# Properties of calcium phosphate coatings deposited and modified with lasers

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Physical, chemical and biological properties of calcium phosphate coatings fabricated by a pulse laser deposition method at room temperature (RT PLD) have been studied. *In vitro* evaluation of RT PLD coatings on bioresorbable polymers (Poly-ε-caprolactone and Poly-L-lactide) have been carried out. It was shown that both polymers support osteoblast growth, with increased cell activity, alkaline phosphatase activity and total protein content on those surfaces that have been coated. The advantages of RT PLD coatings in biomaterials surface optimization are discussed.


## 1. Introduction

Over the last decade laser assisted techniques have been successfully applied to improve surface properties of biomaterials [1, 2]. Since 1992 pulse laser deposition (PLD) techniques have been applied for fabrication of biocompatible coatings on metals [3–8]. Since 1996 such coatings have also been deposited onto polymer surfaces [9, 10] by PLD methods.

The main purpose of Cotell [3], and others [5, 7, 8] was to reproduce within coatings the stoichiometry and crystallinity of ablated HA targets. High substrate temperatures up to $\sim 600\,^{\circ}\mathrm{C}$ and specific gaseous conditions have been used. However, highly crystalline HA has the lowest bioactivity among the calcium phosphates. Thus, HA is not always optimal material for numerous biomedical applications. Fabrication of biphasic calcium phosphate ceramics is one of approach to optimize the properties of biomaterials [11–13]. In this case, the more soluble phase provides high initial bioactivity and the less resorbable component ensures long time stability. It was found that room temperature PLD (RT PLD) gives a mixture of calcium phosphates. Similarly to biphasic ceramic, RT PLD coatings provide a possibility to control coatings composition and, thus, to optimize their biological properties, with the added advantage of being able to be deposited onto polymeric materials.

In this paper, we summarize the results of our studies of both physicochemical and biological properties of RT PLD calcium phosphate coatings on various materials and present our *in vitro* evaluation of laser deposited coatings on bioresorbable polymers.

## 2. Pulse laser deposition

Principles and details of PLD technique have been published elsewhere [14]. Briefly, an hydroxyapatite (HA) target is ablated in a vacuum chamber by the laser pulses (Fig. 1). Ablated products: atoms, ions, and macroparticles are condensed onto the substrate surface placed at some distance from the target. Targets of different densities (80–95% of maximum theoretical HA density) were prepared by pressing and sintering the HA powder (P120 batch type, ''Plasma Biotal'', UK). The PLD process was primarily carried out using a KrF-excimer laser $(\lambda = 248\,\mathrm{nm}, t_{\mathrm{pulse}} = 30\,\mathrm{ns})$, with a TEA-$CO_2$ laser $(\lambda = 10.6\,\mu\mathrm{m}, t_{\mathrm{pulse}} = 100\,\mathrm{ns})$ being used in some experiments [15]. Laser fluence and residual air pressure were varied in ranges $1.5\text{–}10\,\mathrm{J/cm^2}$ and $10^{-2}\text{–}100\,\mathrm{Pa}$, correspondingly. Metals (titanium foil and Ti6Al4V alloy), and polymers (polyethylene, Teflon, silicon rubber, poly-L-lactide (PLA) and poly-ε-caprolactone (PCL)) were used as substrates.

## 3. Coatings specification

The coatings properties were studied with different methods: scanning electron microscopy (SEM), energy dispersive X-ray microanalyses (EDX), atomic force microscopy (AFM), Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction. Coatings adhesion to the substrates was measured using a scratch test. Evaluation of coatings chemical stability has been done by studying their dissolution in phosphate buffered saline (PBS). *In vitro* tests were performed with fibroblast and

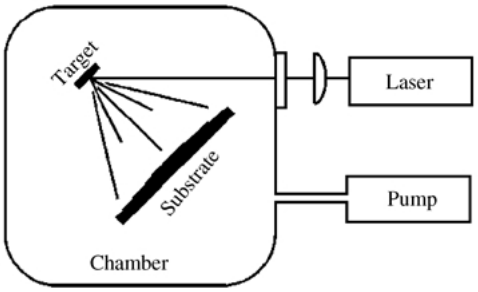


*Figure 1* Laser deposition apparatus. The atmosphere in the chamber, laser fluence and substrate – target separation can be varied to modify the coatings produced.

human osteoblast cell cultures. White rats (180–200 g, ''Wistar'' line) and rabbits (290–300 g, Chinchilla) were used for our *in vivo* study.

## 4. Properties of PLD coatings

### 4.1. Structure

In general, all PLD coatings were dense and smooth films with explosively ejected macroparticles of various sizes integrated into the surface (Fig. 2). The spherical shape of the macroparticles was a result of laser heating and partial melting of the HA particles followed by cooling on the substrate surface. For ''thick'' coatings, thickness $> 3\,\mu m$, the surface structure was defined mainly by macroparticles. The morphology of deposited coatings is controllable by both laser fluence and HA targets density. Macroparticle size distribution reveals that for dense targets, the fraction of large macroparticles, greater than 5 μm in size, increases with laser fluence [16]. For 95% dense targets, the maximum amount of large macroparticles was found to be a fluence of at $9\,J/cm^2$.

### 4.2. Adhesion

Mechanical adhesion of PLD coating to the substrate surface is controlled by deposition condition, type of HA target and prior substrate preparation. Coating adhesion to Ti substrate increases with both laser fluence and target density. The adhesive strength of the coatings deposited at room temperature from the 95% target at 6 and $9\,J/cm^2$ and at residual pressure in chamber less than 10 Pa was found to exceed the elastic strength of the Ti substrate – $1 \times 10^{10}$ Pa. This value is close to $1.7 \times 10^{10}$ Pa, corresponding to adhesion of the crystalline HA coating deposited on substrate at 600 °C [3].

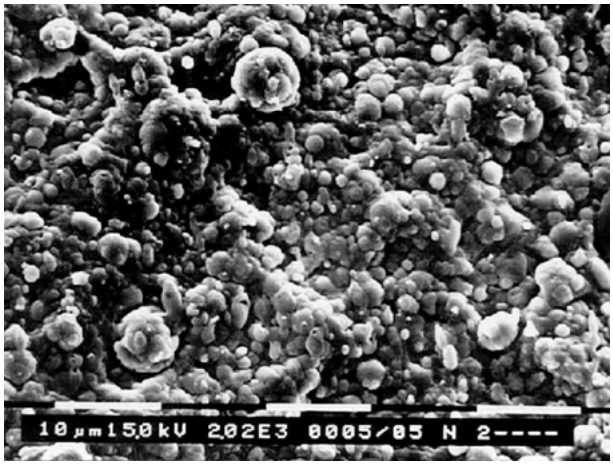


*Figure 2* SEM micrograph of coating surface from an HA target of 95% density, and at a fluence of $9\,J/cm^2$. Note that a very uniform surface coverage is achieved. The surface is formed from both evaporated HA and HA macroparticles that have been ejected from the target. The balance of these components and the size of the HA particles can be controlled.

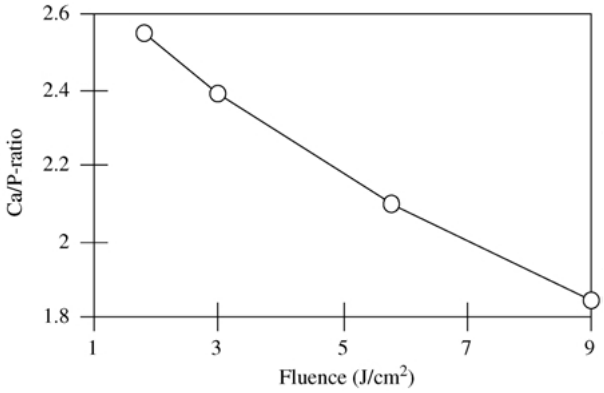


*Figure 3* Ca/P ratio of various HA coatings prepared at a contant 2 Pa residual chamber pressure as measured by EDAX analysis. The ratio can be finely tuned by controlling the applied laser fluence.

### 4.3. Coating composition

Coatings deposited from HA on substrates at room temperature consist of a mixture of amorphous calcium phosphates. The calcium to phosphorus ratio (Ca/P) as a function of laser fluence is presented in Fig. 3. The Ca/P value exceeds 1.67 (Ca/P = 1.67 for stoichiometric HA used for the target material) for all laser fluences. The higher the laser fluence, the closer the coating composition is to that of the initial target material. This can be associated with the increase in density of HA macroparticles at high fluences.

### 4.4. Coatings stability

The rate of dissolution of the coatings in PBS solution has been investigated by FTIR technique. The molecular absorption band near $1000\,cm^{-1}$ is associated with $PO_4$ and careful monitoring of the integrated intensity of this feature has allowed us to determine dissolution rates [17]. Using the same methodology, the dissolution rate of RT PLD coatings is found to be an order of magnitude lower than that of plasma sprayed coatings. Our measurements show that the higher the target density and laser fluence the more resistant to dissolution in PBS are these coatings [16]. Coatings ( $\sim 1\,\mu m$ in thickness) fabricated from the low density (80%) target at $3\,J/cm^2$ were dissolved in PBS in less than 10 h. However coatings deposited from 95% density target at a fluence of $9\,J/cm^2$ are stable in simulated body fluids even after 78 h of soaking.

### 4.5. Coatings bioactivity

Alamar blue and alkaline phosphatase assays were used to assess *in vitro* human osteoblast cell responses to the

different PLD coatings on titanium [16]. Cells were cultured on the coatings for 48 h. These measurements showed that coatings deposited from high density target (95%) supports highest cell activity. Alkaline phosphatase production on coatings deposited from the different targets had the same trend as for Alamar blue assay. Again, the coatings of 95% target supported the highest cell activity both at 6 and at 9 J cm$^2$.

### 4.6. Implantation

The effect of HA coatings deposited with KrF excimer and $CO_2$ lasers on Ti6Al4V alloy implants on the process of implant osteointegration was studied in Alimpiev *et al*. [15] and Antonov *et al*. [18]. The implants, coated with HA and uncoated (control), were inserted into white rats (''Wistar'' line) femurs for periods of 15, 30 and 60 days and rabbits (Chinchilla) for 1, 2 and 4 months. The implants with PLD coatings demonstrate rather larger direct contact area with regenerated bone, and, also, much better osteogenesis than that for uncoated implants for all periods of observation.

### 4.7. Combination of RT PLD with other methods

The PLD technique offers the possibility of being combined with other deposition techniques, such as plasma spray and biomimetics. Our studies demonstrate that PLD calcium phosphate coatings can be used as seed sublayers, to exclude the prenucleation biomimetic cycle [19]. The combination of laser deposited precursors followed by coating deposition by other methods allows the fabrication of highly adhesive coatings even on thermally unstable and hydrophobic polymer surfaces.

## 5. Surface modification

Laser assisted techniques can be applied successfully for surface cleaning, etching, smoothing, etc., including activation of polymers to improve their biocompatibility [1]. Moreover, we have showed that laser radiation can be used for delicate modification of the calcium phosphate coatings on any substrate with no damage of both coating and substrate [20].

## 6. Coatings on bioresorbable polymers. *In vitro* tests

Bioresorbable polymers have been developed as bone substitutes in recent years. These polymers can degrade, whilst avoiding formation of toxic products and can be replaced by natural bone tissue. However, bioresorbable polymers are not bioactive. Using RT PLD we have produced a thin bioactive calcium phosphate coatings on polymer surfaces to overcome this drawback.

### 6.1. Materials and methods

Films of PLA and PCL were prepared by solvent casting. HA coatings ( $\sim$ 1 μm in thickness) were deposited onto the polymer surface by KrF excimer laser ablation of an HA target (95% density). The deposition process was carried out at 1 Pa and at room temperature. The laser fluence on the target was 3, 6 and 9 J cm$^{-2}$.

#### 6.1.1. Cell culture experiments

Samples were placed into sterile 96 well plates. Human osteoblasts (derived from femoral head trabecular bone) were seeded on the coatings at concentration of 20,000 cells per well in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 0.02 M of HEPES buffer, 2 mM of glutamine, 50 μg/ml of ascorbate and 1% (v/v) non essential amino acids. The plates were placed into the tissue culture incubator at 37 °C with 5% $CO_2$ for 48 h. Cell activity was measured by fluorescence using the Alamar Blue dye. Alkaline phosphatase activity and total protein content were measured using a colorimetric method. Relative cell number was determined by fluorometric quantification of DNA. Data for Alamar Blue, alkaline phosphatase and

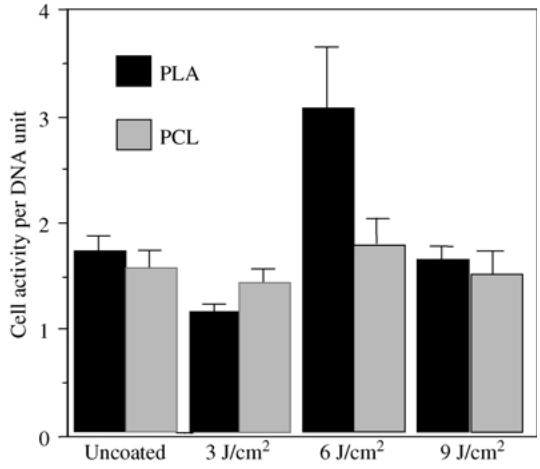


*Figure 4* Osteoblast activity per DNA unit measured using the Alamar Blue assay. The coatings were deposited upon PLA and PCL samples at three different laser fluences 3, 6 and 9 J/cm$^2$.

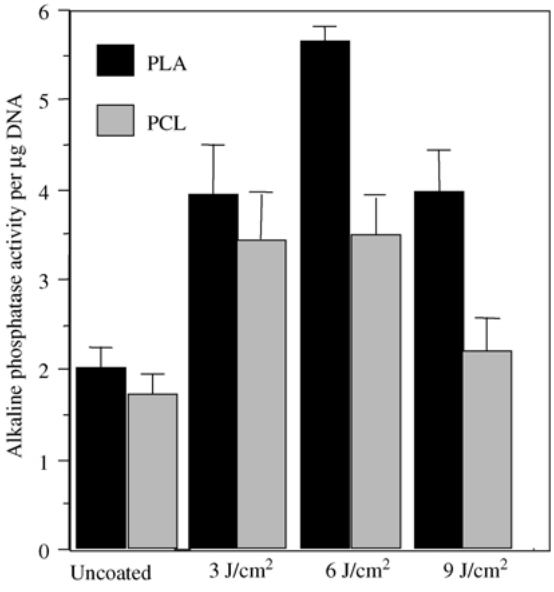


*Figure 5* Alkaline phosphatase activity per DNA unit for PLD coated and uncoated polymers. The coating clearly increases the activity of the cells over and above that observed on the uncoated materials.

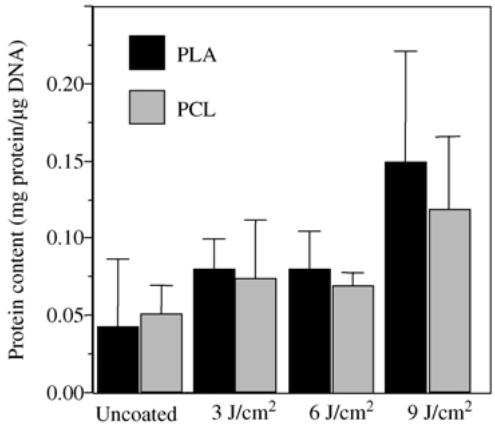


*Figure 6* Protein content of osteoblasts cultured on coated and uncoated polymers expressed as a proportion of the DNA content. Again, the coating clearly has a positive effect over and above that observed on the uncoated materials.

protein tests were expressed as a function of the DNA content.

### 6.2. Results and discussion

The results of cell activity, alkaline phosphatase activity and protein content measurements are shown in Figs. 4–6. Cell activity, alkaline phosphatase activity and protein content were all found to be dependent on laser fluence. Cell activity on the PLA polymer coated with HA using a fluence $6\,J/cm^2$ was significantly greater than that on the other samples ($p < 0.05$), including the uncoated PLA control. This sample also demonstrated the highest alkaline phosphatase activity. However, there was no essential difference between the total proteins content of osteoblasts cultured on different polymers. The main reason of cell activity variations between two polymers may be the different mechanical properties of the coated materials supporting different adhesion of the coatings. This may partially explain why the higher fluence deposition does not automatically provide the highest cell activity. The high energy and larger particles produced by the higher fluences may damage the polymer surfaces resulting in poorer adherence to the polymer substrate.

## 7. Conclusions

- PLD is a powerful and efficient method for fabrication of biocompatible and bioactive coatings on a very wide range of metal and polymer substrates across a wide range of processing temperatures. RT PLD enables the extension of the range of materials that can be used for biomedical applications.
- The high adhesion of RT PLD coatings can exceed the yield strength of the substrate materials (even for metals) providing low residual stress in the coating/substrate interface.
- The PLD technique allows control of the stoichiometry of multi-component coatings. By this technique it is possible to dope the coatings with different species (Si, C, etc.) to optimize coatings composition and properties.
- PLD enables fabrication of optimized microstructures and surface morphologies for coatings on almost any specific substrate with various roughnesses (from mirror-like surface finishes to surfaces with 5–10 μm particulates).
- PLD allows deposition of thin layers (less than hundreds nanometers in thickness). Resorption rate of PLD coatings in living tissue environment are in order of magnitude lower than that of plasma-sprayed ones. Thus, the optimal coating thickness can be decreased up to 0.2–2.0 μm. Thin coating reduces the risks of implant loss caused by premature coating degradation. Such degradation is very common for plasma sprayed coatings.
- PLD may be combined with other deposition techniques such as plasma spray and biomimetics. This allows optimization of coating parameters for different implant applications.
- PLD can be used to fabricate calcium phosphate coatings on both PCL and PLA polymers. Both polymers can support cell growth, with increased cell activity and alkaline phosphatase activity on those surfaces that have been coated. Cell responses to the coatings depends on laser fluence and hence allows for optimization.

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