

# Bioactive Glasses in Bone Tissue Engineering

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## SUMMARY

Bioactive glasses are often used nanomaterials in tissue engineering of bone and soft tissue. Many newly synthesized materials for applications in medicine and dentistry are based on these bioactive substances. Bioactive glass is usually used as a scaffold or as an implant coating on implants and it allows fast formation of apatite layer with positive effect on osteoblasts proliferation. These biomaterials play an important role in dentistry and endodontics. This study is mostly part of the monograph titled "Nanomedicine, the Greatest Challenge of the 21<sup>st</sup> Century", that for two years has received attention from professional and scientific community in various fields. Information presented in this paper highlight structural characteristics of bioactive glasses that have a significant role in bone tissue engineering.

**Keywords:** bioactive glass; tissue engineering; nanomedicine

## INTRODUCTION

Newly synthesized nanomaterials can mimic characteristics of natural tissue, they are cytocompatible and biocompatible and exhibit excellent characteristics for use in tissue engineering and regenerative medicine. The goal of bone engineering is to develop scaffold (matrix) that would provide cellular support and guide formation of bone tissue. Calcium phosphate ceramics and hydroxyapatite are the most studied materials as they generate mineral component of calcified tissue of vertebrates, bioceramic materials and bioactive glasses.

## BIOACTIVE GLASSES

In the early 1970s Hench et al. [1] showed that glasses of composition  $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$  do not form fibrous tissue in contact with living tissue. Instead, they form chemical bond. Primary requirements for these materials were not to contain toxic elements for living organisms and to contain calcium and phosphorus that are the main constituents of the mineral phase of bone. Silicon was used to build a stable network for glass allowing the development of phosphate glasses with silicon rich matrices. First bioactive glass developed by Hench and Walker [2] was bioglass 45S5. This glass was highly active and easily formed bond even with soft tissues. This glass was made up of 45%  $\text{SiO}_2$ , 24.4%  $\text{CaO}$ , 24.5%  $\text{Na}_2\text{O}$  and 6%  $\text{P}_2\text{O}_5$ . Bioglass 45S5 was based on  $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$  system. Its composition was close to the composition of the corresponding eutectic system of  $\text{SiO}_2\text{-CaO-Na}_2\text{O}$ , and significantly different from the typical composition of sodium

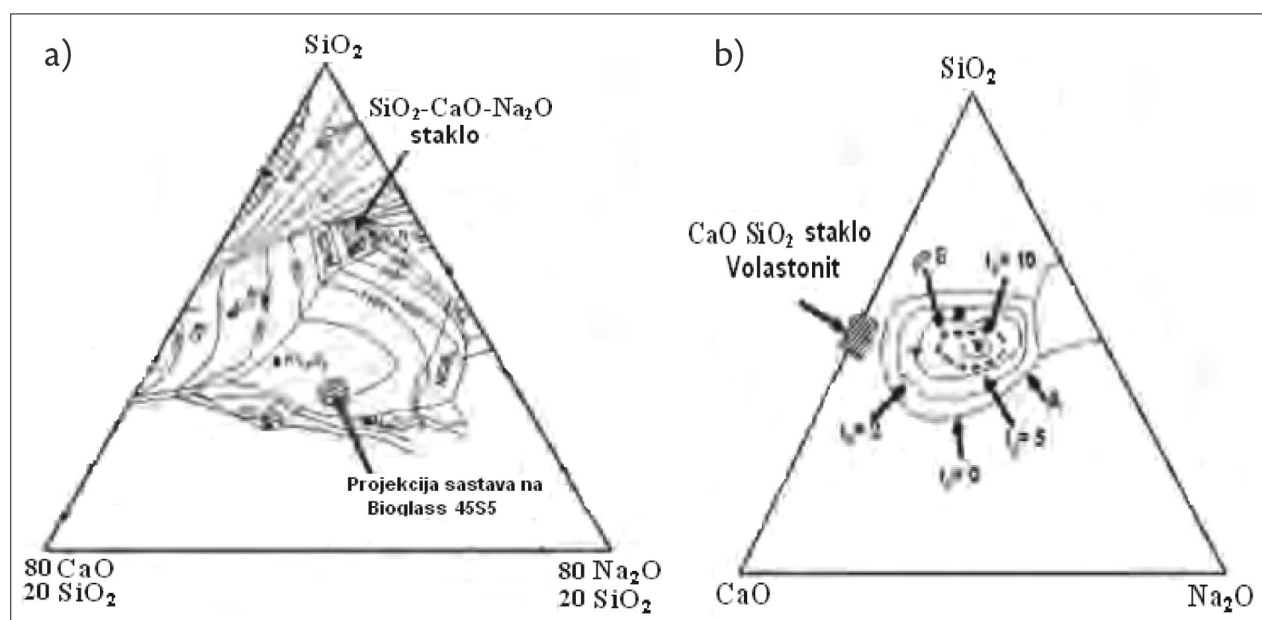
glass, which contains more  $\text{SiO}_2$  [2, 3, 4]. The composition of bioactive glass 45S5 has three microstructural forms (amorphous, partially crystalline and crystalline). This glass was studied by a number of studies *in vitro* and *in vivo*. In some of these studies done on rat femur, 45S5 was used in different forms. It was shown that all implants, regardless of the form, attached to femur during 6 weeks [1].

Starting from bioactive glass 45S5, Hench and Walker [2] developed series of glasses based on the quaternary system  $\text{CaO-SiO}_2\text{-Na}_2\text{O-P}_2\text{O}_5$ , with all containing 6%  $\text{P}_2\text{O}_5$  (Figure 1). Bioactive glass can be classified into two large groups, based on the composition: a) rich in alkali (developed by Hench et al.), containing more than 20% of alkali oxides or b) poor in alkali, with less than 5% of such oxides. Level of bioactivity depends on different composition; therefore they can have different clinical applications [5-8].

## PREPARATION OF BIOACTIVE GLASSES

Bioactive glasses are prepared from pure materials, which quality determines the quality of bioactive glasses. Basic materials are pure quartz or  $\text{SiO}_2$  sand, sodium or potassium carbonate with reactive purity, calcium phosphates that do not contain bound water, or in some rare cases highly pure pseudo-wollastonite ( $\text{CaSiO}_3$ ). Bioglass synthesis is sensitive process that starts with precisely measured constituents mixture melt in Pt or Pt/Rh container. It is important that melt is homogeneous and mixed carefully in order not to lose volatile components such as  $\text{Na}_2\text{O}$  and  $\text{P}_2\text{O}_5$ .

Sol-gel process, often used for bioglass synthesis is based on inorganic and organic soluble salts of constitu-



**Figure 1.** a)  $\text{SiO}_2\text{-CaO-Na}_2\text{O}$  system shows the position of traditional sodium glasses and projection of composition on bioglass 45S5; and b) Section corresponding to the composition of 6%  $\text{P}_2\text{O}_5$  in the system  $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$  and iso-bio-activity lines as a function of composition (wt%) (\* bioglass, \* 45S5, \* cerovital and bioglass) [5].

**Slika 1.** a) Sistem  $\text{SiO}_2\text{-CaO-Na}_2\text{O}$  pokazuje položaj tradicionalnih natrijumovih stakala i projekciju sastava na biostaklo 45S5; b) Sekcija koja odgovara sastavu 6%  $\text{P}_2\text{O}_5$  u sistemu  $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$  i linije izobioaktivnosti kao funkcije sastava u mas% (\* biostaklo, \* 45S5, \* cerovital i biostaklo) [5].

ents. Melting temperature is between 1200 and 1450°C depending on the composition of a given glass. The melt is then poured into a graphite or steel mould, which neither contaminates nor adheres to the glass [8].

## THE MECHANISM OF BINDING BIOACTIVE GLASS TO A LIVING TISSUE

When bioactive glass is bound to bone tissue, biologically active layer of carbonated hydroxyapatite is formed on the surface of implanted material. The ability to bind to bone tissue is associated with chemical reactivity of bioactive glasses in physiological media [8, 9]. Andersson and Kangasniemi [6] found that basic condition for bioactivity of glass is the ability to form a layer of amorphous silica on their surface. Silica gel provides large number of sites with optimal distance between oxygen ions ( $-\text{O}-\text{O}-$ ), which favours the process of chelation. Accordingly, chelation of phosphate ions to silica gel should be the initial step, followed by calcium ions, and new phosphate ions to satisfy electro-neutrality. Later, Andersson and Kangasniemi [6] modified hypothesis of chelation suggesting that active site must contain two phosphate ions attached to two separated silicon atoms, bonded previously with calcium ions.

Surface of implanted bioactive material goes through number of steps regardless of the type of tissue. A series of additional interfacial reaction is needed to complete the process of bioactive glass binding to tissue, however, they are not fully explained yet. According to Hench [5] and Andersson and Kangasniemi [6], the following reactions occur on the interface of tissue-bioactive glass: a) adsorption of biological components on the layer of carbonate hydroxyapatite; b) reaction of macrophages; c) binding of stem cells; d) differentiation of stem cells; e) matrix

production; and f) matrix mineralization. Based on the analysis of the interface of bioactive glass and tissue performed by Wilson et al., it was concluded that relationship between bioactive glass and tissue occurs as result of various chemical and mechanical factors [10]. In fact, collagen fibres form scaffold for micro-nucleation of hydroxyapatite crystals, inside the collagen fibres and at the collagen surface. Hydroxyapatite crystals grow epitaxially through the interface of the implant and bone, meaning that hydroxyapatite crystals are oriented along the collagen fibres [8-11].

Mechanical strength at the interface of bioactive glass and living tissue decreases with increasing thickness of the interface. In addition, the thickness of this layer is proportional to the bio-active index ( $I_B$ ) of the material. 45S5 bioactive glasses that have high  $I_B$ , form 200  $\mu\text{m}$  thick interface with relatively low strength of cohesion. In contrast to bioglass 45S5, glass ceramic Cerabone (A/W\*) with medium  $I_B$  produces interfacial layer of lower thickness (20-25  $\mu\text{m}$ ) but with higher strength [11].

Interfacial strength is time and morphological factors dependent such as changes in surface and interface morphology over time. It affects the degree of mineralization of interfacial tissue and increases modulus of elasticity of a given material with time. In many cases, the interfacial adhesive strength is comparable or even higher than the strength of biological material or bone itself. Therefore, if breakage occurs, bone or implant would break but interface would be rarely affected. Hench [11] found that if bioactive implants are immobilized for the critical time period with tissue they will show strength identical to healthy cortical bone. Biomechanical measurements of femoral implants in primates showed that attachment between ceramic implants covered with bio glass 45S5 and bone is very strong. Fracture that occurs due to torsional

force occurs mainly through the implant or surrounding bone without breaking the interface between them [8-11].

### BIOACTIVE GLASS-CERAMICS

Glass-ceramics is obtained using an appropriate heat treatment of the glass that results in the nucleation and growth of specific crystalline phase with the part of residual vitreous phase. In the first step a variety of inexpensive glass techniques, including casting, blowing, pressing or rolling is used to obtain glass. Through the subsequent crystallization, glass-ceramic gets fine microstructure with little or no residual pores that provides good mechanical properties of the end product.

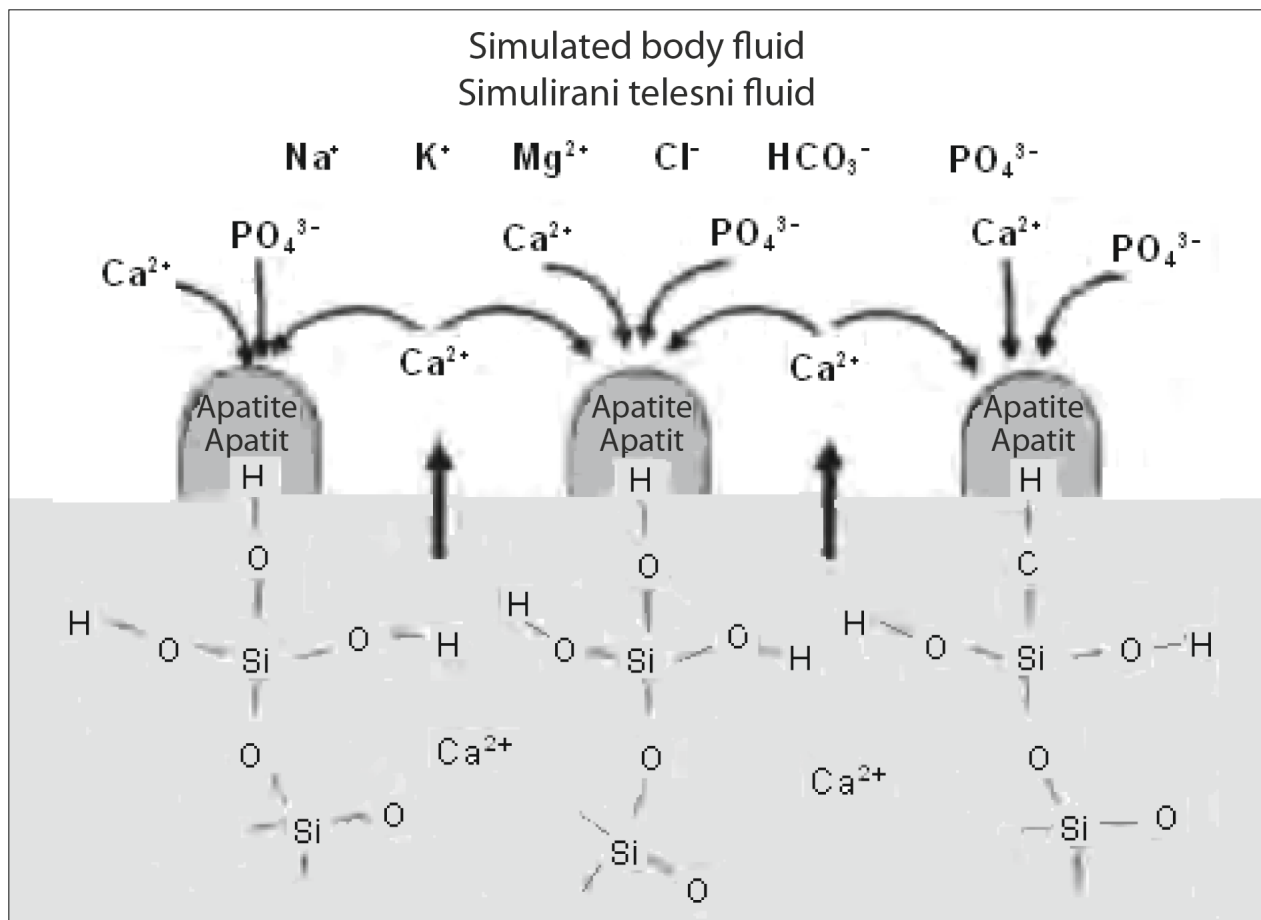
Nucleation of crystallites is critical step for obtaining glass-ceramics with good features. Temperature must increase slowly, usually less than 50°C/min, to avoid straining through volume changes during crystallisation that can cause cracks or even fracture. Slow warming prevents propagation of stresses within viscous residual glass; material becomes increasingly harder with the progression of crystallization. After completion of heating process final product should be cooled to room temperature. Depending on the composition of glass, crystallization may start on the surface that will result in a product of poor strength.

Volume crystallization provides desired microstructural properties after addition of suitable additives (nu-

cleation agents), metals (Cu, Ag, Au, Pt) or metal oxides ( $\text{SnO}_2$ ,  $\text{Ce}_2\text{O}_3$ ,  $\text{TiO}_2$ ) that favor heterogeneous nucleation of crystal phases. Most bioactive glass-ceramics have similar composition to Hench's bioactive glass (bioglass®) with low concentration of alkali oxides [12].

Brömer et al. [12] developed the first glass-ceramics (Ceravital) for clinical use in 1973. Ceravital showed excellent properties even when used for heavily loaded parts of bones and teeth. The modulus of fracture is below 160 MPa (that corresponds to modulus of fracture of human cortical bone) and similar modulus of fracture to densely sintered hydroxyapatite ceramics (115 MPa). In addition, long term *in vivo* tests showed that the weakest point of this material is its stability. Bioactivity of ceravital (5.6) has a value approximately half of bioactive glass 45S5 bioactivity (12.5). Ceravital implants are exclusively used as a substitute for ossicle chain in middle ear, where even materials with inadequate mechanical properties are acceptable.

Ceramics based on the system of hydroxyapatite/wollastonite (A/W) has been shown more suitable than ceravital in clinical applications. This ceramic consists of two crystalline phases: oxy-fluorapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{O}, \text{F})_2$  and wollastonite ( $\alpha$ - $\text{CaSiO}_3$ ) while the rest is glassy phase. This glass-ceramic was developed by Kokubo et al. [13, 14] and in commercial application is known as Cerabone A/W. Special structure of glass-ceramics provides mechanical properties significantly better than



**Figure 2.** The formation mechanism of carbonated hydroxyapatite layer on A/W glass-ceramics according to Kokubo [16].

**Slika 2.** Mehanizam formiranja sloja karbonatnog hidroksiapatita na A/W staklo-keramici prema Kokubu [16]

hydroxyapatite (modulus of fracture of 220 MPa which is two times higher than dense sintered hydroxyapatite) and modulus of fracture higher than that of cortical bone (160 MPa). In addition, their cohesiveness (strength) is 2.0 MPa, and hardness by Vickers is 680 HV. Similarly to bioactive glass, in simulated physical medium hydroxyapatite layer is formed on the surface of the glass-ceramics A/W providing bond between glass-ceramics A/W and bone tissue. Chemical and structural properties of this layer allow osteoblasts proliferation [13, 14].

However, unlike bioactive glass, there is no amorphous layer between the silicon carbonated hydroxyapatite and A/W glass-ceramics as shown in some studies using high performance electron microscopy. Kokubo et al. [13, 14] explained that silanol groups formed on glass-ceramic surface are responsible for the formation of carbonated hydroxyapatite, which provides area for nucleation and bone growth (Figure 2) [15, 16].

After implantation into the bone defect, A/W glass-ceramics form Ca and P-rich layers that allow binding to bone. Bond between these two is that strong that breakage (due to bending) never happens on the A/W bone interface but always occurs within the bone [7]. Good biocompatibility, bioactivity and mechanical properties of glass-ceramics facilitate its processing using diamond discs and burs encouraging its use for reconstruction of iliac peaks, vertebral discs and intervertebral discs and in granular form for filling defects. These applications are available in clinical practice since 1980.

De Aza et al. [7] have developed a type of apatite/wollastonite glass-ceramics called Imaplant. It is different from A/W glass-ceramics by a higher content of  $\text{CaF}_2$ ,  $\text{SiO}_2$  and  $\text{P}_2\text{O}_5$  and lower CaO content. Due to poor mechanical properties the application of this glass-ceramics is limited to maxillofacial implants.

Vogel and Höland [17, 18] at the University of Jena in 1983 developed a series of bioactive glass-ceramics, called Bioverit® I. They are obtained from silicate-phosphate glasses of complex composition, belonging to  $\text{Al}_2\text{O}_3$ - $\text{MgO}$ - $\text{Na}_2\text{O}$ - $\text{K}_2\text{O}$ - $\text{F}$ - $\text{CaO}$ - $\text{P}_2\text{O}_5$  system. After the first series of bioverit glass-ceramics, the same authors have developed another family of machine workable glass-ceramics, bioverit® II. Later one contained significantly less  $\text{P}_2\text{O}_5$  compared to bioverit® I. Bioverit® II also contains fluor-flogopite, talc-like structure (which crystals show curved morphology not found in any natural mineral) and other crystalline compounds, including cordierite ( $\text{Mg}_2[\text{Si}_5\text{Al}_4\text{O}_{14}]$ ). Furthermore, the same authors developed a family of glass-ceramics called bioverit® III that contained phosphate glass but with no  $\text{SiO}_2$  [17, 18].

The composition of crystalline phase in the glassy matrix bioverit® glass-ceramics can be modified by changing the composition of ingredients to transform physical properties and bioactivity of the system. Translucency of material is a function of crystalline phase whereas mechanical workability depends on the content of talc (bioverit® II is more workable than bioverit® I). Additionally, the color of material can be modified using small amounts of oxides, such as  $\text{NiO}$ ,  $\text{Cr}_2\text{O}_3$ ,  $\text{MnO}$ ,  $\text{FeO}$ ,  $\text{Fe}_2\text{O}_3$  etc. By nineties of the last century more than 1,000 im-

plants were made of these glass-ceramics and successfully applied in various medical applications, including orthopedic surgery (acetabulum reconstruction, orbital base repair, reconstruction of the cranial base, rhinoplasty, etc.) [7, 17, 18].

## BIOACTIVE GLASS COATINGS AND COMPOSITES

As mentioned previously the greatest obstacle to wider use of bioactive glasses and glass-ceramics is related to their poor mechanical properties, especially in areas exposed to mechanical loading. This disadvantage was partially overcome using various methods to increase the strength of these materials. One of proposed solutions was to use bioactive glass as a coating on materials with high mechanical strength. This method is used with number of substrates, such as dense alumina, various types of stainless steel, chromium and cobalt alloys and titanium alloys. Titanium alloys are particularly attractive because of its high strength, low modulus of elasticity and good biocompatibility [19].

Coating is prepared by dipping into melted glass or solution suspension or gel (dipping technique), electrophoresis of solution or suspension (covering metal that works as an electrode), biomimetic growth of coatings or flame and plasma spraying. Methods of spraying are often used for deposition of bioactive glass on metal surface. Another application of bioactive glass is for producing composite for reinforcement of bioactive glass with second phase. Such reinforcing phase can be "bioglass fibers" and alumina, organic polymers and metal fibers. Alumina fibers are not the best choice due to negative effect on tissue [20]. Materials reinforced with metal fibers have the greatest potential. Metal fibers reinforce bioglasses and improve their deformability. The most widely used procedure for production of these materials is hot pressing. Ducsheyne and Hench [21] showed composites that consist of 45S5 bioglass and metal fibers AISI 316L of stainless steel. They are obtained by dipping metal fibers into the glass melt. Such composites exhibit improved mechanical strength and ductility with Young's modulus comparable to modulus of human cortical bone.

Other glass-based composites are ceravital reinforced with titanium particles and A/W glass-ceramics reinforced with partially stabilized  $\text{ZrO}_2$  or polyethylene. They can be used as dental implants or can have orthopedic applications due to low modulus of elasticity, good deformability, tensile and fracture resistance as well as good workability [21]. Bioactive glasses are commonly used as ossicle implants in the middle ear of patients with chronic otitis. Merwin et al. demonstrated long-term stability of ossicle implants due to their proper attachment to the tympanic membrane [20]. Ossicular chain transmits sound vibrations from the tympanic membrane to the oval windows. The chain consists of malleus, incus and stapes. Reck et al. showed that ceravital serves the best for complete reconstruction of ossicular chain [22].

Using A/W glass-ceramic reconstruction for iliac hump gave acceptable radiological and clinical results. Animal



**Table 1.** Bioactive glasses and glass-ceramics and their clinical application**Tabela 1.** Bioaktivna stakla i staklo-keramika i njihova klinička primena

| Design Dizajn                      | Material Materijal   | Clinical application Klinička primena                                  |
|------------------------------------|--|--|
| Dense materials Gusti materijali   | Bioglass Biostaklo   | Maxilofacial reconstruction Maksilofacijalna rekonstrukcija            |
|                                    |  | Middle ear reconstruction Rekonstrukcija srednjeg uva                  |
|                                    |  | Vertebral connection Povezivanje kičmenih pršljenova                   |
|                                    | Glass-ceramics Staklo-keramika   | Dental implants Dentalni implantati                                    |
|                                    |  | Maxilofacial reconstruction Maksilofacijalna rekonstrukcija            |
|                                    |  | Spine prosthesis devices Uređaji proteze kičme                         |
| Porous materials Porozni materijal | Bioglass (porous surface) Biostaklo (porozna površina)                       | No application yet Još nema primenu                                    |
|                                    |  |  |
| Coatings Prevlake                  | Bioglass on $Al_2O_3$ (high density) Biostaklo na $Al_2O_3$ (visoke gustine) | Maxilofacial reconstruction Maksilofacijalna rekonstrukcija            |
|                                    |  | Middle ear reconstruction Rekonstrukcija srednjeg uva                  |
|                                    |  | Vertebral connection Povezivanje kičmenih pršljenova                   |
|                                    | Bioglass on stainless steel Biostaklo na nerđajućem čeliku                   | Hip prosthesis Proteza kuka  |
|                                    |  | Implant attachment to bone Kačenje veštačkih delova na kost            |
|                                    |  | Isolation of neural electrodes Izolacija neuronskih elektroda          |
| Composites Kompoziti               | $Al_2O_3$ + bioglass $Al_2O_3$ + biostaklo                                   | No application yet Još nema primenu                                    |
|                                    | Polymers + bioglass Polimeri + biostaklo                                     | Soft tissue-bone attachment; cements Veze mekog tkiva i kosti; cementi |

tests demonstrated that bioglass coating is useful for hip replacement due to its mechanical strength and bioactivity, with no need for bone cement to attach implant to the bone. Various applications of glass-ceramics are presented in Table 1 [2].

## CONCLUSION

Bioactive glasses are nanomaterials that are often used in tissue engineering of bone and soft tissue and this materials are usually used as a scaffold or as a coating on implants. Bioactive glasses allow fast formation of apatite layer with positive effect on osteoblasts and they are usually used in dentistry and endodontics.

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# Bioaktivna stakla u inženjerstvu koštanih tkiva

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## KRATAK SADRŽAJ

Bioaktivna stakla su nanomaterijali koji se dosta često koriste u inženjerstvu koštanih i mekih tkiva. Brojni novosintetisani materijali za primenu u medicini i stomatologiji se baziraju upravo na ovim bioaktivnim supstancama. Bioaktivno staklo se najčešće koristi kao skafold ili prevlaka na implantatima, i omogućava brzo formiranje apatitnog sloja, odnosno povoljno utiče na proliferaciju osteoblasta. Značaj ovih biomaterijala za primenu u stomatologiji i endodonciji zaslužuje posebno mesto, pa će im zato u okviru ovog rada biti posvećena posebna pažnja. I ovaj rad je najvećim delom sastavni deo monografije pod nazivom „Nanomedicina, najveći izazov 21. veka“, koja je već dve godine u žiži interesovanja stručne i naučne javnosti iz različitih oblasti. Informacije predstavljene u radu su vrlo značajne za teorijsko osvetljavanje značaja i strukturnih odlika bioaktivnih stakala, koji su od neprocenljivog značaja za inženjerstvo koštanih tkiva.

**Ključne reči:** bioaktivna stakla; inženjerstvo tkiva; nanomedicina

## UVOD

Novosintetisani nanomaterijali mogu da oponašaju svojstva prirodnih tkiva, izrazito su citokompatibilni i biokompatibilni i pokazuju odlična svojstva za primenu u inženjerstvu tkiva i regenerativnoj medicini. U ovoj oblasti se najdalje otišlo u koštanom tkivnom inženjeringu, gde je pažnja istraživanja usmerena ka razvoju skafolda (matriksa) koji treba da obezbedi ćelijsku podršku i vođenje formiranja koštanog tkiva. U tom smislu se odmaklo s istraživanjima kalcijum-fosfatne keramike i hidroksiapatita, koji čini mineralnu komponentu kalcifikovanih tkiva kičmenjaka, odnosno biokeramičkih materijala i bioaktivnih stakala.

## BIOAKTIVNA STAKLA

Henh (*Hench*) i saradnici [1] su ranih sedamdesetih godina dvadesetog veka pokazali da pri korišćenju stakala sastava  $\text{SiO}_2$ - $\text{CaO}$ - $\text{Na}_2\text{O}$ - $\text{P}_2\text{O}_5$  ne dolazi do stvaranja fibroznog tkiva u kontaktu sa živim tkivima. Umesto toga, formiraju se hemijske veze. Primarni zahtev u odnosu na ove materijale bio je da ne sadrže toksične elemente za žive organizme, već da sadrže kalcijum i fosfor, kao glavne konstituentne mineralnih faza koštanih tkiva. Kao stabilni formirač mreže stakala izabran je silicijum, što je omogućilo razvoj fosfatnih stakala s matricama bogatim silicijumom. Prvo bioaktivno staklo koje su razvili Henh (*Hench*) i Voker (*Walker*) [2] nazvano je biostaklo 45S5. Takvo staklo bilo je visoko aktivno i lako je formiralo veze čak i sa mekim tkivima. To staklo se sastojalo od: 45%  $\text{SiO}_2$ , 24,4%  $\text{CaO}$ , 24,5%  $\text{Na}_2\text{O}$  i 6%  $\text{P}_2\text{O}_5$ . Biostaklo 45S5 se zasniva na sistemu  $\text{SiO}_2$ - $\text{CaO}$ - $\text{Na}_2\text{O}$ - $\text{P}_2\text{O}_5$ . Njegov sastav sličan je sastavu koji odgovara eutektičkoj tački sistema  $\text{SiO}_2$ - $\text{CaO}$ - $\text{Na}_2\text{O}$  i značajno se razlikuje od tipičnog sastava natrijumovog stakla, koje sadrži mnogo više  $\text{SiO}_2$  [2, 3, 4]. Biostaklo 45S5 poseduje tri mikrostrukturne forme (amorfnu, delimično kristalnu i kristalnu). Takvo staklo izučavano je u brojnim istraživanjima u uslovima *in vitro* i *in vivo*. U nekima od tih istraživanja urađenih na femuru pacova korišćena su biostakla tipa 45S5, nezavisno od toga da li se nalaze u staklastoj ili kristalnoj formi. Pokazano je da su se svi

implantati, nezavisno od oblika takvog biostakla, vezali za femur pacova tokom šest nedelja [1].

Polazeći od biostakla 45S5, Henh i Voker [2] su razvili veliku seriju stakala zasnovanu na kvarternom sistemu  $\text{SiO}_2$ - $\text{CaO}$ - $\text{Na}_2\text{O}$ - $\text{P}_2\text{O}_5$ , pri čemu su sva ona sadržavala 6%  $\text{P}_2\text{O}_5$  (Slika 1). Bioaktivna stakla mogu se klasifikovati u dve velike grupe zavisno od toga da li su: a) bogata alkalijama (stakla koja je razvio Henh sa svojim saradnicima), koja sadrže više od 20% alkalnih oksida; ili b) siromašna alkalijama, sa manje od 5% takvih oksida. Takvim staklima odgovaraju različite vrednosti bioaktivnosti, što ukazuje i na njihove moguće specifične primene vezane za različite kliničke potrebe [5-8].

## PRIPREMA BIOAKTIVNIH STAKALA

Bioaktivna stakla se pripremaju iz visoko čistih sirovina, čiji kvalitet ima presudan uticaj na kvalitet biostakla. Osnovni materijali za proizvodnju biostakala su čisti kvarc ili  $\text{SiO}_2$  pesak, natrijum ili kalijum-karbonat reaktivne čistoće, kalcijum-fosfati koji ne sadrže vezanu vodu, ili u nekim ređim situacijama i visoko čisti pseudovolastonit ( $\text{CaSiO}_3$ ). Sam proces dobijanja biostakala odvija se tako što se prvo tačno odmerene količine datih sirovinskih smesa tope u Pt ili Pt/Rh lončiću; pri tome, veoma je važno da rastop bude homogen i da se pažljivo meša, kako se ne bi izgubile isparljive komponente, kao što su  $\text{Na}_2\text{O}$  i  $\text{P}_2\text{O}_5$ .

Sol-gel proces, koji se često koristi u proizvodnji biostakala, zasniva se na primeni organskih i neorganskih rastvornih soli kao sirovina. Temperatura topljenja sirovinske smese je između 1200 i 1450°C, zavisno od sastava datog stakla. Rastop se potom izliva u grafitni ili čelični kalup, koji ne može da ga kontaminira ili da na njemu prijanja staklo [8].

## MEHANIZAM VEZIVANJA BIOAKTIVNOG STAKLA ZA ŽIVA TKIVA

Kod vezivanja bioaktivnog stakla za koštano tkivo biološki aktivni sloj karbonatnog hidroksiapatita se stvara na površini

implantiranog materijala. Sposobnost da se bioaktivna stakla vežu za koštana tkiva povezana je s njihovom hemijskom reaktivnošću u fiziološkom medijumu [8, 9].

Anderson (*Andersson*) i Kangasniemi (*Kangasniemi*) [6] su zaključili da je osnovni uslov za bioaktivnost stakla vezan za formiranje sloja amorfnog silicijum-dioksida na njihovoj površini. Na osnovu toga zaključeno je da silika gel obezbeđuje veliki broj mesta sa optimalnim razdaljinama između jona kiseonika ( $-O-O-$ ), koja favorizuju proces helatacije. Saglasno tome, helatacija fosfatnih jona sa silika gelom trebalo bi da bude polazni korak, koji potom slede kalcijumovi joni, povlačeći za sobom nove fosfatne jone, da bi bio zadovoljen kriterijum elektroneutralnosti naelektrisanja. Kasnije su Anderson i Kangasniemi [6] modifikovali hipotezu helatacije i ukazali na to da aktivno mesto mora da sadrži dva fosfatna jona vezana sa dva razdvojena atoma silicijuma, koja su prethodno vezana sa kalcijumovim jonima.

Površina implantiranog bioaktivnog materijala prolazi kroz niz već opisanih koraka nezavisno o kojim je tkivima reč. Za vezivanje bioaktivnog stakla za tkivo neophodna je serija dodatnih međupovršinskih reakcija, koje zbog nedovoljnog razumevanja biologije još nisu adekvatno opisane. Saglasno Henhu [5] i Andersonu i Kangasniemiju [6], na takvim međupovršinama dešavaju se sledeće reakcije: a) adsorpcija bioloških komponenata na sloju karbonatnog hidroksiapatita; b) reakcija makrofaga; c) vezivanje matičnih ćelija; d) diferencijacija matičnih ćelija; e) proizvodnja (generacija) matriksa; i f) mineralizacija matriksa. Na osnovu analize međupovršine biostakla i tkiva Vilsona (*Wilson*) i saradnika, zaključeno je da veza između biostakala i tkiva nastaje kao posledica kombinacije različitih hemijskih i mehaničkih faktora [10]. Pri tome, izgleda da kolagenska vlakna obezbeđuju skafold s mestima neophodnim za mikronukleaciju hidroksiapatitnih kristala, koji zauzimaju mesta unutar vlakana kolagena na površini kolagena. Hidroksiapatitni kristali rastu epitaksijalno kroz međupovršinu implantata i kosti, što znači da su kristali hidroksiapatita orijentisani duž kolagenskih vlakana [8-11].

Mehanička čvrstoća na međupovršini između bioaktivnog stakla i živog tkiva se smanjuje s povećanjem debljine međupovršine. Pored toga, debljina toga sloja proporcionalna je vrednosti bioaktivnog indeksa ( $I_B$ ) tog materijala. Biostakla 45S5, čiji je  $I_B$  visok, formiraju međupovršinu debelu 200  $\mu m$  s relativno slabom čvrstoćom kohezije. Nasuprot biostaklu 45S5, staklo-keramika Cerabone ( $A/W^*$ ) sa srednjim vrednostima  $I_B$ , stvara međupovršinski sloj znatno manje debljine (20–25  $\mu m$ ), koji je veoma čvrst [11].

Međupovršinska čvrstoća je funkcija zavisna od vremena i morfoloških faktora, kao što su promena površine i morfologije međupovršinske oblasti sa vremenom. Ona utiče na stepen mineralizacije međupovršinskog tkiva i povećanje modula elastičnosti datog materijala sa vremenom. U mnogim slučajevima međupovršinska adheziona čvrstoća uporediva je ili je čak veća od čvrstoće biološkog materijala ili same kosti, tako da se lom dešava ili kroz kost ili kroz implantat, a veoma retko kroz međupovršinu. Henh [11] je utvrdio da, ako se implantati biostakla imobilizuju u nekom kritičnom vremenu njihove veze sa bliskim tkivima, pokazuju čvrstoću veze istovetnu zdravoj kortikalnoj kosti. Biomehanička merenja femoralnih implantata kod primata pokazuju da je veza između keramičkog implantata prekrivenog biostaklom 45S5 i kosti

jaka, a da se lom pod dejstvom torzionih sila dešava uglavnom kroz implantat ili okolnu kost bez kidanja međupovršine kosti i implantata [8-11].

## BIOAKTIVNA STAKLO-KERAMIKA

Staklo-keramika se dobija koristeći odgovarajuću termičku obradu stakla čiji su rezultat nukleacija i rast specifičnih kristalnih faza, uz deo zaostale staklene faze. U prvom koraku procesa dobija se staklo korišćenjem različitih jeftinih tehnika, uključujući livenje, duvanje, presovanje ili valjanje. Kroz efekat naknadne kristalizacije stakla, staklo-keramika zadobija finu mikrostrukturu, koja sadrži malo ili nimalo zaostalih pora, što sve daje kao rezultat dobre mehaničke osobine krajnjeg proizvoda.

Korak nukleacije kristalita uvek je kritičan za dobijanje staklo-keramike dobrih svojstava. Temperatura se mora povećavati lagano, najčešće manje od 5°C u minuti, da bi se izbeglo naprezanje tokom zapreminske promene staklo-keramičkog sistema usled kristalizacije, koje može da izazove nastajanje pukotina ili dovede do lomljenja materijala. Sporo zagrevanje olakšava suzbijanje takvog naprezanja kroz viskozni tok preostalog stakla, pri čemu materijal postaje sve tvrdi sa porastom stepena njegove kristalizacije. Nakon završenog procesa zagrevanja dati proizvod hladi se na sobnoj temperaturi. U zavisnosti od datog sastava stakla, kristalizacija može da započne na površini, što za posledicu ima proizvod slabe čvrstoće.

Zapreminska kristalizacija obezbeđuje željena mikrostrukturna svojstva uz dodatak odgovarajućih aditiva (nukleacionih agensa), metala (Cu, Ag, Au, Pt) ili metalnih oksida ( $SnO_2$ ,  $Ce_2O_3$ ,  $TiO_2$ ) koji podstiču heterogenu nukleaciju kristalnih faza. Većina bioaktivnih staklo-keramika zasniva se na sastavima sličnim Henhovim bioaktivnim staklima (biostaklo<sup>®</sup>), koja većinom sadrže veoma malu koncentraciju alkalnih oksida [12].

Najraniju staklo-keramiku za kliničku primenu razvili su Bremer (*Brömer*) i saradnici [12] 1973. godine pod imenom *ceravital*. Ceravital je pokazao izuzetne osobine čak i pri zameni opterećenih delova kosti i zuba. Njegov modul loma je ispod 160 MPa (modul koji odgovara humanoju kortikalnoj kosti) i ima sličnu vrednost modulu loma gusto sinterovane hidroksiapatitne keramike (115 MPa). Pored toga, u dugotrajnim ispitivanjima *in vivo* pokazano je da je najslabija tačka ovog materijala njegova stabilnost. Bioaktivnost ceravitala ima vrednost približno jednaku polovini bioaktivnosti biostakla 45S5 (5,6 prema 12,5). Ceravitalni implantati se isključivo koriste kao zamena osikularnih lanaca u srednjem uvu, gde su i materijali sa neodgovarajućim mehaničkim osobinama prihvatljivi za primenu.

Keramika koja se pokazala uspešnijom od ceravitala u kliničkim primenama je ona koja se zasniva na sistemu hidroksiapatit-volastonit ( $A/W$ ). Ona se sastoji od dve kristalne faze: oksifluorapatita  $Ca_{10}(PO_4)_6(O, F_2)$  i volastonita ( $\alpha$ - $CaSiO_3$ ), dok njen ostatak čini staklasta faza. Ovu staklo-keramiku razvili su Kokubo (*Kokubo*) i saradnici [13, 14] i u komercijalnoj primeni je pod nazivom *Cerabone A/W*. Posebne strukture staklo-keramike imaju znatno bolje mehaničke osobine od hidroksiapatita (modul loma oko 220 MPa, veći oko dva puta od sinterovanog gustog hidroksiapatita) koje prelaze vrednosti modula loma za kortikalne kosti od 160 MPa. Uz to njihova kohezivnost (jačina) je 2,0 MPa, a Vikersova tvrdoća oko 680 HV. Slično bioaktivnom

staklu, u simuliranom telesnom medijumu stvara se hidroksiapatitni sloj, kao i na površini staklo-keramike A/W. Upravo taj formirani sloj hidroksiapatita omogućuje spajanje staklo-keramike A/W s koštanim tkivom. Zahvaljujući hemijskim i strukturnim odlikama ovog sloja karbonatnog hidroksiapatita, slično kao i kod koštanog tkiva, očekuje se da na međupovršini s kostima proliferuju osteoblasti [13, 14].

Ipak, za razliku od bioaktivnog stakla, nema amornog silicijumovog sloja između karbonatnog hidroksiapatita i A/W staklo-keramike, kao što je to u nekim istraživanjima pokazano metodom visokorezolucijske elektronske mikroskopije. Kokubo i saradnici [13, 14] smatraju da su silanolne grupe formirane na staklo-keramičkim površinama odgovorne za formiranje sloja karbonatnog hidroksiapatita, koji obezbeđuje mesta pogodna za nukleaciju i rast kosti (Slika 2) [15, 16].

Posle implantacije u koštani defekt, A/W staklo-keramika formira Ca- i P-bogate slojeve kojima se veže sa kostima. Vezivanje je tako snažno, da nikad ne dolazi do loma (usled savijanja) na A/W koštanoj međupovršini, nego se lom uvek dešava unutar kosti [7]. Dobra biokompatibilnost, bioaktivnost i mehaničke osobine ove staklo-keramike olakšavaju njenu obradu dijamantskim diskovima i bušenje, i na taj način podstiču njenu primenu u rekonstrukciji ilijskih vrhova, pršljenova i međupršljenih diskova i u obliku granula za punjenje defekata. Takve primene su zastupljene u kliničkoj praksi od 1980. godine.

De Aza i saradnici [7] su razvili vrstu apatit-volastonitne staklo-keramike pod nazivom *Ilmaplant*. Od A/W staklo-keramike razlikuje se po većem sadržaju  $\text{CaF}_2$ ,  $\text{SiO}_2$  i  $\text{P}_2\text{O}_5$  i manjem sadržaju  $\text{CaO}$ . Zbog slabih mehaničkih osobina, primena ove staklo-keramike ograničena je na maksilofacijalne implantate.

Fogel (*Vogel*) i Heland (*Höland*) [17, 18] su 1983. godine na Univerzitetu u Jeni razvili seriju bioaktivnih staklo-keramika koje su nazvane bioverit® I. Takve staklo-keramike dobijene su iz silikatno-fosfatnih stakala kompleksnog sastava, koja pripadaju sistemu  $\text{Al}_2\text{O}_3$ - $\text{MgO}$ - $\text{Na}_2\text{O}$ - $\text{K}_2\text{O}$ - $\text{F}$ - $\text{CaO}$ - $\text{P}_2\text{O}_5$ . Nakon date prve serije bioveritnih staklo-keramika, isti autori su razvili drugu familiju mašinski lako obradivih staklo-keramika, koju su nazvali bioverit® II. Takve staklo-keramike sadrže znatno manje  $\text{P}_2\text{O}_5$  u odnosu na bioverit® I. Bioverit® II takođe sadrži fluorflogopit, strukture slične talku (čiji kristali pokazuju zakrivljenu morfologiju, koja nije registrovana ni kod jednog minerala u prirodi) uz druga kristalna jedinjenja, među kojima poseban značaj ima kordijerit ( $\text{Mg}_2[\text{Si}_5\text{Al}_4\text{O}_{14}]$ ). Na kraju, razvili su familiju staklo-keramika nazvanu bioverit® III, iz fosfatnog stakla koje ne sadrži  $\text{SiO}_2$  [17, 18].

Sastav kristalne faze u staklastoj matrici staklo-keramike bioverit® može se modifikovati promenom sastava, da bi se na taj način modifikovali fizičke osobine i bioaktivnost sistema. Translucencija ovih materijala je funkcija udela kristalnih faza, dok mašinska obradivost zavisi od sadržaja talka (bioverit® II se može bolje obraditi od bioverit® I). Uz to boja materijala može se modifikovati malim količinama oksida, kao što su  $\text{NiO}$ ,  $\text{Cr}_2\text{O}_3$ ,  $\text{MnO}$ ,  $\text{FeO}$ ,  $\text{Fe}_2\text{O}_3$  itd. Do sredine devedesetih godina prošlog veka više od 1.000 implantata je napravljeno na bazi ovih staklo-keramika i uspešno primenjeno u raznim medicinskim postupcima, kao što je ortopedska hirurgija (rekonstrukcija acetabuluma, popravka orbitalne osnove, rekonstrukcija kranijalne osnove, rinoplastika itd.) [7, 17, 18].

## PREVLAKE BIOAKTIVNIH STAKALA I KOMPOZITI

Kao što je prethodno već rečeno, najveća prepreka širem korišćenju bioaktivnih stakala i staklo-keramike vezana je za njihove slabe mehaničke osobine, posebno u zonama izloženim mehaničkom opterećenju. Ovaj nedostatak je delimično prevaziđen korišćenjem različitih metoda da se poveća čvrstoća takvih materijala i olakša njihovo korišćenje kao implantata. Jedno od rešenja je da se koristi bioaktivno staklo kao prevlaka za materijale visoke mehaničke čvrstoće. Ova metoda je korišćena s velikim brojem supstrata, uključujući gustu aluminu, razne tipove nerđajućih čelika, legure hroma i kobalta i titanijumove legure. Legure titanijuma su posebno privlačne zbog svoje visoke čvrstoće, niskog modula elastičnosti i dobre biokompatibilnosti [19].

Kod ovih metoda prevlaka se priprema umakanjem u rastop stakla ili rastvor suspenzije ili gel (tehnika umakanja), elektroforezom iz rastvora ili suspenzije (prekrivajući metal deluje kao elektroda), biomimetskim rastom prevlaka ili plamenim i plazma raspršivanjem. Metode raspršivanja se veoma mnogo koriste za deponovanje bioaktivnog stakla na površinu metala. Druga primena bioaktivnih stakala je u proizvodnji kompozita za ojačanje bioaktivnog stakla sa drugom fazom. Takve ojačavajuće faze mogu biti „vlakna biostakla“ i alumine, organski polimeri i metalna vlakna. Alumina vlakna nisu najpogodnija, jer utiču razarajuće na tkiva [20]. Materijali ojačani metalnim vlaknima imaju najjači potencijal. Metalna vlakna ojačavaju biostakla i poboljšavaju njihovu deformabilnost. Najšire se koristi procedura za proizvodnju ovih materijala toplim presovanjem. Dišejn (*Ducshyne*) i Henh [21] su pokazali u svojim istraživanjima kompozite koji se sastoje od biostakla 45S5 i metalnih vlakana AISI 316L nerđajućeg čelika. Procedura je izvedena umakanjem metalnih vlakana u rastop stakla. Takvi kompoziti pokazuju bolju mehaničku čvrstoću i duktilnost, uz Jangov modul uporediv s Jangovim modulom humanih kortikalnih kostiju.

Drugi kompoziti zasnovani na staklu su ceravital ojačan titanijumovim česticama i A/W staklo-keramika ojačana delimično stabilisanim  $\text{ZrO}_2$  ili polietilenom. Suštinski zahtevi u odnosu na ove materijale su da se koriste kao dentalni implantati ili u ortopedskim primenama, jer poseduju nizak modul elastičnosti, dobru deformabilnost, čvrstoću istezanja i dobar otpor na udar, pri čemu se uz sve to još i lako mašinski obrađuju [21].

Biostakla se najčešće koriste kao implantati s težnjom da sačuvaju alveolarni lanac i delimično ili potpuno zamene osikularni lanac srednjeg uva pacijenata s hroničnim otitisom. Mervin (*Merwin*) i saradnici su pokazali dugotrajnu stabilnost ovih implantata koja proizlazi iz načina njihovog vezivanja s timpaničnom membranom [20]. Osikularni lanac je mala kost u srednjem uvu koja pravi lanac koji prenosi vibracije zvuka od timpanične membrane do jajolikog prozora. Taj lanac se sastoji od čekića, nakovnja i uzengije. Posle duge kliničke primene Rek (*Reck*) i saradnici su pokazali da je ceravital najefikasniji kao proteza za potpunu rekonstrukciju osikularnog lanca [22].

Korišćenje A/W staklo-keramike za rekonstrukciju ilijske grbe dalo je prihvatljive radiološke i kliničke rezultate. Testovi na životinjama pokazali su da su prevlake biostakla korisne za trajne proteze kuka, jer kombinuju dobru mehaničku čvrstoću i bioaktivnost, zbog čega pri njihovom korišćenju nije potrebno primeniti koštane cimente za kačenje implantata za kost. Razne mogućnosti primene staklo-keramika date su i u tabeli 1 [2].



## ZAKLJUČAK

Bioaktivna stakla su nanomaterijali koji se dosta često koriste u inženjerstvu koštanog tkiva. Najčešće se koriste kao skafoldi

ili prevlake na implantatima. Omogućavaju brzo stvaranje apatitnog sloja, odnosno povoljno utiču na osteoblaste, pa se često primenjuju u stomatologiji, odnosno endodonciji.