Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XVI/B 2017

Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XVI/B 2017

PERIODONTAL BONE SUBSTITUTES

Aversa Raffaella*, Petrescu Relly Victoria, Apicella Antonio*, Petrescu Florian Ion T.****

*Advanced Material Lab, Department of Architecture and Industrial Design, Second University of Naples, 81031 Naples (CE) Italy, e-mail: raffaella.aversa@unina2.it; antonio.apicella@unina2.it

** Polytechnic University of Bucharest, Faculty of Transport, 313 Splaiul Independentei, 060042 Bucharest (CE) Romania, e-mail: petrescuvictoria@yahoo.com; fitpetrescu@gmail.com

Abstract

Bioactive Materials have been used since decades but the researches on these materials are still continuing in phase. This material got extra ordinary attention by the scientist and researchers. Bioactive material has ability to bind itself chemically with natural bone tissues. Bioactive materials bring revolution in the field of bone repair and implantology. Bioactive materials have also ability to effect on gene activation of osteoblastic cells that enhance proliferation, resulting rapid bone formation. At last the techniques through which bioactive materials are used to deposits on the implant, to create bond between implants and the bone. Cost evaluation is the very essential part that classifies the use of material commercially.

Key words: Biomaterials, Ceramics, Titanium, Scaffold, Implants

INTRODUCTION

The earliest bioactive materials which were used within the body were identified as called Prostheses (Hench and Thompson, 2010). These Prostheses had to be standardized according to the physical properties of living tissues. Professor Bill Bonfield et al. (1981) was the pioneer of researching mechanical properties of living tissues, its skills were especially centered on bone to make Prosthesis. The basic objective of making the Prosthesis was to achieve a combination of physical properties of living tissue with minimal toxic response to the surrounding structures (Hench and Thompson, 2010). These prosthesis had the limitation of stress shielding and bone resorption. Professor Bill Bonfeild explore the concept of Bioactive materials and design bio composite that matches more to the mechanical properties of living tissues and removed the limitation i.e., resorption of the underlying bone structure (Hench and Thompson, 2010). The Bio active mechanism is the procedure through which living tissues are attached and integrated to an artificial implant with a chemical bond (Tilocca, 2009).

There are many applications of bioactive materials in tissue engineering (Tilocca, 2009). Tissue engineering is the art and science of biological substitution through which tissue function is restored. This is achieved with the formation of biological scaffold provide structural support to the tissue which later filled with number of cells and implantations (Chen et al., 2012). The requirements of scaffold materials to fulfill the demand of tissue engineering, are biocompatibility, the material doses not respond on unresolved inflammatory reaction, mechanical properties must be sufficient to prevent surface failure, controllable interconnected porosity which can help to grow cells and support vascularization (Chen et al., 2012). About 90% porosity with 100micrometer is essential for cell growth and proper vascularization (Chen et al., 2012). Bone has natural combination of inorganic calcium phosphatase appetite and a biological polymer called Collagen in which associates are deposited (Chen et al., 2012; Buzea et al., 2015; Petrescu and Petrescu, 2016).

In tissue engineering 3-dimensional scaffold is formed which is fabricated with natural or artificial materials exhibit high porosity and pore interconnectivity (Hoppe et al., 2011; Maeno et al., 2005; Sachot et al., 2013). The function of scaffold is not only to provide structural support to the bony structure but also to enhance cell proliferation and differentiation of Osteoblastic cell (Hoppe et al., 2011; Aversa et al., 2016). Several Inorganic Bioactive materials could form a desired porous scaffold with suitable mechanical properties. According to the researched literature the ionic dissolution is the key procedure through which inorganic material behavior in forming scaffold and interact with living tissue can be understood in vitro and Vivo. Some inorganic elements such as Sr, Cu, Co, Zn was already present in the human body and play anabolic effect on bone metabolism (Hoppe et al., 2011). Introduction of therapeutic ions in the scaffold material for increase its bioactivity (Sachot et al., 2013). The release of ions after exposure of physiological environments is effected on the bioactivity of scaffold related to osteogenisis and angiogenesis (Hench and Wilson, 1993; Hoppe et al., 2011; Hutmacher, 2000; Okuda et al., 2007).

MATERIAL AND METHOD

ROLE OF INORGANIC IONS IN BONE METABOLISM

Human bone has natural process of healing through the process of remodeling. Remodeling is the process of deposition and resorption of bone tissue by Osteoblastic and Osteoclastic cell activities. As remodeling occurs, Osteoblastic cells produced new bone cells and Osteoclastic bone cells destroyed or resorbed existing bone. Failure in maintaining the balance of

remodeling results in multiple problems like Osteoporosis and Arthritis (Habib *et al*., 2007; Petrescu et al., 2016).

The remodeling procedure is regulated by few growth factors, hormones and inorganic ions such as Calcium (Ca) (Heinemann *et al*., 2013; Julien *et al*., 2007; Liu, 2003; Saltman and Strause, 1993), Phosphorous(p) (Heinemann *et al*., 2013; Julien *et al*., 2007), Silicon (Si) (Liu, 2003), Strontium(Sr) (Liu, 2003), Zinc(Zn) (Liu, 2003; Saltman and Strause, 1993), Boron(B), Vanadium(V), Cobalt (Co), Magnesium(Mg) (Cepelak et al., 2013), Magneese (Mn, Copper(Cu) (Liu, 2003; Saltman and Strause, 1993). Inorganic ions dissolution plays a very important role in the process of bone healing (Mouriño *et al*., 2012). Metal ions act as an enzyme co-factored effect on signaling pathways to stimulate the metabolic effect on tissues engineering (Hoppe *et al*., 2011). Metal ions play important role as therapeutic agent in hard and soft tissue engineering. Ca and P ions are the part of the main component of inorganic apatite of human bone ($Ca_{10}(PO_4,CO_3)6OH_2$) (Bielby *et al*., 2005; Habib *et al*., 2007; Hoppe *et al*., 2011; Mouriño *et al*., 2012). Bioactive Material has ability to release inorganic ions and contributes in natural bone metabolism (Bielby *et al*., 2005; Habib *et al*., 2007; Karageorgiou and Kaplan, 2005; Maeno *et al*., 2005).

BIOACTIVE MATERIALS

First Generation Biomaterials (Table 1): Early biomaterials were used to replace damage or missing living structure that's why biomaterial assumed to have compatible physical properties similar to the natural structure with minimal tissue reaction or toxic effect on tissue. Most of the materials were bioinerts (Sundar et al., 2012; Petrescu et al., 2015).

Second Generation Biomaterials (Table 1): During early 70s bioactive material such as bioactive glass, ceramic glass and composites were introduced in the field of tissue engineering. These materials make a chemical bond with natural tissue and elicit tissue generation by enhancing production of tissue forming cells, through the ion dissolution process from the surface of materials (Sundar et al., 2012). Second Generation bio materials also includes resorbable biomaterial such as calcium phosphates.

It has ability to breaks down chemically and reabsorb to equivalent ratio of that regrowth tissue (Shirtliff and Hench, 2003; Gramanzini et al., 2016). The material tissue bonding involves 11 steps of reacting. First 5 steps involves surface material reaction of ion exchange which followed by poly condensation reaction. This surface reaction provides a layer of hydroxyapatite layer that equivalent to the inorganic layer of natural bone tissue.

Third Generation Biomaterials (Table 1): The concept of resorbable materials and bioactive material is merged to form third generation bioactive resorbable glass and ceramic material that can activate gens in tissue engineering (Shirtliff and Hench, 2003). Bioactive materials are used in powder, solution or micro particles form to stimulate tissue repair (Sorrentino et al., 2007; 2009). The release of chemicals in the form of ions dissolution from the bioactive materials and growth factors such as bone morphogenic protein that enhance the cell proliferation (Hench and Polak, 2002; Sundar et al., 2012) due to osteo conduction and osteoproduction process. The surface reaction of material that gives ions dissolution responsible in intracellular and extracellular response (Hench and Polak, 2002; Sundar et al., 2012).

Cell Cycle and Gene Activation: Osteoblastic cell differentiation and proliferation is controlled by the activation of a synchronized sequence of genes which undergo mitosis of cells after that the synthesis of extracellular matrix by bone cells occur (Polak and Hench, 2005). There is genetic control of cellular response to the bioactive material also present. When human Osteoblastic cells expose to ionic dissolution of bioactive material seven families of genes are activated. These activated genes express protein that effect on differentiation and proliferation of osteoblast (Sundar et al., 2012). The ion dissolution (Fig. 1) of bioactive materials that enhance cell repair at molecular level by creating scaffold on the damage bone tissue (Polak and Hench, 2005; Sundar et al., 2012). After construction of scaffold it is necessary to build blood vessels in it.

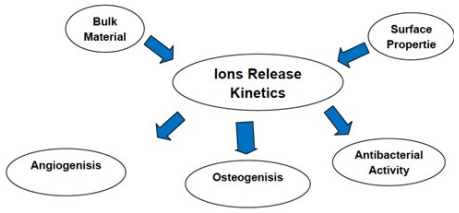

Fig. 1.Diagrammatic representation of the kinetics of ion release and its role in different biological process

Table 1

First, second and third generations of bioactive materials with their applications

Generation	Material	Difference in function
First	Bio inert	Replace tissues without reaction with tissues
Second	Bioactive	Making chemical bond with tissues
Third	Bioactive plus resorbable	Gene activation

Third Generation bioactive materials are also useful in making vascularization in scaffold. This is revolution in molecular biology it makes connection between inorganic materials with living tissue (Sundar et al., 2012). The materials used in scaffold are synthetic polymers such as Polysaccharides, Poly (x-hydroxy ester), hydrogels or thermoplastic elastomers (Boccaccini and Ma, 2014; Rezwan et al., 2006) and other important materials are bioactive ceramic such as calcium phosphate and bioactive glasses or glass ceramic (Boccaccini and Ma, 2014; Rezwan et al., 2006) composites of polymers and ceramics are being produced to enhance mechanical scaffold stability and to improve tissue interaction (Bielby et al., 2005; Kim et al., 2004). Polymers are the chain of molecules which has repeated unit in it. Repeated unit make polymers differ it from other small molecules. Monomer, the elimination of small molecules such as water and HCL during polymerization (Ratner et al., 2004). The syntheses of polymers are of two methods, additional polymerization chain reaction and condensation polymerization (Ratner et al., 2004). Polymers are in amorphous or semi crystalize form. Its crystalline state can be increased by short side group and chain regularity. Its crystallization increase its mechanical property which determines the thermal behavior and also increases its fatigue strength (Ratner et al., 2004).

The most important property of polymers to use as biomaterial is the stress at the point of breakage or failure. Failure means catastrophic (complete breakage). Viscoelastic property also represented by its thermal behavior (Perillo et al., 2010). Linear amorphous Polymer with increase temperature 5-10°C, converted from stiff glass to leathery material (Boccaccini and Ma, 2014; Ratner et al., 2004).

Saturated Polymer: The most often used for 3D scaffold biodegradable synthetic polymers, saturated polymers includes Poly-x-hydroxy esters, poly (lactic acid) PLA and poly (glycolic acid) (PGA) as well as poly (lactic-Co glycolide) (PLGA) Co polymer (Rezwan et al., 2006). The heterogeneous degradation contributes in neutralization of the carboxylic end group at the surface and diffusion of soluble oligomers from the surface towards inside (Rezwan et al., 2006), this helps to reduce acidity on the surface layer. The degradation rate is increased due to the auto catalyzing of the carboxyl end group. Hydrolysis of amorphous polymer such as PDLLA is more frequent because of it less crystalline property. PDLLA application used for scaffold formation in tissue engineering (Boccaccini and Ma, 2014; Mano et al., 2004; Rezwan et al., 2006).

Unsaturated Polymer: Polypropylene fumarate is an unsaturated polyester. Its degraded products, propylene glycol and fumaric acid, are biocompatible and also removed from the body. The double bond at the back-bone of polymer that become cross linkage causes hardening in it. Its mechanical

properties depend on its molecular weight. Polypropylene fumarate is used for scaffold in tissue engineering (Hedberg et al., 2005; Mano et al., 2004; Rezwan et al., 2006).

Polyhydroxyalkanoates (PHB, PHBV, P4HB, PHBHHx, PHO): Polyhydroxyalkanoates (PHA) are produced by microorganism and aliphatic poly esters. Due to its biodergrable and thermoprocesseble properties it is used as biomaterials. PHA, particularly poly-3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly-4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and poly-3-hydroxyoctanoate were used in tissue engineering. For obtaining desirable application PHA may use by blending with other polymers, enzymes. The challenge is to have a cost effective industrial production for some PHA polymers due to their lengthy and expensive exploration process (Rezwan et al., 2006).

Surface Bioeroding Polymers: These polymers undergo heterogeneous hydrolysis interaction with water. Having surface eroding property these polymers have minimal toxic effect, having mechanical integrity and increase bone growth in porous scaffold (Apicella and Hopfenberg, 1982; Rezwan et al., 2006).

Ceramic Materials: Ceramic materials were used in daily routine. Ceramics are solid which inorganic and non-metallic in nature. They present in both crystalline and monocrystalline form. Glasses and glass-ceramic are subclasses of ceramic (Rezwan et al., 2006; Morales-Hernandez et al., 2012).

Bioactive Glass: Although, the first Bioactive glass 45S5 was discovered by L. Hench in 1969, Bioactive glasses with the composition of SiO2, P2O5, Na2O, CaO started to be clinically use only from 1985 (Brauer, 2015).

The clinical success depends on its properties of degradation in solution forming surface layer of hydroxycarbonate appetite, making bond with bone and ultimately replaced by natural tissues (Döhler et al., 2016). It is biocompatible in vivo. It has tendency to crystallize, which makes processing into sintered porous scaffolds (Döhler et al., 2016; Gorustovich et al., 2010). It tends to show a lower solubility, degradation and bioactivity.

Bioactive mats used for healing application and soft tissue repair, making pours scaffold and reinforcing degradation of polymers. Bioactive glass also helps in preparation of glass fiber-reinforced polymers to get composites with anisotropic properties, which can be used in degradable fixation devices for bone fractures (Döhler et al., 2016; Gorustovich et al., 2010). It also provides help in bone regeneration bactericidal action orvascularization (Saiz et al., 2002; Rezwan et al., 2006).

Hybrid ceramo-polymeric materials have been also developed (Schiraldi et al., 2004; Aversa et al., 2009) with improve biocompatibility and mechanical properties.

Structure of Bioactive Glass: The degradation of Bioactive glass in physiological solution that form hydroxyl appetite layer which allow bonding between glass and the bone which enhance bone regeneration instead of just bone replacement (Rezwan et al., 2006). All this procedure is strongly supported by the specific structure of bioactive glass with both the polymerization of phosphate and silicate (Cormack and Tilocca, 2012).

There are long intervals between temperature variables from super cold liquid to solid glass that is a crystalline solid. At high temperature decrease its viscosity. Oxides glass is manufactured by melting of precursors (Jones and Clare, 2012). Bioactive glass particle size also effect on the resorption and formation of bone. Smaller the size may affect more rapid resorption and involve in substitution of new bone than the larger particles (Cormack and Tilocca, 2012).

Effect of PH and heat on Bioactive Glass: Bioactive glass has an ability to make bond with bone tissues by releasing ions, to form appetite layer. Ions release process increases in low pH and the formation apatite layer become faster through which cells adhere and proliferate (Shah et al., 2014). Bioactive glass has tendency to crystallize on heating that reduce its capability of making appetite. If Potassium is substituted with sodium and fluoride is added to it thus increasing calcium alkalication ration, the crystallization process at sintering scaffold and degradation process forming appaite in few hours (Shah et al., 2014).

Gene Expression: Bioactive glass has ability to effect on gene expression profiling of human osteoblasts. Ionic products of Bioglass® 45S5 dissolution increases the level of 60 transcript of twofold or more and regulates RCLgene. A c-myc responsive growth related gene and also control cell cycle regulators such as G1/S specific cyclin D1 and apoptosis regulators including calpain and defender against cell death (DAD1). It also contributes in gene regulation of cell surface receptors CD44 and integrin β1, various extracellular matrix regulators including metalloproteinases-2 and 4 and their inhibitors TIMP-1 and TIMP-2. It shows Bioactive glass has property to enhance the osteo productive process (Xynos et al., 2001; Yamamuro et al., 1991).

Bioactive Silicate Glass: The biological activity Hench Glass depends on the partial dissolution of silicate network and reactivity of the glass surface. Silicate glass is amorphous solid in nature. It is structurally covalent bond of SiO_4 linked with (BO) oxygen atom (Lee et al., 2016). Crest bone and surgeries related to implants (Kumar et al., 2011). A very frequent change of Ca and Na modifier occurs at high temperature, the fast migration of Ca

and Na can be seen and at high temp phosphate and silicate network also effected (Kim et al., 2004).

Composite Bioactive Material: The composite of polymer and bioglass is achieve to get benefits of both types of materials for the reinforcement of porous scaffold (Schiraldi et al., 2004; Rezwan et al., 2006).

Metal Bioactive Material

Titanium: Titanium is biocompatible to human body tissue. It has its physical properties which makes it more desirable material than other alloys. As compared to the gold alloy is four time less. Titanium is a light metal and has resistant to corrosion. It is strong and ductile metal. It helps and encourage surrounding bone to grow that enhance rapid healing (Cortizo et al., 2006; Smith, 1981). New glassy metals alloy and hybrid metals-polymeric systems (trabecular sintered Titanium scaffolds) may be designed for optimum mechanical properties for osseointegration (Apicella and Aversa, 2016; Aversa et al., 2016).

Bioactive Materials Coating Techniques: There is essential to understand the specific technique through which materials are deposited. Calcium phosphates are the largest group of materials most widely used for this purpose (Neifar et al., 2016). Dry Deposition Techniques: Dry deposition techniques are physical coating techniques deal with the deposition of calcium phosphates (Kokubo et al., 2016; Annunziata et al., 2008) Among different types of techniques plasma spraying technique is most widely used commercially (Annunziata et al., 2008).

Plasma-Spraying (PS) technique: In this technique, the precursor material is deposited on the target metal (implant) through plasma hot jet. If this procedure is performed in atmospheric pressure (Atmospheric Plasma Spraying, APS) or it is performed under vacuum (Vacuum Plasma Spraying, VPS) or under reduced pressure (Low Pressure Plasma Spraying, LPS).

Radio Frequency (RF) Magnetron Sputtering: Sputtering is the technique through atoms or molecules are ejected and bombarded from vacuum chamber on to the target forming layer of precursor material with high energy ions (Perrotta et al., 2015).

Pulsed Laser Deposition (PLD): PLD is the vapor deposition method through which focused pulse laser is subjected to the target and a thin layer of film CaP is deposited on the target and create these product Ca4P2O9, Ca3(PO4)2, CaO, P2O5 and H2O (Rezwan et al., 2012). Forming high-energy plasma cloud is composed of Electron, atoms, ions, molecules, and molecular clusters and, in some cases, droplets and target fragments.

Wet Deposition Technique: Wet deposition technique is the alternative of physical deposition technique. Which deals and preserves the activity of bioactive molecules. It has advantage of simple setup, minimal chemical preparations and coating of 3D implants (Rezwan et al., 2012).

Biomimetic Deposition Method: This procedure is performed under physiological temperature and pressure in which pre heated substrate is immersed in so called Simulated Body Fluid (SBF) to obtain coated with Calcium Phosphate (CaP) layer on to the substrate.
SOL–GEL Technique: Sol-Gel technique is applied to provide alternative to physical deposition techniques that enhance bone attachment to the materials and increase the process of bone healing. In this technique the layer of bioactive ceramic material is applied to form bioactive surface layer that prevents corrosion in metal. This coated material makes a bond with the existing bone and also control the release of metal ions into the tissue (Beketova et al., 2016). The first material which is used as a coating layer on the metal is synthetic Hydroxyl apatite Ca10(Po4)6(OH)2. During coating an adherence between the layer and the metal is also required. Electrophoresis, hot pressing and sputtering methods can deposit the coating. The Sol-Gel technique can be used as an alternative to plasma spraying process. In comparison of two methods, there are some differences in which the main one is cost effectiveness (Beketova et al., 2016).

Due to the poor mechanical strength of hydroxyapatite, it cannot be used in bulk material instead it can be used as a coating of a thin layer on metals to achieve bioactive material properties. As compared to the melting method, Sol-Gel method is a low temperature reaction. Hydroxyapatite has the same composition of natural bone tissues and it enhances bone growth as its bioactive behavior works without any immune response from the body.

The Sol-Gel technique is based on colloidal suspension of solid particles (1-500 nm) in size in solution to make Gel (Sol). This Sol-Gel layer is applied on the target by spraying, spin coating or dip coating methods. After drying only Sol-Gel transition is left.

DISCUSSION

Biomaterials were used to replace damaged bones since several years. The materials used in the early years have been chosen to be bio inert and not interacting with bone tissues. Further on, bioactive materials were introduced. The big difference was to make chemical and mechanical interactions with the bone tissue (Apicella et al., 1993; Schiraldi et al., 2004; Apicella et al., 2010; 2011; 2015; Aversa et al., 2009; 2016).

Bone tissue is the combination of inorganic component and organic matrix. Bioactive material structure is similar to the inorganic component of bone, such as CaP and HA. These materials, after degradation in aqueous medium, releases ions that help in bone repair. Polymers and bio glass are main types that took the main attention of researchers.

Techniques through which bioactive materials are deposited on the implant are remarkably the revolution, in the field of implantology. Bioactive materials can be deposited on the metal to achieve bioactive surface bonding, the bone with the advantages of strength of metal. Different techniques were discussed and advantages and disadvantages were also discussed but Sol-Gel technique is the latest technology with good prognosis.

CONCLUSIONS

The earliest bioactive materials which were used within the body were identified as called Prostheses. These Prostheses had to be standardized according to the physical properties of living tissues. Professor Bill Bonfield et al. was the pioneer of researching mechanical properties of living tissues, its skills were especially centered on bone to make Prosthesis. The basic objective of making the Prosthesis was to achieve a combination of physical properties of living tissue with minimal toxic response to the surrounding structures. These prostheses had the limitation of stress shielding and bone resorption.

Bioactive materials are most latest materials which are still undergo in research and bring new technology to make it commercial material and give benefit to humanity with its low cost and easy availability.

The double bond at the back-bone of polymer that become cross linkage causes hardening in it. Its mechanical properties depend on its molecular weight. Titanium is biocompatible to human body tissue. It has its physical properties which makes it more desirable material than other alloys.

REFERENCES

1. Annunziata, M., L. Guida, L. Perillo, R. Aversa and I. Passaro *et al*., 2008. Biological response of human bone marrow stromal cells to sandblasted titanium nitride-coated implant surfaces. J. Mater. Sci. Mater. Med., 19: 3585-3591. DOI: 10.1007/s10856-008-3514-2.
2. Apicella, A. and R. Aversa, 2016. Factors affecting chemo-physical and rheological behaviour of Zr_{44}-Ti_{11}-Cu_{10}-Ni_{10}-Be_{25} metal glassy alloy supercooled liquids. Am. J. Eng. Applied Sci. DOI: 10.3844/ajeassp.2016.98.106
3. Apicella, A., B. Cappello, M.A. Del Nobile, M.I. La Rotonda and G. Mensitieri *et al*., 1993. Poly(Ethylene oxide) (PEO) and different molecular weight PEO blends monolithic devices for drug release. Biomaterials, 142: 83-90. DOI: 10.1016/0142-9612(93)90215-N
4. Apicella, A. and H.B. Hopfenberg, 1982. Water-swelling behavior of an ethylene–vinyl alcohol copolymer in the presence of sorbed sodium chloride. J. Applied Polymer Sci., 27: 1139-1148. DOI: 10.1002/app.1982.070270404
5. Apicella, D., R. Aversa, M. Tatullo, M. Simeone and S. Sayed *et al*., 2015. Direct restoration modalities of fractured central maxillary incisors: A multi-levels

validated finite elements analysis with *in vivo* strain measurements. Dental Mater., 31: e289-e305. DOI: 10.1016/j.dental.2015.09.016
6. Apicella, D., M. Veltri, P. Balleri, A. Apicella and M. Ferrari, 2011. Influence of abutment material on the fracture strength and failure modes of abutment-fixture assemblies when loaded in a bio-faithful simulation. Clin. Oral Implants Res., 22: 182-188. DOI: 10.1111/j.1600-0501.2010.01979.x
7. Apicella, D., R. Aversa, E. Ferro, Ianniello, D. Ianniello and A. Apicella, 2010. The importance of cortical bone orthotropicity, maximum stiffness direction and thickness on the reliability of mandible numerical models. J. Biomed. Mater. Res. Part B Applied Biomater., 93: 150-163. DOI: 10.1002/jbm.b.31569
8. Aversa, R., D. Apicella, L. Perillo, R. Sorrentino and F. Zarone *et al*., 2009. Non-linear elastic three-dimensional finite element analysis on the effect of endocrown material rigidity on alveolar bone remodeling process. Dental Mater., 25: 678-690. DOI: 10.1016/j.dental.2008.10.015
9. Aversa, R; Parcesepe, D; Petrescu, RV; Chen, G; Petrescu, FIT; Tamburrino, F; Apicella, A; 2016 Glassy Amorphous Metal Injection Molded Induced Morphological Defects, American Journal of Applied Sciences, 13(12):1476-1482.
10. Beketova, A., N. Poulakis, A. Bakopoulou, T. Zorba and L. Papadopoulou *et al*., 2016 Inducing bioactivity of dental ceramic/bioactive glass composites by Nd:YAG laser. Dent Mater., 32: e284-e296. DOI: 10.1016/j.dental.2016.09.029
11. Bielby, R.C., R.S. Pryce, L.L. Hench and J.M. Polak, 2005. Enhanced Derivation of Osteogenic Cells from Murine Embryonic Stem Cells after Treatment with Ionic Dissolution Products of 58S Bioactive Sol–Gel Glass. Tissue Eng., 11: 479-488. DOI: 10.1089/ten.2005.11.479
12. Boccaccini, A.R. and P.X. Ma, 2014. Tissue Engineering using Ceramics and Polymers. 1st Edn., Woodhead Publishing, Elsevier, ISBN-10: 9780857097163, pp: 728.
13. Bonfield, W., M.D. Grynpas, A.E. Tully, J. Bowman and J. Abram, 1981. Hydroxyapatite reinforced polyethylene — a mechanically compatible implant material for bone replacement. Biomaterials, 2: 185-186. DOI: 10.1016/0142-9612(81)90050-8
14. Brauer, D.S., 2015. Bioactive glasses—structure and properties. Angew. Chem. Int. Ed. Engl., 54: 4160-4181. DOI: 10.1002/anie.201405310
15. Buzea, E., F.l. Petrescu, L. Nanut, C. Nan and M. Neacsa, 2015. Mechatronic system to determine the concentration of carotenoids, analele Univers. Craiova Biologie Horticultura Tehn. Prel. Prod. Agr. Ing. Med., 20: 371-376.
16. Čepelak I., Slavica Dodig, Ognjen Čulić, 2013, Magnesium-more than a common cation. Med. Sci., 39: 47-68.
17. Chen, Q., C. Zhu and G.A. Thouas, 2012. Progress and challenges in biomaterials used for bone tissue engineering: Bioactive glasses and elastomeric composites. Progress . Biomater., 1: 1-22. DOI: 10.1186/2194-0517-1-2
18. Cormack, A.N. and A. Tilocca, 2012. Structure and biological activity of glasses and ceramics. Philos. Trans. Math. Phys. Eng. Sci., 370: 1271-1280. DOI: 10.1098/rsta.2011.0371
19. Cortizo, A.M., M.S. Molinuevo, D.A. Barrio and L. Bruzzone, 2006. Osteogenic activity of vanadyl(IV)–ascorbate complex: Evaluation of its mechanism of action. Int. J. Biochem. Cell Biol., 38: 1171-1180. DOI: 10.1016/j.biocel.2005.12.007
20. Döhler, F., D. Groh, S. Chiba, J. Bierlich and J. Kobelke *et al*., 2016. Bioactive glasses with improved processing. Part 2. Viscosity and fibre drawing, J. Non-Crystalline Solids, 432A: 130-136. DOI: 10.1016/j.jnoncrysol.2015.03.009

21. Gorustovich, A.A., J.A. Roether and A.R. Boccaccini, 2010. Effect of bioactive glasses on angiogenesis: A review of *in vitro* and *in vivo* evidences. Tissue Eng. Part B Rev., 16: 199-207. DOI: 10.1089/ten.TEB.2009.0416
22. Gramanzini, M., S. Gargiulo, F. Zarone, R. Megna and A. Apicella *et al*., 2016. Combined microcomputed tomography, biomechanical and histomorphometric analysis of the peri-implant bone: A pilot study in minipig model. Dental Mater., 32: 794-806. DOI: 10.1016/j.dental.2016.03.025
23. Habib, N., N.Y. Levičar, M. Gordon, L. Jiao and N. Fisk, 2007. Stem cell repair and regeneration. World Sci., 2: 304- 304. DOI: 10.1142/9781860948312
24. Hedberg, E.L., C.K. Shih, J.J. Lemoine, M.D. Timmer and M.A. Liebschner *et al*., 2005. *In vitro* degradation of porous poly(propylene fumarate)/poly(dl-lactic-co-glycolic acid) composite scaffolds. Biomaterials, 26: 3215-3225. DOI: 10.1016/j.biomaterials.2004.09.012
25. Heinemann, S., C. Heinemann, S. Wenisch, V. Alt and H. Worch *et al*., 2013. Calcium phosphate phases integrated in silica/collagen nanocomposite xerogels enhance the bioactivity and ultimately manipulate the osteoblast/osteoclast ratio in a human co-culture model. Acta Biomaterialia, 9: 4878-4888. DOI: 10.1016/j.actbio.2012.10.010
26. Hench, L.L. and J.M. Polak, 2002. Third-generation biomedical materials. Science, 295: 1014-1017. DOI: 10.1126/science.1067404
27. Hench, L.L. and I. Thompson, 2010. Twenty-first century challenges for biomaterials. J. Royal Society Interface, 7: S379-S391. DOI: 10.1098/rsif.2010.0151.focus
28. Hench, L.L. and J. Wilson, 1993. An introduction to bioceramics. World Sci., 1: 396-396. DOI: 10.1142/2028
29. Hoppe, A., N.S. Güldal and A.R. Boccaccini, 2011. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials, 32: 2757-2774. DOI: 10.1016/j.biomaterials.2011.01.004
30. Hutmacher, D.W., 2000. Scaffolds in tissue engineering bone and cartilage. Biomaterials, 21: 2529-2543. DOI: 10.1016/S0142-9612(00)00121-6
31. Jones, J.R. and A.G. Clare, 2012. Bio-Glasses. An Introduction. 1st Edn., Wiley, Chichester, ISBN-10: 1118346475, pp: 320.
32. Julien, M., D. Magne, M. Masson, M. Rolli-Derkinderen and O. Chassande *et al*., 2007. Phosphate stimulates matrix Gla protein expression in chondrocytes through the extracellular signal regulated kinase signaling pathway. Endocrinology, 148: 530-537. DOI: 10.1210/en.2006-0763
33. Karageorgiou, V. and D. Kaplan, 2005. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials, 26: 5474-5491. DOI: 10.1016/j.biomaterials.2005.02.002
34. Kim, H.W., J.C. Knowles and H.E. Kim, 2004. Development of hydroxyapatite bone scaffold for controlled drug release via poly(ϵ-caprolactone) and hydroxyapatite hybrid coatings. J. Biomed. Mater. Res. Part B: Applied Biomater., 70: 240-249. DOI: 10.1002/jbm.b.30038
35. Kokubo, Y., Kisara, K., Yokoyama, Y., Ohira-Akiyama, Y., Tada, Y., Hida, A., Kawano, Y. (2016). Habitual dietary protein intake affects body iron status in Japanese female college rhythmic gymnasts: a follow-up study. SpringerPlus, 5(1), 862. http://doi.org/10.1186/s40064-016-2569-7
36. Kumar, P.G., J.A. Kumar, N. Anumala, K.P. Reddy and H. Avula *et al*., 2011. Volumetric analysis of intrabony defects in aggressive periodontitis patients

following use of a novel composite alloplast: A pilot study. Quintessence Int., 42: 375-384. PMID: 21519556
37. Lee, J.H., S.J. Seo and H.W. Kim, 2016. Bioactive glass-based nanocomposites for personalized dental tissue regeneration. Dent Mater. J., 35: 710-720. DOI: 10.4012/dmj.2015-428
38. Liu, Z.X., 2003. Calcium Support Nutrients for enhancing the absorption, utilization and function of calcium. Compliments of Coral Advantage. http://www.coraladvantage.com/supportnutrients.pdf
39. Maeno, S., Y. Niki, H. Matsumoto, H. Morioka and T. Yatabe *et al*., 2005. The effect of calcium ion concentration on osteoblast viability, proliferation and differentiation in monolayer and 3D culture. Biomaterials, 26: 4847-4855. DOI: 10.1016/j.biomaterials.2005.01.006
40. Mano, J.F., R.A. Sousa, L.F. Boesel, N.M. Neves and R.L. Reis, 2004. Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: State of the art and recent developments. Composi. Sci. Technol., 64: 789-817. DOI: 10.1016/j.compscitech.2003.09.001
41. Morales-Hernandez, D.G., D.C. Genetos, D.M. Working, K.C. Murphy and J.K. Leich, 2012. Ceramic identity contributes to mechanical properties and osteoblast behavior on macroporous composite scaffolds. J. Funct. Biomat., 23: 382-397. DOI: 10.3390/jfb3020382
42. Mouriño, V., J.P. Cattalini and A.R. Boccaccini, 2012. Metallic ions as therapeutic agents in tissue engineering scaffolds: An overview of their biological applications and strategies for new developments. J. Royal Society Interface, 9: 401-419. DOI: 10.1098/rsif.2011.0611
43. Neifar, M., Chouchane, H., Mahjoubi, M., Jaouani, A., & Cherif, A. (2016). Pseudomonasextremorientalis BU118: a new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolourization. 3 Biotech, 6(1), 107. http://doi.org/10.1007/s13205-016-0425-7
44. Okuda, T., K. Ioku, I. Yonezawa, H. Minagi and G. Kawachi *et al*., 2007. The effect of the microstructure of β-tricalcium phosphate on the metabolism of subsequently formed bone tissue. Biomaterials, 28: 2612-2621. DOI: 10.1016/j.biomaterials.2007.01.040
45. Perillo, L., R. Sorrentino, D. Apicella, A. Quaranta and C. Gherlone *et al*., 2010. Nonlinear visco-elastic finite element analysis of porcelain veneers: A submodelling approach to strain and stress distributions in adhesive and resin cement. J. Adhesive Dentistry, 12: 403-413.
46. Perrotta, V, R. Aversa, C. Misiano and Apicella, 2016. The compatibility of ion plating plasma assisted technologies for preservation antique ceramics. Athens.
47. Petrescu, RV; Aversa, R; Apicella, A; Petrescu, FIT; 2016 Future Medicine Services Robotics, American Journal of Engineering and Applied Sciences, 9(4):1062-1087.
48. Petrescu, F.l., E. Buzea, L. Nănuţ, M. Neacşa and C. Nan, 2015. The role of antioxidants in slowing aging of skin in a human, Analele Univers. Craiova Biologie Horticultura Tehn. Prel. Prod. Agr. Ing. Med., 20: 567-574.
49. Petrescu, F.I., Petrescu, R.V., 2016 Protecting the Environment through Green Energy, Analele Universitatii din Oradea, Fascicula: Protectia Mediului 26A:149-154.
50. Polak, J. and L. Hench, 2005. Gene therapy progress and prospects: In tissue engineering. Gene Therapy, 12: 1725-1733. DOI: 10.1038/sj.gt.3302651

51. Ratner, B.D., A.S. Hoffman, F.J. Schoen and J.E. Lemons, 2004. Biomaterials Science: An Introduction to Materials in Medicine. 1st Edn., Academic Press, Amsterdam, ISBN-10: 0125824637, pp: 851.
52. Rezwan, K., Q. Chen, J. Blaker and A.R. Boccaccini, 2006. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials, 27: 3413-3431. DOI: 10.1016/j.biomaterials.2006.01.039
53. Sachot, N., O. Castaño, M.A. Mateos-Timoneda, E. Engel and J.A. Planell, 2013. Hierarchically engineered fibrous scaffolds for bone regeneration. J. Royal Society Interface. DOI: 10.1098/rsif.2013.0684
54. Saiz, E., M. Goldman, J.M. Gomez-Vega, A.P. Tomsia and G.W. Marshall *et al*., 2002. *In vitro* behavior of silicate glass coatings on Ti6Al4V. Biomaterials, 23: 3749-3756. DOI: 10.1016/S0142-9612(02)00109-6
55. Saltman, P.D. and L.G. Strause, 1993. The role of trace minerals in osteoporosis. J. Am. College Nutrit., 12: 384-389. DOI: 10.1080/07315724.1993.10718327
56. Schiraldi, C., A. D' Agostino, A. Oliva, F. Flamma and A. De Rosa *et al*., 2004. Development of hybrid materials based on hydroxyethylmethacrylate as supports for improving cell adhesion and proliferation. Biomaterials, 25: 3645-3653. DOI: 10.1016/j.biomaterials.2003.10.059
57. Shah, F.A., D.S. Brauer, R.M. Wilson, R.G. Hill and K.A. Hing *et al*., 2014. Influence of cell culture medium composition on *in vitro* dissolution behavior of a fluoride-containing bioactive glass. J. Biomed. Mater. Res. A., 102: 647-654. DOI: 10.1002/jbm.a.34724
58. Shirtliff, V. and L. Hench, 2003. Bioactive materials for tissue engineering, regeneration and repair. J. Mater. Sci., 38: 4697-4707. DOI: 10.1023/A:1027414700111
59. Smith, W.F., 1981. Structure and Properties of Engineering Alloys. 1st Edn., Mc Graw Hill, New York.
60. Sorrentino, R., R. Aversa, V. Ferro, T. Auriemma and F. Zarone *et al*., 2007. Three-dimensional finite element analysis of strain and stress distributions in endodontically treated maxillary central incisors restored with diferent post, core and crown materials. Dent Mater., 23: 983-993. DOI: 10.1016/j.dental.2006.08.006
61. Sorrentino, R., D. Apicella, C. Riccio, E.D. Gherlone and F. Zarone *et al*., 2009. Nonlinear visco-elastic finite element analysis of different porcelain veneers configuration. J. Biomed. Mater. Res.-Part B Applied Biomater., 91: 727-736. DOI: 10.1002/jbm.b.31449
62. Sundar, V., R.P. Rusin and C.A. Rutiser, 2012. Bioceramics: Materials and Applications IV. Proceedings of a Symposium to Honor Larry Hench at the 105th Annual Meeting of The American Ceramic Society. 1st Edn., John Wiley and Sons, Hoboken, ISBN-10: 1118406079, pp: 182.
63. Tilocca, A., 2009. Structural models of bioactive glasses from molecular dynamics simulations.
64. Xynos, I.D., A.J. Edgar, L.D. Buttery, L.L. Hench and J.M. Polak, 2001. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass® 45S5 dissolution. J. Biomed. Mater. Res., 55: 151-157. DOI: 10.1002/1097-4636(200105)55:2<151::AID-JBM1001>3.0.CO;2-D