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# Stimulated in vitro bone-like apatite formation by a novel laser processing technique

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**Abstract**

Hydroxyapatite is a mineral widely studied as an artificial replacement material in dentistry and medicine due to its chemical and crystallographic resemblance to bone and tooth minerals. New trend of stimulating the hydroxyapatite formation in vitro has evolved by applying various sources of external energy. Lasers have been widely used in biomaterials area as clean and powerful sources of energy and have diverse bioapplications. They are excellent tools for creating micrometer-scale structures by precise and flexible irradiation of small and complex shapes. Using a hierarchical approach that mimics the natural material formation processes, we developed a method to produce materials with controlled physical structure at both micro- and nanometer scale. Materials organized on multiple length scales bear a closer resemblance to biological matrices than those with single scale features, and thus materials with multi-scale organization should be more advantageous in biomedical applications. In the applied laser–liquid–solid interaction method, micrometer scale architecture with precisely controlled size and shape is induced by laser irradiation of various surfaces and then nanostructured hydroxyapatite is grown preferably on the micrometer-sized areas by a biomimetic approach. The method results in an enhanced calcium phosphate formation on the materials' surfaces which further facilitates the growth of a thicker hydroxyapatite layer. In this work we present our method and the application of optical emission spectroscopy for the in situ monitoring of the processes during the laser–liquid–solid interaction. Furthermore, tests with osteoblast-like cells reveal the biocompatibility of the hydroxyapatite layers obtained as a result of the laser–liquid–solid interaction method.


*Keywords:* Bone-like apatite; Hydroxyapatite; Synthesis; Stimulated growth; Laser processing; Nanostructured surfaces; Porous materials; Optical diagnostics

## 1. Introduction

Hydroxyapatite (HA, chemical formula $Ca_{10}(PO_4)_6(OH)_2$) is widely studied as an artificial bone and teeth replacement material in dental and orthopedic implants, as coating of hard tissue implants, bone fillers, and for drug delivery, due to its chemical and crystallographic resemblance to bone and tooth minerals [1]. HA is known to have an appropriate biological activity when implanted in living organisms and this property has attracted growing interest in the last decades. The goal of the biomaterials synthesis and processing nowadays is to mimic the way materials have been created in nature. Organisms in nature create perfect fine mineralized structures with diverse biological functions and very often from simple salt solutions through interactions between inorganic and organic substances [2,3]. Stimulated by fascinating natural examples, such as bones, teeth, cartilage, shells and corals, attempts are being made to develop synthetic, biomimetic nanocomposites by simulating the basic principles of biomineralization. Following this goal, many researchers have been exploring the potential of a simple immersion method in an aqueous supersaturated solution, known as simulated body fluid (SBF) [4] in order to mimic the process of biological apatite formation. A disadvantage of the method is the long time required to produce calcium phosphate (CaP) coatings. Therefore, a new trend of applying external energy to stimulate the HA formation in vitro has evolved: ultrasound and electrical field, ultraviolet and microwave irradiation have been used with this purpose [5–7]. Fang et al. [5] applied

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ultrasound to accelerate the formation of HA in aqueous solutions at low temperature and managed to reduce the time for the HA formation from hours to minutes. Another research group [6] reported the formation of bio-resembling apatite crystals from SBF on $TiO_2$ under illumination with ultraviolet light and following soaking of the samples for 10 days in the SBF. There is also a tendency of using microwave energy for acceleration of specific chemical reactions [7]; under the microwave influence, precipitation of HA from aqueous medium was obtained within an hour. Recently, a novel method involving laser–liquid–solid interaction (LLSI) for the facilitated synthesis of HA by using a laser irradiation of materials simultaneously immersed in the supersaturated SBF has been proposed [8–13].

The idea of using laser light was based on the fact that over the past two decades, laser sources of energy have proved to be major tools for surface processing of large variety of metals, ceramics and polymers, as well as for processing of living tissues and synthesis of biocompatible materials. Lasers have been utilized as exceptional contactless, fully adaptable sources of clean energy. Bioapplications, such as pulsed laser deposition and processing of HA [14,15], biofilms modification [16], bone defect healing [17], modification of root canal dentine [18], laser sterilization of dental implants [19] are already well established. Lasers have also been employed to increase the long-time performance of Ti dental implants through assuring cleanness, specific microrelief and stable oxide layer on the surfaces [20]. Particularly, pulsed lasers have unique qualities that can be applied to process biomaterial thin films. Pulsed lasers have proven to be an invaluable tool in the research and development of new thin film materials because of the unique nature of the laser-material interaction.

The proposed in our work method of LLSI was based on the simultaneous interaction between a scanning pulsed laser beam and a liquid precursor solution (SBF or distilled water as a control solution) in the presence of a solid substrate [8–13]. We used metal, semiconductor and insulator as substrates. Metals (Ti, Ti alloys, Co–Cr–Mo alloys, austenitic stainless steels, tantalum and zirconium) are most frequently used for implants [21–23]; they form protective nm-thick native oxide layer on their surfaces which determine their excellent corrosion resistance and decreases the leaching of metal ions from the implant surface. These properties make metal materials appropriate for implantation in the human body. Silicon (Si) is one of the most abundant elements on earth, and it is also present in the human blood plasma in the form of orthosilicic acid [$Si(OH)_4$] which is vital for the optimum growth and development of bones and collagen in the living organism [24]. Glasses are rich in $SiO_2$ which yields the formation of surface Si-$OH^-$ groups in the SBF, which on their turn ensure active sites for the formation of HA [25]. Further, porous and nanostructured materials (porous Si, polycrystalline Si and CdSe/$SiO_x$ nanostructures), obtained by using well-established nanotechnologies were also utilized in our work, since nanosized objects, as well as porous structures are known to promote bone and tissue ingrowth into open pores and they also allow to be easily biodegraded and bioresorbed [24,26–31].

Materials structural properties at micro-and nanometer scale influence the cellular responses [27,28]. It has been shown that materials organized on multiple length scales bear a closer resemblance to biological matrices than those with single scale features, and thus materials with multi-scale organization should be more advantageous in biomedical applications [27,28]. Using an approach that mimics the natural material formation processes, the method of LLSI was utilized to produce materials with controlled physical structure at both micro- and nanometer scale. By applying this process, various microdesigns can be realized on the material surface by a precise scanning system. The LLSI results in an enhanced CaP formation on the material surface, compared to the traditional method of prolonged soaking in SBF, and this result is attained as a single one-step and time-sparing process. The new laser processing method can yield a significant progress in the materials coating with bone-like apatite in terms of nucleation rate, simplicity and availability to coat complex shapes. It is carried out under a room temperature and atmospheric pressure, i.e. it does not require the presence of buffer gasses or vacuum conditions. However, the mechanisms of the enhanced CaP formation are not clear enough. Observation of the interaction of the laser beam with the substrates immersed in the solution by an optical diagnostic technique was expected to give us more details on the mechanisms of the LLSI process. For this purpose we used optical emission spectroscopy (OES) as a plasma diagnostic tool for in situ monitoring during the LLSI process. The technique was used to observe the emission from excited species and was applied for a first time in such a complex interaction like 'laser light–solution–solid substrate'.

Materials, compatible with cells are important in medical applications and therefore the interactions of the cells with the materials have been intensively investigated [32]. Cells are highly sensitive to topography, roughness, chemistry, surface charge, and hardness [33,34]. Cell–material interactions in vitro may be approximated by the process of cell adhesion and spreading, which is a convenient way to determine the biocompatibility of a material. In our study, the CaP layer grown on glass substrates by applying the LLSI process was tested in terms of its cell compatibility with osteoblast-like cells.

## 2. Experimental

### 2.1. Materials

#### 2.1.1. Stainless steel, silicon, silica glass

Samples from stainless steel AISI 316 (further named SS), *n*-type silicon with (1 0 0) orientation (named S), and silica glass Herasil (named SG) subjected to standard mechanical treatment [35] were prepared. All samples were subjected to LLSI process and subsequent soaking in the SBF solution at 37 °C for up to 24 h. One group of samples was taken out of the SBF immediately after the end of the LLSI in order to study the instantaneous effect of the laser irradiation. In both cases, the samples were finally washed with distilled water and dried in air before investigations.

#### 2.1.2. CdSe/$SiO_x$ nanostructures

CdSe/$SiO_x$ nanostructures were prepared by multi-step sequential evaporation of SiO and CdSe layers (each having