contrast to ibuprofen, limited drug solubility effects within the *in-situ* formed PLGA implants do not seem to play a major role.

4.2. Investigation of the solvent's impact

4.2.1. Global observations

The second part of the characterization of the ISFI was to evaluate the impact of the solvent in the liquid formulation of the implant on the resulting properties of the implant after solvent exchange. Solvents were selected according to their water solubility as well as their safety/toxicity profile (see section 1.4.2.1.2.). Four highly water miscible solvents were investigated: N-methyl pyrrolidone (NMP), dimethylsulfoxyde (DMSO), 2-pyrrolidone (2-Pyr) and ethyl lactate (Eth Lact). NMP, 2-Pyr and DMSO were chosen according to their already extensive use in ISFI formulations (107). Ethyl lactate was chosen for its ability to dissolve PLGA and its wide use as both a food and pharmaceutical additive (128).

Table 9: Solvents investigated and their main characteristics
[data providing from PubChem (https://pubchem.ncbi.nlm.nih.gov/)]

	Viscosity, cP at 20°C	Log P	Solubility in water, M	Safety profile GHS Classification
NMP	1.65	-0.38	10.09	H315/H319/H335/H360D
DMSO	2.47	-1.35	12.8	H315/H319/H335
2-pyrrolidone	13.3	-0.85	11.75	H302/H315/H318/H319/H335
Ethyl lactate	2.71	-0.18	8.46	H226/H318/H335

In this study, chlorhexidine was replaced by another very promising non antibiotic, antibacterial agent, CIN-102. Its compostion (table 9) is very close to cinnamon essential oil but is "*a well-defined, synergistic blend of trans-cinnamaldehyde, trans-2-methoxycinnamaldehyde, cinnamyl acetate, linalool, b-caryophyllene, cineol and benzyl benzoate* (116)*".*

Table 10: Composition of CIN-102 (116).

Component	% (w/w)
Trans-cinnamaldehyde	86.7
Trans-2-methoxycinnamaldehyde	5.35
Cinnamyl acetate	2.5
Linalool	2.4
β-Caryophyllene	1.7
Cineol	1
Benzyl benzoate	0.35

Figure 20 show the simultaneous release of cinnamaldehyde (the main component of CIN-102) and the anti-inflammatory drug ibuprofen upon exposure to water. The black curves illustrate ibuprofen release and the blue ones cinnamaldehyde release. As in the previous study the initial drug loadings were varied as follows: 1.5% / 1.5% (squares), 5.3% / 5.3% (crosses) and 16.1% / 16.1% (circles) (referring to the *in-situ* forming implant formulations without solvent = “theoretical” implant masses). Different solvents were used for the formulation: a) NMP, b) DMSO, c) 2-pyrrolidone, and d) ethyl lactate. The following observations can be made:

- All *in-situ* forming implants are able to control simultaneously the release of cinnamaldehyde and ibuprofen over several weeks.
- The ibuprofen release is always slower than cinnamaldehyde release.
- cinnamaldehyde and ibuprofen release profiles and rates are very similar irrespective of the type of solvent used and the initial drug loading.
- Ibuprofen release is almost complete within thirty days for 1.5 and 5.3% loadings, which is remarkably different from the release kinetics of implants where ibuprofen was combined with Chlorhexidine (Figure 20 vs. Figure 14).

The systems were characterized in more details in order to better understand these observations. First, cinnamaldehyde release is discussed in more details, followed by ibuprofen release.

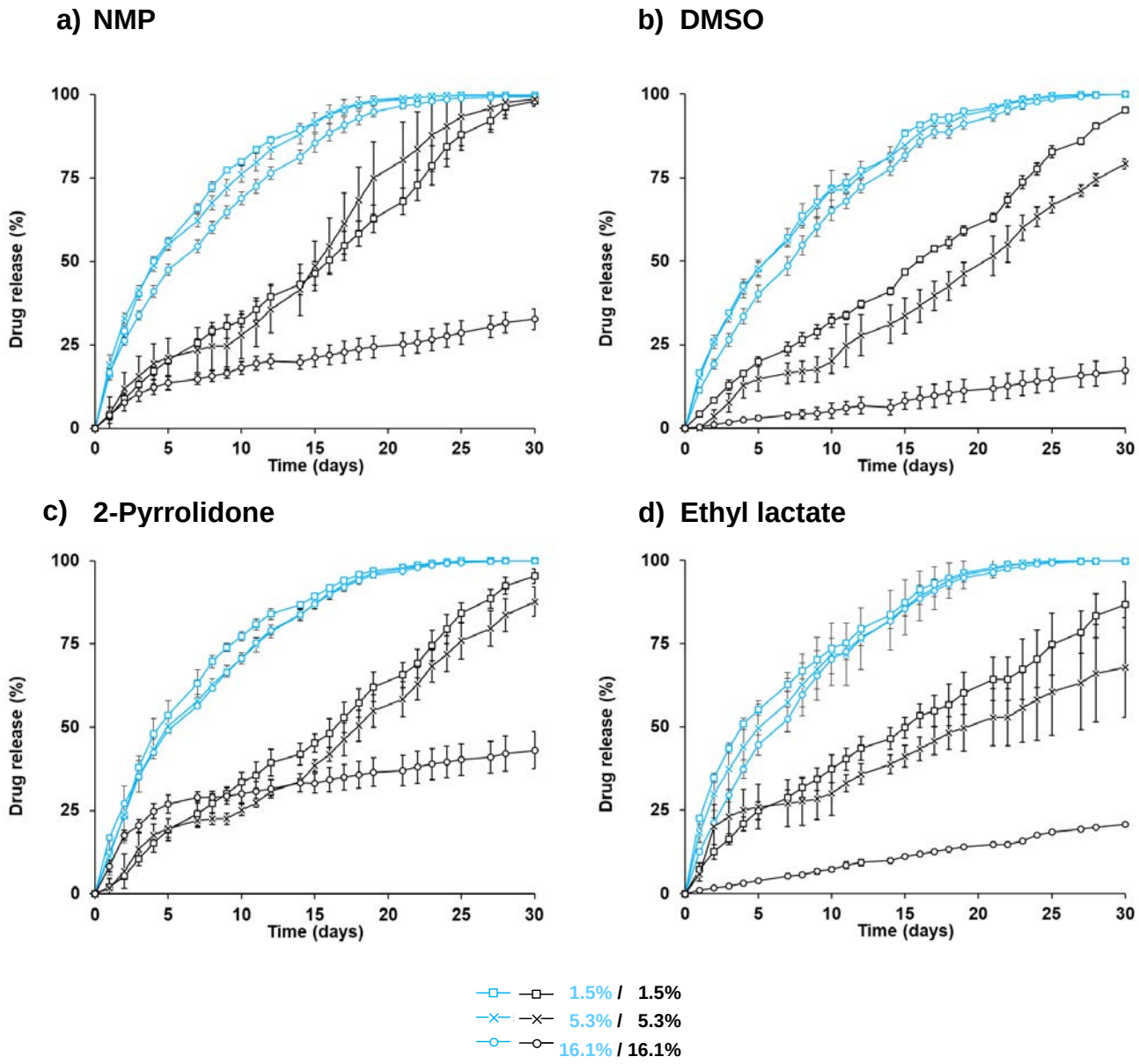


Figure 20: Dual drug release from in-situ forming implants upon exposure to release medium (i.e.. water) loaded with CIN-102 / Ibuprofen free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. The blue curves show CIN-102 release, the black curves ibuprofen release.

4.2.2. Cinnamaldehyde release

Very interestingly, cinnamaldehyde release was overall very similar irrespective of the drug loading and type of solvent. Moreover, although cinnamaldehyde is an oil and is well soluble in all solvents (> 200 mg/mL), no burst effect was observed. The drug slowly diffuses out of the implant and is almost completely released after 20 days. Surprisingly, the viscosity of the solvent does not either impact the release kinetics. 2-Pyrrolidone is characterized by a much higher viscosity than the 3 other investigated solvents and thus, it could have been expected that this higher viscosity would have slowed down the diffusion of the drug and water in the solidifying matrix (107).

Interestingly, there was also no clear correlation between the dynamic changes in the implants' mass upon exposure to the release medium and cinnamaldehyde release (Figure 21 vs. Figure 20). It can clearly be seen at a first glance that the type of solvent and drug loading have a significant impact on the mass change (Figure 21) and appearance of the implants (Figure 23) whereas cinnamaldehyde release was absolutely not impacted. For example, a substantial increase in wet mass is observed with NMP and 2-pyrrolidone at 1.5% / 1.5 % during the first two days, whereas systems with 5.3 % / 5.3 % and 16.1 % / 16.1 % lost wet mass in this period. Thus major changes in the wet mass do not correlate with cinnamaldehyde release. It is also interesting to notice that all implants prepared with ethyl lactate were all loosing mass upon contact with the release medium, and this phenomenon happened to a similar extent and rate irrespective of the drug loading. This might be attributed to a lower hydrophilicity of ethyl lactate than the 3 other solvents (Cf Table 9: higher log P and lower miscibility with water), which probably translate in a denser matrix after solvent exchange. But again the release kinetics of cinnamaldehyde are similar for implants prepared with ethyl lactate or the 3 other solvents.

Although cinnamaldehyde is in the liquid state and presents a high solubility in all the investigated solvents (> 200 mg/mL), its release is controlled over 20 days. This might be attributed to a very high affinity of the drug for the polymer. Further DSC studies should also be performed to confirm whether cinnamaldehyde might also act as a plasticizer for the PLGA.

These observations are very interesting as it shows that the release profiles are very robust despite tremendous changes in the drug loading and the solvent used for the formulation.

4.2.3. Ibuprofen release

Figure 20 shows the drug release kinetics from implants loaded with CIN-102 / Ibu free acid upon exposure to the release medium (*i.e. water*) during 30 days. Except for Eth Lact, which shows a drug release around 80%, it can be seen a total drug release at low drug loadings (1.5%) within 30 days with the three other solvents. A similar trend is observed with medium drug loading (5.3%), although a more "burst like" effect can be seen during the first five days, at least for Eth Lact (Figure 20 d) and in a minor way for NMP (Figure 20 a). At higher drug loadings, the kinetics change: a slow linear drug release can be seen with DMSO and Eth Lact (Figure 20 b and d) whereas a burst effect is clearly seen during the first five days with 2-Pyr followed by a linear slow phase two release (Figure 20 c) and this trend is also observed with NMP, although less pronounced (Figure 20 a).

Figure 21 shows the dynamic mass change of the implant upon exposure to the release medium during eleven days. Interestingly, implants formulated with Eth Lact do not swell unlike implant's formulated with other solvents (*i.e.* NMP, DMSO and 2-Pyr). The implants formulated with Eth Lact exhibit a decrease in mass directly after implant's formation and their mass change is importantly affected 24h after exposure to the release medium. This phenomenon seems independent of the drug loading and can be macroscopically seen (Figure 23).

Figure 22 shows the pH of the release medium (*i.e.* water) after contact with the formulation. Because of the absence of buffer in water and the degradation products of PLGA (*i.e.* lactic acid and glycolic acid), the release medium becomes rapidly acidic and remains acidic during the whole observation period of 30 days. It can be seen that the pH values are almost always below 4. This is in agreement with previously observed pH values with chlorhexidine / ibuprofen loaded implants (Figure 16), suggesting that acidification of the release medium is mostly due to PLGA and potentially ibuprofen, but independent of chlorhexidine. Surprisingly, only implants using NMP and 2-Pyr at 5.3% drug loadings lead to an increase in pH values about 6 at the end of the observation period (Figure 21, left hand, blank squares).

Figure 23 shows the macroscopic pictures of the implants during the first ten days of release. These pictures are in good agreement with the dynamic mass changes measured gravimetrically (Figure 21).

In contrast with the previous association of Ibu with Chx, it is interesting to see that Ibu, when associated with CIN-102, is completely released within 30 days at low drug loadings (1.5%) and almost completely released at higher concentrations (5.3%), only high drug loadings (16.1%) exhibit an incomplete release, probably due to ibuprofen low solubility in acidic aqueous medium, leading to saturation effect.

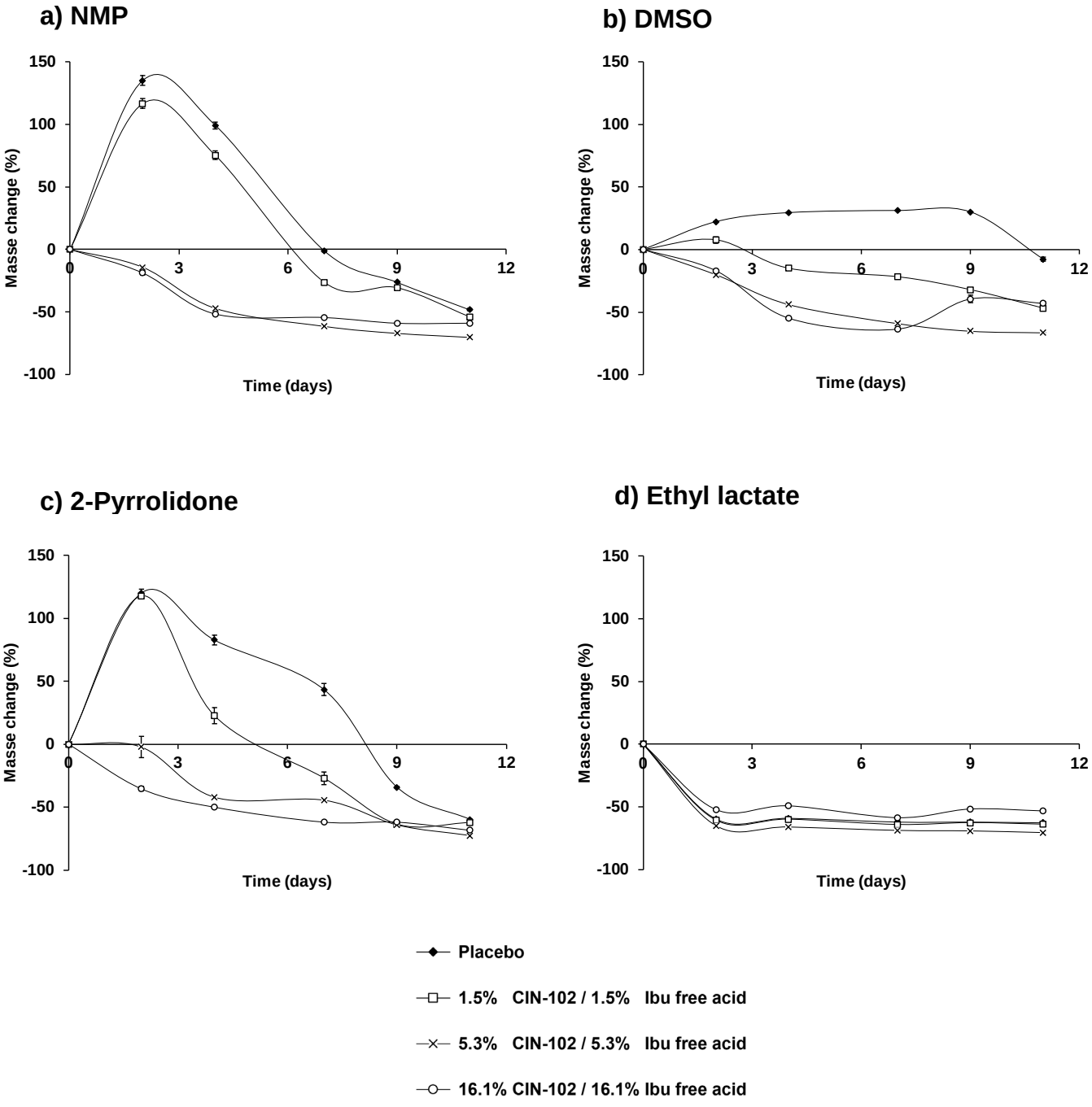


Figure 21: Dynamic changes in the (wet) mass of the in-situ forming implants during drug release. The systems were loaded with CIN-102 / ibuprofen free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug-free implants were studied.

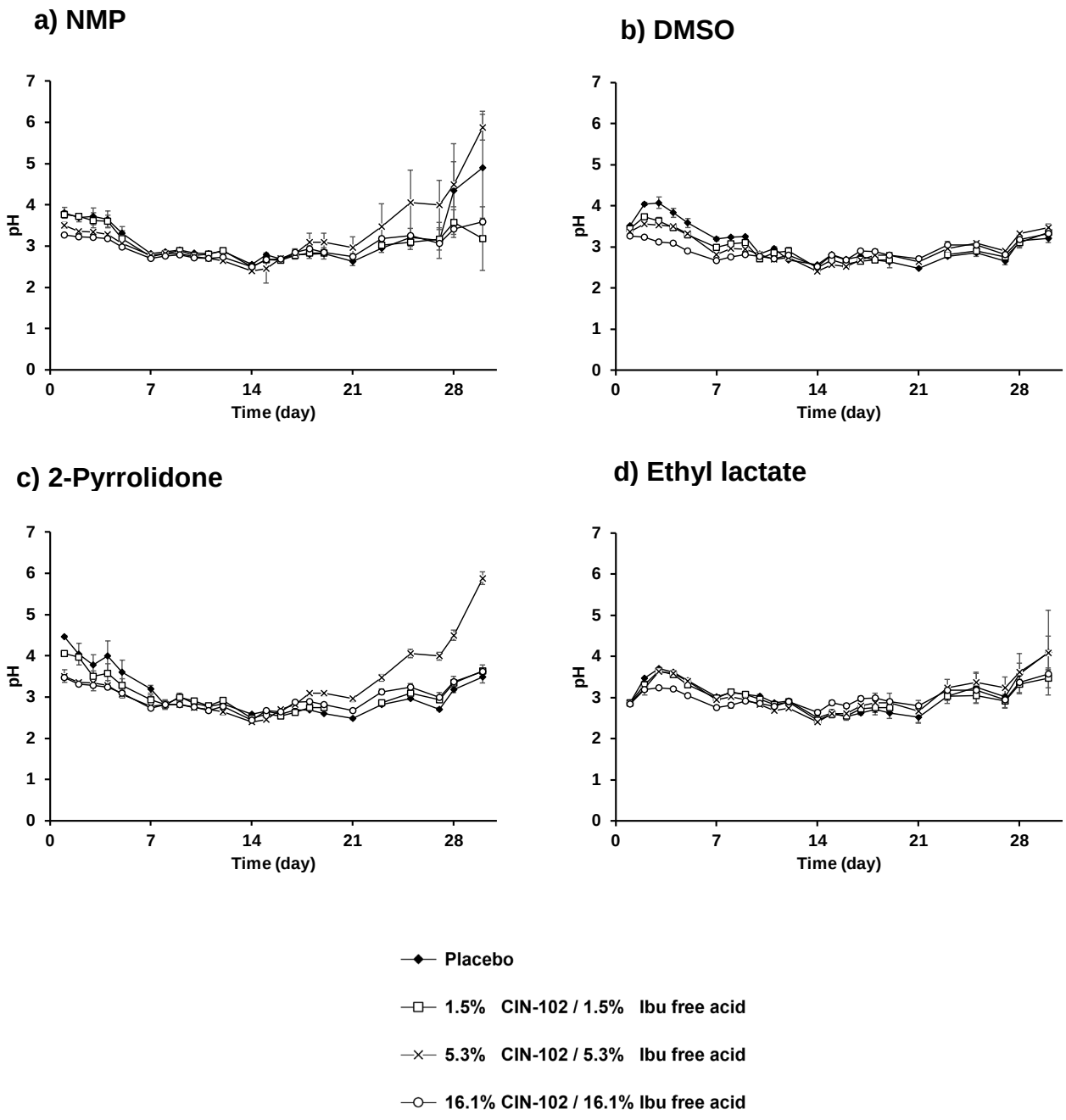


Figure 22 : Dynamic changes in the pH of the bulk fluid (water) during drug release from in-situ forming implants loaded with CIN-102 / Ibuprofen free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated.

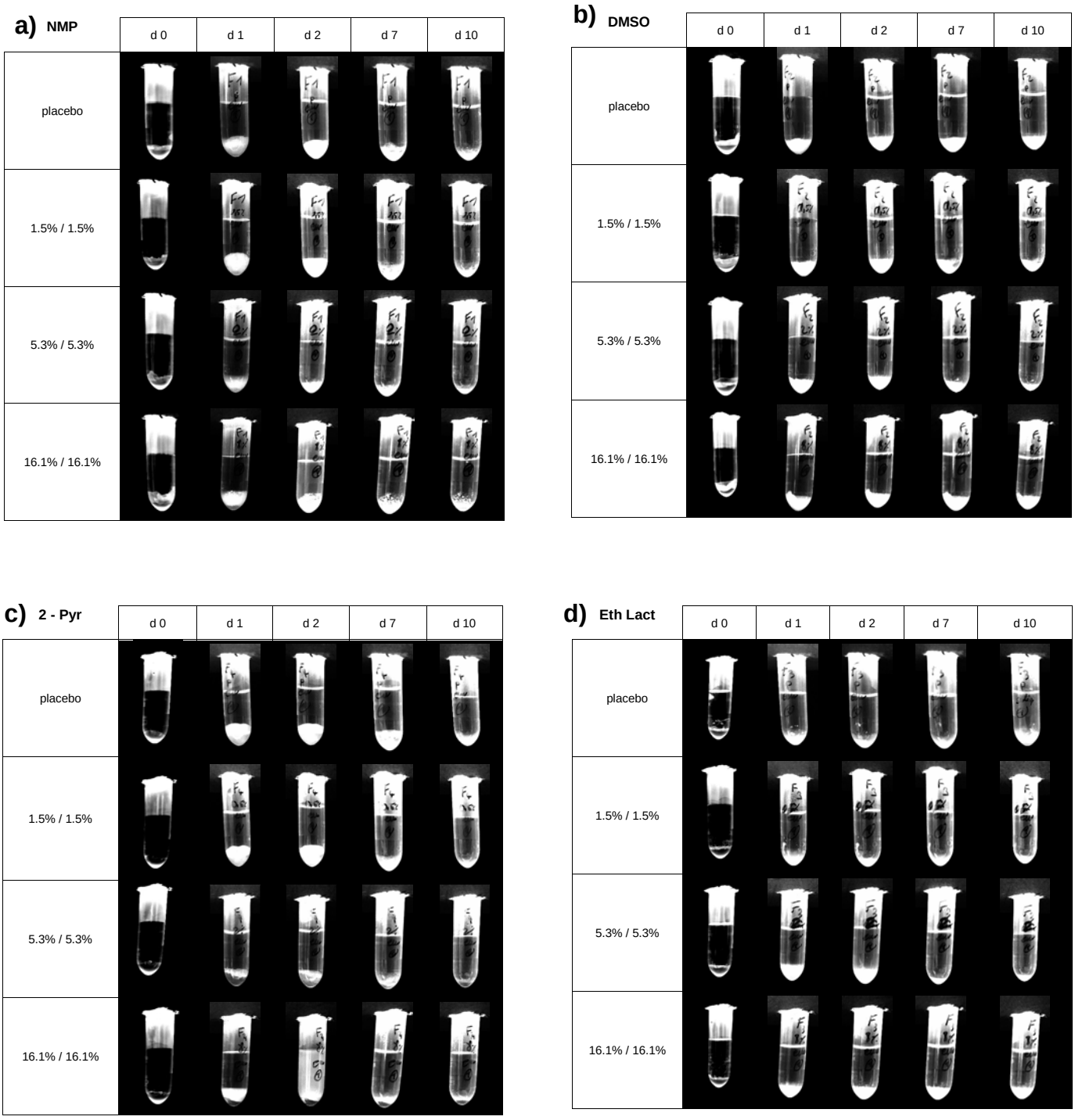


Figure 24: Macroscopic pictures of in-situ forming implants loaded with CIN-102 / Ibu free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate during the first ten days upon exposure to water (37 °). The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug-free implants were studied.

4.3. Investigation of the release medium impact

4.3.1. Global observations

Given the clinical use that is promised for this specific DDS, it was important to assess the release kinetics in a release medium that was closer to the *in-vivo* environment. For that purpose, a specific medium was used for this study. Based on the literature, an artificial saliva was made to assess the physico-chemical characterization of the implant. Several artificial saliva have been described, the formulation of the “complete saliva” described by *Pratten et al* (129) was used. This medium was chosen because considered as relevant and relatively recently described. Thus, it may be more accurate than far more complex synthetic saliva previously described (130). Finally, its formulation is easier than the first one described which comprises more than twenty different ingredients.

Although quite “simplified”, the artificial saliva used is a complex medium, comprising ionic species and larger molecules issued from beef extract and yeast extract. Also, the medium is buffered with urea solution, making it more likely to resemble to the *in-vivo* conditions (131). The preparation of artificial saliva is explained in section 2.2.2. It is important to note the presence of beef extract and particularly yeast extract, this late one being likely a source of biological activity in the medium.

Therefore, the release study was conducted the same way as previously described (see section 2). Drug release of CIN-102 as an antiseptic agent and Ibu as an anti-inflammatory substance upon exposure to artificial saliva can be seen on Figure 24. The black curves exhibit ibuprofen release and the blue ones CIN-102 release.

Some observations can be made:

- The extended release was again achieved over more than two weeks for both API.
- The ibuprofen release is, in most cases, slower than CIN-102 release.
- CIN-102 release rates are rather similar with every solvent used with an exception for 2-PYR at low drug loading.
- CIN-102 exhibits always the same release profile, consisting of a burst and a rapid following phase 2, reaching quickly a plateau.
- Ibuprofen is totally released within thirty days, for every formulation and drug loadings.
- Ibuprofen exhibits a very typical tri-phasic release profile in every case.

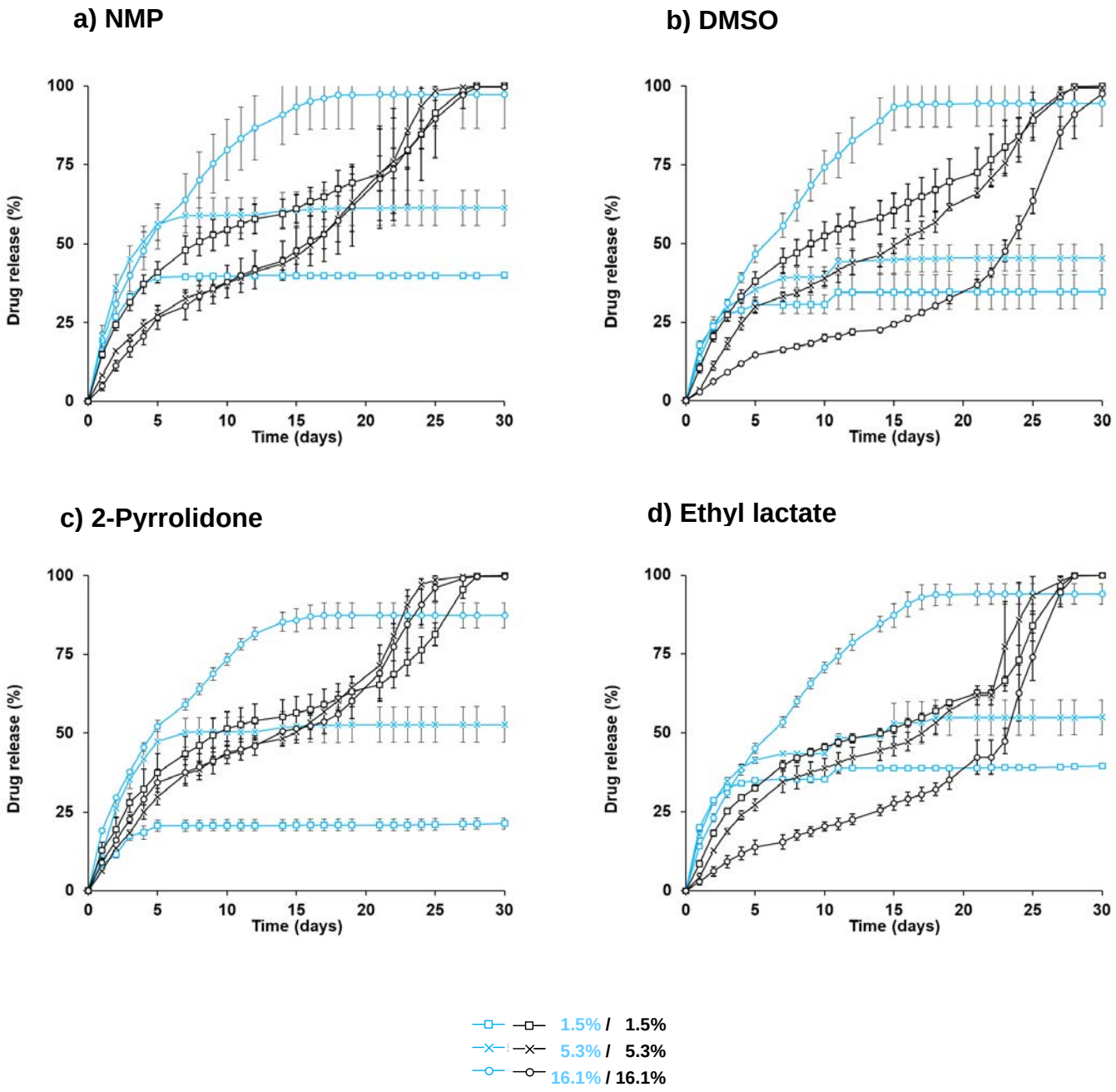


Figure 26: Dual drug release from in-situ forming implants upon exposure to release medium (i.e. artificial saliva) loaded with CIN-102 / Ibuprofen free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. The blue curves show CIN-102 release, the black curves ibuprofen release.

4.3.1. CIN-102 Release

Figure 25 shows the drug release kinetics of CIN-102 from ISFI loaded with CIN-102 / Ibuprofen free acid at different drug loadings in artificial saliva. When observing the release kinetics of CIN-102 in artificial saliva, it exhibits a burst effect in the first days followed by a rapid phase two release. On the contrary to release in water, it can be seen with artificial saliva that a plateau is quickly reached, not necessary at 100% release. This can be explained because cinnamaldehyde is quite unstable and very sensitive to oxidation (Figure 25). Thus, we found in the release medium what was very probably cinnamic acid. The observed pics were compared to pure cinnamic acid, which correspond perfectly in retention time via HPLC-UV. Although mass spectrometry and NMR would have been necessary to confirm the exact structure of the product, this is already a strong indication that cinnamic acid is likely to form in this medium. Indeed, artificial saliva comprises yeast extract that is probably not free from enzymatic activity. Thus, it would also explain why the CIN-102 release is higher at higher drug loading, since the release medium volume stay the same (therefore, the enzymatic activity) while the drug load is higher, allowing for a higher drug release.

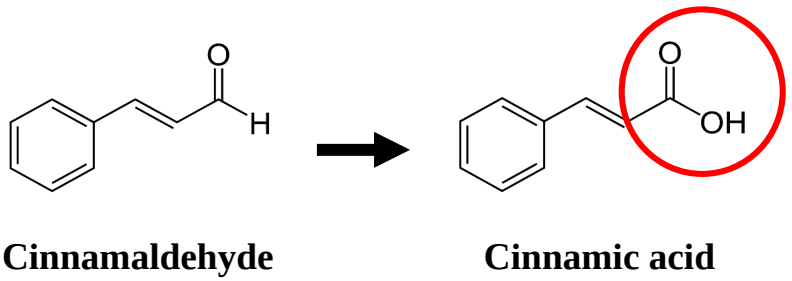


Figure 27: Chemical structures of cinnamaldehyde and cinnamic acid.

Figure 26 shows the mass change monitoring of the implant upon exposure to the release medium (*i.e.* artificial saliva) during eleven days. It can be seen that formulations made using NMP and 2-Pyr exhibit pretty much the same behavior, with a swelling of the implant at low drug loading (*i.e.* 1.5%) during the first two days followed by a mass decrease (Figure 26 a and d). At higher drug loadings (*i.e.* 5.3% and 16.1%), no swelling is observed and the implants immediately loose mass. Interestingly, implants using ethyl lactate as a solvent were all loosing mass immediately upon exposure to the release medium, in contrast with the other solvents were the DDSs firstly exhibit a swelling the first two days. This fact does not correlate with the drug release kinetic of CIN-102 (Figure 24 d) which is the pretty much the same for all the solvents used.

Figure 28 shows macroscopic pictures of the implants upon exposure to the release medium during the ten first days of release. It can be seen that implants using NMP does form completely within 24h at every drug loading.

This API does not show major difference in release kinetics in function of the solvent used.

This phenomenon is not surprising as a rapid release of any essential oil from this type of formulation can be expected for the previous cited reasons. As an oil at atmospheric pressure and room temperature, this physical state will not change to a solid state at higher temperature (*i.e.* body temperature, 37°C). Thus, it is not prompt to be trapped into the polymer matrix. Therefore, in function of the oil affinity, which has greater chances to be higher for the solvent than for water, it is more likely to show a burst during the implant formation.

This type of release is interesting though, as it shows that it is quite constant and robust despite all the changes made: the kinetic stay the same despite tremendous variations in the drug loading and the solvent used for the formulation. Moreover, regarding the application it is used for, a quick release is not necessarily useless: after a scaling and root planning, it is important to have high antiseptic concentrations during the first days in order to avoid a bacterial recolonization of the site, which is achieved by this type of release kinetics. Once a few days have passed, the immune system will manage to recover and prevent the recolonization.

It is interesting to note that CIN-102, although complex to analyse due to its nature of a molecules blend and the instability of cinnamaldehyde, would be a really good candidate. Indeed, this blend of molecules has a broad spectrum antibacterial activity that has not been exploited yet, therefore no bacterial resistance is known for such substance. Finally, even if cinnamaldehyde seems unstable, its metabolites and/or derivatives are not deprived of anti-bacterial activity (132).

Further investigations on the antimicrobial activity of CIN-102 loaded ISFI would be of major importance, as it is a new substance that has never been tested yet on bacteria involved in periodontal diseases. So far, it has been only tested against colonic *enterobacteria,* with a specific oral dosage form (*i.e.* mini-tablets or coated pellets loaded with CIN-102). Nevertheless, a proof of concept was performed *in-vivo* by reducing the intestinal mucosal bacterial count in a rat induced model of inflammatory bowel disease. As *enterobacteria* are mostly Gram negative rod shapped facultative anaerobic bacteria, and because they are from the gastro intestinal tract, CIN-102 is therefore very interesting for periodontitis treatment. It can be suggested that bacteria present in the buccal flora may react similarly to CIN-102 activity, although it has to be proven, specifically with periodontopathogens like bacteria from the red complex (*i.e. P. gingivalis, T. denticola and T. forsythia*) but also others involved in the disease like *Fusobacterium nucleatum* or *Aggregatibacter actinomycetemcomitans.*

4.3.2. Ibuprofen Release

Drug release kinetics of ibuprofen can be seen on Figure 24 (black curves). In artificial saliva, ibuprofen releases are completely different from what it is in water. With the four solvents and at all concentrations tested, release of ibuprofen is triphasic. There is an Initial burst during the first five days, then a slower and linear release rate during approximately two weeks, and a final burst release around three weeks. This difference in release rate compared to water as a release medium is explained by the composition of the artificial saliva (AS). Indeed, this one is buffered and slightly basic (pH between 7 and 8), due to the presence of urea solution into it.

Drug release kinetics of ibuprofen are quite similar for all drug loading tested when formulated with 2-Pyr (Figure 24 c). When NMP is used, a small drug loading (0.5%) leads to a higher release rate compared to 5.3% and 16.1% which show a similar release rate (Figure 24 a); whereas when Eth Lact is used, the opposite trend is observed, with a slower release rate at 16,1% drug loading and a similar higher release rate is observed with 5.3% and 1.5% drug loadings (Figure 24 d). Finally, ISFI made up of DMSO shows a correlation between drug loading and relative release rate: the higher the drug loading is, the slower the release rate is (Figure 24 b).

It is interesting to note that this final burst occurs between days 21 and 25 (closer to day 21), with every solvents and concentrations tested. This is a strong indication that the polymer may be the main factor contributing to ibuprofen release rate in these conditions. Although, release rates are still influenced by the drug loading and the solvent used.

Another important point to note is that in these conditions, every formulation tested for drug release were completely degraded and/or solubilized into the release medium within thirty days. All analyses were matching: no more drug was detected on HPLC, gravimetric analysis showed complete mass loss of the implants (at day 30, data not shown on Figure 26) and pH measurements of the artificial saliva were similar to its initial value (approx. 7, depending on the batch produced, data not shown).

pH variations of artificial saliva during drug release from ISFI can be seen on Figure 27. pH of the release medium (here AS) is most of the time comprised between 6 and 7, at least for low drug loadings (*i.e. 1.5 and 5.3%*). At higher drug loadings (16.1%), we can observe a lower pH comprised between 4 and 5, no matter the solvent used for the formulation. This indicates that ibuprofen is potentially responsible of this acidification. Interestingly, the pH of the release medium observed at day 1 with ethyl lactate is more acidic (pH<5) compared to the three other solvents (pH around 6). This phenomenon cannot be explained by a superior burst effect of ibuprofen release with Eth Lact compared to other solvents (Figure 24 d vs Figure 24 a, b and c).

Ethyl lactate, has been reported to be hydrolysed in lactic acid and ethanol in presence of water. This reaction is influenced by different parameters like temperature, water to ethyl lactate ratio or catalyst concentration. Interestingly, acids catalyse this

reaction, and the reaction undergoes a thermodynamic equilibrium. Most importantly, the presence of a catalyst increases the conversion rate but is not obligatory, and the reaction occurs within hours (133,134). It is therefore very likely that ethyl lactate is responsible for the quick acidification of the release medium observed during the first days of release.

However, this acidity rapidly followed by an increase of pH and values tend to be comprised between 6 and 7 after three days of release. Please note that the drastic diminutions in pH mediums observed as peaks on days 14 and 21 correlates with the weekends. Medium was renewed every 24h except on Sundays were the laboratory was not accessible, thus explaining this pH decrease after 48h in contact with the formulation.

Dynamic mass changes (Figure 26) do not explain the relative release rates observed with ibuprofen. For example, with a 1.5% drug loading, the relative release rates are rather similar with the four solvents used (Figure 24 a, b, c and d, dark curve, crosses) whereas dynamic mass change of the implants show an important increase of mass for NMP and 2-Pyr based implants during the first three days, compared to a slight mass loss with DMSO and an important mass loss with Eth Lact (Figure 26 left hand vs right hand, white squares). At the opposite, at 16.1% drug loadings, implants exhibit quite similar dynamic mass changes (Figure 24, dark curves, white circles), whereas ibuprofen relative release rates are lower for DMSO and Eth Lact based implants compared to NMP and 2-Pyr based ones (Figure 26 right hand vs left hand).

It can be concluded though that using artificial saliva is a better release medium because of its buffer capacity, since ibuprofen solubility is pH dependant. Here, with all the formulations tested (three concentrations, four solvents, each in triplicate), we observed an entire ibuprofen release on an extended period of time of one month. This is a strong indication of the potential for ibuprofen to be used in PLGA based ISFI. Further studies would be necessary in order to define more precisely what are the underlying release mechanisms for ibuprofen. Also, studies regarding more closely to the wound healing process would be interesting to see if this triphasic release profile of ibuprofen, over a month-time period, is perfectly adapted to periodontal wound healing. However, to the best of our knowledge, this *in-vitro* release time can be estimated to be an upper limit of what should be expected *in-vivo*, thus it is truly believed that the implant should not stay into the periodontal pocket for a too long period of time, which would cause the host to consider it as a foreign body, causing other troubles.

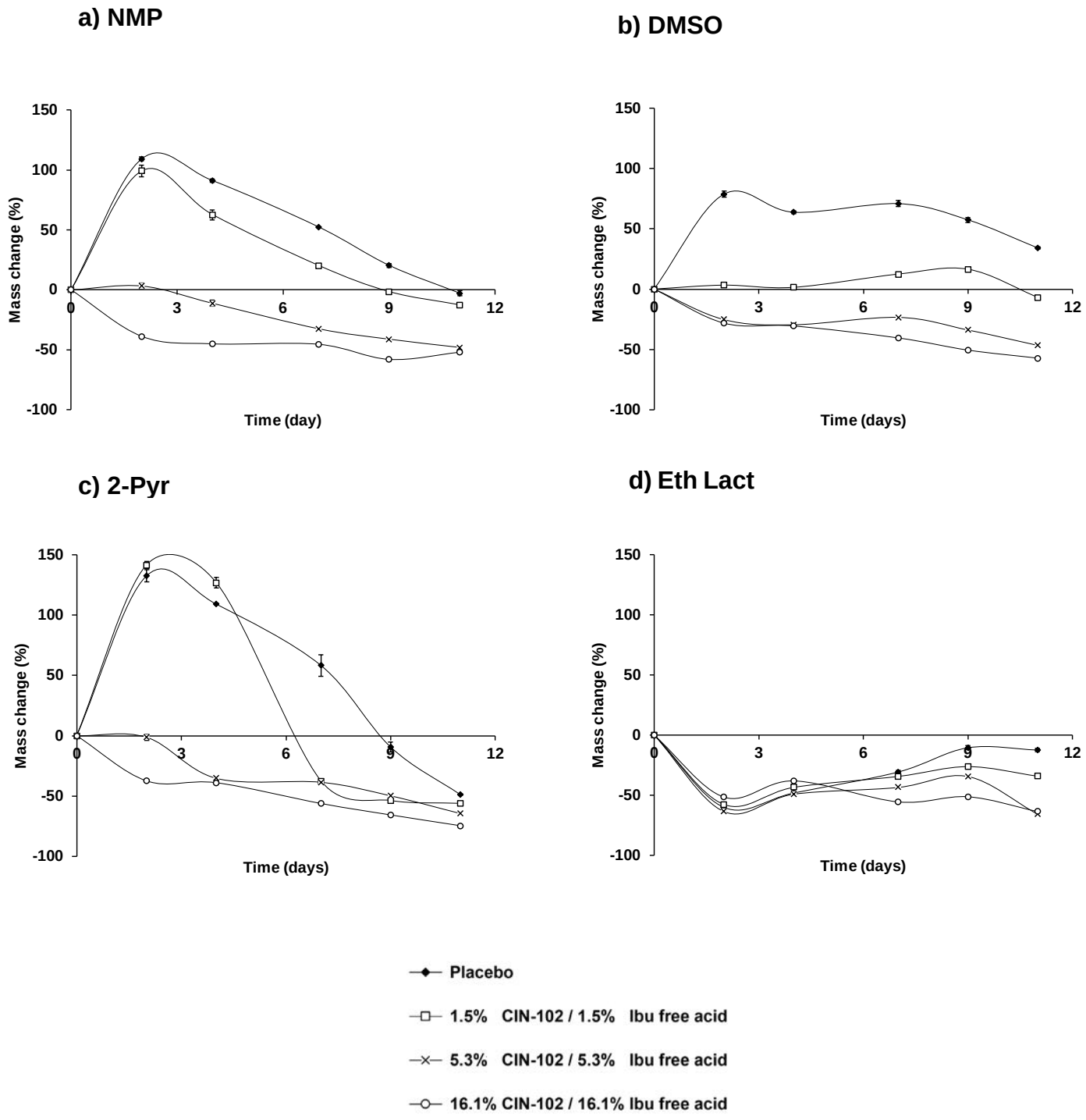


Figure 29: Dynamic changes in the (wet) mass of the in-situ forming implants during drug release. The systems were loaded with CIN-102 / ibuprofen free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug-free implants were studied.

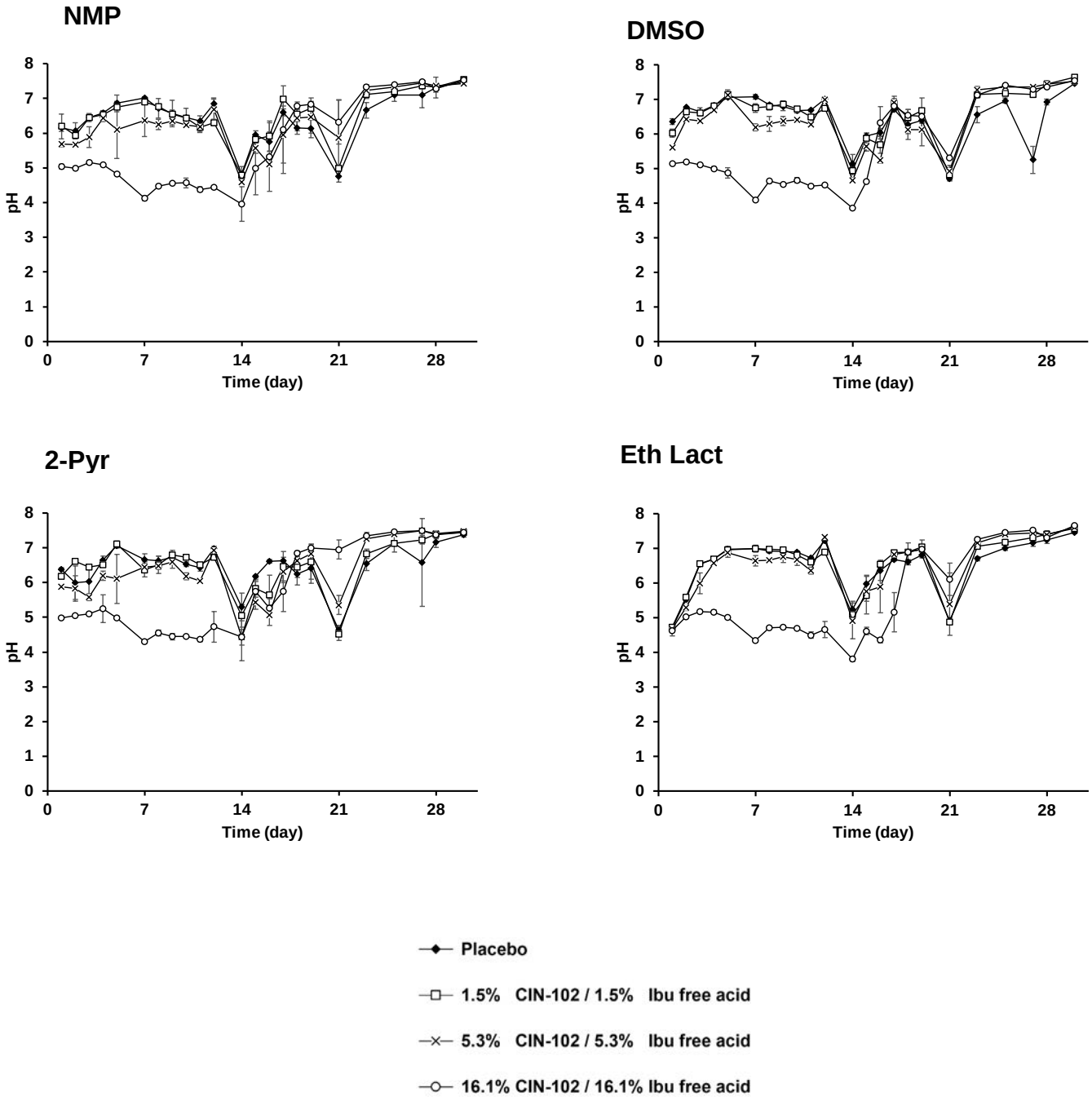


Figure 31: Dynamic changes in the pH of the bulk fluid (artificial saliva) during drug release from in-situ forming implants loaded with CIN-102 / Ibuprofen free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) 2-Pyr: 2-pyrrolidone, and d) Eth Lact: ethyl lactate. The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug free implants were studied.

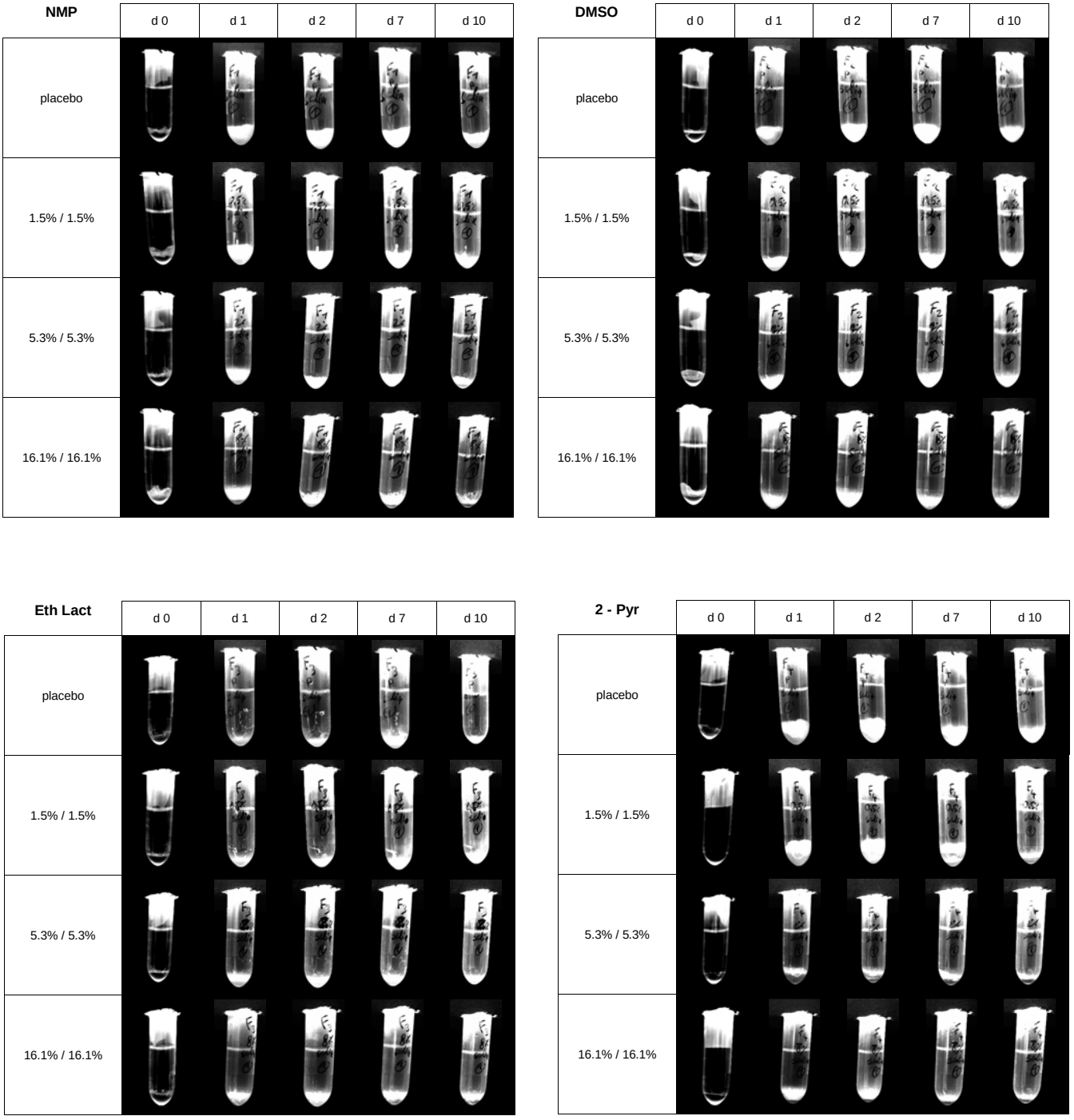


Figure 32: Macroscopic pictures of in-situ forming implants loaded with CIN-102 / Ibu free acid and formulated with: a) NMP: N-methyl pyrrolidone, b) DMSO: dimethylsulfoxyde, c) Eth Lact: ethyl lactate, and d) 2-Pyr: 2-pyrrolidone during the first ten days upon exposure to artificial saliva (37 °). The initial drug loadings were 1.5% / 1.5%, 5.3% / 5.3%, 16.1% / 16.1%, as indicated. For reasons of comparison, also drug-free implants were studied.

5. GENERAL CONCLUSION AND PERSPECTIVES

This study highlighted the feasibility of *in-situ* forming implants drug delivery system to treat periodontal pockets as adjunct to scaling and root planning. First, it was shown that ISFI is effective in delivering two active pharmaceutical ingredients simultaneously in a time controlled manner. These *in-vitro* release studies gathered important data about drug release kinetics from this drug delivery system. It was shown that extended release over a minimum of two weeks was achieved with every drug combination between chlorhexidine dihydrochloride or digluconate associated with ibuprofen “free acid” or lysinate, at drug loadings up to 16.1% w/w (based on the total liquid formulation without solvent = “theoretical” concentrations).

It was shown that the combination of these two drugs (Chx diHCl or diglu and Ibu “free acid” or lysinate) results in a dual release of the active substances at different release rates. Importantly, the drug release kinetics could be modified in function of the drugs salts used in the formulations.

Another important result is that ISFI formulations tested have shown to be completely degraded *in-vitro*. Therefore, the release durations mentioned in this study are upper limits, as *in-vivo* conditions encompass many biological features that are most certainly able to degrade the implant faster than *in-vitro* like enzymatic activity or presence of radical oxygen species for example. It is generally admitted that *in-vivo* drug release occurs faster than *in-vitro* (making *in-vitro*/*in-vivo* relationship and/or correlation [IVIV/R] – [IVIV/C] a great challenge).

Regarding the impact of the solvent used for the formulation, it was shown that different solvents can be used while keeping rather similar drug release profiles, at least in these conditions:

- Ibuprofen used in the formulation is under “free acid” form, which exhibit low solubility in water at low pH (below its pka of 4.4). In this regard, it would be interesting to evaluate the impact of the combination between different ibuprofen salts associated with different solvents.

- The antiseptic agent is a molecule blend, comprising only very lipophilic compounds. Even if only cinnamaldehyde was quantified, it can be assumed that other molecules of the blend will have the same behavior considering their chemical structure, exhibiting almost no hydrogen donor or acceptor bond.

Finally, in the last section was evaluated the drug release kinetics of the implants loaded with CIN-102 and ibuprofen in artificial saliva. This part is in good agreement with what was previously observed (*i.e.* a complete release of the implants in-vitro) but also and more importantly with what we previously admitted, namely that *in-vivo* release rates are nearly always faster *in-vivo*: here, the implants are totally degraded within a month and 100% of ibuprofen is released, no matter the solvent and the drug

loadings were. However, CIN-102 exhibits an unexpected tendency, that is resolutely believed to be its oxidation into cinnamic acid.

It brings a new question as to know if cinnamic acid is effective against periodontopathogens. It was found in the literature that cinnamic acid was already used as a pharmacophore in medicinal chemistry as a starting block material, in order to produce more potent derivatives, since cinnamic acid has already been reported multiple times to exhibit different pharmaceutical activities. But its activity against bacteria of the red complex for example or its potential use in periodontal diseases as an antibacterial adjunct to scaling and root planning remains to be assessed, at least *in-vitro* against collection's strains or, if possible, against fresh sampled strains from affected patients.

This last concept has to be particularly emphasized. Indeed, working on collection bacteria strains may have the advantage to make "reproducible" experiments, whilst the major drawback with these strains is that they are cultivated for many years in test tubes, undergoing numerous subcultures to maintain them alive, transforming them slowly but surely into "comfortably numb" bacteria... Thus, it is resulting from this process that bacteria do not have the same pathogenicity as the wild ones, because they don't need their virulence factors anymore to survive, which can make them respond sensitively to some antibacterial agents *in-vitro* while clinical wild strains encountered in infected patients may express several biological activities in order to resist from exterior aggressions

However, these results strongly indicate that further tests and evaluations of the CIN-102 / ibuprofen loaded drug delivery system are required in order to assess its potential antibacterial activity. It would be the strict minimum regarding the application of this specific drug delivery system.

Regarding the initial wish list for the implant's properties (cf section 2. Purpose of this work), many of the parameters have been assessed. These were sometimes compared to already commercialized products in order to improve the desired properties. Therefore, mechanical properties have been assessed with an ISFI of our own compared to both Chlo-site® and Parocline® and shown good bio adhesive properties directly toward the dental surface and also good syringeability (135). These experiments have proven that this type of DDS is suitable for intrapocket administration with ease of administration and a good adhesion on the dental root.

A second important point to note is the proof of concept that has been assessed *in-vivo* for this specific formulation. Here, the formulation was tested in an experimental periodontitis mouse model (*i.e.* periodontal disease induced by *P. gingivalis* ligature of the gum). An ISFI loaded with Chx diHCl 1.5% / Ibu free acid 1.5% has proven to be effective against unloaded drug ISFI and untreated mice at 7 and 15 days post-treatment, histomorphometrically and on inflammatory cell scoring. These experiments suggest that this DDS could be suitable for other drug delivery such as

pro-regenerative drugs, thus allowing a more efficient adjuvant therapy for periodontitis treatment (136).

Also, considering the *in-vitro* releases studies, some conclusions can be made. Using water as a release medium is very far from reality but is very simple. It allows for fast experimentation but most importantly, easy analysis, thus allowing to make extensive experimentations and to collect preliminary data prior to deal with some issues in depth. In contrast, artificial saliva is more relevant but is also heavier to handle. The medium preparation and conservation are quite sensitive as it is a good culture broth. But most of all, samples analysis is much longer to prepare and carry out.

An important fact has to be pointed out: there is no existing standard protocol to test the drug release from such specific DDSs. All the standard apparatus (*i.e.* USP I to VII) are indeed made to test more common drug dosage forms, in particular *per os* forms. Thus, the dissolution volumes and many other parameters do not represent the conditions required to test these new types of DDSs. It really represents an issue as these parameters can drastically influence the release kinetics from the system. Therefore, some research teams focus on developing specifically new dissolution chambers for this particular application (*i.e.* periodontal pocket drug delivery), using 3D printing to build small volumes dissolution chambers and comparing with standard apparatus (137). This study is particularly interesting as it shows that new technologies allows to tune quite finely *in-vitro* systems to mimic as close as possible *in-vivo* conditions.

Finally, these results are promising. Indeed, combining all the new insights and knowledge resulting from this transdisciplinary work involving so many fields allows for faster development of new optimized devices. Dental surgeons know the patient's needs and the conditions required for the product to be used effectively. Pharmacists can develop new DDSs considering these facts and physicists, chemists, biologists and microbiologists working together can bring many data to characterize these DDSs and optimize them. It is really important to note and emphasis on that particular aspect of the research: these news DDSs are related to so many aspects of science that it involves *de facto* the work of multi-disciplinary teams in order to progress.

6. RÉSUMÉ DÉTAILLÉ

Les maladies parodontales se définissent comme une destruction des tissus de soutien de la dent (la gencive, le ligament et l'os alvéolaire). A la différence des pathologies dentaires à proprement parler (telles que les caries par exemple), les dents ne sont pas affectées par ces maladies, mais bien leur structure d'ancrage. Ces pathologies sont parmi les plus fréquentes dans les populations humaines, quelles qu'elles soient. Menant *in-fine* à la perte dentaire (c'est la première cause de perte dentaire chez l'adulte en occident), elles sont également impliquées dans de nombreuses autres pathologies.

Les recherches menées sur ce sujet depuis une vingtaine d'années montrent, sinon des liens de causalité directe, au moins des corrélations fortes entre le statut parodontal et la survenue ou l'aggravation d'autres pathologies. Celles-ci balayent un large éventail : on retrouve ainsi le diabète, les maladies inflammatoires chroniques de l'intestin, la polyarthrite rhumatoïde, les pré-éclampsies, la maladie d'Alzheimer ainsi que de nombreux troubles métaboliques... Il convient de souligner la relation étroite, mutuelle et réciproque entre le statut parodontal et le diabète : cette pathologie entrainant une élévation du taux de glucose sanguin créé un environnement favorable au développement bactérien, celui-ci entrainant une réponse inflammatoire à bas bruit propice à l'augmentation de la résistance insulinique.

Bien que les liens de causalité entre parodontite et pathologies systémiques ne soient pas toujours directement établis selon des études spécifiquement discriminantes (la conception et réalisation d'études cliniques étant onéreuses, elles ne sont pas spécifiquement établies dans ce but précis), il semble de plus en plus clair que les parodontites engendrent plusieurs conséquences. Elles sont à la fois génératrices d'un statut inflammatoire plus ou moins important et généralisé, tout en étant une « porte d'entrée » pour les bactéries, susceptibles de générer des infections à distance.

Les maladies parodontales regroupent donc un grand nombre de pathologies, dont la classification ne cesse d'évoluer. Le plus souvent chroniques, elles sont complexes à définir et à prendre en charge. Bien que connues depuis longtemps, elles n'ont fait l'objet d'études que tardivement. Ainsi, ce n'est qu'en 1965 que Loë et Coll mettent en évidence la relation directe entre la présence de certaines bactéries avec les poches parodontales. L'hypothèse du facteur bactérien émise ici sera le socle de nombreuses études permettant la discrimination de bactéries parodontopathogènes. Parmi les centaines d'espèces bactériennes présentes dans la cavité buccale, certaines ne sont retrouvées que dans des lésions parodontales déjà avancées. C'est le cas, par exemple, de *Porphyromonas gingivalis*, Cocci à Gram négatif, strictement anaérobie, opportuniste et équipée d'un redoutable arsenal enzymatique propice à la destruction des tissus parodontaux. Elle forme un groupe de bactéries appelé « complexe rouge » avec deux autres espèces que sont *Treponema denticola* et *Tannerella forsythia.* Cette dénomination de complexe rouge a été émise pour la première fois en 1998 par Socranski *et al*, elle est depuis largement utilisée. En 1999 apparaît la première classification des maladies parodontales par l'AAP

(American Association of Periodontology), qui sera la référence en la matière jusqu'en 2017, avant d'être remaniée pour proposer une approche plus clinique des pathologies. En effet, cette première classification adoptait une approche systématique, tandis que la nouvelle est basée sur une approche plus clinique, évaluant les maladies par stades et grades, permettant une meilleure adéquation avec la prise en charge des patients. Les bactéries ont donc été les premières à être tenues responsables du développement des pathologies parodontales, d'où un nombre élevé d'études sur ces bactéries spécifiques et leurs implications de mieux en mieux connues. Il faut souligner la complexité de l'écosystème buccal : si certaines bactéries ont des rôles établis, leurs interactions ne sont pas toutes élucidées, pas plus que les multiples relations bactéries-cellules hôtes.

Ainsi, le facteur bactérien n'est pas le seul en cause dans la pathologie : en réponse à l'agression microbienne, le système immunitaire de l'hôte génère de nombreuses réactions et produits. Seulement, certaines bactéries ont développé la capacité d'échapper à la réponse immune. Par des moyens complexes et subtils, *P. gingivalis* est par exemple capable de manipuler le système du complément pour éviter sa lyse. *P. gingivalis* est également capable de modifier les capacités d'adhésion des leucocytes, diminuant leur pouvoir de phagocytose. Les mécanismes immunitaires, initialement impliqués dans la destruction des bactéries pathogènes, finiront dans certains cas par être directement responsables de la destruction tissulaire. Celle-ci, de nature auto-immune donc, semble représenter une part importante de la pathogénie.

C'est donc l'inadéquation de la réponse immunitaire de l'hôte, d'une part, et l'aptitude de certaines bactéries impliquées dans la pathologie à échapper à cette réponse, d'une autre, qui sont le socle principal du développement de la maladie. A cela peuvent s'ajouter de nombreux autres facteurs qui peuvent être liés au mode de vie, tels que l'alimentation, l'hygiène bucco-dentaire, la consommation de tabac, le stress ; ou non tels que des facteurs génétiques, qui peuvent avoir une influence sur le statut parodontal (le plus généralement pour les pathologies dites agressives, se déclenchant chez le sujet jeune et dont l'évolution est très rapide).

Le traitement de référence de la parodontite, ou « gold standard », consiste à éliminer mécaniquement la plaque dentaire ainsi que le tartre situé sous la gencive (donc sur la racine dentaire) au cours d'une opération appelée surfaçage radiculaire. Ce geste n'est pas à proprement parler invasif, dans la mesure où l'accès à la poche parodontale peut se faire sans nécessiter systématiquement une lésion tissulaire préalable (celle-ci, éventuelle, sera une conséquence du geste dite iatrogène, sans être une étape préalable indispensable à sa réalisation). De ce fait, les recommandations officielles ne préconisent pas toujours l'utilisation d'antibiotiques, et ils sont plutôt limités à des cas bien précis (patients à haut risque d'endocardite infectieuse, immunodépression…). Ainsi, l'utilisation d'antibiotiques devrait être limitée à un cadre stricte de prise en charge nécessitant la réalisation d'un acte chirurgical (lambeau d'assainissement, comblement osseux, etc).

Dans beaucoup de cas cependant, bien que les recommandations officielles ne préconisent pas toujours l'utilisation d'antibiotiques par voie systémique, ceux-ci sont en pratique très souvent prescrits en plus du geste. Des rapports effectués par les caisses d'assurance maladie mettent en évidence la différence entre les recommandations officielles et la réalité de la pratique clinique. Compte tenu des nombreux effets indésirables liés à leur usage excessif, en plus de favoriser l'émergence de bactéries résistantes, il apparaît nécessaire de développer de nouveaux médicaments permettant de diminuer leur utilisation.

Les antibiotiques utilisés dans le cadre des maladies parodontales sont, de fait, des antibiotiques à spectre large, agissant sur les bactéries à Gram négatif. Il en résulte des effets secondaires potentiellement très délétères, au regard d'une efficacité modérée. En effet, l'utilisation d'Amoxicilline associée à l'acide clavulanique en première intention n'ayant une action que modérée vis-à-vis des souches bactériennes à Gram négatif, l'utilisation d'autres antibiotiques tels que la spiramycine associée au metronidazole (par exemple Birodogyl®) est très courante, bien que recommandée en seconde intention. Les conséquences sont multiples, notamment sur la flore intestinale. Or, de nombreuses recherches et études cliniques menées ces dernières années démontrent toute l'importance de la flore microbienne intestinale dans de nombreux rôles physiologiques, et nombreuses sont les études qui corrèlent une diminution de la diversité microbienne intestinale avec des pathologies systémiques aux conséquences parfois lourdes.

Afin d'éviter l'usage d'antibiotiques par voie orale, qui génèrent des effets secondaires potentiellement graves, de nombreuses recherches ont été menées sur des dispositifs locaux. Ces systèmes permettent le traitement de la poche parodontale, au niveau local, au moyen de différentes formes galéniques à libération contrôlée, destinées à être mise en place dans la poche après l'étape de surfaçage radiculaire.

Ainsi, différents médicaments ou dispositifs médicaux (selon la législation et le cadre dans lequel ils ont été mis sur le marché), ont été conçus. Tandis que certains sont peu répandus (notamment en raison du bénéfice thérapeutique relativement faible à l'égard du coût de ces thérapeutiques, tandis que d'autres n'ont pas su rester sur le marché, notamment en raison de limites techniques liés à leur utilisation.

C'est le cas de l'Actisite®, retiré quelques années après son introduction sur le marché en 1994. Il s'agit d'une fibre composée d'ethyl-vinyl-acetate (EVA) chargée en minocycline, destinée à être enroulée autour de la racine dentaire après lithotritie plus surfaçage, et libérant le principe actif sur une période prolongée d'environ dix jours. Sa difficulté d'utilisation, liée à sa pose délicate, ainsi que la nature insoluble du polymère utilisé (EVA) qui requérant une seconde intervention pour retirer le système, ont rapidement révélé que le système n'apportait pas de solution satisfaisante à la pratique clinique.

D'autres systèmes à libération locale et contrôlée existent sur le marché, présentées sous différentes formes galéniques. On peut citer par exemple :

- l’Arestin® aux US, se présentant sous la forme de microparticules polymériques biodégradables à base de Poly-(Lactic-Co-Glycolic Acid) (PLGA) chargées en minocycline, destinées à être injectées directement dans la poche parodontale.
- L’Atridox®, gel biodégradable composé de Poly-(Lactic-Acid), aussi appelé Poly (DL-lactide), en solution dans du N-methyl pyrrolidone (NMP) et chargé avec de l’hyclate de doxycycline.
- Le Parocline®, gel biodégradable composé d hydroxyéthylcellulose, chlorure de magnésium, copolymère d'ammoniométhacrylate de type B (Eudragit RS), triacétine, glycérol.

Le point commun entre tous ces médicaments à libération locale contrôlée, malgré leurs formes galéniques variées, est l’utilisation d’antibiotiques. Ce type de système peut être pensé rationnellement comme un moyen finalement efficace pour sélectionner des souches résistantes. En effet, les durées de libération étant de l’ordre de dix à quinze jours, et compte tenu de la vitesse de multiplication bactérienne dans un milieu propice à leur croissance, la libération d’antibiotiques à des concentrations potentiellement inférieures aux concentrations minimales inhibitrices ou bactéricides (CMI/CMB) des souches impliquées dans la pathologie pendant une longue durée peut avoir deux conséquences loin d’être négligeables : elle augmente à la fois le risque de sélection de souches préalablement plus résistantes tout comme en favorisant la mutation de souches préalablement sensibles.

Il convient également de noter que dans le cas des maladies parodontales, les bactéries s’organisent sous la forme de plaque dentaire. Plus précisément, il s’agit d’un biofilm bactérien, composé d’une matrice majoritairement polysaccharidique dans laquelle les bactéries vont s’organiser sous la forme d’un écosystème complexe, entretenant de très nombreuses interactions. Ce phénomène de formation de biofilm au niveau des tissus parodontaux entraine deux conséquences majeures :

- Protégées de leur environnement par leur matrice polysaccharidique, les bactéries seront globalement plus résistantes à tout type d’agression extérieure, les rendant beaucoup moins sensibles aux agents antibactériens comparées aux bactéries sous forme planctonique.
- Sous cette forme « enclavées » dans la matrice extracellulaire, les bactéries vont présenter une activité métabolique moindre. Par conséquent, elles seront également beaucoup moins sensibles aux antibiotiques

De ce fait, il convient dans la mesure du possible d’utiliser au maximum des agents antiseptiques. De par leur mécanisme d’action, ils agissent physiquement sur la paroi bactérienne pour la rendre perméable et induire la lyse de la cellule.

Ainsi, d’autres systèmes à libération locale et contrôlée de principes actifs ont été développés. On retrouve ici le Periochip®, dispositif de petite taille composé d’un film de gélatine contenant de la chlorhexidine, destiné à être placé dans la poche après la

lithotritie parodontale. Ce dispositif étant de taille unique, il n'est pas toujours capable de combler l'entièreté de la poche dans le cas de lésions profondes. Il existe également le Chlo-site®, un gel préformé à base de gomme xanthane contenant de la chlorhexidine sous forme de deux sels en association : le dichlorhydrate et le digluconate de chlorhexidine. Le principal désavantage de cette formulation est lié au comportement de l'implant après injection dans la poche parodontale : il se rétracte, laissant alors un espace non comblé pouvant être recolonisé par les bactéries.

On constate donc que malgré les nombreuses améliorations apportées à ce type de système, il reste encore de multiples aspects à optimiser. Dans l'idéal, il convient d'éviter l'utilisation d'antibiotiques et de privilégier l'utilisation de molécules antibactériennes reposant sur un autre mécanisme d'action comme les antiseptiques. Enfin, les propriétés mécaniques de l'implant peuvent également être améliorées afin d'éviter des phénomènes d'expulsion prématurée et incontrôlée du système en dehors de la poche parodontale, entrainant pas conséquent une exposition non fiable du médicament au site d'action.

L'objectif principal de cette thèse était donc de développer des implants innovants se formant *in-situ* pour le traitement des parodontites, tout du moins spécifiquement pour le traitement des poches parodontales en adjuvant de la lithotritie mécanique associée au surfaçage radiculaire. Ce travail été effectué dans le cadre d'un projet interdisciplinaire IMPERIO (*in-situ* forming implants for periodontitis treatment) financé par l'Agence Nationale de la Recherche (ANR) en collaboration avec des pharmaciens, dentistes, microbiologistes, immunologistes et physiciens. Dans ce contexte, le but de mon travail était de développer des implants se formant *in-situ* et d'élucider les mécanismes de libération des principes actifs sous-jacents à ce type de systèmes, afin de faciliter leur optimisation. Afin de répondre à ces objectifs, l'implant se formant *in-situ* devrait idéalement posséder les propriétés suivantes :

- Facilité d'injection (formulation liquide).
- Ecoulement rapide de la formulation liquide dans l'entièreté de la poche parodontale, permettant l'adaptation de la géométrie et de la taille de l'implant à la morphologie spécifique de la poche parodontale, variable selon le patient (traitement personnalisé).
- Présentant un temps de résidence fiable au sein de la poche.
- Permettant la délivrance contrôlée et simultanée d'un agent antiseptique associé à un agent anti-inflammatoire pendant des durées optimisées.
- Etre biocompatible et biodégradable.
- Présenter des propriétés mécaniques appropriées, plus spécifiquement avec une texture « chewing-gum like ».

Sur la base d'expérimentations préliminaires menées au sein du laboratoire U1008 et de données bibliographiques spécifiques à ce type de système, une formulation a été développée comprenant les éléments suivants :

- PLGA RG 502 H*:* polymère principal, compose la matrice structurelle de l'implant après échange de solvant.
- HPMC: second polymère, hydrophile. Augmente l'adhérence de l'implant à la surface de la racine dentaire.
- ATBC: plastifiant.
- PAs: Principes Actifs

L'innovation majeure de cette étude consiste à associer deux principes actifs au sein du même système à libération contrôlée. En effet, selon nos connaissances actuelles de l'état de l'art, il n'existe aucun système à libération locale et prolongée associant un antiseptique et un anti-inflammatoire, permettant leur libération simultanée afin de traiter les deux composantes de la parodontite. De plus, l'utilisation d'un antiseptique au lieu des antibiotiques utilisés actuellement, peut aider à combattre l'émergence de bactéries résistantes aux antibiotiques.

Ce travail étudie l'impact de différents facteurs sur les cinétiques de libération des principes actifs. Les trois paramètres étudiés sont :

- ➔ Le type de se de principe actif incorporé dans la formulation. **(I)**
- ➔ Le type de solvant utilisé dans la formulation. **(II)**
- ➔ Le type de milieu de libération **(III)**

Afin de mieux comprendre les mécanismes sous-jacent de libération des substances actives de ce type de formulation, différents paramètres ont été suivis et mesurés durant les études de libération :

- ✓ Mesure des quantités de principes actifs libérés (HPLC-UV/Vis)
- ✓ Absorption d'eau dans l'implant (par gravimétrie)
- ✓ Dégradation du PLGA (par GPC/SEC)
- ✓ Mesure du pH du milieu de libération
- ✓ Apparence macroscopique

(I) Quatre combinaisons de sels de principes actifs ont été réalisées en utilisant les sels hydrophiles des principes actifs (chlorhexidine digluconate [Chx diHCl] et ibuprofène lysinate [Ibu Lys]) ainsi que les formes hydrophobes de ces principes actifs (chlorhexidine dichlorhydrate [Chx diglu] et ibuprofène « acide libre » [Ibu]). Lescombinaisons suivantes ont donc été étudiées pour leur cinétiques de libération à partir des implants se formant *in-situ* dans de l'eau purifiée à 37°C.

[Chx diHCl] / [Ibu] – [Chx diHCl] / [Ibu lys] – [Chx diglu] / [Ibu] – [Chx diglu] / [Ibu lys]

Les principes actifs sont incorporés toujours selon la combinaison suivante : antiseptique / anti-inflammatoire. Les concentrations testées sont de 1.5%, 5.3% et 16.1% (m/m, basées sur la formulation totale sans solvant = concentrations théoriques)

✓ Les principaux résultats sont :

➔ Les implants se formant *in-situ* sont tous capables de libérer les deux principes actifs de manière contrôlée, simultanément sur une durée de plusieurs semaines.

➔ Augmenter les concentrations initiales des deux substances actives dans la formulation entraine généralement une diminution de la vitesse de libération relative de l'ibuprofène.

➔ Quatre solvants miscibles à l'eau ont été choisis pour formuler les implants : le N-methylpyrrolidone (NMP), le 2-pyrrolidone (2-Pyr), le dimethyl sulfoxyde (DMSO) ainsi que le lactate d'ethyle (Eth Lact).

➔ La chlorhexidine est libérée plus rapidement que l'ibuprofène dans la majorité des cas.

➔ La libération de l'ibuprofène n'est pas fondamentalement affectée par son type de sel utilisé, ni par son principe associé, malgré des différences majeures en terme de solubilité (*e.g.* facteur > 5000, cf. tableau 8)

➔ L'impact du type de sel du principe actif et de la combinaison des principes actifs n'a qu'un impact globalement modéré malgré les différences majeures de solubilités des principes actifs (*e.g.* facteur > 10 dans le NMP, facteur > 200 dans l'eau, cf. tableau 8)

(II) L'utilisation de chlorhexidine présente des avantages certains, et nombreux, de par son efficacité largement établie et son utilisation très répandue. Cependant, il a été choisi de tester une autre substance antiseptique, cette fois neutre. En effet, la chlorhexidine est un biguanide chargé deux fois positivement (bication), à l'origine de nombreuses interactions, notamment avec les éléments sanguins et les milieux complexes. Il est bien établi que la présence de sang diminue l'efficacité de la chlorhexidine. Le but étant d'insérer la formulation dans la poche parodontale après lithotritie, et donc en présence de sang et de débris cellulaires divers, il est souhaitable d'envisager des alternatives, afin d'évaluer les propriétés de la formulation en présence d'autres agents antiseptiques. Le composé testé est donc le CIN-102, définit comme « *un mélange chimiquement bien déterminé, mélange synergique développé par Septeos. La composition du CIN-102 ressemble à celle de l'huile essentielle de cannelle (table 10). Son composant majoritaire est le cinnamaldehyde (86.7% m/m). le CIN-102 possède une activité antibactérienne à spectre large et il n'est pas connu de résistance bactérienne à ce composé à ce jour »* (116). L'étude de libération des principes actifs a été réalisée comme précédemment décrite.

- ✓ Les principaux résultats sont :

➔ Les implants se formant *in-situ* sont tous capables de libérer les deux principes actifs (Cinnamaldehyde et ibuprofène) de manière contrôlée, simultanément sur une durée de plusieurs semaines.

➔ Le cinnamaldehyde est libéré plus rapidement que l'ibuprofène dans la plupart des cas

➔ Les vitesses de libération relatives du cinnamaldehyde sont quasiment les mêmes pour tous les solvants utilisés, à l'exception de la formulation comprenant du 2-Pyr à faible dosage (1.5%)

➔ Le cinnamaldehyde présente toujours le même profil de libération, à savoir une cinétique d'ordre 1 (Burst initial suivi d'une libération rapide et totale du PA)

➔ L'ibuprofène est **entièrement** libéré en trente jours, quelle que soit la formulation utilisée et la concentration en actifs de celle-ci.

➔ L'ibuprofène est presque entièrement libéré aux faibles dosages (1.5% et 5.3%), cependant, il semble encore y avoir un effet de saturation du milieu la plus haute concentration, très probablement lié au fait de la solubilité particulièrement faible de l'ibu à pH acide, de son haut dosage et, par conséquent, de l'absence de conditions sink.

(III) Le milieu de libération ayant un impact considérable sur la vitesse de solubilisation des principes actifs, la réalisation d'un test de libération dans un milieu simulant les conditions rencontrées *in-vivo* a été faite en créant de la salive artificielle, selon une recette bien établie par une équipe de chercheurs travaillant sur le sujet depuis les années 1960. Ainsi, le milieu contient de nombreux ingrédients, tels que de l'extrait de bœuf, des levures, des sels minéraux divers, de la mucine (gastrique porcine) … Tout ceci en fait un milieu de libération relativement complexe et, même s'il reste éloigné des réelles conditions *in-vivo*, il se rapproche tout de même un peu plus des conditions qu'est susceptible de rencontrer l'implant lorsqu'il va libérer ses principes actifs. L'étude de libération des principes actifs a été réalisée comme précédemment décrite.

- ✓ Les principaux résultats sont :

➔ Comme précédemment, la libération contrôlée a été supérieure à deux semaines avec les deux principes actifs

➔ Le cinnamaldehyde est libéré plus rapidement que l'ibuprofène dans la plupart des cas

➔ Les vitesses de libération relatives du cinnamaldehyde sont quasiment les mêmes pour tous les solvants utilisés, à l'exception de la formulation comprenant du 2-Pyr à faible dosage (1.5%)

➔ L'ibuprofène est **entièrement** libéré en trente jours, quelle que soit la formulation utilisée et la concentration en actif de celle-ci.

➔ L'ibuprofène présente un profil de libération triphasic particulièrement spécifique dans tous les cas : pour tous les solvants utilisés à toutes les concentrations.

En conclusion globale, ce type de système s'avère particulièrement intéressant et prometteur pour le traitement adjuvant des poches parodontales après lithotritie/détartrage et surfaçage radiculaire. Les études *in-vitro* montrent que le système peut être chargé avec un ou plusieurs principes actifs et permettre la libération simultanée de deux actifs de manière contrôlée dans le temps sur une durée supérieure à 2 semaines, ce qui est une durée cible idéale dans le contrôle de la cicatrisation parodontale après curetage + surfaçage. La modulation de la vitesse de libération du système peut être réalisée en modifiant certains paramètres que l'on peut incrémenter relativement aisément au niveau de la formulation : nature des principes actifs, nature des polymères, et d'autres excipients comme les plastifiants.

Il reste cependant de nombreuses recherches à effectuer avant de parvenir à mettre au point quelque chose de vraiment idéal, qui soit réellement supérieur aux autres produits présents sur le marché, ou autrement dit qui apporte une réelle amélioration du service médical rendu. En effet, c'est la clef du remboursement des médicaments en France. Si certains autres dispositifs tel que le Chlo-site® ont facilement obtenus une AMM, c'est parce qu'il a été réglementé en tant que dispositif médical et non pas en tant que médicament, selon le fait qu'il ne possède pas d'activité pharmacologique propre puisque c'est un dispositif contenant un antiseptique qui agit sur les bactéries présentes sur la surface dentaire, même si elle est sous-gingivale. C'est la raison de la création de ce projet IMPERIO (implants se formant *in situ* pour le traitement de la parodontite) multidisciplinaire. Réunissant 5 laboratoires autour d'une même table, la force de ce projet réside dans sa transversalité, en faisant intervenir de concert chirurgiens-dentistes, microbiologistes, immunologistes, physiciens, médecins spécialisés dans l'expérimentation animale sur des modèles spécifiques de régénération parodontale, pharmaciens spécialisés en imagerie médicale ou formulation galénique.

Parmi ces recherches à effectuer, il s'agit principalement de caractériser plus précisément l'activité antibactérienne des implants se formant *in-situ.* Le premier type de formulation conçu au laboratoire U1008 a d'abord été analysé au sein de l'unité pour évaluer ses propriétés mécaniques (135), ses propriétés physico-chimiques, en insistant particulièrement sur les analyses des cinétiques de libération des principes actifs du système et enfin l'élaboration d'une preuve de concept *in vivo* avec le laboratoire partenaire U1109 de Strasbourg (136). Cependant, l'activité bactéricide de la chlorhexidine n'est plus un secret, et c'est plutôt l'absence d'efficacité antimicrobienne qui aurait soulevé des interrogations.

La deuxième formulation quant à elle, a pu être caractérisée pour ses profils de libération mais aucune preuve de son efficacité antibactérienne n'a été mise en évidence. Ainsi, ce serait idéal de pouvoir réaliser des tests similaires à ceux faits sur l'implant contenant de la chlorhexidine comme antiseptique.

Ainsi les possibilités d'extrapolation de ce type de système à libération prolongée à d'autres principes actifs sont immenses, et les recherches s'intensifient de plus en plus, surtout ces quelques dernières années, autour de ces polymères biodégradables et biocompatibles comme le PLGA, pourtant découvert il y a plusieurs décennies, puis laissés sur la touche avant de revenir sur le devant de la scène pour leur possibilités nombreuses, offrant des variations fines et subtiles des cinétiques de libération, ainsi que la possibilité de traiter des zones particulièrement précises.

7. ANNEXES

TABLE 1A Classification of periodontitis based on stages defined by severity (according to the level of interdental clinical attachment loss, radiographic bone loss and tooth loss), complexity and extent and distribution

Periodontitis stage		Stage I	Stage II	Stage III	Stage IV
Severity	Interdental CAL at site of greatest loss	1 to 2 mm	3 to 4 mm	≥5 mm	≥5 mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (15% to 33%)	Extending to mid-third of root and beyond	Extending to mid-third of root and beyond
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local	Maximum probing depth ≤4 mm Mostly horizontal bone loss	Maximum probing depth ≤5 mm Mostly horizontal bone loss	In addition to stage II complexity: Probing depth ≥6 mm Vertical bone loss ≥3 mm Furcation involvement Class II or III Moderate ridge defect	In addition to stage III complexity: Need for complex rehabilitation due to: Masticatory dysfunction Secondary occlusal trauma (tooth mobility degree ≥2) Severe ridge defect Bite collapse, drifting, flaring Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	Add to stage as descriptor	For each stage, describe extent as localized (<30% of teeth involved), generalized, or molar/incisor pattern			

The initial stage should be determined using clinical attachment loss (CAL); if not available then radiographic bone loss (RBL) should be used. Information on tooth loss that can be attributed primarily to periodontitis – if available – may modify stage definition. This is the case even in the absence of complexity factors. Complexity factors may shift the stage to a higher level, for example furcation II or III would shift to either stage III or IV irrespective of CAL. The distinction between stage III and stage IV is primarily based on complexity factors. For example, a high level of tooth mobility and/or posterior bite collapse would indicate a stage IV diagnosis. For any given case only some, not all, complexity factors may be present, however, in general it only takes one complexity factor to shift the diagnosis to a higher stage. It should be emphasized that these case definitions are guidelines that should be applied using sound clinical judgment to arrive at the most appropriate clinical diagnosis.

For post-treatment patients, CAL and RBL are still the primary stage determinants. If a stage-shifting complexity factor(s) is eliminated by treatment, the stage should not retrogress to a lower stage since the original stage complexity factor should always be considered in maintenance phase management.

7.2. **Annexe 2: Periodontitis classification: grades (138)**

TABLE 1B Classification of periodontitis based on grades that reflect biologic features of the disease including evidence of, or risk for, rapid progression, anticipated treatment response, and effects on systemic health

Periodontitis grade			Grade A: Slow rate of progression	Grade B: Moderate rate of progression	Grade C: Rapid rate of progression
Primary criteria	Direct evidence of progression	Longitudinal data (radiographic bone loss or CAL)	Evidence of no loss over 5 years	<2 mm over 5 years	≥2 mm over 5 years
	Indirect evidence of progression	% bone loss/age	<0.25	0.25 to 1.0	>1.0
		Case phenotype	Heavy biofilm deposits with low levels of destruction	Destruction commensurate with biofilm deposits	Destruction exceeds expectation given biofilm deposits; specific clinical patterns suggestive of periods of rapid progression and/or early onset disease (e.g., molar/incisor pattern; lack of expected response to standard bacterial control therapies)
Grade modifiers	Risk factors	Smoking	Non-smoker	Smoker <10 cigarettes/day	Smoker ≥10 cigarettes/day
		Diabetes	Normoglycemic/ no diagnosis of diabetes	HbA1c <7.0% in patients with diabetes	HbA1c ≥7.0% in patients with diabetes

Grade should be used as an indicator of the rate of periodontitis progression. The primary criteria are either direct or indirect evidence of progression. Whenever available, direct evidence is used; in its absence indirect estimation is made using bone loss as a function of age at the most affected tooth or case presentation (radiographic bone loss expressed as percentage of root length divided by the age of the subject, RBL/age). Clinicians should initially assume grade B disease and seek specific evidence to shift towards grade A or C, if available. Once grade is established based on evidence of progression, it can be modified based on the presence of risk factors. CAL = clinical attachment loss; HbA1c = glycated hemoglobin A1c; RBL = radiographic bone loss.

7.3. Annexe 3 : HPLC methods

7.3.1. API concentration measurement

Separation module : Waters Alliance W2690/5

Detection module : Waters Alliance W2489

Mobile phase:

-Solution A: phosphate buffer (Et_3N 0.5%, Na_2HPO_4 115 mM, pH 2.5 adjusted with phosphoric acid) : ACN (70:30)

-Solution B: Acetonitrile (ACN)

Column: Phenomenex Gemini® 5 µm C18 110Å, 100 × 4.6 mm LC column

Flow rate: 1.5 mL/min

Column temperature: Ambient

Injection volume: 20 µL

7.3.1.1. Chlorhexidine

Mode : Isocratic

Time (min)	Flow (mL/min)	% A	% B
	1.5	100	0

Detection wavelenght : λ = 239 nm

Retention time: R_t = 2.28 min

Standards range concentrations : 1 – 100 µg/mL

7.3.1.2. Ibuprofen

Mode : Isocratic

Time (min)	Flow (mL/min)	% A	% B
	1.5	55	45

Detection wavelenght : λ = 245 nm

Retention time: R_t = 2.47 min

Standards range concentrations : 1 – 100 µg/mL

7.3.1.3. Cinnamaldehyde

Mode : Isocratic

Time (min)	Flow (mL/min)	% A	% B
	1.5	60	40

Detection wavelenght : λ = 290 nm

Retention time: R_t = 2.28 min

Standards range concentrations : 1 – 100 µg/mL

7.3.1.4. Chlorhexidine + Ibuprofen

Mode: Gradient

Time (min)	Flow (mL/min)	% A	% B
0	1.5	100	0
4.00	1.5	100	0
4.01	1.5	55	45
10.00	1.5	55	45
10.01	1.5	100	0
13.00	1.5	100	0

Detection wavelength: λ = 239 nm

Retention time: R_t Chx = 2.50 min ; R_t Ibu = 6.85 min

Standards range concentrations : [Chx] = [Ibu] = 1 – 100 µg/mL

7.3.1.5. Cinnamaldehyde + Ibuprofen

Mode: Isocratic

Time (min)	Flow (mL/min)	% A	% B
	1.5	60	40

Detection wavelength: Dual λ_{cinna} = 290 nm & λ_{Ibu} = 245 nm

Retention time: R_t Cinna = 1.70 min ; R_t Ibu = 2.85 min

Standards range concentrations: [Cinna] = 0.5 – 50 µg/mL ; [Ibu] = 1 – 100 µg/mL

7.3.2. Polymer M_w measurement

Separation module : Waters Alliance W2690/5

Detection module : Waters Alliance RI

Mobile phase: 100% THF

Column: PLGel 5 µm MIXED-D (7.5×300 mm; Agilent Technologies, Interchim, Montluçon, France).

Flow rate: 1.5 mL/min

Column temperature: 40°C

Injection volume: 50 µL

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9. PUBLICATIONS & PRESENTATIONS RESULTING FROM THIS WORK

Presentations (posters)

- K. Agossa, E. Delcourt-Debruyne, T. Rongthong, M. Lizambard, J. Siepmann, F. Siepmann. Textural properties and bioadhesion of an *in-situ* implant for the treatment of periodontal pocket 4th Congress on Innovation in Drug Delivery Site-Specific Drug Delivery, Antibes-Juan-les-Pins, Septembre 2016.

- T. Rongthong, M. Lizambard, J. Siepmann, F. Siepmann. Novel *in-situ* forming implants loaded with antiseptic and anti-inflammatory drugs for periodontitis treatment. 4th congress on innovation in drug delivery, Antibes-Juan-les-Pins, 25-28 Septembre 2016.

- T. Rongthong, M. Lizambard, J. Siepmann, F. Siepmann. Chlorhexidine-loaded, *in-situ* forming PLGA implants for periodontitis treatment: importance of the type of salt and loading. 2nd European conference on pharmaceutics: drug delivery, 3-4 April 2017, Krakow, Poland.

- M. Lizambard, T. Menu, J. Siepmann, F. Siepmann. Chlorhexidine/ibuprofen-loaded, *in-situ* forming implants for periodontitis treatment: Ibuprofen free acid vs. ibuprofen lysinate. 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, 19-23 Mars 2018.

- M. Lizambard, T. Menu, J. Siepmann, F. Siepmann. Chlorhexidine/ibuprofen-loaded, *in-situ* forming implants for periodontitis treatment: Chlorhexidine diHCl vs. digluconate. 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, 19-23 Mars 2018.

- M. Lizambard, T. Menu, J. Siepmann, F. Siepmann. *In-situ* forming PLGA implants for periodontitis treatment: importance of the drug loading. 3nd European conference on pharmaceutics: drug delivery, 25-26 March 2019, Bologna, Italy.

- M. Lizambard, M. Fossart, C. Neut, J. Siepmann, F. Siepmann. *In-situ* forming PLGA implants for periodontitis treatment: importance of the type of solvent. 3nd European conference on pharmaceutics: drug delivery, 25-26 March 2019, Bologna, Italy.

Presentations (Talks)

- M. Lizambard, T. Menu, J. Siepmann, F. Siepmann. *In-situ* forming PLGA implants loaded with chlorhexidine and ibuprofen for the treatment of periodontitis. Pharmaceutical Solid State Research Cluster (PSSRC), 12th Annual Symposium, Leuven, Belgium, 2018.

Articles

- K. Agossa, M. Lizambard, T. Rongthong, E. Delcourt-Debruyne, J. Siepmann, F. Siepmann. Physical key properties of antibiotic-free, PLGA/HPMC-based *in-situ* forming implants for local periodontitis treatment. Int J Pharm. (2017).

- Batool F, Agossa K, Lizambard M, Petit C, Bugueno IM, Delcourt-Debruyne E, Benkirane-Jessel N, Tenenbaum H, Siepmann J, Siepmann F, Huck O. In-situ forming implants loaded with chlorhexidine and ibuprofen for periodontal treatment: Proof of concept study in vivo. Int J Pharm. (2019).

- M. Lizambard, T. Menu, M. Fossart, C. Bassand, K. Agossa, O. Huck, C. Neut, F. Siepmann. In-situ forming implants for the treatment of periodontal diseases: Simultaneous controlled release of an antiseptic and an anti-inflammatory drug. Int J Pharm. (2019).

Abstract

Periodontal diseases are highly prevalent and widely spread in all human populations. Their consequences are multiples, strongly impacting general quality of life, general health and represent also a global economic burden. They are known to be triggered by a bacterial infection followed by an inadequate inflammatory body response. These mechanisms result in the destruction of the supporting tissues of the tooth (gingiva, ligament and alveolar bone), finally leading to tooth lost. Moreover, periodontal health status has been shown to be correlated with various other conditions and it is believed to worsen other systemic conditions like diabetes mellitus for example. Gold standard treatment of periodontitis is a procedure called scaling and root planning (SRP), consisting of a subgingival mechanical removal of dental plaque and/or calculus. In clinical practice, systemic treatments such as antibiotics are often added to SRP, causing various side effects, without being particularly optimal. Thus, the need to improve the management of periodontitis can be met by developing local drug delivery systems. The aim of this work was to develop and physico-chemically characterise an *in-situ* forming implant (ISFI). This particular drug delivery system (DDS) is a liquid formulation that will form a solid implant upon injection into the periodontal pocket, releasing two active pharmaceutical ingredients at a controlled release rate. In order to avoid the rise of bacterial resistances against antibiotics, this specific DDS is loaded with antiseptic drugs (*e.g* chlorhexidine). Secondly, to promote periodontal wound healing, an anti-inflammatory agent (*e.g.* ibuprofen) is added to the formulation. This work will firstly describe the state of the art regarding these DDSs, with an emphasis on ISFIs. Then, the experimental section will describe the formulations and methods used to characterize the resulting implants. The effects of the formulation parameters on the resulting physico-chemical properties of the systems and in particular the release kinetics will be discussed with respect to the clinical use requirements for this kind of DDS.

Key-words: Periodontitis, PLGA, Chlorhexidine, Ibuprofen, Controlled release, *In-situ* forming implant.

Résumé

Les maladies parodontales, de prévalence élevée, sont largement répandues à travers les populations humaines. Leurs conséquences sont multiples, altérant fortement la qualité de vie comme la santé globale et ont un impact économique important. Elles sont la conséquence d'une infection bactérienne à laquelle est associée une réponse inflammatoire inadéquate. Ces mécanismes entrainent la destruction des tissus de soutien de la dent (gencive, ligament, os alvéolaire) menant à la perte dentaire. De plus, il est prouvé que le statut parodontal est associé à de nombreuses autres pathologies et on estime que la parodontite aggrave certaines autres pathologies comme le diabète par exemple. Le traitement de référence consiste en l'élimination mécanique de la plaque dentaire sous-gingivale, opération appelée surfaçage radiculaire. En pratique, ce geste est souvent accompagné de la prise d'antibiotiques par voie systémique, engendrant de nombreux effets indésirables et dont l'efficacité reste à améliorer. Ainsi, la nécessité d'améliorer la prise en charge de la maladie peut être comblée par le développement de systèmes à libération locale de substances actives. L'objectif de ce travail consiste à développer et caractériser physico chimiquement un implant se formant *in-situ* (ISFI). Ce système très spécifique est une formulation liquide destinée à former un implant solide après injection dans la poche parodontale, libérant deux substances actives de manière contrôlée. Afin d'éviter l'augmentation des résistances bactériennes vis-à-vis des antibiotiques, ce dispositif est formulé avec des antiseptiques (ex. chlorhexidine). Ensuite, afin de favoriser la cicatrisation parodontale, un agent anti-inflammatoire est également utilisé (ex. ibuprofène). Ce travail décrit dans un premier temps l'état des lieux sur ce type de dispositif et en particulier les implants se formant *in-situ.* Ensuite, la section expérimentale décrit la formulation des implants et les méthodes utilisées pour les caractériser. Les effets des paramètres de formulation sur les propriétés physico-chimiques des systèmes et en particulier les cinétiques de libération sont discutées au regard des besoins de la pratique clinique pour ce type de dispositif.

Mots-clefs: Parodontite, PLGA, Chlorhexidine, Ibuprofène, Libération contrôlée, Implants se formant *in-situ.*