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Systematic Review

Antimicrobial Properties of Strontium Functionalized Titanium Surfaces for Oral Applications, A Systematic Review

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Abstract: The aim of this systematic review was to assess the current scientific evidence of the antimicrobial potential of strontium (Sr) when used to functionalize titanium (Ti) for oral applications. Out of an initial list of 1081 potentially relevant publications identified in three electronic databases (MEDLINE via PubMed, Scopus, and Cochrane) up to 1 February 2021, nine publications based on in vitro studies met the inclusion criteria. The antimicrobial potential of Sr was investigated on different types of functionalized Ti substrates, employing different application methods. Nine studies reported on the early, i.e., 6–24 h, and two studies on the late, i.e., 7–28 days, antimicrobial effect of Sr, primarily against *Staphylococcus aureus* (*S. aureus*) and/or *Escherichia coli* (*E. coli*). Sr-modified samples demonstrated relevant early antimicrobial potential against *S. aureus* in three studies; only one of which presented statistical significance values, while the other two presented only the percentage of antimicrobial rate and biofilm inhibition. A relevant late biofilm inhibition potential against *S. aureus* of 40% and 10%—after 7 and 14 days, respectively—was reported in one study. Combining Sr with other metal ions, i.e., silver (Ag), zinc (Zn), and fluorine (F), demonstrated a significant antimicrobial effect and biofilm inhibition against both *S. aureus* and *E. coli*. Sr ion release within the first 24 h was generally low, i.e., below 50 μg/L and 0.6 ppm; however, sustained Sr ion release for up to 30 days, while maintaining up to 90% of its original content, was also demonstrated. Thus, in most studies included herein, Sr-functionalized Ti showed a limited immediate (i.e., 24 h) antimicrobial effect, likely due to a low Sr ion release; however, with an adequate Sr ion release, a relevant antimicrobial effect, as well as a biofilm inhibition potential against *S. aureus*—but not *E. coli*—was observed at both early and late timepoints. Future studies should assess the antimicrobial potential of Ti functionalized with Sr against multispecies biofilms associated with peri-implantitis.

Keywords: strontium; dental implant; titanium surfaces; antimicrobial; peri-implantitis

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1. Introduction

One of the major challenges in everyday dental clinical practice is the biological complications surrounding dental implants, due to bacterial attachment to and biofilm formation on the implant reconstruction and/or the implant surface itself [1–3]. A long-standing infection often leads to inflammatory destruction of the surrounding bone, a condition known as peri-implantitis. Peri-implantitis is characterized clinically by increased pocket probing depth, bleeding on probing and/or suppuration, along with progressive

loss of peri-implant bone [3,4], which can eventually lead to implant loss [5,6]. Peri-implantitis is rather common, with mean prevalence of 22% on the patient level [7] and 26% on the implant level, for implants in function for at least 5 years [8]. The current management protocol for peri-implantitis most often involves surgery, with the aim of gaining access to the implant surface to remove the attached biofilm [9], and is commonly combined with systemic administration of antibiotics [10]. Even though the available treatment protocols may stop further disease progression and/or facilitate regeneration of the lost peri-implant bone, there is limited evidence on the long-term efficiency of peri-implantitis therapy. Currently available long-term data show a considerable rate of relapse [11]. Therefore, development of prevention strategies is pertinent.

A key aspect in dealing with biomaterial infection is targeting biofilm adhesion and growth; thus, efforts have been made towards developing antimicrobial coatings striving to prevent biofilm attachment and colonization on the implant surface [12–16]. Various implant surface functionalization approaches, e.g., with antibiotics, antimicrobial peptides, and metal ions, have been investigated for their antimicrobial potential, as well as their impact on the tissues surrounding the implant [17,18]. Several in vitro studies have indicated that metal ions, e.g., silver (Ag) [19], zinc (Zn) [20], and strontium (Sr), alone or in combination with other metal ions [21], exert an antimicrobial effect against bacteria associated with peri-implantitis. Mechanisms of action involve the interaction of these metal ions with the bacterial cell wall and a subsequent change in the structure of the cell membrane and/or DNA damage, leading to cell death or inhibition of bacterial proliferation [22,23]. The possible impact of such approaches on the surrounding tissues and how to optimize the antimicrobial potential of ions are still matters requiring further investigation. For instance, despite the excellent antimicrobial effect of Ag ions against several types of bacteria known to play an active role in peri-implantitis, toxic side effects to human cells at certain threshold levels of Ag have been observed [24]. A commonly used strategy to overcome such possible negative effects of a specific metal ion is to incorporate additional secondary metal ions or other bioactive compounds [25]. Sr is one of the elemental ions that have been co-administered with Ag and found to improve the biocompatibility, without interfering with the antimicrobial properties of Ag-modified Ti implant surfaces [26,27]. Recently, the antimicrobial potential of strontium hydroxide against bacteria associated with peri-implantitis was investigated [28], and the results indicated that strontium hydroxide, at certain concentrations, has a bacteriostatic, as well as bactericidal, effect. Sr was also found to possess inherent antimicrobial potential, in vitro, when incorporated in the formulation of bioglass [29,30]. Furthermore, Sr was shown to promote osseointegration of Ti implants and to have a positive effect on bone regeneration [31–33]. Sr has also been used as a treatment for osteoporosis, acting by inhibiting osteoclast activity and accelerating osteoblast proliferation [34–36].

Nevertheless, the antimicrobial effect of Sr—when applied on Ti substrates using different application methods, concentrations, ion release rates, and assessment intervals—has not been assessed in a comprehensive manner. Therefore, this study aimed to systematically review the literature to assess the current evidence of the antimicrobial potential of Sr when used to functionalize Ti surfaces.

2. Materials and Methods

2.1. Protocol Development

This review employed a detailed protocol in accordance with the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) statement [37]. A literature search was carried out to answer the following focused research question: Do Ti surfaces, functionalized with Sr, possess antimicrobial potential against bacteria associated with biomaterial infections, as demonstrated in in vitro and in vivo studies?

2.2. Search Strategy for Identification of Studies

Three different electronic databases (MEDLINE via PubMed, Scopus, and Cochrane) were used for the literature search of studies published from 1973 until 1 February 2021 (Figure 1). The search strategy was limited to English language publications. Two independent reviewers (HA and FB) searched the literature to identify the relevant studies with a combination of three blocks of search terms, including various MeSH terms. Block one included the following terms: 'Titanium', 'Ti', 'Implants', 'Surfaces', 'Disks', 'Wafers', and 'Sheets'. Block two included the following terms: 'Strontium', 'Sr', 'Metal ions', and 'Ions'. Block three included the following terms: 'Anti-Bacterial Agents', 'Antibacterial', 'Antimicrobial', 'Bacteriostatic', 'Bactericidal', and 'Viability'. The Boolean operator 'OR' was used to combine the search terms within each block. The search terms in all blocks were then combined by the Boolean operator 'AND':

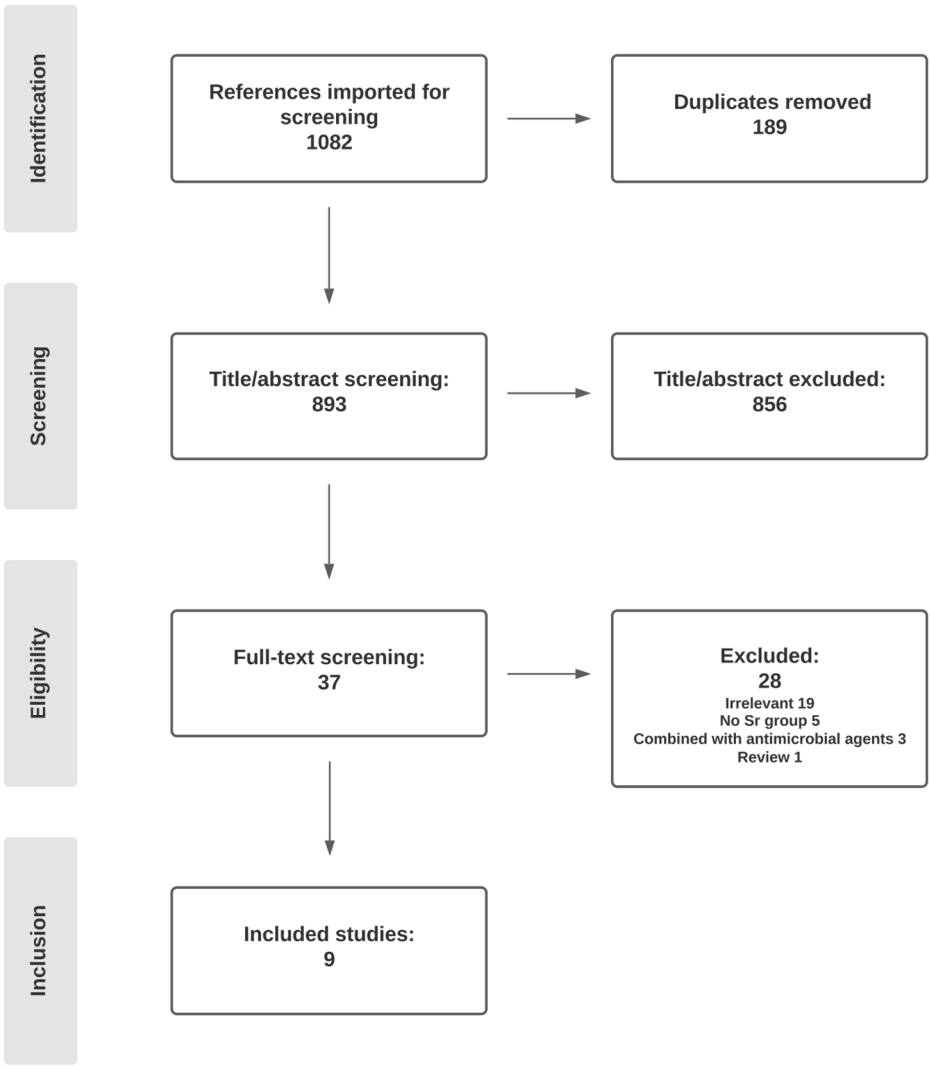

Figure 1. Flow chart of the search process and results.

'(Titanium OR Ti OR Dental Implants OR Surfaces OR Disks OR Wafers OR Sheets) AND (Strontium OR Sr OR Metal ions OR ions) AND (Anti-Bacterial Agents OR Antibacterial OR Antimicrobial OR Bacteriostatic OR Bactericidal OR Viability)'.

The literature search was completed manually by searching reference lists of the relevant articles for undetected studies possibly suitable for inclusion. Titles, abstracts, and full texts of the possibly relevant studies were independently screened by the two observers (HA and FB). Disagreements were resolved by discussion to reach consensus.

2.3. Study Selection Criteria

Titles and abstracts were initially screened, and relevant articles were identified and selected for full-text review. Studies were considered relevant according to the following specific inclusion criteria: (a) original studies (in vitro, in vivo), in the English language, investigating the antimicrobial potential (bactericidal effect and/or interruption of bacterial growth) of Ti surfaces functionalized with Sr, against planktonic cells and/or biofilm, utilizing one or more test methods; (b) Sr was used as the only antimicrobial agent in at least one of the experimental groups; (c) the method of Ti surface functionalization, type of Ti substrate, type of the tested bacteria, type of antimicrobial test, and testing timepoints/period were clearly presented.

2.4. Data Extraction

An overview table, including the general characteristics and summary of relevant information of the included studies, was created. The table includes the authors' names, type of study, type of Ti substrate used, active antimicrobial agents tested, and method of applying the agent to the Ti substrate. Furthermore, the articles were divided into three subgroups: Sr used with Ag and compared with an Sr only group, Sr compared with other metal ions, i.e., Ag or Zn, and Sr used as an active antimicrobial agent along with different metal ions. A summary of the methods used, type of bacteria tested against, and the main outcomes was also created.

2.5. Risk of Bias and Relevance Assessment

The included studies were assessed by implementing a purpose-made tool including 6 criteria: (a) using at least 2 antimicrobial testing methods, (b) evaluating the antimicrobial effect at different time points, (c) testing the antimicrobial effect against a multispecies consortium, (d) performing antimicrobial test against bacteria in biofilm, (e) testing both gram-positive and -negative bacteria, and (f) testing against bacteria commonly associated with peri-implantitis. Studies were categorized based on fulfilment of the aforementioned criteria into: Green, Yellow, or Red if they satisfied 5–6, 3–4, or $\leq$2 criteria, respectively.

3. Results

3.1. Search Results

A total of 1082 articles were initially identified and screened for potentially relevant studies (Figure 1), utilizing the Covidence online platform (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia). Subsequently, 189 articles were excluded as duplicates and 856 were excluded after title and abstract screening, either because Sr was not among the substances investigated or its antimicrobial potential was not tested. A total of 37 articles were selected for full-text reading, 28 of which were excluded due to: 19 irrelevant studies (no antimicrobial testing), five studies with no Sr-only coating group, three studies combined Sr with antimicrobial agents and one was a review (on antimicrobial prosthesis coatings [38]). Furthermore, the results of manually searching the reference lists of the included articles did not result in any additional relevant articles. In total, nine in vitro articles fulfilled the inclusion criteria and were included in the current review.

3.2. Surface Substrates and Application Methods

Among the included studies, commercially pure Ti was used in seven studies, while alloyed Ti, i.e., Ti-6Al-4V, was used in two studies (Table 1). The identified studies investigated various substrates: four studies used Ti disks, while Ti-coated wafers, Ti plates, Ti "substrate", Ti wire cut into specimens, and Ti "coupons" were used in each of the remaining five studies. Among the different included studies, Sr was applied either alone or in combination with other metal ions on Ti substrates using different application methods. Application methods of Sr included micro-arc oxidation [26,39,40], incorporation into hydroxyapatite (HA) coatings using either hydrothermal processes [41,42] or plasma spray-

ing [43], or by using co-incident micro-blasting (CoBlast) [44] in addition to calcium (Ca) using alkali-and-heat treatment [45,46]. Moreover, Ti substrates were functionalized with Sr alone or Sr in combination with Ag [26] or Zn [40], HA coatings comprising different metal ions, either doped or incorporated into the HA coatings [41–44], or as Sr combined with calcium (Ca) alone [45,46] or with other metals: cobalt (Co), fluorine (F), and phosphate (P) [39].

Table 1. General characteristics of included studies.

Author/Year	Type of Investigation	Substrate	Material	Tested Ions	Method of Application
He, et al. 2016 [26]	In vitro	Ti wafers	cp Ti	Sr/Ag	Magnetron sputtering and micro-arc oxidation
Geng, et al. 2016 [41]	In vitro	Ti plates	Grade 5 Ti alloy	Sr/Ag/HA coating	Hydrothermal method
Geng, et al. 2017 [42]	In vitro	Ti discs	cp Ti	Sr/Ag/HA coating	Hydrothermal method
Fielding, et al. 2012 [43]	In vitro	Ti substrate	Grade 2 cp Ti	Sr/Ag in HA coating	Plasma spray
Masamoto, et al. 2021 [45]	In vitro *	Ti disks	cp Ti	CaSr/CaSrAg	Alkali-and-heat treatment
Okuzu, et al. 2021 [46]	In vitro	Ti disks	cp Ti	CaSr/CaSrAg	Alkali-and-heat treatment
Zhao, et al. 2019 [40]	In vitro	Ti specimens	cp Ti	MT-Zn/Sr	Micro-arc oxidation
O'Sullivan, et al. 2010 [44]	In vitro	Ti coupons	Grade 5 Ti alloy	HA- Sr/Ag/Zn	CoBlast technology
Zhou, et al. 2016 [39]	In vitro	Ti disks	cp Ti	Sr, Ca, Co, P, F	Micro-arc oxidation

* This study also included in vivo antimicrobial testing; however, a CaSr group was not included in the in vivo part, and thus is not included here.Ti: Titanium; Sr: Strontium; Ag: Silver; Zn: Zinc; HA: Hydroxyapatite; MT: Microporous coating; Ca: Calcium; P: Phosphate; Co: Cobalt; F: Fluorine; cp: commercially pure

3.3. Bacterial Strains and Methods of Antimicrobial Analysis

Employing different methods, eight studies investigated the antimicrobial effect against *Staphylococcus aureus* (*S. aureus*) as a representative of Gram-positive bacteria, while five of these studies also investigated *Escherichia coli* (*E. coli*), representing Gram-negative bacteria (Table 2). Only one study investigated the antimicrobial effect against *Pseudomonas aeruginosa* (*P. aeruginosa*), a Gram-negative bacterium [43]. The plate counting method was used for both early and late detection of antimicrobial activity, while both the agar diffusion test and the bacterial viability assay were used to assess early antimicrobial effects (Table 2).

Table 2. Antimicrobial outcome of coated Ti surfaces.

Author	Active Agent	Bacteria Tested	Method of Analysis/Time of Experiment	Intergroup Comparison *		Antimicrobial Significance of Sr-Related Samples Compared with Non-Functionalized Controls **
He, et al. [26]	Ti/Sr/Ag	*S. aureus* *E. coli*	Plate counting method (6 h) Immersion and culturing (1, 7, 14, 21, and 28 days) Bacteria viability assay (24 h) (*S. aureus* only)	Ti = Sr < Sr/Ag 0.40 ≈ Sr/Ag 0.83 Sr < Sr/Ag 0.40 < Sr/Ag 0.83 Ti = Sr < Sr/Ag 0.40 ≈ Sr/Ag 0.83		Sr: no antimicrobial effect observed Sr: no antimicrobial effect observed Sr: no antimicrobial effect observed
Geng, et al. [41]	Sr/Ag/HA	*S. aureus* *E. coli*	Agar diffusion test (24 h)	HA = Sr < Ag0.3 < 10Sr Ag0.3 = Ag0.1 = Ag0.5		Sr: no zone of inhibition observed
Geng, et al. [42]	Sr/Ag/HA	*S. aureus* *E. coli*	Plate counting method (24 h) Agar diffusion test (24 h)	Ti = HA = 10 Sr * < Sr/Ag = Ag0.1 Ti = HA = 10 Sr * < Sr/Ag = Ag 0.1		10Sr: no antimicrobial effect observed 10Sr: no zone of inhibition observed
Fielding, et al. [43]	Sr/Ag/HA	*P. aeruginosa*	Bacterial viability assay (24 h)	HA = Sr-HA < Sr/Ag-HA = Ag-HA		Sr-HA: limited antimicrobial effect
Masamoto, et al. [45]	CaSr/CaSrAg	*S. aureus*	Colony forming unit assay (24 h)	Ti < CaSr < CaSrAg		CaSr: limited antimicrobial effect
Okuzu, et al. [46]	CaSr/CaSrAg	*S. aureus* *E. coli*	Colony forming unit assay (24 h)	*S. aureus*	Attached: Ti < CaSr < CaSrAg Planktonic: Ti < CaSr < CaSrAg	CaSr: limited antimicrobial effect CaSr: limited antimicrobial effect
				E. coli	Attached: Ti = CaSr < CaSrAg Planktonic: CaSr < Ti < CaSrAg	CaSr: no antimicrobial effect observed CaSr: no antimicrobial effect observed
			Bacterial viability assay (24 h)	*S. aureus* *E. coli*	Ti ≈ CaSr < CaSrAg Ti ≈ CaSr < CaSrAg	CaSr: no antimicrobial effect observed CaSr: no antimicrobial effect observed
Zhao, et al. [40]	MT-Zn/Sr	*S. aureus*	Plate counting method (24 h)	Ti < MT < MT-Sr < MT-Zn/Sr < MT-Zn		MT-Sr: antimicrobial rate ca. 55%
O'Sullivan, et al. [44]	HA-Sr/Ag/Zn	*S. aureus*	Modified ASTM, immediately or after 30 days of incubation for ion release.	Immediately After 30 days	Zn-HA < Sr-HA ≈ AgA Zn-HA < Ag-HA < Sr-HA	Sr-HA: antimicrobial rate = 49% Sr-HA: antimicrobial rate ca. 49%
			Anticolonization activity (1, 7, and 14 days)	Day 1 Day 7 Day 14	Zn-HA ≈ Sr-HA ≈ Ag-HA Zn-HA ≈ Sr-HA < Ag-HA Zn-HA ≈ Sr-HA < Ag-HA	Sr-HA: biofilm inhibition ca. 40% Sr-HA: biofilm inhibition ca. 40% Sr-HA: biofilm inhibition ca.10%
Zhou, et al. [39]	Sr, Ca, Co, P, F	*S. aureus* *E. coli*	Plate counting method (24 h)	Ti = TiCaP = Sr-TiCaP < SrCo-TiCaP < SrCoF-TiCaP		Sr-TiCaP: no antimicrobial effect observed SrCo-TiCaP: antimicrobial rate ca. 40%, ($p < 0.01$)

* Intergroup comparison of the antimicrobial outcome of all samples tested in the study, arranged from lowest to highest. ** Refers to the outcome of the group testing Sr as the only antimicrobial agent. Ti: Titanium; Sr: Strontium; Ag: Silver; Zn: Zinc; HA: Hydroxyapatite; Ca: Calcium; P: Phosphate; Co: Cobalt; F: Fluorine; MT: Microporous coating; Zn-HA: Zinc-substituted hydroxyapatite; Sr-HA: Strontium-substituted hydroxyapatite; Ag-HA: Silver-substituted hydroxyapatite; ASTM: Antimicrobial standard testing method; ZOI: zone of inhibition

3.4. Antimicrobial Effect of Sr

Table 2 shows a summary of the findings from the antimicrobial assays, specific methods of antimicrobial analysis, and the observation time, as reported in the included studies. Sr-modified samples demonstrated relevant early antimicrobial potential against *S. aureus* in three studies; only one presented statistical significance values [39], while the other two presented only the percentage of antimicrobial rate and biofilm inhibition [40,44]. Further, O'Sullivan et al. showed a potent antimicrobial effect of Sr, superior to that of both Zn and Ag, after immersing Sr-coated samples for 30 days in phosphate buffered saline (PBS) for ion release induction [44]. By comparing freshly prepared coupons with coupons that had been eluted for 30 days, they demonstrated that only Sr-functionalized substrates maintained 49% antimicrobial activity. Additionally, using the plate counting method, Zhao, et al. showed a superior antimicrobial rate of Sr-modified micro-porous surfaces compared with non-functionalized Ti surfaces; three other studies indicated that both uncoated Ti and Sr-functionalized Ti samples showed similar bacterial growth patterns, and the agar plates were found to harbor a near confluent population of bacterial colonies [26,41,42]. Only two studies [26,44] investigated the long-term antimicrobial effect of Sr-functionalized surfaces (Table 2). O'Sullivan et al. demonstrated the anticolonization capacity of Sr against a biofilm of *S. aureus* at day 7 and 14. In contrast, He, et al. did not observe a similar effect, utilizing a bacterial viability assay and immersion and culturing methods, against either *S. aureus* or *E. coli*.

When Sr was combined with other metal ions, i.e., Co and/or F, a reduction in colonies was observed [39]. Furthermore, studies that used the bacterial viability assay showed largely viable cells for the Ti and Sr samples, compared with when Ag was added to the surface in combination with Sr [26,43,46]. Additionally, other studies showed that combining Sr with Ag did not impact the antimicrobial potential of Ag and resulted in a similar outcome as when adding solely Ag to the Ti substrate [41,42]. Likewise, an antimicrobial effect when combining Sr with Ag was also observed after 28 days (Table 2) [26]. However, Sr-functionalized surfaces did not show any antimicrobial effect against *E. coli* for any of the included studies, at either early or late timepoints (Table 2). The in vivo antimicrobial potential of combined Sr/Ag-modified surfaces was investigated by Masamoto et al. [45]; however, Sr only-coated samples were not included and therefore the outcome of the test was not included in the analysis.

3.5. Sr Ion Release

In the study by He, et al., Sr presented sustained stable release over 28 days of observation, while Ag showed a rapid release, especially during the first day. A gradual cumulative increase in the concentration of Sr was observed up to 7–14 days; on the first day, the concentration of Sr was only 0.6 ppm, followed by a gradual increase until reaching 1 ppm at day 14, and then a steady release of 1.1 ppm until day 28 [26]. Similarly, Geng, et al. reported that Sr ion release was below 50 μg/L in the first 24 h, followed by a cumulative increase reaching 150 μg/L at day 3, until reaching approximately 400 μg/L at day 7 [41].

Although combining Ag and Sr metal ions did not affect the release rate, in the first 7 days, of either metal ion significantly [42], surfaces functionalized with a combination of Sr/Ag presented slightly lower release rates compared with Ag alone [41,43]. Within the first 24 h, HA-coating containing Sr had a low release profile (less than 1 ppm), yet Sr release and cumulative concentration started to increase rapidly after 24 h up to 7 days (approximately 1.8 ppm) [41,43]. Furthermore, the Sr reservoir was maintained at more than 90% of its initial concentration while over 90% of the Ag concentration was released over a 30-day period [44]. In vivo blood serum ion concentration showed stable levels of Sr over a period of 28 days after placement in the right femoral bone of rats, ranging between 0.8 and 1 ppm; this value was almost three times higher than that of Ag [45].

3.6. Risk of Bias and Relevance Assessment

The results of risk of bias and relevance assessment, based on the predetermined set of six parameters, are presented in Table 3. For risk of bias, only two studies partially fulfilled the criteria and seven did not fulfil any or only fulfilled one criterion. For the relevance assessment, two studies fulfilled the criteria, four partially fulfilled the criteria, and three did not fulfil any or only fulfilled one criterion. An overall assessment is also presented in Table 3. For the overall assessment, only one study fulfilled five out of the six criteria [26], while five studies fulfilled three to four criteria, and three fulfilled two or less criteria.

Table 3. Risk of bias and relevance assessment of the included studies.

Study	Two Antimicrobial Tests	Different Time Points for Assessment	Multi-Species Consortia	Risk of Bias *		Gram+ & Gram− Bacteria	Bacteria Common in Peri-Implantitis	Testing Against Biofilm	Relevance Assessment *		Overall Assessment **
He, et al. [26]	✓	✓	×			✓	✓	✓			
Geng, et al. [41]	✓	×	×			✓	✓	×			
Geng, et al. [42]	✓	×	×			✓	✓	×			
Fielding, et al. [43]	×	×	×			×	×	×			
Masamoto, et al. [45]	✓	×	×			×	✓	×			
Okuzu, et al. [46]	✓	×	×			✓	✓	✓			
Zhao, et al. [40]	✓	×	×			×	✓	×			
O'Sullivan, et al. [44]	✓	✓	×			×	✓	✓			
Zhou, et al. [39]	✓	×	×			✓	✓	×			

* For risk of bias and relevance assessments: Green, fulfilled all 3 criteria; Yellow, fulfilled only 2 criteria; and Red, fulfilled ≤ 1 criteria.
** For overall assessment: Green, fulfilled 5–6 criteria; Yellow, fulfilled 3–4 criteria; and Red, fulfilled ≤ 2 criteria.

4. Discussion

The nine studies included in this review showed large variation regarding the methods of applying Sr on Ti surface substrates and the methods used for antimicrobial analysis. Nevertheless, the results of the current systematic review revealed that, in most studies, Sr-functionalized Ti demonstrated a limited immediate (i.e., within 24 h) antimicrobial effect, likely due to a low Sr ion release; with an adequate Sr ion release, however, a relevant bactericidal and bacteriostatic potential was observed at both early and late timepoints.

Most included studies argued that the important timepoint for preventing Ti implant infections is 6–12 h post-surgery; hence, most included studies investigated only the early antimicrobial effect of the functionalized surfaces. Indeed, the mechanism of biofilm formation starts with initial protein adhesion and bacterial cell attachment, followed by cell proliferation and aggregation, and eventual formation of a mature biofilm [47]. However, it is widely recognized that peri-implantitis is caused by a dysbiotic cluster of bacteria in a biofilm attached to the Ti surface [48]; this can occur long after implant placement, with the implants having been in the oral cavity for several years. In fact, with the development of modern modified implant surfaces, it has become apparent that surface topography is a crucial factor in biofilm formation on dental implants [49], and it was recently argued that Ti by itself might even favor dysbiosis [50]. Hence, even though modified implant surfaces enhance the osseointegration process, their impact on bacterial adhesion cannot be ignored; this further substantiates the importance of developing long-lasting antimicrobial coatings. It was demonstrated that, although the submucosal biofilm profiles of patients suffering from peri-implantitis presents great complexity and heterogeneity, several specific bacterial species appear more frequently at infected implants [51]. Examples of species commonly discussed in the literature are *Porphyromonas gingivalis* (*P. gingivalis*) [52], *Ag-*

gregatibacter actinomycetemcomitans (*A. actinomycetemcomitans*), *S. aureus*, *Streptococcus mitis* (*S. mitis*) [53], *Staphylococcus epidermidis* (*S. epidermidis*) [54], *Fusobacterium nucleatum* (*F. nucleatum*) [55], and *E. coli* [56]. In fact, the multispecies biofilms associated with peri-implant infections were revealed to be more resistant to antimicrobial treatment and to present a great behavioral shift of the microbial community [57]; this must be represented adequately in antimicrobial investigations by using multispecies biofilms, thus allowing for proper assessment of possible antimicrobial technologies.

The antimicrobial ability of metal ions, in general, can be attributed to the ability of ions to be released into the surrounding environment [58,59]. A sustained uniform release is preferable for long-lasting antimicrobial properties [59,60]. By contrast, a rapid short-term release may not only compromise the longevity of the antimicrobial effect, but may exert a toxic effect on the surrounding vital tissue [61]. In the current systematic review, for the early 6–24 h testing methods, the data indicated that surface functionalization with metal ions presenting rapid release (i.e., Ag) had the most potent antimicrobial effect. Nonetheless, O'Sullivan et al. demonstrated that a decrease in Ag ion release, after 30 days of immersion, was found to be associated with a significant reduction in the antimicrobial effect, maintaining only 9% of its original antimicrobial effect [44], while the antimicrobial effect of Sr remained unchanged at almost 50%. Furthermore, during the first 30 days, the reservoir of Ag ions nearly depleted completely, while less than 10% of Sr ions were released, compared with their respective original reservoirs. This finding indicates that, for long-term prevention strategies, Sr may be a more reasonable option. Moreover, several cytotoxic reactions were observed in association with the use of Ag ions, including a significant decrease in cell viability, proliferation, and metabolic activity, thus, indicating an eminent risk of using Ag in proximity to biological tissues [62]. Surprisingly, Ag at a subtoxic concentration was found to negatively affect adipogenic and osteogenic differentiation of human mesenchymal stem cells (hMSCs) [63]. On the other hand, monitoring lactate dehydrogenase (LDH) to determine the cytotoxic effect of Sr ions not only indicated that Sr release was safe over 28 days of observation, but also that lower levels of LDH, compared with uncoated Ti, were present; this may indicate improved biocompatibility of Sr-functionalized surfaces [39]. Both He, et al. and Fielding, et al. demonstrated that combining Sr with Ag did not adversely impact release of any of the ions. Similar data were also reported by a recent investigation, where combining Sr with other metal ions (i.e., Ca, P, F, and Co) did not affect the release profiles of the specific ions over a period of 30 days; specifically, all surface modifications showed sustained stable cumulative release up to 30 days [21].

The specific method used for functionalizing the Ti surface is critical in determining release properties, but also may have a negative impact on the Ti surface itself. With the aim to achieve long-lasting antimicrobial properties, various methods have been reported for the application of Sr with Ag, Zn, and copper (Cu) on titanium substrates. For example, plasma spraying is a well-known surface modification technique that allows deposition of, e.g., HA coatings on Ti surfaces. One major disadvantage of using this technique, however, is the potential decomposition of the HA coating over time [64]. Another approach is micro-arc oxidation (MAO) [65], which is considered an efficient technique that generates highly stable surfaces. Nonetheless, MAO can be influenced negatively by many factors, such as the type of electrolytes used, the composition of the resulting oxide matrix, and discharge parameters which, in turn, affect the mechanical properties, microstructure, and morphology of the resulting surface [65,66]. Yet another approach is the co-incident micro-blasting process (CoBlast), where an abrasive material, along with the desired dopant, is directed in a co-incident manner on the surface of the substrate. This method was found suitable for modifying the surface of Ti substrates with different Sr-substituted bioceramics [44,67]. The amount of Sr release from samples modified using the CoBlast technique, reported by O'Sullivan, et al. [44], for coupon samples incubated over a period of 30 days in 2 mL PBS was similar to the in vivo Sr serum concentration reported by Masamoto, et al. [45], using alkali heat treatment and ranging between 0.8–1 ppm. Likewise, He, et al., by em-

ploying magnetron sputtering and micro-arc oxidation techniques, presented a release rate of 1.1 ppm over a period of 28 days for Sr-functionalized Ti wafers incubated in 20 mL PBS. Ion release data obtained from substrates modified using plasma spraying doped in 5 mL PBS, presented by Fielding, GA. et al., demonstrated slightly higher release rates, for the collective metal ions (i.e., Sr and Ag) in HA coating, of about 1.7 ppm. However, the release rate of Sr-only was not presented [43]. On the other hand, modifying Ti substrates with the hydrothermal treatment method indicated a stable daily Sr release of 50 µg/L, up to 7 days, from functionalized plates immersed in 10 mL PBS [41]. Due to the great variation in terms of substrates and their dimensions, the amount of solution used for immersion, and the methods of analysis, comparing Sr release to determine the best method for application among the included surface functionalization techniques is not possible. Nonetheless, understanding the limitations of the previously mentioned approaches is crucial for optimizing functionalization of Ti substrates with Sr. Further, one should consider any coating, without a recharge system, only as a temporary preventive approach.

Although various metal ions have been investigated in early research, Ag remains the most widely studied metal ion due to its well-documented antimicrobial capacity [68,69]. However, as demonstrated, the negative impact on biological tissues resulting from adding Ag to Ti surfaces is still an issue of concern [70]. Hence, adding another biocompatible metal ion with antimicrobial capacity and bone formation enhancement potential to the surface of the Ti implant, i.e., Sr, is not only a rational alternative, but may be considered a necessary step for the development of a novel antimicrobial and biocompatible surface modification for dental implants. Indeed, in vivo investigation of Sr/Ag combination as a surface functionalization on Ti substrates demonstrated a significant potential to serve that purpose [27]. Interestingly, even though the earlier mentioned study by O'Sullivan et al. demonstrated an antimicrobial potential of Sr-functionalized surfaces, the authors argued that the mechanism of action is still not fully known or understood, which, in turn, provides an opportunity for future research to further elaborate on this matter. Moreover, Sr is known to enhance bone formation through different mechanisms, e.g., improving osteoblast differentiation, increasing preosteoblast proliferation, collagen synthesis, and improving the mineralization of the bone matrix [71]. It has previously been used in the treatment of osteoporosis, showing enhancement in bone mineral density and bone quality, and was associated with a notable reduction in bone fractures in patients [34,72]. In a recent preclinical in vivo study, systemically administered Sr ranelate seemed to promote bone formation in grafted bone defects in healthy and osteoporotic rats [73], while grafting of a Sr-loaded bone substitute significantly enhanced bone formation, compared with non-loaded controls in another preclinical study [33]. In recent systematic reviews it was found that both systemically or locally administered Sr ranelate significantly improves implant osseointegration and peri-implant bone quality in both healthy and osteoporotic conditions [74,75]. Modifying Ti dental implants with Sr displayed significantly higher bone to implant contact (BIC), increased bone formation and, interestingly, improved the biomechanical properties of the implant compared with non-coated implants [76]. Thus, functionalization of Ti implants with Sr seems to exert a dual effect, i.e., enhancement of bone regeneration/osseointegration and antimicrobial activity, which is relevant both in dental and orthopedic applications.

In this context and to evaluate the risk of bias and relevance of the studies included herein, a purpose-made tool was developed and implemented. It revealed that the current evidence is limited by the fact that studies investigated the antimicrobial effect of Sr using single-species models. Further, most of the studies used too short of an observation timepoint (i.e., up to 24 h); although it is important to ensure that implants are capable of preventing infections from occurring intra- and post-surgically, peri-implantitis usually develops at a later timepoint and therefore any possibly relevant effect of an antimicrobial agent should be assessed over an extended period of time. Hence, for relevant testing of the antimicrobial capacity of Ti surfaces functionalized with Sr, multispecies biofilm testing methods using various and extended timepoints should be considered. To allow for direct

comparison of various test methods and specific technologies, it is suggested that Sr ion concentration in the medium is reported, along with the Sr ion release from a given surface area over time, e.g., as $\mu g/cm^2$. Additionally, information on the physical properties and stability of the examined surfaces, as well as on the impact on mammalian cells and/or tissues, is missing and should be generated.

5. Conclusions

In most studies included herein, Sr-functionalized Ti showed a limited immediate (i.e., 24 h) antimicrobial effect, likely due to a low Sr ion release; however, with an adequate Sr ion release, a relevant antimicrobial effect as well as a biofilm inhibition potential against *S. aureus*—but not *E. coli*—was observed at both early and late timepoints. Nevertheless, combining Sr with other metal ions (i.e., Ag, Zn, and F) demonstrated antimicrobial activity against both bacterial species. Future studies should assess the antimicrobial potential of Ti functionalized with Sr against multispecies biofilms associated with peri-implantitis.

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