DEVELOPMENT OF A NOVEL BIOCERAMIC ROOT CANAL SEALER AND ITS EFFECT ON THE DISLODGEMENT RESISTANCE AND DENTINAL TUBULE PENETRATION

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Galvin Sim Siang Lin

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LIST OF SYMBOLS

%	Percentage
^{o}C	Degree Celsius
mol	Mole
mm	Millimetre
µm	Micrometre
nm	Nanometre
ml	Millilitre
L	Litre
g	Gram
wt.%	Weightage
cm^{-1}	Wavelength
kV	Kilovolt
mA	Milliampere
nA	Amps
mW/cm^2	Milliwatts per square centimetre
N	Newtown
MPa	Megapascal
mm^2	Square millimetre
r	Coefficient correlation
α	Alpha
β	Beta

LIST OF ABBREVIATIONS

Al	Aluminium
ANOVA	Analysis of variance
Ba	Barium
BCE	Before Christian era
BG	Bioactive glass
β-TCP	β-tricalcium phosphate
C	Carbon
Ca	Calcium
$CaAl_2Si_2O_8$	Calcium aluminosilicate
CaCl	Calcium chloride
$CaCO_3$	Calcium carbonate
$Ca(NO_3)_2 \cdot 4H_2O$	Calcium nitrate tetrahydrate
CaO	Calcium oxide
$CaOH_2$	Calcium hydroxide
$CaSiO_3$	Calcium silicate
CEJ	Cemento-enamel junction
CEM	Calcium enriched mixture
$C_{12}H_{14}CaO_{12}$	Calcium alginate
C_2H_6O	Ethanol
CHX	Chlorhexidine
Cl	Chloride
CLSM	Confocal laser scanning microscopy
C=O	Carbonyl group
C-O-O	Carboxylate
COOH	Carboxylic acid
C_2S	Dicalcium silicate
C_3S	Tricalcium silicate
CSH	Calcium silicate hydrate
EDS	Energy dispersive spectroscopy
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron

FTIR	Fourier transform infrared spectroscopy
GDL	D-gluconic acid δ-lactone
GIC	Glass ionomer cement
H	Hydrogen
HA	Hydroxyapatite
HCA	hydroxycarbonate apatite
HCl	Hydrochloric acid
H_3PO_4	Phosphoric acid
HRTEM	High-resolution transmission electron microscopy
IR	Infrared
ISO	International Organization for Standardization
K	Potassium
LED	Light emitting diode
Micro-CT	Micro-computed tomography
Mg	Magnesium
Mg-PSZ	Partial magnesium oxide-stabilized
Mn	Manganese
MTA	Mineral trioxide aggregate
N	Nitrogen
Na	Sodium
$NaC_6H_7O_6$	Sodium alginate
Na_2O	Sodium oxide
NaOCl	Sodium hypochlorite
NiTi	Nickel-titanium
O	oxygen
O-H	Hydroxide
P	Phosphorus
PCL	polycaprolactone
PET	Polyethylene terephthalate
P_2O_5	Phosphorus pentoxide
rpm	Round per minute
SEM	Scanning electron microscopy
Si	Silicon
SiO_2	Silicon dioxide

Si-O-H	Silanol group
SPSS	Statistical Package for the Social Sciences
Sr	Strontium
TEM	Transmission electron microscopy
TEOS	Tetraethyl orthosilicate
TEP	Triethyl phosphate
USM	Universiti Sains Malaysia
XRD	X-ray diffraction
Y-TZP	Partial yttria-stabilized
Zn	Zinc
ZOE	Zinc oxide eugenol
Zr	Zirconium
ZrO_2	Zirconium dioxide

PEMBANGUNAN SEALER KANAL AKAR BIOSERAMIK BARU DAN KESANNYA TERHADAP RINTANGAN DISLODGEMENT DAN PENEMBUSAN TUBULE DENTINAL

ABSTRAK

Kajian ini bertujuan untuk merekabentuk dan mencirikan algin-gabungan kaca bioactif 58S kalsium-silikat sealer kanal akar (Bio-G) baru dan menilai kesannya terhadap rintangan dislodgement dan penembusan tubule dentinal ke dinding dentinal akar berbanding dengan sealer berasaskan bioseramik yang tersedia secara komersial (BioRoot RCS dan iRoot SP). Tiga kumpulan serbuk kaca bioaktif (BG) 58S disintesis menggunakan kaedah sol-gel dan dicirikan menggunakan SEM, HRTEM dan FTIR untuk pengoptimuman: BG-1 (tiada ammonia), BG-2 (3 ml ammonia), dan BG-3 (5 ml ammonia). BG-3 yang dioptimum digunakan untuk mencipta serbuk sealer Bio-G eksperimen dengan penambahan kalsium-silikat, zirkonia dioksida, kalsium karbonat dan serbuk asid alginik. Sementara itu, cecairnya terdiri daripada larutan 5% kalsium klorida. 0-5% algin Bio-G sealer eksperimen kemudiannya dibandingkan dengan BioRoot RCS dan iRoot SP. Spesimen bahan sealer campuran (n=5 setiap kumpulan) disediakan dan diletakkan dalam inkubator demi penetapan sebelum pencirian di bawah SEM, HRTEM, EDS, FTIR dan XRD. Seterusnya, seratus tujuh puluh enam premolar mandibular dbahagikan secara rawak (n=16 setiap kumpulan): kawalan, gutta-percha + 0-5% algin Bio-G, gutta-percha + BioRoot RCS, dan kumpulan gutta-percha + iRoot SP, dengan pengecualian kumpulan kawalan dalam corak pelekat dan ujian penembusan tubule dentinal. Mereka diinstrumen, diobturasi dan diletakkan dalam inkubator selama 72 jam untuk membolehkan penetapan sealer. Untuk ujian penembusan tubule dentinal, sealer dicampur dengan 0.1% pewarna rhodamine B.

Selepas itu, gigi dipotong menjadi keratan rentas tebal 1 mm pada tahap 5 mm dan 10 mm dari apex akar. Kekuatan ikatan push-out, corak pelekat dan ujian penembusan tubule dentinal dilaksanakan. Sealer bio-G eksperimen mendedahkan zarah bersaiz mikro yang tidak teratur dengan kandungan oksigen, silikon, dan kalsium yang lebih tinggi, serta kesan aluminium dan klorida. Sementara itu, penemuan FTIR dan XRD mencadangkan bahawa semua sealer mengandungi hidrat kalsium silikat, kalsium karbonat, dan zirkonium dioksida, manakala kalsium aluminium silikat oksida dikesan dalam 0-5% algin Bio-G. 5% algin Bio-G menunjukkan kekuatan ikatan push-out min tertinggi (p<0.05) dengan corak lekatan yang memuaskan, manakala iRoot SP menunjukkan tahap penembusan sealer tertinggi (p<0.05). Di samping itu, tiada persatuan penting yang diperhatikan antara rintangan dislodgement dan penembusan tubule dentinal (p>0.05). Kesimpulannya, sealer Bio-G baru ini menunjukkan taburan saiz zarah yang memuaskan dan tahap ketulenan yang boleh diterima. Bio-G yang diperbadankan algin menunjukkan corak pelekat yang menggalakkan dengan rintangan dislodgement dan nilai penembusan tubule dentinal yang setanding dengan sealer berasaskan bioseramik yang dikomersialkan.

DEVELOPMENT OF A NOVEL BIOCERAMIC ROOT CANAL SEALER AND ITS EFFECT ON THE DISLODGEMENT RESISTANCE AND DENTINAL TUBULE PENETRATION

ABSTRACT

The present study aimed to fabricate and characterise novel algin-incorporated bioactive glass 58S calcium-silicate root canal sealer (Bio-G) and evaluate its effect on the dislodgement resistance and dentinal tubule penetration to root dentinal walls in comparison to commercially available bioceramic-based sealers (BioRoot RCS and iRoot SP). Three groups of bioactive glass (BG) 58S powders were synthesised using sol-gel method and characterised using SEM, HRTEM and FTIR for optimisation: BG-1 (no ammonia), BG-2 (3 ml of ammonia), and BG-3 (5 ml of ammonia). Optimised BG-3 was used to fabricate the powder form of experimental Bio-G sealer with the addition of calcium silicate, zirconia dioxide, calcium carbonate and alginic acid powder. Meanwhile, the liquid form composed of 5% calcium chloride solution. The experimental 0-5% algin Bio-G sealers were then compared with BioRoot RCS and iRoot SP. Standardised disc specimens of mixed sealer materials (n=5 per group) were prepared and placed in an incubator to allow setting before characterising under SEM, HRTEM, EDS, FTIR and XRD. Next, one-hundred-and-seventy-six mandibular premolars were randomly assigned (n=16 per group): control, gutta-percha + 0-5% algin Bio-G, gutta-percha + BioRoot RCS, and gutta-percha + iRoot SP groups, with the exclusion of the control group in adhesive pattern and dentinal tubule penetration tests. They were instrumented, obturated and placed in an incubator for 72 hours to allow sealer set. For the dentinal tubule penetration test, sealers were mixed with 0.1% of rhodamine B dye. Subsequently, teeth were cut into a 1-mm-thick cross-section at

5-mm and 10-mm levels from the root apex, respectively. Push-out bond strength, adhesive pattern and dentinal tubule penetration tests were performed. Experimental Bio-G sealer revealed irregular micro-sized particles with a higher content of oxygen, silicon, and calcium, as well as trace of aluminium and chloride. Meanwhile, FTIR and XRD findings suggested that all sealers predominantly contained calcium silicate hydrate, calcium carbonate, and zirconium dioxide, while calcium aluminium silicate oxide was detected in 0-5% algin Bio-G. 5% algin Bio-G showed the highest mean push-out bond strength ($p<0.05$) with more favourable adhesion pattern, while iRoot SP showed the greatest sealer penetration ($p<0.05$). In addition, no significant association was noted between the dislodgement resistance and dentinal tubule penetration ($p>0.05$). In conclusion, the present novel Bio-G sealer demonstrated desirable particle size distribution and acceptable degree of purity. Algin-incorporated Bio-G showed favourable adhesive pattern with comparable dislodgement resistance and dentinal tubule penetration values to commercialised bioceramic-based sealers.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Root canal treatment aims at removing microorganisms in the infected tooth and facilitating apical tissue healing, thereby preserving the tooth without extraction. Successful root canal treatment requires an adequate three-dimensional seal of the root canal system with biocompatible filling materials to prevent bacterial reinfection through microleakage (Lin *et al.*, 2021). However, gutta-percha core material cannot bond and adapt well to the root canal walls. Thus, a root canal sealer is required to establish a fluid-tight seal in the root canal system and enhance the bond strength to prevent dislodgement of the root filling material (Washio *et al.*, 2019).

The ideal properties of a root canal sealer include excellent adhesion between the sealer material and the root canal wall, able to establish a hermetic seal, no shrinkage, insoluble in tissue fluids, dimensionally stable, biocompatible, ease of application, not causing tooth discolouration, antibacterial, acceptable setting time, and easy to remove during retreatment (Wang *et al.*, 2018). Currently available commercialized root canal sealers can be broadly classified as zinc oxide eugenol-based, calcium hydroxide-based, glass ionomer-based, epoxy resin-based, methacrylate-resin-based, silicone-based, and more recently, bioceramic-based sealers. However, no existing sealer has satisfied all the ideal criteria.

Bioceramic root canal sealers based on calcium silicate were first introduced in the 1990s at Loma Linda University to offset the drawbacks of resin-based sealers (Camilleri, 2008). This type of bioceramic cement is known as Mineral Trioxide Aggregate (MTA), which is hydraulic in nature and sets in the presence of water

(Camilleri, 2008). Nonetheless, MTA contains some amount of heavy metals such as chromium, lead and arsenic, and it exhibits poor flowability (Parirokh and Torabinejad, 2010; Schembri *et al.*, 2010). Hence, new generation of pure bioceramic-based sealers, such as BioRoot RCS (Septodont, Saint Maur-des-Fossés, France) and iRoot SP (Innovative Bioceramix, Inc., Vancouver, British Columbia, Canada), were introduced with the absence of heavy metal oxides or other additives (Al-Haddad and Che Ab Aziz, 2016; Siboni *et al.*, 2017; Wu *et al.*, 2021).

These bioceramic-based sealers will set with hydration reaction to produce calcium silicate hydrate and calcium hydroxide, which further reacts to form a hydroxyapatite layer in the presence of tissue fluid (Saghiri *et al.*, 2017). Bioceramic sealer also forms precipitation of calcium phosphate or calcium carbonate along the root canal wall interface, known as 'mineral infiltration zone' (Al-Haddad and Che Ab Aziz, 2016). In addition, they allow micromechanical interaction of mineral tag-like structures between the sealer and root canal walls (Al-Haddad and Che Ab Aziz, 2016), which enhance their bonding and sealing capabilities (Lin *et al.*, 2021; Lin *et al.*, 2020). Nonetheless, there are still controversial findings in the literature regarding the bond strength of bioceramic-based sealers (Donnermeyer *et al.*, 2018), and some studies discovered that bioceramic-based sealers exhibited high cohesive failure (DeLong *et al.*, 2015; Ersahan and Aydin, 2010).

Another important factor when evaluating root canal sealers is their ability to penetrate deep into the dentinal tubule to form a strong physical barrier, thus improving the retention of the root filling materials and enabling residual bacteria to be entombed in the root canals (El Hachem *et al.*, 2019). Furthermore, with the ability of the sealers to penetrate deep into the tubules, it can be expected that their antibacterial effect will

work better if present (Wang *et al.*, 2014). There is still an elusive answer to whether bioceramic-based sealers can penetrate deeper into the dentinal tubules as data obtained from the literature varies across studies (Aktemur Turker *et al.*, 2018; Arikatla *et al.*, 2018; Kim *et al.*, 2019). Hence, a search for a more advanced bioceramic-based root canal sealer with better adhesion and bond strength to root dentine walls as well as excellent dentinal tubule penetration depth is needed.

Recently, inorganic bioactive glass (BG) or bioglass powder, has gained attention among researchers as it has been found to stimulate more bone regeneration, promote osteogenesis, angiogenesis and wound healing which could possibly boost the healing process of the infected periapical tissues (Mokhtari *et al.*, 2018). The first BG introduced was the 45S5 bioglass using a melt-prepared technique which later led to the discovery of sol-gel synthesis (Faure *et al.*, 2015). Sol-gel derived BG 58S which composes of 59% SiO_2, 36% CaO, and 5% P_2O_5, has been reported to have excellent biocompatibility, bioactivity, and antibacterial activity as compared to its 45S5 bioglass counterpart (Gong *et al.*, 2012; Mortazavi *et al.*, 2010).

Bioceramic root canal sealers based on BG particles have been commercialized recently and the most well-known is GuttaFlow BioSeal (Coltène/Whaledent AG, Altstätten, Switzerland). Nevertheless, the bond strength of GuttaFlow BioSeal has been reported to be inferior to epoxy resin-based and zinc oxide eugenol-based sealers (Dem *et al.*, 2019; Khalil *et al.*, 2019; Marques Ferreira *et al.*, 2022). The dentinal tubule penetration capability of GuttaFlow BioSeal was also found to be inferior to other bioceramic-based sealers such as iRoot SP and Endosequence BC (Brasseler, Savannah, Georgia, USA) (Akcay *et al.*, 2016; Kanwar *et al.*, 2020). Moreover, the incorporation of nano silver particles with antibacterial intention in GuttaFlow BioSeal

has also evoked considerable controversy due to the damaging effect towards the cellular systems (McShan *et al.*, 2014).

Algin, also known as alginic acid, is a hydrophilic polysaccharide commonly derived from seaweeds that forms a viscous gel-like structure when hydrated (Ching *et al.*, 2017; Lin *et al.*, 2022). It can form salts which are known as alginates. Alginate is a non-toxic, readily available, biocompatible, and non-immunogenic marine biopolymer (Yadav *et al.*, 2015). Alginates have many free hydroxyl and carboxyl groups that are distributed along their backbone, making them highly reactive and amenable for strong cross-linkage with other particles. It is widely used in medical applications including wound healing, drug delivery and tissue engineering (Lee and Mooney, 2012). However, the use of alginate in the field of endodontics, particularly for root canal sealers, is still considered new.

Alginate gelation occurs by the binding of divalent cations to create an insoluble and tightly bound configuration that can result in a compact gel network. Therefore, one may hypothesize that adequate sealing of the root canal system can be accomplished with this compact gel-like framework. Furthermore, algin can form strong intermolecular cross-linking irrespective of temperature with shorter setting time which is of prime interest in the current clinical practice as this will further enhance the intermolecular cohesiveness, preventing the dislodgement of material from the root canal walls and making it operator-friendly due to its fast setting (Ching *et al.*, 2017). Instead of shrinking, alginates display slight expansion owing to their hydrophilic functional groups (Lee and Mooney, 2012), and one can postulate that this slight expansion can compensate for the voids that exist in the root canal system, thus giving a tight seal.

It would thus be of clinical interest to combine the desired biological effects of both BG 58S and calcium silicate with relatively low-cost natural biopolymer alginate to form a strong hybrid root canal sealer which has the potential not only to provide excellent bioactivity, biocompatibility, but also high bond strength with strong cohesive force between the molecules to resist dislodgment from root canal walls. This novel sealer is expected to provide a three-dimensional tight seal in the root canals and further improve the clinical performance of root canal treated teeth.

1.2 Problem statement and justification of the study

An ideal root canal sealer should not only possess excellent biocompatibility and bioactivity with apatite-like structure formation, but also requires a strong bonding with the root canal walls to prevent dislodgement through gap formation at the sealer-wall interface. Inferior bond strength and dentinal tubule penetration of root canal sealer could lead to bacterial reinfection and treatment failure. However, the currently available bioceramic sealers demonstrated low bond strength with a poor seal in the root canals despite their desirable biological properties (Carvalho *et al.*, 2017; Dem *et al.*, 2019; Pawar *et al.*, 2014). For instance, bioceramic-based sealers, such as BioRoot RCS, demonstrated lower bond strength than epoxy resin-based sealer (Donnermeyer *et al.*, 2019). Similarly, Amin SA *et al*. (2012) found that the bond strength of resin-based sealer was higher than iRoot SP and MTA Fillapex (Angelus Indústria de Produtos Odontológicos S/A, Londrina, Brasil) (Amin *et al.*, 2012).

Most calcium silicate bioceramic-based sealers were found to exhibit poor cohesiveness between the molecule particles with a long setting time (Donnermeyer *et al.*, 2018). Also, the dentinal tubule penetration of bioceramic-based sealers was found to be sparse with mixed results when compared to other types of sealer (Arikatla *et al.*,

2018; El Hachem *et al.*, 2019; Kim *et al.*, 2019). A previous study showed more sealer penetration in epoxy resin-based sealer than MTA Plus (Avalon Biom Inc., Bradenton, FL, USA) and BioRoot RCS (Arikatla *et al.*, 2018). Such a finding is also in line with that published by Yang *et al*. (2021) suggesting that resin-based sealer showed greater sealer penetration depth than iRoot SP (Yang *et al.*, 2021). Moreover, the currently available BG bioceramic-based sealer, GuttaFlow BioSeal, consists of nano silver particles which have raised concerns among clinicians regarding their cytotoxicity.

A new biomaterial with acceptable biological properties, strong bonding to dentinal walls and excellent sealer penetration depth should be developed. It can be anticipated that the incorporation of eco-friendly algin into bioceramic-based root canal sealer to form a novel organic-inorganic hybrid root canal sealer (Bio-G) is able to demonstrate better adhesion to root dentine walls with lesser heavy metals and the absence of silver particles and resin monomers. This study will also give rise to a new generation of root canal sealer and provide novel insight and understanding of algin incorporated Bio-G sealer, which could be an alternative to replace currently available bioceramic-based root canal sealers.

Furthermore, the introduction of algin may further enhance the intermolecular network resulting in a strong cohesion bonding to resist dislodgement, thereby achieving a tight seal, and preventing bacterial reinvasion into the root canal system. As a result, prompt periapical tissue healing can be foreseen in patients receiving root canal treatment with the novel Bio-G sealer and potentially improve the patients' quality of life.

1.3 Research objectives

1.3.1 General objective

To synthesise and characterize novel algin incorporated bioactive glass 58S calcium-silicate root canal sealer (Bio-G) and evaluate its properties.

1.3.2 Specific objectives

1. To synthesise BG 58S powder using a one-pot sol-gel method.
2. To characterise BG 58S using scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HRTEM), Fourier transform infrared spectroscopy (FTIR), and x-ray diffractometer (XRD).
3. To fabricate the powder and liquid forms of novel Bio-G root canal sealer containing different weightage of algin in its composition.
4. To characterise and compare the particle size and surface morphology of novel Bio-G sealer containing different algin weightage with commercially available bioceramic-based sealers, namely BioRoot RCS and iRoot SP, using SEM and HRTEM.
5. To identify and compare the elemental content, chemical and phase composition of novel Bio-G sealer containing different algin weightage with BioRoot RCS and iRoot SP using energy dispersive spectroscopy (EDS), FTIR, and XRD.
6. To evaluate and compare the dislodgement resistance of novel Bio-G sealer containing different algin weightage with commercialised BioRoot RCS and iRoot SP using push-out bond strength test.
7. To compare and classify the adhesive pattern of novel Bio-G sealer containing different algin weightage with commercialised BioRoot RCS and iRoot SP after push-out bond strength test.

8. To determine and compare the dentinal tubule penetration of novel Bio-G sealer containing different algin weightage with commercialised BioRoot RCS and iRoot SP using confocal laser scanning microscope (CLSM).

1.4 Research questions

1. Can BG 58S be successfully synthesised through one-pot sol-gel method?
2. Can synthesised BG 58S be characterised using SEM, HRTEM, FTIR and XRD?
3. Can the powder and liquid forms of novel Bio-G sealer containing different algin weightage be successfully fabricated?
4. Is there any difference in the particle size and surface morphology of novel Bio-G sealer containing different algin weightage when compared to commercialised BioRoot RCS and iRoot SP using SEM and HRTEM?
5. Is there any difference in the element content, chemical and phase composition of novel Bio-G sealer containing different algin weightage when compared to commercialised BioRoot RCS and iRoot SP using EDS, FTIR and XRD?
6. Is there any difference in terms of dislodgement resistance of novel Bio-G sealer containing different algin weightage as compared to BioRoot RCS and iRoot SP using push-out bond strength test?
7. Is there any difference in terms of adhesive patterns of novel Bio-G sealer containing different algin weightage as compared to BioRoot RCS and iRoot SP?
8. Is there any difference in terms of dentinal tubule penetration depth of novel Bio-G sealer containing different algin weightage as compared to BioRoot RCS and iRoot SP using CLSM?

1.5 Research null hypotheses

1. BG 58S can be successfully synthesised through one-pot sol-gel method.
2. BG 58S can be characterised using SEM, HRTEM, FTIR and XRD.
3. The powder and liquid forms of novel Bio-G sealer containing different algin weightage can be successfully fabricated.
4. Novel Bio-G sealer does not consist of homogeneous aggregates of micro to nano-sized particles which are not comparable to BioRoot RCS and iRoot SP under SEM and HRTEM.
5. Novel Bio-G sealer does not contain similar elemental content, chemical and phase compositions with those of BioRoot RCS and iRoot SP under EDS, FTIR and XRD.
6. There is no significant difference in terms of dislodgement resistance to root dentinal walls between novel Bio-G sealer and other commercialised bioceramic-based sealers, namely BioRoot RCS and iRoot SP.
7. There is no significant difference in the adhesive pattern between novel Bio-G sealer and other commercialised bioceramic-based sealers, namely BioRoot RCS and iRoot SP.
8. There is no significant difference in terms of mean and maximum dentinal tubule penetration depths between novel Bio-G sealer and other commercialised bioceramic-based sealers, namely BioRoot RCS and iRoot SP.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction chapter

This chapter highlights the current evidence in the literature that forms the foundation of the present study. It starts with an introduction to ceramics, followed by the history of ceramics. This chapter then emphasises on bioceramics with their common classifications. Evidence on bioactive glass and calcium silicate was also analysed in the current chapter. Under the heading of bioactive glass, specific type of bioactive glass, BG 58S, and the synthesis method of bioactive glass were critically reviewed.

The next heading exposed the general characteristics and properties of ceramics, as well as the structural characterisations of bioceramics, such as X-ray diffraction, infrared spectroscopy, scanning electron microscopy and transmission electron microscopy. Next, the literature on porous bioceramics was reviewed, followed by the biomedical and dental applications of ceramics. The chapter then introduced the applications of bioceramics in endodontics, as well as their ideal physico-chemical and biological properties in endodontic applications.

In the subsequent heading, the chapter highlights root canal sealers and explored in detail bioceramic-based sealers and the uses of bioactive glass in root canal sealers. After that, a literature review on algin and the organic-inorganic natural hydrogel polymer from algin was introduced. This is followed by the roles of alginate in biomedical and endodontic applications. Finally, the chapter ends with summarising the current evidence on the dislodgement resistance, failure pattern and dentinal tubule penetration of different bioceramic-based root canal sealers.

2.2 Bioceramics

A non-viable substance used in a medical device that interacts with living processes is called biomaterial (Vallet-Regí, 2010). Biomaterial can also be defined as "any substance, other than a drug, or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system, which treats, augments, or replaces any tissue, organ, or function of the body", as per the definition put forth at the Conference of the European Society for Biomaterials in 1993 (Biomaterials, 1993). Therefore, ceramics used in medical, dental or biological applications on the human body are categorised as "bioceramics" (Huang and Best, 2014).

Furthermore, a bioceramic can also be referred to as a biomaterial that is composed of polycrystalline materials that exhibit distinctive hardness, brittleness, resilience, stiffness, corrosion, and wear resistance, as well as low density (Huang and Best, 2007). Even though research is being conducted to enhance the properties of bioceramics, its definition is still up for debate. The majority of clinical uses for bioceramics include repairing the skeletal system, which includes the bones, joints, and teeth, as well as enhancing both hard and soft tissue (Huang and Best, 2014).

One of the most significant bioceramics is aluminium oxide, which was initially viewed as a component of many conventional ceramic products (Carter and Norton, 2013a). Porcelain was the first bioceramic material that was used as dental crown in the 1800s. Thereafter, in the early 1900s, Plaster of Paris was used in the field of dentistry (Taira *et al.*, 1989). Although alumina ceramics were suggested for use in medicine in 1932, the area of bioceramics did not truly take off until the 1970s with the introduction of the first hip implants made of alumina balls and cups (Carter and Norton, 2013d).

2.3 Classifications

Ceramics were previously classified as traditional and advanced ceramics (Carter and Norton, 2013c). Clay and silica are examples of traditional ceramics. Special, technical, or engineering-driven ceramics are classified as advanced ceramics. They have superior physical and mechanical properties. Advanced ceramics have only been produced in the last 100 years, in contrast to traditional ceramics, which have been used for over 25000 years.

2.3.1 Classifications of bioceramics

2.3.1(a) Classification based on tissue reaction

Currently available bioceramics come in three specific types which are bioinert, bioactive, and bioresorbable (Huang and Best, 2014; Washio *et al.*, 2019). Bioinert bioceramics are those that do not cause any tissue responses when they interact with a physiological system. Bioinert ceramics have high chemical stability, superior mechanical strength, and are able to initiate contact reaction with the bone tissue when they are implanted in the living bone which is known as "contact osteogenesis" (Yamamuro, 2004).

Alumina and zirconia are examples of bioinert ceramics. However, zirconia ceramics offer stronger flexural strength, higher fracture toughness, and a slightly lower Young's modulus than alumina ceramics (Huang and Best, 2014). Zirconia ceramics can be either yttria-stabilized (Y-TZP) or partial magnesium oxide-stabilized (Mg-PSZ). Although alumina and zirconia ceramics have considerable mechanical strength and acceptable biocompatibility, they are not directly bonded to host tissue and are mostly inert (Best *et al.*, 2008).

Bioactive ceramics, on the other hand, have the ability to cause osteoconduction and form chemical bonding with bone tissue, which is known as "bonding osteogenesis" (Yamamuro, 2004). A variety of bioactive ceramics, including glasses and glass ceramics, can promote the development of bone at their surfaces and produce an interface that prolongs the functional life of tissue. Examples of bioactive ceramics are hydroxyapatite (HA), BG, and other glass-ceramics (Vallet-Regí, 2010).

The bioactive bioceramics currently in use are either biological or synthetic. Biological-origin bioceramics include natural coral, HA, bovine bone apatite or marine algae-derived apatite. Synthetic-origin bioceramics encompass silica-based or phosphate-based glass, and calcium sulphate or plaster of Paris (LeGeros *et al.*, 2008). Biodegradable ceramics are ceramics that are progressively absorbed and replaced by bone tissue (Tan *et al.*, 2013). They also show similar patterns to contact osteogenesis, but the interaction between bioresorbable ceramics and the bone is not as stable as that of bioinert ceramics (Yamamuro, 2004). Calcium phosphate and calcium silicate are examples of bioresorbable ceramics. Nonetheless, overlapping classifications among bioactive and biodegradable-based bioceramics still exists in the literature (Salinas and Vallet-Regí, 2013).

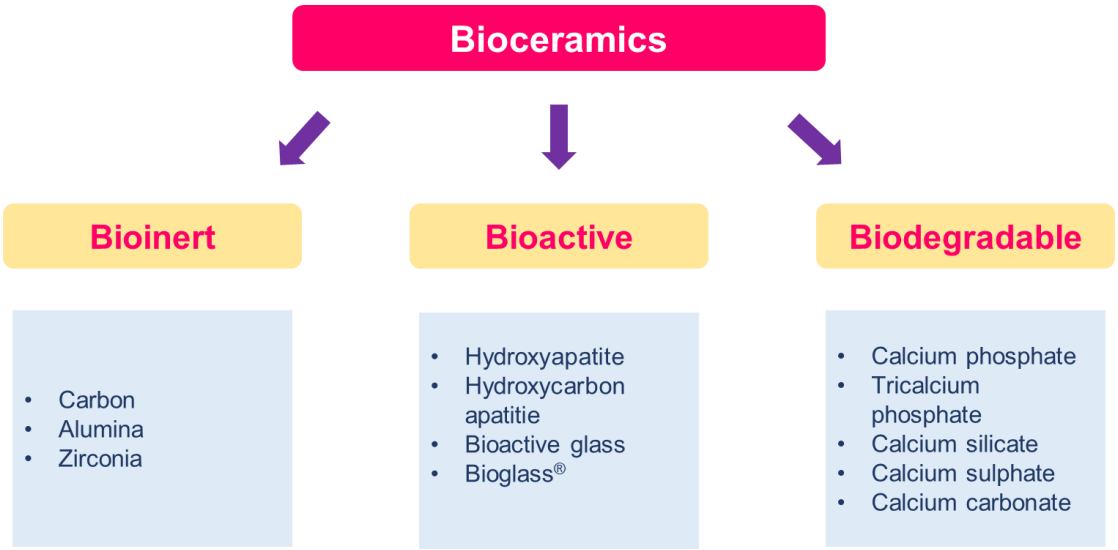

Figure 2.1 Classification of bioceramics into bioinert, bioactive and biodegradable (Yamamuro, 2004)

2.3.1(b) Classification based on generation

Some researchers classified bioceramics into different generations (Daculsi *et al.*, 2003). Alumina and zirconia are examples of the first generation of bioceramics. Their excellent mechanical properties, especially high wear resistance, were the essential factors of first-generation bioceramics (Best *et al.*, 2008; Skallevold *et al.*, 2019). BG, HA, and calcium phosphate-based cement formed the second generation of bioceramics. Second generation bioceramics can bond and integrate with the surrounding living bones without creating fibrous tissue, inflammation, or toxicity (Skallevold *et al.*, 2019; Washio *et al.*, 2019).

In addition, the second generation also features bioresorbable biomaterials that have the potential to dissolve when tissues are regenerated and recovered (Daculsi *et al.*, 2003). What makes second-generation bioceramics so special is that BG has sparked a revolution in healthcare equipment and paved the way for new medicine powered by biomaterials (Jones *et al.*, 2016). Finally, the third generation of bioceramics utilized

both bioactive and bioresorbable materials to form a temporary three-dimensional porous scaffold that can stimulate tissue regeneration (Vallet-Regí, 2010). The ultimate purpose of the third generation of bioceramics is to provide an organizing framework of scaffolding that allows living cells to undergo their natural processes.

In short, the first generation of inert bioceramics intended to serve as artificial bone grafts, whereas the second generation of both bioactive and bioresorbable bioceramics was designed to replicate certain biomineralization-related roles. The third generation of bioceramics is designed to provide a sufficient scaffolding structure to help bone cells accomplish their restorative activities (Vallet-Regí, 2014).

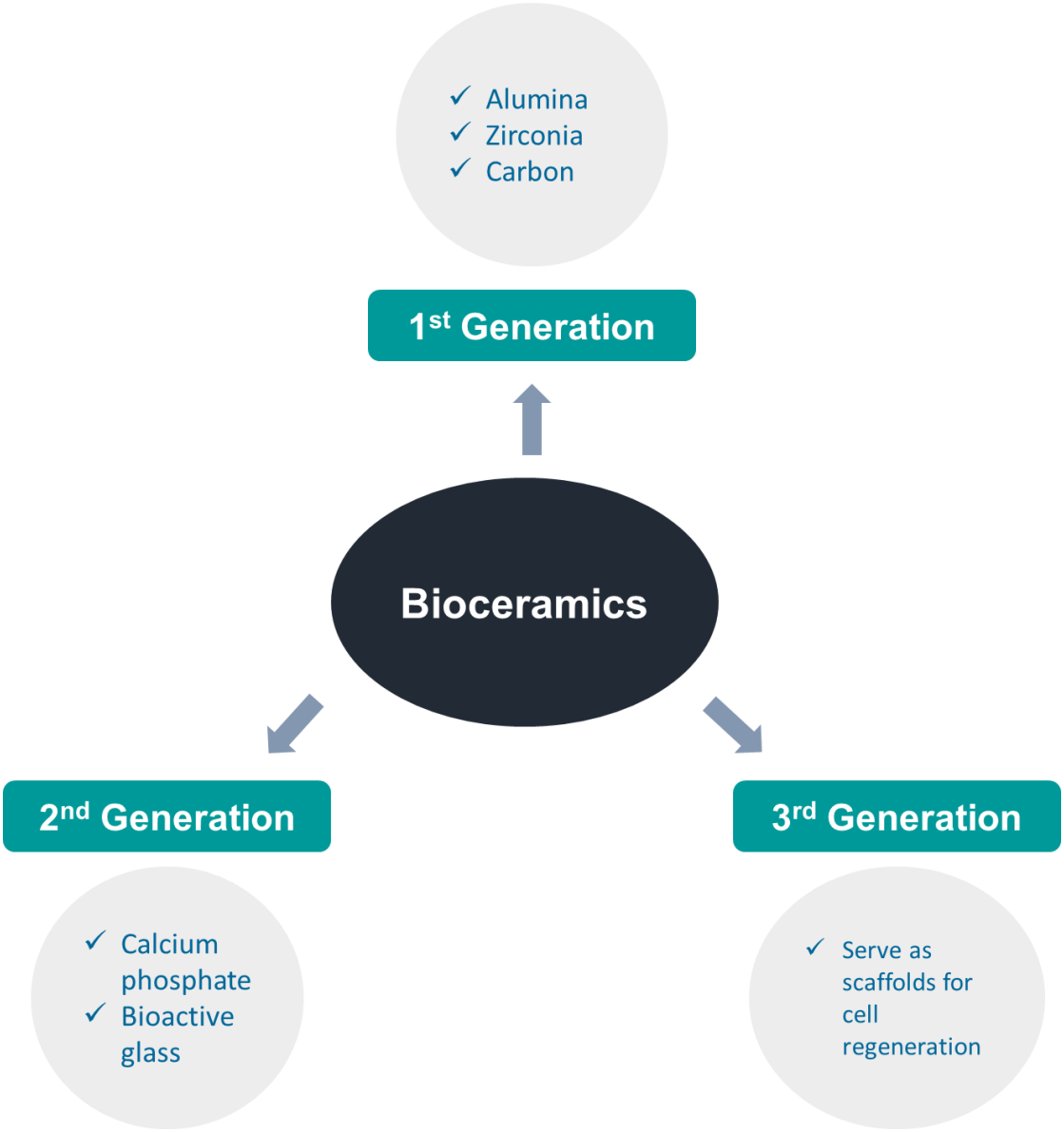

Figure 2.2 Classification of bioceramics according to different generations (Daculsi *et al.*, 2003)

Moreover, bioceramics have been classified based on their origin (natural or synthetic), composition (alumina-based, zirconium-based, carbon-based, calcium phosphate-based, silica-based), and crystallinity (crystalline or amorphous) (Gul *et al.*, 2020).

2.3.1(c) Classification based on origin

"Natural bioceramics" are bioceramics produced naturally and derived from diverse living or dead creatures, such as biogenic silica, natural pearls, molluscs shells, bones, and teeth. Meanwhile, "Synthetic bioceramics" are created artificially which include calcium phosphate-based materials, HA, zirconia, alumina, BG etc. (Brundavanam *et al.*, 2017).

2.3.1(d) Classification based on composition

Alumina-based ceramics are brittle solids that break under direct force and exhibit lower hardness than diamonds. However, it has great compressive strength and outstanding dimensional stability under heat, pressure, and shock (Boutin, 2014). Aluminium-based ceramics have undergone several forms of improvement such as leucite-reinforced, glass-infiltrated, sintered alumina, and high-purity alumina ceramic cores (Chaar *et al.*, 2015).

On the other hand, zirconia is a type of polycrystalline ceramic that does not include glass and has all its atoms arranged in regular crystalline arrays. At various temperatures, zirconia crystals can form either monoclinic, cubic, or tetragonal phase (Miyazaki *et al.*, 2013). When zirconia is fired, it is in the tetragonal phase, which transforms into the monoclinic phase upon cooling to room temperature (Badami and Ahuja, 2014). It has mechanical characteristics similar to those of stainless steel and has a high stress tolerance (Abd El-Ghany and Sherief, 2016). Besides, zirconia ceramic shows high strength, improved aesthetics, and excellent biocompatibility.

Carbon-based bioceramics compose of elements such as diamond, graphite, vitreous carbon, amorphous carbon, and pyrolytic carbon. Graphene, carbon nanotubes, diamond-like carbon, and turbo-static carbons are among the carbon types used in biomedical equipment (Venkatesan *et al.*, 2014). Among the different carbon allotropes, graphene is considered a novel nanomaterial whose potential applications are being investigated in the fields of biomedical sciences, and biotechnology (Li *et al.*, 2018; Saghatforoush *et al.*, 2014).

Another form of bioceramic is calcium phosphate-based which is analogous to the inorganic component of teeth and bones (Pina and Ferreira, 2012). Although calcium phosphates tend to form chemical bonds with bones and surrounding tissues, they also help to stabilise mechanical load-bearing materials. In the fields of orthopaedics and dentistry, calcium phosphates are widely utilised for bone tissue engineering (Habraken *et al.*, 2016). The most widely utilised calcium phosphate-based bioceramics are HA and β-tricalcium phosphate (β-TCP).

HA derived from calcium phosphates having calcium and phosphate atomic ratios between 1.5 and 1.67 (Eliaz and Metoki, 2017). HA is a durable and biocompatible bioceramic material that can promote osteointegration (Pina and Ferreira, 2012). Over time, β-TCP will resorb and facilitate osteogenesis. Unfortunately, β-TCP is not suitable for load-bearing applications due to its poor mechanical strength (Shi *et al.*, 2018). However, when combined with bioinert polymers, it creates a composite that speeds up implant attachment while also facilitating chemical interaction with bone (Huang and Best, 2014; Pina and Ferreira, 2012).

Calcium silicates such as calcium silicate glass, β-calcium silicate, α-calcium silicate, dicalcium silicates, and tricalcium silicates are silica-based bioceramics that could chemically integrate with bony structures and facilitate prompt apatite production in physiological fluid (Li *et al.*, 2014). MTA is an example of calcium silicate-based bioceramics composed mainly of calcium silicate, calcium oxide, tricalcium aluminate, and silicate oxide (Ha *et al.*, 2017). During hydrolysis, MTA will produce calcium hydroxide and calcium silicate hydrate.

Aluminosilicate glasses are another sub-class of calcium silicate-based bioceramics that have refractive indices of 1.65 with moderate coefficients of thermal expansion, and extremely high glass transition temperatures (Huang and Best, 2014). Glass ionomer cement (GIC), a type of dental restorative material is an example of aluminosilicate glass bioceramic (Hatrick *et al.*, 2011). Moreover, another sub-class of calcium silicate-based bioceramics is silicon nitride. Due to its distinctive microstructure, which consists of a thin layer of refractory glass grain boundary, it demonstrates outstanding strength, toughness, and crack propagation limitation potentials (McEntire *et al.*, 2015).

2.3.1(e) Classification based on crystallinity

"Crystalline bioceramics" are bioceramics that have a lattice structure and are produced by the crystallisation process, such as HA. On the other hand, “amorphous bioceramics”, such as bioactive glass, are bioceramics without a lattice structure (Gul *et al.*, 2020). However, crystalline BG may be undesirable due to its lower bioactivity (Ghaebi Panah *et al.*, 2021).

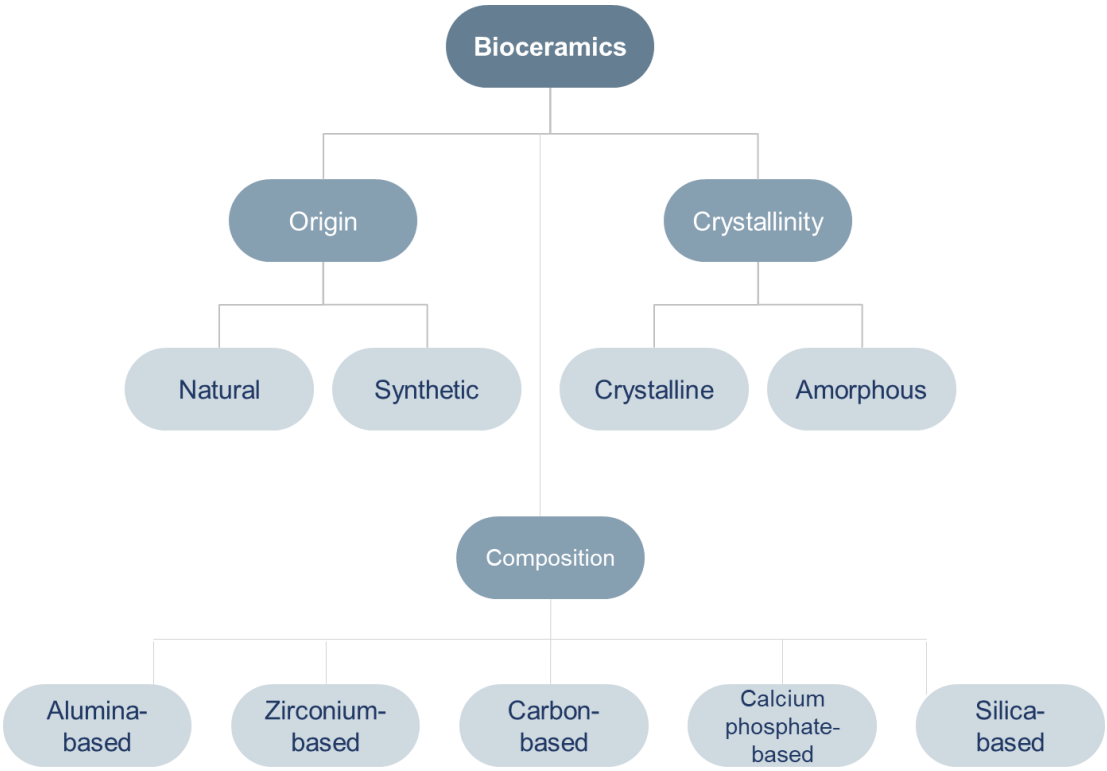

Figure 2.3 Classification of bioceramics according to different origins, compositions and crystallinities (Gul *et al.*, 2020)

2.3.2 Bioactive glass (BG)

BGs are amorphous silicate-based substances that may bond to bones and encourage the development of new bones while gradually disintegrating in the body (Jones, 2008). BG is a type of bioceramic that has been the subject of extensive research attention in dental biomaterials. The first bioactive glass was discovered in 1969 at the University of Florida by Professor Larry Hench (Akademi, 1969; Hench, 2006; Jones, 2013). Professor Hench started his research to find a material that could adhere to the bone, but the challenge was that after implantation, all implant materials available at that moment, activated fibrous encapsulation rather than creating a stable interface or tissue bond (Hench, 2006; Jones, 2013).

Professor Hench then discovered a degradable glass with the composition of 46.1 mol.% SiO_2, 24.4 mol.% sodium oxide (Na_2O), 26.9 mol.% CaO and 2.6 mol.% P_2O_5, which later termed as BG 45S5 or Bioglass® (Hench, 2006). BG 45S5 is a silica-based glass that can form strong bonding with the surrounding bone that could not be removed without fracturing them (Izquierdo-Barba *et al.*, 2013; Rahaman, 2014). Replacement of the ossicles (small bones in the middle ear) was the first area in which BG was successfully used. Furthermore, BG 45S5 was claimed to be able to bond with bone tissue well and promote bone development (Hench and Paschall, 1973).

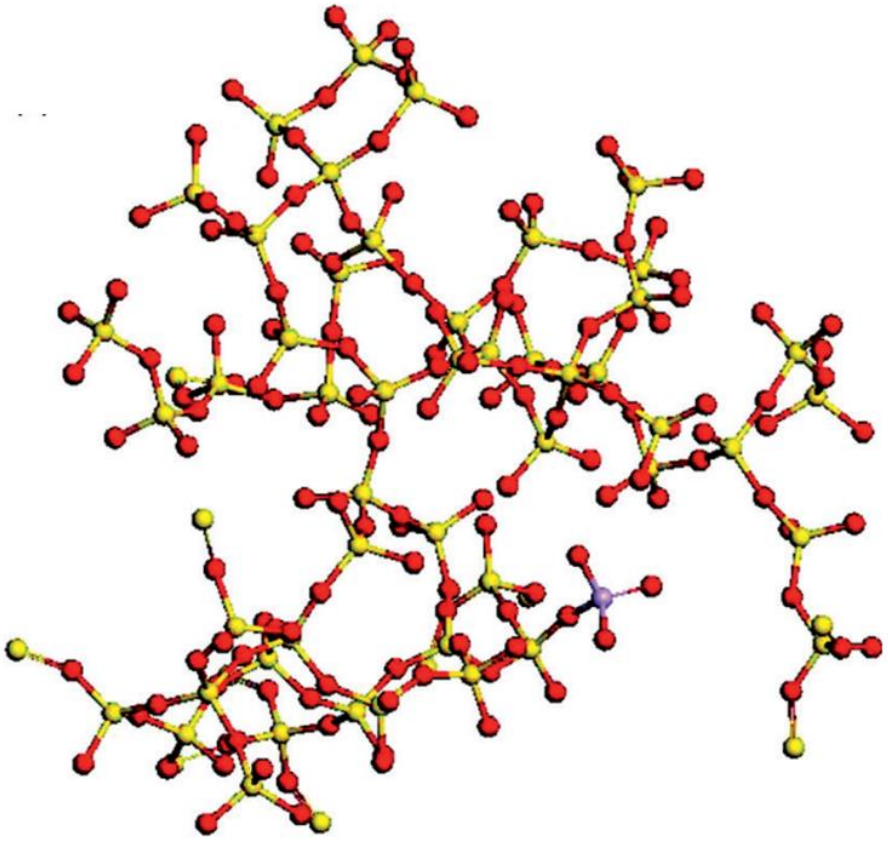

Figure 2.4 Structure of BG 45S5. Image adapted from (Brauer, 2015)

Although BG 45S5 is still the gold standard among all BG types, it has certain drawbacks as a scaffold biomaterial (Rahaman, 2014). The challenge of processing BG 45S5 glass into porous 3-dimensional scaffolds by sintering constructions made of particles is one limitation. As a result, the scaffold frequently has low strength, which led to the invention of advanced BG particles, such as BG 58S and BG 6P53B (Bui and Dang, 2019; Yang, 2018). All existing BGs at the moment are silicates, and all new BGs are based on the same four elements found in BG 45S5 (Carter and Norton, 2013a).

Composition (wt%)	45S5	13-93	6P53B	58S	70S30C	13-93B1	13-93B3	$P_{50}C_{35}N_{15}$
Na_2O	24.5	6.0	10.3	0	0	5.8	5.5	9.3
K_2O	0	12.0	2.8	0	0	11.7	11.1	0
MgO	0	5.0	10.2	0	0	4.9	4.6	0
CaO	24.5	20.0	18.0	32.6	28.6	19.5	18.5	19.7
SiO_2	45.0	53	52.7	58.2	71.4	34.4	0	0
P_2O_5	6.0	4.0	6.0	9.2	0	3.8	3.7	71.0
B_2O_3	0	0	0	0	0	19.9	56.6	0

Figure 2.5 Examples of BGs in different compositions. Image adapted from (Rahaman, 2014)

BGs feature a low density, random two-dimensional sheet-like structure due to the low concentration of silicon dioxide (Brauer, 2015). BG has a poor fracture toughness and is mechanically fragile owing to its glass structure (Baino, 2018). To create a homogenous BG melt, the component oxides are combined in the appropriate ratios and heated to high temperatures to form glass after cooling (Fernandes *et al.*, 2018). After the initial glass dissolution, the process of bone bonding is due to the formation of hydroxycarbonate apatite (HCA) coating on the surface of the glass (Jones, 2008).

HCA is analogous to bone minerals and interacts with the collagen fibrils to integrate with the bone tissue (Hench *et al.*, 1971). In short, bioactive material is described as a material that has the ability to stimulate bonding to the host bone tissue, whereas bioceramic is a term that includes glasses, glass–ceramics and ceramics. 'Bioglass®' was a trademark for the original BG 45S5 composition by the University of Florida, and therefore, it can be used only in regard to the composition of 45S5 instead of generic term for BGs (Jones, 2013). It is worth noting that Bioglass® development has sparked the creation of other novel glass and glass ceramic compositions.

BG-based ceramics are primarily amorphous materials that only exhibit short-range regularity because the atoms do not have enough time to arrange themselves into a periodic pattern during the quick cooling process that creates these glasses (Huang and Best, 2014). BG particles differ from conventional soda-lime silica glasses in three ways: SiO_2 concentration of less than 60%, high levels of Na_2O and CaO, and a high $CaO:P_2O_5$ ratio (Hench, 2006). Generally, the rapid surface reactivity and capacity to alter chemical composition to enable bonding with a range of tissues, are among the advantages of using BG particles. Nonetheless, their low Young's modulus and bending strength are drawbacks of BG (Huang and Best, 2014).

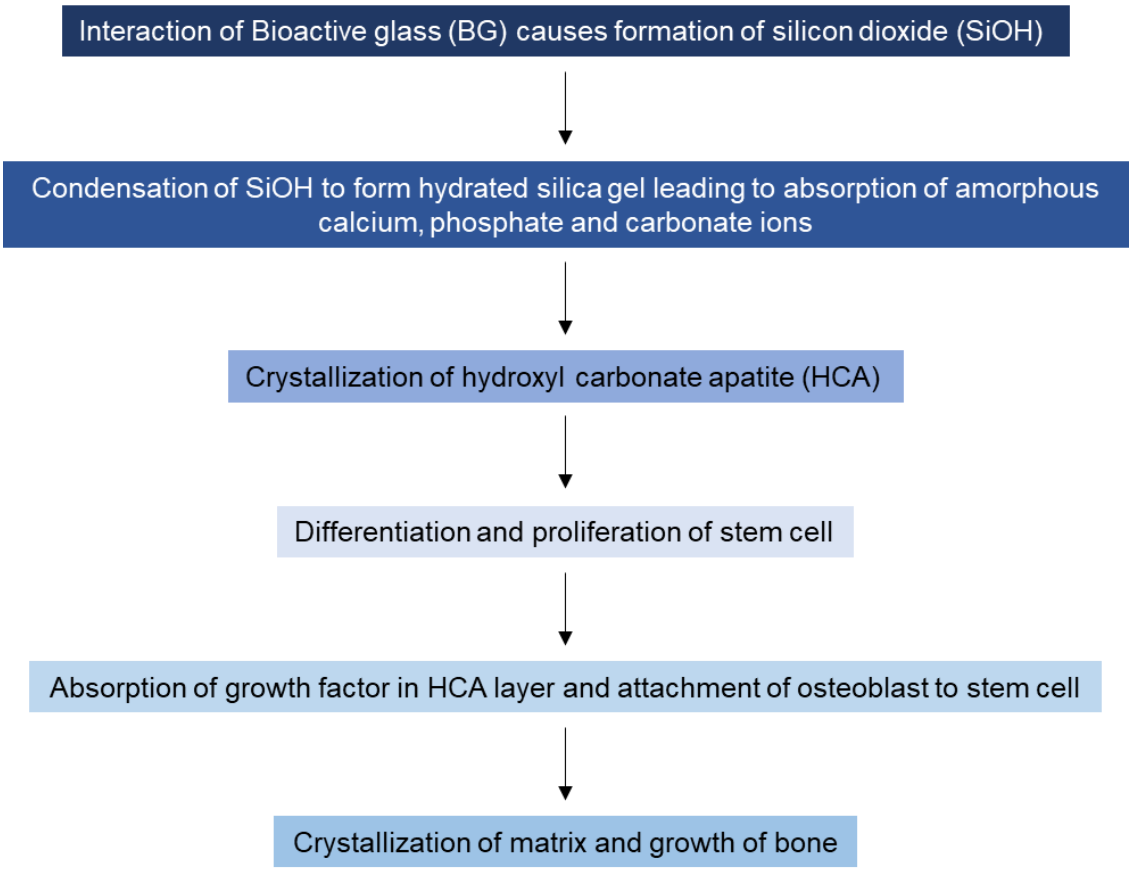

Figure 2.6 Reaction between bioactive glass and bone interface

Different BG compositions have been established with a focused application in bone and tissue regeneration since the discovery of Bioglass®. The osteogenic properties are primarily attributed to the release of silicate (Si^{4+}) and Ca^{2+} ions, which serve as triggers for osteogenic gene expression activation and a spur for osteoblast metabolism and homeostasis of the bone (Jones *et al.*, 2016). Although such properties were achieved across several BG formulations, those containing CaO above 25 mol %, such as 45S5 Bioglass®, S53P4, or 58S, are the most commercially used for bone and tissue regeneration since greater calcium content induces greater mineralization of the cells (Izquierdo-Barba *et al.*, 2013; Jones, 2013).