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Protected Laser Evaporation/Ablation and Deposition of Organic/Biological Materials: Thin Films Deposition for Nano-biomedical Applications

Gianina-Florentina Popescu-Pelin,
Carmen-Georgeta Ristoscu, Maria Badiceanu and
Ion N. Mihailescu

Abstract

This chapter reviews the laser ablation of delicate organic/biological substances by matrix-assisted pulsed laser evaporation (MAPLE). It is shown that direct ablation in this case is possible but sometimes not workable at all in adverse conditions. The considered solution is the protection by a prevalent dissolving/suspending component that can allow for a "shielded" ablation by the frozen solvent followed by its gradual evaporation by melting, evaporation and evacuation by pumping system. We extend the study to the case of non-UV absorbing solvents, e.g., water, when the primary interaction between laser and solute ignites evaporation process at a lower ablation threshold due to reduced pressure inside irradiation chamber. We called this case as "generalized" MAPLE interaction. Relevant examples are provided and critically analyzed in view of potential applications for nanobiomedicine, biosensors, advanced implants and chemical technologies.

Keywords: laser interaction and ablation mechanisms of organic/biological compounds, thin films deposition, nanobiomedicine, protected ablation, functional organic/biological layers

1. Introduction

Laser ablation of "delicate," organic and/or biological materials is reviewed. Particular attention was recently paid to this field stimulated by the progress of laser sources, the advance of "smart targets" and new applications in key technological areas like chemistry environment,

biology, and medicine at micro- and nanoscale. The ablation of organic/biological materials was initiated and promoted under "protection" and investigated in various stages after generation, during propagation in vacuum or different ambiances till the final deposition of expulsed material on substrates of interest. Appropriate models were developed for describing the coupling of radiation with the organic/biological materials, bare or protected by a matrix, while complementary techniques were used for obtaining exhaustive information about the ablated substance in plasma plume, liquid, and final solid states. The progress was possible on this basis of a new generation of composite thin films for applications in drug delivery systems, biosensing, and advanced coatings for metallic implants.

The "protected" ablation and transfer of organic and biological substances can be obtained by multipulse laser irradiation. In this case, the "delicate" solute substance is dissolved in more solvents while the obtained mixture is frozen. The resulting "icy" target is then submitted to the laser ablation. The solute is ablated and transferred under the protection of a frozen solvent layer. During transfer, the icy layer is gradually melted, evaporated, and evacuated by the vacuum system whereas the solute reaches the final destination with the minimum or no perturbation or damage and is deposited in the form of a thin film. The technique was invented in Naval Research Laboratory, Washington D.C. Refs. [1, 2] describe it as matrix-assisted pulsed laser evaporation (MAPLE) and is extensively applied since 2000 for the ablation and deposition of a large class of compounds with application in many top field domains, like nanobiomedicine, photocatalysis, synthesis of hard layers, and so on [3]. The choice of an appropriate solvent plays a key role in MAPLE. It is generally accepted now that the main requirements for these selections are: laser fluence must have proper values, lower than in pulsed laser deposition(PLD); incident laser energy must be the majority absorbed by solvent molecules and not by organic molecules of the base material (0.5–10) wt%; frozen solvent must be characterized by a high absorption at working laser wavelength; solvent has to be selected so that the organic material presents a good solubility; solvent has to present a high freezing point; and solvent must not produce chemical reaction under laser radiation exposure. For illustration, we give in **Figure 1** a general scheme of a MAPLE setup and the involved fundamental physical-chemical processes in ablation of the frozen target.

The main difference between MAPLE and "classical" pulsed laser deposition (PLD) [4, 5] consists of target preparation and the laser interaction (ablation) mechanisms. This makes the ablation process in case of MAPLE substantially different to that in PLD. Fundamental mechanism and processes in MAPLE ablation were studied in [6, 7]. The ablation of organic/ biological materials is followed by time-resolved plume imaging [8] in many cases with spatial and/or temporal resolution. The expulsed substance was characterized by combined techniques like time program desorption mass spectrometry and atomic spectroscopy [9, 10].

Nevertheless, the selection of the perfect solvent is not easy and sometimes impossible because of the limits introduced by very toxic characters of various solvents, available quantities and prices, and good mixing between a solvent and solute, as being the most important.

Therefore, we refer in Section 2 to a simple and cheap solution of using solvents by hand when all the aforementioned criteria are properly applied.
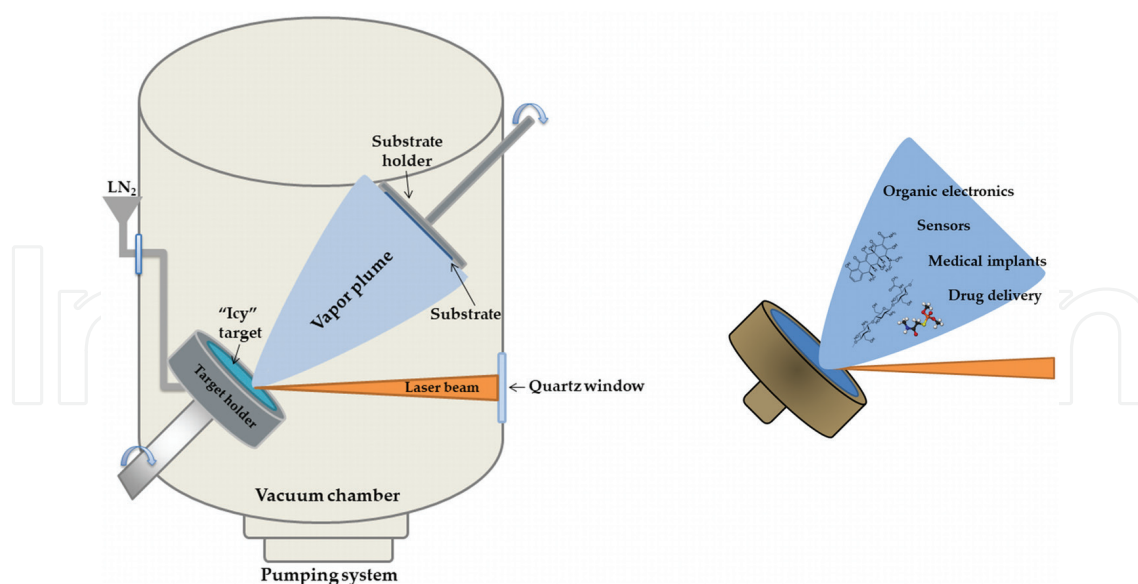
Figure 1. Schematic of MAPLE setup and interaction mechanism.

Selected information are introduced about ablation and deposition of polysaccharides (tri-acetate-pullulan), enzyme (urease), proteins (fibro- and vitro-nectines), and biopolymers (papain, lysozyme, poly(lactic-co-glycolic acid). All structures were studied by physical-chemical methods and assessed biologically by *in vitro* and more recently *in vivo* studies. Recent literature results are mentioned and evaluated critically.

2. "Generalized" MAPLE mechanism

Smausz *et al*. [11] synthesized urease thin films by MAPLE and PLD from frozen water solutions of urease (1–10 wt%) and pure urease pellets. The KrF* excimer laser fluence was varied between 300 and 2200 mJ/cm^2. The FT-IR spectra of the PLD films at the same fluence range evidenced no significant difference as compared to the MAPLE ones (**Figure 2**). This can be considered an unexpected result, because the direct irradiation, and in particular higher fluence, is predicted to severely affect the molecular structure of the biomaterial. Measurements of absorption coefficient indicated that the idea of absorbing matrix does not work when using water solvent, that is, the relatively high absorptivity of matrix is not a general requirement for a successful MAPLE experiment. Accordingly, the laser energy is absorbed by the organic molecules and the heat is transferred to the surrounding solvent, while the matrix protects the delicate organic material from overheating and consequently thermal decomposition.

Different direct measurements or numerical simulations inferred absorption coefficients below 1 m^{-1} around 250 nm wavelength [12]. For the concrete case of a 1 wt% frozen urease solution [11], this is about two orders of magnitude lower, indicating that the photons are generally absorbed by the urease molecules instead of the water solvent. It has to be mentioned that pure ice could not be ablated in vacuum chamber at 1 J/cm^2 fluence. This is,
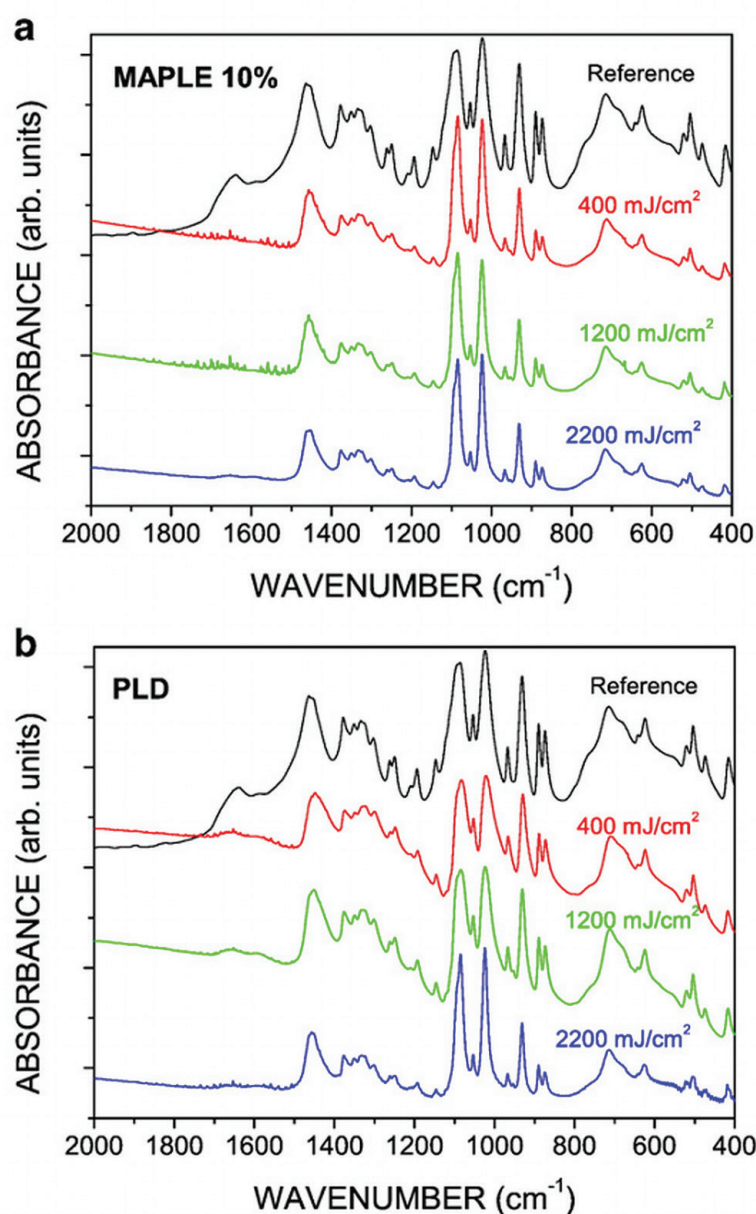
Figure 2. FT-IR spectra of urease thin films deposited by 10% MAPLE and conventional PLD at different fluences (reproduced with permission from [11]).

however, in contradiction with the principle of "classical" MAPLE based on an absorptive matrix. In our opinion [11], the laser energy is absorbed by the organic molecules (and/or molecule cluster) (**Figure 3a** and **b**), leading to an increase in their temperature, thus melting and heating the water in their close vicinity (**Figure 3c**). Because of the working pressure in the reaction chamber, the molten 0°C water starts boiling, the upper ~micrometer layer evaporates from the surface (**Figure 3d**) carrying away the urease molecules, while the deeper layers refroze. Accordingly, the ablation of the delicate material can be achieved at lower temperature (well below the denaturation threshold of 90°C) than in the case of bulk material ablation. Similar ablation process was reported in [13] for aqueous solutions of absorptive materials.
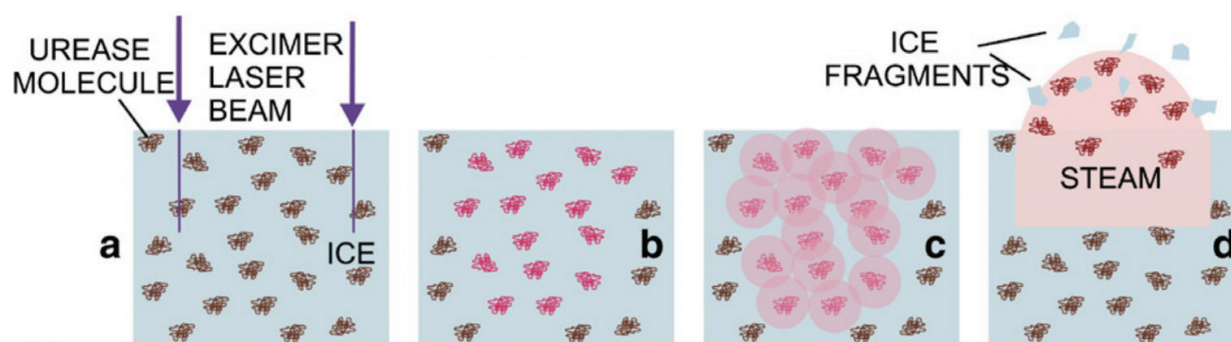
Figure 3. Scheme of the ablation process of the frozen water solution of urease: the laser energy is absorbed by the urease molecules (a) increasing their temperature (b). Part of the heat is transferred to the surrounding ice medium (c), the upper layer evaporates and the deeper layers refreeze (d) (reproduced with permission from [11]).

Experiments using water as MAPLE matrix were performed at 355 nm [14]. In this case, the absorption of ice in UV-visible range is close to the local minimum. This confirms that successful MAPLE deposition can be accomplished without the principal absorption of matrix material, e.g., water.

3. Relevant examples

3.1. Polysaccharides

Polysaccharides, described as complex molecules, are an interesting class of materials due to their biological and chemical properties such as biodegradability, nontoxicity, biocompatibility, nonimmunogenicity, and increased chemical reactivity [15–17]. Moreover, most polysaccharides are of natural origin (plants, animals, and microorganism), and depending on the sources, they can vary with respect to the molecular weight and structure [18, 19]. The presence of glycosaminoglycans (part of the extracellular matrix) in the composition of natural polysaccharides is an important feature, which proved to increase the wound healing process by binding to proteins at hierarchical peculiarity [17, 20, 21]. Since the biological activity of polysaccharides is dependent on their properties, the further advancement of polysaccharide-based nanomedicine, which is a current direction of interest, proposes the development of alternative methods to produce polysaccharides with reliable features [17, 19].

Tissue engineering and drug delivery are also two directions of permanent interest for the medical field, and so a variety of polysaccharides have been used in order to bring new solutions to the encountered issues [17]. In this respect, there were reported in the literature the benefits of alginate [22–24], gellan [25, 26], dextran [27–29], hyaluronic acid [30], chitosan [31, 32], and pullulan [29, 33–35] for specific applications [17].

3.1.1. Pullulan

Pullulan, with the molecular formula $(C_6H_{10}O_5)_n$, is a neutrally charged polysaccharide, which is soluble in water and produced by yeast-like fungus *Aureobasidium pullulans* [16, 33]. Due to its unique structural features, excellent mechanical properties and biocompatibility,

and to the high hydration capacity, the native pullulan and its derivatives possess various biomedicinal and pharmaceutical applications [33, 36]. The application of pullulan in tissue engineering involves the surface modification practices. Consequently, the surface properties of this biopolymer could be enhanced by replacing, on its hydroxyl groups, the desired chemical moieties [33].

Furthermore, thin films of pullulan, which are biodegradable, biocompatible, and with good mechanical properties, can be synthetized and used in various biomedical applications [33]. One processing method is MAPLE technique that allows for the deposition of high-quality pullulan films, e.g., triacetate-pullulan, cinnamate-pullulan and tosylate-pullulan [35, 37, 38]. The laser processing of such organic materials in the form of thin films requires the preservation of pullulan molecular structure and functionality [10]. Cristescu *et al*. synthetized MAPLE thin films of pullulan and its derivatives from a frozen target of 2 wt% biopolymer in dimethyl sulfoxide, under the irradiation with a KrF* excimer laser source [35, 37].

Based on the biofunctionality of this biopolymer, Bulman *et al*. proved that the addition of pullulan enhanced mesenchymal stem cells (MSCs) retention at a diseased cartilage surface, increasing MSC therapeutic efficiency and acting as a cellular adhesive [39]. In 2016, Atila *et al*. reported for the first time the production of pullulan and cellulose acetate scaffolds (in various combinations) by wet electrospinning technique, in order to fulfill the requirements of an engineered-tissue construct [40]. As for the use of pullulan in drug delivery systems, a relatively new study revealed the synthesis and efficiency of a system based on pullulan and poly(β-amino)ester for gene co-delivery (hepatoma-targeted) and chemotherapy agent [41].

These are only a small part of pullulan applications that demonstrate its significance for the present and future research directions, offering a wide field of activity due to its versatile composition.

3.2. Proteins

Proteins are macromolecules distinguished from polysaccharides by their content of approximately 20 amino acid monomers and can be found in all biological systems, from inferior prokaryotes to complex eukaryotes [42–44]. According to the chemical properties, amino acids are classified as non-polar aliphatic (hydrophobic), non-polar aromatic (hydrophobic, except tyrosine), polar uncharged (hydrophilic), polar negatively charged at pH 7, polar positively charged at pH 7, and sulfur-containing (maintain the structure of the protein) groups [45]. The attachment of each amino acid to the central carbon by a different side group leads to the unique character of proteins [43].

Furthermore, proteins comprise a significant number of reactive groups that ensure flexibility by their chemical modification [42]. This statement is strengthened by the fact that proteins have multiple sites for chemical interaction, which can allow for the improvement and tailoring of their properties [46]. Proteins are known to occur essentially in aqueous or membrane environments, are insoluble in non-polar solvents, and cover a wide range of polymeric compounds [43, 44]. Thus, van der Waals, hydrogen bonding, electrostatic, hydrophobic, and

disulfide cross-link interactions between the amino acid units are connections responsible for the structural modifications of proteins along the polymeric chain [43].

The specific requirements of various applications can be fulfilled due to the possible adjustment of the properties of proteins [42]. One of the most discussed and studied biomedical applications of proteins are diagnostic imaging [47–49], therapeutic delivery [47, 50, 51], and tissue engineering [52–54].

The progress on the processing of different protein materials (e.g., silk, gelatin, collagen, casein, keratin, etc.) into coatings with applicability in tissue engineering, cell adhesion, implants proved to be a key factor for the improvement of the medical field [43, 55, 56]. The presence of the different side groups attached to the central carbon in the protein structure is an advantage for film fabrication or even for its improvement (stability) because proteins can be submitted to chemical and/or mechanical modifications [42, 46]. Furthermore, protein-based films or coatings are known to be biodegradable, being thus a source of nitrogen (proteins contain about 16% nitrogen by mass) which contributes as fertilizers [44, 46].

As a component, proteins of the extracellular matrix (ECM), which is present within all tissues and organs, fibronectin (FN), vitronectin (VN), and collagen I (Col1) proved their important roles in wound-healing processes [57].

Our own interest was focused on the study of FN and VN in which *in vitro* have been shown to increase the attachment of specific cells for a designed substrate [58].

3.2.1. Fibronectin

Fibronectin is one of the most important and intensively studied ECM proteins, exists as a dimer, induces mineralization, and is a soluble circulating protein in body fluids (like plasma) [59–61]. At physiological pH (7.4), FN proved to be negatively charged due to its acidic isoelectric point (pI = 5.5–6.0) [59]. From the biological point of view, FN plays a key role in the adhesion, spreading, migration, differentiation, and proliferation of various cells and support the accumulation of multiple growth factors (GF) [59, 60]. In addition, FN can enhance the GF growth-promoting function [60]. Due to its stimulus in cell attachment and migration processes, FN also known as a multifunctional extracellular glycoprotein, is extensively studied and used as a coating in tissue engineering [62].

In 2013, the fabrication of functional FN patterns onto Ti substrates by using the laser direct write (LDW) technique was described by Grigorescu *et al*. [63]. In this study, the authors validated the suitability of LDW technique for FN molecules' transfer, preserving the compositional protein features and functionality [63]. The integrity preservation of the transferred FN was evaluated by ATR–FTIR measurements, and one could observe no structural modifications between FN dropcast and the LDW ribbon (**Figure 4**). The characteristic amide I and II bands are clearly visible and correspond well to the similar bands of the positive control consisting of a FN solution dropcasted onto a Si substrate (**Figure 4**—curve 5). The spectrum presented in **Figure 4**—curve 2 corresponds to a negative control of a heat-denatured protein sample. One can clearly notice that the two proteic bands are no longer visible in this case.
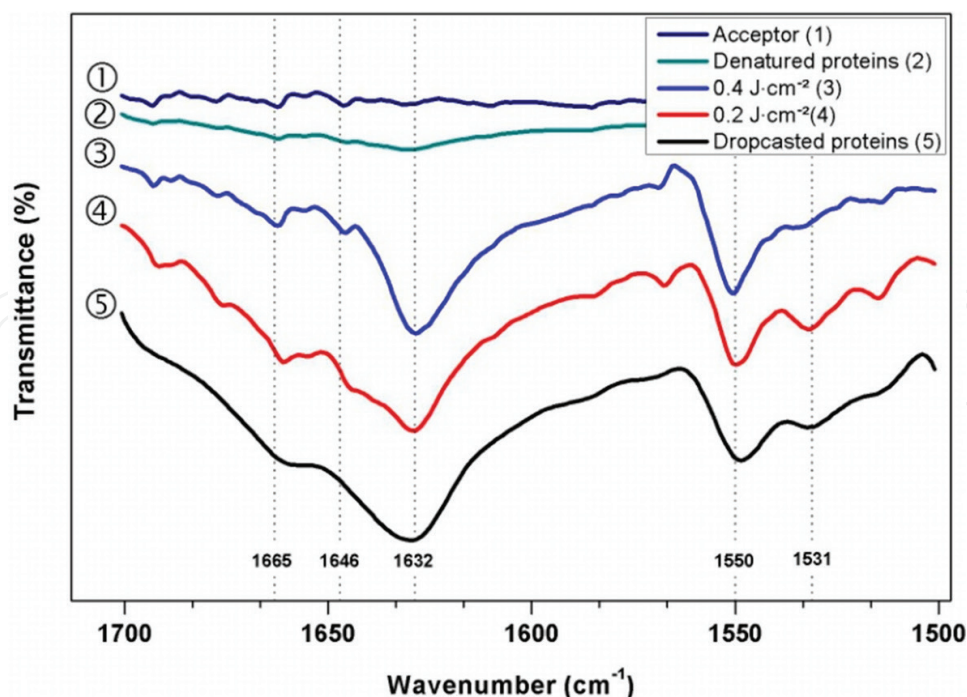
Figure 4. Representative ATR-FTIR spectra of Si acceptor (1), a negative control of heat-denatured proteins (2), proteins transferred at 0.4 J/cm^{-2} (3), proteins transferred at 0.2 J/cm^{-2} (4), and a positive control consisting of dropcasted proteins (5).

This implies that proteins exposed at a temperature of 100°C for 1 min lose their secondary structure. However, any heat damage susceptible to occur is strictly localized and does not influence the secondary structure of the proteins in a detectable manner, which is majoritarily preserved after the transfer.

In addition, Western blot assays were conducted onto a control-purified FN solution and that of protein desorbed from the LDW acceptor. One can observe similar molecular weights between the tested solutions (**Figure 5**) [63].

In vitro tests performed with MC3T3-E1 preosteoblast cells validated the transfer of secondary structure and functionality. Particular attention was paid to the presence and availability of the cell binding domain within the FN molecules, as evidenced by immunostaining with specific monoclonal antibodies followed by microscopic observations. MC3T3 osteoblasts preferentially attached onto the FN features (**Figure 6a** and **b**). The spreading of cells close to the FN features was slightly enhanced in comparison with cells onto the bare substrate. The cellular morphology was generally influenced by the presence of the FN spots. Thus, the cells situated in the close vicinity of FN exhibited an asymmetric aspect with elongated cytoplasmic extensions, whose tip-adhesion site ended in most cases on a FN region. Cells situated completely onto the FN features present a stronger polarity, with longer filopodia, which suggest an improved fixation on the substrate. A characteristic representation of such cellular behavior is depicted in **Figure 6e** and **f**, wherefrom one can observe the actin filaments (**Figure 6f**) oriented along the FN formations (**Figure 6e**). In all cases, cell nuclei did not exhibit significant morphological modifications such as fragmentations or condensation, which could suggest
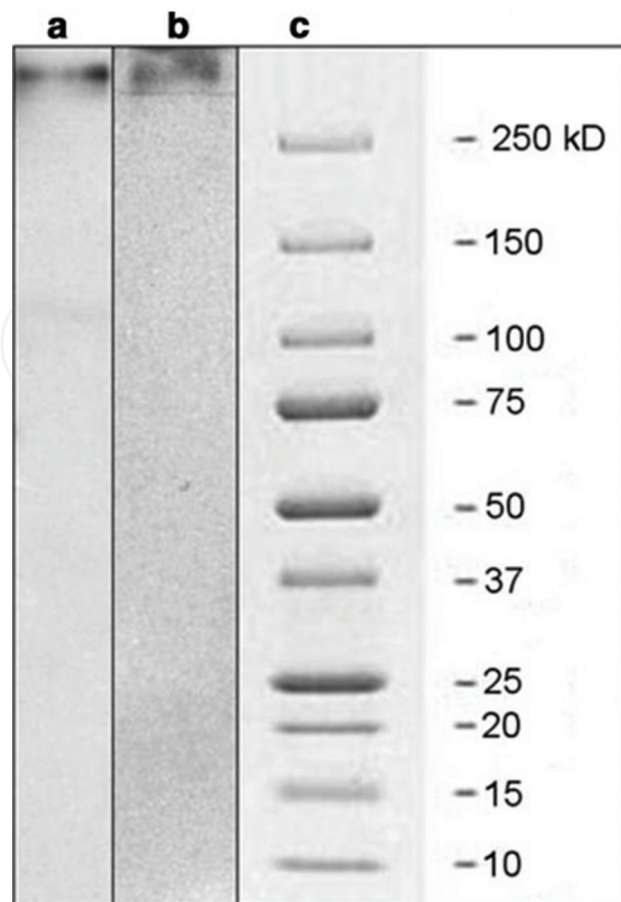
Figure 5. Western blot profiles obtained for the control-purified FN solution (a) and that of protein desorbed from the LDW acceptor (b) compared with the BioradAllBlue calibration molecular weight reference proteins (c) (reproduced with permission from [63]).

cellular apoptosis. The *in vitro* tests revealed the full functionality of the transferred protein features in both deposition regimes, 0.2 or 0.4 J/cm^2. However, it was observed that slight differences concerning the local uniformity, as well as the aspect repeatability of the protein features, do exist. For obtaining uniform, highly repetitive features, lower fluences are definitely desirable.

The biological response of the transferred FN was also assessed by fluorescence microscopic measurements after the seeding of Swiss 3 T3 fibroblasts cells onto the tested samples [63]. The shape of the imprinted FN features strongly influenced the morphology of the adherent Swiss 3 T3 fibroblasts cells. A weaker attachment and a round shape of the attached cells onto the Ti control was observed, whereas for the transferred FN patterns, they were clearly spread, actin filaments being organized according to the FN spots aspect [63]. These results are in good agreement with those obtained in case of preosteoblast MC3T3 cells.

The biochemical characterization of the FN structures open new perspectives on the fabrication of complex biomaterials including proteins (with high molecular weight) for use in biomedical applications (e.g., drug delivery, regenerative tissue engineering) [63].
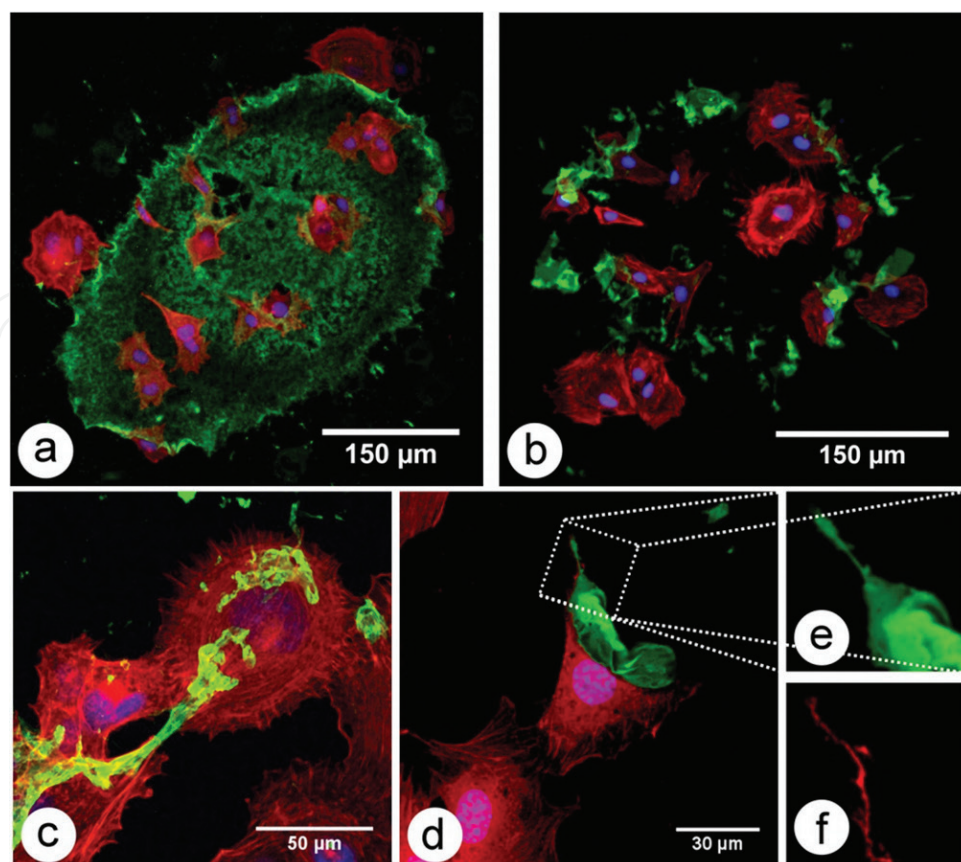
Figure 6. Characteristic microscopic aspect of MC3T3 cells in the neighboring region to laser transferred fibronectin features. (a) Osteoblasts attached to the FN features obtained at 0.2 J/cm². (b) Osteoblasts attached to the FN features transferred at 0.4 J/cm². (c) Details of cytoskeleton actin fibers oriented along the FN subfeatures obtained at 0.4 J/cm². (d) Specific modified cell morphology in the close vicinity of FN (0.4 J/cm²), where a certain bending of the protein subfeature can be deduced as a result of the cellular activity. (e and f) Detailed image of a cytoplasmic extension (filopodia) placed along a FN subfeature. Confocal scanning microscopy was conducted using TRITC-coupled phalloidin for the F-actin, DAPI for the DNA, and FITC-coupled antibodies for the FN.

MAPLE technique was also applied in the synthesis of fibronectin coatings. A recent report of Sima *et al*. describe the fabrication, in two steps, of a new hybrid biomimetic thin structure (inorganic-organic bilayer) using two different laser techniques (PLD for hydroxyapatite (HA) and MAPLE for FN deposition, respectively) [54]. The authors quantified by temperature-programmed desorption mass spectrometry the deposited FN over the HA layer and verified its biological efficiency by multiple cellular studies [54].

In a recent study published in 2015, Agarwal *et al*. reported the enhanced implant-bone integration in healthy and osteoporotic rats by applying FN coatings obtained by immersion of clinical grade stainless steel implants in purified solutions of FN for 30 min [64]. Following the same direction on integration of biomedical implants with tissue, other research groups attained the idea of producing an interface able to mimic the ECM and improve cell behavior [59]. Gand *et al*. proposed the synthesis of a layer-by-layer film by direct incorporation of FN (as anionic polymer) into poly(L-lysine) (PLL) (positively charged) in order to obtain an FN-enriched structure, capable to mimic the ECM [59].

From reported data, one can notice the fast development on FN processing, thus the principal objective being the chemical preservation and the improvement of the biological response.

3.2.2. Vitronectin

A similar and important constituent of the interstitial ECM, vitronectin, is readily adsorbed at the interface of biomaterials and is known for its adhesive functions during development and angiogenesis [65, 66]. VN is the main glycoprotein adsorbed from the serum onto synthetic polymers and was identified as an essential adhesion and spreading mediator in many cells [65]. Given its multifunctional physiological activity, vitronectin is also described as S protein, epibolin, or "serum spreading factor" [65–67].

In order to prevent fibrosis and to support migration of adjacent cells, VN have the capability to trigger the enzymatic degradation of provisional ECM, influencing thus the fate of implants. In addition, it encompasses an approach for improving the endothelization of implants [66]. As reported [68], VN and FN are vital for the *in vitro* adhesion of osteoblast cells.

In 2009, Whitlow *et al*. reported the cover of a platinum aneurysm coil with gold (Au) or VN by using an electrostatic self-assembly process trying to evaluate the degree of neointimal change associated with the applied ultrathin layer [69]. The neointimal response revealed the improved reaction in case of Au and VN coil groups as compared with the uncoated platinum group [69].

Later, in 2011, Sima *et al*. reported the transfer and immobilization of VN by MAPLE technique on a collector coated with an HA layer (by PLD). In this study, the biological response was evaluated by using human osteoprogenitor (HOP) cells, and the obtained results revealed the improved adherence, spreading, and growth in respect with HA coatings [70].

The vitronectin multifunctionality makes it an attractive biopolymer for the tissue engineering when used as a surface coating and moreover, opens new directions for research.

3.3. Enzymes

Enzymes are proteins that consist of one or more polypeptide chains and have an active site, thus determining their specificity and flexibility [71, 72]. Enzymes are also known as biocatalysts that accelerate a chemical reaction without interfering their equilibrium [71, 73]. Enzymes are recognized for their high catalytic activity and excellent selectivity for the targeted substrate, being thus described as optimal biorecognition molecules [74]. As biosensor components, enzymes are considered the shortest lived elements because they progressively lose activity [74].

For a long time, enzymes have been used in microbial processes, helping (by fermentation process) in the preparation of cheeses, wines, and other milk products [71]. In course of time, the interest of some researchers leads to the use of enzyme in the medical field, in the diagnosis, and in the treatment of various diseases [75]. In this respect, they try to study, understand, and obtain the basic information on toxicology, immunological reactions, and chemical stability of the organism *in vivo* [76].

Various deposition techniques could be applied in order to immobilize different enzymes onto a solid holder. This task is not an easy one due to the fact that one can deal with the transfer of complex molecules [77]. A possible approach for enzyme immobilization could be MAPLE technique recognized for its capability to transfer soft materials, avoiding the changes of the chemical and biological properties.

3.3.1. Papain

Papain, a proteolytic enzyme, found naturally in papaya is of key importance in various vital biological processes [78]. Due to its proteolytic and elastolytic properties, papain exhibits antibacterial, antifungal, and anti-inflammatory activities [79].

The use of papain as a coating for surface improvement in different medical devices is still at the beginning. In 2013, Motoc *et al.* [80] reported the deposition of a simple HA (Ti/HA or papain Ti/papain) and a bilayer (consisting of HA and papain synthetized by PLD and MAPLE, respectively: Ti/HA/papain). The authors tried to functionalize the titanium surface in two steps, enhancing the bioactivity (HA layer) and activating the localized drug with antimicrobial and anti-inflammatory effects (papain layer) [80]. In order to evaluate the stoichiometric transfer of papain, the used powders and the obtained coatings were analyzed by FTIR. The spectra revealed the presence of the proteins characteristic bands (**Figure 7**) [80].

Moreover, the osteoblasts-like SaOs2 human cells were used in order to evaluate the biological performances of papain coatings, by adhesion (immunofluorescence microscopy: **Figure 8**) and proliferation tests (MTS assay: data not shown here) [80].

Taking into account the positive results, one can consider the papain-based coatings as potential implant material with good antimicrobial properties and improved integration.

3.3.2. Lysozyme

Lysozyme, a small and stable lytic enzyme, is found in nature, being present not only in almost all secretions, body fluids (tears, saliva, and sweat), and tissues (nasal cavity) of the living organisms, but also in some plants, bacteria, or egg white [81, 82]. It has a specific hydrolytic activity against the cell walls of liable bacteria [81, 83]. Thus, lysozyme increases the permeability of Gram-positive bacteria and causes the burst of cells [82, 84]. Oppositely, the antimicrobial efficiency of lysozyme against the Gram-negative bacteria is limited and even lacks toward eukaryotic cell walls [82]. Additionally, there are studies that reveal the potential of lysozyme to inhibit tumor formation and growth (anticancer agent) [85, 86].

Due to its multifunctionality and recognized antimicrobial efficiency, lysozyme is used, more and more, in the biomedical field. A special attention was paid to the development of medical devices by surface functionalization with lysozyme or composites based on it, which can also act as drug delivery systems.

In this respect, Visan *et al.* reported the deposition of composite coatings based on lysozyme embedded in a mixture of polyethylene glycol (PEG) and polycaprolactone (PCL) by both MAPLE and dip-coating techniques. The authors demonstrated the antimicrobial efficiency
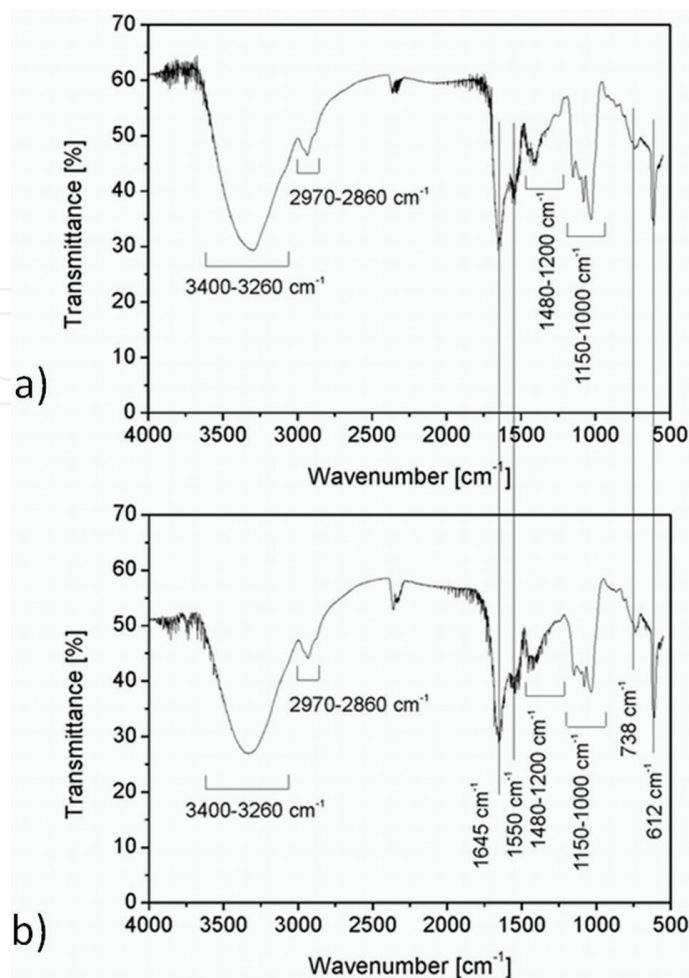
Figure 7. FTIR micrographs of the papain dropcast (a) and of the thin films obtained by MAPLE (b) for 5 wt% papain in distilled water and a laser fluence of 0.75 J/cm^2 (reproduced with permission from [79]).

of PCL:PEG-lysozyme composite coatings, which was in good agreement with the viability results with osteoblasts and MSC cells [87].

The incorporation of lysozyme into polymeric matrices was also studied by Grumezescu *et al*. This time, poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (P(3HB-3 HV)) and PEG bio-polymers were used as core shells for lysozyme, and the composite material was deposited by MAPLE in the form of thin films. Both P(3HB-3 HV)/PEG/Lys and P(3HB-3 HV)/Lys coatings proved a significant antimicrobial activity and an improved biocompatibility [88].

Such complex coatings based on lysozyme are promising for the nanobiomedical field due to their potential use as bone implants.

3.3.3. Urease

Urease, a non-redox metalloenzyme, known also as nickel-dependent enzyme, induces the hydrolysis of urea into ammonium and carbon dioxide [89–91]. Urease can also provide a defense mechanism against pathogens by controlling the nitrogen content in the biological
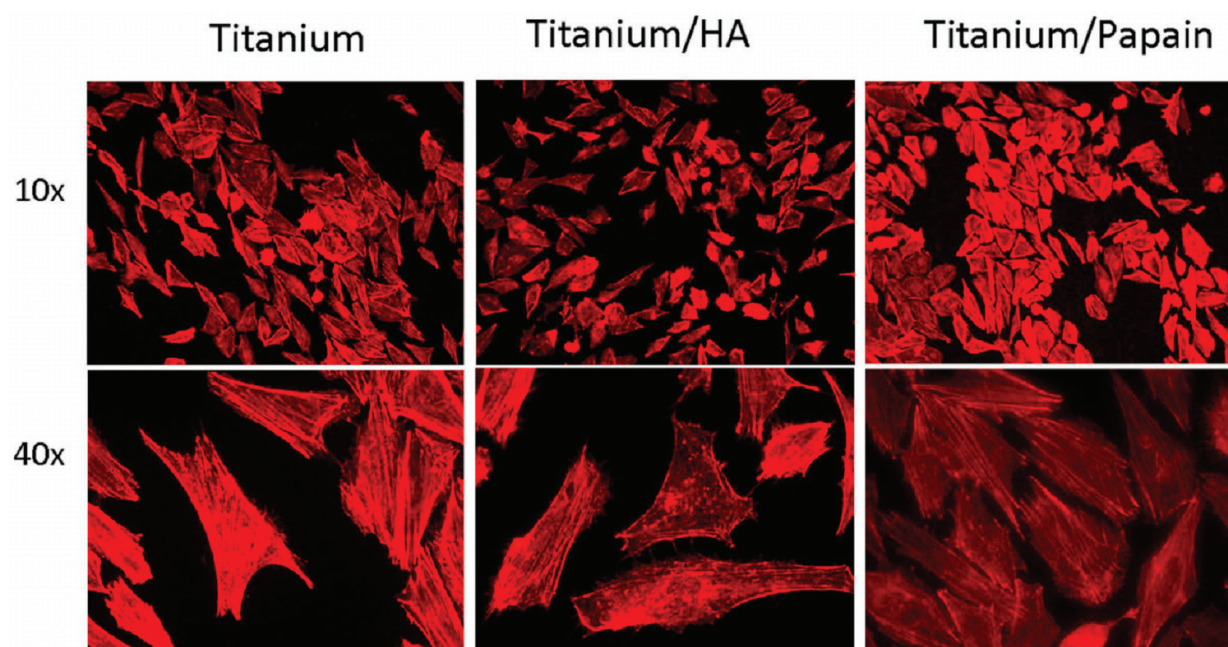
Figure 8. Fluorescence microscopic images of human SaOs2 cells grown on bare Ti, Ti/HA, and Ti/papain for 5 wt% papain in distilled water and a laser fluence of 0.75 J/cm² (reproduced with permission from [79]).

environments [92]. In addition, urease can be isolated from a selection of organisms, comprising bacteria, fungi, and plants [91]. Urease is a key enzyme used to determine the amount of urea in biological solutions (blood), where urea being toxic above certain concentrations [93, 94]. The removal of urea from waste water, food, and fruit juices is also relevant for domains such as environmental analyses and the food industry [94]. The presence of urea can be determined by electrochemical or optical methods, on the basis of the formed ammonia [90].

The use of urease in applications such as clinical diagnosis, environmental analysis and detection of food adulteration requires the maintenance of the enzyme stability, functionality and activity as close as possible to its natural state [95].

The use of urease in biosensing applications was and still is an area of interest for a relatively large number of scientists. In 2010, György *et al.* reported the immobilization of urease (from "icy" targets with different urease concentrations) in the form of thin films by using MAPLE technique. The authors proved the stoichiometric transfer of urease, and further, the enzymatic activity was preserved. The kinetic and enzymatic activity of the immobilized urease was evaluated by using the Worthington assay method (**Figure 9**) [90]. This study proved that the thin film of urease could be active in urea disintegration if it is obtained under optimum deposition conditions [90].

Furthermore, the key factor in the achievement of a high-sensitive biosensing unit could be considered the optimum combination between biomolecules and nanomaterials [96]. In this respect, Siqueira *et al.* [96] studied the electrochemical properties of urea-based electrolyte-insulator-semiconductor (EIS) sensor for urea detection. The authors described the synthesis of LbL films for urease immobilization on capacitive EIS chips by using polyamidoamine
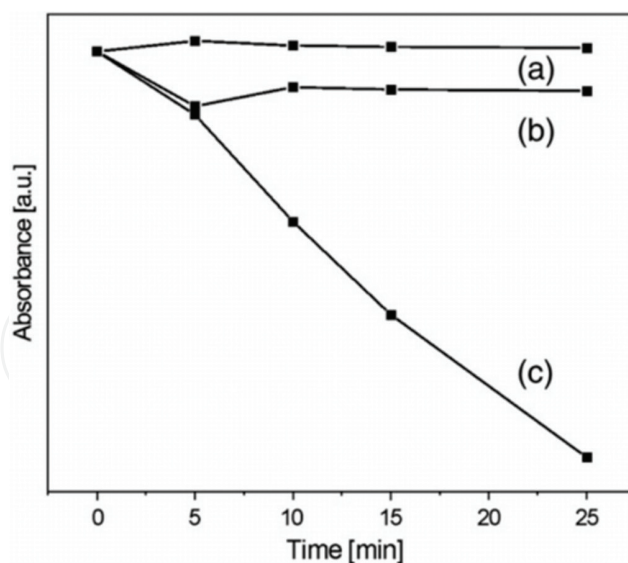
Figure 9. The kinetic assay of urease thin film for (a) 1, (b) 3.5, and (c) 10 wt% concentrations in the frozen composite targets deposited at 0.4 J/cm² laser fluence (reproduced with permission from [89]).

(PAMAM)/carbon nanotubes (CNT) as stabilizing matrices. The achievement of PAMAM/CNT LbL films for urease immobilization opens new directions of research in the field of biosensors, with the possibility of extension to other enzymes [96].

4. Conclusions

Direct laser ablation of organic/biological materials was considered for a long time inaccessible because of risks of decomposition and irreversible damages. The recent progress of "soft" ablation laser techniques makes possible the safe expulsion and transfer of materials, from target to substrate for synthesis of structures of various bio-, nano-, and more recently meta-materials. This opened the access toward pulsed laser technologies utilization for the ablation of "delicate" simple and composite materials. Systematic complementary investigations demonstrated that, under proper irradiation conditions and "special protection" as ensured by cryogenic utilization, the preservation is possible for basic material composition, structure, morphology, and more likely functionality. Simultaneous or subsequent ablation of organic/biological materials was reached for new top applications in technology and in particular in nanobiomedicine. The chapter is based on recent original results of the authors and a selection of the relevant existing new data from specialized literature.

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Author details

Gianina-Florentina Popescu-Pelin[1,2], Carmen-Georgeta Ristoscu[1], Maria Badiceanu[1,2] and Ion N. Mihailescu[1]*

*Address all correspondence to: ion.mihailescu@inflpr.ro

1 National Institute for Lasers, Plasma and Radiation Physics, Magurele-Ilfov, Romania

2 Faculty of Physics, University of Bucharest, Magurele-Ilfov, Romania

References

[1] McGill RA, Chrisey DB. Method of producing a film coating by matrix assisted pulsed laser deposition. Google Patents; 2000

[2] Chrisey D, Pique A, McGill R, Horwitz J, Ringeisen B, Bubb D, et al. Laser deposition of polymer and biomaterial films. Chemical Reviews. 2003;**103**(2):553-576

[3] Jelinek M, Kocourek T, Remsa J, Cristescu R, Mihailescu IN, Chrisey DB. MAPLE applications in studying organic thin films. Laser Physics. 2007;**17**(2):66-70(5)

[4] Ristoscu C, Mihailescu IN. Biomimetic coatings by pulsed laser deposition, chapter 7. In: Belegratis M, Schmidt V, editors. Laser Technology in Biomimetics: Basics and Applications, Springer—Biological and Medical Physics, Biomedical Engineering. Berlin Heidelberg: Springer-Verlag; 2013. p. 163-191 ISBN 978-3-642-41340-7

[5] Nelea V, Jelinek M, Mihailescu IN. Biomaterials: New issues and breakthroughs for biomedical applications, chapter 18. In: . Pulsed Laser Deposition of Thin Films: Applications-Lead Growth of Functional Materials. Wiley & Sons; Hoboken, New Jersey; 2007. p. 421-459

[6] Leveugle E, Zhigilei LV. Molecular dynamics simulation study of the ejection and transport of polymer molecules in matrix-assisted pulsed laser evaporation. Journal of Applied Physics. 2007;**102**(7):074914

[7] Zhigilei LV, Lin Z, Ivanov DS, Leveugle E, Duff WH, Thomas D, Sevilla C, Stephen JG. Atomic/molecular-level simulations of laser–materials interactions, chapter 3. In: Miotello A, Ossi PM, editors. Laser-Surface Interactions for New Materials Production Tailoring Structure and Properties, Series: Springer Series in Materials Science. Vol. 130. Springer Heidelberg Dordrecht London New York; 2010. p. 43-79

[8] Bubb DM, Johnson SL, Collins B, Haglund RF. Thermal confinement and temperature-dependent absorption in resonant infrared ablation of frozen liquid targets. The Journal of Physical Chemistry. 2010;**114**:5611

[9] Itina TE, Zhigilei LV, Garrison BJ. Matrix-assisted pulsed laser evaporation of polymeric materials: A molecular dynamics study. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 2001;**180**:238-244

[10] Caricato AP. MAPLE and MALDI: Theory and Experiments, Chapter 12. In: Castillejo M, Ossi PM, Zhigilei L, editors. Lasers in Materials Science. Springer Cham Heidelberg New York Dordrecht London; 2014. p. 295-323

[11] Smausz T, Megyeri G, Kékesi R, Vass C, György E, Sima F, Mihailescu IN, Hopp B. Comparative study on pulsed laser deposition and matrix assisted pulsed laser evaporation of urease thin films. Thin Solid Films. 2009;**517**:4299-4302

[12] Warren SG, Brandt RE, Grenfell TC. Visible and near-ultraviolet absorption spectrum of ice from transmission of solar radiation into snow. Applied Optics. 2006;**45**:5320-5334

[13] Esenaliev RO, Karabutov AA, Podymova NB, Letokhov VS. Laser ablation of aqueous solutions with spatially homogeneous and heterogeneous absorption. Applied Physics B—Springer. 1994;**59**:73-81

[14] Purice A, Schou J, Kingshott P, Dinescu M. Production of active lysozyme films by matrix assisted pulsed laser evaporation at 355 nm. Chemical Physics Letters. 2007;**435**:350-353

[15] Ikuma K, Madden AS, Decho AW, Lau BLT. Deposition of nanoparticles onto polysaccharide coated surfaces: Implications for nanoparticle–biofilm interactions. Environmental Science: Nanomaterials. 2014;**1**:117-122

[16] Uthaman S, Lee SJ, Cherukula K, Cho C-S, Park I-K. Polysaccharide-coated magnetic nanoparticles for imaging and gene therapy. BioMed Research International. 2015;**14**: Article ID 959175

[17] Donald C. Aduba Jr, Hu Yang. Polysaccharide fabrication platforms and biocompatibility assessment as candidate wound dressing materials. Bioengineering. 2017;**4**:1. DOI: 10.3390/bioengineering4010001.

[18] Samal PK, Dangi JS. Isolation, preliminary characterization and hepatoprotective activity of polysaccharides from *Tamarindus indica* L. Carbohydrate Polymers. 2014;**102**:1-7

[19] Doh K-O, Yoon Yeo. Application of polysaccharides for surface modification of nanomedicines. Therapeutic Delivery. 2012;**3**(12):1447-1456

[20] Senni K, Pereira J, Gueniche F, Delbarre-Ladrat C, Sinquin C, Ratiskol J, Godeau G, Fischer A-M. Dominique Helley and Sylvia Colliec-Jouault marine polysaccharides: A source of bioactive molecules for cell therapy and tissue engineering. Marine Drugs. 2011;**9**:1664-1681. DOI: 10.3390/md9091664

[21] Lohmann N, Schirmer L, Atallah P, ElkeWandel RAF, Werner C, Simon JC, Franz S, Freudenberg U. Glycosaminoglycan-based hydrogels capture inflammatory chemokines and rescue defective wound healing in mice. Science Translational Medicine. 2017;**9**:eaai9044

[22] Sun J, Tan H. Alginate-based biomaterials for regenerative medicine applications. Materials. 2013;**6**:1285-1309

[23] Wang Y, Wang X, Shi J, Zhu R, Zhang J, Zhang Z, Ma D, Hou Y, Lin F, Yang J, Mizuno M. A biomimetic silk fibroin/sodium alginate composite scaffold for soft tissue engineering. Scientific Reports. 2016;**6**: Article number 39477

[24] Lu L, Qi Y, Tian J, Zhou C. Alginate/hydroxyapatite hydrogel as biodegradable in situ forming scaffold. In: Peng Y, Weng X, editors. 7th Asian-Pacific Conference on Medical and Biological Engineering. IFMBE Proceedings. Vol. 19. Berlin, Heidelberg: Springer; 2008

[25] Oliveira JT, Martins L, Picciochi R, Malafaya PB, Sousa RA, Neves NM, Mano JF, Reis RL. Gellan gum: A new biomaterial for cartilage tissue engineering applications. Journal of Biomedical Materials Research Part A. 2009;852-863

[26] Cengiz IF, Miguel J, Oliveira, Reis RL. Tissue engineering and regenerative medicine strategies for the treatment of osteochondral lesions, chapter 2. In: Ratib O, Choi H, editors. 3D Multiscale Physiological Human, Nadia Magnenat-Thalmann. London: Springer-Verlag; 2015. p. 25-47

[27] Sun G, Mao JJ. Engineering dextran-based scaffolds for drug delivery and tissue repair. Nanomedicine (Lond). 2012;**7**(11):1771-1784. DOI: 10.2217/nnm.12.149

[28] Liu J, Qi C, Tao K, Zhang J, Zhang J, Luming X, Jiang X, Zhang Y, Huang L, Li Q, HongjianXie JG, Shuai X, Wang G, Wang Z, Wang L. Sericin/dextran injectable hydrogel as an optically trackable drug delivery system for malignant melanoma treatment. ACS Applied Materials & Interfaces. 2016;**8**(10):6411-6422

[29] Schlaubitz S, Mohammed Derkaoui S, Marosa L, Miraux S, Renard M, Catros S, Visage C, Letourneur D, Amédée J, Fricain J-C. Pullulan/dextran/nhamacroporous composite beads for bone repair in a femoral condyle defect in rats. PLoS ONE. 2014;**9**(10):e110251. DOI: 10.1371/journal.pone.0110251

[30] Maurice NC. Hyaluronic Acid for Biomedical and Pharmaceutical Applications. Smithers Rapra Technology, eBook, 238 pages; 2014

[31] Visan A, Stan GE, Ristoscu C, Popescu-Pelin G, Sopronyi M, Besleaga C, Luculescu C, Chifiriuc MC, Hussien MD, Marsan O, Kergourlay E, Grossin D, Brouillet F, Mihailescu IN. Combinatorial MAPLE deposition of antimicrobial orthopedic maps fabricated from chitosan and biomimetic apatite powders. International Journal of Pharmaceutics. 2016;**511**(1):505-515

[32] Riva R, Ragelle H, des Rieux A, Duhem N, Jérôme C, Préat V. Production chitosan derivatives. In: Jayakumar R, Prabaharan M, Muzzarelli RAA, editors. Drug Delivery and Tissue Engineering, Chapter 2 in Chitosan for Biomaterials II. Advances in Polymer Science. Berlin Heidelberg: Springer Verlag; 2014. p. 19-44. DOI: 10.1007/12_2011_137.sau

[33] Singh RS, Kaur N, Rana V, Kennedy JF. Pullulan: A novel molecule for biomedical applications. Carbohydrate Polymers. 2017;**171**:102-121

[34] Wong VW, Rustad KC, Galvez MG, Neofytou E, Glotzbach JP, Januszyk M, Major MR, Sorkin M, Longaker MT, Rajadas J, Geoffrey C. Gurtner "engineered pullulan–collagen composite dermal hydrogels improve early cutaneous wound healing". Tissue Engineering: Part A. 2011;**17**(5-6):631-644

[35] Cristescu R, Dorcioman G, Ristoscu C, Axente E, Grigorescu S, Moldovan A, Mihailescu IN, Kocourek T, Jelinek M, Albulescu M, Buruiana T, Mihaiescu D, Stamatin I, Chrisey DB. Matrix assisted pulsed laser evaporation processing of triacetate-pullulan polysaccharide thin films for drug delivery systems. Applied Surface Science. 2006;**252**:4647-4651

[36] Ponnusami V, Gunasekar V. Production of pullulan by microbial fermentation. In: Ramawat KG, Mérillon J-M, editors. Polysaccharides: Bioactivity and Biotechnology. Springer Cham Heidelberg New York Dordrecht London; 2014. p. 1-13. DOI: 10.1007/978-3-319-03751-6_58-1

[37] Cristescu R, Stamatin I, Mihaiescu DE, Ghica C, Albulescu M, Mihailescu IN, Chrisey DB. Pulsed laser deposition of biocompatible polymers: A comparative study in case of pullulan. Thin Solid Films. 2004;**453-454**:262-268

[38] Jelinek M, Cristescu R, Axente E, Kocourek T, Dybal J, Remsa J, Plestil J, Mihaiescu D, Albulescu M, Buruiana T, Stamatin I, Mihailescu IN, Chrisey DB. Matrix assisted pulsed laser evaporation of cinnamate-pullulan and tosylate-pullulan polysaccharide derivative thin films for pharmaceutical applications. Applied Surface Science. 2007;**253**:7755-7760

[39] Sarah E, Bulman, Coleman CM, Mary Murphy J, Medcalf N, Ryan AE, Barry F. Pullulan: A new cytoadhesive for cell-mediated cartilage repair. Stem Cell Research & Therapy. 2015;**6**:34. DOI: 10.1186/s13287-015-0011-7.

[40] Atila D, Keskin D, Tezcaner A. Crosslinked pullulan/cellulose acetate fibrous scaffolds for bone tissue engineering. Materials Science and Engineering C. 2016;**69**:1103-1115

[41] Liu Y, Wang Y, Zhang C, Zhou P, Yang L, An T, Sun D, Zhang N, Wang Y. Core−shell nanoparticles based on pullulan and poly(β-amino) ester for hepatoma-targeted codelivery of gene and chemotherapy agent. ACS Applied Materials & Interfaces. 2014;**6**(21):18712-18720

[42] Catherine Guérin, Jean-Luc Audic. Egg-protein-based films and coatings, chapter 31 In: Rainer Huopalahti, Rosina López-Fandiño, Marc Anton, Rüdiger Schade editors. Bioactive Egg Compound. Berlin Heidelberg: Springer-Verlag; 2007. pp. 265-273

[43] John MK. Proteins as raw materials for films and coatings: Definitions, current status, and opportunities, chapter 1. In: Gennadios A, editor. Protein-based Films and Coatings. Boca Raton-London-New York-Washington D.C.: CRC Press LLC; 2002

[44] Cozzone AJ. Proteins: Fundamental chemical properties, encyclopedia of life sciences. Macmillan Publishers Ltd, Nature Publishing Group; 2002. p. 10

[45] Revest P. Biochemistry and cell biology, chapter 2. In: Naish J, Revest P, Court DS, editors. Medical Sciences. Saunders: Elsevier; 2009. p. 15-88

[46] Dangaran K, Tomasula PM, Qi P. Structure and function of protein-based edible films and coatings, chapter 2. In: Huber KC, Embuscado ME, editors. Edible Films and Coatings for Food Applications. New York: Springer; 2009. p. 25-56. DOI: 10.1007/978-0-387-92824-1_2

[47] Gagner JE, Kim W, Chaikof EL. Designing protein-based biomaterials for medical applications. Acta Biomaterialia. 2014;**10**:1542-1557

[48] McCann TE, Kosaka N, Choyke PL, Kobayashi H. The use of fluorescent proteins for developing cancer-specific target imaging probes, chapter 13. In: Hoffman RM, editor. In Vivo Cellular Imaging Using Fluorescent Proteins. Vol. 872. New York: Humana Press Springer Science+Business Media, eBook; 2012. p. 191-204. DOI: 10.1007/978-1-61779-797-2_13

[49] Löfblom J, Feldwisch J, Tolmachev V, Carlsson J, Ståhl S, Frejd FY. Affibody molecules: Engineered proteins for therapeutic, diagnostic and biotechnological applications. FEBS Letters. 2010;**584**:2670-2680

[50] Rehman K, Hamid Akash MS, Akhtar B, Tariq M, Mahmood A, Ibrahim M. Delivery of therapeutic proteins: Challenges and strategies. Current Drug Targets. 2016;**17**(10):1172-1188

[51] Yin L, Yuvienco C, Montclare JK. Protein based therapeutic delivery agents: contemporary developments and challenges. Biomaterials. 2017;**134**:91-116

[52] Gomes S, Leonor IB, Mano JF, Reis RL, Kaplan DL. Natural and genetically engineered proteins for tissue engineering. Progress in Polymer Science. 2012;**37**(1):1-17

[53] Sayin E, TürkerBaran E. Vasif Hasirci Protein-based materials in load-bearing tissue-engineering applications. Regenerative Medicine. 2014;**9**(5):687-701

[54] Sima F, Davidson PM, Dentzer J, Gadiou R, Pauthe E, Gallet O, Mihailescu IN, Anselme K. Inorganic–organic thin implant coatings deposited by lasers. ACS Applied Materials & Interfaces. 2015;**7**:911–920

[55] Borkner CB, Elsner MB, Scheibel T. Coatings and films made of silk proteins. ACS Applied Materials & Interfaces. 2014;**6**(18):15611-15625

[56] Wang H-J, Di L, Ren Q-S, Wang J-Y. Applications and degradation of proteins used as tissue engineering materials. Materials. 2009;**2**:613-635

[57] Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. Journal of Cell Science. 2010;**123**:4195-4200

[58] Li M, Mills DK, Cui T, McShane MJ. Cellular response to gelatin- and fibronectin-coated multilayer polyelectrolyte nanofilms. IEEE Transactions on Nanobioscience. 2005;**4**(2):170-179

[59] Gand A, Tabuteau M, Chat C, Ladam G, Atmani H, Tassel PRV, Pauthe E. Fibronectin-based multilayer thin films. Colloids and Surfaces B: Biointerfaces. 2017;**156**:313-319

[60] Andreeva NV, Leonova OG, Popenko VI, Belyavsky AV. Controlled formaldehyde fixation of fibronectin layers for expansion of mesenchymal stem cells. Analytical Biochemistry. 2016;**514**:38-41

[61] Sangkert S, Kamonmattayakul S, Chai WL, Meesane J. A biofunctional-modified silk fibroin scaffold with mimic reconstructed extracellular matrix of decellularized pulp/collagen/fibronectin for bone tissue engineering in alveolar bone resorption. Materials Letters. 2016;**166**:30-34

[62] Li G, Yang P, Huang N. Layer-by-layer construction of the heparin/fibronectin coatings on titanium surface: Stability and functionality. Physics Procedia. 2011;**18**:112-121

[63] Grigorescu S, Hindie M, Axente E, Carreiras F, Anselme K, Werckmann J, Mihailescu IN, Gallet O. Fabrication of functional fibronectin patterns by nanosecond excimer laser direct write for tissue engineering applications. Journal of Materials Science Materials in Medicine. 2013;**24**:1809-1821

[64] Agarwal R, Gonzalez-García C, Torstrick B, Guldberg RE, Salmeron-Sanchez M, García AJ. Simple coating with fibronectin fragment enhances stainless steel screw osseointegration in healthy and osteoporotic rats. Biomaterials. 2015;**63**:137-145

[65] Schleicher I, Parker A, Leavesley D, Crawford R, Upton Z, Xiao Y. Surface modification by complexes of vitronectin and growth factors for serum-free culture of human osteoblasts. Tissue Engineering. 2005;**11**(11/12):1688-1698

[66] Toromanov G, Gugutkov D, Gustavsson J, Planell J, Salmeron-Sanchez M, Altankov G. Dynamic behavior of vitronectin at the cell–material interface. ACS Biomaterials Science Engineering. 2015;**1**(10):927-934

[67] Yamada KM. Fibronectin and other cell interactive glycoproteins, chapter 4. In: Hay ED, editor. Cell Biology of Extracellular Matrix. 2nd ed. USA: Springer; 1991. p. 111-146

[68] McConigle J, Thomas J. Webster functional tests for biocompatability, chapter 20. In: Glocker D, Ranade S, editors. Medical Coatings and Deposition Technologies. Wiley Scrivener Publishing, eBook; 2016. p. 671-702

[69] Whitlow CT, Geer CP, Mattern CWT, Mussat-Whitlow BJ, Yazdani SK, Berry JL, Lalli JH, Claus RO, Challa VR, Morris PP. Endovascular histologic effects of ultrathin gold- or vitronectin-coated platinum aneurysm coils in a rodent arterial occlusion model: A preliminary investigation. AJNR American Journal of Neuroradiology. 2009;**30**:85-90

[70] F. Sima, P. Davidson, E. Pauthe, O. Gallet, K. Anselme,·IN. Mihailescu. Thin films of vitronectin transferred by MAPLE, Applied Physics A 105:611-617 (2011).

[71] Mäntsälä P, Niemi J. Enzymes: The biological catalysts of life, chapter 1. In: Hanninen OOP, Atalay M, editors. Physiology and Maintenance: Enzymes: The Biological Catalysts of Life, Nutrition and Digestion. Vol. 2. EOLSS Publisher Co. Ltd., Oxford, United Kingdom; 2009. p. 1-21

[72] Nature of Biology 2. Enzymes and biochemical pathways [Internet]. 2016. Available from: http://www.wiley.com/legacy/Australia/PageProofs/c03EnzymesAndBiochemicalPathways_web.pdf [Accessed: 2017-07-30]

[73] Pratul KA. Enzymes: An integrated view of structure, dynamics and function. Microbial Cell Factories. 2006;**5**:2-12. DOI: 10.1186/1475-2859-5-2

[74] Putzbach W, Ronkainen NJ. Immobilization techniques in the fabrication of nanomaterial-based electrochemical biosensors: A review. Sensors (Basel). 2013;**13**(4):4811-4840

[75] Leitgeb M, Knez Ž, Vasić K. Micro- and nanocarriers for immobilization of enzymes, chapter 2. In: Stanciu SG, editor. Micro and Nanotechnologies for Biotechnology. Croatia: InTech; 2016. p. 21-58

[76] Costa SA, Helena SA, Reis RL. Enzyme immobilization in biodegradable polymers for biomedical applications, chapter 17. In: Reis RL, San Román J, editors. Biodegradable Systems in Tissue Engineering and Regenerative Medicine. CRC Press, eBook; 2004. p. 301-323

[77] Popescu C, Roqueta J, Pérez delPino A, Moussaoui M, Nogués MV, György E. Processing and immobilization of enzyme Ribonuclease A through laser irradiation. Journal of Materials Research. 2011;**26**(6):815-821

[78] Amri E, Mamboya F. Papain, a plant enzyme of biological importance: A review. American Journal of Biochemistry and Biotechnology. 2012;**8**(2):99-104

[79] Manohar CM, Prabhawathi V, Sivakumar PM, Doble M. Design of a papain immobilized antimicrobial food package with curcumin as a crosslinker. PLoS One. 2015;**10**(4):e0121665

[80] Motoc MM, Axente E, Popescu C, Sima LE, Petrescu SM, Mihailescu IN, Gyorgy E. Active protein and calcium hydroxyapatite bilayers grown by laser techniques for therapeutic applications. Journal of Biomedical Materials Research A. 2013;**101A**(9):2706-2711

[81] Park S-I, Daeschel MA, Zhao Y. Functional properties of antimicrobial lysozyme–chitosan composite films. Journal of Food Science. 2004;**69**(8):M215-M221

[82] Liburdi K, Benucci I, Esti M. Lysozyme in wine: An overview of current and future applications. Comprehensive Reviews in Food Science and Food Safety. 2014;**13**:1062-1073

[83] Dzhavakhiya VG, Shcherbakova LA. Creation of disease-resistant plants by gene engineering, chapter 16. In: Dyakov YT, Dzhavakhiya VG, Korpela T, editors. Comprehensive and Molecular Phytopathology part 3: Practical Use of Molecular Studies. Elsevier, eBook; 2007. p. 439-466

[84] Oliver WT, Wells JE. Lysozyme as an alternative to growth promoting antibiotics in swine production. Journal of Animal Science and Biotechnology. 2015;**6**(1):35

[85] Pacor S, Giacomello E, Bergamo A, Clerici K, Zacchigna M, Boccu E, Sava G. Antimetastatic action and lymphocyte activation by the modified lysozyme mPEG-Lyso in mice with MCa mammary carcinoma. Anticancer Research. 1996;**16**:2559-2564

[86] Mahanta S, Paul S, Srivastava A, Pastor A, Kundu B, Chaudhuri TK. Stable self-assembled nanostructured hen egg white lysozyme exhibits strong anti-proliferative activity against breast cancer cells. Colloids and Surfaces B: Biointerfaces. 2015;**130**:237-245

[87] Visan A, Cristescu R, Stefan N, Miroiu M, Nita C, Socol M, Florica C, Rasoga O, Zgura I, Sima LE, Chiritoiu M, Chifiriuc MC, Holban AM, Mihailescu IN, Socol G. Antimicrobial polycaprolactone/polyethylene glycol embedded lysozyme coatings of Ti implants for osteoblast functional properties in tissue engineering. Applied Surface Science. 2017;**417**:234-243

[88] Grumezescu V, Holban AM, Sima LE, Chiritoiu MB, Chiritoiu GN, Grumezescu AM, Ivan L, Safciuc F, Antohe F, Florica C, Luculescu CR, Chifiriuc MC, Socol G. Laser deposition of poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) – Lysozyme microspheres based coatings with anti-microbial properties. International Journal of Pharmaceutics. 2017;**521**(1-2):184-195

[89] Mazzei L, Cianci M, Musiani F, GáborLente MP, Ciurli S. Inactivation of urease by catechol: Kinetics and structure. Journal of Inorganic Biochemistry. 2017;**166**:182-189

[90] György E, Sima F, Mihailescu IN, Smausz T, Hopp B, Predoi D, Sima LE, Petrescu SM. Biomolecular urease thin films grown by laser techniques for blood diagnostic applications. Materials Science and Engineering C. 2010;**30**:537-541

[91] Stanisçuaski F, Carlini CR. Plant ureases and related peptides: understanding their entomotoxic properties. Toxins. 2012;**4**:55-67

[92] Raza Shah M, Soomro ZH. In: Sharma R, editor. Urease Inhibition, Enzyme Inhibition and Bioapplications. InTech. Available from: http://www.intechopen.com/books/enzyme-inhibition-and bioapplications/urease-inhibition; 2012 ISBN: 978-953-51-0585-5

[93] Doğaç YI, Teke M. Synthesis and characterisation of biocompatible polymer-conjugated magnetic beads for enhancement stability of urease. Applied Biochemistry and Biotechnology. 2016;**179**(1):94-110

[94] Gyorgy E, Mihailescu IN. Enzyme-based biosensors for trace detection, chapter 9. In: Vaseashta A, editor. Life Cycle Analysis of Nanoparticles: Risk, Assessment, and Sustainability. DEStech Publications, eBook; 2015. p. 231-248

[95] Saxena A, Bhattachary A, Kumar S, Epstein IR, Sahney R. Biopolymer matrix for nano-encapsulation of urease—A model protein and its application in urea detection. Journal of Colloid and Interface Science. 2017;**490**:452-461

[96] José R. Siqueira Jr, Denise Molinnus, Stefan Beging, Michael J Schoning Incorporating a hybrid urease-carbon nanotubes sensitive nanofilm on capacitive field-effect sensors for urea detection. ACS Analytical Chemistry. 2014;**86**:5370-5375