



HAL
open science

Bioactivity assessment of bioactive glasses for dental applications: A critical review

Caroline Mocquot, Nina Attik, Nelly Pradelle-Plasse, Brigitte Grosgeat,
Pierre Colon

► To cite this version:

Caroline Mocquot, Nina Attik, Nelly Pradelle-Plasse, Brigitte Grosgeat, Pierre Colon. Bioactivity assessment of bioactive glasses for dental applications: A critical review. *Dental Materials*, 2020, 10.1016/j.dental.2020.03.020 . hal-02909004

HAL Id: hal-02909004

<https://hal.science/hal-02909004v1>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

BIOACTIVITY ASSESSMENT OF BIOACTIVE GLASSES FOR DENTAL APPLICATIONS: A critical review

**Caroline Mocquot^{1,2}, Nina Attik^{1,3}, Nelly Pradelle-Plasse^{1,2}, Brigitte Grosogeat^{1,3,4},
Pierre Colon^{1,2}**

¹Univ Lyon - Claude Bernard Lyon 1, UMR CNRS 5615, Laboratoire des Multimatériaux et Interfaces, F-69622 Villeurbanne, France

²Département d'Odontologie Conservatrice - Endodontie. Université de Paris, Faculté dentaire, Hôpital Rothschild, Assistance Publique-Hôpitaux de Paris, France

³Univ Lyon, Université Claude Bernard Lyon 1, Faculté d'Odontologie, 69008 Lyon, France

⁴Hospices civils de Lyon, Service d'Odontologie, 69007 Lyon, France

* Corresponding author: Dr Caroline MOCQUOT, Laboratoire des Multimatériaux et Interfaces/Faculté d'Odontologie - Université de Lyon 1, 11 rue Guillaume Paradin, 69372 LYON Cedex 08, France
caroline.mocquot@etu.univ-lyon1.fr

BIOACTIVITY ASSESSMENT OF BIOACTIVE GLASSES FOR DENTAL APPLICATIONS: A critical review

1. Introduction

Minerals ions can be removed from hydroxyapatite (HA) crystals of dental hard tissues, enamel and dentin, in case of erosive attack or carious lesions, this process is demineralization. Restoring these mineral ions to the HA crystals is called remineralization [1]. The contemporary approach to caries management has drastically changed: operative strategies are abandoned in favor of biologic approaches based on individual caries risk, preventive dentistry, and non-invasive or minimal options [2-3]. Remineralization is a non-invasive treatment to preserve dental tissues after an acidic attack. *In situ* remineralization could occur with a dissolution reprecipitation process in enamel or in dentin, but new tertiary dentin could also be produced by stimulation of pulp cells [4]. BAG are involved in both processes, but some studies have focused only on the first mechanism because others have focused on the second mechanism; thus, the term bioactivity for BAG remains unclear. BAG were introduced in 1969 by professor Larry Hench (BAG 45S5), the composition was 45% silica (SiO_2) – 24.5% calcium oxide (CaO) - 24.5% sodium oxide (Na_2O) – 6% phosphate (P_2O_5). Initially, BAG were used for medical application for their ability to form a bond with bone [5]. Nowadays, the original composition (45S5) and manufacturing method of BAG are modified to create mesoporous BAG, to change particle size (nano or micro-scale) or to add additives [6]. BAG are considered bioactive materials; however, a better definition of the bioactive effect is required to improve formulations or incorporation of particles in scaffolds [7-8].

It is generally accepted that a bioactive material is a material able to induce specific biological activity and stimulate a beneficial response from the body bonding to the host tissue [5;9]. Regarding BAG, Lebecq et al. defined bioactivity by following the crystalline hydroxy-carbonate apatite (HCA) layer growth on the surface of glass particles [10]. According to other definitions, the bioactivity is the ability to form mineral deposits in biological conditions [11].

In the dental field, the definition of bioactivity depends on the clinical use from the ability to induce reprecipitation of HA on the surface of enamel and dentin to cellular effects induced by the release of biologically active substances and ions. There is no consensus on bioactivity's definition. Vallittu et al. suggested limiting the terms 'bioactive' with respect to dental materials only to scientifically

proven materials and material combinations that release substantial quantities of ions for specific biomineralization in the clinical environment of the material [12].

The main objective of this review was to identify and classify the different methodologies used for the BAG bioactivity quantification regarding dental hard tissues, to determine the key parameters considered for these studies and therefore, to influence dental research and clinical practice on an international basis.

2. Materials and methods

The website COVIDENCE was used to import references and view duplicates, titles, abstracts, and full texts.

2.1 Inclusion and exclusion criteria

To identify relevant studies, the following inclusion and exclusion criteria were defined.

The search strategy was conducted to include research articles that have evaluated the bioactivity of BAG (without additives, scaffolds, or other constituents other than SiO₂, CaO, Na₂O, or P₂O₅) with the perspective of dental applications. BAG with phosphoric acid (PPA) or polyacrylic acid (PAA) were included because these adjunctions were used to modify the consistency of BAG (from powder to paste) and not the composition.

The search strategy was conducted to exclude case reports, abstracts, literature review articles, retrospective studies, editorials, opinions, surveys, guidelines, conferences and commentary articles, and publications in a language different from English or French.

The series of inclusion and exclusion criteria were established by a consensus of all authors after discussions and while considering the research question—How to evaluate the bioactivity of bioactive glasses for dental applications?—and the objectives of the study.

2.2 Search strategy

Four databases were screened: PubMed, Embase, Cochrane Library, and Scopus. The references cited in the articles included were also checked. The published scientific articles from May 1999 to January 2020 were systematically assessed for this review.

Search strategies were developed and performed in the four electronic databases. The search terms were divided in three parts:

- Teeth, for example, dental, enamel, dentin, dental, dentistry, teeth, and tooth.
- Bioactive glasses, for example, bioactive glass, bioglass, 45S5, and nanobioactive glass.
- Bioactivity, for example, remineralization, bioactivity, bioactive, and biomineralization.

2.3 Paper selection

The titles and abstracts of all articles identified by the electronic search were read and assessed by two authors (CM and BG) until January 2020. All titles and abstracts were examined and selected in accordance with the eligibility criteria. Those that appeared to fulfil the inclusion criteria, or with insufficient data in the title and the abstract, were selected for full analysis. The two authors were also the two reviewers and assessed the full text articles independently. Any disagreement on the eligibility of studies included was resolved through discussion and consensus.

2.4 Data extraction

One author (CM) extracted the data using a prepiloted data collection form, and a second author (BG) verified data extraction independently for completeness and accuracy. Data obtained were as follows: general study details (first author, date of publication); population: BAG investigated (e.g. glass label, size of particles if mentioned, technique used to elaborate BAG, BAG's composition, comparison with); and the objectives, criteria, methods (solutions, time of storage, and analysis), and outcomes of studies. Any potential conflict was resolved by a joint discussion between the two authors.

3. Results

3.1 Study selection

The initial electronic search using the keyword combination returned 1078 articles. After the removal of duplicates, 708 records were examined. The title and abstracts of the remaining 708 papers were screened, and 563 papers were excluded because they were irrelevant to the inclusion

criteria. Finally, 145 relevant papers were scrutinized by downloading the papers and reading the full text. A consensus between the two authors was reached to determine which studies fully fulfilled the selection criteria. Ninety-eight papers were excluded after reading the full text. Thirteen papers were found from the references of the selected papers, and these were also assessed in a similar manner. Finally, 60 studies were included in this review (Fig. 1).

3.2 Main characteristics of selected articles

A wide range of analysis of bioactivity was presented from the included studies. The most common analysis was the formation of apatite by *in vitro* evaluation in solutions. Other analysis corresponded to enamel and/or dentin remineralization *in vitro*, mineralization ability of cells *in vitro*, and pulp capping or pulpotomy *in vivo*. Data obtained were as follows: BAG investigated, criteria, methods, and main results.

BAG investigated

The majority of BAG were made by melt-quench (n=20) and sol-gel techniques (n=15). There were 5 BAG made by the EISA (Evaporation Induced Self-Assembly) process and 3 by flame spray synthesis.

The size of particles was not systematically specified. Eighteen studies stipulated that BAG had a micrometric size, especially 38 or 45 μm . Eight BAG had nanometric size (BGn/nBG/BGNR/NBG), from 10 to 590 nm and included 34, 20-60, 100, 510 nm. Some BAG were mesoporous (MBG/MBGs).

The composition of BAG was mainly traditional BAG 45S5 (n=29 studies). Other studies changed the concentration of SiO, CaO, Na₂O, or P₂O₅ to modify the characteristics and bioactivity of BAG. Five BAG were made only with SiO₂ and CaO: 90 or 85 or 70 (%) – 10 or 15 or 30 (%), respectively. Nineteen BAG were made without Na₂O.

Comparison with:

The BAG without adjuvants were compared with the following:

- 1: Control group (artificial saliva, demineralized water...).
- 2: BAG with adjuvants (fluoride (n=9), potassium (n=2), strontium (n=2), magnesium (n=1), zinc (n=1), bismuth oxide (n=1), ampicilline (n=1), siRNA (Small Interfering

Ribonucleic Acid) (n=1), barium (n=1)...), or scaffolds (chitosan [CS] or polycarbolactone [PCL]).

- 3: Oxide alone (ZnO), antiseptic (chlorhexidine [CHX]), desensitizing agents (with oxalate) or bioactive products (caseinphosphopeptide-amorphouscalciumphosphate [CPP-ACP], stannous fluoride, mineral trioxide aggregate (MTA), formocresol, ferric sulfate, Biodentine™, biosilicate, calcium phosphate cement, calcium hydroxide [CH], BAG modified with soda lime spherical glass or laser irradiation, coating glasses...) or restorative materials: dental adhesive, glass porcelain system, glass ionomer cement/resin-modified glass ionomer cement (GIC/RMGIC).
- 4: Laser irradiation.

Objectives, Criteria, and Methods – Results in terms of objectives

Mechanism of apatite formation on BAG surface *in vitro* in solutions:

The majority of articles evaluated the bioactivity of BAG in solutions *in vitro* (Table 1). Simulated body fluid (SBF) (n=23) was the main solution used; however, a large part of the research used Tris buffer solution (TBS) (n=7) and one study used Hank's solution. Two studies used SBF and TBS.

The objectives and criteria of these studies were to evaluate the formation of apatite *in vitro*. There was no consensus on contact time: 15, 20, or 30 minutes; 1, 3, 4, 6, 8, 9, or 12 hours; 1, 2, 3, 5, 7, 9, 17, or 30 days; and 2, 3 or 4 weeks. Studies used the following analysis techniques:

- *Microscopic observations:* Scanning electron microscopy (SEM), field emission-SEM (FESEM), and transmission electron microscopy (TEM).
- *Chemical or structural analysis:* Energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), solid state nuclear magnetic resonance (NMR), Raman spectroscopy, inductively coupled plasma (ICP), and UV-VIS spectrophotometer.
- *Association between microscopy and chemical or structural analysis:* SEM-EDX.

The main analysis techniques were SEM, FTIR, XRD, and solid state NMR (Fig. 2).

Results lead to the following conclusion: apatite formation occurs by contact with BAG and solutions.

BAG capacity to remineralize enamel and/or dentin *in vitro*:

To evaluate the ability of BAG to remineralize dental tissues, 16 studies used human dentin, 9 studies used human enamel, and 2 studies bovine enamel *in vitro* (Table 2).

The objectives were to assess BAG capacity to remineralize enamel and/or dentin *in vitro*. Many criteria were used, for example, mineral changes (Ca/P ratio, presence of Ca, P, Si, O, or Na), surface and subsurface microhardness, roughness, formation of the pellicle layer or globular HA, improvement of mechanical properties, surface morphologies, tubular obstruction for dentin, tubular fluid. No consensus was reached on contact time: 10 or 30 seconds; 5 or 10 minutes; 1, 2, 3, 5, 7, 10, 14, 21, 24, or 30 days; and 3 or 6 weeks. To evaluate the ability of BAG to remineralize enamel or dentin *in vitro*, studies used the following analysis techniques:

- *Microscopic observations*: SEM, SEM/three-dimensional stereophotographs, FESEM, TEM, and confocal laser scanning microscopy (CLSM).
- *Chemical or structural analysis*: EDX, FTIR, XRD, Solid State-NMR, ICP-atomic emission spectroscopy, attenuated total reflectance-FTIR (ATR-FTIR), electron microprobe analysis (EMPA), micro-computed tomography (CT), atomic force microscope (AFM), thermogravimetric analysis (TG), fluid filtration/transport system, optical coherence tomography (OCT), focused ion beam, profilometry analysis, X-ray photoelectron spectroscopy (XPS), and Raman spectroscopy.
- *Association between microscopy and chemical or structural analysis*: SEM-EDX and FESEM-EDX.
- *Evaluation of mechanical properties*: Nanoindentation, ultramicroindentation analysis (UMIS), microhardness, and cryofracture examination.

Fig. 3 shows the repartition of different analysis techniques.

According to the studies, BAG were bioactive materials for therapeutic remineralization of dental hard tissues.

In vitro effect of BAG on the mineralization capacity of cells:

Five studies evaluated the bioactivity of BAG in contact with cells *in vitro* (Table 3).

The objectives were to investigate the *in vitro* effects of BAG on the mineralization capacity of cells. The calcium precipitation and the matrix mineralization of cells were the criteria. Four studies used human dental pulp cells (hDPCs) [13-16] and one study used stem cells derived from deciduous teeth (SHEDs) [17]. The contact time was 21 days to 4 weeks. Alizarin red S was used for technical analysis. For all studies, BAG increased mineralized nodule formation.

In vivo evaluation of dentin mineralization and/or dental pulp response:

Three studies evaluated the dental pulp response with BAG by using pulp capping or pulpotomy in human or rat pulp *in vivo* (Table 4). The objectives were *in vivo* evaluation of dentin mineralization and/or dental pulp response. The criteria were the presence or absence of a dentin bridge associated with the pulp status (no or mild, moderate, or severe inflammation, necrosis, abscess, resorption). BAG were compared with Calcium Hydroxide (CH) and MTA. The contact time extended from 2 weeks to 2 months. Histological analysis, hematoxylin and eosin staining or Masson's trichrome were for the technical analysis. BAG was used in Wang et al., especially the nanoscale version, and greater induction of pulp cell migration, surface attachment, proliferation, and odontogenic differentiation were demonstrated [15]. In Salako et al., BAG induced an inflammatory response at 2 weeks with the resolution of inflammation at 4 weeks in molars rats [18]. Moreover, MTA performs as an ideal pulpotomy agent and causes dentin bridge formation while maintaining normal pulpal histology, compared with BAG. According to Haghgoo et al., pulp inflammation was observed in BAG samples with dentin bridge formation but not internal resorption or abscess [19-20]. Unlike the literature, Haghgoo et al. demonstrated MTA and BAG could be used for vital pulp therapy (direct capping) of primary teeth.

Three articles combined two methodologies to assess the bioactivity of BAG:

- Haghgoo et al. used TBS and human dentin: both *in vitro* [19].
- Wang et al. (2014) used hDPCs cells *in vitro* and molar pulp rats *in vivo* [13].
- Wang et al. (2016) used SBF and hDPCs: both *in vitro* [15].

4. Discussion

4.1 Study selection

For study selection, especially search terms, the term 'glass' was observed in many studies of tricalcium silicates and bioceramics but not for those of BAG. Additionally, many studies were excluded after the title or abstract screening. Thirteen studies were observed in the references of other articles, and many of them were in chemistry and physics reviews; therefore, they were not mentioned in the study selection with medical electronic databases.

4.2 Methodologies linked to biomaterial chemistry

4.2.1 Particles sizes and morphology in relation to the manufacturing method

The melt-quench and sol-gel processes are the most common manufacturing techniques for BAG. According to Lee et al. melted glass exhibited a greater size (30–60 μm) without pore formation. The average particle size of sol-gel bioglass was still smaller. Moreover, sol-gel bioglass exhibited a porous structure with a large surface area [21-22]. These results were in accordance with Curtis et al.: melt-derived possessed an irregular particle morphology and had a mean size of $3.30\pm 0.42 \mu\text{m}$. The sol-gel bioglass particles were spherical, with a mean size of $0.65\pm 0.19 \mu\text{m}$ [23]. Dentin treated with melt-derived bioglass exhibited a tightly adherent continuous apatite layer. Treatment with nBAG resulted in particle deposition within tubules and formation of apatite rods, which were tightly adherent to tubule walls and continuous to a measured depth of 270 μm [23]. Other techniques have been used, specially to create MBG or nBG. For example, EISA (Evaporation Induced Self-Assembly) combines the sol-gel technique and supramolecular chemistry to obtain MBG [24]. Using flame spray synthesis, BAG synthesis occurred by combining and mixing the corresponding liquid metal precursors and feeding the mixture into a flame reactor [25]. According to MaćKović et al., nBAG (flame spray synthesis) have a higher surface area and, thus, higher reactivity compared with conventional μBAG (melt-quench): coprecipitation of nanocrystalline and large calcite was observed by TEM [25]. In contact with hDPCs, nBAG can induce the mineralization of cells more efficiently compared with regular BAG. Because of the higher specific area, n58S released more Si and Ca ions into the medium compared with 58S and 45S5 in a short time [14]. These results were confirmed by Wang et al.: in contact with pulp cells, nBAG increased mineralized nodules formation [13]. nBAG were promising particles for dental applications and might be a better potential candidate for dentin–pulp complex regeneration. In cases of direct pulp capping, a continuous layer of dentin-like tissue was observed.

4.2.2 Glass composition - $\text{SiO}_2/\text{Na}_2\text{O}/\text{CaO}$ ratio

BAG 45S5 were used in most studies because of the original composition of BAG made by Larry Hench in 1969. BAG 45S5 were able to form carbonated HA in solution and to precipitate a layer of apatite at the enamel and dentin interfaces. According to Plewinski et al., calcium silica and

calcium carbonate layers were found on amorphous bioglass 45S5 after 7 and 14 d (stored in SBF). However, apatite formation after storage was observed only on the crystallized 45S5 samples [26]. Dong et al. compared 45SiO₂ with 58SiO₂ and 77SiO₂ (both without sodium oxide). They found that an enamel surface formed a homogenous and dense mineralized layer with the treatment of 45SiO₂ and 58SiO₂ samples. The average thickness was 4 and 2.5 μm, respectively. The surface roughness decreased with the increase of silicon content, and the mechanical strength of the enamel samples decreased gradually. BAG with 77SiO₂ showed a loose, uneven remineralized layer, whereas 45SiO₂ and 58SiO₂ formed a homogenous, dense mineralized layer on surface enamel [27]. 45SiO₂ paste had the best mineralization ability, best mechanical strength, and satisfactory surface roughness [27]. In the included articles, they were BAG with only SiO₂ and CaO; notably, CaO was fundamental in the first step of HA formation because of an exchange with H⁺ ions [28]. According to Curtis et al. and El-Fiqi et al., it was possible to create nBAG with SiO₂ and CaO [23;29]. These SiO₂-CaO BAG formed apatite rods emanated from within the tubules [23]. According to Gunawidjaja et al., 90SiO₂-10CaO BAG and 85SiO₂-15CaO BAG displayed similar *in vitro* behavior [30]. In other studies, BAG was made without Na₂O, and indeed, high sodium content was unnecessary for bioactivity [30-33]. The porosity increased when calcium oxide increased, compared with sodium oxide. Indeed, the enhanced fluxing in compositions with high sodium interfered with the textural features by reducing the porosity because of fusion of pores [34]. Porosity was a critical parameter to increase surface area and hence bioactivity. Moreover, molecules, drugs, or growth factors could be included in the pores.

4.2.3 Glass composition: adjuvants

Some studies used BAG with adjuvants to conduct comparisons with traditional BAG. Three methodologies were used for these assessments: in solution *in vitro*, remineralization of enamel and/or dentin *in vitro*, and mineralization of cell *in vitro*.

Fluoride was often added to BAG. According to Moawad et al., the formation of HA and hence bioactivity was significantly influenced by the mol% of CaF₂ in the initial glass composition [35]. Addition of fluoride ions to the glass, by maintaining the ratio of all other components constant, maintains the polymerization of the silicate network, network connectivity, and bioactivity of BAG [36-37]. By contrast, substituting CaO (or Na₂O) for CaF₂ increases the polymerization of the SiO₂ network, resulting in decreased solubility, bioactivity, and even cell compatibility [36]. Incorporation of fluoride resulted in the formation of fluoroapatite (FAp) compared with fluoride-free glasses [35;38]. This parameter was notable for dental application because FAp was more

resistant to acidic attack than fluoride, had lower solubility compared with HA, and was more chemically stable than HA or carbonated HA [39-40]. For the oxide addition or substitution, in Nommeots-Nomm et al., phosphorous reduction and CaP layer formation were much faster in SBF containing BAG with K₂O compared with BAG containing K₂O-MgO or K₂O-MgO-SrO [41]. In other studies, BAG with Sr formed apatite to increase dissolution resistance and tubules' obliteration [42-43]. For magnesium, according to Souza et al., partial replacement of CaO by MgO in the BAG did not influence the rate of precipitation of the initial ACP layer when the glass was exposed to SBF [44].

4.2.4 Bag versus other materials

BAG have been compared with RMGIC as liner materials for dentin remineralization *in vitro*. According to Schmidlin et al., the dentin exposed resulted in significantly higher hardness and elastic modulus values with BAG compared with corresponding dentin RMGIC-interfaces [45]. GIC have been used for comparison with BAG. According to Gjorgievska et al. bioactivity of BAG and GIC were confirmed by ion exchange with the surrounding tooth tissue [46]. In 2003, bioactivity of BAG was compared with MTA, formocresol (FC), and ferric sulfate (FS) [18]. FC and FS are pulpotomy agents; however, their use today is uncommon. According to Salako et al. and Haghgoo et al., MTA induced less inflammation than BAG in contact with dental pulp and formed more dentin bridge [18;47]. MTA was progressively replaced by Biodentine™ in restorative procedures. Based on this review, 1 study compared BAG and Biodentine™ as dentin substitutes [46]. In this study, Biodentine™ performed well as a dentin substitute. The use of BAG was limited to the size of particles (< 700 µm) to allow for a better adaptation to cavity walls. Notably, BAG may also be considered for use as dentin substitute [46]. According to Ubaldini et al., BAG and biosilicate promoted increased mineral matrix ratios on the bleached and control dentin samples using MicroRaman Spectroscopy. Microtensile bond strength of dentin resin bonding interfaces was significantly improved when the bleached or control dentin surfaces had been covered by BAG and/or biosilicate [48].

4.3 Methodologies to assess bioactivity

4.3.1 *In vitro* solution - mechanism of apatite formation on bag surface

Studies used mainly SBF (n=24) and TBS (n=7) to assess the mechanism of apatite formation on

BAG surface *in vitro*. Microscopic observations and chemical or structural analysis allowed for the following conclusion: BAG formed apatite *in vitro*.

According to Mathew et al., results obtained from BAG after being soaked in SBF or TBS revealed a biomimetically grown surface layer of calcium phosphate comprising domains of nanocrystalline HCA [49]. Gunawidjaja et al. observed ACP and HCA growth at the surface of BAG in SBF and TBS. When the authors examined the ion release, the TBS grains displayed a 60 μm thick domain of almost pure silica, and their SBF counterparts were verified as small but had significant amounts of Ca and P. This finding is attributed to more extensive local deposits of ACP at BAG when they are subjected to SBF, because the latter medium is already supersaturated with respect to apatite [33]. These results were in accordance with the literature.

For other authors, the choice of SBF solution for testing the bone bonding ability of materials was arbitrary, and there was no correlation between bioactivity in SBF solution and *in vivo*. The supersaturation of SBF in calcium and phosphate can induce false-positive results [50]. Mneimne and al. recommended the use of TBS without Ca^{2+} and PO_4^{3-} because saliva in the mouth is diluted after liquid uptake and no longer saturated with these ions [40]. TBS was used to avoid the possible interference of phosphate ions during measurement [51]. However, according to Cerruti and al., TBS solutions and SBF were comparable to evaluate the dissolution of BAG [52]. For Kokubo and al., SBF was useful to predict the bioactivity of a material [53]. One study used Hank's balanced salt solution [54]. According to Gandolfi et al., Hank's solution can be used for cells' culture; however, it is a solution with a low concentration of phosphate and reduced the rate of apatite precipitation and increased the time required for superficial apatite formation [55].

Bioactivity evaluation in solution provides the best incomplete information because it is irrespective of biological processes and context. However, for Zadpoor, three quarters of the *in vitro* studies of ability successfully predicted *in vivo* performance of the same materials [56]. The ability to form apatite in solution could be a bioactive parameter [57]. According to some definitions of bioactivity and the description of protocol in articles, the answer is *yes*. However, because bioactive required a biological reaction induced by BAG, apatite formation in a solution is the reactivity of BAG and not bioactivity [52].

4.3.2 *In vitro* remineralization of enamel and/or dentin

To assess the BAG capacity to remineralize enamel and/or dentin *in vitro*, the literature used a combination of microscopic observations, chemical or structural analysis, and evaluation of mechanical properties. One or 2 studies used AFM, CLSM, CT, EMPA, fluid filtration of dentin,

focused ion beam, nanoindentation, TG, UMIS, or XPS (fig. 3). According to the studies included, BAG remineralized demineralized enamel:

- Improved mechanical properties (mechanical strength, surface roughness, microhardness).
- Increased mineral content.
- High mineral volume recovery.
- Coverage with a layer of brushite crystals.
- Ultrastructural changes.

For the dentin, BAG formed apatite at the interface and

- Decreased the degree of decalcification of dentin during the mineralization process in one study [51].
- Promoted the increase of mineral matrix ratios and the appearance of new interface peaks, indicating a chemical interaction.
- Remineralized artificially carious dentin.
- Allowed deposition in the tubules with obliteration by precipitation of apatite (increase of the % of tubular occlusion – reduce dentin permeability).
- Increased hardness and elastic modulus values of the dentin.

Precipitations of deposits inside the tubules were a barrier against bacterial penetration and therefore prevented pulp inflammation. Dentin remineralization is more complex than enamel remineralization because dentin has a heterogenous structure. Electronic microscopy could evaluate the inter- or intrafibrillar remineralization, and mineral formation must be assessed by measurements of mechanical properties [58]. According to the 27 articles in this review, standard BAG appears to be promising agents of remineralization.

To use BAG in contact with dental hard tissues, the bioactivity of BAG in contact with pulp cells must be assessed. Notably, carious lesions increase the permeability of dentin. Permeability involves the passage of fluids, ions, molecules, particulate matter, and bacteria into and through a substance or tissue under different and variable conditions. The permeability of dentin is essential to support the physiology and reaction patterns of the pulp–dentin organ [59]. Enamel carious lesions can also lead to a pulp–dentin complex reaction. Notably, the examination of enamel lesions and pulp–dentin organs confirmed not early odontoblast cell reactions and a difference in tertiary dentin formation as a response to slow and active lesion activity [60]. Enamel is a microporous solid, the carious process and response of the dentin–pulp complex can frequently start before it is breached [61].

4.3.3 *In vitro* effect of bag on the mineralization capacity of cells

All the studies that assessed the bioactivity of BAG in contact with cells used Alizarin red staining to evaluate matrix mineralization.

According to these studies, cells in contact with BAG led to increases in relative calcium concentration and precipitation and increases in mineralized nodules formation.

Five studies used hDPCs. These cells may represent a different population of differentiated and undifferentiated cells with great potential for dental pulp repair and dentin regeneration. They can be successfully used to evaluate new biomaterials for dental applications [62].

SHEDs were identified as a population of highly proliferative, clonogenic cells capable of differentiating into a variety of cell types, for example, neural cells, adipocytes, and odontoblasts [59].

The other factors used to assess biocompatibility could be studied to evaluate bioactivity: collagen production, protein expression (DSPP, DMP-1), expression of several mineralization-related genes (osteocalcin, osteonectin, osteopontin, and bone sialoprotein) and promotion of alkaline phosphatase. These factors could be responsible for the initiation and modulation of dentin mineralization [13]. Moreover, a bioactive product can be toxic. Therefore, Alizarin red staining is insufficient to predict the behavior of BAG in contact with cells. The *in vivo* studies were reported as the best means to evaluate the bioactivity of bioactive products [64].

4.3.4 *In vivo* remineralization of enamel and/or dentin

No study of the *in vivo* remineralization of enamel and/or dentin using BAG was observed. A likely explanation for this result is the difficulties of evaluating *in vivo* remineralization. The International Caries Detection and Assessment System (ICDAS) criteria, optical boundary depth by OCT system, enamel decalcification index, quantitative light-induced fluorescence, and digital photography were mainly used for visual characterization of *in vivo* remineralization of white spot lesions (WSLs) [65-66]. These methods were used as quantitative methodologies for CPP-ACP but not for pure BAG [67-70].

The gold standard technique to quantify changes in the mineral content of early carious lesions was transverse microradiography (TMR). However, TMR requires preparation of thin sections and therefore cannot be used to monitor a WSL undergoing *in vivo* treatment [60]. This technique can be used *in situ* with removable mid-palatal appliances [67].

Thus, no study investigated the *in vivo* remineralization capacity of dental tissues by using pure BAG.

4.3.5 *In vivo* evaluation of dentin mineralization and/or dental pulp response

Histological analyses are required to characterize the bioactivity of BAG in direct or indirect contact with pulp cells. Pulp inflammation, presence of resorption, dentin bridge formation, and organization of odontoblast-like cells were commonly reported.

According to Salako et al. and Haghgoo et al., BAG induced an inflammatory response from the dental pulp [18-20]. Fluctuations in pH can lead to an unfavorable microenvironment for dental pulp tissues and cause inflammation and pulp tissue necrosis [71]. Haghgoo et al. demonstrated formation of a dentin bridge but did not observe internal resorption and abscess [19-20]. According to Wang et al., a continuous layer of dentin-like tissue with uniform thickness, a well-organized dentinal tubule structure, and polarizing odontoblast-like cells aligned along were generated on the nano-BAG layer [13]. These results are in accordance with Salako et al.: BAG can interact with pre-existing odontoblasts and underlying dental pulp cells [18].

4.3.6 Association of two categories of methodologies

Three articles combined two categories of methodologies to assess the bioactivity of BAG:

- Forsback et al. used *in vitro* solution and *in vitro* dentin remineralization [51]. According to these assessments of bioactivity, bioactive glass S53P4 can be used as a therapeutic material for mineralization of dentin and its tubules in a physiological environment.
- Wang et al. (2014) used *in vitro* capacity mineralization of cells and *in vivo* evaluation of dentin mineralization and/or dental pulp response [13]. According to these methodologies, BAG, especially nano-BAG, induced the odontogenic differentiation and dentin formation of dental pulp cells and is a potential material for pulp repair and dentin regeneration.
- Wang et al. (2016) used *in vitro* solution and *in vitro* capacity mineralization of cells [15]. After incubation in SBF, a crystalline structure precipitated on the surfaces of BAG and in contact with cells, and mineralized nodules were generated in the BAG group. Both methodologies confirmed the bioactivity of BAG.

4.4 Prospects

Based on this review, we suggested a classification of methodologies to improve the understanding of the bioactive properties investigated (fig. 4).

The assessment of the bioactivity of BAG was specific because two types of mineralization processes occurred: *in situ* reprecipitation, as fluoride, CPP-ACP or GIC, and stimulation of pulp cells, as tricalcium silicate materials. This specificity complicates the evaluation of bioactivity *in vivo* and *in vitro*; however, it is a fundamental notion that offers many clinical perspectives.

Regarding the types of clinical indications from enamel lesion remineralization to pulp capping, different aspects of BAG bioactivity were explored. Nevertheless, no study investigated the combination of reactivity of particles and cell stimulation effects in the dentin–BAG interface; thus, further research through *in vivo* and clinical studies could fill that gap in the literature.

Careddu and Duncan proposed a literature review topic: a comparison of pulpal response to tricalcium silicate materials in the laboratory and clinic [72]. To complete the evaluation of pulp cell behavior in contact with BAG, other markers should be assessed: the expression of inflammatory and odontoblast differentiation markers, percentage of inflammatory cells, and vascular density in the pulp tissue after pulpotomy [72-73]. Notably, the interrelations between inflammation and regeneration are of particular significance within the dental pulp tissue inextensible environment [74].

5. Conclusions

According to all the *in vitro* studies included, BAG is a bioactive material. Bioactivity ranged from reactivity and apatite reprecipitation to pulp cell stimulation. However, the literature provides no consensus on the results of *in vivo* studies and no comparison between protocols to assess their bioactivity compared with other bioactive products.

A clear classification of the methods used to assess the bioactivity of BAG could improve the elaboration of protocols and rank the impact of different studies and the bioactive effects.

Funding

This research received no specific grant from funding agencies in the public, commercial, or nonprofit sectors.

Acknowledgements

The authors thank Clémence Agostini, librarian of the interuniversity health library (*Université de Paris*), for her assistance with the research strategy of the study. The authors also thank Charlène Chevalier (*Université Claude Bernard Lyon1*) for her help for the teeth illustrations of the graphical abstract.

References

- [1] E.A. Abou Neel, A. Aljabo, A. Strange, S. Ibrahim, M. Coathup, A.M. Young, L. Bozec, V. Mudera, Demineralization-remineralization dynamics in teeth and bone, *Int. J. Nanomedicine*. 11 (2016) 4743–4763.
- [2] N.B. Pitts, A.I. Ismail, S. Martignon, K. Ekstrand, G.V. Douglas, C. Longbottom, ICCMS Guide for Practitioners and Educators, in: 2014.
- [3] F. Schwendicke, J.E. Frencken, L. Bjørndal, M. Maltz, D.J. Manton, D. Ricketts, K. Van Landuyt, A. Banerjee, G. Campus, S. Doméjean, M. Fontana, S. Leal, E. Lo, V. Machiulskiene, A. Schulte, C. Splieth, A.F. Zandona, N.P.T. Innes, Managing Carious Lesions: Consensus Recommendations on Carious Tissue Removal, *Adv. Dent. Res.* 28 (2016) 58–67.
- [4] European Society of Endodontology (ESE) developed by: H.F. Duncan, K.M. Galler, P.L. Tomson, S. Simon, I. El-Karim, R. Kundzina, G. Krastl, T. Dammaschke, H. Fransson, M. Markvart, M. Zehnder, L. Bjørndal, European Society of Endodontology position statement: Management of deep caries and the exposed pulp, *Int. Endod. J.* 52 (2019) 923–934.
- [5] L.L. Hench, The story of Bioglass, *J. Mater. Sci. Mater. Med.* 17 (2006) 967–978.
- [6] F. Lizzi, C. Villat, N. Attik, P. Jackson, B. Grosogeat, C. Goutaudier, Mechanical characteristic and biological behaviour of implanted and restorative bioglasses used in medicine and dentistry: A systematic review, *Dent. Mater.* 33 (2017) 702–712.
- [7] A.A. Taha, M.P. Patel, R.G. Hill, P.S. Fleming, The effect of bioactive glasses on enamel remineralization: A systematic review, *J. Dent.* 67 (2017) 9–17.
- [8] D. Fernando, N. Attik, N. Pradelle-Plasse, P. Jackson, B. Grosogeat, P. Colon, Bioactive glass for dentin remineralization: A systematic review, *Mater. Sci. Eng. C Mater. Biol. Appl.* 76 (2017) 1369–1377.
- [9] D. Williams, *Definitions in biomaterials*, Amsterdam-Oxford-New York-Tokyo: Elsevier, 1987.
- [10] I. Lebecq, F. Désanglois, A. Leriche, C. Follet-Houttemane, Compositional dependence on the in vitro bioactivity of invert or conventional bioglasses in the Si-Ca-Na-P system, *J. Biomed. Mater. Res. A.* 83 (2007) 156–168.
- [11] R.G. Hill, Predicting the bioactivity of glasses using the network connectivity or split network models, *J Non-Cryst Solids.* 357 (2011) 3884–3887.
- [12] P.K. Vallittu, A.R. Boccaccini, L. Hupa, D.C. Watts, Bioactive dental materials -Do they exist and what does bioactivity mean?, 34 (2018) 693–694.

- [13] S. Wang, X. Gao, W. Gong, Z. Zhang, X. Chen, Y. Dong, Odontogenic differentiation and dentin formation of dental pulp cells under nanobioactive glass induction, *Acta Biomater.* 10 (2014) 2792–2803.
- [14] W. Gong, Z. Huang, Y. Dong, Y. Gan, S. Li, X. Gao, X. Chen, Ionic extraction of a novel nano-sized bioactive glass enhances differentiation and mineralization of human dental pulp cells, *J. Endod.* 40 (2014) 83–88.
- [15] S. Wang, Q. Hu, X. Gao, Y. Dong, Characteristics and effects on dental pulp cells of a polycaprolactone/submicron bioactive glass composite scaffold, *J. Endod.* 42 (2016) 1070–1075.
- [16] M. Huang, R.G. Hill, S.C.F. Rawlinson, Zinc bioglasses regulate mineralization in human dental pulp stem cells, *Dent. Mater.* 33 (2017) 543–552.
- [17] T.C. Camilo Lopez, I.M. Alves Diniz, L.S. Ferreira, J. Marchi, R. Borges, S.P. Harumi Miyagi de Cara, R. D’Almeida-Couto, M.M. Marques, Bioactive glass plus laser phototherapy as promise candidates for dentine hypersensitivity treatment, *J. Biomed. Mater. Res. PART B-Appl. Biomater.* 105 (2017) 107–116.
- [18] N. Salako, B. Joseph, P. Ritwik, J. Salonen, P. John, T. Junaid, Comparison of bioactive glass, mineral trioxide aggregate, ferric sulfate, and formocresol as pulpotomy agents in rat molar, *Dent. Traumatol.* 19 (2003) 314–320.
- [19] R. Haghgoo, N.J. Naderi, Comparison of Calcium Hydroxide and Bioactive Glass after Direct Pulp Capping in Primary Teeth, *Front. Dent.* (2007) 155–159.
- [20] R. Haghgoo, M. Ahadvand, Evaluation of pulpal response of deciduous teeth after direct pulp capping with bioactive glass and mineral trioxide aggregate., *Contemp. Clin. Dent.* 7 (2016) 332–335.
- [21] B.-S. Lee, S.-H. Kang, Y.-L. Wang, F.-H. Lin, C.-P. Lin, In vitro study of dentinal tubule occlusion with sol-gel DP-bioglass for treatment of dentin hypersensitivity, *Dent. Mater. J.* 26 (2007) 52–61.
- [22] V. Farano, J.-C. Maurin, N. Attik, P. Jackson, B. Grosogeat, K. Gritsch, Sol-gel bioglasses in dental and periodontal regeneration: A systematic review, *J. Biomed. Mater. Res. PART B-Appl. Biomater.* 107 (2019) 1210–1227.
- [23] A.R. Curtis, N.X. West, B. Su, Synthesis of nanobioglass and formation of apatite rods to occlude exposed dentine tubules and eliminate hypersensitivity, *ACTA Biomater.* 6 (2010) 3740–3746.
- [24] D. Fernando, P. Colon, M. Cresswell, C. Journet, N. Pradelle-Plasse, P. Jackson, B. Grosogeat, N. Attik, The influence of precursor addition order on the porosity of sol-gel bioactive glasses, *Dent. Mater.* 34 (2018) 1323–1330.
- [25] M. Mačković, A. Hoppe, R. Detsch, D. Mohn, W.J. Stark, E. Spiecker, A.R. Boccaccini, Bioactive glass (type 45S5) nanoparticles: In vitro reactivity on nanoscale and biocompatibility, *J. Nanoparticle Res.* 14 (2012).
- [26] M. Plewinski, K. Schickle, M. Lindner, A. Kirsten, M. Weber, H. Fischer, The effect of crystallization of bioactive bioglass 45S5 on apatite formation and degradation, *Dent. Mater.* 29 (2013) 1256–1264.
- [27] Z. Dong, J. Chang, Y. Zhou, K. Lin, In vitro remineralization of human dental enamel by bioactive glasses, *J. Mater. Sci.* 46 (2011) 1591–1596.
- [28] G. Kaur, G. Pickrell, N. Sriranganathan, V. Kumar, D. Homa, Review and the state of the art: Sol-gel and melt quenched bioactive glasses for tissue engineering, *J. Biomed. Mater. Res. B Appl. Biomater.* 104 (2016) 1248–1275.
- [29] A. El-Fiqi, T.-H. Kim, M. Kim, M. Eltohamy, J.-E. Won, E.-J. Lee, H.-W. Kim, Capacity of mesoporous bioactive glass nanoparticles to deliver therapeutic molecules, *NANOSCALE.* 4 (2012) 7475–7488.
- [30] P.N. Gunawidjaja, R. Mathew, A.Y.H. Lo, I. Izquierdo-Barba, A. García, D. Arcos, M. Vallet-Regí, M. Edén, Local structures of mesoporous bioactive glasses and their surface alterations

- in vitro: Inferences from solid-state nuclear magnetic resonance, *Philos. Trans. R. Soc. Math. Phys. Eng. Sci.* 370 (2012) 1376–1399.
- [31] O.M. Goudouri, E. Kontonasaki, A. Theocharidou, L. Papadopoulou, N. Kantiranis, X. Chatzistavrou, P. Koidis, K.M. Paraskevopoulos, Modifying a dental ceramic by bioactive glass via the sol-gel route: Characterization and bioactivity investigation, *Mater. Chem. Phys.* 125 (2011) 309–313.
- [32] P.N. Gunawidjaja, A.Y.H. Lo, I. Izquierdo-Barba, A. García, D. Arcos, B. Stevansson, J. Grins, M. Vallet-Regí, M. Edén, Biomimetic Apatite Mineralization Mechanisms of Mesoporous Bioactive Glasses as Probed by Multinuclear ^{31}P , ^{29}Si , ^{23}Na and ^{13}C Solid-State NMR, *J. Phys. Chem. C* 114 (2010) 19345–19356.
- [33] P.N. Gunawidjaja, I. Izquierdo-Barba, R. Mathew, K. Jansson, A. García, J. Grins, D. Arcos, M. Vallet-Regí, M. Edén, Quantifying apatite formation and cation leaching from mesoporous bioactive glasses in vitro: a SEM, solid-state NMR and powder XRD study, *J. Mater. Chem.* 22 (2012) 7214–7223.
- [34] D. Fernando, N. Attik, M. Cresswell, I. Mokbel, N. Pradelle-Plasse, P. Jackson, B. Grosgeat, P. Colon, Influence of network modifiers in an acetate based sol-gel bioactive glass system, *Microporous Mesoporous Mater.* 257 (2018) 99–109.
- [35] H.M.M. Moawad, H. Jain, Development of nano-macroporous soda-lime phosphofluorosilicate bioactive glass and glass-ceramics, *J. Mater. Sci. Mater. Med.* 20 (2009) 1409–1418.
- [36] D.S. Brauer, M. Mneimne, R.G. Hill, Fluoride-containing bioactive glasses: Fluoride loss during melting and ion release in tris buffer solution, *J. Non-Cryst. Solids.* 357 (2011) 3328–3333.
- [37] A.A. Taha, P.S. Fleming, R.G. Hill, M.P. Patel, Enamel Remineralization with Novel Bioactive Glass Air Abrasion, *J. Dent. Res.* 97 (2018) 1438–1444.
- [38] D.S. Brauer, N. Karpukhina, M.D. O'Donnell, R.V. Law, R.G. Hill, Fluoride-containing bioactive glasses: Effect of glass design and structure on degradation, pH and apatite formation in simulated body fluid, *Acta Biomater.* 6 (2010) 3275–3282.
- [39] F.A. Shah, Fluoride-containing bioactive glasses: Glass design, structure, bioactivity, cellular interactions, and recent developments, *Mater. Sci. Eng. C Mater. Biol. Appl.* 58 (2016) 1279–1289.
- [40] M. Mneimne, R.G. Hill, A.J. Bushby, D.S. Brauer, High phosphate content significantly increases apatite formation of fluoride-containing bioactive glasses, *Acta Biomater.* 7 (2011) 1827–1834.
- [41] A. Nommeots-Nomm, S. Labbaf, A. Devlin, N. Todd, H. Geng, A.K. Solanki, H.M. Tang, P. Perdika, A. Pinna, F. Ejeian, O. Tsigkou, P.D. Lee, M.H.N. Esfahani, C.A. Mitchell, J.R. Jones, Highly degradable porous melt-derived bioactive glass foam scaffolds for bone regeneration, *Acta Biomater.* 57 (2017) 449–461.
- [42] S.M. Salman, S.N. Salama, H.A. Abo-Mosallam, The role of strontium and potassium on crystallization and bioactivity of $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ glasses, *Ceram. Int.* 38 (2012) 55–63.
- [43] M. Saffarpour, M. Mohammadi, M. Tahriri, A. Zakerzadeh, Efficacy of Modified Bioactive Glass for Dentin Remineralization and Obstruction of Dentinal Tubules, *J. Dent. Tehran Iran.* 14 (2017) 212–222.
- [44] M.T. Souza, M.C. Crovace, C. Schröder, H. Eckert, O. Peitl, E.D. Zanotto, Effect of magnesium ion incorporation on the thermal stability, dissolution behavior and bioactivity in Bioglass-derived glasses, *J. Non-Cryst. Solids.* 382 (2013) 57–65.
- [45] P.R. Schmidlin, M. Zehnder, T. Imfeld, M.V. Swain, Comparative assessment of hardening of demineralized dentin under lining materials using an ultramicroindentation system, *J. Biomed. Mater. Res. - Part B Appl. Biomater.* 83 (2007) 199–205.
- [46] E.S. Gjorgievska, J.W. Nicholson, S.M. Apostolska, N.J. Coleman, S.E. Booth, I.J. Slipper, M.I. Mladenov, Interfacial properties of three different bioactive dentine substitutes, *Microsc.*

Microanal. 19 (2013) 1450–1457.

- [47] R. Haghgoo, M. Ahmadvand, Evaluation of pulpal response of deciduous teeth after direct pulp capping with bioactive glass and mineral trioxide aggregate, *Contemp. Clin. Dent.* 7 (2016) 332–335.
- [48] A. Ubaldini, R.C. Pascotto, F. Sato, V.O. Soares, E.D. Zanotto, M.L. Baesso, Effects of Bioactive Agents on Dentin Mineralization Kinetics After Dentin Bleaching, *Oper. Dent.* (2019).
- [49] R. Mathew, P.N. Gunawidjaja, I. Izquierdo-Barba, K. Jansson, A. García, D. Arcos, M. Vallet-Regí, M. Edén, Solid-State ³¹P and ¹H NMR Investigations of Amorphous and Crystalline Calcium Phosphates Grown Biomimetically From a Mesoporous Bioactive Glass, *J. Phys. Chem. C.* 115 (2011) 20572–20582.
- [50] M. Bohner, J. Lemaitre, Can bioactivity be tested in vitro with SBF solution?, *Biomaterials.* 30 (2009) 2175–2179. h
- [51] A.-P. Forsback, S. Areva, J.I. Salonen, Mineralization of dentin induced by treatment with bioactive glass S53P4 in vitro, *Acta Odontol. Scand.* 62 (2004) 14–20.
- [52] M. Cerruti, D. Greenspan, K. Powers, Effect of pH and ionic strength on the reactivity of Bioglass® 45S5, *Biomaterials.* 26 (2005) 1665–1674.
- [53] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity?, *Biomaterials.* 27 (2006) 2907–2915.
- [54] C. Ashok raja, S. Balakumar, P. Bargavi, P. Rajashree, B. Anandkumar, R.P. George, U. Kamachi Mudali, Decoration of 1-D nano bioactive glass on reduced graphene oxide sheets: Strategies and in vitro bioactivity studies, *Mater. Sci. Eng. C Mater. Biol. Appl.* 90 (2018) 85–94.
- [55] M.G. Gandolfi, G. Ciapetti, P. Taddei, F. Perut, A. Tinti, M.V. Cardoso, B. Van Meerbeek, C. Prati, Apatite formation on bioactive calcium-silicate cements for dentistry affects surface topography and human marrow stromal cells proliferation, *Dent. Mater.* 26 (2010) 974–992.
- [56] A.A. Zadpoor, Relationship between in vitro apatite-forming ability measured using simulated body fluid and in vivo bioactivity of biomaterials, *Mater. Sci. Eng. C.* 35 (2014) 134–143.
- [57] S. Ferraris, S. Yamaguchi, N. Barbani, M. Cazzola, C. Cristallini, M. Miola, E. Vernè, S. Spriano, Bioactive materials: In vitro investigation of different mechanisms of hydroxyapatite precipitation, *Acta Biomater.* 102 (2020) 468–480.
- [58] L.E. Bertassoni, S. Habelitz, J.H. Kinney, S.J. Marshall, G.W. Marshall, Biomechanical perspective on the remineralization of dentin, *Caries Res.* 43 (2009) 70–77.
- [59] I.A. Mjör, Dentin permeability: the basis for understanding pulp reactions and adhesive technology, *Braz. Dent. J.* 20 (2009) 3–16.
- [60] L. Bjørndal, S. Demant, S. Dabelsteen, Depth and activity of carious lesions as indicators for the regenerative potential of dental pulp after intervention, *J. Endod.* 40 (2014) S76–81.
- [61] L. Bjørndal, S. Simon, P.L. Tomson, H.F. Duncan, Management of deep caries and the exposed pulp, *Int. Endod. J.* 52 (2019) 949–973.
- [62] L.B. Mestieri, A.L. Gomes-Cornélio, E.M. Rodrigues, L.P. Salles, R. Bosso-Martelo, J.M. Guerreiro-Tanomaru, M. Tanomaru-Filho, Biocompatibility and bioactivity of calcium silicate-based endodontic sealers in human dental pulp cells, *J. Appl. Oral Sci. Rev. FOB.* 23 (2015) 467–471.
- [63] M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P.G. Robey, S. Shi, SHED: stem cells from human exfoliated deciduous teeth, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 5807–5812.
- [64] H. Pan, X. Zhao, B.W. Darvell, W.W. Lu, Apatite-formation ability – Predictor of “bioactivity”?, *Acta Biomater.* 6 (2010) 4181–4188.
- [65] M. Sugiura, Y. Kitasako, A. Sadr, Y. Shimada, Y. Sumi, J. Tagami, White spot lesion remineralization by sugar-free chewing gum containing bio-available calcium and fluoride: A double-blind randomized controlled trial, *J. Dent.* 54 (2016) 86–91.
- [66] Y. Kitasako, A. Sadr, Y. Shimada, M. Ikeda, Y. Sumi, J. Tagami, Remineralization capacity

- of carious and non-carious white spot lesions: clinical evaluation using ICDAS and SS-OCT, *Clin. Oral Investig.* 23 (2019) 863–872.
- [67] D.J. Manton, G.D. Walker, F. Cai, N.J. Cochrane, P. Shen, E.C. Reynolds, Remineralization of enamel subsurface lesions in situ by the use of three commercially available sugar-free gums, *Int. J. Paediatr. Dent.* 18 (2008) 284–290.
- [68] N.J. Cochrane, G.D. Walker, D.J. Manton, E.C. Reynolds, Comparison of quantitative light-induced fluorescence, digital photography and transverse microradiography for quantification of enamel remineralization, *Aust. Dent. J.* 57 (2012) 271–276.
- [69] J. Wang, Y. Yan, X. Wang, Clinical evaluation of remineralization potential of casein phosphopeptide amorphous calcium phosphate nanocomplexes for enamel decalcification in orthodontics, *Chin. Med. J. (Engl.)*. 125 (2012) 4018–4021.
- [70] O.B. Al-Batayneh, E.I. Bani Hmood, S.N. Al-Khateeb, Assessment of the effects of a fluoride dentifrice and GC Tooth Mousse on early caries lesions in primary anterior teeth using quantitative light-induced fluorescence: a randomised clinical trial, *Eur. Arch. Paediatr. Dent. Off. J. Eur. Acad. Paediatr. Dent.* (2019).
- [71] C.-Y. Cui, S.-N. Wang, H.-H. Ren, A.-L. Li, D. Qiu, Y.-H. Gan, Y.-M. Dong, Regeneration of dental-pulp complex-like tissue using phytic acid derived bioactive glasses, *RSC Adv.* 7 (2017) 22063–22070.
- [72] R. Careddu, H.F. Duncan, How does the pulpal response to Biodentine and ProRoot mineral trioxide aggregate compare in the laboratory and clinic?, *Br. Dent. J. Advance Online Publication* (2018).
- [73] C.S. Lopes, M.A. Junqueira, L. Cosme-Silva, C. de O.R. Pegoraro, C.C.D. Garbelini, T.M. Oliveira, N.S. Martins, J.D.S. Neves, V.T. Sakai, Initial inflammatory response after the pulpotomy of rat molars with MTA or ferric sulfate, *J. Appl. Oral Sci. Rev. FOB.* 27 (2019) e20180550.
- [74] T. Giraud, C. Jeanneau, C. Rombouts, H. Bakhtiar, P. Laurent, I. About, Pulp capping materials modulate the balance between inflammation and regeneration, *Dent. Mater.* 35 (2019) 24–35.
- [75] H. Takadama, H.-M. Kim, T. Kokubo, T. Nakamura, Mechanism of Biomineralization of Apatite on a Sodium Silicate Glass: TEM–EDX Study In Vitro, *Chem. Mater.* 13 (2001) 1108–1113.
- [76] K. Lin, Y. Tseng, Y. Mou, Y. Hsu, C. Yang, J. Chan, Mechanistic study of apatite formation on bioactive glass surface using P-31 solid-state NMR Spectroscopy, *Chem. Mater.* 17 (2005) 4493–4501.
- [77] O.M. Goudouri, E. Kontonasaki, X. Chatzistavrou, L. Papadopoulou, P. Koidis, K.M. Paraskevopoulos, Investigation of the bioactivity of dental ceramic/bioactive glass composites prepared by the sol gel route, in: Prado, M and Zavaglia, C (Ed.), *Bioceram.* 21, Int Soc Ceram Med, 2009: pp. 119–122.
- [78] D. Mohn, M. Zehnder, T. Imfeld, W.J. Stark, Radio-opaque nanosized bioactive glass for potential root canal application: Evaluation of radiopacity, bioactivity and alkaline capacity, *Int. Endod. J.* 43 (2010) 210–217.
- [79] A. Bachar, C. Mercier, A. Tricoteaux, S. Hampshire, A. Leriche, C. Follet, Effect of nitrogen and fluorine on mechanical properties and bioactivity in two series of bioactive glasses, *J. Mech. Behav. Biomed. Mater.* 23 (2013) 133–148.
- [80] I. Farooq, M. Tylkowski, S. Müller, T. Janicki, D.S. Brauer, R.G. Hill, Influence of sodium content on the properties of bioactive glasses for use in air abrasion, *Biomed. Mater. Bristol.* 8 (2013).
- [81] S.K. Arepalli, H. Tripathi, V.K. Vyas, S. Jain, S.K. Suman, R. Pyare, S.P. Singh, Influence of barium substitution on bioactivity, thermal and physico-mechanical properties of bioactive glass, *Mater. Sci. Eng. C.* 49 (2015) 549–559.
- [82] A. Kirsten, A. Hausmann, M. Weber, J. Fischer, H. Fischer, Bioactive and thermally

- compatible glass coating on zirconia dental implants, *J. Dent. Res.* 94 (2015) 297–303.
- [83] Z. Abbasi, M.E. Bahrololoum, R. Bagheri, M.H. Shariat, Characterization of the bioactive and mechanical behavior of dental ceramic/sol-gel derived bioactive glass mixtures, *J. Mech. Behav. Biomed. Mater.* 54 (2016) 115–122.
- [84] R. Mathew, C. Turdean-Ionescu, Y. Yu, B. Stevansson, I. Izquierdo-Barba, A. García, D. Arcos, M. Vallet-Regí, M. Edén, Proton Environments in Biomimetic Calcium Phosphates Formed from Mesoporous Bioactive CaO-SiO₂-P₂O₅ Glasses in Vitro: Insights from Solid-State NMR, *J. Phys. Chem. C Nanomater. Interfaces.* 121 (2017) 13223–13238.
- [85] A. Anand, B. Kundu, V.K. Balla, S.K. Nandi, Synthesis and Physico-Chemical Characterization of Different Mesoporous Bioactive Glass Nanopowders: in-vitro SBF Activity and Cytotoxicity, *Trans. Indian Ceram. Soc.* 77 (2018) 106–117.
- [86] A.A. Taha, R.G. Hill, P.S. Fleming, M.P. Patel, Development of a novel bioactive glass for air-abrasion to selectively remove orthodontic adhesives, *Clin. Oral Investig.* 22 (2018) 1839–1849.
- [87] S.E. Efflandt, P. Magne, W.H. Douglas, L.F. Francis, Interaction between bioactive glasses and human dentin, *J. Mater. Sci. Mater. Med.* 13 (2002) 557–565.
- [88] O.E. Hassanein, T.A. El-Brossy, An Investigation about the Remineralization Potential of Bio-active glass on Artificially Carious Enamel and Dentin using Raman Spectroscopy., (2006) 12.
- [89] M. Vollenweider, T.J. Brunner, S. Knecht, R.N. Grass, M. Zehnder, T. Imfeld, W.J. Stark, Remineralization of human dentin using ultrafine bioactive glass particles, *Acta Biomater.* 3 (2007) 936–943.
- [90] A.S. Bakry, H. Takahashi, M. Otsuki, A. Sadr, K. Yamashita, J. Tagami, CO₂ Laser Improves 45S5 Bioglass Interaction with Dentin., *J. Dent. Res.* 90 (2011) 246–250.
- [91] S. Sauro, T.-F. Watson, I. Thompson, Ultramorphology and dentine permeability changes induced by prophylactic procedures on exposed dentinal tubules in middle dentine, *Med. Oral Patol. Oral Cirurgia Bucal.* 16 (2011) e1022-1030.
- [92] Z. Wang, T. Jiang, S. Sauro, D. Pashley, M. Toledano, R. Osorio, S. Liang, W. Xing, Y. Sa, Y. Wang, The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: an in vitro study., *Aust. Dent. J.* 56 (2011) 372–381.
- [93] A.S. Bakry, H. Takahashi, M. Otsuki, J. Tagami, The durability of phosphoric acid promoted bioglass-dentin interaction layer, *Dent. Mater.* 29 (2013) 357–364.
- [94] A.S. Bakry, H.Y. Marghalani, O.A. Amin, J. Tagami, The effect of a bioglass paste on enamel exposed to erosive challenge, *J. Dent.* 42 (2014) 1458–1463.
- [95] A.S. Bakry, H. Takahashi, M. Otsuki, J. Tagami, Evaluation of new treatment for incipient enamel demineralization using 45S5 bioglass, *Dent. Mater.* 30 (2014) 314–320.
- [96] H. Milly, F. Festy, T.F. Watson, I. Thompson, A. Banerjee, Enamel white spot lesions can remineralise using bio-active glass and polyacrylic acid-modified bio-active glass powders, *J. Dent.* 42 (2014) 158–166.
- [97] H. Milly, F. Festy, M. Andiappan, T.F. Watson, I. Thompson, A. Banerjee, Surface pre-conditioning with bioactive glass air-abrasion can enhance enamel white spot lesion remineralization, *Dent. Mater.* 31 (2015) 522–533.
- [98] C.N. Carvalho, L.G. Freire, A.P.L. de Carvalho, M.A.H. Duarte, J. Bauer, G. Gavini, Ions Release and pH of Calcium Hydroxide-, Chlorhexidine- and Bioactive Glass-Based Endodontic Medicaments, *Braz. Dent. J.* 27 (2016) 325–331.
- [99] N.A. El-Wassefy, Remineralizing effect of cold plasma and/or bioglass on demineralized enamel., *Dent. Mater. J.* 36 (2017) 157–167.
- [100] J. Zhang, V. Boyes, F. Festy, R.J.M. Lynch, T.F. Watson, A. Banerjee, In-vitro subsurface remineralisation of artificial enamel white spot lesions pre-treated with chitosan, *Dent. Mater.* 34 (2018) 1154–1167.
- [101] J. Zhang, R.J.M. Lynch, T.F. Watson, A. Banerjee, Remineralisation of enamel white spot lesions pre-treated with chitosan in the presence of salivary pellicle, *J. Dent.* 72 (2018) 21–28.

- [102] E.M.R. Lee, R. Borges, J. Marchi, C. de P. Eduardo, M.M. Marques, Bioactive glass and high-intensity lasers as a promising treatment for dentin hypersensitivity: An in vitro study, *J. Biomed. Mater. Res. B Appl. Biomater.* 0 (n.d.).
- [103] D. Dionysopoulos, K. Tolidis, T. Sfeikos, Effect of air-abrasion pre-treatment with bioactive glass 45S5 on enamel surface loss after erosion/abrasion challenge, *Dent. Mater.* (2019).

IDENTIFICATION

Records identified through database searching
(n=1078)

PubMed (n=570)

Scopus (n=331)

Cochrane (n=57)

Embase (n=119)

SCREENING

Records after duplicates
removed
(n=708)

Records excluded
after title,
language and
abstract screening
(n=563)

Full-text articles assessed
for eligibility
(n=145)

Full-text articles
excluded (n=98)

- BAG with scaffold (n=48)
- BAG with adjuvants or other constituent (n=14)
 - No BAG as remineralization solution (n=8)
- No evaluation of BAG's bioactivity (n=17)
- No dental application (n=4)
- Foreign Language (n=7)

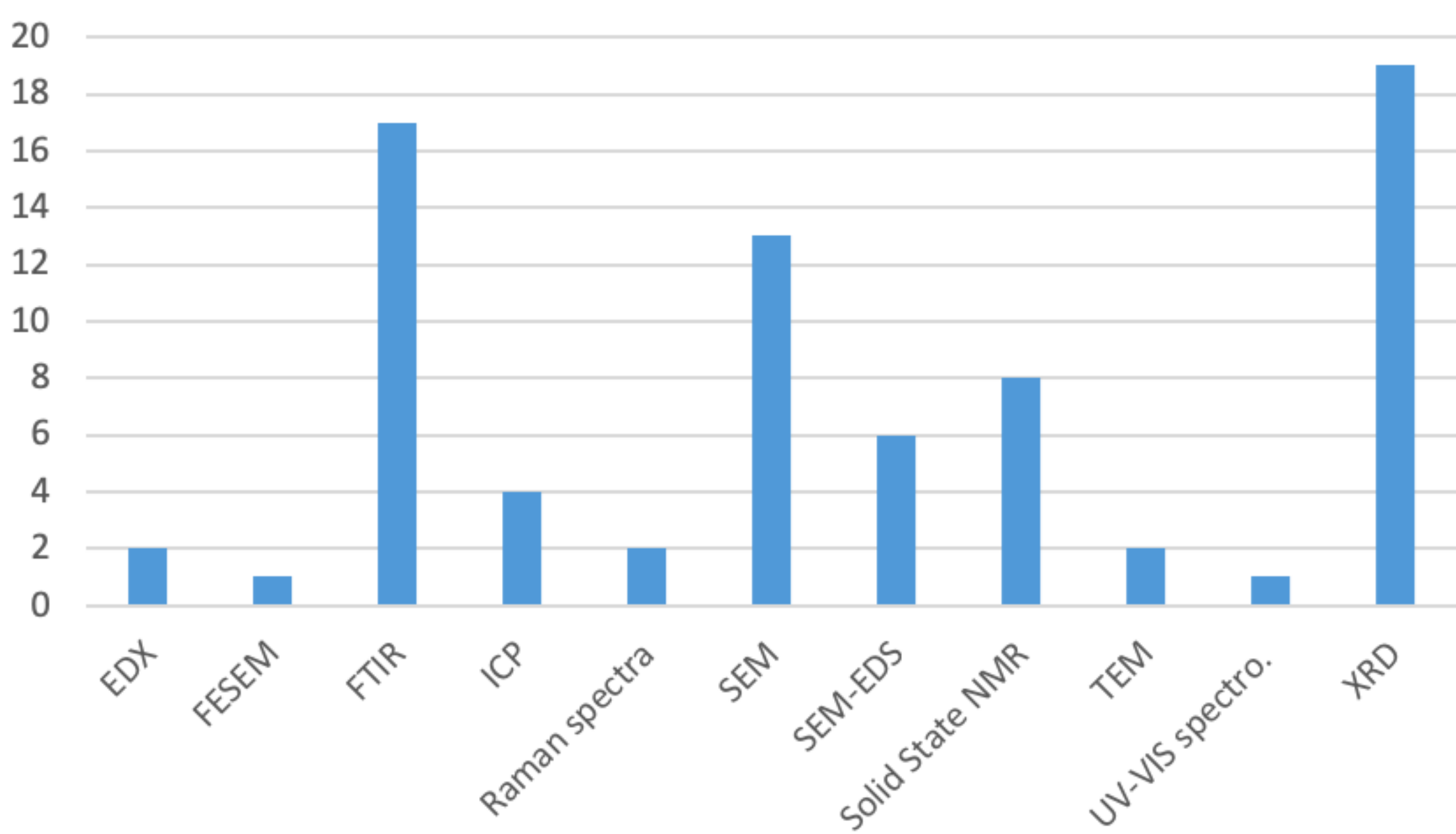
ELIGIBILITY

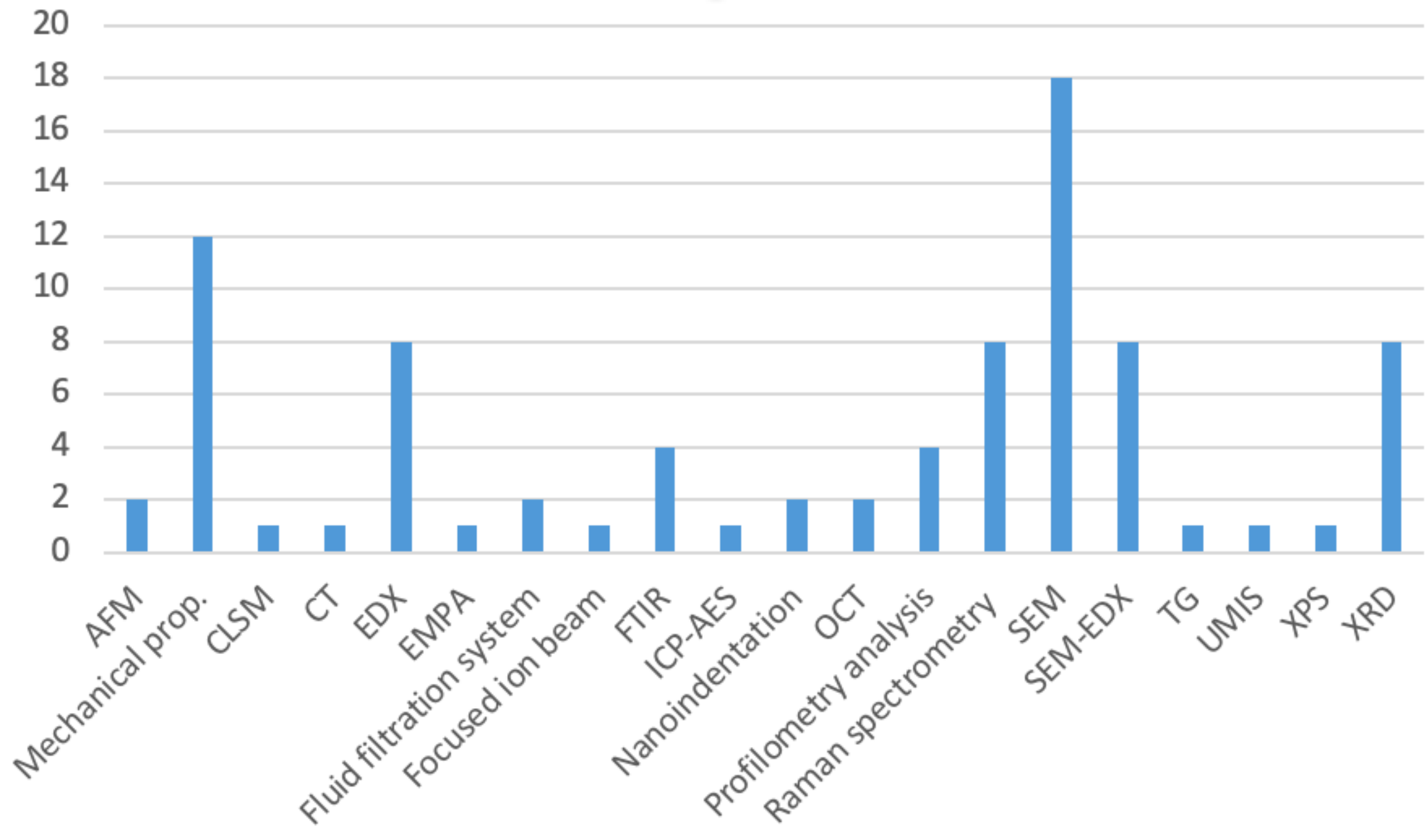
Included articles
(n=47)

Additional records
identified through
reference lists
(n=13)

INCLUDED

Studies included in qualitative synthesis
(n=60)





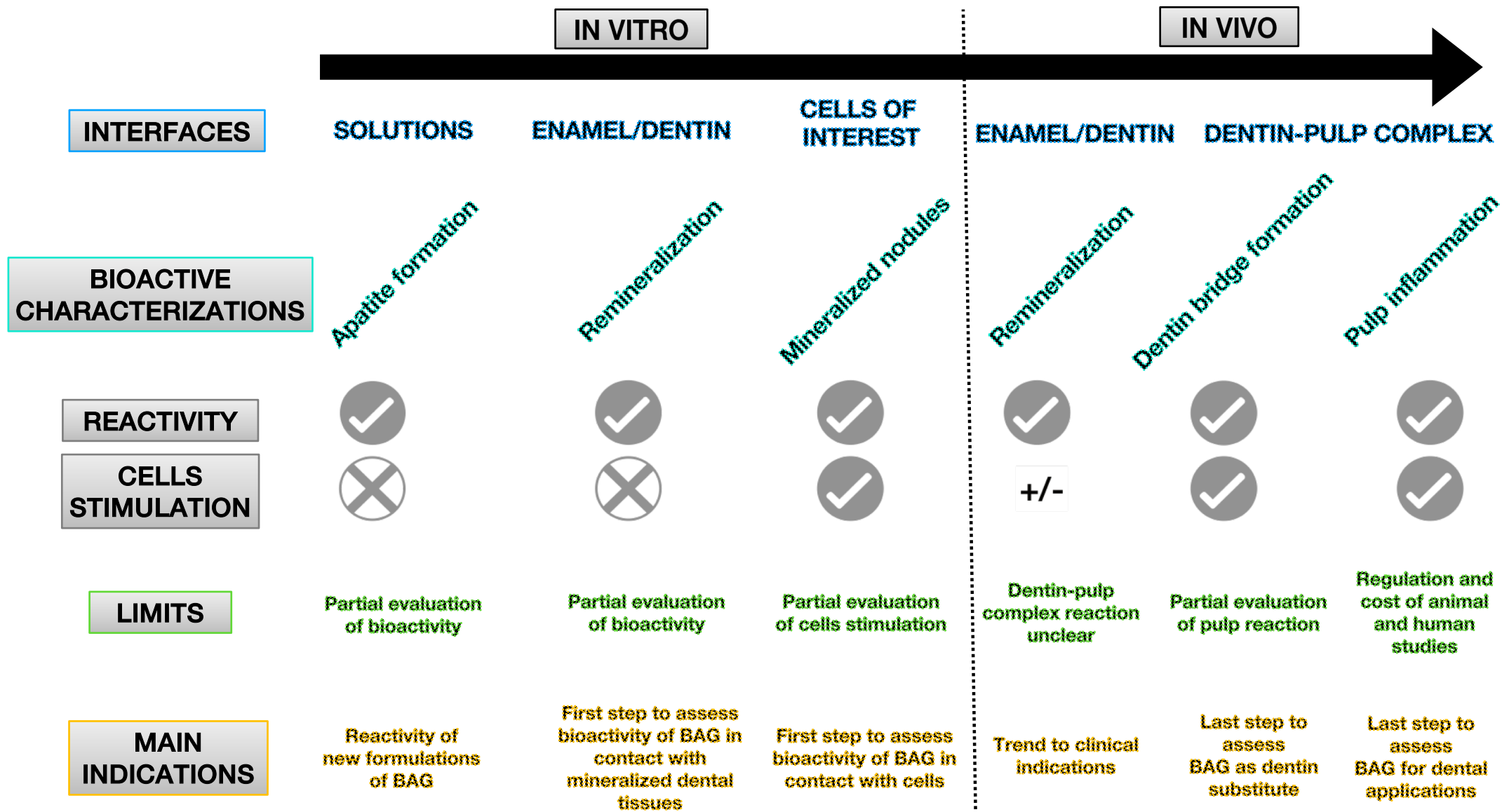


Table 1. Selected articles: *In vitro* mechanism of apatite formation on BAG surface in solutions

BAG compared with 1 Control group; 2 BAG with adjuvants; 3 oxide alone, Antibacterial, desensitizing agents, bioactive, or restorative materials; 4 Laser. Analysis: 1 Microscopic observations; 2 Chemical or structural analysis; 3 Mechanical properties evaluation; 4 Histological analysis (_ = not specified in the article / m = minutes; h = hours; d = days; w = weeks; m = months)

REFER ENCES	BAG INVESTIGATED						COMPARE D WITH				CRITERIA	METHODS				MAIN RESULTS		
	Glass Label – size	Manufac turing methods	Glass Composition by mol%				1	2	3	4		Interface	Contact time	Analysis				
			SiO ₂	Ca O	Na ₂ O	P ₂ O ₅								1	2		3	4
TAKA DAMA – 2001 [75]	Glass specimen	MELT-QUENCH	80	0	20	0					Apatite formation	SBF	12 h – 1, 5, 10, 17, 28 d	X	X			Na ₂ O-SiO ₂ glass induced mineralization of apatite on its surface in the SBF.
FORSB ACK – 2004 [51]	S53P4 (<45 μm)	_	53	20	23	4	X				Silica concentration	TBS	Time up to 24 h		X			After a short period of immersion, silica is dissolved from both types of glasses, but the amount of silica released is much higher from S53P4 than from CG.
	Control glass (CG) (<45 μm)	_	_	_	_	_												
LIN – 2005 [76]	GEL-SILICA Glasses	SOL-GEL	70	30	0	0					Apatite formation	SBF	20 m – 1, 3, 6, 12, 21.5 h	X	X			After 1 h of SBF soaking, there was a significant increase in the phosphorus density of the phosphorus species on the glass surface. Presumably, an amorphous layer with constituents similar to HA had formed on the glass surface.

GOUD OURI – 2009 [77]	BG	SOL- GEL	60	36	0	4			X		Formation of apatite	SBF	6, 12, 24, 48 h	X	X		Apatite formation from pure BAG is compared with dental glass ceramic composites products in SBF solutions. Level of bioactivity is evaluated through the kinetic of apatite formation. Porous particles improved the level of bioactivity.
MOAW AD – 2009 [35]	48S	MELT- QUENC H	48	yCaO – zNa ₂ O : y+z = 49,3		2. 7			X		Formation of HA layer	SBF	7 d		X		Diffraction peaks for the surface layer on 48S4F (4%CaF ₂) glass are stronger than those for the layer on 48S glass. This simple comparison suggests that the presence of fluoride ions likely enhanced the bioactivity of BAG.
BRAUE R – 2010 [38]	GLASS A – 38 μ m	MELT- QUENC H	49.4 7	23 .0 8	26 .8 9	1. 07			X		Apatite deposition	SBF	3 d – 1, 2 w		X		Incorporation of fluoride that resulted in the formation of FAp compared with fluoride-free glasses.
GUNA WIDJA JA – 2010 [32]	MBG – S85	MELT- QUENC H	85	10	0	5					Formation of amorphous calcium phosphate layer and crystallization into HCA	SBF	30 m – 1, 4 h - 1, 3, 7 d		X		The phosphorus content increased only marginally over the first 4 h of SBF exposure relative to the initial value of S85. The 2 formations of ACP and HCA partially occurred simultaneously, and the former was most active over the initial 24 h of SBF immersion and the latter dominating between 3 and 7 days (d).
MOHN – 2010 [78]	45S5 – 34 nm	FLAME SPRAY SYNTH ESIS	45	24 .5	24 .5	6			X		Formation of carbonated hydroxyapatite	SBF	7 d	X	X		Radiopaque BAG with bismuth oxide had higher <i>in vitro</i> bioactivity regarding the formation of carbonated HA.
BRAUE R – 2011 [36]	GLASS A	MELT- QUENC H	48.4 7	23 .0 8	26 .3 8	1. 07			X		Apatite formation	TBS	1, 3, 7, 14 d		X		Glasses formed apatite in TBS. However, Glass A.1 (0.94% CaF ₂) showed more clearly pronounced peaks and higher peak intensities in XRD compared with fluoride-free glass A.

GOUD OURI – 2011 [31]	BG	SOL- GEL	60	36	0	4			X		Apatite formation	SBF	6, 12 h - 1, 2, 3, 6, 9 d - 2, 4, 8, 12, 18 d	X	X			The lower rate of bioactivity of the dental ceramic with BG in comparison with the powdered BG samples was a result of the lower surface area of the specimens compared with powdered BG samples.	
MATH EW – 2011 [49]	MBG	EISA process	85	10	0	5					Apatite formation	SBF – TBS	16 h	X	X			HA, and the CaP clusters, were present in the pristine MBG pore walls.	
MNEI MNE – 2011 [40]	A – (<38 μm)	MELT- QUENC H	49.4	23	26	1.					Apatite deposition	TBS	3, 6, 9 h – 1, 3, 7 d		X			An increase in phosphate or fluoride content allowed for apatite formation at lower pH.	
	A2 – (38 μm)		38.1	25	29	6.													
EL- FIQI – 2012 [29]	BGn	SOL- GEL	85	15	0	0			X		Apatite formation	SBF	Differen t times up to 28 d	X	X			A SBF test of the mesoporous BGn confirmed their excellent apatite-forming ability.	
GUNA WIDJA JA – 2012 [30]	S90	EISA process	90	10	0	0					Formation of HCA	SBF	Variable intervals between 0.5 h and 7 d		X			The S90 and S85 MBGs displayed similar in vitro behavior, whereas the Ca-rich S58 specimen reacted more similarly to melt-prepared bioactive glasses.	
	S85		85	10	0	5													
	S58		58	37	0	5													
GUNA WIDJA JA – 2012	S85	/	85	10	0	5					Growth of CaHA – Amounts of amorphous CaP and HA	SBF – TBS	Between 1 h and 7 d	X	X			Whereas the TBS grains displayed a 60 μm thick domain of almost pure silica, their SBF counterparts verified small but significant amounts of Ca and P throughout all particles of diameter >20 μm . This finding is attributed	

NSKI – 2013 [26]	(Amorphous – crystallized samples)	QUENCH		.2	.1	2						d				on amorphous BAG after 7 and 14 d. Apatite formation was observed only on the crystallized 45S5 samples after storage.
SOUZA – 2013 [44]	45S5	MELT-QUENCH	46.3	26.9	24.3	2.5		X		Formation of an apatite-like layer	SBF	4, 8, 16 h - 1, 2, 4, 8, 16 d	X	X		Partial replacement of CaO by MgO in the BAG had no influence on the kinetic of precipitation of the initial ACP layer when glass was exposed to SBF.
AREPALLI - 2015 [81]	Ba-0	–	46.1	26.9	24.3	2.6		X		Formation of HCA layer	SBF	1, 3, 7, 14, 30 d	X	X		The formation of a hydroxyl carbonate apatite layer was observed on the surface of the barium containing BAG after immersion in SBF. However, an increase in barium content in the glass samples decreased the tendency of calcite formation.
KIRSTEN – 2015 [82]	45S5	–	45.53	24.8	23.1	6.29		X		Surface reaction layers (HCA)	SBF	1, 3.5, 7, 17, 28 d	X	X		45S5 and BAG with partial substitution of Na ₂ O and CaO by K ₂ O and MgO exhibited an Si-rich layer of approximately 20 μm and a CaP-rich layer of approximately 10 μm after 14 d of storage in SBF. HCA was found after 3.5 to 7 d on both glasses.
ABBAS I – 2016 [83]	58S (<40 μm)	SOL-GEL	–	–	–	–		X		Formation of an apatite layer	SBF	6h - 1, 3, 6, 9 d	X	X		The 58S has a high surface area and can release ions very fast in the solution (high bioactivity). Dental ceramic/58S BAG mixtures also exhibit high degrees of bioactivity despite the presence of leucite with non-bioactive behavior.
WANG – 2016 [15]	smBG (510-590 nm) – 45S5	–	–	–	–	–		X		Apatite formation	SBF	1, 5 d	X			Crystalline apatites were precipitated on the polycarbolactone (PCL)/submicron BG (smBG) group and pure smBG surfaces after incubation. The thickness of the apatite layer on the PCL/smBG group was higher than for the smBG samples.

MATH EW – 2017 [84]	S90	EISA process	90	10	0	0				ACP formation/conversi on into HCA – Distinct Ca, Si and P contents	SBF	0.25, 1, 4, 8, 24 h - 3, 7, 15, 30 d		X					HCA formation occurred after 4h of SBF exposure, except for P-free S90 MBG (after 8h).
	S85		85	10	0	5													
	S58		58	37	0	5													
ANAN D – 2018 [85]	MBG (nano)	EXPERI MENTA L TECHNI QUE	–	–	0	–				Carbonated HA forming ability	SBF	24, 48 h	X	X					Formation of hydroxyapatite layer on different surfactants was evaluated: non-ionic surfactant pluronic and ionic surfactant hexadecyltrimethyl-ammonium bromide in contrast with non-ionic surfactant polyethylene glycol and citric acid.
ASHO K – 2018 [54]	BGNR – 45S5	SOL- GEL	45	24 .5	24 .5	6			X	Formation of hydroxyl carbonated apatite layer (HCA)	Hank's solution	3, 7 d	X	X					On the 3 rd day, the formation of the HCA layer is relatively higher in nano hybrids of BGNR/rGO (reduced graphene oxide sheets) compared with that of BGNR. Similar levels of HCA formation in BGNR were observed only on the 7 th day.
TAHA – 2018 [86]	45S5 (Sylc™) air- abrasion	MELT- QUENC H	46.1	26 .9	24 .4	2. 6			X	Apatite formation	TBS	1, 3, 6, 9, 24 h		X					BAG with CaF ₂ formed apatite faster (6 h) than 45S5 (Sylc™) (24 h) in TBS.

Table 2. Selected articles: *In vitro* BAG remineralization of enamel and/or dentin

BAG compared with 1 Control group; 2 BAG with adjuvants; 3 Oxide alone, antibacterial, desensitizing agents, bioactive, or restorative materials; 4 Laser. Analysis: 1 Microscopic observations; 2 Chemical or structural analysis; 3 Mechanical properties evaluation; 4 Histological analysis. (_ = not specified in the article / s = seconds; m = minutes; h = hours; d = days; w = weeks; m = months)

REFER ENCES	BAG INVESTIGATED						COMPARE D WITH				CRITERIA	METHODS				MAIN RESULTS		
	Glass Label – size	Manufac turing methods	Glass Composition by mol%				1	2	3	4		Interface	Contact time	Analysis				
			SiO ₂	Ca O	Na ₂ O	P ₂ O ₅								1	2		3	4
EFFLA NDT – 2002 [87]	45S5	MELT- QUENC H	45	24 .5	24 .5	6		X			Apatite formation	Human dentin	5, 21, 24 d	X	X			Ions from glass penetrated dentin. Presence of apatite at the interface between glass and dentin was observed.
FORSB ACK – 2004 [51]	S53P4 (<45 μm)	_	53	20	23	4	X				Occlude dentin tubules - Formation of CaP	Human Dentin	14 d	X	X			Pretreatment with BAG decreased the degree of decalcification of dentin during the mineralization process.
	Control glass (CG) (<45 μm)	_	_	_	_	_												
HASSA NEIN – 2006 [88]	S53P4	_	53	20	23	4	X		X		Topography of the remineralized areas – Nature of the compounds	Human enamel and dentin	10 d	X	X			BAG has the potential to remineralize artificial carious lesions in enamel and dentin: formation of hydroxyapatite, sealing of enamel pores, and plugging of dentinal tubules.

											deposited – Degree of remineralization							
LEE – 2007 [21]	DP- BAG with 30% PPA	SOL- GEL MELT- QUENC H	39.6	40	8. 4	12					Dentinal tubule occlusion	Human dentin	3 d	X	X			Percentage of tubular occlusion with DP-BAG was 53.2%–65.4% and significantly better than Seal & Protect (41.2%).
SCHMI DLIN – 2007 [45]	S53P4 (≤ 45 μm)	–	–	–	–	–			X		Improve mechanical properties – Higher hardness	Human Dentin	3 w			X		Hardness and elastic modulus values of the dentin subjacent to empty microcavities and counterparts lined were significantly higher with BAG compared with the RMGIC.
VOLLE NWEID ER – 2007 [89]	45S5 NBG – 45S5 BG (PerioGl as® 90- 710 μm)	FLAME SPRAY SYNTH ESIS (NBG)	/	/	/	/					New mineral precipitated – Improve mechanical properties	Human dentin	1, 10, 30 d	X	X	X		After treatment with nano-BAG for 10 or 30 d, a pronounced increase in mineral content of the dentin was observed. A substantially higher remineralization rate was induced by nanometer-sized vs. micrometric.
CURTI S – 2010 [23]	70S30C (0.65 μm)	SOL- GEL	70	30	0	0					Tubule occlusion - Formation of an apatite layer	Human dentin	24 h	X	X			Treatment with nanobioglass resulted in particle deposition within tubules and formation of apatite rods.
	45S5 (3.30 μm)	MELT- QUENC H	45	24 .5	24 .5	6												
BAKR Y - 2011 [90]	45S5 with 50 % PAA	–	45	24 .4	24 .5	6			X		Tubule orifice Closure – Recover mechanical	Human dentin	24 h	X	X	X		45S5 could occlude the dentinal tubule orifices with calcium phosphate crystals.

											properties							
DONG – 2011 [27]	45S5	MELT-QUENCH	44.8	26	23	5.	X				Mineralized layer – Surface roughness – Nano-hardness and Nano-reduced elastic modulus	Human enamel	7 d	X	X	X		Enamel surface formed a homogenous, dense mineralized layer with the treatment of 45S and 58s samples. 77S treatment showed a loose and uneven remineralized layer. These results also indicated that the level of silicon content of BAG played a key role in dental enamel remineralization.
	58S	SOL-GEL	62.8	27	0	9.												
	77S	SOL-GEL	73.7	16	0	9.												
	A2 – (38 μm)		38.1	25	29	6.												
SAURO - 2011 [91]	45S5 (Sylc™)	–	–	–	–	–			X	Dentin permeability – Hydroxyapatite precipitation	Human dentin	24, 48 h	X	X		X	45S5 (Sylc™) was the only substance (compared with prophylactic materials) able to reduce dentin permeability after immersion in remineralising solution and to show hydroxyapatite precipitation.	
WANG – 2011 [92]	BAG (30-90 μm)	–	45	24	24	5.			X	Mineral variation of the surface – Apatite formation – Roughness of the surface	Human dentin	7 d	X	X			Results showed a significant increase of the mineral matrix area ratio in dentin specimens treated with BAG and M-BAG (with magnesium). Both formulations have similar potential in dentine remineralization.	
BAKR Y – 2013 [93]	45S5 with 50% PPA	/	45	24	24	6			X	Dentin permeability – Examination of surfaces (chemical nature - crystalline	Human dentin	24 h	X	X	X		Application of 45S5 BAG paste to dentin occluded patent dentinal tubule orifices with a layer of calcium phosphate crystals.	

MILLY – 2015 [97]	BAG 45S5 slurry (2 – 6 – 12 µm)	–	–	–	–	–	X	X		Average roughness – Optical changes – Structural changes of WSL	Human enamel	Twice daily (5 min per application) for 21 d	X	X	X		The pretreatment enhanced the remineralization of WSL treated with BAG 45S5 slurry: increased mineral content, improved mechanical properties and ultrastructural changes.
CARVALHO – 2016 [98]	Bio-Gran+distilled water	EXPERIMENTAL TECHNIQUE – MELTING MIXTURE	42.3	28.3	22.8	6.6		X		Release of Ca, Na and P ions	Human dentin	10 min – 24 h - 7, 14, 21, 30 d		X			CH had the highest level of Ca ions release at 30 d. The BAG released more Na and P ions and presented an alkaline pH immediately and after 30 d.
EL-WASSEFY – 2017 [99]	45S5 with 50% PPA	–	45	24.4	24.5	6	X	X	X	Changes in the mineral content – High microhardness	Bovine enamel	24 h	X	X	X		Treating demineralized enamel with cold plasmas before BAG application ensued a significant high mineral volume recovery and microhardness of demineralized region.
SAFFARPOUR – 2017 [43]	BG	–	64	26	0	10		X		Chemical structure – Presence of globular HA crystals – Tubular obstruction	Human dentin	7, 14, 21 d	X	X			Dentinal tubules were partially occluded by BG and BG modified with 5% Strontium, and they almost completely obstructed after the use of BG modified with 10% Sr. Addition of 10% Sr to BG enhanced apatite formation. Addition of 5% Sr to BG stabilized the apatite lattice and increased the remineralization.
TAHA	45S5	NC	NC	N	N	N		X		Intensity of light	Human	24 h	X	X	X		BAG with CaF ₂ enhanced enamel remineralization

- 2018 [37]	(Sylc™) air- abrasion			C	C	C					backscattering – Roughness – Hardness – Mineral precipitate-like deposits – Presence of Ca, P, O and Na	enamel					more effectively than 45S5. Surface roughness and intensity of light backscattering similar to that of sound enamel were observed following treatment with fluoride-containing BAG.
ZHAN G – 2018 [100]	45S5 (Novami n®) – 45S5 (Novami n®)+PA A	NC	NC	N C	N C	N C	X	X	X		Change of surface mineral content – Improve mechanical properties – Identify the type of newly formed minerals – Surface morphologies and Ca/P ratio	Human enamel	7 d	X	X	X	BAG/PAA presented significantly higher mineral regain compared with negative control on lesions surfaces. Chitosan (CS) improved the remineralization efficacy using either a BAG slurry alone or BAG/PAA complexes as both surface and subsurface showed an increased tendency in mineral content assessed and greatest hardness recovery.
ZHAN G – 2018 [101]	45S5 (Novami n®) – 45S5 (Novami n®)+PA A	NC	NC	N C	N C	N C	X	X	X		Mineral content/changes – Sub/surface microhardness - Formation of pellicle layer	Human Enamel	7 d	X	X	X	Chitosan-BG exhibited denser subsurface structure than BG, and in CS-BG+PAA, the crystals were bigger in size but were more enamel-like compared with BG+PAA, as shown in SEM observations.
LEE – 2019 [102]	BG + PPA	SOL- GEL	58	33	0	9	X		X	X	Tubules obstructions – Formation of HA	Human dentin	5 min	X	X		BG with PPA prevented the formation of cracks and degradation of collagen fibers caused by CO ₂ irradiation and promoted obliteration of the

Table 3. Selected articles: *In vitro* effects of BAG on the mineralization capacity of cells

BAG compared with: 1 Control group; 2 BAG with adjuvants; 3 Oxide alone, antibacterial, desensitizing agents, bioactive, or restorative materials; 4 Laser. Analysis: 1 Microscopic observations; 2 Chemical or structural analysis; 3 Mechanical properties evaluation; 4 Histological analysis (_ = not specified in the article / d= days; w = weeks)

REFER ENCES	BAG INVESTIGATED						COMPARE D WITH:				CRITERIA	METHODS				MAIN RESULTS						
	Authors Date	Glass Label – size	Manufac turing methods	Glass Composition by mol%				1	2	3		4	Interface	Contact time	Analysis							
				SiO ₂	Ca O	Na ₂ O	P ₂ O ₅								1		2	3	4			
GONG – 2014 [14]	45S5 – 1 and 10 μm	MELT- QUENC H	45	24 .5	24 .5	6	X					Matrix mineralization	hDPCs	21 d					X	All BG extractions, especially nano-58S, increased mineralized nodules formation.		
	58S BG – 2–20 μm	SOL- GEL	58	33	0	9																
	Nano- 58S BG – 10-100 nm		58	33	0	9																
WANG – 2014 [13]	58Sm (micro)- BG 58Sn (nano)-	SOL- GEL	58	33	0	9	X					Calcium concentrations	hDPCs	4 w					X	Relative calcium concentrations increased significantly in the m-BG and n-BG groups and were particularly higher in the n-BG group compared with the control group.		

	BG																		
WANG – 2016 [15]	smBG (510-590 nm) – 45S5	–	–	–	–	–			X		Mineralized nodules	hDPCs	4 w					X	More mineralized nodules were generated in the polycarbolactone (PCL)/submicron BG (smBG) group than for smBG.
HUAN G – 2017 [16]	Zn0BG ($\leq 38 \mu\text{m}$)	MELT-QUENC H	38.5	29	26	6.3	3		X		Mineralized nodule formation and composition	hDPSCs	3, 5 w					X	Formation of mineralized nodules was observed in the 0ZnBG group; however, it was higher in the ZnBG group.
LOPEZ – 2017 [17]	BG	SOL-GEL	57	26	0	17	X		X	X	Calcium precipitation	SHEDs	21 d					X	Desensitizing agent alone or with laser, and BG alone or with laser, stained positively with Alizarin red staining. BG + laser had significantly higher mineral matrix deposition than BG alone.

Table 4. Selected articles: *In vivo* evaluation of dentin mineralization and/or dental pulp

BAG compared with 1 Control group; 2 BAG with adjuvants; 3 Oxide alone, antibacterial, desensitizing agents, bioactive, or restorative materials; 4 Laser. Analysis: 1 Microscopic observations; 2 Chemical or structural analysis; 3 Mechanical properties evaluation; 4 Histological analysis (_ = not specified in the article / w = weeks; m = months)

REFER ENCES	BAG INVESTIGATED						COMPARE D WITH:				CRITERIA	METHODS				MAIN RESULTS		
	Glass Label – size	Manufac turing methods	Glass Composition by mol%									Interface	Contact time	Analysis				
			SiO ₂	Ca O	Na ₂ O	P ₂ O ₅								1	2		3	4
SALAK O – 2003 [18]	S53P4	_	53	20	23	4			X		Inflammation - Necrosis – Cells observations – Dentin bridge formation	Molars rats – Pulpotomy	2, 4 w				X	BAG induced an inflammatory response at 2 weeks (w) with resolution of inflammation at 4 w. Most of the 4 w-old BAG samples showed regular pulpal histology, with some dilated blood vessels. Complete pulpal necrosis of some specimens. No dentin bridge formation.
WANG – 2014 [13]	58Sm (micro)-BG 58Sn (nano)-BG	SOL-GEL	58	33	0	9	X			Mineralized tissue formation	Molars pulp rats	2, 6 w				X	2 w/n-BG group: newly generated matrix was observed around particles. 6 w/n-BG group: new dentin-like tissue was formed with uniform thickness and well-organized dentinal tubule structure. 6 w/m-BG group: pulpo–dentinal complex-like tissue was also generated (thinner than that in n-BG group).	
HAGH GOO – 2016 [20]	BAG	_	_	_	_	_			X	Pulp statue (Inflammation, necrosis, abscess, resorption) - Dentin bridge formation	Human dentin and pulp	2 m				X	2 m: In the BAG group, inflammation was observed in 3 patients (n=22); internal resorption and abscess were not observed. Dentinal bridge formation was observed in 5 patients in the BAG group (n=22).	

BIOACTIVITY ASSESSMENT OF BAG

IN VITRO

Apatite formation on BAG surface in SOLUTIONS



BAG capacity to remineralize ENAMEL and/or DENTIN



Effect of BAG on the CELLS mineralization capacity



IN VIVO

BAG capacity to remineralize ENAMEL and/or DENTIN



Evaluation of MINERALIZED DENTIN FORMATION and/or DENTAL PULP RESPONSES



BIOACTIVE CHARACTERIZATIONS

?

LIMITS

?

MAIN INDICATIONS

?