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Enzymatic crosslinking of silk fibroin – simple or complex?

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There are numerous ways of chemical crosslinking, however there is drawback for it – high toxicity and neutralisation, which comes after crosslinking. Nevertheless, there is safer way to make a dense polymer matrice from silk fibroin (SF) using enzymes [1].

Our research focuses on silk fibroin crosslinking with horseradish peroxide (HRP), which is an enzyme obtained from horseradish root [2], and hydrogen peroxide (H_2O_2). The main principle of this method is to crosslink tyrosine groups in SF. Both HRP and H_2O_2 concentrations and addition sequence has an affect on hydrogel structure, that we studied in the process of hydrogel preparation. Unfortunately, our hydrogels did not form as expected, so gelatin (G) was added to improve hydrogel elasticity and density.

In results, elastic, porous SF/G/HRP hydrogels were obtained. Gel fraction was up to 20% and hydrogels were stable in deionized water for a day, after which they started to dissolve. Overall, a non-toxic crosslinking method was used to form a stable SF matrice.

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Characterization of chemically cross-linked hydrogels based on ε-polylysine and hyaluronic acid

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Abstract: Development and investigation of multifunctional biomaterials is a main key area in fields of biomedicine. Hydrogels are considered as promising bio-tool for tissue engineering applications. However, several aspects as hydrogel base, cross-linking approach, viscoelastic properties, porosity, and adhesion tendency are still uncertain in the development of hydrogels. Although each year surgery field faces bacterial infection problems including bacterial hitting on biomaterial surface or surgical site which entails recovery and regeneration embarrassments. Considering this, achieving both physicochemical and antibacterial properties within requirements of biocompatibility is typical challenging topic. Present paper reveals study of novel chemically cross-linked hydrogels based on natural biopolymers: antibacterial ε-polylysine and bio-intrinsic hyaluronic acid. FT-IR, SEM, and further techniques were used for molecular structure, morphology, gel fraction and swelling behavior to ascertain the presence of newly formed hydrogel matrix and characterize physicochemical properties. Further studies were obtained to determine antibacterial activity against *E.coli* as Gram- bacteria and *S.aureus* as Gram+ bacteria. This work provides insights into developing bioactive antibacterial hydrogels as injectable biomaterial for tissue engineering.

Gallium substituted calcium phosphates: A review

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Calcium phosphates (CaPs) are widely used as biomaterials for bone regeneration. The most used synthetic bone substitutes are hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$) and tricalcium phosphate (TCP; $Ca_3(PO_4)_2$). CaPs provides such properties as osteoconductivity and biodegradability. Additionally, there is infection risk after implantation of CaPs due to the lack of antibacterial properties. Furthermore, antibiotic resistant of bacteria increases every year leading to the development of alternative materials to fight infections.

Metal ions have been studied in the past and can be used as alternative due to the antibacterial properties against bacteria. Gallium (III) (Ga^{3+}) deposition was detected in the bone tissue, relating to its linkage with bone remodelling on active metaphyseal growth plates and healing fractures [1]. Additional to that, Ga^{3+} have been studied from 1970's as a therapeutic agent of the effective treatment of autoimmune diseases. Furthermore, it plays an important role in the bone-tumour imaging, hypercalcemia treatment and it has shown antibacterial properties [2].

CaP substituted with Ga (III) ions empower long-term local ion release, thus avoiding negative effect of systematic introduction of gallium base drugs. Systemic administration (oral or parenteral) of Ga (III) compounds results in an elevated concentration of Ga in kidneys and liver, possibly generating toxicity issues [2].

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Development of analytical method for determination of cannabidiol content in liposomes and release medium

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Cannabidiol (CBD) is a non-psychoactive compound derived from Cannabis plant. It has potential for wide range of medical applications (1,2). The main disadvantage of CBD is its poor solubility in aqueous solutions, which affects its absorption into body fluids, thus reducing the therapeutic efficacy (3). To overcome this limitation and to improve the absorption of CBD in the body, it can be incorporated in liposomes (1). Liposomes are spherical drug carriers that form from phospholipids which are hydrated with an aqueous medium. To obtain liposomes natural and synthetic phospholipids can be used. During liposome formation phospholipid molecules are arranged in two layers so that their hydrophilic heads are oriented towards the aqueous phase while the hydrophobic tails are oriented towards the inside of the bilayer. Cholesterol is also commonly introduced into the liposome structure to improve the stability of the phospholipid bilayer and reduce its fluid permeability. These drug carriers have a unique property that distinguish them among other drug delivery systems - liposomes can encapsulate both hydrophilic and hydrophobic substances (4,5).

To evaluate the efficacy of the CBD-containing liposome system, an ultra-performance liquid chromatography (UPLC) analytical method for CBD determination and quantification was developed and validated in this study.

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α-Tricalcium Phosphate Bone Cements as Efficient Drug Delivery Systems for Cancer Treatment

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Bone cancer causes fractures and bone pain [1], with bisphosphonates being the most typical drugs used for its treatment [2]. Their intravenous administration, however, results in systemic toxicity [3], while the amount of drug reaching the cancer site is limited [4]. Therefore, local drug delivery systems are pivotal for effective treatment. Calcium phosphate bone cements have been preferred for tissue engineering and drug delivery, due to their biocompatible and osteoconductive properties [5], as well as their injectability [5,6]. Furthermore, their porosity facilitates the drug release, while their setting reaction is non-exothermic, favoring the loading of temperature-sensitive drugs [7]. Recently, among the calcium phosphates, α-tricalcium phosphate (α-TCP) has gained increased attention due to its ability to hydrolyze to calcium deficient hydroxyapatite at the physiological conditions of the human body [8]. Additionally, as α-TCP hardens by a dissolution-precipitation mechanism [9], during its setting, a network of micro-channels is formed, facilitating the drug loading. Moreover, its high solubility promotes the required biodegradability for the cement. Although a large number of drugs has been incorporated into the α-TCP cements produced by the solid-state reaction method [10], the drug entrapment into the α-TCP cements fabricated by novel synthesis - as well as such system *in vitro* and *in vivo* performance - is still unexplored.

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Chitosan and fucoidan carrier systems for sustained drug delivery

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Fucoidan is a sulfated polysaccharide derived from brown marine algae and has a broad spectrum of pharmacological and osteogenic differentiating activities. It modulates the effect of various growth factors on cell proliferation and increases the bioactivity of mesenchymal stem cells (1). Fucoidan is a non-toxic and biocompatible material used for drug delivery and preparation of scaffolds for bone and cartilage tissue engineerings (2). On the other hand, chitosan is a cationic polysaccharide with antimicrobial activity, thereby limiting the development of various bacteria (3). Fucoidan-chitosan carrier systems such as microparticles and microgels can be used in maxillofacial surgery for cell and growth factor delivery. The main advantage of drug delivery systems is that by controlling the drug release, high drug concentrations can be achieved locally and reduced side effects in other tissues. For optimal drug use, it is first necessary to study the interaction between fucoidan and chitosan, when they are capable of forming particles and when they are gels. By understanding this mechanism, it will be possible not only can to determine what types of drugs be included in delivery systems, but also to control the time of their release depending on the local location.

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Inflammatory process-related gene expression in the brain after closed and open head injury in mice

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The weight-drop (WD) model is a widely used experimental set-up to study the pathophysiology of closed-head injury. However, there is a substantial variability regarding biochemical and functional outcomes between laboratories. The aim of the study was to evaluate and compare the inflammatory response after WD injury in the brain and correlate these findings with the presence and severity of skull fractures. Male Swiss-Webster mice were subjected to WD injury with either a 2- or 5-mm cone tip and neurological outcome was assessed at 2 h and 24 h postinjury. The expression of inflammatory genes was measured at 12 h and 1, 3, and14 days postinjury. The cone with a 2 mm tip caused skull fractures in 33% of animals, while only 10% of animals had fractures when using the 5 mm cone. Mice with skull fractures had a more pronounced upregulation of inflammatory gene expression in the brain and worse functional outcome. The data from animals with skull fractures must be analyzed separately from those without skull fractures to reduce the heterogeneity (i.e., inflammatory response in the brain) of the WD model.

Metformin decreases the plasma concentration of gut microbiota metabolite trimethylamine N-oxide in an experimental model of type 2 diabetes

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Trimethylamine N-oxide (TMAO) is gut microbiota-derived metabolite associated with development of cardiometabolic diseases. This study aimed to investigate the effects of metformin, the most widely prescribed medication for the treatment of type 2 diabetes, *in vitro* on the microbial metabolism of choline, a dietary precursor of TMAO, and plasma TMAO levels *in vivo* in an experimental model of diabetes.

Metformin was administered to diabetic db/db mice and nondiabetic controls at a dose of 250 mg/kg for 8 weeks. Mice were also fed choline-rich diet to mimic high TMAO precursor intake. Moreover, the effects of metformin on bacterial growth and the production of TMAO precursor trimethylamine (TMA) *in vitro* were tested.

Diabetic mice presented 10-13-fold higher plasma concentrations of TMAO than nondiabetic mice, and metformin treatment resulted in a twofold decrease in TMAO levels. When choline was administered to facilitate TMAO production *in vivo*, metformin decreased plasma TMAO by ~50%. Metformin selectively affected the growth of various bacterial genera *in vitro* and significantly decreased the production of TMA.

Our data provide evidence that metformin suppresses bacterial TMA production and significantly decreases TMAO levels in db/db mice. These data also warrant further investigation of TMAO for diabetes research applications.

Isolation and characterization of gingival mesenchymal stromal cells (GMSC) for soft tissue regeneration

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With the rise in the numbers of oral cancer patients, there is an increase in oncological surgeries. This creates demand for alternative, less invasive methods of regeneration of soft tissue after surgical removal of malignant tumours. Mesenchymal stem cell (MSC) therapy is a promising solution for improved post-surgery healing of oncological patients' soft tissue.

GMSC are multipotent cells that are being isolated from gingival connective tissue and are distinguished by their quick self-renewal abilities and impeccable regeneration quality of damaged tissue. Gingival tissue is easily accessible and so the biopsy extraction is minimally invasive. Therefore GMSC are being used in the research of soft tissue regeneration.

Gingival tissues were obtained from 12 patients. This study was approved by the Ethics Committee of the Riga Stradins University (Nr.6-1/12/47). During the research, GMSC were isolated from tissue by enzymatic digestion. The obtained cells were then characterized according to the criteria of MSC population: 1) the adherence to plastic surfaces, 2) specific cell-surface antigen expression, 3) potential to differentiate into other cell types. The obtained cell cultures are adherent, express standardized MSC markers, and differentiate into osteoblasts, adipocytes and chondroblasts. The isolated cell populations meet the proposed set of criteria and thus can be defined as MSC. Obtained GMSC cells will be further used to develop a novel injectable hydrogel for soft tissue regeneration.

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Cold sintering of weakly crystalline apatite: influence of crystallinity and water content in the structure on its sintering ability

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The sintering ability of calcium phosphates at low temperatures using the cold sintering method (CS) is significantly influenced by the degree of crystallinity of the material and the amount of water in its structure.

The aim of this study was to find how crystallinity and water content in low crystalline apatite affect its sinterability by CS.

Low crystalline apatite with different crystallinity and water content was obtained by the dissolution-precipitation method, by leaving the synthesized precipitates in the synthesis solution for a different time periods. Afterward, the precipitates were filtered, rinsed and dried. CS was performed using a laboratory press and a pressing die with inner diameter of 13 mm.

Synthesized material and sintered samples were characterized by various analytical methods. X-ray diffraction patterns showed that crystallization begun in the third hour after the synthesis. Weakly crystalline hydroxyapatite was obtained 24 h after the synthesis. Thermogravimetry results showed that by increasing the holding time of the precipitates in the synthesis solution, the water amount in their structure decreases, also affecting their sinterability. Sintering did not affect the crystallinity of the synthesized material. The relative density of all sintered samples exceeded 90%.

Decreased cardiac content of long-chain acylcarnitines in TMLHE$^{-/-}$ mice prevents ischaemia-reperfusion-induced cardiac damage

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Accumulation of long-chain acylcarnitines (LCAC) is known to disrupt mitochondrial function, therefore, increasing myocardial damage during ischemia-reperfusion. We investigated how a decrease of carnitine and acylcarnitine biosynthesis can attenuate cardiac damage during myocardial infarction. For this purpose, trimethyllysine dioxygenase, the first enzyme of carnitine/acylcarnitine synthesis, gene knockout (TMLHE$^{-/-}$) mice model was developed, and myocardial infarct size, mitochondrial respiration and ROS production was determined in TMLHE$^{-/-}$ and wild type animals in *ex vivo* setting. Up to a 10-fold decrease in carnitine and acylcarnitine content was observed in TMLHE$^{-/-}$ mice cardiac tissue and plasma. Infarct size in TMLH$^{-/-}$ animals was by 35.8% lower compared to controls. A 57.8% decrease in OXPHOS coupling efficiency and a 3-fold increase in ROS production was observed in wild type cardiac fiber mitochondria from the risk area compared to the non-risk area. In comparison, OXPHOS coupling efficiency in TMLHE$^{-/-}$ mice mitochondria was preserved and the increase of ROS production was 2 times lower compared with that of wild type mice. In conclusion, TMLHE$^{-/-}$ mice model has significantly lower LCAC levels in plasma and cardiac tissue, which preserves mitochondrial function and reduces myocardial infarct during ischemia-reperfusion.

Amplification of the rat Insulin 2 gene coding sequence using Reverse Transcription-PCR

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As diabetes is predicted to affect 10.9% of the world population by 2040 and accessibility to insulin therapy has been declining in recent years, the need for a more permanent alternative to repeated insulin injections might become imperative [1; 2]. Rodent models of diabetes have been useful for studying the effects of novel pharmacotherapies, but most mechanisms for induction of diabetes involve generating animals requiring insulin therapy for survival [3; 4]. Under standard protocol, this entails administration of human insulin [5], but studies have shown the human protein to be less effective at glycaemic control in diabetic rats than its rat orthologue, encoded by the *Insulin2 (Ins2)* gene [6]. Hence, using rat insulin 2 for maintaining diabetic rat strains might be more cost-effective, as lower doses would be necessary, and reduce any side effects from administering a foreign protein. Since rat insulin 2 is not available for purchase commercially, the aim of this project was to design and test primers for the amplification of the rat *Ins2* coding sequence using Reverse Transcription-PCR (RT-PCR). The coding sequence was amplified successfully and could further be used for molecular cloning and expression in an appropriate bacterial host to mass-produce rat insulin 2.

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Bioinks for bone regeneration

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Bone tissue engineering is a multidisciplinary field that deals with the fabrication of scaffolds for bone and cartilage regeneration. A scaffold provides a 3D conformation for cells to attach and proliferate, that initiates the formation of tissue construct. Bioprinting is a relatively new approach that has potential in the development of such scaffolds. The major advantage of this technique is the ability to develop patient-specific scaffolds of complex architecture which is difficult to achieve by other fabrication techniques. Bioink is made up of biomaterials and cells which is a vital component of bioprinting. The biomaterial provides favorable mechanical, physiochemical, and biological properties that stimulate cellular response (e.g., proliferation, migration, and differentiation). Moreover, the biomaterials also govern the printability and structural fidelity of the printed construct. In recent years many biomaterials are explored for achieving a high level of flexibility and strength. This review is focused on bioink advancement for bone regeneration.

The cultivation of *Chelidonium majus* L. influences both the phytochemical composition and the cytotoxic activity of aqueous ethanol extracts

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Chelidonium majus L. (*Papaveraceae*) is a widely spread medicinal plant in Europe and is traditionally used to treat gastric, liver, and skin diseases. The aim of this study was to investigate how cultivation affects both the phytochemical composition and the cytotoxic activity of aqueous ethanol extracts of *C. majus*. LC/MS methods were used to analyze the qualitative and quantitative composition of alkaloids and flavonoid derivatives. The cytotoxic activities of the *C. majus* extracts were determined in B16-F10, HepG2, and CaCo-2 cells. The total content of alkaloids in extracts prepared from cultivated *C. majus* was significantly higher than that of wild samples. Coptisine was the dominant compound in wild- grown *C. majus* extracts, however, chelidonine was found to be the dominant alkaloid after cultivation. *C. majus* cultivation did not significantly affect the total content of flavonol glycosides. Extracts prepared from cultivated specimens showed higher cytotoxicity than extracts from wild-grown plants. HepG2 and CaCo-2 cells were less sensitive than melanoma cells. The observed differences in the phytochemical composition of the *C. majus* extracts resulted in a significant increase in cytotoxic activity.

Nanoparticle with improved pharmacokinetics for better solid tumor penetration

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Nanoparticles (NP) play an important role in improving cancer therapy techniques. Most research on NP shows that the delivery efficiency to tumors is approximately 1% of the injected dose. Low delivery efficiency to solid tumors is associated with low biodistribution, tumor penetration and poor pharmacokinetics of NP.

This paper presents new NP that can avoid fast clearance and therefore have a better chance for tumor accumulation. To measure the pharmacokinetic characteristics of our improved NP, we performed a study in healthy BALB/c mice (n=6). Blood samples were collected at different time points and organs were harvested after 2 weeks post injection. Analysis of blood samples revealed that our NP has unprecedented pharmacokinetics with a half-life more than 24 hours. NP are differently distributed among organs, higher concentrations can be observed in adipose tissue, liver and spleen. Lower concentrations were in kidneys, lungs, heart, and almost none of the NP can be found in brain tissue.

These results suggest that an increase in the half-life of NP leads to an improved biodistribution that can provide NP with a better chance to accumulate in solid tumors. The findings of this study have several implications for NP-based cancer therapies or better cancer diagnostics.

3D bioprinting of calcium phosphates/biopolymer composite systems for bone tissue regeneration: review

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To date, due to the population accelerated aging and prolonged lifetime, bone has been ranked as the second most transplanted tissue. Thus, the expected bone graft market value will reach $4 billion by 2027. In recent years, to improve patient health outcomes, increased efforts have been focused on the developing novel three-dimensional (3D) bioprinted scaffolds for bone tissue regeneration [1]. The number of publications on 3D printing for bone tissue engineering has skyrocketed over the past decades, starting from 5 publications in 2005 up to 530 in 2020. However, only a few of them focus on 3D printing of calcium phosphate (CaP)/polymer composites for bone tissue regeneration, and even lower number is assigned to the CaP containing 3D bioprinted constructs.

For 3D bioprinting, bioinks are used to protect cells from mechanical damage, while a highly hydrated environment provides a physiologically suitable environment after the printing [2]. In general, bioinks for 3D bioprinting must: 1)support cell viability, 2)form 3D structure and 3)flow under modest pressure (30 to >6mPa/s for extrusion-based 3D bioprinting) [3]. Such biopolymers as chitosan, alginate and hyaluronic acid are mostly used for the bioink preparation. Furthermore, in order to develop patient-specific treatment and improve construct tissue integration, biopolymers can be combined with cell cultures (gBMSCs, gEPCs, hMSCs, e.c.) and CaP (biphasic-calcium phosphate, hydroxyapatite and calcium-deficient hydroxyapatite, e.c.).

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Lateral alveolar ridge augmentation. Long-term stability of biomaterials used in common practice in maxillofacial surgery. Preliminary results

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Aim: To evaluate in lateral alveolar ridge augmentation used biomaterial long-term stability by using 3D radiographic volumetric analysis.

Methods: Riga Stradins University Institute of Stomatology (RSU IS) computerized archive was used to select patients with history of lateral alveolar ridge augmentation surgery.

Results: From 1182 patient charts with history of alveolar ridge augmentation in RSU IS, 523 patients were selected based on primary exclusion criteria: (1) pre-surgery CBCT, post-surgery CBCT and follow-up CBCT; (2) all medical data available in patients' charts. After applying more selective exclusion criteria - alveolar ridge augmentation without any additional bone augmentation surgeries, 26 patients were selected for further 3D radiographic analysis.

Further analysis and expected results: Currently in progress of volumetric analysis using 3D superimposition of pre-surgical, post-surgical and follow-up CBCT. This study expects retrospective results that can be used as bases for future alveolar ridge augmentation clinical trials to better evaluate most stable augmentation biomaterial for better long-term stability.

Impact of hyaluronic acid content on hyaluronic acid/ε-polylysine hydrogels properties

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Hyaluronic acid (HA) is a natural polysaccharide that exhibits excellent properties such as biocompatibility, viscoelasticity, and total biodegradability. When the pH is approximately 7.4, HA displays an anionic charge, thus it is able to form the bonds through carboxyl groups and, for example, amino groups (1). ε-Poly-L-lysine is a natural, cationic polypeptide that becomes more popular in the field of biomedicine because of its antibacterial, non-toxic, water-soluble, and biodegradable aspects (2). Thus, the main objective of the current research was to prepare hyaluronic acid/ε-polylysine hydrogels and characterize them. HA/ε-PL hydrogels, with different HA content (from HA/ε-PL ratio 10:90 wt% to HA/ε-PL ratio 90:10 wt%), were cross-linked and characterized. The swelling behavior of prepared samples was measured by immersing lyophilized HA/ε-PL composites in deionized water at 37 °C for 4 weeks. The gel-forming process was determined by using the AntonPaar rheometer at 1 % strain, with a constant 1 Hz frequency. During the research it was establish that at least 40 wt% of HA is necessary to form HA/ε-PL composites, that are stable in aqueous medium for at least 4 weeks. Also, it was established that HA/ε-PL hydrogels were formed immediately after the liquid phase addition to the solid phase as G' value exceeded G" value.

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Conversion of Biowastes into Bioceramics

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Present work aimed to utilize different biowastes for the preparation of silicate bioceramics. Diopside ($CaMgSi_2O_6$), forsterite (Mg_2SiO_4), and wollastonite ($CaSiO_3$) were synthesized by the solid-state method. Decomposition of rice husk followed by alkali treatment, acid precipitation resulted in the extraction of silica whereas powdered eggshells were used as a calcium source. Diopside and forsterite were found to possess secondary phases (SiO_2, Enstatite) after calcination while pure wollastonite was obtained at 1100 ^oC. Moreover, the utilization of biowastes reduced the melting point of the diopside. Wollastonite revealed good apatite deposition whereas forsterite showed poor biomineralization ability. The existence of calcium in wollastonite favored remarkable biomineralization while the presence of magnesium in forsterite resulted in delayed apatite deposition. The biowaste-derived ceramics exhibited compatibility with the mammalian blood cells. The interaction of diopside with multipotent mesenchymal stromal cells (MMSCs) resulted in a negligible increase in the apoptosis level in the culture medium as compared to the control sample.