Chapter 9

Hyaluronic-Based Antibacterial Hydrogel Coating for Implantable Biomaterials in Orthopedics and Trauma: From Basic Research to Clinical Applications

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Additional information is available at the end of the chapter

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Abstract

Bacterial colonization of implanted biomaterials remains one of the most challenging complications in orthopedics and trauma surgery, with extremely high social and economic costs. Antibacterial coating of implants has been advocated by many experts as a possible solution to reduce the burden of implant-related infection and several different solutions have been proposed in the last decades. However, while most of the investigated technologies have shown their efficacy *in vitro* and/or *in vivo*, only few were able to reach the market, due to clinical, industrial, economic and regulatory issues. Hyaluronic acid composites have been previously shown to possess antifouling capabilities and have been used in various clinical settings to reduce bacterial adhesion and mitigate biofilm-related infections. Recently, a fast-resorbable, hyaluronic-based hydrogel coating was developed to protect implanted biomaterials in orthopedics, trauma and maxillofacial surgery. Preclinical and clinical testing did show the safety and efficacy of the device that can be intraoperatively loaded with one or more antibiotics and directly applied by the surgeon to the implant surface, at the time of surgery. Here, we review the current evidence concerning this very first antibacterial coating of implants and outline the economic impact of the possible large-scale application of this technology.

Keywords: coating, hydrogel, hyaluronic acid, DAC, infection, implant, orthopedic, trauma, prosthesis, prevention

1. Introduction

Up to 80% of human bacterial infections are biofilm-related, according to the U.S. National Institutes of Health [1]. Among these, implant-related infections in orthopedics and trauma still have a tremendous impact [2]. In fact, periprosthetic joint infection (PJI) (**Figure 1**) is among the first reasons for implant failure [3], posing challenging diagnostic and therapeutic dilemmas [4] and with high economic and social costs [5–7].

Similarly, surgical site infections after osteosynthesis, with a reported incidence ranging from 3.9 to 10% for closed fractures [8–11] and even more after open fractures [12], are associated with high morbidity and possible mortality raise [9] and elevated costs [13].

Whenever a biomaterial is implanted, a competition between host and bacterial cells occurs for surface colonization. In the event of bacterial adhesion to an implant, immediate biofilm formation starts, making the bacteria extremely resistant to host's defense mechanisms and to antimicrobials [14–16]. According to recent evidence, fully formed biofilm can be found few hours after the first bacterial adhesion [17]; thus, the destiny of an implant is decided at the very time of surgery.

To reduce or prevent bacterial adhesion and biofilm formation, a number of different antimicrobial finishing or coatings of implants are under study [18]. However, their clinical application appears particularly challenging, due to the many requirements they need to fulfill [19].

Hyaluronic acid (HA) is mucopolysaccharide, occurring naturally in mammals. It is abundant in skin and in connective tissues, being one of the main components of extracellular matrices. HA has several clinical applications in dermatology, esthetic surgery, dentistry, urology, orthopedics and ophthalmology [20]. In fact, due to its high biocompatibility, and nonimmunogenicity, hyaluronic acid is considered as an ideal biomaterial for medical and pharmaceutical applications [21, 22].

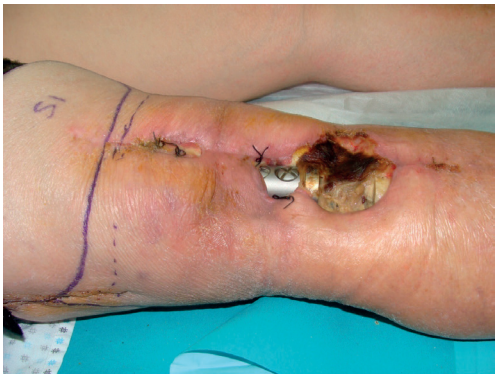

Figure 1. Infected, exposed, knee prosthesis in a 60-year-old woman. Approximately one million joint replacements are performed annually in Europe, and infection is currently among the first three most common reasons for failure of implants. Septic complications are associated with prolonged and complex medical and surgical treatments, often leading to implant removal. Poor functional results, possible infection recurrence, risk of amputation and increased mortality rate are all well known and feared consequences of periprosthetic and implant-related infections. Direct costs of treatment of periprosthetic infection exceeds 100,000 euros, per case, according to a recent analysis [7].

Local application of hyaluronic-based compounds has been demonstrated to be protective against various infectious agents, depending on HA concentration and molecular weight; furthermore, HA's ability to reduce bacterial adhesion and biofilm formation has been recently reported [23].

High biocompatibility, safety profile and antiadhesive properties make HA and its composites a possible non-antibiotic option to reduce the impact of biofilm-related infections in various clinical settings. However, the use of HA in its pure form as an antibacterial coating does not appear suitable, due to its rapid degradation by hyaluronidases, enzymes naturally occurring in the human and animal body. Furthermore, due to its high hydrophilicity, a coating produced with a hydrogel of HA alone would not have sufficient mechanical stability when a prosthesis is implanted in the body, which is an essentially water-based environment.

To overcome these limits, a combination of HA with another biocompatible and biodegradable polymer, polylactic acid (PLA), was investigated [24]. In fact, PLA is a synthetic polyester, approved in the U.S.A. by the Food and Drug Administration (FDA) and widely used for orthopedic implants [25]. PLA unlike HA, shows a hydrophobic character; therefore, its presence could be exploited to control in appropriate way the hydrophilic and mechanical properties of a hydrogel based on HA, thus slowing down the susceptibility to hydrolysis.

Here, after an overview of the antiadhesive and antibiofilm properties of HA, we summarize the development of a CE-marked, patented hydrogel coating, based on HA grafted to PLA (DAC®, "Defensive Antibacterial Coating," Novagenit Srl, Mezzolombardo, Italy). Some of the most relevant preclinical and clinical results that made this device the very first resorbable antibacterial coating for large-scale clinical applications in orthopedic, trauma, dentistry and maxillofacial implants are also briefly reported.

2. Antiadhesive and antibiofilm properties of HA

Pavesio et al. [26] were probably the first to describe HA nonfouling properties and its ability to resist bacterial adhesion, with particular reference to *Staphylococcus epidermidis* [27], proposing coated polymeric medical devices to reduce implant-related infections. In particular, a hydrophilic HA overlayer, linked to the surface of polymethylmethacrylate intraocular lenses (IOLs), was shown to be able to significantly reduce the adhesion of *Staphylococcus epidermidis* to the implant surface [28].

In line with this observation, Kadry and coworkers, reported the ability of hyaluronan to reduce bacterial adhesion to IOLs of a *S. epidermidis* wild strain [29]; based on these findings, the authors proposed the use of HA as an antiadhesive, adjuvant therapy, in combination with antibiotics in irrigating solutions for bacterial ocular infections.

More recently, Drago et al. reported on the *in vitro* antiadhesive and antibiofilm activity of HA toward bacterial species commonly isolated from respiratory infections [30]. In this experimental study, HA was shown to be able to reduce bacterial adhesion to a cellular substrate in a concentration-dependent manner. The antibiofilm action, exerted by HA in ear, nose and throat districts, has been recently reviewed [31]. The authors conclude that "its efficacy in treating

rhinosinusitis, whether or not associated with polyposis, is well documented, as well as results from its effects on mucociliary clearance, free radical production and mucosal repair."

HA has also been reported to exert bacteriostatic, dose-dependent effect on different planktonic microorganisms [32, 33]. Radaeva et al. showed the inhibiting activity of HA with respect to some *Pseudomonas* species [34], while Ardizzoni and coworkers [23] investigated the effects of HA on 15 ATCC bacterial strains, representative of clinically relevant bacterial and fungal species. According to their results, different microbial species and strains are differently affected by HA. In particular, staphylococci, enterococci, *Streptococcus mutans*, two *Escherichia coli* strains, *Pseudomonas aeruginosa*, *Candida glabrata* and *C. parapsilosis* showed a dose-dependent growth inhibition, while no HA effects were detected in *E. coli* ATCC 13768 and *C. albicans*, and *S. sanguinis* was favored by the highest HA dose.

Carlson and coworkers [33] compared the potential bacteriostatic effect of collagen type I, hyaluronic acid, hydroxyapatite, polylactic acid and polyglycolic acid on some of the most common orthopedic bacterial pathogens (*S. aureus*, *S. epidermidis*, *β-hemolytic Streptococcus* and *Pseudomonas aeruginosa*): HA had the most significant bacteriostatic properties on the studied organisms. Similarly, Pirnazar et al. [32] did demonstrate the bacteriostatic effect of HA in different concentrations and molecular weight on oral and nonoral microorganisms (*Staphylococcus aureus*, *Propionibacterium acnes*, *Actinobacillus actinomycetemcomitans*, *Prevotella oris* and *Porphyromonas gingivalis*). The authors concluded that the clinical application of hyaluronan in the form of membranes, gels or sponges may reduce bacterial contamination of the surgical wound, thereby lessening the risk of postsurgical infection and promoting more predictable regeneration.

Concerning orthopedic applications, Harris and Richards [35] showed how coating titanium with sodium hyaluronate significantly decreased the density of *S. aureus* adhering to the surfaces and proposed its potential use to protect osteosynthesis, orthopedic or dental implants.

In a recent review, focused on the use of polysaccharide-based coatings to prevent biofilm formation, hyaluronic acid was discussed as one of the most promising [36]; displaying hydrophilic characteristics, this coating was in fact reported to reduce adhesion of *S. aureus*, *S. epidermidis* and *E. coli* by several orders of magnitude compared to unmodified surfaces.

3. Clinical applications of HA to prevent bacterial adhesion

Several clinical local applications of HA to reduce the impact of biofilm-related infections have been reported with favorable results and no adverse events [37].

Torretta et al. [38] recently described topical administration of hyaluronic acid in children with recurrent or chronic middle ear inflammations and chronic adenoiditis.

Other studies have documented the positive effect of topical HA in chronic urinary tract infections (UTI). At variance with current antibiotic treatments, aimed at eradicating pathogens, HA local administration targets bacterial adherence to the bladder mucosa [39–42]. Damiano et al., in a prospective, randomized, double-blind, placebo-controlled study, showed a significant reduction of 77% ($P < 0.0002$) in the UTI rate per year in HA-treated patients, compared to controls. Moreover, mean time to UTI recurrence was significantly prolonged (185.2 ± 78.7

vs. 52.7 ± 33.4 days, $P < 0.001$) after HA treatment, compared with placebo [43]. No adverse events were reported. A recent multicenter European study confirmed the efficacy of intravescical administration of combined HA and chondroitin sulfate (CS) for the treatment of female recurrent urinary tract infections [44].

In dentistry, the effect of the application of HA-containing gels in early wound healing after scaling and root planing (SRP) on clinical variables, subgingival bacteria and local immune response was investigated [45, 46]. Eick et al. [47] reported on 34 individuals affected by chronic periodontitis and treated with full-mouth SRP; in the test group (n = 17), a 0.8% hyaluronan-containing gel was introduced into all periodontal pockets during SRP and a 0.2% HA gel was applied by the patients onto the gingival margin twice daily during the following 2 weeks, while the control group (n = 17) was treated with SRP only; no placebo was used. Probing depth (PD) and clinical attachment level (CAL) were recorded at baseline and after 3 and 6 months, and subgingival plaque and sulcus fluid samples were taken for microbiologic and biochemical analysis. The changes in PD and the reduction of the number of pockets with PD ≥ 5 mm were significantly higher in the test group after 3 ($P = 0.014$ and 0.021) and 6 ($P = 0.046$ and 0.045) months. Six months after SRP, the counts of *Treponema denticola* were significantly reduced in both groups (both $P = 0.043$), as were those of Campylobacter rectus in the test group only ($P = 0.028$). *Prevotella intermedia* and *Porphyromonas gingivalis* increased in the control group. No adverse effects of HA were observed during the study.

4. Synthesis of DAC® HA-g-PLA hydrogel coating

Composed of covalently linked hyaluronan and poly-D,L-lactide, the "Defensive Antibacterial Coating" (DAC®, Novagenit Srl, Mezzolombardo, Italy) was specifically developed in order to protect implanted biomaterials used in orthopedics, traumatology, dentistry and maxillofacial surgery from bacterial colonization [24, 48] (**Figure 2**).

Figure 2. DAC® HA-g-PLA, fast-resorbable, hydrogel coating. Composed of covalently linked hyaluronan and poly-D, L-lactide, the "Defensive Antibacterial Coating" (DAC®, Novagenit Srl, Mezzolombardo, Italy) is the first antibacterial-coating cleared for clinical use in orthopedics, trauma, dentistry and maxillofacial surgery in Europe.

Preparation of the hydrogel was performed according to a patented procedure [49]. In particular, HA-g-PLA copolymer was dispersed in an appropriate volume of twice distilled water, and the mixture was stirred vigorously at the vortex to obtain a gelatinous and transparent hydrogel with a polymer concentration between 3% (w/v) and 10% (w/v).

The synthesis of HA-g-PLA copolymer was performed as previously reported [50, 51] (**Figure 3**). Briefly, a low weight–average molecular weight HA (HALMW) was made soluble in organic solvents by transformation to its tetrabutylammonium (TBA) salt. The synthesis of the N-hydroxysuccinimide (NHS) derivative of PLA (i.e., PLA-NHS) was performed as reported elsewhere [52]. In particular, 2.4 g of PLA was dissolved in 30 ml of anhydrous dichloromethane with an excess of DCC and NHS for 24 h at room temperature, and then the solution was precipitated in ethanol and the recovered solid was dried under vacuum. 1H NMR of PLA-NHS ($CDCl_3$) showed: δ 1.5 and δ 1.6 (d, 3H, —O—CO—CH(CH_3)—OH; d, 3H, —O—CO—CH(CH_3)—O—),

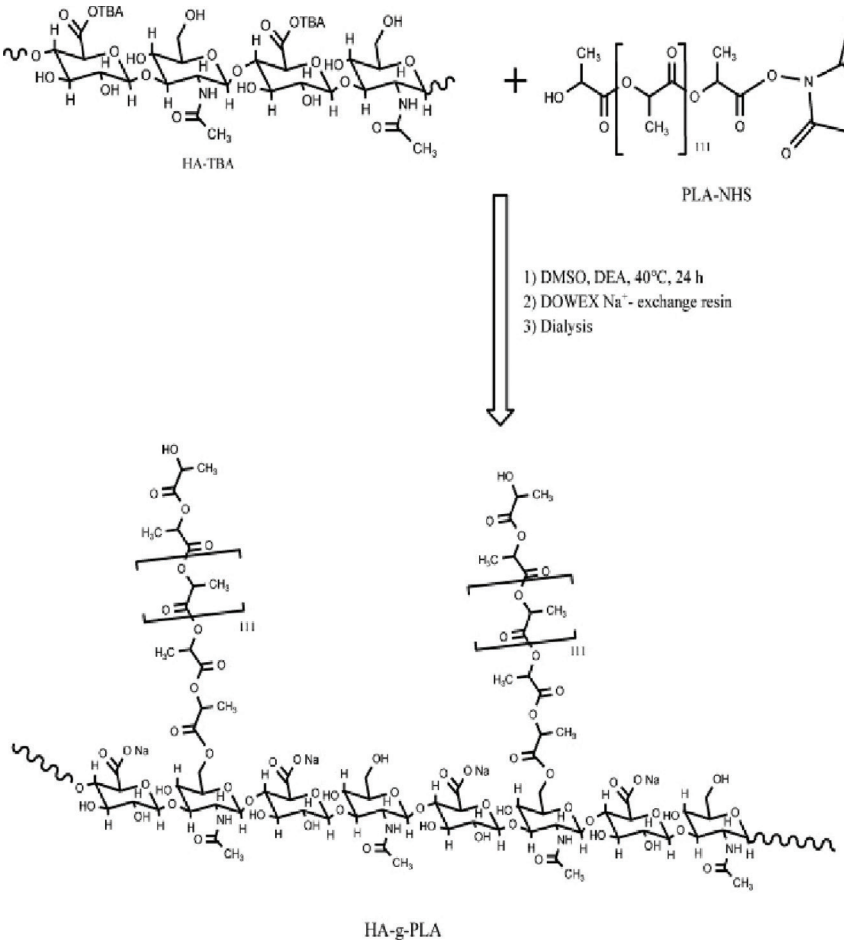

Figure 3. Principal steps in the synthesis of HA-g-PLA copolymer.

δ 2.80 (m, 4H, ─OC─CH_2─CH_2─CO─); δ 4.3 and δ 5.2 (m, 1H, ─O─CO─**CH**(CH_3)─OH; m, 1H, ─O─CO─**CH**(CH_3)─O). The synthesis of HA-g-PLA copolymer was carried out as follows: 600 mg of HA-TBA was dissolved in 48 ml of anhydrous dimethyl sulfoxide (DMSO) and then 576 μl of DEA, as a catalyst, was added. A suitable amount of PLA-NHS (dissolved in 6 ml of anhydrous DMSO) was added according to $X = 1$, X being equal to moles of PLA-NHS/moles of HA repeating units. The PLA-NHS solution was added drop by drop to the HA-TBA solution in about 1 h. The reaction was carried out under argon at 40°C for 24 h. After this time, the TBA was exchanged with Na^+ using a Dowex 50 W × 8-200 resin, and then the eluate was dialysed against distilled water, by using spectra/por tubing with a cutoff of 14,000 Da and then freeze-dried. The sample has been characterized by FT-IR and 1H NMR analyses. FT-IR spectrum (KBr) of HA-g-PLA showed a broad band centered at 3450 cm^{-1} (ν as OH + ν as NH of HA), bands at 1757 (ν as COO of PLA), 1623 (amide I of HA), 1456 (δ as CH_3 of PLA), 1382 (δ as CH_3 of PLA), 1189 (ν as C─O─C ester group of PLA), 1089, 1048 (ν C─O alcoholic and ether of HA) cm^{-1}. 1H NMR of HA-g-PLA (DMSO-d_6/D_2O 90:10) spectrum showed: δ 1.25 and δ 1.45 (2d, ─O─CO─CH(CH_3)─O─ of PLA); δ 1.85 (s, 3H, ─NH─CO─CH_3 of HA) δ 5.1 ppm (m, ─O─CO─CH(CH_3)─ of PLA). The % degree of grafting (DG) has been calculated as: %DG = (moles PLA chains/moles of HA repeating units) × 100. The degree of grafting was determined by comparing the integral of the peaks at δ 1.25–1.45 attributed to protons of methyl groups of PLA with the integral of peaks at δ 1.85 attributed to protons of NHCO**CH_3** belonging to N-acetylglucosamine residue of HA and resulted to be 7 ± 1 mol%.

5. DAC® hydrogel *in vitro* activity

5.1. Cell compatibility assay

In vitro cell compatibility of DAC® HA-g-PLA hydrogel (polymer concentration 6%, w/v) was evaluated using human dermal fibroblasts. The viability of cells cultured in direct or indirect contact with HA-g-PLA hydrogel was comparable with that of the control well, showing that the hydrogel does not release in the culture medium substances that interfere with cell viability and they do not cause a decrease in the cell viability after direct contact with them [24]. Further *in vitro* and *in vivo* biocompatibility studies were performed on the DAC® hydrogel and on the DAC® kit, in accordance to ISO standards, all showing no cytotoxicity, genotoxicity, sensitization, irritation or intracutaneous reactivity, systemic toxicity (acute), subchronic toxicity or interference with bone or periimplant tissues.

Furthermore, as degradation of DAC® HA-g-PLA hydrogel occurs via deesterification of hyaluronic acid and polylactic acid, it gives raise exclusively to the starting macromolecules, whose degradation pathways in the human body are widely known and whose use as implantable class III medical devices is largely accepted and tested safe.

5.2. Antiadhesive and antibiofilm activity

Both the ability of the DAC® HA-g-PLA hydrogel to reduce bacterial adhesion and biofilm formation were extensively studied *in vitro*.

Reductions of adhered bacteria on sterile titanium discs, coated with DAC® hydrogel, equal to 86.8, 80.4, 74.6 and 66.7% vs. untreated discs were observed after 15, 30, 60 and 120 min of incubation, respectively [37]. In another experiment, the ability to dislodge previously adhered bacteria was investigated. Once again, the results showed that DAC® hydrogel treatment of discs reduced the amount of adhered bacteria in respect to control discs after 15, 30, 60 and 120 min by 84.0, 72.8, 72.3 and 64.3%, respectively [37].

Concerning more specifically the antibiofilm activity, DAC® hydrogel showed similar or superior *in vitro* activity, compared to various antibacterials and a synergistic activity when used in combination [48]. In one experimental setting, *S. epidermidis* and *S. aureus* were grown on chrome-cobalt devices in 6-wells polystyrene plates containing TSB for 24 h at 37°C. The plates were incubated at 37°C in ambient air, until a visible biofilm was obtained. Gentamycin and vancomycin were tested at a final concentration of 20 mg/mL. Similarly, when mixed with the hydrogel, 60 mg of gel powder was reconstituted with 1 mL of water for injections containing gentamicin or vancomycin at 20 mg/mL concentration. The amount of biofilm at each time was determined before hydrogel and antibiotic agents' addition and after 0.5, 1, 2, 4, 6, 24 and 48 h of incubation by a spectrophotometric assay. At each time point, both gentamicin and vancomycin showed only a partial inhibition of biofilm formation (ca. 30–40% for gentamicin; ca. 40–50% for vancomycin), with minor difference between the two studied microorganisms. On the other side, the hydrogel alone resulted in a significant reduction of biofilm of ca. 50%, in comparison to the untreated controls, while a combination of the hydrogel with either antibacterial coating resulted in a larger reduction of biofilm formation (approximately 75–80% in comparison with untreated controls).

Both these experimental studies show the ability of the DAC® hydrogel to significantly reduce bacterial adhesion and biofilm formation of common bacterial pathogens, thus potentially providing an effective protection of the implant; however, these data also point out how, in the clinical setting, in the absence of an adequate immune response from the host and/or of sufficient local levels of antibiotics, a passive antiadhesive coating [18] like HA can be overcome by the remaining bacteria in a time-dependent manner. For this reason, any passive antiadhesive coating of implants [53] should probably better be seen as a tool to reduce and delay bacterial adhesion and biofilm formation to a variable degree, also depending on the local environment, the contaminating bacterial species and initial bacterial load; this activity of the coating may represent a key additional advantage to the host's cells to win the competition with the microorganisms that may eventually be present. However, the known intrinsic limits of all passive coatings ground the idea of adding antibacterial agents to the protective hydrogel, in order to minimize the possibility for planktonic bacteria, which may eventually remain in the local environment, to colonize the implant at a second stage, when the coating has been hydrolyzed or covered by host's proteins.

5.3. Antibiotic release studies

Although designed as a "stand alone" product, the DAC® hydrogel was also tested concerning its ability to entrap and eventually release locally various antibacterial agents. As outlined above, the rationale for this combination lies in the pathogenesis of implant-related infections and on the specificities of passive protective coatings. In fact, biofilm formation is a multistep

process that schematically includes the bacterial adhesion to a substrate, the subsequent immediate release of signals from adherent bacteria that triggers biofilm production and, finally, the biofilm construction and progressive consolidation. Acting mainly as a physical and antiadhesive barrier, DAC® hydrogel may reduce or prevent the first phase of the process, provided that the number of living bacteria is not too high and that they are not able to overcome or hydrolyze the hydrogel [54]; moreover, for an effective prevention of bacterial colonization of the coated implant, it is necessary that, while bacteria are in the more vulnerable planktonic state, they are completely removed or killed by the host's immune system and/or by the local chemicophysical environment. This is why, even in the presence of DAC® coating protection, systemic antibiotic prophylaxis is still to be considered necessary. In fact, if not eliminated, the remaining floating microorganisms may successfully colonize the implant once the protective coating has been hydrolyzed (a phenomenon that is expected to happen normally within 3 days from application for the DAC hydrogel) or after the implanted biomaterial has been covered by host's proteins (fibrin, fibronectin, etc.), which may also work as suitable for bacterial adhesion. In this scenario, the possibility to add also an antibacterial drug to the coating may further contribute to reduce the planktonic microorganisms, enhancing the overall protection offered by the DAC hydrogel (**Figure 4**).

To test the hydrogel ability to entrap antibacterial drugs, vancomycin and tobramycin had been originally chosen as examples of antibiotic molecules [24]. Both these antibiotics have been added to the hydrogel just before its use, a solution that offers several advantages. First of all, this allows to add the antibiotic when it is needed, thus avoiding the problems of shelf-life and any long-term compatibility with the hydrogel; secondly, in this way, it is possible to choose the optimal antibiotic in the specific case, taking into account the patient's specificity (e.g., known intolerances to specific antibacterial agents) or of the specific intervention; finally, the dosing of the antibacterial agent on a case-by-case basis can be decided by the clinician. The results of the *in vitro* study clearly showed how the investigated antibacterial hydrogel coating, applied on a titanium disc, at a concentration in polymer in the range 2–8% (w/v) and a concentration in drug equal to 1 or 2% (w/v) is able to release vancomycin or tobramycin, or of their association, for up to 72 h, with an amount of drug released that is hundred or thousand times higher than the minimum inhibitory concentration (MIC), in a time- and dose-dependent manner.

Similar results were founded by testing several other antibacterial compounds or their combinations, including vancomycin, teicoplanin, rifampicin, daptomycin, tigecycline, cefazolin, gentamicin, tobramycin, amikacin, meropenem, levofloxacin, etc. (cf. **Figure 5**), at concentrations ranging from 2 to 10% [48].

In summary, all examples reported above show that DAC® hydrogel is potentially able to entrap and release suitable quantities of antibacterial agents just after the implant of the coated prosthesis. The high initial burst effect of the released drug may ensure the most efficient action at the time that is critical for the destiny of the biomaterial. Moreover, during the entire drug release period, the antibiotic concentration released by the hydrogel remains greater than MIC, thus further ensuring effectiveness of the drug released in proximity to the prosthesis.

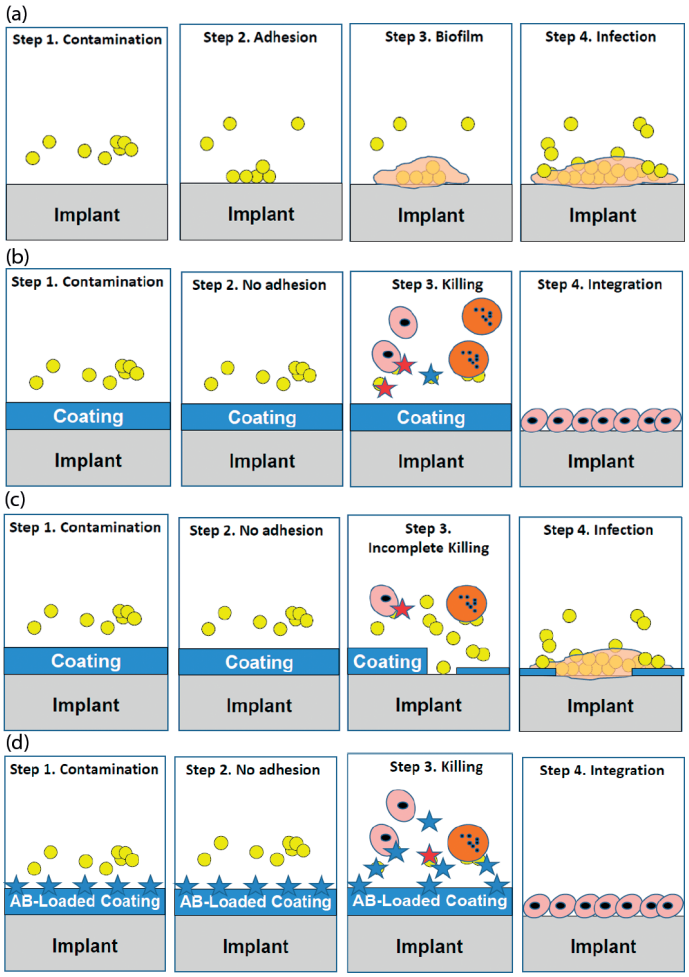

Figure 4. Rationale for intra-operative mixing of DAC® hydrogel coating with antibacterial agents. Schematic representation of different scenarios. (a) Noncoated implants may get colonized by biofilm-forming bacteria (yellow circles) and infection will develop. (b) Antiadhesive coating may reduce/prevent bacterial adhesion, while the immune system (orange circles and red stars) and the systemically administered antibiotics (blue star) kill planktonic microorganisms. (c) However, if bacterial load is large enough, or if immune response and local antibiotic levels are inadequate, surviving bacteria may eventually colonize the implant, once the coating has been hydrolyzed or covered by host's proteins. (d) To prevent this, the antibacterial hydrogel may be loaded, at the time of surgery, with antibiotic agents (blue stars) that may be locally released, contributing to eliminate all remaining planktonic bacteria.

5.4. DAC® hydrogel coating ability

For any device candidate to act as a coating of orthopedic and trauma biomaterials, mechanical adherence to the implant surface plays a key role. In particular, DAC® hydrogel has been designed to be spread manually at the time of surgery and to not interfere with the usual surgical techniques of press-fit insertion of an implant. The ability of DAC® hydrogel to completely cover even sand-blasted titanium surface and resist scraping has been confirmed by

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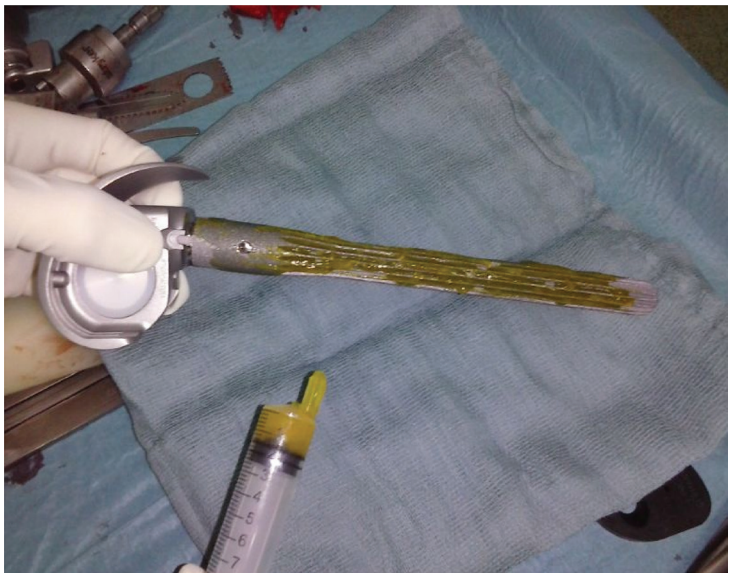

Figure 5. Tigecycline-loaded DAC® hydrogel coating, applied at surgery on a knee revision prosthesis. The hydrogel, which comes in a powder form, in a prefilled syringe, is designed to be reconstituted at the time of surgery with water for injection. The surgeon may decide to add a single or a combination of antibiotics to the water for injection, to further enhance implant protection.

scanning electron microscopy (SEM) analysis [37]. This is an important requirement in order to reduce the exposed surface of a biomaterial, thus creating a uniform coating of the surface and leaving no pores or cracks that could eventually be colonized by planktonic bacteria.

The resistance to scraping and declothing has also been tested in the animal model and in human femurs, simulating a press-fit insertion of a cementless implant [24, 48]. Both studies demonstrated the ability of the hydrogel coating to resist insertion, with 60% to more than 80% of the hydrogel remaining adherent to the entire implant surface, while the remainder being retrieved along the inner surface of the medullary canal.

6. DAC® hydrogel *in vivo* activity

Safety and efficacy of the DAC® hydrogel have been investigated in several animal studies.

Concerning efficacy, in an acute model of highly contaminated implant-related infection in the rabbit, Giavaresi and coworkers [55] found that a vancomycin-loaded DAC® coating was associated with local bacterial load reduction ranging from 72 to 99%, compared to uncoated controls.

In another large investigation in the rabbit model, Boot et al. [56] showed, at longer follow-up and without systemic antibiotic prophylaxis, the ability of vancomycin-loaded DAC®-coated implants to significantly resist infection, compared to uncoated controls. Both studies did also reveal the absence of local or systemic side effects. In line with this observation, a more

recent paper, focused on the impact on bone healing and implant osteointegration, reported no detrimental effects of vancomycin-loaded DAC® or of DAC® alone [57].

Another study on a rat model of acutely infected osteosynthesis did provide evidence that vancomycin-loaded DAC®-coated plates and screws not only are associated with a significant reduction of infection but also protect from the occurrence of septic nonunion, compared to uncoated implants [58]. This study is the very first demonstration that bone healing in a contaminated fracture can be improved by using an osteosynthesis coated with a fast-resorbable, antibiotic-loaded hydrogel.

7. Clinical results and applications

The DAC® hydrogel received the CE mark at the end of year 2013. The available kit (www.coatingdac.com or www.dac-coating.com) is composed of a prefilled syringe, containing 300 mg sterile DAC® powder, that is filled at surgery with a solution of 5 mL sterile water for injection, eventually mixed with the desired antibiotic(s); this allows to obtain, in approximately 3–5 min, the antibiotic-loaded hydrogel, at a DAC® concentration of 6% (w/v) and at an antibiotic concentration usually ranging from 20 mg/mL to 50 mg/mL, depending on the surgeon's choice. The surgeons can choose the antibiotic from among a list of antibacterials previously tested as being compatible with the hydrogel (Novagenit SRL, data on file). A few minutes after reconstitution, the hydrogel can be directly spread onto the implant, which is then inserted into the body in the usual way. If necessary, once reconstituted, the hydrogel may remain at ambient temperature for up to 4 h.

Two large multicenter, randomized, prospective clinical trials were undertaken in Europe, within the 7th European Framework Programme (project # 277988), funded by the European Commission.

In a first trial, a total of 380 patients, scheduled to undergo primary (n = 270) or revision (n = 110) total hip (N = 298) or knee (N = 82) joint replacement with cementless or hybrid (partially cemented) implants were included [59, 60]. The patients were randomly assigned, in six European orthopedic centers, to receive an implant either with the DAC® coating, intraoperatively loaded with antibiotics (treatment group), or without the coating (control group). Pre- and postoperative assessment of clinical scores, wound healing, laboratory tests and X-ray exams were performed at fixed time intervals. Overall, 373 patients were available at a mean follow-up of 14.5 ± 5.5 months (range 6–24). On average, a volume of 8.3 mL hydrogel was used to coat an implant. The most often used antibiotics were vancomycin and gentamicin at a concentration of 5% and 3.2%, respectively. Fifteen patients received an implant with a combined vancomycin and meropenem antibiotic coating; four patients received an implant coated with teicoplanin 5% or ceftazidime 5% or amphotericin B 5%, all in a second-stage procedure for previous infection. Eleven surgical site infections were observed in the control group and only one in the treatment group (6% vs. 0.6%; P = 0.003). No local or systemic side effects related to the DAC® hydrogel coating were reported and no detectable interference with implant osteointegration was noted.

In the other multicenter, prospective study, 256 patients, undergoing osteosynthesis for a closed fracture, were randomly assigned, in 5 European orthopedic centers, to receive the antibiotic-loaded DAC® coating or to a control group, without coating. At a mean follow-up of

Reference	Number of patients	Follow-up (months)	DAC-treated SSI rate (%)	Controls SSI rate (%)	P	Side effects
Romanò et al. [58]	380	14.5 ± 5.5	0.6	6	0.003	None
Malizos et al. [60]	256	18.1 ± 4.5	0	4.6	<0.02	None

SSI, surgical site infection.

Table 1. Summary of the main results of the published clinical multicenter trials on DAC® hydrogel coating in orthopedics and trauma.

18.1 ± 4.5 months (range 12–30), 253 patients were available for evaluation. On average, 5.7 mL (range: 1–10 mL) of DAC® hydrogel was needed to coat the implant. Gentamicin and vancomycin were the most used antibiotics, at concentration of, respectively, 4% or 2%. Six surgical site infections (4.6%) were observed in the control group compared to none in the treated group ($P < 0.02$). No local or systemic side effects related to DAC® hydrogel coating were observed and no detectable interference with bone healing was reported [61, 62] (cf. **Table 1**).

Preliminary results of the possible use of the DAC® hydrogel coating in one-stage exchange of infected prosthesis did also recently show the efficacy and safety of the device in this challenging application [63]. Further studies are currently under way concerning joint replacement in bone tumors, spine surgery, exposed fractures and dentistry.

8. Economic impact

Periprosthetic joint infections (PJI) are associated with increased costs for public health systems, mainly due to additional surgeries, prolonged hospitalization, increased length of rehabilitation and increased use of antibiotics [64]. Moreover, PJIs are associated with an increase in morbidity and mortality [65]. Unless novel, effective measures are taken to reduce the incidence of surgical site infections (SSIs), these complications will become an accruing burden to the health care system in the next two decades [66, 67].

Cost-effectiveness of antibacterial coatings of joint prostheses can be calculated, comparing their direct and indirect hospital costs with those of unprotected implants, taking into consideration the expected surgical site infection rate and using a decision-analytic modeling approach, as previously described by Diaz-Ledezma et al. [68] and Kapadia et al. [69].

Table 2 reports an algorithm used to calculate the overall economic impact of DAC® hydrogel coating. The following variables are included for calculation: average cost and number of primary joint replacements; average cost of the antibacterial coating per patient; incidence of PJI and expected reduction of infection rate with the use of the coating; average cost of PJI treatment and expected number of cases.

Various scenarios can be simulated with the reported algorithm, depending on the relative value given to each variable.

Considering the undiscounted price of DAC® hydrogel at our institution of € 585.00 per package and two packages of DAC® as the standard use per patient, it can be calculated that, if the

	Without ABC	With ABC
Joint replacement, average cost per patient	a	
Number of joint replacements/year	b	
Total cost of joint replacements/year	c = a*b	
ABC, cost per patient	0 (zero)	d
% of expected PJI	e	
% reduction of PJI with ABC	f	
Expected number of infections	g = b*(e/100)	h = b*(e/100)*(1-f/100)
PJI treatment, cost per case	i	
Costs for all septic complication treatments/year	j = g*i	k = h*i
Overall costs for joint replacement incl. septic complications/year	l = c+j	m = c+k
Total costs for ABC		n = b*d
Total costs	o = l	p = m+n
Balance (Medical costs with ABC/without ABC)	q = o-p	
% Balance (Medical costs with ABC/without ABC)	q' = (p/o)*100	

Table 2. Algorithm used to estimate the economical impact of antibacterial coating technologies.

	Without ABC		ABC
Joint replacement, average cost per patient		8,000 €	
Number of joint replacements/year		1,000	
Total cost of joint replacements/year		8,000,000 €	
ABC, cost per patient	0 €		**1,170 €**
% of expected PJI		2.6	
% reduction of PJI with ABC		90	
Expected number of infections	26.0		2.6
PJI treatment, cost per case		50,000 €	
Costs per all septic complication treatment/year	1,300,000 €		130,000 €
Costs for joint replacement incl. septic complications/year	9,300,000 €		8,130,000 €
Total costs for ABC			1,170,000 €
Total costs	**9,300,000 €**		**9,303,000 €**
Balance		**0 €**	
% Balance		**100.0%**	

Table 3. In this simulation, assuming an average cost of primary joint replacement of € 8000 per patient, an average cost of DAC® of € 1170 per case (i.e., two packages per patient), an expected reduction of postsurgical infections by using the coating of 90% and an average cost of PJI treatment of € 50,000 [6, 74], it can be calculated that DAC® is in economical balance if used in a population of patients with an expected periprosthetic infection rate, without the coating, of 2.6%.

coating is able to reduce surgical site infection by 90% [60], DAC® is in economical balance if applied to a population of patients with an expected rate of septic complications (without the coating) of 2.6% (**Table 3**).

According to a similar calculation, if applied on a large scale, to a selected population of patients with at least one risk factor for infection and an expected incidence of infection, without the coating, of 5%, DAC® would provide, in a medium size country, like Italy (approximately 160,000 joint replacements per year), annual direct cost savings of approximately € 43,200,000 (or 1080 € per patient). An expected incidence of postsurgical infection of 5% applies to patients with at least one risk factor for infection, which is at least 25% of all patients undergoing joint replacement [70, 71].

The present analysis is very conservative. One package of DAC® is in fact sufficient in the vast majority of primary implants. Secondly, recent studies point out how the long-term average cost of PJI is much higher than € 50,000, largely exceeding € 100,000 per patient [13]; finally, the algorithm does not include indirect costs, like those deriving from treatment complications, functional inability, work loss and compensation, medicolegal costs, increased mortality rate and quality of life reduction.

9. Conclusions

Biofilm- and implant-related infections represent a dramatic and increasing burden worldwide. Available data show that hyaluronic acid has a proven *in vitro* antiadhesive/antibiofilm effect against some of the most common pathogens, and HA has been used safely, alone or in combination with other polymers, with satisfactory results in different conditions associated with biofilm-related chronic infections. Clinical data in various applications, including dentistry, urology, wound management, dermatology and orthopedics, paved the way to the possible use of HA as a protective coating barrier of implants.

The chemical derivatization of hyaluronic acid with polylactic acid allows the formation of graft copolymers, which, when contacted with an aqueous medium, can be used to produce hydrogels, like the recently CE-marked DAC®, with appropriate characteristics for easy preparation and application at the time of surgery. Resulting medicated hydrogel is transparent, easily spreadable over a surface, like a titanium prosthesis, and has a specifically designed duration; moreover, it has proven, peculiar, antiadhesive and antibiofilm capabilities. If required, it may also be easily loaded, at surgery, with antibacterial agents that will be released over the following hours or few days in effective high local concentrations. In fact, as a passive protective barrier, DAC® hydrogel has some limits. Among others, the antiadhesive/antibiofilm effect is limited and may vary, depending on the type of the microorganism, the bacterial load, the local environment, etc.; moreover, HA protection may be neutralized by the possible ability of some bacteria to produce hyaluronidase, an enzyme that catalyzes the degradation of hyaluronic acid [52], while collagen and hyaluronan may even become possible ligands for microbial attachment in particular situations, or the coating can be covered by other host's proteins to which bacteria may anchor [72, 73]. To overcome some of these limits, possible loading of

the hyaluronic-based hydrogel with antibiotics is technically feasible and has been found safe in various preclinical and clinical settings, being a possible option for clinicians.

In fact, both *in vitro* and *in vivo* studies did confirm the safety and efficacy of the hydrogel coating with and without loaded antibacterials.

Clinical results also clearly point out the efficacy of the DAC® coating to significantly reduce early postsurgical infection after joint replacement or internal osteosynthesis, without any detectable local side effect both concerning wound and bone healing. Moreover, no changes in organ-specific serum markers or systemic unwanted effects were noted. The high biocompatibility of its basic constituents and the short time (less than 3 days) needed for a complete hydrogel resorption make the possible occurrence of longer term side effects quite unlikely.

Finally, economical consideration points out the high cost-to-benefit ratio of the large-scale use of DAC® coating, especially in a population with at least one risk factor for infection.

The versatility of the device and its safety profile may open the way to application in other surgical fields that share similar infection risk as orthopedics and trauma.

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