Alumina applied in bone regeneration: porous α-alumina and transition alumina

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Abstract

Alumina is a polymorphic bioceramic that has been extensively investigated for application in bone regeneration. Dense α-alumina has been considered a suitable biomaterial for dental and orthopedic implants due to its superior mechanical properties. However, its use is limited due to its high inertia in a biological environment. Recent investigations have focused on its distinct phases and surface characteristics through the control of morphology, physical properties, and chemical composition to enhance bioactivity. This article presents a brief review of the developments in porous α-alumina and amorphous-γ-alumina transition. Most studies have shown that composites and alumina coated with bioactive materials, high surface area, and hydroxylated surface can significantly improve biological properties. Cellular responses such as fixation, growth, and proliferation, as well as biomineralization, are the main studies to validate improvements in bioactive properties.
Keywords: porous α-alumina, alumina transition, bioactivity, bone tissue.

INTRODUCTION

Biomaterials have been extensively studied due to their ability to repair bone defects. This is a common clinical problem that has grown significantly worldwide [1] as a result of trauma, cancer, infection, and arthritis [2]. Those materials can be constituted of biometal [3], biopolymer [4], bioceramic [5], and biocomposites [6]. Bioceramics materials are used in many medical procedures. They are constituted of biocompatible ceramic that ranges from bioinert to bioactive materials [7, 8]. The latter interacts with the body tissues by chemical bonding between them [9]. Bioceramics materials can be resorbable and non-resorbable. For resorbable, the material serves as a temporary scaffold, which allows the regeneration and formation of new bone tissue, and it is later replaced by the body without the need for surgery to remove it [10]. On the other hand, bioinert materials do not interact with the body tissue and the corporal biological environment [9]. However, they play an important role in bone implants due to their high chemical stability and excellent mechanical properties [7]. Among inert bioceramics, alpha-alumina (α-Al_2O_3) has received considerable attention in medical and dental implants for many decades [11, 12]. In general, an alumina device with acceptable implant properties has a surface coated with bioactive and bioabsorbable materials [13-16]. In addition, biocomposites with bioactive material

and bioinert alpha-alumina have been also extensively observed [9, 17-19]. This is essential to make a device that integrates into the host tissue before the fibrous capsule formation [9].

The great interest in alumina-based materials in the last century is related to the improvement of the mechanical properties of the final material [20], in which the alumina content ensures biocompatibility, bioinertia, and mechanical strength, while a coating/presence of bioactive material is responsible for bioactivity [21]. However, modifying alpha-alumina surfaces such as topography and chemical properties may influence initial cellular behavior, resulting in proliferation, adhesion, and differentiation of osteoblast-like cells [22] and fibroblastic cells [23]. Despite several efforts involving the use of alumina-based materials in bone repair devices, there are still debates about the effect of alumina on bioactivity and osseointegration. Alumina varies from the amorphous phase to α-alumina (the most thermodynamically stable crystalline phase), where other metastable crystalline forms are also possible (χ, η, δ, $\varkappa$, θ, γ, ϱ), which are commonly called transition aluminas [24]. They differ in surface features, notably topography, surface area, and reactivity.

The most reported alumina phase is α-alumina [25], and recently porous α-alumina has been used as support for bioactive materials in composite systems. On the other hand, γ-alumina has a particular interest because of its high specific surface area, pore morphology, and nanometric surface roughness [26]. These properties provide a certain activity for γ-alumina, which promotes an increase in the bioactivity

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of the material. Moreover, amorphous alumina, as well as γ-alumina, has a high specific surface area [27] and presented bioactivity in several studies. These findings indicate that the most important factors to improve the properties of alumina as a biomaterial are the increase in specific surface area, roughness, and porosity at the micro- and nano-scale. These characteristics have been the reason for several efforts to expand the application of alumina through the modification of its physical surface structure. However, no recent work has tried to cover the new directions of alumina contributing to the increased bioactivity of biomaterials. Then, in this review, we highlight a brief overview of amorphous/γ-alumina and porous α-alumina materials, emphasizing their possible bioactive response of bone cells for application in bone tissue regeneration.

Therefore, the search was carried out in the Web of Science Core Collection (Clarivate Analytics). It was first provided a search of publications between 2010 and 2021 using as keywords: alumina and bone (704 articles), Al_2O_3 and bone (358 articles), alumina and scaffolds (0 articles), Al_2O_3 and scaffolds (267 articles), alumina and biomaterial (138 articles), Al_2O_3 and biomaterial (97 articles), alumina and bioactivity (156 articles), and Al_2O_3 and bioactivity (150 articles). The exclusion criteria were as follows: repeated articles in the listing; articles that have not been published in journals (such as conference papers, retractions, congress abstracts, etc.), and articles that did not have at least two keywords in the abstract besides the keyword bone. A total of 298 articles was reached. The abstracts of these articles were evaluated by two independent reviewers to determine whether or not the articles were related to the topic of the proposed review article. The articles that were selected by the two reviewers were considered for the next step. Then, reviewers and authors discussed the relevance of each article and which ones should be part of this article. The selected articles were all read by the authors and another exclusion criterion was applied: articles without a clear description of the type of alumina used or that did not present data that could determine the type of alumina studied/analyzed. It should be noted that articles before 2010 were cited in this review article due to the need to substantiate the information mentioned throughout the text.

ALUMINA

Alumina, also known as corundum [28], is a ceramic material widely used in various applications due to its excellent mechanical and physicochemical properties, such as high hardness, high wear resistance, low thermal conductivity, high corrosion resistance, high chemical and electrical resistance [24, 28, 29]. In most published papers, authors refer to the α-alumina (α-Al_2O_3) phase simply as alumina (Al_2O_3) or corundum. Nevertheless, alumina can feature amorphous and several polymorphic phases depending on the starting minerals and the synthesis temperature, as illustrated in Fig. 1 [30-32]. As can be seen, for the diaspore mineral, the alpha phase forms at

approximately 550 °C, and no polymorphism is observed. While for the other minerals, the presence of polymorphic alumina is observed and the metastable form transforms into the α phase from 1100 to 1200 °C through different transition sequences [25]. These sequences of crystalline structure transformations can vary depending on the precursor used, the particle size of the original material, the presence of impurities, and temperature conditions of calcination/process, which notably affect the agglomeration state and/or aggregation of the particles and their sizes [33].
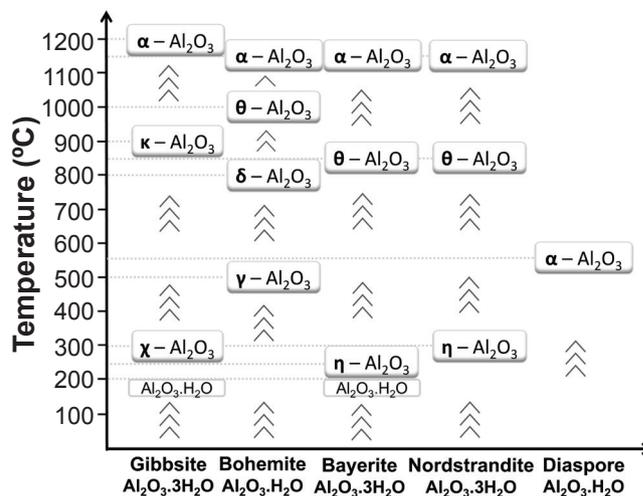
Figure 1: Phase transformations of aluminum hydroxide from different minerals as a function of calcination temperature (based on data of refs. [30-32]).

The metastable crystallographic forms are also called transition alumina (χ, η, δ, ϰ, θ, γ, ϱ) and present crystalline structures and morphology very different from α-alumina. Although the alpha phase is thermodynamically the most stable, the other phases also have great technological importance due to their high specific surface area and the nature of their surfaces, which give them important properties for applications such as catalyst support [34], paint formulations [35], water treatment [36] and biomaterials [37]. As a biomaterial, several studies have been carried out to analyze the interaction between alumina and the components of the body [38, 39]. Most studies are focused on bone regeneration and many *in vitro* studies using alumina are based primarily on cell adhesion and functionality. Several types of cells have been used for these tests, such as macrophages, fibroblasts, osteoblasts, and pre-osteoblasts, where cytotoxicity is assessed by cell culture, immunocytochemical analysis, and protein synthesis or bone markers, such as collagen and alkaline phosphatase. Studies have found that there is no toxicity of alumina against cells growing on it [40]; however, biological responses depend on the characteristics of alumina and the presence or absence of bioactive agents, forming composites with alumina.

In addition to the aforementioned properties, α-alumina and amorphous-γ-alumina have been recognized for clinical applications because of their biocompatibility and long-term

Table I - Alumina-based materials with potential bioactive features.

Alumina phase	Associated component	Bioactive propose	Ref.
Porous alumina	Coated with hydroxyapatite and bioglass	Osseointegration	[7]
Porous alumina	Coated with hydroxyapatite and bioglass	*In vivo* (rat tibiae/osseointegration)	[9]
Porous α-alumina	Coated with tricalcium phosphate	Bioactivity, osteoconductivity, and cellular proliferation	[11]
Porous alumina	Coated with carbonated hydroxyapatite + Au	*In vitro* cell viability and cells growth (human osteoblast cell line, HFB4)	[12]
Nanowhisker alumina	Reinforcement within poly(e-caprolactone)	Cell adhesion and proliferation (human bone marrow stromal cells, hMSCs)	[13]
Alumina powder	Added to hydroxyapatite composite	*In vitro* apatite-forming-ability (SBF)	[14]
Porous alumina	Composite with hydroxyapatite	*In vitro* apatite-forming-ability (SBF)	[15]
Dense alumina	Chitosan-coated	HGF-1 cell proliferation	[16]
Porous α-alumina powder	Composite with bovine hydroxyapatite	*In vitro* cell viability (L929 cell line)	[17]
Porous alumina nanoparticle	Silk fibroin blend	Osteogenic differentiation of rabbit adipose-derived stem cell	[19]
Alumina nanowire	Filler for poly(3-hydroxybutyrate)-chitosan composite	Proliferation and viability of MG-63 cell and alkaline phosphatase secretion	[20]
Nanopatterned alumina	-	*In vitro* proliferation of MG-63 cell	[22]
Alumina nanoparticle	Ultra-high molecular weight polyethylene, zirconia	*In vitro* fibroblastic cell line NHDF viability	[23]
α-alumina powder	Seeding agent for hydroxyapatite and bioglass composite	*In vitro* bioactivity in phosphate-buffered saline (PBS) solution	[28]
Dense α-alumina powder	Selenium-doped carbonated hydroxyapatite	-	[42]
Nanoporous alumina	-	*In vivo* inflammatory response after subcutaneous implantation (RayBioVR Mouse Inflammation Antibody Array G series I)	[46]
Alumina	Coated with hydroxyapatite	*In vitro* cell viability and cell growth human fibroblast cell line	[47]
γ-alumina buffer layer	Nano-structured hydroxyapatite composite	-	[48]
Porous alumina	Coated with fluorapatite	-	[49]
Porous alumina	Al_2O_3-doped Ta_2O_5 film	*In vitro* human osteoblast-like MG-63 cell culture, adhesion, and proliferation	[50]
Nanoporous α-alumina	-	Cell adhesion, viability, and osteogenic potential pre-osteoblastic MC3T3-E1 cell	[51]
Porous alumina	Calcium phosphate and strontium ion	-	[52]
α-alumina particle	Hydroxyapatite/poly-ε-caprolactone composite	Dermal fibroblast normal human, neonatal (HDFn) cell culture and viability	[53]
Porous α-alumina	Fluorapatite + TiO_2 composite	-	[54]
Porous alumina	Coated with platinum, titanium, and tantalum	Cell behavior fibroblast and hematopoietic stem cell (HSC)	[55]
Nano alumina powder	Hydroxyapatite	Cell culture and plasma protein adsorption	[56]
Porous alumina	Hydroxyapatite	-	[57]
Nano γ-alumina	Silk fibroin/chitosan composite	*In vitro* biodegradation and biomineralization	[58]

stability in wet physiological environments [41, 42]. These factors are important because the human body possesses a highly corrosive environment. Then, it is no observed biological rejection in the body during the application/use of alumina. However, the most use of α-alumina is as an implant in a conventional form, which is unable of binding to soft and hard tissues, and it does not interact with body fluids to promote apatite deposition and osseointegration [41, 43]. The chemical stability of α-alumina results in a very thin fibrous layer covering it [43, 44]. As a result, α-alumina implants can undergo loosening and develop fibrosis, also known as fibrous encapsulation. This is a protective mechanism, where fibrous tissue forms to isolate the implant from the biological system, which may lead to complete encapsulation of the implant over time [45]. Therefore, it is reason that the use of pure α-alumina is a big challenge, and the great interest to use alumina is as a base for bioactive composites or as a support for the deposition of bioactive materials [21].

Table I lists studies on γ and α dense alumina and porous alumina pure or with bioactive materials that focus on their potential biological applications. Hydroxyapatite is the most common component in alumina compounds/composites and is used both as a coating and as a component for alumina composite. It is also observed that monolithic alumina without any bioactive component presented bioactive features. However, in these cases, cell response is strongly influenced by nanoscale surface features. For instance, nanoporous anodic alumina (pore size 200 nm) presented an important role in inflammatory cell response [46], and alumina with nanoscale topographical features with low aspect ratios was able to enhance the proliferation of the osteoblast-like cell line MG-63 [22].

Porous α-alumina and α-alumina composites

Dense α-alumina surfaces do not promote bone union due to their bioinertness. Efforts have been made to make it bioactive by the use of bioactive materials in the development of coatings, modified surfaces, or composite systems. In all cases, the main objective is to develop an innovative biomaterial that combines superior mechanical and bioactive properties. Modified surfaces can be achieved by generating a hydroxylated surface [59]. For hydroxylated surface, an increase in aluminol, Al-OH, groups under specific conditions are achieved and these groups promote bioactivity in cell culture tests [59] and become nucleation centers for apatite growth [41]. For instance, Alonso et al. [41] reported the nucleation and growth of apatite using a sintered α-alumina subjected to acid 'piranha' treatment. This pretreatment generates a highly hydroxylated surface, which increases the capacity of α-alumina to adsorb Ca ions. This modification was an important condition for calcium phosphate nucleation and apatite growth on alumina *in vitro*. The results suggest that pretreatment with the piranha solution seems to be a fast and efficient alternative to bioactivate the α-alumina surface.

At the end of the last century and the beginning of this century (1998-2001), Webster et al. [60-63] started several studies involving the adhesion of osteoblasts to the alumina surface with nanometric grain sizes. Although these studies provide evidence that nanophase alumina can promote osseointegration, only in the last 10 years, there has been researched into the use of nanostructured alumina for more effective bone regeneration purposes. Furthermore, there is no review that emphasizes the alumina nanostructure to enhance its bioactivity properties. In biocomposites, α-alumina serves as a matrix or even as a filler to enhance the mechanical properties of biopolymer matrices, because of its high Young's modulus and tensile strength. Normally, alumina used as reinforcement for biocomposites is at the nanometer scale, such as nanoparticles, nanowhiskers [13], nanowires [21], and nanofibers to improve tissue structure. Dong et al. [13] introduced Al_2O_3 nanowhiskers into the poly(e-caprolactone) (PCL) matrix. Al_2O_3 nanowhiskers at low concentrations were uniformly distributed in a polymer matrix, leading to entanglements and good interfacial adhesion between the components. In comparison with pure PCL, the mechanical properties of the composite improved significantly. Tensile strength increased from 3.4 to 7.3 MPa and the tensile modulus increased from 8.5 to 16.1 MPa when 10 wt% Al_2O_3 whiskers were added. Even though no differences in hMSC 5077-GFP cell morphology and cell density were observed, the authors conclude that the Al_2O_3-whisker reinforced the PCL membrane and supported cell attachment and growth.

Recently, Toloue et al. [20] showed a bioactivity character of nanostructured alumina-based material. They prepared scaffolds of polyhydroxybutyrate-chitosan/alumina (PHB-CTS/alumina) for tissue engineering. The alumina nanowires content varied from 1-5 wt%. Results demonstrated that 3 wt% of alumina were adequate to increase up to >10 fold the tensile strength of the PHB-CTS scaffold. PHB, PHB-CTS, and PHB-CTS/alumina scaffolds were compared to their bioactivity, MG-63 cells proliferation and viability, and alkaline phosphatase secretion. It was observed that the formation of calcium phosphate sediments occurred only on the surface of alumina-containing scaffolds after 7 and 28 days of immersion in simulated body fluid (SBF). For the other tests, the results showed that proliferation and viability of MG-63 cells and alkaline phosphatase secretion essay were significantly higher on scaffolds containing alumina than that of the PHB or PHB-CTS. Thus, the authors concluded that alumina nanowires influenced cellular behavior, and improved the bioactivity and the mechanical properties of the scaffold. Unlike the bulk form, nano alumina has shown bioactive nature [56]. This evidences its importance as a biomaterial for tissue engineering. However, the content of nanostructured alumina is limited to a low concentration to avoid agglomeration and, consequently, to reduce its properties. The surface features on the proliferation of osteoblast-like cell line MG-63 were also strongly influenced by nanoscale surface features with low aspect ratios <0.1 [22]. In this case, Wittenbrink et al. [22] irradiated ultra-flat

single-crystalline Al_2O_3 substrates with low-energy noble gas ions to induce the self-organized formation of a periodic ripple pattern. The patterns with a periodicity of 179 nm (height 11.5 nm) showed significantly higher cell viability after days 3 and 7 compared to nanoripples with 24 nm (height 0.7 nm) periodicity and to the polished sample. The authors concluded that controlling the surface topography of alumina substrate at the nanoscale is an important step in tailoring the cellular response.

Another way of producing α-alumina with bioactive properties is related to the control of the pore content of the device, leading to better bone bonding. Thus, dense α-alumina bioceramics are chosen for load-bearing applications, while porous α-alumina-based bioceramics are more suitable for accelerating bone tissue growth [64]. The surface properties of porous α-alumina have been adjusted to a micro-scale to produce an optimal biocompatible and bioactive material. The most common porous structures used as supports in bone repair or regeneration of damaged tissue are scaffolds [8], owing to their similarity to trabecular bone structure [11] and their topographically patterned surfaces that are similar to the extracellular matrix (ECM). They have a 3D structure that promotes new tissue ingrowth, cell adhesion, and cell growth and allows nutrients and oxygen to flow within the pores [42]. The porosity is a factor of great importance for the performance of the material in the ability of infiltration and proliferation of cells. The size and amount of the pores must be carefully controlled. This means that the pore size should be suitable for cell proliferation and growth [58] and the porosity must be as high as necessary for a good performance of biological activities due to cell size, migration requirements, and transport. In addition, small pores may impair tissue oxygenation leading to a hypoxic condition. However, the porosity should not be so high as to impair the mechanical properties of the scaffold [14]. In some cases, an ideal pore size ranges from 100 to 400 μm and volumetric porosity of 70-90% [11].

Costa et al. [64] were one of the first researchers to develop micro-macroporous alpha-alumina scaffolds with high potential to be used in bone tissue engineering. The scaffolds were coated with calcium phosphate doped with Zn^{2+} and showed pore sizes from 150 to 800 μm. Biocompatibility was evaluated by VERO cell spreading and fixation assays. SEM imaging revealed the propagation and adhesion of VERO cells on the surface of the scaffold. In addition, the samples showed good biocompatibility and no cytotoxicity. Years later, Kim et al. [11] developed both uncoated alumina and tricalcium phosphate (TCP)-coated alumina scaffold with the classic 3D sponge-like open cellular morphology. Most pore sizes were found to range from 300 to 400 μm. They showed to be highly interconnected and homogeneously dispersed throughout the scaffold. *In vitro* studies demonstrated both pore size and surface roughness of alumina-based scaffold had a significant effect on adhesion, proliferation, and differentiation of human osteoblast-like cells (MG-63 cells). The scaffold was also biocompatible with fibroblast L-929 cells. Cell growth was supported *in vitro* and no cytotoxicity was observed.

Pazarlioglu and Salman [14] evaluated the effect of 5% of alumina (Al_2O_3) additive on *in vitro* bioactivity properties of hydroxyapatite (HA) composite. They mixed commercial alumina and HA powders, with grain size at micro-scale, using a conventional ball milling at 180 rpm for 2 h to obtain the HA-Al_2O_3 composites. Based on the highest mechanical strength values, they chose HA-Al_2O_3 composites sintered at 1200 °C. SBF solution was used to investigate the bioactivity property. Results demonstrated that apatite layers covered the entire surface formed on HA-Al_2O_3 composites only after 30 days of immersion into SBF as it was in the monolithic HA. Nevertheless, Bartonickova et al. [15] produced a hydroxyapatite HA-alumina scaffold having a composition of HA/alumina=90/10 (w/w) with a porosity of about 64%. They mixed synthesized HA powder and commercial alumina powder with a foaming agent to the prepared suspensions, which were magnetic stirred to initialize the *in situ* foaming process. Then, dried samples were sintered at 1250 °C. For bioactivity analysis, the *in vitro* asses also using SBF solution showed that HA/alumina composite was significantly more covered in comparison with pure HA (porosity of about 46%) after 28 days of immersion. These differences mean that the biological behavior depends on the preparation method, chemical composition, and surface properties of hydroxyapatite-alumina binary ceramics. However, both two mentioned *in vitro* testing indicated that the HA-alumina composite has suitable application for tissue engineering.

Bioglass has also been used to coat porous alumina scaffolds. Kido et al. [7] and Camilo et al. [9] proposed an approach to evaluate the effects of porous alpha-alumina coated with bioglass and hydroxyapatite (HA) on bone regeneration. They implanted the substrates in a model of rat tibial bone. Kido et al. [7] investigated their effects on bone regeneration for 30, 60, 90, and 180 days and genotoxicity for blood, liver, and kidney cells. The results indicated that bioglass/HA coated alumina implants did not possess any genotoxic potential. After 180 days, the newly formed bone significantly increased. Then, the scaffolds produced were able to stimulate bone formation and growth in rat tibial defect models. Camilo et al. [9] investigated their effects on bone regeneration only for 28 days. Histological analysis was carried out and images show direct contact between bone and scaffold implanted after 28 days. The new bone tissue was observed on the surface and in the pores containing sizes in the range of 100-400 μm. All results indicated that bioglass/HA coated alumina implants may be promising candidates to promote osseointegration and bone regeneration.

Zirconia is another biomaterial able to form a composite with alumina. Hadjicharalambous et al. [65] prepared porous sintered alumina, zirconia, and zirconia/alumina [80 wt% Zr(Y)O_2-20 wt% Al_2O_3] composite with similar porosity (50-60%). They investigated their mechanical properties and the extent of MC3T3-E1 (osteoblast-like) cells from newborn mouse calvaria response by *in vitro* assay. Results demonstrated that after 7 days of cell culture,

all three materials, including pure porous alumina, have proliferated significantly and formed a layer of living cells. Stanciuc et al. [66] also produced zirconia-reinforced alpha-alumina scaffolds (porous systems) to verify processing, characterization, and interaction with human primary osteoblasts. Scaffolds with pore sizes of 273±15 μm were obtained by the robocasting method followed by sintering at 1350 °C. Primary osteoblast cells were cultured in the scaffolds for 30 days, being homogeneously distributed throughout the entire thickness of the scaffold. The study carried out indicated that the scaffolds can serve as a substrate for the growth of bone cells.

For alumina with an organic molecule, a blend with silk fibroin (SF)/alumina nanoparticles scaffold was developed by Zafar et al. [19]. They assessed the osteogenic differentiation of rabbit adipose-derived stem cells (rADSCs). The content of alumina played an important role in the physicochemical properties of the bulk SF scaffold, which did not show a toxicity effect on the proliferation and attachment of rADSCs *in vitro*. The microporous structure of the scaffold with 10% of alumina had acceptable mechanical strength, water uptake capacity, and biomineralization capability. Moreover, SF scaffold containing 10% NA encouraged cells to produce more ALP and deposit calcium phosphate. Thus, it confirms that alumina serves as a suitable component for SF compounds, which made it a good substrate for proliferation and osteogenic differentiation of ADSCs applicable for bone tissue engineering.

Amorphous and γ alumina

The most popular process to produce amorphous nanoporous alumina for tissue engineering application is by electrochemical anodization of pure aluminum and aluminum alloys [50]. In this process, a thin film of the aluminum substrate is immersed into an acid electrolyte (mainly sulphuric acid) and anodized under controlled conditions [67]. Tunable morphology, pore diameters, and depths may be obtained by varying the electrical conditions, temperature, and the nature of anodizing solution [50]. Nanoporous anodic aluminum oxide (AAO), also known as porous-anodic-alumina (PAA) provides a useful substrate in tissue engineering with a suitable environment for tissue migration and proliferation. It can be coated with bioactive materials, combined with other biocompatible materials, and also used as a 'mold' for biodegradable materials [18]. Ferraz et al. [68] studied the effects of alumina nanotopography on monocyte/macrophage responses. They cultured human mononuclear cells on anodized alumina substrates with pore diameters in the range of 20-200 nm. Cell adhesion and cell viability results indicated that a significant difference in cell responses and cytokine release was porosity-dependent. Based on the results, when increasing the pore size, few cells were detected on the alumina surface. However, a moderately higher number of cells were detected on alumina surfaces with 20 nm porous structures.

Domagalski et al. [67] manufactured anodic alumina nanotubes (AANTs) with varying pulse periods, current density levels, and various post-anodization treatments under galvanostatic mode in sulfuric acid electrolyte modified with 10% (v) ethanol. The geometric features and physicochemical properties of the alumina nanotubes were achieved by the combination of different anodization conditions. *In vitro* cell cultures have demonstrated the suitability of porous alumina for the growth of osteoblastic cells. Nano-porous alumina was also produced by anodization of aluminum oxide (AAO) on a titanium substrate by Walpole et al. [43]. These authors produced two different pore sizes and porosities on anodic alumina that was carried out by varying the voltage of the anodization process such as 25 and 160 V in phosphoric acid. Anodized alumina was also obtained varying the potential at 5, 10, and 20 V and then re-anodized, respectively, at 8, 15, and 25 V. These samples were self-organized arrays of nano-mounds that were synthesized from the mixture of Al_2O_3 and Ta_2O_5 by anodization process by Fohlerova et al. [50]. The nano-mounds systematically increased from 10 to 40 nm as the potential was increased. To assess the influence of the nano-mound size on cell growth, differentiation, and proliferation, MG-63 osteoblast-like cells were investigated under supplementation with 10% fetal bovine serum (FBS) and serum-free media. In the presence of FBS, cells showed better initial adhesion on the lowest nano-mound (10 nm). In the absence of FBS, the adhesion, proliferation, and cell growth were greater on the nano-mounds with 20 and 40 nm. Fohlerova et al. [50] stated that the cells were able to sensitively recognize the features of surface structuring without pre-adsorbed proteins. They attributed this to the relation between the composition, sizes, and spacing of the mixed oxide nano-mounds. Commercial nanoporous anodized alumina membranes with different pore sizes around 200, 100, and 20 nm were studied by Song et al. [69]. The effects of nanoporous alumina substrates on the viability of MG-63 cells cultured were compared with cover glass. Results indicated that smaller pore-sized alumina (20 nm) had more cell numbers of MG-63 on the surface, making it more advantageous for cell growth. The same behavior was observed for cell proliferation and adhesion, which increased as decreased the pore size. In contrast to cell number analysis, after incubation for 4 weeks, the mineralization was increased with the increase of pore size, and the highest was detected in cells cultured on 200 nm sized alumina substrate. Results also showed that MG-63 cells viability on nanoporous alumina substrates was higher than that of the traditional bioactive cover glass.

In addition to the anodizing process, high-purity amorphous and γ-alumina phases can be acquired by the calcination process of boehmite [AlO(OH)] or bayerite [Al(OH)$_3$] obtained from a wet chemical route, with the hydrolysis of aluminum alkoxides [70]. The desired phase is achieved by controlling the temperature of calcination. Karin [27] synthesized γ-alumina with an anionic charged surface and with an amorphous and nanoporous character. Due to its high surface area and hydroxyl-rich surface, γ-alumina showed high adsorption capacity for cationic organic

molecules. Thus, the author suggested that this material has great potential to find biomedical applications, in addition to being able to be modified or functionalized to diversify its applications. The structural properties of γ-alumina also depend on the method of synthesis and the nature of the aluminum precursors. Two different mesoporous γ-alumina was obtained by calcining γ-AlOOH which was synthesized by using aluminum chloride and aluminum nitrate, both with HMTA ($C_6H_{12}N_4$) as precipitant. Short nanofiber morphology was obtained for both samples. However, their sizes are slightly different, where, the length of nanofibers is in ranges from 200 to 500 nm, and the diameter is about 50-100 nm. These samples show BET surface area of 200.898 and 273.302 $m^2.g^{-1}$ and pore volume of 0.121 and 0.682 $cm^3.g^{-1}$, respectively. In this study, mesoporous γ-alumina was also obtained by calcining γ-AlOOH which was synthesized by using aluminum sulfate as an Al source. Different kinds of precipitants such as sodium carbonate and urea were used. Cluster-shaped and flower-like morphology were observed when the precipitant was selected as sodium carbonate and urea, respectively. For these samples, BET surface area of 206.089 and 212.236 $m^2.g^{-1}$ and pore volume of 0.290 and 0.723 $cm^3.g^{-1}$, respectively, were observed. All results revealed that the kinds of Al resources and even precipitant agents can control the morphologies and physical properties of γ-alumina effectively [71].

Moreover, a high specific surface area also can be attributed to the small particle size, pore morphology, and nanometric surface roughness [26]. These properties provide certain bioactivity for γ-alumina. As well as γ-alumina, the amorphous phase also has a high specific surface area. Although both phases have similar properties, for some processes amorphous alumina is the most indicated due to its greater reactivity. These findings suggest that the most important factors to improve the properties of alumina as a biomaterial are the increase in specific surface area, roughness, and porosity. As a biomaterial, the high surface area of γ-alumina has a very important role in apatite formation due to its high content of Al-OH groups. This is because γ-alumina hydrates easily at room temperature to form $Al(OH)_3$. Under specific pH, AlO$^-$ sites are formed by Al-OH groups dissociated and calcium ions, Ca^{++}, are attached to alumina by ion exchange on Al-OH to form Al-O-Ca$^+$. Then, at high pH, phosphate ion (PO_4^-) can be adsorbed to form Al-O-Ca-PO_4 [40]. On the other hand, the mechanism described by Bartonickova et al. [15] shows that the apatite formation on a hydroxyapatite/alumina composite surface is the ion exchange on Al-OH groups in presence of phosphoric acid ($H_2PO_4^{2-}$), with PO_4^- interacting to form Al-O-PO_3^- + OH^-.

Teimouri et al. [58] produced a porous scaffold of γ-alumina-based composite. The scaffold was prepared by freeze drying and was constituted with silk fibroin/chitosan/nano γ-alumina composite. They varied the γ-alumina nanopowders content from 5% to 15%. The results showed that the higher the γ-alumina content, the greater compressive strength and modulus, and water-uptake capacity. For cell viability tests, γ-alumina-based composite showed to be higher than that without γ-alumina, as fibroin/chitosan scaffold blended. They attributed that the proliferation of HGF fibroblasts cells was more active within the silk fibroin/chitosan/nano γ-alumina composite scaffolds, due to the formation of appropriate active binding sites for proteins during culture periods, resulting in a stimulation of cellular proliferation more efficiently. Results also indicated that the inclusion of γ-alumina enhanced the biomineralization and bioactivity of the scaffold. Then, a bone-like apatite layer was formed on the surface of the composite scaffolds after soaking in simulated body fluid (SBF). For cell attachment, the presence of 15% of γ-alumina also showed great results. Moreover, cells seeded onto the SF/CS/nano γ-alumina scaffold were found to be non-toxic. Thus, according to the authors, nano γ-alumina as a component for the silk fibroin/chitosan composite improved the characteristics of the scaffold, making it a suitable material for tissue engineering applications.

Despite the properties of amorphous and γ-alumina substrates providing a bioactive character, and being interesting support to apatite deposition and cell attachment, proliferation, and differentiation, there are still few published studies focusing on it and more studies are needed for a better evaluation and understanding of the bioactive character of these materials.

CONCLUSIONS

Alumina-based materials are interesting inert bioceramics, which are easily manipulated to promote bioactivity. These materials can be produced with distinct phase and surface structures by controlling the synthesis conditions and calcination temperature. The main technological phases obtained are amorphous, γ-alumina, and α-alumina. For inert α-alumina, the most useful approach for tissue engineering is porous scaffold design, which can be combined with bioactive materials and their surface morphologies can be tailored to better interact with bone tissue and biological fluids, resulting in improved cell responses. On the other hand, pure amorphous and γ-alumina present an active surface due to their high surface area and hydroxylated surface character, which can promote bonding with several ions and compounds. The control of porosity and pore sizes are also possible and are important to improve cell responses. Results reported for these three alumina phases include fixation, viability, growth, and proliferation of osteoblastic cells, especially MG-63-like cells, in addition to apatite deposition in contact with SBF. Therefore, alumina-based biomaterials can be bioactive, biocompatible and a promising candidate for bone tissue regeneration.

ACKNOWLEDGMENTS

REFERENCES

[1] S.S. Pazarlioglu, S. Salman, J. Aust. Ceram. Soc. **56**, 2 (2020) 413.

[2] G. Turnbull, J. Clarke, F. Picard, P. Riches, L. Jia, F. Han, B. Li, W. Shu, Bioact. Mater. **3** (2018) 278.

[3] Z. Zheng, B. Chen, N. Fritz, Y. Gurumukhi, J. Cook, M. Ates, N. Miljkovic, P. Braun, P. Wang, J. Energy Storage **30** (2020) 101502.

[4] Y. Luo, F. Xue, K. Liu, B. Li, C. Fu, J. Ding, Mater. Des. **201**, 3 (2021) 109484.

[5] C. Paredes, F. Martínez-Vazquez, H. Elsayed, P. Colombo, A. Pajares, P. Miranda, J. Eur. Ceram. Soc. **41** (2021) 892.

[6] X. Li, Y. Xiong, H. Zhang, R. Gao, Mater. Lett. **282** (2021) 128670.

[7] H.W. Kido, D.A. Ribeiro, P.D. Oliveira, N.A. Parizotto, C.C. Camilo, C.A. Fortulan, E. Marcantonio, V.H.P.D. Silva, A.C. Muniz Renno, J. Biomed. Mater. Res. A **102**, 7 (2014) 2072.

[8] E. Cichoń, K. Haraźna, S. Skibiński, T. Witko, A. Zima, A. Ślósarczyk, M. Zimowska, M. Witko, B. Leszczyński, A. Wróbel, M. Guzik, J. Mech. Behav. Biomed. Mater. **98** (2019) 235.

[9] C.C. Camilo, C.A.E. Silveira, R.S. Faeda, J.M.D.D. Almeida Rollo, B.D.M. Purquerio, C.A. Fortulan, J. Appl. Biomater. Funct. Mater. **15**, 3 (2017) 223.

[10] Y.C. Chen, P.Y. Hsu, W.H. Tuan, C.Y. Chen, C.J. Wu, P.L. Lai, J. Mater. Med. **32**, 13 (2021) 12.

[11] Y.H. Kim, J.M. Anirban, H.Y. Song, H.S. Seo, B.T. Lee, J. Biomater. Appl. **25**, 6 (2011) 539.

[12] M.K. Ahmed, R. Ramadan, M. Afifi, A.A. Menazea, J. Mater. Res. Technol. **9**, 4 (2020) 8854.

[13] Z. Dong, Y. Wu, Q. Wang, C. Xie, Y. Ren, R.L. Clark, J. Biomed. Mater. Res. A **100** (2012) 903.

[14] S. Pazarlioglu, S. Salman, J. Aust. Ceram. Soc. **7** (2019) 43202.

[15] E. Bartonickova, J. Vojtisek, J. Tkacz, J. Porizka, J. Masilko, M. Moncekova, L. Parizek, Mater. Tehnol. **51**, 4 (2017) 631.

[16] U.T. Kalyoncuoglu, B. Yilmaz, S.G. Koc, Z. Evis, P.U. Arpaci, G. Kansu, Clin. Implant Dent. Relat. Res. **20**, 6 (2018) 1022.

[17] A. Yelten, O. Karal-Yilmaz, Z.P. Akguner, A. Bal-Ozturk, S. Yilmaz, Gazi Univ. J. Sci. **33**, 4 (2020) 690.

[18] E. Davoodi, M. Zhianmanesh, H. Montazerian, A.S. Milani, M. Hoorfar, J. Mater. Sci. Mater. Med. **31**, 7 (2020) 59.

[19] B. Zafar, F. Mottaghitalab, Z. Shahosseini, B. Negahdari,

[20] E.B. Toloue, S. Karbasi, H. Salehi, M. Rafienia, Mater. Sc. Eng. C **99** (2019) 1075.

[21] S. Punj, J. Singh, K. Singh, Ceram. Int. **47**, 20 (2021) 28059.

[22] I. Wittenbrink, A. Hausmann, K. Schickle, I. Lauria, R. Davtalab, M. Foss, A. Keller, H. Fischer, Biomaterials **62** (2015) 58.

[23] D. Duraccio, V. Strongone, M.G. Faga, F. Auriemmac, F.D. Mussanod, T. Genovad, G. Malucelli, Eur. Polym. J. **120** (2019) 109274.

[24] T. Ren, L.N.N. Nforbi, R. Kanakala, O.A. Graeve, Inorg. Chem. **57**, 6 (2018) 3035.

[25] A. Mavrič, M. Valant, C. Cui, Z.M. Wang, J. Non-Cryst. Solids **521** (2019) 119493.

[26] M. Urbonavicius, S. Varnagiris, L. Pranevicius, D. Milcius, Materials **13**, 6 (2020) 1.

[27] M.R. Karim, Open Colloid Sci. J. **4**, 1 (2011) 32.

[28] A. Yelten, S. Yilmaz, Ceram. Int. **45** (2019) 15375.

[29] A.H. De Aza, J. Chevalier, G. Fantozzi, M. Schehl, R. Torrecillas, Biomaterials **23** (2002) 937.

[30] M. Bodaghi, A.R. Mirhabibi, H. Zolfonun, M. Tahriri, M. Karimi, Phase Transit. **81**, 6 (2008) 571.

[31] S. Lamouri, M. Hamidouche, N. Bouaouadja, H. Belhouchet, V. Garnier, G. Fantozzi, J.F. Trelkat, Bol. Soc. Esp. Ceram. V. **56**, 2 (2017) 47.

[32] I. Levin, D. Brandon, J. Am. Ceram. Soc. **81**, 8 (1998) 1995.

[33] A. Eftekhari, B. Movahedi, G. Dini, M. Milani, J. Eur. Ceram. Soc. **38** (2018) 3297.

[34] Z. Zhang, R.W. Hicks, T.R. Pauly, T.J. Pinnavaia, J. Am. Chem. Soc. **124**, 8 (2002) 1592.

[35] E. Pérez, I.A. Ibarra, A. Guzmán, E. Lima, Spectrochim. Acta A Mol. Biomol. Spectrosc. **172** (2017) 174.

[36] Y. Wang, W. Li, X. Jiao, D. Chen, J. Mater. Chem. A **1** (2013) 10720.

[37] A. Amiri, A.V. Bekker, G.D. Ingram, I. Livk, N.E. Maynard, Chem. Eng. Res. Des. **91**, 3 (2013) 485.

[38] H. Akasaka, M. Konishi, N. Gawazawa, S. Ohshio, I. Toda, H. Saitoh, Appl. Surf. Sci. **258**, 24 (2012) 9526.

[39] C. Piconi, G. Maccauro, F. Muratori, E.B. Del Prever, J. Appl. Biomater. Biomech. **1**, 1 (2003) 19.

[40] A. Nevarez-Rascon, S. González-Lopez, L.S. Acosta-Torres, M.M. Nevarez-Rascon, E. Orrantia-Borunda, Dent. Mater. J. **35**, 3 (2016) 392.

[41] J.C. Alonso, O.C. Alonso, J.G. Paz, Ceram. Int. **44** (2018) 1751.

[42] M.K. Ahmed, R. Ramadan, S.I. El-Dek, V. Uskoković, J. Alloys Compd. **801** (2019) 70.

[43] A.R. Walpole, Z. Xia, C.W. Wilson, J.T. Triffitt, P.A. Wilshaw, J. Biomed. Mater. Res. A **90**, 1 (2009) 46.

[44] A.A. Aguiar, N.B. de Lima, F.J.C. Braga, W. Rossi, A.A. Couto, R. Baldan, Adv. Struct. Mater. **61** (2016) 141.

[45] H.H. Al-Moameri, Z.M. Nahi, D.R. Rzaij, N. Al-Sharify, J. Eng. Sustain. Dev. **24**, 5 (2020) 28.

[46] S. Pujari, A. Hoess, J. Shen, A. Thormann, A. Heilmann, L. Tang, M. Karlsson-Ott, J. Biomed. Mater. Res. A **102**, 11

(2014) 3773.

[47] M.F.H.A. El-Kader, M.K. Ahmed, M.T. Elabbasy, M. Afifi, A.A. Menazea, Surf. Coat. Technol. **409** (2021) 126861.

[48] M. Khalid, M. Mujahid, A.N. Khan, R.S. Rawat, J. Mater. Sci. Technol. **29**, 6 (2013) 557.

[49] H.F. Ghorbel, A. Guidara, Y. Danlos, J. Bouaziz, C. Coddet, Mater. Sci. Eng. C **71** (2017) 1090.

[50] Z. Fohlerova, A. Mozalev, J. Biomed. Mater. Res. B **106**, 5 (2018) 1645.

[51] F. Mussano, T. Genova, F.G. Serra, M. Carossa, L. Munaron, S. Carossa, Int. J. Mol. Sci. **19**, 2 (2018) 528.

[52] A.D.R. Silva, W.R. Rigoli, D.C.R. Mello, L.M.R. Vasconcellos, E.M.J.A. Pallone, A. Lobo, Int. J. Appl. Ceram. Technol. **16**, 2 (2019) 562.

[53] A.K. Monrreal-Rodríguez, J.A. Garibay-Alvarado, C.L. Vargas-Requena, S.Y. Reyes-Lopez, Results Mater. **6** (2020) 100091.

[54] A. Guidara, K. Chaari, J. Bouaziz, JOM **73**, 8 (2021) 2431.

[55] G. Hasemann, U. Betke, M. Krüger, H. Walles, M. Scheffler, Materials **14**, 11 (2021) 2813.

[56] M. Akmal, M. Hassan, M. Afzal, H.J. Ryu, Mater. Chem. Phys. **260** (2021) 124187.

[57] A.V.M. Esteves, M.I. Martins, P. Soares, M.A. Rodrigues, M.A. Lopes, J.D. Santos, Mater. Chem. Phys. **276** (2022) 125417.

[58] A. Teimouri, R. Ebrahimi, A.N. Chermahini, R. Emadi, RSC Adv. **5**, 35 (2015) 27558.

[59] H. Fischer, C. Niedhart, N. Kaltenborn, A. Prange, R. Marx, F.U. Niethard, R. Telle, Biomaterials **26** (2005) 6151.

[60] T.J. Webster, R.W. Siegel, R. Bizios, in Proc. 11th Int. Symp. Ceram. Med., R.Z. LeGeros, J.P. LeGeros (Eds.), World Scient., New York (1998) 273.

[61] T.J. Webster, R.W. Siegel, R. Bizios, Biomaterials **20** (1999) 1222.

[62] T.J. Webster, R.W Siegel, R. Bizios, Nanostruct. Mater. **12** (1999) 983.

[63] T.J. Webster, L.S. Schadler, R.W. Siegel, R. Bizios, Tissue Eng. **7**, 3 (2001) 291.

[64] H.S. Costa, M.M. Pereira, G.I. Andrade, E.E.B. Stancioli, H.S. Mansur, Mater. Res. **10**, 1 (2007) 27.

[65] C. Hadjicharalambous, A. Buyakov, S. Buyakova, S. Kulkov, M. Chatzinikolaidou, Biomed. Mater. **10**, 2 (2015) 25012.

[66] A.M. Stanciuc, C.M. Sprecher, J. Adrien, L.I. Roiban, M. Alini, L. Gremillard, M. Peroglio, J. Eur. Ceram. Soc. **38**, 3 (2018) 845.

[67] J.T. Domagalski, E. Xifre-Perez, A. Santos, J. Ferre-Borrull, L.F. Marsal, Microporous Mesoporous Mater. **303** (2020) 110264.

[68] N. Ferraz, J. Hong, M. Santin, M.K. Ott, Int. J. Biomater. **2010** (2010) 402715.

[69] Y. Song, Y. Ju, Y. Morita, G. Song, J. Biosci. Bioeng. **116**, 4 (2013) 509.

[70] A. Boretskaya, Il'dar Il'yasov, A. Lamberov, A. Popov, Appl. Surf. Sci. **496** (2019) 143635.

[71] N. Xu, Z. Liu, S. Bian, Y. Dong, W. Li, Ceram. Int. **42**, 3 (2016) 4072.