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Review

# Hyaluronic Acid and Its Composites as a Local Antimicrobial/Antiadhesive Barrier

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## Abstract

Living in biofilms is probably the most common condition for bacteria and fungi and biofilm-related infections account for the majority of bacterial infectious diseases worldwide.

Among others biofilm-related infections, those associated with implanted biomaterials have an enormous and still largely underestimated impact in orthopaedics and trauma, cardio-surgery and several other surgical disciplines.

Given the limited efficacy of existing antibiotics in the prevention and treatment of bacterial biofilms, new strategies are needed to protect implants and host tissues, overcoming the striking ability of the microorganisms to adhere on different surfaces and to immediately protect themselves by forming the biofilm matrix.

Adhesion is a necessary first step in microbial colonization and pathogenesis and provides a potential target for new preventive and treatment approach.

Among various polymers, tested as antibacterial coatings, hyaluronic acid and some of its composites do offer a well-established long-term safety profile and a proven ability to reduce bacterial adhesion and biofilm formation.

Aim of the present review is to summarize the available evidence concerning the antiadhesion/antibiofilm activity of hyaluronic acid and some of its derivatives to reduce/prevent bacterial adhesion and biofilm formation in various experimental and clinical settings.

Key words: Hyaluronic Acid, Biofilm, Adhesion, Bacteria, Infection, Implant

## Introduction

According to the U.S. National Institutes of Health, up to 80% of human bacterial infections involve biofilm-associated microorganisms [1].

Among these, implant-related infections do still have a tremendous impact in orthopaedics and trauma [2], with high social and economic costs [3, 4], posing challenging diagnostic and therapeutic dilemmas [5].

In fact, peri-prosthetic joint infection (PJI) remains one of the most feared complications in orthopaedic surgery and among the first reasons for implant failure [6].

Moreover, given the increasing number of hip and knee arthroplasties performed, the prevalence of this complication is rising, with increasing costs for national health systems and increasing biological costs for the patients, such as loss or reduced joint function and deterioration in their physical and psychological health [7].

According to the widely accepted model of the 'race for the surface' for PJI development, host and bacterial cells compete for surface colonization, with a

low probability of bacterial attachment if host cells adhere to implant first, and vice versa. In the event of bacterial adhesion to an implant, immediate biofilm formation starts, making the bacteria resistant to host defense mechanisms [8].

In addition, the matrix protects the biofilm cells from various microbicidal agents and stresses, including dehydration, toxins, ultraviolet light, chemical disinfectants, temperature and osmotic shock, and lead them to increased resistance against antimicrobials [9, 10].

To address the limited efficacy of existing antibiotics in the treatment of established bacterial biofilms, novel approaches are required to prevent bacterial adhesion and biofilm formation [11].

Adhesion is a necessary first step in microbial colonization and pathogenesis and provides a good theoretical target for new preventive and treatment strategies [12].

Bacterial adhesion to surfaces can be divided into a first, reversible phase and a second, irreversible phase. The first bacterial adhesion occurs between bacterial adhesins and surface receptor sites [13].

Once an implant is inserted into the body, it is covered by a conditional protein layer composed of host proteins, such as albumin and complement, that act as a reservoir of several receptors for bacterial adhesive ligands, mediating adhesion of free-floating bacteria to the surface of the biomaterials [14, 15]; these first adhesions are, however mechanically and biologically unstable.

Few minutes after this first, reversible phase, bacterial clusters attached to the surface starts to express biofilm related genes, produce glycocalyx and form mature biofilm, thus transforming the adhesion from reversible to irreversible [16].

Full-formed biofilm can be found few hours after the first bacterial adhesion [17].

Antimicrobial surface coatings can be based on an anti-adhesive principle that prevents bacteria to adhere and form biofilms [18].

In fact, some polymer coatings, like the hydrophilic polymethacrylic acid, polyethylene oxide or protein-resistant polyethylene glycol can be applied to the surface of titanium implants and result in significant inhibition of bacterial adhesion [19 – 22].

Hydrophobic and superhydrophobic surface treatment technologies have also shown a great repellent antibacterial effect in preclinical studies [23 – 25].

However, clinical application of completely novel coating technologies and compounds, not otherwise previously tested in humans, appears particularly challenging [26].

Bacterial colonization can also be blocked by an inhibitor interfering with ligand–receptor interaction for bacterial attachment. One of these inhibitors could be Hyaluronic Acid (HA), a glycosaminoglycan made up of glucuronic acid and N-acetylglucosamine disaccharide units. It is a uniform, linear and unbranched molecule, with highly variable length and molecular weight (up to 106 Da). It is abundant in skin (up to 56%) and in connective tissues, with a turnover ranging from several hours to a few days depending on tissues.

Hyaluronic acid constitutes one of the main components of extracellular matrices. Because of its biological properties, HA has several clinical applications (aesthetic surgery, dermatology, dentistry, orthopedics and opthalmology) [27].

Extensive studies on the chemical and physicochemical properties of HA and its physiological role in humans, together with its versatile properties, such as its biocompatibility, non-immunogenicity, biodegradability, and viscoelasticity, have proved that it is an ideal biomaterial for medical and pharmaceutical applications [28, 29].

Among its various properties, several studies have recently shown the ability of HA to protect against various infectious agents [30], depending on HA concentration and molecular weight [31, 32], while more recently HA interference on bacterial adhesion and biofilm formation has been extensively investigated [33].

Given its high biocompatibility and well known safety profile and the anti-adhesive capabilities, HA and its composites represent an attractive, non-antibiotic, option to mitigate the impact of biofilm-related infections in various clinical settings including implant-related infections.

Aim of this review is to provide an update of the current evidence concerning HA ability to reduce/prevent bacterial adhesion and biofilm formation.

## Anti-adhesive and anti-biofilm properties of hyaluronic acid and its composites

Nearly two decades ago, Pavesio et al. [34] were probably the first to describe the ability of HA to resist bacterial adhesion, with particular reference to *Staphylococcus epidermidis,* and its non-fouling properties [35], proposing coated polymeric medical devices (e.g., intraocular lenses, stents and catheters) 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 prevent fibroblasts adhesion and to greatly reduce

*Staphyloccous epidermidis* adhesion to the implant surface [36].

The impact of slime dispersants and anti-adhesives on *in vitro* biofilm formation on IOLs was further investigated by Kadry and co-workers [37], using a *Staphyloccous epidermidis* wild strain, isolated from a patient with endophtalmitis; the authors reported the ability of hyaluronan to reduce bacterial adhesion to IOLs to ≥30%, compared with untreated control cells. The authors suggested the use of adjuvant therapy such as dispersants or anti-adhesives, in addition to the antibiotics in irrigating solutions for bacterial ocular infections.

More recently, the *in vitro* antiadhesive and antibiofilm activity of hyaluronic acid towards bacterial species commonly isolated from respiratory infections was investigated by Drago et al. [33].

In this study, the interference exerted on bacterial adhesion was evaluated by using Hep-2 cells, while the antibiofilm activity was assessed by means of spectrophotometry after incubation of biofilm with hyaluronic acid and staining with crystal violet.

The experimental findings clearly demonstrated how hyaluronic acid is able to interfere with bacterial adhesion to a cellular substrate in a concentration-dependent manner. Moreover, *Staphylococcus aureus* biofilm was found to be more sensitive to the action of HA, compared to that produced by *Haemophilus influenzae* and *Moraxella catarrhalis*.

Concerning more specifically the antimicrobial activity, HA has also been shown to exert varied bacteriostatic, but not bactericidal, dose-dependent effects on different microorganisms in the planktonic phase [31, 38].

In this regard, Radaeva et al. reported the inhibiting activity of HA with respect to some *Pseudomonas* species [39], while Ardizzoni and co-workers [30] investigated the effects of HA on 15 ATCC bacterial strains, representative of clinically relevant bacterial and fungal species. Their results showed that different microbial species and, sometimes, different strains belonging to the same species, are differently affected by HA. In particular, staphylococci, enterococci, *Streptococcus mutans*, two *Escherichia coli* strains, *Pseudomonas aeruginosa*, *Candida glabrata* and *C. parapsilosis* displayed a HA dose-dependent growth inhibition, while no HA effects were detected in *E. coli* ATCC 13768 and *C. Albicans* and *S. sanguinis* was favoured by the highest HA dose.

Comparing the potential bacteriostatic effect of some of the most commonly used biomatrix materials (collagen type I, hyaluronic acid, hydroxyapatite, polylactic acid and polyglycolic acid) on the growth over the first 12h of exposure of some of the most common orthopaedic bacterial pathogens (*Staphylococcus aureus, Staphylococcus epidermidis, β-hemolytic Streptococcus, Pseudomonas aeruginosa)*, Carlson and co-workers [38] found that HA had the most significant bacteriostatic properties on the studied organisms. None of the materials exhibited a purely bactericidal effect on the bacterial strains studied.

Pirnazar et al. [31] investigated the potential bacteriostatic effect of hyaluronic acid in different concentrations and molecular weight on oral and non-oral microorganisms (*Staphylococcus aureus, Propionibacterium acnes, Actinobacillus actinomycetemcomitans, Pavotella oris* and *Porphyromonas gingivalis*) with potential application in dentistry surgery; The results showed that different hyaluronan solutions exerted varied bacteriostatic effects on all the bacterial strains. The authors concluded that the clinical application of hyaluronan in form of membranes, gels, or sponges during surgical therapy may reduce bacterial contamination of the surgical wound, thereby lessening the risk of postsurgical infection and promoting more predictable regeneration.

Concerning possible orthopaedic applications, in 2004 Harris and Richards [40] showed the visualization and quantification of *S. aureus* adhering to a variety of different treated/coated titanium surfaces. In their study, coating titanium with sodium hyaluronate significantly decreased the density of *S. aureus* adhering to the surfaces and its potential use in osteosynthesis, orthopaedics or dental applications was suggested out.

In a very recent review on polysaccharide-based coatings, that have been proposed over the last ten years to impede biofilm formation on material surfaces exposed to bacterial contamination, hyaluronic acid was discussed as one of the most studied, with demonstrated non-fouling properties on glass surfaces [41]; displaying hydrophilic characteristics (contact angle of 22°), this coating was in fact reported to reduce adhesion of *S. epidermidis* and *E. coli* by several orders of magnitude compared to the unmodified glass slide. Similarly, adhesion of *S. aureus* on Ti foils functionalized with hyaluronic acid-catechol was lower than on pristine substrates.

Based on HA antiadhesive properties, a novel HA-based hydrogel has been recently proposed, in order to protect implanted biomaterials in orthopaedics, trauma and dental surgery from bacterial colonization [42]; this fast-resorbable hydrogel coating, composed of covalently linked hyaluronan and poly-D,L-lactide ("Defensive

Antibacterial Coating", DAC®, Novagenit Srl, Mezzolombardo, Italy), has been found to have a synergistic antibiofilm activity with various antibacterials and able to be effectively manually spread onto the surface of various biomaterials commonly used in orthopaedics, trauma and dental surgery [43] (Fig. 1).

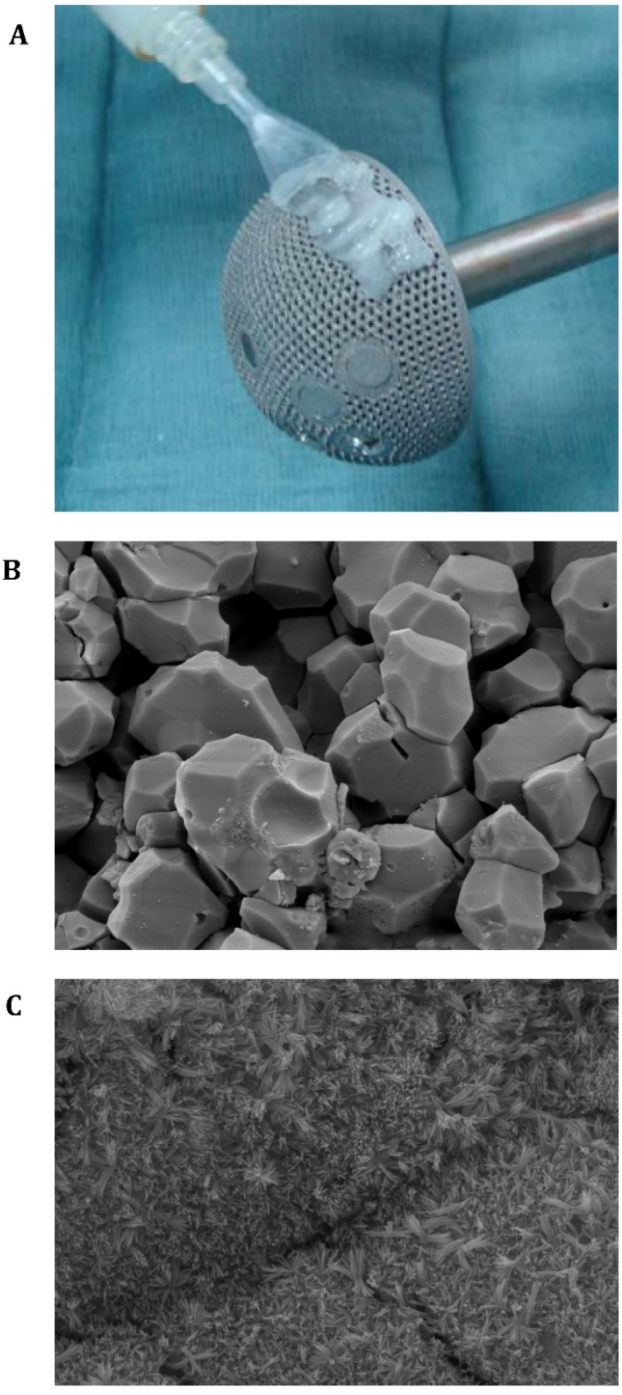


**Figure 1.** DAC® hydrogel application on a titanium acetabular cup prosthesis (A). The hydrogel comes in powder form in a prefilled syringe and is designed to be reconstituted at the time of surgery with water for injection. Scanning Electron Microscope (SEM) analysis of sandblasted titanium samples surface (magnification 10,000x) without the hydrogel coating (B) or after DAC® coating (C) and mechanical scraping of the hydrogel to test its ability to resist press-fit insertion. Note the complete and uniform coverage of the titanium surface.

The ability to completely cover even sand-blasted titanium surface and resist scraping has in fact been confirmed by scanning electron microscopy (SEM) analysis (cf. Fig. 1). 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.

In unpublished experiments (Novagenit Srl, data on file), in order to evaluate DAC® ability to prevent bacterial adhesion, 200 mg of hydrogel were homogenously spread on the surface of sterile titanium discs. Hydrogel-coated substrates and uncoated substrates (controls) were then placed into sterile 6-wells polystyrene plates and overlaid with a standardized inoculum ($10^8$ CFU/mL) of bacterial cells for 15, 30, 60 and 120 minutes. Afterwards, non-adherent bacteria were removed by rinsing with sterile saline. The remaining adhered cells were detached by adding a solution of 0.1% w/v dithiothreitol (DTT) (Sigma-Aldrich, Milan, Italy) to each well and stirring for 15 minutes at room temperature. Then, 100 µL of each sample were plated onto Tryptic Soy agar (TSA; Merck, Darmstadt, Germany) and incubated at 37°C for 24 hours for CFU counts. Ten discs were used for each condition and each time interval.

The results showed that the adhesion density of *S. aureus* on titanium discs pre-treated with DAC®, was significantly lower than adhesion on untreated controls at each time point (Fig. 2). In particular, reductions of adhered bacteria equal to 86.8%, 80.4%, 74.6% and 66.7% vs untreated discs were observed after 15, 30, 60 and 120 minutes of incubation, respectively, while an increase of adhesion density during time was observed for both control and pre-treated discs (Fig. 3).

Further analyses were conducted to show the ability to dislodge previously adhered bacteria; to this aim, titanium discs were placed into sterile 6-wells polystyrene plates and overlaid with a standardized inoculum ($10^8$ CFU/mL) of bacterial cells in order to allow the adhesion of bacterial cells. Afterwards, 200 mg of hydrogel were spread on the surface of contaminated titanium discs in order to remove previously adhered bacteria. Contact times were 15, 30, 60 and 120 minutes. Untreated substrates were used as controls. Non-adherent bacteria were removed by rinsing with sterile saline, while the remaining adhered cells were detached by adding 0.1% DTT as previously described. Then, 100 µL of each sample were plated onto TSA and incubated at 37°C for 24 hours for CFU counts. Ten discs were used for each condition and each time interval.

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 minutes of 84.0%, 72.8%, 72.3% and 64.3%, respectively (Fig.4). Once again, an increase of adhesion density during time was observed for both control and treated discs (Fig. 5).

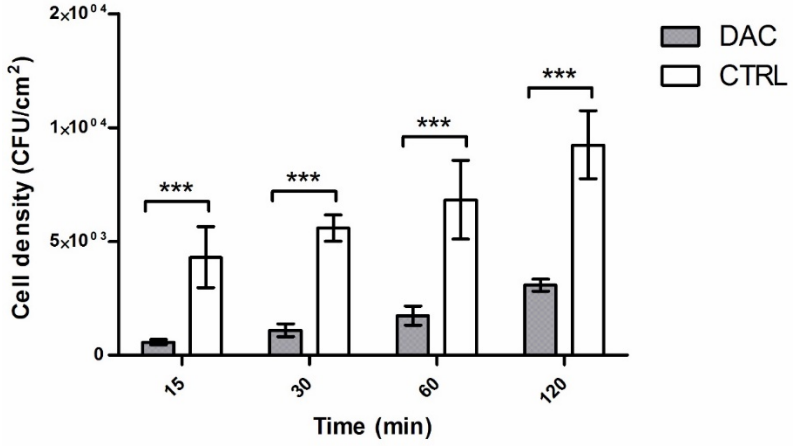


**Figure 2.** Adhesion densities of *S. aureus* (mean CFU/cm² ± standard deviation) to discs pre-treated with DAC® ("Defensive Antibacterial Coating", Novagenit Srl, Mezzolombardo, Italy) and controls at 15, 30, 60 and 120 min; *** P < 0.001 (two-way ANOVA followed by Bonferroni post hoc test).

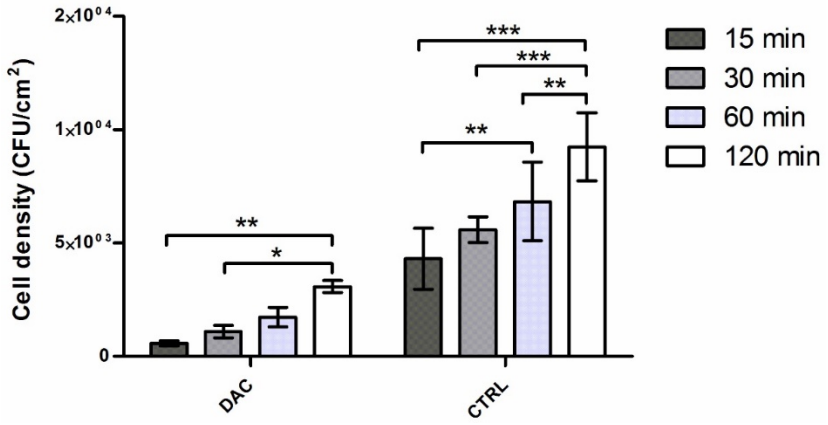


**Figure 3.** Adhesion densities of *S. aureus* (mean CFU/cm² ± standard deviation) over time in pre-treated with DAC® and control discs at 15, 30, 60, 120 min; * 0.01 < P <0.05, ** 0.001 < P < 0.01, *** P < 0.001 (two-way ANOVA followed by Bonferroni post hoc test).

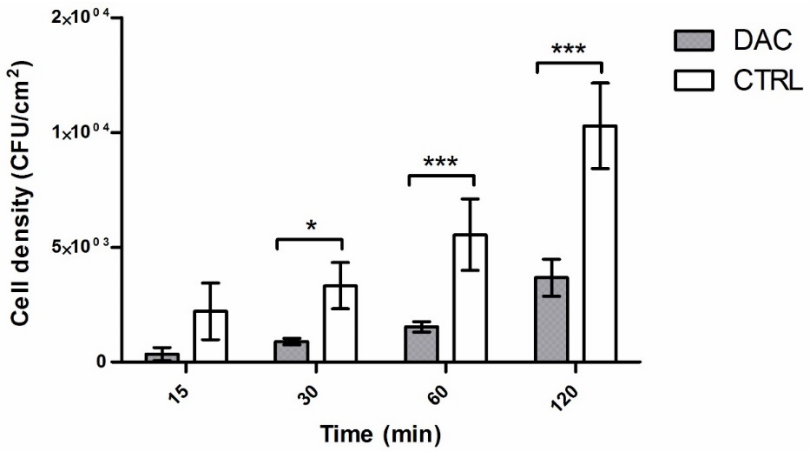


**Figure 4.** Adhesion densities on discs with of *S. aureus* (mean CFU/cm² ± standard deviation) applied before DAC treatment and controls at 15, 30, 60, 120 min; * 0.01 < P <0.05, *** P < 0.001 (two-way ANOVA followed by Bonferroni post hoc test).

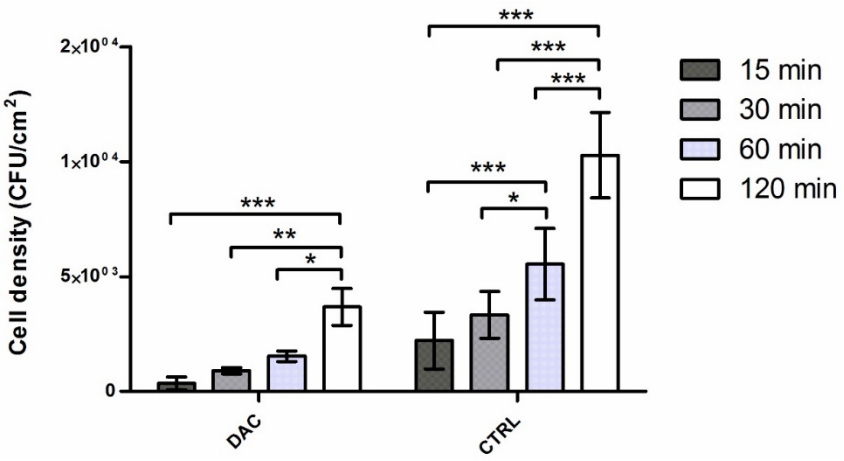


**Figure 5.** Adhesion densities over time on discs with of *S. aureus* (mean CFU/cm² ± standard deviation) applied before DAC treatment and controls at 15, 30, 60, 120 min; * 0.01 < P <0.05, ** 0.001 < P < 0.01, *** P < 0.001 (two-way ANOVA followed by Bonferroni post hoc test).

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 (Fig. 6) [43]. In the experimental setting *S. epidermidis* and *S. aureus* were grown on chrome-cobalt devices in 6-wells polystyrene plates containing TSB for 24 hours at 37°C. Then, growth medium was removed together with non-adherent bacteria and new broth added. 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 were reconstituted with 1 mL of water for injections containing gentamicin or vancomycin at 20 mg/mL concentration.

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 hours of incubation by a spectrophotometric assay. In particular, at each time, broth was removed and biofilm stained with Crystal Violet. The excess stain was then rinsed off with distilled water and air-dried. After elution of the stain from implants with absolute ethanol, the amount of biofilm was quantified by reading optical density (O.D.) at a wavelength of 595 nm against blank (consisting of ethanol). Amount of biofilm at each time was compared with that formed on the same type of implant before treatment. Each assay was performed in duplicate and repeated for three times.

At each time point, both for gentamycin 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 resulted in a larger reduction of biofilm formation (approximately 75 to 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 [44] 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 may still provide an additional advantage to the host's cells to first colonize the implanted biomaterial and win the competition with the microorganisms that may eventually be present, thus contributing to reduce the occurrence of implant-related infections.

## Clinical applications of hyaluronic acid to prevent bacterial adhesion

Several clinical local applications of HA to reduce the impact of biofilm-related infections have been reported, in different clinical settings, with favourable results and no adverse events.

Torretta et al. [45] recently described topical administration of hyaluronic acid in children with recurrent or chronic middle ear inflammations and chronic adenoiditis.

In this prospective, single-blind, randomised

controlled study, otoscopy, tympanometry and pure-tone audiometry in children which received the daily topical administration of normal 0.9% sodium chloride saline solution (control group) or 9 mg of sodium hyaluronate in 3 mL of a 0.9% sodium saline solution was performed. The final analysis was based on 116 children (49.1% boys; mean age, 62.9 ± 17.9 months): 58 in the control group and 58 in the study group. At the end of follow-up, the prevalence of patients with impaired otoscopy was significantly lower in the study group (P value = 0.024) compared to baseline but not in the control group. In comparison with baseline, the prevalence of patients with impaired tympanometry at the end of the follow-up period was significantly lower in the study group (P value = 0.047) but not in the control group. The reduction in the prevalence of patients with conductive hearing loss (CHL) (P value = 0.008) and those with moderate CHL (P value = 0.048) was significant in the study group, but not in the control group. The mean auditory threshold had also significantly improved by the end of treatment in the study group (P value = 0.004) but not in the control group.

Several studies have also reported the beneficial effect of topical HA in chronic urinary tract infections (UTI). In contrast to traditional antibiotic therapy, which aims at eradicating pathogens, treatment with HA targets bacterial adherence to the bladder mucosa with the presumption that a damaged glycosaminoglycan mucous layer facilitates bacterial adherence and therefore recurrent UTI [46].

Among others [47, 48], Lipovac and colleagues evaluated the efficacy of nine HA bladder instillations over 6 months in 20 women with a history of recurrent UTI. Their status was assessed prospectively but compared with a retrospective review of patients' charts. The number of infections per year per patient was significantly reduced (from 4.99±0.92 to 0.56±0.82, p>0.001) and the mean time to recurrence (from 76.7±24.6 to 178.3±25.5 days, p>0.001) was prolonged significantly. Nevertheless 65% of treated patients were free of recurrences until the end of study (47.6 weeks) [49].

Damiano et al. were able to provide a higher level of evidence by reporting a prospective, randomized, double-blind, placebo-controlled study, in which a significant reduction of 77% (p<0.0002) in the UTI rate per patient per year versus placebo was observed at the end of the study. Moreover, mean time to UTI recurrence was significantly prolonged (185.2±78.7 versus 52.7±33.4 days, p<0.001) after treatment compared with placebo. Overall urinary symptoms and quality of life measured by questionnaires significantly improved compared with placebo [50]. No adverse events were reported.

Very recently a multicentre European study confirmed the efficacy of intravesical administration of combined hyaluronic acid and chondroitin sulphate (CS) for the treatment of female recurrent urinary tract infections [51]. A total of 276 adult women received intravesical administration of HA+CS or standard of care (antimicrobial/ immunoactive prophylaxis/ probiotics/cranberry). At follow-up, 181 patients treated with HA+CS and 95 patients treated with standard of care from 7 centres were available. The crude and adjusted ORs (95% CI) for bacteriologically confirmed recurrence within 12 months were 0.77 (0.46 to 1.28) and 0.51 (0.27 to 0.96), respectively.

Studies were also undertaken to determine the effect on clinical variables, sub-gingival bacteria and local immune response brought about by application of hyaluronan-containing gels in early wound healing after scaling and root planing (SRP) in dentistry [52, 53].

In the study reported from Eick et al. [54], 34 individuals with chronic periodontitis were evaluated after full-mouth SRP. The exclusion criteria were: antibiotics intake in the 6 months before the study, periodontal treatment received during the previous year, pregnancy, nursing, smoking, chronic diseases such as diabetes mellitus or rheumatoid arthritis, and allergy to ingredients in the drug. In the test group (n=17), a 0.8% hyaluronan-containing gels (HA) 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. In both groups, PD and CAL were significantly reduced (P<0.001).

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.

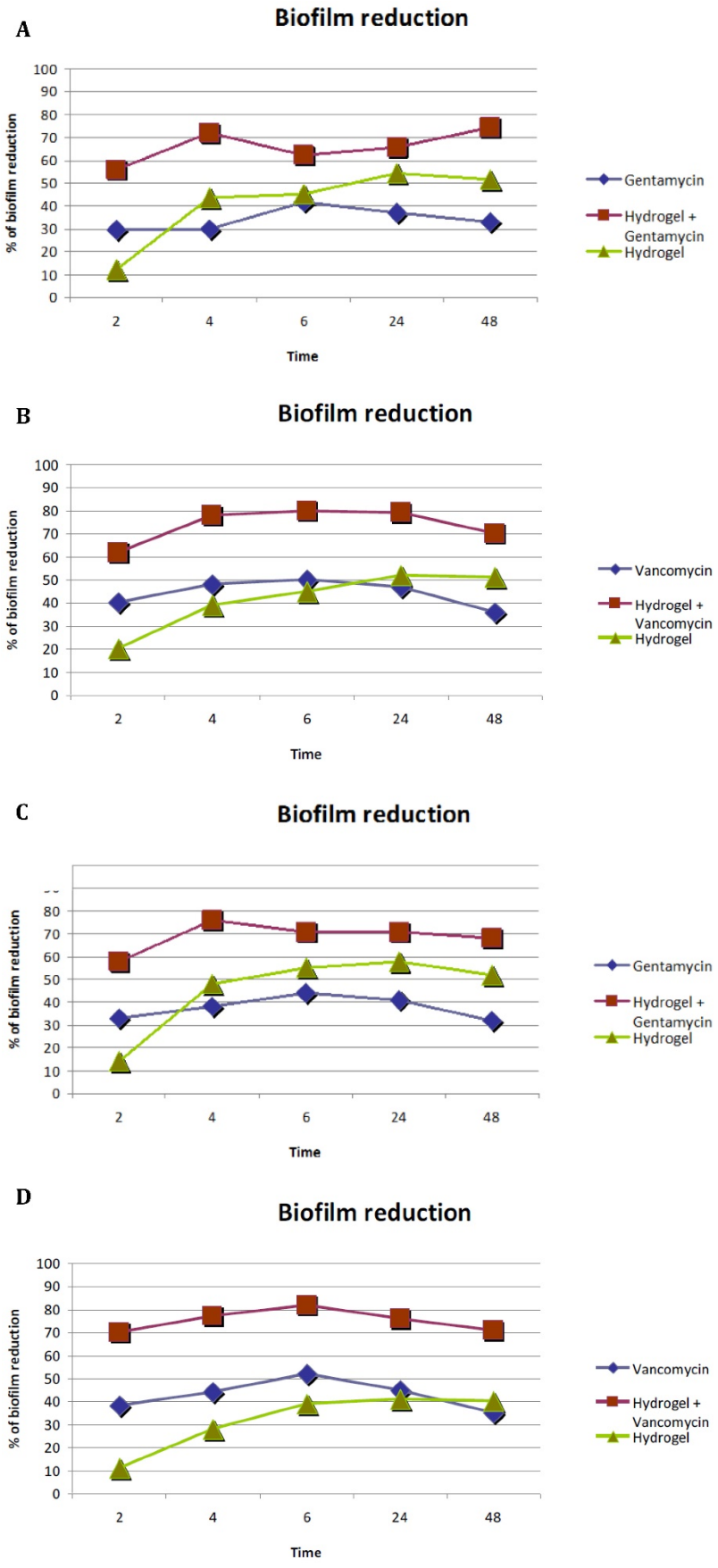


**Figure 6.** Comparison of the efficacy of DAC hydrogel, gentamicin, vancomycin or a combination thereof, on biofilm formation reduction of *Staphylococcus aureus* (A. and B.) and *Staphylococcus epidermidis* (C. and D.) over time (hours). Note that the hydrogel alone is able to provide an equal or superior biofilm reduction compared to commonly used antibiotics, while a synergistic effect is observed using a combination of the hyaluronic acid based hydrogel and the antibiotic compounds.

## Conclusion

Although to date no surface modification has been reported to be able to fully prevent bacterial adhesion and biofilm formation [55], available data show that hyaluronic acid has a proven *in vitro* antiadhesive/antibiofilm effect against some of the most common pathogens and it 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, allow to consider the potential use of HA as a protective coating barrier of implants particularly safe and feasible on a large scale basis.

While antibacterial coatings to mitigate the occurrence of implant- and biofilm-related infections are regarded as one of the most needed technology, currently only few and insufficient options are available for clinical use in orthopedics and trauma surgery [18].

Considering the pathogenesis of implant-related infections, any protection offered by a fully biocompatible antiadhesive barrier, like HA and some of its derivatives, could be extremely useful to reduce the tremendous burden of implant-related infections.

On the other hand, it should be noted that hyaluronic acid as a passive protective barrier 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 [56], while collagen and hyaluronan may even become possible ligands for microbial attachment in particular situations [57, 58].

To overcome at least some of these limits, possible loading of hyaluronic-based hydrogels with antibiotics is technically feasible and has been proposed by different authors [59 – 62], being a possible option for future developments and large scale clinical applications, provided that regulatory requirements can be met.

## Competing Interests

The authors have declared that no competing interest exists.

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