# Chapter 4
# Implant Surface Modifications and Osseointegration

**Nishant Chakravorty, Anjali Jaiprakash, Saso Ivanovski, and Yin Xiao**

**Abstract** Osseointegration and osteogenic differentiation are important determinants of clinical outcomes involving implants in orthopaedics and dentistry. Implant surface microstructure and hydrophilicity are known to influence these properties. Recent research has focused on several modifications of surface topography and chemistry aimed at improving bone formation to achieve faster and better healing. Topographically modified titanium implant surfaces, like the sandblasted, large-grit, acid-etched (SLA) surface and chemically modified hydrophilic SLA (modSLA) surface, have shown promising results when compared with smooth/polished titanium surfaces. Although most studies consider an average roughness (Ra) of 1–1.5 μm to be favourable for bone formation, there is no consensus regarding the appropriate roughness and chemical modifications necessary to achieve optimal osseointegration. Studies on microstructurally modified surfaces have revealed intricate details pertaining to the molecular interactions of osteogenic cells with implant surfaces. The in vivo and in vitro findings from these studies highlight the ability of modified titanium surfaces to support the establishment of a native osteogenic niche for promoting bone formation on the implant surfaces. Improved osteogenic properties of modified surfaces are evidenced in vitro by the differential regulation of the molecular transcriptome on such surfaces. Recent studies indicate that post-transcriptional modulators like microRNAs also play an important role in osteogenic regulation on implant surfaces. In this chapter, we discuss the current concepts and considerations in orthopaedic and dental implant research and the new knowledge in the field, which will assist in the development of novel approaches and designs of future implant devices.

**Keywords** Implants • Osseointegration • Surface modification • Surface topography • Cell signaling • Osteogenic differentiation • Molecular regulation

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## 4.1 Introduction

Orthopaedic and dental implants have become important treatment options for replacement and restoration of missing/damaged parts of bones and teeth. The science of orthopaedic and dental implants has come a long way since its beginning which possibly dates back to around 600 AD when the Mayan civilisation started using seashells and stones as endo-osseous implants. Implant structures and surfaces have subsequently undergone a multitude of modifications. The quest for superior clinical outcomes has led researchers to a continually evolving search for the ideal implantation material. The choice of materials to be used as implants depends on several properties, such as mechanical stability, elasticity, biocompatibility, hydrophilicity, corrosivity, etc. Several materials have been used as implants for the human body. The ready availability and established processing methods initially made stainless steel a popular material for orthopaedic implants. The progressive search for improved implants led to the use of alloys made out of iron and other metals like nickel, cobalt and molybdenum. Titanium with its significant potential to support bone regeneration with little evidence of rejection has been the material of choice clinically for a long time now.

The primary aim of implant research is to create materials with functional designs that serve the purpose of achieving structural and functional restoration of a body part, and in the context of bone integration, we need to understand the phenomenon known as osseointegration. The term 'osseointegration' was coined by the Swedish orthopaedic surgeon Per-Ingvar Brånemark in the 1950s, when he observed difficulty in the removal of titanium chambers implanted in animals for vital microscopic studies [1]. Osseointegration may be defined as the structural and functional amalgamation of the load-bearing surface of an implant with the surrounding bone tissue. Osseointegration is a biological process guided by a highly regulated cascade of molecular steps that lead to osteogenic differentiation and new bone formation on the implant surfaces. Several research studies have emphasised the importance of surface topography on the bone-to-implant contact [2–6]. The present chapter discusses the current knowledge and concepts pertaining to the surface topography of implants, with a focus on modified titanium implant surfaces as a model to understand the mechanisms of osteogenic differentiation and osseointegration.

## 4.2 Discovery of Osseointegration

A chapter on osseointegration without a tribute to Professor Per-Ingvar Brånemark and his pioneering work with the implant material titanium would be an injustice to the topic itself. Titanium was first identified as an element in 1791 by Revd. William Gregor when he was examining samples of black sands sent to him from the valley of Manaccan located in the Lizard peninsula, Cornwall, in England

[7]. Based on his experiments, Gregor was able to identify an unknown metallic substance in the samples; however, he was unable to reduce it to its metallic form. He had originally named it as 'manaccanite' after the region from where he had identified the element. Later in 1795, Martin Heinrich Klaproth from Germany isolated a metal from an abundant ore called 'rutile' and named this metal as 'titanium'. Klaproth also analysed the new element Gregor had identified and found it to be the same as titanium and recognised Gregor as the scientist who discovered the new metal. Subsequently, scientists realised the properties and significance of this material, and it is now widely utilised in various areas like aircrafts, naval ships, spacecrafts, missiles, jewellery, etc.

After one-and-a-half century of the discovery of titanium, its importance as an implant material was discovered by Professor Per-Ingvar Brånemark. During the 1950s and 1960s, Brånemark was studying the formation of blood vessels in the bone marrow using in vivo models. Micro-optical devices enveloped in titanium cases were incorporated into tissues to observe the human microcirculation. He subsequently observed extreme difficulty in removal of these devices. These were the initial observations that led to the discovery of the phenomenon of osseointegration. A modified experimental setup for the rabbit ear chamber was subsequently conducted, whereby a titanium implant with a central canal and a transverse opening was placed into the bone to enable the bone and blood vessels to grow into the chamber [1]. These observations led to the findings that the integration of titanium with live tissue could enable a prolonged fixation of dental prostheses and eventually unfolded the path to the magnanimous clinical success of titanium for dental implants and reconstructive surgeries.

On 9 April 2002 the International Society of Osseointegration and the Associated Brånemark Osseointegration Centers (ABOC) visited Manaccan and unveiled a plaque made of titanium commemorating the discovery of titanium and recognising the scientific achievements of the two great scientists – Revd. William Gregor and Professor Per-Ingvar Brånemark.

## 4.3 Titanium and Its Modifications

Titanium is the 22nd element in the periodic table and has an atomic mass of 47.867 and its symbol is Ti (Fig. 4.1). It is classified in group 4 (transition elements) of the periodic table along with zirconium, hafnium and rutherfordium. The element is strong and lustrous with a metallic silver shade. It is usually found in the form of its ores: rutile ($TiO_2$), anatase ($TiO_2$) and ilmenite (Fe, $TiO_3$). Ilmenite is the most abundant form of titanium. Four grades of commercially pure titanium and two alloys of titanium are recognised by the American Society for Testing and Materials (ASTM) Committee F04 on Materials for Surgical Implants. Commercially pure titanium is graded as between I and IV; and the ASTM recognised that titanium alloys are Ti-6Hl-4 V and Ti-6Hl-4 V extra low interstitial (ELI) [8]. Essential

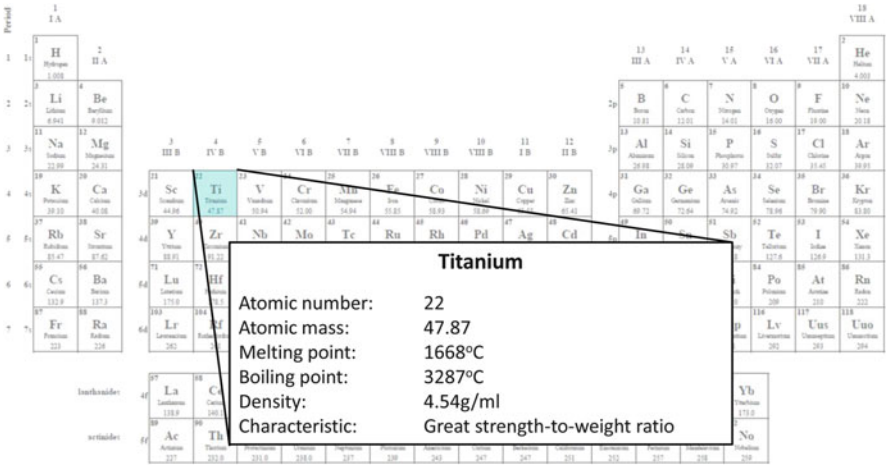


**Fig. 4.1** Titanium – the element of choice for orthopaedic and dental implants

qualities that make titanium a highly compatible material in the field of implantology include:

1. High strength-to-weight ratio – titanium is 60 % the density of steel but has tensile strengths reaching as high as 63,000 psi (434.4 MPa).
2. High melting point (1668 °C) – commercial titanium alloys have the capability of safely withstanding temperatures of up to 600 °C.
3. Resistance to corrosion – usually due to the formation of a titanium dioxide layer on the surface.
4. Ability to be passivated and therefore be resistant to corrosion with acids.
5. Inert to body fluids.
6. Relatively low modulus of elasticity akin to human bone.
7. Ability to osseointegrate.

Titanium has certain shortcomings like low resistance to wear and notch sensitivity. However, its advantages outweigh the disadvantages, and therefore it is considered one of the most suitable elements for implants.

### *4.3.1 Surface Modifications*

Implant research has focused on technologies to improve osteoinduction (ability to induce differentiation of undifferentiated cells towards osteogenic lineage), osteoconduction (allowing growth of bone on the surface of the implant) and osseointegration [9]. Surface modification techniques have been an integral part of implant research. Implant surface quality has been considered one of the most important factors implicated in successful osseointegration [3]. The most common

parameters used in describing surface roughness are $R_a$, $R_q$, $R_z$ and $R_t$ (in - two-dimensional) and their three-dimensional counterparts: $S_a$, $S_q$, $S_z$ and $S_t$.

$R_a/S_a$ Arithmetic mean deviation (average roughness) of the roughness profile ($R_a$) or of a surface ($S_a$ in 3D)

$R_q/S_q$ Root mean square deviation of profile ($R_q$) or of a surface ($S_q$ in 3D)

$R_z/S_z$ Arithmetic mean deviation of the sum total of 10 (5 of the lowest peaks and 5 of the highest peaks) of the profile ($R_z$) or the surface ($S_z$)

Several different methods are employed to alter surface topographies. Some of the most common techniques include polishing, surface blasting, plasma spraying, anodic oxidation, etching and chemical coatings. A brief description of some of the most commonly employed techniques for modifying surfaces is outlined below.

#### 4.3.1.1 Topographical Modifications

Polishing

Polishing is a technique used to smoothen implant surfaces. The most common method of polishing is machining. Electropolishing is another technique often utilised to prepare polished surfaces, whereby metal is electrochemically removed by oxidation and subsequent dissolution into the electrolyte. Polished surfaces usually have an average roughness measuring $<0.5$ μm. Turning and milling are other techniques employed to modify surfaces, which would result in regular grooves on the implant surfaces and are known as 'minimally rough' surfaces.

Blasting

Blasting is an abrasive technique usually employed to roughen a smooth or polished surface. The most common method of blasting surfaces is known as 'sandblasting' which is usually a dry process of thrusting a jet of abrasive media like alumina, corundum, crushed glass, silica and steel grit on to the surface at accelerated velocity. Blasting surfaces with alumina particles of 25–75 μm have been shown to create isotropic surface modifications with $R_a/S_a$ around 1.1–1.5 μm in contrast to blasting with 250-μm particles that creates anisotropic surfaces with deviation around 2 μm [10]. Blasted surfaces have been observed to have improved cellular adhesion and osteoblastic differentiation [11]. Proper cleaning of blasted surfaces after the process is essential as retained particles of blasting materials like alumina can impair bone formation [12].

| | |
|---|---|
| Etching | Etching is a subtractive process using strong acids or alkalis capable of eroding the surface of implants to create roughened surfaces. Hydrochloric acid, hydrofluoric acid and sulphuric acid are the most commonly used acids for etching implant surfaces. A mixture of acids is also used often to erode surfaces to make them more conducive for bone formation. Alkaline etching is commonly performed using sodium hydroxide solutions. Varying the concentration of the acid/alkali in use, time of exposure and temperature are important considerations during the process of etching. |
| Oxidation | Oxidation is the technique of chemically converting the titanium surface into an oxide layer, thereby increasing the surface titanium dioxide coating. This has been observed to impart improved biocompatibility to the implant surface. An oxide layer is allowed to deposit on the titanium surface placed at the anode, and this leads to a thicker layer of titanium oxide on the surface. |
| Bioactive coating | Several bioactive coating methods have been attempted on titanium surfaces to improve the efficacy of implants. The key property that makes bioactive coating technique an attractive modification option is their ability to exert a particular response in the biological system. The most common coatings on titanium surfaces include hydroxyapatite, calcium phosphate, bone morphogenetic proteins and collagen. Hydroxyapatite coating is a very commonly used method owing to its excellent biocompatibility and ability to bond with the surrounding bone. |
| Titanium plasma spraying | Plasma spraying technology is a 'non-bioactive' coating technique used to create porous titanium surfaces that can favour ingrowth of bone. A jet of molten titanium is sprayed on to the implant surface during the process of titanium plasma spraying (TPS). TPS is an additive procedure that imparts a roughened surface architecture. TPS implants have been observed to have variable $R_a$, and some studies have reported improved osseointegration of TPS compared to smooth surfaces [13]. Plasma spraying technique is also used to create hydroxyapatite coatings. Although plasma spraying is an established method, controlling the variables involved in the process is quite complicated, and small variations may lead to completely different properties than expected. There are several other techniques of coating implant surfaces like sputter coating, pulsed laser deposition, dip coating, electrophoretic deposition and thermal spraying. Deposition |

techniques have an inherent drawback that they take a long time.

Laser ablation — Laser ablation is a subtractive technique commonly used to create microstructures with improved physical properties like toughness, rigidity and corrosion resistance. This technique has the ability to generate nanostructures on the titanium surfaces [14].

#### 4.3.1.2 Physicochemical Modifications

Physicochemical modifications involve changing the surface free energy, surface charge and hydrophilicity. Studies have revealed improved osseointegration and bone formation on hydrophilic surfaces [15–18]. Certain topographical modifications of surfaces also impart changes in surface charge. Sandblasting techniques have been shown to create a negative surface charge. A negative surface charge is known to improve cellular attachment [19]. Modifications of surface energy have been showing to increase the hydrophilicity and thereby help adsorption of proteins necessary for cellular growth and development [20]. Titanium is highly reactive to fluoride ions. Fluoride-treated titanium surfaces have been found to enhance osteoblastic differentiation [21].

Several combinations of these techniques have been used in the field of implantology to achieve better clinical outcomes and success. Sandblasting and acid-etching techniques have been commonly used to modify dental implants with improved clinical success. Recent research has further focused on combining the topographical methods with the physicochemical modifications leading to the development of topographically and chemically modified titanium implant surfaces like the modSLA surface.

### *4.3.2 Micro-roughened Surfaces*

Wennerberg et al. [22–24] classified implant surfaces based on their surface topography into the following subtypes:

1. Smooth (<0.5 μm)
2. Minimally rough (0.5–1 μm)
3. Intermediately rough (1–2 μm)
4. Rough (2–3 μm)

There has been a consensus among majority of the scientific reports that suggest improved bone-to-implant contacts with higher surface roughness (micro-roughness). Wennerberg and Albrektsson's suggested guidelines for evaluation of implant surfaces based on topography [25] advocated that the positive correlation of surface roughness with bone formation works in a particular range: $R_a/S_a$ value from 1 to 1.5 μm. However, Shalabi et al.'s systematic review on surface roughness

and healing of the bone in 2006 [26] did not substantiate this. Their assessment of the literature on implant fixation/bone formation and surface roughness revealed a positive correlation between bone formation and surface roughness of implants from $R_a/S_a$ 0.5 μm to 8.5 μm. Machined and polished titanium implant surfaces are usually used as control surfaces to test the efficacy of modified surfaces in terms of osseointegration and other improved functionalities.

Recent reports have also demonstrated the importance of nanoscale roughness to improve bone-forming properties. Newer modifications have also focused on modulating the surface free energy to enhance the wettability and hydrophilicity. Improving surface hydrophilicity has been demonstrated to increase osteogenic differentiation in vitro and osseointegration in vivo [18, 20, 27, 28]. Newer advances in implant surface technology have enabled researchers to incorporate nanostructural modifications to implant surfaces. 'Nano' modifications are conventionally defined as alterations in the range of 1–100 nm. Topographical manipulations in the nanoscale have been found to have a positive influence on the phenomenon of osseointegration and osteogenic differentiation [29].

The implication of topographical and chemical modification of titanium implants on clinical outcomes is documented by the success of micro-roughened dental implants like sandblasted, large-grit and acid-etched (SLA) surface and its successor the chemically modified hydrophilic modSLA surfaces. Both SLA and modSLA surfaces are micro-roughened surface, and the SLA surface has largely been considered the gold standard in implant dentistry. Recent studies have demonstrated the presence of nanoparticles on the modSLA surfaces [30–32]. Studies on the various implant surface modifications have enabled us to begin to unravel the molecular mechanisms of osseointegration.

### *4.3.3 Properties of Topographically Modified Implant Surfaces*

Osseointegration is a biological phenomenon that involves the interaction of osteoblastic cells with their microenvironment. The biological response that takes place at the interface between implant surfaces and osteogenic cells is the key to the phenomenon of osseointegration. It is important to understand the physiology of the cellular response to implant surfaces, especially in light of the properties conferred on to the newer implant surfaces by virtue of their modifications. The native niche of osteoblastic cells is interspersed with proteins and structural elements at the micro- and nanoscale. Therefore modifying implant surfaces topographically (at the micro- and nanoscale) results in structural features that influence cells to directly interact with such surfaces. Micro- and nanoscale roughness allows surfaces to have an increased surface area, which allows such surfaces to adhere greater amounts of proteins and mediators necessary for growth and differentiation. Hydrophilic surfaces have the capability to attract and retain proteins necessary for growth and

differentiation of cells. Cells differentiating towards an osteoblastic lineage have been observed to show higher expression of integrins and focal adhesion proteins on micro-roughened titanium surfaces, when compared to smooth surfaces [33].

### *4.3.4 The SLA and modSLA Titanium Surfaces*

The sandblasted, large-grit and acid-etched (SLA) titanium and its successor, the chemically modified hydrophilic modSLA surfaces, designed by Institut Straumann AG (Waldenburg, Switzerland) are micro-roughened titanium implant surfaces known to have improved osseointegrative and osteoconductive properties compared to their smooth-surfaced counterparts. These surfaces have proven to be remarkable features for investigating and elucidating the mechanisms of osteogenic differentiation in vitro and osseointegration in vivo. A brief discussion about the method of preparation of these surfaces and their physical and chemical characteristics is worth detailing in this section of the chapter.

#### 4.3.4.1 Method of Fabrication

The acronym 'SLA' was first used by Buser et al. in their 1991 publication [2] where they demonstrated higher bone-to-implant contact on titanium implant surfaces prepared by sandblasting using large-grit particles and etching using hydrochloric and sulphuric acid. The modSLA surface (commercially known as SLActive surface, Fig. 4.2a) was described for the first time by Buser et al. in 2004 [34], where they demonstrated increased bone apposition to modSLA surfaces compared to the SLA surfaces. The modSLA surface is essentially a physicochemically modified version of the SLA surface (Fig. 4.2b) that allows maintenance of the initial hydrophilicity of microstructured titanium surfaces after their fabrication. Both the SLA and modSLA surfaces are commercially produced by Institut Straumann (Basel, Switzerland) and have achieved the status of industry standards, with many other companies in the field of implant dentistry adopting the methods to fabricate surfaces similar to SLA and modSLA.

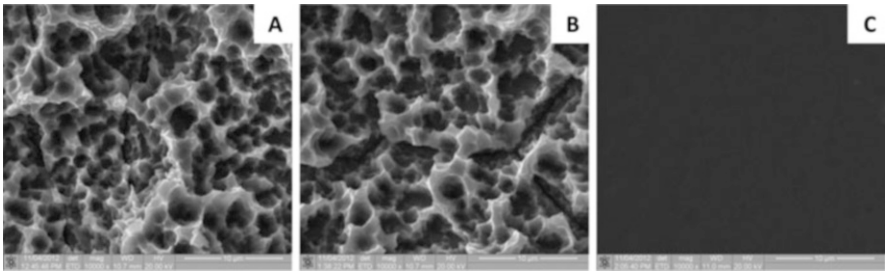


**Fig. 4.2** Scanning electron microscope (SEM) images of (**a**) modSLA, (**b**) SLA and (**c**) smooth (SMO) titanium surfaces (10,000× magnification)

The SLA surface is achieved by sandblasting grade II commercially pure titanium surfaces using 250–500-μm large-grit corundum (crystalline form of $Al_2O_3$) and subsequently acid etching using a hot solution of hydrochloric and sulphuric acids, thereby combining the principles of blasting and acid etching. This technique gives rise to a micro-roughened topography of the titanium surface. Titanium, on exposure to air, gets converted to titanium dioxide ($TiO_2$) which in turn may get hydroxylated upon exposure to water. Variations in net charges may lead to ionic interactions on the surface, and exposure to air may also lead to contamination of the $TiO_2$, which may collectively lead to decrease in the initial hydrophilicity developed after the topographical modification [20].

The modSLA surface was introduced as a successor to the SLA surface, wherein a chemical modification was introduced to the SLA surface immediately post-production, so that the hydrophilicity generated can be maintained. Baier and Meyer's publication in 1988 on the future directions of implant surface preparations discussed the importance of surface cleaning methods on the retention of high surface energy [35]. A similar concept was employed in the creation of the modSLA titanium implant surfaces. The surfaces are produced in the same way as the SLA surface using sandblasting and acid-etching technique. Immediately after generation of the surface, they are rinsed in a nitrogen-protected environment and then stored in an isotonic sodium chloride solution in a nitrogen environment [20]. This has been observed to retain the hydrophilic properties of the surface.

#### 4.3.4.2 Characteristic Features of modSLA and SLA Surfaces

As a result of their proven superior osseointegration properties, the modSLA and SLA surfaces have been the subject of extensive investigation. They provide us with a suitable model to study the molecular mechanisms of osteogenic differentiation and osseointegration in an in vitro setting. The essential attribute that makes these surfaces suitable for studying molecular interactions lies in the fact that they do not provoke any chemical interactions with cells and therefore provide us with physiologically relevant models to study the intricate mechanisms of osteoblastic differentiation.

Topographical features of the modSLA and SLA surfaces appear similar, when observed using conventional scanning electron microscope (SEM) and atomic force microscopy (AFM). Both the surfaces are observed to have micro-roughness with $S_a$ values varying between $1.15 \pm 0.05$ μm for SLA and $1.16 \pm 0.04$ μm for modSLA [20]. The roughness values observed in different studies seem to vary slightly, e.g. Vlacic et al. have described an $S_a$ value of 1.8 μm [36], whereas Olivares-Navarrete et al.'s study demonstrated $R_a$ to be around 3.22 μm [37]. Using AFM, we observed RMS roughness values in the range of 1.6–2.1 μm for the modSLA and SLA surfaces, in contrast to mirror-finished polished titanium surfaces that showed RMS values in the range 0.006–0.009 μm [38].

The roughness features of modSLA and SLA surfaces are not seen to be distributed evenly throughout the surfaces and therefore seem to indicate that

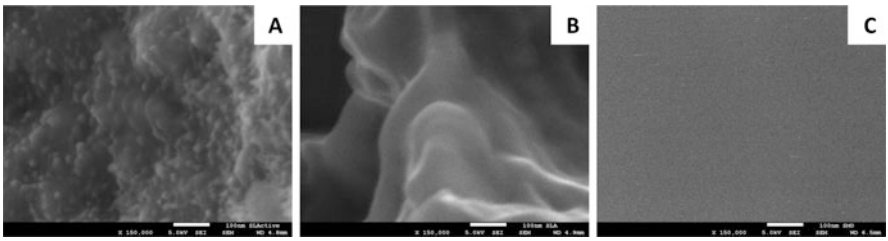


**Fig. 4.3** High-resolution SEM images of (**a**) modSLA, (**b**) SLA and (**c**) smooth (SMO) titanium surfaces depicting the presence of nanostructures only on the modSLA surface (150,000× magnification)

uniformity in the topographical pattern is not a prerequisite for improved osseointegration and osteodifferentiation on implant surfaces. Recent studies using high-resolution SEM imaging techniques have observed the presence of nanostructures interspersed on the micro-roughened modSLA surface [30, 31, 39]. We also have observed similar nanostructures on the modSLA surfaces using high-resolution SEM imaging (Fig. 4.3) which corroborates with others. Chemical analysis using X-ray photoelectron spectroscopy (XPS) has confirmed that these nanostructures are not the result of crystallisation of sodium chloride present in the isotonic solution used to store the modSLA surfaces [31]. Wennerberg et al. further observed the formation of similar nanostructures when freshly prepared sandblasted and acid-etched titanium surfaces were stored in water (instead of isotonic saline solution) [31]. The impact of these nanostructures on the superior clinical outcomes of modSLA surfaces has not been established as yet.

Chemical analyses of the modSLA and SLA surfaces have demonstrated titanium, oxygen, nitrogen and carbon as the key elements present [40]. Traces of sodium and chlorine have also been observed in modSLA surfaces in some studies [28, 41] which could be a result of storing them in saline solution. The modSLA surface has a contact angle close to 0°, which exemplifies its extraordinary hydrophilic nature.

## 4.4 Biology of Healing on Implant Surfaces

Bone formation and healing on implant surfaces are multifaceted processes involving several factors and considerations. Several factors contribute towards successful osseointegration and bone formation on implant surfaces. Some of the key factors that play an important role in this process are summarised in Table 4.1

Insertion of any implant into the human body is akin to any other wound healing process. Bone healing starts immediately after an implant is inserted into the human body. The first tissue to come in contact with the implant surface is blood and its components. The large accessible surface area provided by micro-roughened implant surfaces allows for greater adsorption of proteins. Hydrophilic implant

**Table 4.1** Key factors important for the process of osseointegration

| Factors influencing osseointegration |
|---|
| Material biocompatibility |
| Implant shape and design |
| Surface characteristics – roughness, hydrophilicity, surface energy |
| Age of the patient |
| Pre-existing conditions – systemic diseases like diabetes, cancer, immunosuppressive conditions, hypersensitive conditions, infections, bone diseases like osteoporosis, osteomalacia, Paget's disease, etc. |
| Condition of the implant bed – poor bone quality, presence of debris, local infection, etc. |
| Surgical technique – minimal tissue damage is favourable for osseointegration |
| Relative mobility of implant – higher degree of movement of implant in the initial stages of healing inhibits osseointegration |
| Timing of loading |

surfaces further improve the process of protein adsorption. The contact of blood with implants gives rise to a cascade of reactions involving coagulation, inflammation, release of chemoattractants and eventually recruitment, proliferation and differentiation of mesenchymal stem cell and pre-osteoblasts.

Coagulation of blood on the implant surface leads to the conversion of fibrinogen to fibrin. Fibrin forms a mesh-like scaffold on the implant surface that can retain other proteins and mediators to allow for appropriate progression of the healing process. Platelet activation and release of proteins like adenosine diphosphate (ADP), platelet-derived growth factor (PDGF), histamines, platelet factor 4 and transforming growth factor-β (TGF-β) and serotonin are important steps in the process of implant healing and osseointegration. The aggregation of platelets is instrumental in clot formation and eventually in the formation of the fibrin mesh network. Activated platelets have von Willebrand factor (VWF) and glycoprotein IIb/IIIa (GP IIb/IIIa) receptors on their surface. Fibrinogen binds to the GP IIb/IIIa receptors, leading to platelet aggregation. The process of activation of thrombin from prothrombin is catalysed by activated platelets. Thrombin in turn helps in the stabilisation of the fibrin network and therefore the platelet plug [42] (Fig. 4.4).

Leucocytes are next in line in the inflammatory response that happens upon the insertion of any implant. Neutrophils are the first white blood cells (WBCs) that are recruited to the site within 24–48 h. After this duration, monocytes and macrophages become the dominant cells at the implant site. Activation of leucocytes leads to release of inflammatory mediators that include cytokines like interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor (TNF)-α.

The extent and duration of the inflammatory response to any biomaterial define the biocompatibility of the material [43]. Inflammation is essentially a biological response to any injury to the body, and it may progress through the phases of acute inflammation, chronic inflammation and granulation tissue formation.

In cases of biocompatible materials, the acute inflammatory process eventually evolves into the bone formation phase of the wound healing and thereby integration of the implant material. Persistence of any inflammatory stimulus may lead to a

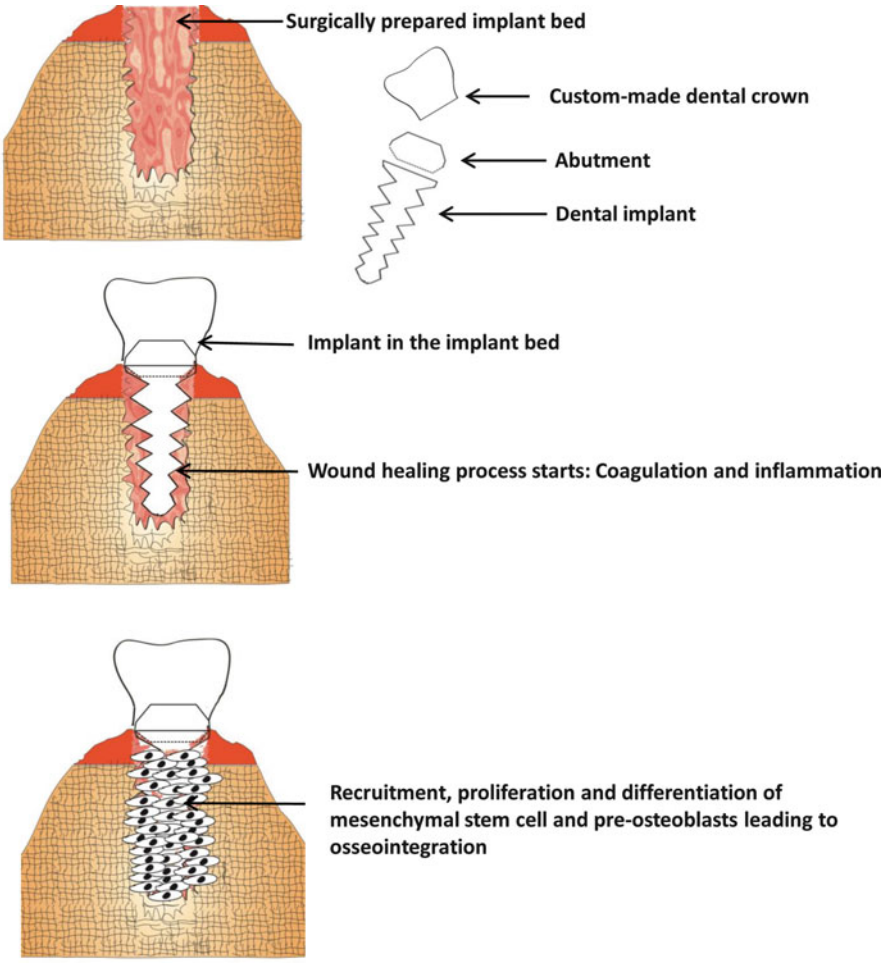


**Fig. 4.4** The biology of implant healing process and osseointegration

chronic inflammatory response. The post-implantation inflammatory response may keep progressing depending on the properties of the material and may lead to a chronic inflammatory condition. The fundamental cells determining the nature of the inflammatory response are macrophages, lymphocytes and fibroblasts. Chronic inflammation may eventually lead to healing by fibrosis and scar tissue formation – an outcome not very conducive to bone formation. Higher levels of cytokines and inflammatory mediators have been observed to be deterrents to osteogenic differentiation in vitro [44] and bone formation in vivo [45].

The initial inflammatory response and haematoma formation are essential for the recruitment and differentiation of mesenchymal and osteoprogenitor cells which eventually leads to a successful osseointegration of the implant. This stage of

healing leads to osteogenesis, deposition and integration of mineralised matrix on the surface of the implant. From this stage onwards, bone remodelling takes over giving rise to well-defined lamellar bone on the implant surface, thereby finally leading to bone bonding or, in other words, osseointegration.

## 4.5 Molecular Regulation of Osteogenic Differentiation and Osseointegration on Implant Surfaces

Multipotent mesenchymal stem cells (MSCs) have the capability to differentiate into osteogenic, chondrogenic or adipogenic cell types and other cell types such as myocytes, marrow stroma and tendons [46]. The precise cellular signaling mechanisms involved in osteogenic differentiation of progenitor cells remain indeterminate. However, before delving deeper into the phenomena of osteogenic differentiation and osseointegration on implant surfaces, it would be useful to briefly discuss normal bone architecture and physiology.

### *4.5.1 Bone Cells*

The essential living components of bone tissue include three different types of cells – osteoblasts, osteocytes and osteoclasts. Some of the terminally differentiated osteoblast cells eventually get trapped in the lacunae of the matrix and form osteocytes.

#### 4.5.1.1 Osteoblasts

Osteoblasts are the key bone cells that form the building blocks of the bone and are responsible for the deposition of mineralised matrix. These are mononucleate cells which have the potential to form osteoid (organised un-mineralised portion of a typical bone, characteristically composed of type I collagen). Subsequently they are also responsible for the mineralisation of the osteoid.

Osteoblasts arise from osteoprogenitor cells present in the human body (usually located in the deeper layers of the periosteum and bone marrow). Studies have demonstrated the presence of several niches of osteoprogenitor cells in the human body [47–50]. Multiple reports support the presence of mesenchymal progenitor cells with potential to differentiate into various cell types including osteoblasts. These cells under specific culturing or growing conditions give rise to osteoblasts.

Osteoprogenitor cells are known to express the RUNX2/Cbfa1 transcription factor. These osteoprogenitor cells differentiate to osteoblasts and start expressing gene markers that include Osterix, Col1, BSP, M-CSF, ALP, osteocalcin,

osteopontin and osteonectin. Mature osteoblasts are cuboidal in shape. Osteoblasts have cytoplasmic projections that allow them to form communications with adjacent osteoblasts and osteocytes. Osteoblasts become flattened and elongated upon maturation. These cells usually form a single layer of cells on the surface of the bone. However, in cases where there is active bone formation, they may be present in the form of layers.

The principal function of osteoblasts is bone formation. As mentioned earlier, osteoblasts are the cells that facilitate the deposition of mineralised matrix in bones. They are also responsible for the synthesis of various bone-related proteins and polysaccharides. These cells also play an important role in bone remodelling by maintaining a balance between bone formation and resorption during new bone formation. Rarely, osteoblasts are also known to initiate the bone resorption process. Mature osteoblasts synthesise several phenotypic markers, including type I collagen, alkaline phosphatase (ALP) and osteocalcin (Fig. 4.5).

#### 4.5.1.2 Osteocytes

Osteocytes are the most abundant type of bone cells (90–95 %) and cannot proliferate further to form new cells. They are known to function as mechanosensors of bone [51].

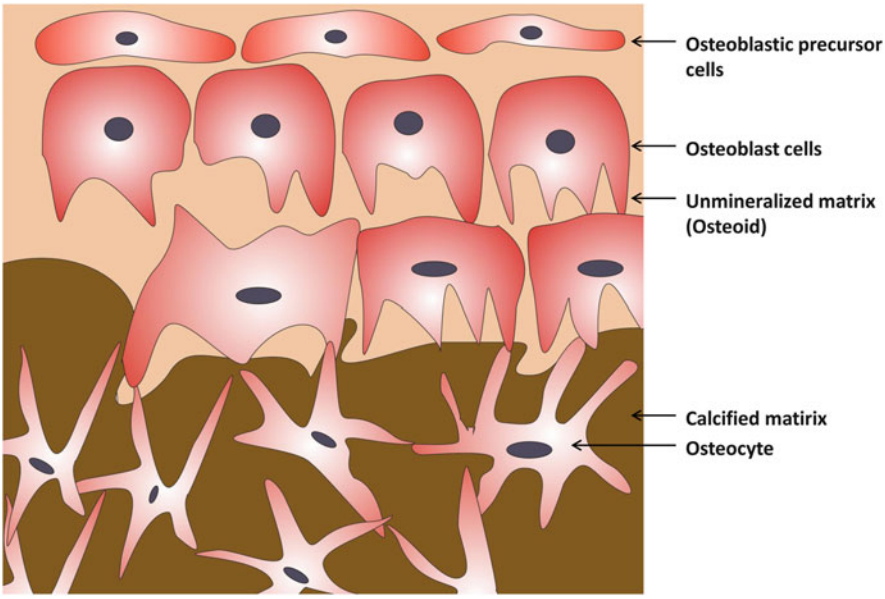


**Fig. 4.5** Origin of osteoblasts in humans. The osteoblast precursors proliferate and give rise to osteoblasts which in turn lay the bone matrix and also give rise to the osteocytes

#### 4.5.1.3 Osteoclasts

Osteoclasts are the cells responsible for bone resorption and thereby help in bone remodelling. These cells are formed by the fusion of mononuclear cells of the monocyte/macrophage lineage. The interplay between the osteoblasts and osteoclasts is responsible for maintaining normal bone homeostasis in the human body.

### *4.5.2 Osseointegration and New Bone Formation*

The phenomenon of osseointegration is initiated simultaneously with the healing phase. Schenk and Buser have divided the process of osseointegration into three different stages [52]. Firstly, the implant surface is internalised by the formation of woven bone. They stated that the woven bone tends to form in two different patterns – 'distance osteogenesis', which occurs when the woven bone forms from the surrounding bone moving towards the implant surface, and 'contact osteogenesis', where osteogenesis occurs via direct deposition of new bone on the implant surface itself.

The second stage of osseointegration conditions the implant for its load-bearing function, wherein the woven bone laid on the implant surface slowly changes to a well-defined lamellar pattern. Lamellar bone consists of concentrically (in compact bones) or parallelly (in spongy bones) organised lamellae of collagen fibres and needs a firm base to be laid onto. The newly formed woven bone, existing bone and the implant surface supposedly provide the solid structure on which lamellar bone can be formed.

Schenk and Buser further described that bone remodelling takes place in the last stage of osseointegration which involves a balance and coordination between the osteoclastic resorptive activity and osteoblastic formative activity. The osseointegrative activities on dental implants, according to Schenk and Buser, vary with the different regions of the implant depending on the type of bone the region is in contact with. The coronal part of the implant integrates with cortical bone, whereas the remainder of the implant is in contact with cancellous bone and bone marrow. The compact structure of the cortical bone provides much of the initial stability, whereas the spongy cancellous bone ensures greater exposure to the vascular network and osteogenic cells.

Osteogenic differentiation and osteoinduction have been recognised as important processes leading to the formation of new bone during fracture healing and implant osseointegration. It has been argued that pre-existing osteoblasts play a minor role in bone formation in the regions of fracture healing or implant placement [53, 54]. The surrounding niche created in the region of the implant placement is rich in biochemical mediators that assist in recruitment and subsequent differentiation of cells towards osteogenesis, similar to fracture healing. Therefore, the process of osteogenic differentiation on implant surfaces provides important cues

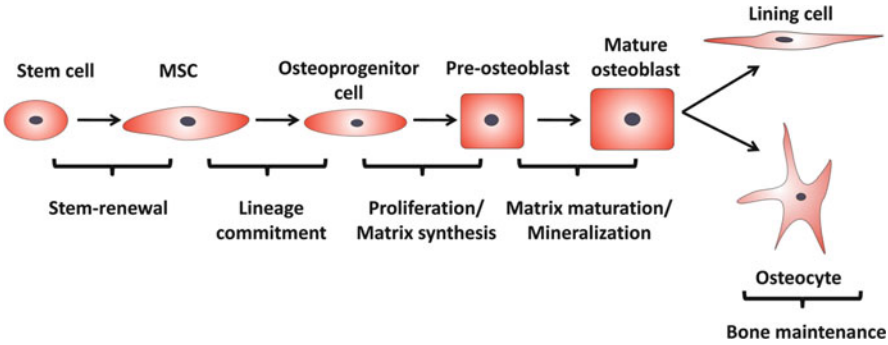


**Fig. 4.6** Stages of osteoblastic differentiation (*MSC* mesenchymal stem cell)

in regard to the biocompatibility features of the material and the surface. Indeed, the implant surfaces with proven superior osseointegrative properties like the modSLA and SLA titanium surfaces have enabled us to learn some of the intricate details regarding the process of osteogenesis.

The process of osteogenic differentiation progresses through the stages of lineage commitment, pre-osteoblast cells, mature osteoblasts and ultimately forming the terminal bone cell – the osteocyte. Committed pre-osteoblasts are the first to express alkaline phosphatase (ALP-early marker of osteoblast differentiation). Mature osteoblasts express high levels of ALP and are involved in the production of extracellular matrix (ECM). Several genes and factors are involved in the process of commitment of mesenchymal stem cells to osteoblastic differentiation. The TGF-β/BMP molecules appear to play an important role in the process of commitment of MSCs to osteoprogenitor cells [55, 56] (Fig. 4.6).

### *4.5.3 Modulation of Molecular Pathways on Topographically Modified Implant Surface*

Several studies have been conducted to observe the differences in osteogenic properties of different titanium surfaces. Most studies suggest that there is a similar initial cell attachment to all of the surfaces [40, 57]. Brett et al.'s study (2004) on the pattern of gene expression in osteoblasts cultured on different titanium surfaces (SLA, SMO and titanium plasma sprayed) revealed that surface roughness of Ti had profound effect on the pattern of gene expression by bone cells. Topographically modified titanium surfaces like the modSLA and SLA surfaces have been observed to stimulate cell signaling pathways. The BMP-2 gene is seen to be highly upregulated (greater than fivefold change) in osteoprogenitor cells as early as 24 h of culture on SLA surfaces, when compared with SMO surfaces. The osteogenic response of modSLA is considered to be better than the hydrophobic SLA surface, possibly because of the activation of WNT5A molecule [57].

#### 4.5.3.1 Cell Signaling Pathways and Osteogenesis

Regulation of cellular interactions, differentiation and maturation are mediated by several factors, conditions and activation of different cell signaling pathways. The integrin signaling pathway is responsible for communication and adhesive interactions of cells with the implant surface, and activation of integrins leads to activation of different biological processes. Osteogenesis and osseointegration are among the various processes that become activated subsequent to stimulation of the integrin signaling pathway. Mesenchymal stem cells (MSC) undergo proliferation, and under specific conditions they become committed towards osteogenesis and thereby form mature osteoblast cells. Osteoprogenitor cells undergo a process of differentiation giving rise to osteoblasts and ultimately to osteocytes. Several cell signaling pathways are known to be instrumental in the process of osteogenic differentiation. The most important cell signaling pathways considered to be imperative to the process of osteodifferentiation are the TGF-β/BMP, Wnt, hedgehog and fibroblast growth factor (FGF) and Notch signaling.

##### TGF-β/BMP Pathway

The TGF-β/BMP pathway is identified as one of the most important molecular pathways that are influential in guiding the differentiation process. The bone morphogenetic proteins (BMPs) are a part of the transforming growth factor (TGF-β) superfamily of ligands, and they work through the SMAD receptors. BMP-2 and BMP-4 knockout mice do not even survive the gastrulation phase of the embryo due to failure of mesenchymal tissue induction [58]. The BMP molecules interact with the BMP receptors (BMPRs), leading to the activation of SMADs which ultimately enter the nucleus and activate several downstream transcription factors such as Dlx5, Cbfa1, Osx, etc. Several pathways have been shown to interact with the TGF-β/BMP pathway [59].

##### Wnt Signaling Pathway

The Wnt family of growth factors is an important pathway known to regulate growth and differentiation of tissues and organs. The canonical Wnt/β-catenin pathway has been found to be of vital significance for regulation of bone mass [60]. In fact, mutations in LRP5, a protein co-receptor in Wnt signaling, have been seen to produce osteoporosis–pseudoglioma features [61]. Moreover, the production of antagonists for the Wnt pathway, like DKK-1, is correlated with the osteolytic features seen in multiple myeloma [62].

The canonical Wnt/β-catenin pathway is probably the best understood pathway. β-catenin is a transcription factor that is central to the functioning of the pathway. The interaction of the TCF (T-cell factor) with SMAD4 potentially connects the

Wnt and the BMP signaling pathways. The canonical Wnt/β-catenin pathway has been found to be significant for regulation of bone mass [60]. The conservation of the β-catenin molecule is the prime factor responsible for the activation of downstream genes which in turn leads to the activation of SMAD4 and thereby raises the possibility of the BMP and the Wnt pathway to be closely related in the process of osteogenic differentiation.

The non-canonical Wnt pathways include the planar cell polarity (PCP) and Wnt/$Ca^{2+}$ pathways. The distinguishing feature of the non-canonical pathways is that they are independent of β-catenin, LRP5/6 co-receptor and Dsh-DEP domain. Non-canonical Wnt pathways haven't been studied in extensive details, especially in relation to osteogenic differentiation. However, the studies on investigating interaction of osteoprogenitor cells with implant surfaces have revealed the upregulation of non-canonical Wnt pathway during osteogenic differentiation [32, 36, 63, 64].

### Hedgehog Signaling Pathway

The hedgehog gene family consists of three members among which the Sonic (Shh) and Indian (Ihh) hedgehogs have been shown to be involved in skeletal development and repair. The hedgehog–BMP interaction is quite conserved in the process of differentiation [58]. Shh is seen to regulate BMP-2 expression in chicken limb buds [65]. The hedgehogs via their interaction with the BMP molecules might be important in the regulation of osteogenesis.

### Fibroblast Growth Factor (FGF) Signaling Pathway

The FGF signaling pathway works through the tyrosine kinase receptors (FGFR1, FGFR2, FGFR3 and FGFR4) and leads to the activation of different cellular processes. FGFR1 signaling is known to activate osteogenic differentiation in osteoprogenitor cells. FGF ligands act on the FGF receptors and lead to downstream molecular processes. One of the key transcription factors RUNX2 is known to be phosphorylated under the influence of FGF2 ligand via the mitogen-activated protein kinase (MAPK) pathway [66].

### Notch Signaling Pathway

The Notch pathway is a pathway for cellular communication that helps communication between neighbouring cells. The Notch pathway is known to work through its four receptors, NOTCH1, NOTCH2, NOTCH3 and NOTCH4. Notch signaling is believed to regulate osteogenic differentiation through its interaction with the BMP-2-mediated cell signaling pathway [67] and has been shown to induce the expression of Osterix.

#### 4.5.3.2 Insights into the Molecular Regulation on modSLA and SLA Surfaces

Topographically modified surfaces are well recognised in the field of implant dentistry for their improved osteogenic properties. Several research groups across the world have been trying to explore the possible molecular mechanisms involved in accruing these osteogenic features. These surfaces have also been used as models to study the process of osteogenesis in vitro as they are considered to provide a substrate to osteoprogenitor cells that is akin to the native niche seen in vivo.

The SLA and modSLA surfaces have been seen to activate the integrin signaling pathway in cells capable of differentiating towards osteogenic lineage like mesenchymal stem cells and osteoprogenitor cells. Higher expression of integrins α2 and β1 has been observed on these surfaces [68, 69]. Integrin molecules are presumed to be important for anchorage of cells to surfaces, and this is possibly the first step before the process of cellular differentiations begins. We have observed higher expression of genes encoding integrins α2 (ITGA2) and β1 (ITGB1) within 24 h of culturing osteoprogenitor cells on modSLA and SLA surfaces compared to SMO surfaces [38]. Cell signaling pathways are stimulated subsequent to the activation of integrins and anchorage of cells to these surfaces. Studies on these surfaces have revealed stimulation of the key osteogenic pathways, TGF-β/BMP and Wnt [32, 36, 37, 64, 70].

The Wnt signaling pathway is subdivided into the canonical Wnt/β-catenin and the non-canonical Wnt pathways. The non-canonical Wnt is further subclassified into different pathways among which the planar cell polarity (PCP) and Wnt/$Ca^{2+}$ pathways have been best known. The role of the Wnt/β-catenin pathway in osteogenesis and new bone formation is well established. However, the results from studies on the modSLA and SLA surfaces revealed upregulation of the Wnt/$Ca^{2+}$ pathway during osteogenic differentiation [32, 63, 64]. Ivanovski et al.'s work assessing the transcriptional profile during osseointegration in human subjects also showed activation of the TGF-β/BMP and Wnt pathways [18]. In addition to these cell signaling pathways, their study also observed the upregulation of the Notch pathway [18]. The Notch pathway has also been shown to be important for osteogenic differentiation in other studies [67], yet it generally has received scant attention in the context of osteogenesis. Our studies exploring the differential regulation of cell signaling pathways when osteoprogenitor cells are cultured on modSLA and SLA surfaces have also shown an early stimulation of the Notch pathway along with the TGF-β/BMP and Wnt (especially the non-canonical Wnt/$Ca^{2+}$ pathway) [32].

The modSLA and SLA surfaces have also been shown to increase the expression of osteogenic markers like alkaline phosphatase (ALP), osteocalcin (OCN), osteopontin (SPP1) and RUNX2 [57, 71–73]. These in vitro observations confirmed their in vivo osteogenic properties. The sequential stimulation of different cell signaling pathways eventually leads to the activation of osteogenic transcription factors and ultimately leads to osteogenic differentiation. We also explored the

regulation of microRNAs, which are small RNA molecules that have the potential to modulate the expression of messenger RNA (mRNA) molecules, on the modSLA and SLA surfaces. Several microRNAs that are known to mediate cell development and differentiation were seen to be downregulated on both the modSLA and SLA surfaces in comparison to the SMO surfaces [38]. Bioinformatic target predictions for the downregulated miRNAs using an online tool, TargetScan, have revealed several genes of the TGF-β/BMP and Wnt/$Ca^{2+}$ pathway as potential targets. Inhibitors of osteogenesis were found to be potential targets for miRNAs that were found to be upregulated.

## 4.6 Conclusion

Osseointegration is a phenomenon that is integral to the successful incorporation of orthopaedic and dental implants into the human body. In this chapter we have presented the current concepts in the field of implant surface modification and osseointegration. The modSLA and SLA micro-roughened titanium dental implant surfaces are known for their improved osteogenic and osseointegration properties, thereby highlighting the importance of topographically modified surfaces. These surfaces have been studied in-depth by researchers across the globe. Findings from such studies have enabled us to learn a great deal about the molecular mechanisms involved in osteogenic differentiation and osseointegration on micro-roughened implant surfaces. Such implant surfaces have also helped us in exploring the various signaling pathways involved in osteogenesis and therefore have provided us with a model to study the molecular mechanisms involved in osteogenic differentiation in vitro without using chemical mediators to induce differentiation.

The positive impact of micro-roughened titanium implants on osseointegration is proven. However, with the advent of nanotechnological modifications and the recent evidence from nano-topographically modified implant surfaces that show better osseointegration and bone formation, it is apparent that we haven't been able to identify all the factors and underlying mechanisms responsible for successful osseointegration and osteogenic differentiation. This in turn means that we need to explore these processes and their molecular regulation in greater depths. It is also clear that research focused on further exploring modifications of implant surfaces is ongoing, and this will likely lead to the development of different kinds of surfaces that will enable us to learn more about the process of osseointegration.

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# Chapter 5
# Advances in Bioglass and Glass Ceramics for Biomedical Applications

**Besim Ben-Nissan, Andy H. Choi, and Innocent Macha**

**Abstract** Tissue engineering and advanced biomedical technologies have proved to be potential to improve the quality of human life. During the last four decades, the capability to engineer or repair new functional tissues has been a very effective approach to improve the quality of life of patients. Since its discovery by Hench and co-workers in the 1960s, bioglasses and glass ceramics have attracted considerable attention of many researchers because of their unique properties which can easily be tailored by manipulating its compositions and morphology. Over the years, many questions concerning its interactions with both hard and soft tissues have been answered with a multidisciplinary team of surgeons, scientists and engineers. Many clinical Bioglass® and other similar structures and compositions are being used for bone augmentation and restoration, in orthopaedic, dental and maxillofacial surgery and in general in the field of tissue engineering. They have proved to be efficient and effective, some with outperformance over other bioceramic and metal prostheses. It is our aim in this chapter to present the development of these important biomaterials focusing on the history, synthesis, properties, modern characterisation methods and the current development of nano- and biocomposite materials for clinical applications.

**Keywords** Bioglass • Glass ceramics • A-W glass • Osteosimulation • Sol–gel

## 5.1 Introduction

When a person suffers from a pain, the main concern for that individual is relieving the pain and returning to a healthy and functional lifestyle. Degeneration and diseases often result in the replacement of skeletal parts, such as the knees, hips, finger joints, elbows, vertebrae and teeth, and repair of the mandible surgically.

It is anticipated that the growth in these areas will continue due to a number of factors, for instance, the need due to the ageing population, improvements in

*Dedicated to Prof Larry Hench who has given us the 'Bioglass' and beyond.*

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technology and lifestyle, a better understanding of body functionality, an increasing preference by younger to middle-aged candidates for undergoing surgery, improved aesthetics and the need for better functions [1].

By definition, a biomaterial is a nondrug substance which is ideal to be placed in a system that can replace or enhance the roles of bodily organs or tissues. These materials are able to be in contact with bodily fluids and tissues while showing little or if any adverse reactions for prolonged periods of time.

The major key factors that are pertinent for the success of an implant are its biocompatibility and biofunctionality. Engineers and surgeons have identified, even at the initial stages of this field, the problems related to the design and materials selection that resulted in premature loss of implant function through mechanical failure, corrosion or inadequate biocompatibility of the component. Depending on the applications, bioactive glass and glass ceramics in addition to ceramic materials are ideal candidates with respect to the above functions, except for their brittle behaviour under functional loading.

In this chapter, our aim is to examine the general definitions of glass as well as the preparation methods, properties and applications of glass, glass ceramics and bioactive glasses currently available and in use. We will also introduce the development and progress of the commercially available and currently investigated bioglasses and glass ceramics. Furthermore, their chemistry, bioactivity and mechanisms of their bonding and interactions within a physiological environment, their preparation methods and their applications in the biomedical field will also be covered.

## 5.2 Glass and Glass Ceramics

Glass is an amorphous, hard and brittle material created from the molten product of oxides. The molten material is normally cooled rapidly in order to prevent crystallisation or devitrification.

For over thousands of years, glass has been known to mankind. A natural glass produced from silicate magna called obsidian was known to prehistoric people long before how to make glass was discovered. The Phoenicians are thought by many to have been the first people to make glass [1].

It is now possible from the means of how glass is manufactured to predict and control the properties. Much of this control derives from the purity and use of appropriate raw materials. The choice of raw materials is generally based on their glass-making properties, which will be discussed in the following sections.

## *5.2.1 Raw Materials*

### 5.2.1.1 Glass Formers

In general, glass formers are oxides which can be turned into a glass without the need to use any other oxides. However, they require very high temperatures in order to melt initially. Silica ($SiO_2$) normally obtained from sand is the most common type of glass former. Other examples of glass formers include $B_2O_3$ and $P_2O_5$.

### 5.2.1.2 Modifiers

Modifiers, as their name suggests, are materials that may alter the properties of the glass-forming oxides. They are also the major groups of compounds typically added to silica. In addition, they may also be employed to avoid defects in the final glass products. Generally, two types of modifiers are added to glass-forming oxides: fluxes and stabilisers.

#### Fluxes

In chemical terms, fluxes are the components that change the underlying properties of the oxides when added to glass formers. For instance, the melting point of glass-forming oxides can be lowered by the addition of fluxes.

Some of the most common fluxes include oxides of sodium ($NaO_2$) and potassium ($K_2O$). In particular, the viscosity of the glass can be lowered through the addition of boric oxide ($B_2O_3$), which in turn increases the fluidity and thus permitting the compounds to move with a greater degree of freedom.

#### Stabilisers

Stabilisers can be used to improve the chemical durability of glass as well as prevent the crystallisation of oxides. In certain applications, crystallisation may be undesirable due to its effect on light scattering, hence a reduction in transparency. Stabilisers, similar to fluxes, may also affect the working temperature of glass formers. Examples of stabilisers include oxides of aluminium ($Al_2O_3$), calcium (CaO) and magnesium (MgO).

#### Refining and Melting Agents

During the conventional melt-based manufacturing of glass, small bubbles are detrimental as the properties of the glass are significantly affected by their presence.

To decrease the number of bubbles, compounds such as sodium nitrate, sodium sulphate, sodium chloride, calcium fluoride and carbon are added to the glass, and the glass is said to be refined. On the other hand, the purity and close control of additives are critically important during the synthesis of glasses for biomedical applications due to the issues of toxicity and biocompatibility.

## 5.3 Types of Glasses

The specific properties of glass can be obtained through its chemical composition. An indication of some types of glasses produced in this manner, together with the desired properties for a number of engineering applications, is given in Table 5.1.

### *5.3.1 Aluminosilicate Glasses*

Aluminosilicate glasses are hard, usually have a good chemical resistance and do not devitrify readily. They also have high heat shock resistance and can withstand heat even better than borosilicate glasses. One specific type of aluminosilicate glass is used in the production of E-glass fibres (also contains CaO).

### *5.3.2 Borosilicate Glasses*

Boron oxide ($B_2O_3$) serves as both a glass former and a modifier. Boron oxide also produces a glass with a low coefficient of thermal expansion, which results in a glass that is better equipped to deal with thermal shock. A common trade name for

**Table 5.1** Types of glasses showing their chemical composition in weight percentage

| Component | Soda–lime glass | Lead glass | Borosilicate glass | Aluminosilicate glass | High-silica glass Vycor® | 45S5 Bioglass® |
|---|---|---|---|---|---|---|
| $SiO_2$ | 70–75 | 53–68 | 73–82 | 57 | 96 | 45 |
| $Na_2O$ | 12–18 | 5–10 | 3–10 | 1.0 | – | 24.5 |
| $K_2O$ | 0–1 | 1–10 | 0.4–1 | – | – | – |
| CaO | 5–14 | 0–6 | 0–1 | 5.5 | – | 24.5 |
| PbO | – | 15–40 | 0–10 | – | – | – |
| $B_2O_3$ | – | – | 5–20 | 4.0 | 3 | – |
| $Al_2O_3$ | 0.5–2.5 | 0–2 | 2–3 | 20.5 | – | – |
| MgO | 0–4 | – | – | 12.0 | – | – |
| $P_2O_5$ | – | – | – | – | – | 6 |

borosilicate glasses is Pyrex, and they are often used in the areas where temperature differences are an issue. Crystallisation is prevented by the presence of alumina ($Al_2O_3$) in large quantities. They also improve the chemical durability and the hardness of the glass.

### 5.3.3 Lead Glasses

Lead glass is often referred to as crystal glass as a result of an improvement in machinability, thus permitting the glass to be more easily engraved. It also gives the glass a heaviness and blue appearance.

One of the most important properties of lead glass is its high refractive index, which gives brilliance when properly cut or graved. The melting point and hot working (shaping) temperature of the glass are lowered as the lead oxide (PbO) acts as a flux and a modifier to acceptable levels. Radiation shielding is another useful application for the lead glasses.

Flint and crown glasses are some of the older terms associated with glass. Flint glass was a term originally used to describe lead glass since flint was used as a source of good-quality silica free from colour. It is now more loosely used to describe glasses with good colour. Crown glasses are alkali–lime–silica based, such as soda–lime glass.

### 5.3.4 Soda–Lime Glasses

The presence of soda ($Na_2O$) in glass lowers the melting point of the glass, and the lime (CaO) keeps the glass from crystallising.

### 5.3.5 Glass Ceramics

By definition, a glass ceramic is essentially a glass in which the formation of nuclei is enhanced by using specific compositions, which are self-nucleating, or by adding an additional nucleating agent [2]. Very small crystals are contained inside the resultant material. A number of factors influence the glass ceramics' final properties: crystal orientation; intergranular bonding; percentage of crystallinity, of crystalline phase distribution and of any remaining glassy phase; and grain size. In the past, by controlling the base composition, the choice of nucleant (nucleating agent) and an appropriate heat treatment schedule, above-mentioned factors have been successfully controlled [2]. In the early days, work on glass ceramics was focused on the lithia–silica ($Li_2O$–$Si_2O$) system. Later on, alumina was presented to destabilise the basic composition ($Li_2O$–$Si_2O$–$Al_2O_3$). β-Spodumene, a polymorph

of $LiAlSiO_4$, is precipitated to inhabit most of the volume of the glass ceramic, such as in a Pyroceram system. In this system, $Si^{4+}$ is replaced by $Al^{3+}$ in the network structure, and $Li^+$ is held in close proximity to maintain the charge balance. The preceding system was an $MgO–Al_2O_3–SiO_2$ system, in which the lithia is replaced completely by MgO. Nucleation is accomplished by $TiO_2$, $ZrO_2$ and $SnO_2$. In another composition, $Na_2O$ is used to replace the lithia.

Extensive investigation has been carried out on glasses and glass ceramics and fabricated as bioactive or surface-active biomaterials with additions of CaO and $P_2O_5$ to their base compositions. A key advantage of phosphate-based materials is their chemical relationship with carbonated apatite which is one of the main constituents of bone and teeth. The structures of phosphate glasses and glass ceramics are based on the networks of corner-sharing phosphate tetrahedra. In addition, apatite–mullite glass ceramics based on $SiO_2–Al_2O_3–P_2O_5–CaO–CaF_2$ compositions have also been developed and observed to form fluorapatite and mullite with a specific heat treatment procedure.

### 5.3.6 Machinable Glass Ceramics

In order to improve the machinability of glass ceramics, the base composition ($MgO–Al_2O_3–SiO_2$) can be modified by replacing $Li_2O$ with a mixture of $MgF_2$ and $K_2O$. By reheating these specific glasses in the temperature range of 650–1150 °C, machinable glass ceramics are produced with an emphasis of inducing a randomly oriented dispersion of tetra-silicic mica crystals. These crystals have the chemical formula of $KMg_{2.5}Si_4O_{10}F_2$ and a structure similar to the tri-silicic mica fluorophlogopite, $KMg_3AlSi_3O_{10}F_2$. Hence, the structure is analogous to the natural mica mineral phlogopite. It is relatively easy for rotation or cleavage to occur in the $K^+$ planes, and since the crystals in the glass ceramic are in random orientations, the propagating cracks are continuously deflected in different directions within the material which leads to a rapid absorption of the propagation energy. The fracture paths follow the mica–glass interfaces or mica cleavage planes, removing very small fragments during the process, so that a good machined finish is easily obtained [2].

### 5.3.7 Bioglasses and Glass Ceramics

Since the discovery by Hench and Wilson [3] of the bioglasses which bond to living tissue (Bioglass®), various types of bioactive glasses and glass ceramics with different functions such as high machinability, mechanical strength and fast setting ability have been developed.

Glasses that are primarily based on silica ($SiO_2$) which may also contain small amounts of other crystalline phases have been examined for implantation purposes.

The most successful and prominent application is Bioglass®, which is described in detail in various comprehensive reviews [4–6].

The first-generation bioactive glass compositions lie in the $Na_2O$–$CaO$–$P_2O_5$–$SiO_2$ system. In 1971, the first development of such a bioglass began when 45S5 Bioglass® with a composition of 45 % $SiO_2$, 24.5 % CaO, 24.5 % $Na_2O$ and 6 % $P_2O_5$ by weight was proposed [7].

It was suggested by Hench [4] and Vrouwenvelder et al. [8] that when compared to hydroxyapatite (HAp), 45S5 Bioglass® has greater osteoblastic activity which is accredited to a rapid exchange of alkali ions with hydronium ions at the surface. This in turn led to the formation of a silica-rich layer over a period of time. The migration of $Ca^{2+}$ and $PO_4^{3+}$ is permitted on this layer to the silica-rich surface where they combine with soluble calcium and phosphate ions from the solution and the formation of an amorphous $CaO$–$P_2O_5$ layer takes place. Upon the interaction with OH, $CO_3^{2+}$ and F from solution, this layer will then undergo crystallisation. Andersson and Kangasniemi [9] have also observed a similar phenomenon in bioglass with slightly modified compositions.

Glass ceramics from a similar composition with various degrees of crystallinity was prepared by Li et al. [10]. They discovered that the formation of an apatite layer was directly influenced by the amount of glassy phase that still remains, with total inhibition when the glassy phase constitutes less than about 5 weight percent (wt%). These specific glasses (for instance, Bioglass®) have been accepted as bioactive materials as a result of their surface activity; and they have been utilised for non-load-bearing applications. Bioglasses® have been used successfully in clinical applications as artificial middle ear bone implants and alveolar ridge maintenance implants [3] and recently as toothpaste additives.

A bioactive glass with precipitated crystalline apatite and reduced alkaline oxide content can be produced by using a specific heat treatment method. The resultant glass ceramic is referred to as Ceravitals, and it has been shown to have a higher mechanical strength but lower bioactivity compared to Bioglass®.

Kokubo et al. [11] produced a glass ceramic named A-W glass ceramic (Cerabone A-W) that contains oxyfluorapatite ($Ca_{10}(PO_4)_6(OH,F_2)$) and wollastonite ($CaO.SiO_2$) in an $MgO$–$CaO$–$SiO_2$ glassy matrix. It was reported in the early 1990s that the A-W glass ceramic spontaneously bonded to living bone without the formation of fibrous tissue around the glass. They have also developed a bioactive and machinable glass ceramic containing apatite and phlogopite ((Na,K) $Mg_3(Al\text{-}Si_3O_{10})(F)_2$) called Bioverits that were utilised in the past in such clinical applications as the artificial vertebra [11]. Currently the production and application of A-W glass are only restricted to research, whereas its commercial production has been discontinued.

## 5.4 Synthesis of Bioactive Glass

Bioactive glasses have been manufactured using conventional glass technology. The glass components of oxides or carbonates in the form of grains are mixed, melted and then homogenised at a temperature between 1250 and 1400 °C [12]. Bulk implants are produced when the molten glass is cast into steel or graphite moulds. It is often necessary for a final grind and polish to achieve the required tolerances. Even though this process has been changed in many ways in order to avoid grinding and polishing by producing different particle sizes directly, still the processes have many advantages.

Nanoparticles and nanofibres of bioactive glass have been made available several years ago, and they have been used either alone or combined with polymers in the form of a nanocomposite in the biomedical field. In the following section, various processing methods used to fabricate nanoscale bioactive glasses are presented.

### *5.4.1 Microemulsion Techniques*

Microemulsions are thermodynamically stable dispersions of oil and water stabilised by a surfactant and, in many cases, a cosurfactant. The microemulsions can be of the droplet type, either with spherical oil droplets dispersed in a continuous medium of water or vice versa with spherical water droplets dispersed in a continuous medium of oil. Researchers have discovered that the key in controlling polydispersity and nanoparticle size is provided by adjusting the microemulsion and/or operation variables [13, 14]. It has been well known that this method is an ideal technique which is also capable of obtaining nanometre-sized inorganic particles with minimum agglomeration [15, 16].

On the other hand, the main disadvantages of the microemulsion techniques are the usage of a large quantity of oil and surfactant phases and the low yield in production [17]. Only a limited number of papers are currently available on the production of nanosized bioactive particles using this approach even though microemulsion technique provides an alternative means for synthesising several types of organic and inorganic nanometre-sized particles compared to other production methods [18, 19].

### *5.4.2 Laser Spinning Techniques*

Extensive experimental work has been carried out over the past few years in the development of laser spinning techniques with definite control of the results to fabricate tailored products [20–22]. Recently, researchers have for the first time

developed a novel technique for producing bioglass nanofibres, by using 'laser spinning' [23]. In this technique, a small quantity of precursor material is melted using a high-energy laser, to produce a superfine filament that is then lengthened and cooled by a powerful gas current.

The advantages of laser spinning technique include that the process is relatively fast and the nanofibres are produced within several microseconds. It is also able to produce glass nanofibres of compositions that would be difficult to obtain using other methods. The diameters of the fibres produced from laser spinning technique range from hundreds down to tenths of microns; in addition the types of products vary from disordered maths to continuous filaments [21]. On the other hand, the major drawback of laser spinning is that high energy is required during the production process, which consequently increases the production cost.

Laser spinning technique has been demonstrated to be an efficient approach for the production of nanofibres of bioactive glasses and new nanostructures with potential for tissue engineering scaffolds, as fillers in bone defects and as reinforcing agents in nanocomposites. The capability of the laser spinning technique to produce nanofibres with a wide range of compositions makes evident its potential to create nanofibres with different rates of bioresorption to control the release of active ions that have the potential to stimulate the gene expression and cellular response necessary for tissue regeneration [22, 23].

### *5.4.3 Gas Phase (Flame Spray) Synthesis*

Flame spray technology has been used by ancient Chinese in Chinese ink artwork and with painting on cave walls [24]. Currently metal–organic precursor compounds are used by flame spray technology to generate nanoparticles at temperatures above 1000 °C. The formation of molecular nuclei is the basic principle of all gas phase synthesis methods. This is followed by condensation and coalescence that induce the subsequent growth of nanoparticles in high-temperature regions during the process [17, 25].

Numerous studies have been conducted in relations to the understanding of the dynamics and key variables of flame spray process as well as how they can be controlled in order to obtain nanoparticles of given size range and chemical compositions [26, 27]. Athanassiou et al. [27] discovered that the metal–carboxylate system is a very convenient precursor as it permits the synthesis of oxide nanoparticles of almost any composition. Furthermore, metal–organic salts are highly stable in air and tolerate humidity, and above all they are fully miscible among each other. Accordingly, the production of any type of nanoparticulate mixed oxides with high chemical homogeneity is allowed using this process.

Using the flame spray technique, bioactive glass nanoparticles in the 20–50-nm range were successfully produced by Vollenweider et al. [28], and they reported within the dentin samples a pronounced increase in mineral content which suggested rapid remineralisation. Mohn et al. [29] also demonstrated the ability

of the flame spray technique to synthesise radio-opaque bioactive glass nanoparticles for potential root canal application.

Compared to other gas phase techniques, the advantage is the precursors do not require an additional energy despite the fact that the flame spray technique is an energy-intensive approach.

### *5.4.4 Sol–Gel Bioglass*

The sol–gel processing of ceramics and glass materials began more than 150 years ago on silica gel [30, 31]. Preliminary studies on sol–gel indicated that under acidic conditions, the hydrolysis of $Si(OC_2H_5)_4$, tetraethyl orthosilicate (TEOS), resulted in $SiO_2$ in the form of a glass-like material [30] that could be drawn into fibres. In the early days, the silica gels were dried for more than a year in order to avoid the gel fracturing into a fine powder, and because of this, the whole process lost technological interest.

A considerable amount of attention has been attracted as the result of the formation of Liesegang rings [32] which led to numerous investigations carried out by researchers on the problem of the periodic precipitation phenomena which result in the formation of Liesegang rings and the growth of crystals from gels.

Using the sol–gel method, various types of coatings and films have also been developed. In particular are the antireflection coatings of indium tin oxide (ITO) and related compositions applied to glass window panes [33]. Compared with traditional glass melting or ceramic powder methods, the motivation for the sol–gel processing is first and foremost the potentially higher homogeneity and purity and the lower processing temperatures associated with the approach [33].

For the past two decades, the production of bioglass using the sol–gel process has become an interesting research field [33–38]. Sol–gel process involves the synthesis of an inorganic network by mixing the metal alkoxides in solution, followed by hydrolysis, gelation and low-temperature firing to produce a dense and stable glass powder. The network structure of the gel can be modified by controlling hydrolysis and polycondensation reactions during productions. Hence, structural variation can be produced without compositional changes (c.f. Fig. 5.1).

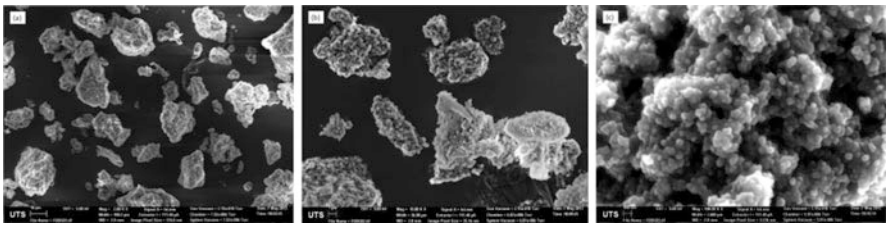

**Fig. 5.1** SEM images of sol–gel-derived agglomerated 45S5 Bioglass® nanoparticles at (**a**) magnification *x*2, (**b**) magnification × 10 and (**c**) magnification × 100

Using gels, bioactive glasses can be prepared by sintering at temperatures between 600 and 700 °C, which reduces most of the disadvantages of high temperature processing with much better control over purity. Furthermore, by either modifying the microstructure or composition through processing parameters, a broader range as well as better control of bioactivity can be achieved [39].

Li et al. [12] reported that $SiO_2$–CaO–$P_2O_5$ powders produced by sol–gel are more bioactive than the melt-derived glasses of the same composition. In addition, Sepulveda et al. [40] examined the rates of dissolution and formations of surface layer on sol–gel and melt-derived bioglass, and they noticed the melt-derived 45S5 Bioglass® exhibited a lower rate than the 58S sol–gel bioglass powder. The high bioactivity of the sol–gel-derived materials is related to the microstructural features of the gels, i.e. grain and pore size associated with the large surface area, higher rate of dissolution and negative surface charge [41]. Furthermore, the sol–gel-derived bioactive glass has been proposed as alternative to glasses produced by melt and quenching methods, as they exhibit excellent degradation/resorption properties, more rapid bone bonding, improved homogeneity and purity and higher rate of apatite layer formation [12].

## 5.5 Biological and Adhesion Properties of Bioactive Glass

A certain compositional range of bioactive glasses containing $SiO_2$, $Na_2O$, CaO and $P_2O_5$ in specific proportions has demonstrated proper bonding of glass to bone. As mentioned earlier, there are three compositional changes separating them from soda–lime–silica glasses: high $Na_2O$ and CaO content, less than 60 % $SiO_2$ and a high CaO/$P_2O_5$ ratio. Highly reactive surfaces are created from these compositional features when exposed to an aqueous medium. On the other hand, the amount of $SiO_2$ in bioactive glasses ranging between 45 and 60 % and as a result of repeated hot working can easily lead to problems in the formation of phase separation and crystallisation of the glassy material [7, 42]. Crystallisation of the material can cause a reduction in the rate of bioactivity of the glass [43], and a glassy phase of incontrollable composition is the result of partial crystallisation. Crystallisation of a bioactive glass can be controlled by its chemical composition [44, 45].

It has been reported that a new generation of bioactive glasses in the $Na_2O$–$K_2O$–MgO–CaO–$B_2O_3$–$P_2O_5$–$SiO_2$ system can be repeatedly heated without the risk of devitrification [46]. Hence, microspheres can be produced and sintered into porous implants of different shapes and sizes [47]. The porosity of a bioactive glass body does not only noticeably increase the total reacting surface of the glass but also allows a three-dimensional formation of the healing bony tissue. The mechanical strength and porosity of the bioactive glass implants can be controlled with different sintering times and temperatures [48]. To achieve the best mechanical strength of the sintered implant, the glass must retain its amorphous structure during the heat treatment.