Bare laser-synthesized plasmonic Au and TiN nanoparticles as functional additives to polymer nanofiber platforms for tissue engineering applications

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Abstract. Exhibiting strong optical absorption in the visible – near-infrared, plasmonic nanomaterials can be used as transducers in optical biosensing, contrast agents in bioimaging and synthesizers of photothermal therapy. Such functionalities promise their employment as functional elements in tissue engineering platforms, but such applications typically require ultraclean nanomaterials to minimize toxicity problems, which is not easy using conventional chemical synthesis routes. We recently demonstrated the possibility of fabricating ultraclean bare (ligand-free) plasmonic Au and TiN nanoparticles by ultrashort laser ablation in liquid ambient. Exempt of any toxic contaminants and exhibiting a series of imaging and therapeutic functionalities, these nanomaterials present promising objects for various biomedical applications. Here, we review our recent progress in the co-electrospinning of laser-synthesized Au and TiN nanoparticles with polymers to form functionalized matrices for tissue engineering.

Introduction

Tissue engineering is a new interdisciplinary field, which employs functional structured scaffolds to mimic mesoporosity and nanoscale morphology of natural extracellular matrices with the possibility to provide advanced therapeutic properties for diagnostics and treatment of diseases [1-3]. Electrospun nanofibers present one of most promising scaffold systems for such applications, but their clinical applications are still challenging due to numerous issues, including difficulty of shaping, rapid degradation rate or even biocompatibility and biodegradability concerns.

Many above-stated problems can be solved by the employment of inorganic nanoparticles (NPs) as functional additives of nanofiber-based matrices. Considerable progress in nanofabrication technologies has contributed to the synthesis of advanced multifunctional NPs for biomedical tasks including drug delivery, imaging and cell labelling [4-6]. Applications of such NPs in tissue engineering could dramatically enhance physicochemical properties of scaffolds and contribute to their proper integration into tissue-specific microenvironments [7,8]. For instance Silver (Ag) [9,10], Gold (Au) [12,13] and iron oxide [14,15] NPs present non-exhaustive functional objects, which are intensively exploited in tissue engineering tasks using a variety of polymeric scaffolds. However, almost all currently employed NPs are synthesized by conventional chemical or electrochemical routes, which involve hazardous products (e.g., HF, nitrate salts, chloride, citrate, etc.) and various ligands [1,8]. The presence of these products can lead to surface contamination by residual toxic products, which is not consistent with targeted biomedical applications.
Laser ablation has recently appeared as a novel powerful tool for the synthesis of nanomaterials for biomedical applications. This technique is based on the natural production of nanoclusters under the action of laser radiation on a solid target [15], followed by their release into a gaseous or liquid medium to form either a nanostructured film [16,17] or a colloidal nanoparticle solution [18-22]. In this case, solutions of NPs can be stable even in a bare (ligand-free) state and contamination-free [22,23], which opens up avenues for their successful use in biological systems in vitro and in vivo [24,25]. We recently elaborated the technique of femtosecond (fs) laser ablation in water and organic media, which makes efficient control over the size characteristics of NPs from a variety of materials possible including plasmonic Au [26,27] and TiN NPs [28,29]. In fact, capable of supporting collective oscillations of free electrons (surface plasmons), such plasmonic nanostructures can offer a number of unique properties, including strong resonant scattering and absorption, and dramatic near-field enhancement, which makes them very promising candidates for a plethora of biomedical applications. For instance, having a strong and broad plasmonic peak around 640–700 nm with a significant tail over 800 nm, even for small NPs sizes (<7 nm), bare laser-synthesized TiN NPs seem to be an extremely promising functional element for hybrid nanofiber platforms. As shown in recent studies, TiN NPs have very low toxicity in vitro and in vivo, as well as initiating a strong photothermal therapeutic effect under near-infrared laser irradiation in the region of relative tissue transparency [28,29]. Au NPs are also of great interest as they can provide biodentification channel based on Surface Enhanced Raman Scattering (SERS) [26]. Such functional properties of laser-synthesized plasmonic nanomaterials promise their successful applications as additives in polymer nanofiber platforms for tissue engineering.

This mini-review describes our recent research findings on the incorporation of Au and TiN NPs as functional additives in electrospun nanofibers based on biocompatible and biodegradable polymer electropun-nanofibers.

Experimental methods
For the fabrication of Au and TiN NPs, we used methods of femtosecond laser fragmentation in water and organic ambient, which were earlier developed in [26-29]. A target of gold or titanium nitride (99.99%, GoodFellow, Cambridge, UK) was placed at the bottom of the glass vessel filled with deionized water (18.2 MΩcm). A beam from a Yb:KGW laser (Amplitude Systems, 1025 nm, 480 fs, 1 kHz) was focused on the surface of the target, while the target was moved constantly in the focusing plane to minimize ablation from the same area on the target surface. A high-resolution transmission electron microscopy (HR-TEM) system (JEOL JEM 3010) was used in imaging and diffraction modes in order to examine size distributions of formed NPs and their crystalline structure.

For the fabrication of nanofibers, we used an electrospinning system from IME Technologies, WG Waalre, Netherlands. The flow rate for all solutions was fixed at 0.2 or 0.3 mL h⁻¹. The collector was covered in aluminum foil and rotated at 2500 rpm. The voltages applied to the spinneret and the collector were fixed at 10 kV and −2 kV, respectively. The electrospinning process was conducted at 18 °C, in 80% relative humidity.

A DSM 982 Gemini Zeiss system (Zeiss, Jena, Germany) was used to obtain high magnification micrographs after the functionalization process. A FTIR/ATR technique was applied to establish specific vibrational frequency of pristine nanofibers and its changes due to functionalization.

Experimental results
The hybrid electrospun nanofibers functionalized with Au and TiN NPs were tested with biocompatible and biodegradable polymers based on chitosan poly(ethylene oxide) (PEO) [30,31] and polycabrolactone [32] formulations, respectively. Chitosan is one of the most exploited polymers in tissue engineering applications due to its biocompatibility, biodegradability and bioactivity (anti-microbial, scar reduction, wound healing, etc.). Furthermore, the presence of amino groups on chitosan surface can enable interactions with a variety of biological species (e.g. lipids, proteins, DNA, etc.), making these nanofibers promising host structure for tissue engineering. As a first approach, we functionalized biologically-derived polymer based on nanofibers chitosan(PEO) by Au NPs [33,34].

Owing to excitations of plasmons, electric field is strongly enhanced in the vicinity of metal surface, which can be used in various applications, including biosensing, imaging, photothermal therapy, gene
and drug delivery. Such NPs formulations thus look very promising as functional additives. Fig. 1a shows typical transmission electron microscopy image of laser-synthesized Au NPs and their corresponding size distribution. One can see that the NPs were spherical in shape and had the mean size of 40 nm. It should be noted that solutions of Au NPs in deionized water had exceptional stability due to electrostatic effect arising as a result of negative charge of Au NPs [22].

![Typical HR-TEM images of Au NPs (a) with corresponding SEM of hybrid chitosan(PEO) nanofibers prepared with different 30 wt% of Au NPs (b) Adapted from [33].](image)

Figure 1: Typical HR-TEM images of Au NPs (a) with corresponding SEM of hybrid chitosan(PEO) nanofibers prepared with different 30 wt% of Au NPs (b) Adapted from [33].

At optimized chitosan:PEO ratio, the Au NPs were thus tested in the polymer solution for electrospinning. Physicochemical analyses were then conducted on obtained nanofibers based on microscopic, thermal and analytical methods. Fig. 1b presents a Scanning Electron Microscopy image of chitosan-based nanofibers decorated by laser-synthesized Au NPs. One can see that the Au NPs were properly attached via electrostatic interaction and homogenously dispersed on the nanofiber surface, while the presence of Au NPs did not affect the morphology of fiber networks and their chemical properties. Besides, we observed a slight reduction of the fiber diameter, compared to electrospun nanofibers without nanoparticles, which was explained by the effect of NPs physico-chemical characteristics of electrospinning process, while the mean size of nanofibers was 190 nm. In addition, as follows from thermogravimetric (TGA) analysis shown in Fig. 2a, functionalized nanofibers exhibited better thermal stability at higher temperatures promising an improved bioactive performance and sensitivity toward temperature, which could be exploited for therapeutic applications based on hyperthermia of cells and tissues. We also studies safety of nanofibers functionalized by Au NPs using MTT tests. As shown in Fig. 2b, we did not reveal any sign of substantial toxicity.

![TGA thermogram curves of chitosan(PEO) nanofibers functionalized by Au NPs at different percentage; (b) Dependence of viability of HaCaT cells on concentration of hybrid nanofibers loaded with 30 wt% of AuNPs (blue curve). Adapted from ref [33]](image)

Figure 2: (a) TGA thermogram curves of chitosan(PEO) nanofibers functionalized by Au NPs at different percentage; (b) Dependence of viability of HaCaT cells on concentration of hybrid nanofibers loaded with 30 wt% of AuNPs (blue curve). Adapted from ref [33]
Figure 3: Typical HR-TEM images of TiN NPs (a) with corresponding SEM of hybrid PCL nanofibers prepared with TiN NPs (b) [21].

Figure 4: Metabolic activity measured using the MTS assay (A), proliferation using dsDNA assay (B), and viability using live/dead assay (C) for 3T3 fibroblasts immobilized of nanofibers with different concentrations of TiN NPs. Tissue culture plastic (TCP) was chosen as a reference to provide the highest absorbance in MTS tests. * means statistical difference related to all other samples. No significant differences were observed in either cell proliferation or cell viability tests. All assays show results as a mean and standard deviation. Adapted from [21].

In the second part of our study, we used nanofibers structures based on polycabrolactone (PCL) [35]. Based on the optimized parameters of PCL solutions, we then performed electrospinning together with laser-synthesized TiN NPs, which have strong and broad plasmonic peak around 640–700 nm even for small NPs sizes (<7 nm) and can be relevant additives for therapeutic in tissue engineering. Fig. 3a shows typical transmission electron microscopy image of laser-synthesized TiN NPs and their corresponding size distribution. As shown in the figure, TiN NPs were spherical in shape and had the
mean size of 25 nm. Similarly to Au counterparts, solutions of these nanoparticles are stable due to electrostatic repulsion effect of negatively charged NPs [28,29]. As shown in Fig. 3b, the co-electrospinning of PCL together with TiN NPs led to the production of PCL nanofibers decorated with TiN. It is interesting that the mean thickness of the nanofibers increased when the nanoparticles were used in the electrospinning. Indeed, mean diameter increased from 400 nm ± 210 nm to 1.1 µm ± 192 nm, while the diameters of the formed nanofibers did not depend on the concentration of TiN NPs.

The potential of hybrid nanofibers in biomedical applications was further examined by biological assessment. As shown in Fig. 4, TiN NPs-decorated nanofibers demonstrated good biocompatibility with increased metabolic activity on day 10 and the highest increase of dsDNA content on day 15. The obtained results evidence that novel hybrid matrices based on PCL nanofibers and TiN NPs can serve as excellent candidates for tissue engineering, drug delivery agents and cancer theranostics.

Conclusion:
In conclusion, demonstrated the possibility of fabrication of polymer nanofibers decorated by ligand-free plasmonic Au and TiN nanoparticles by co-electrospinning different polymers (chitosan(PEO), polycabrolactone (PCL)) together with Au and TiN nanoparticles prepared by methods of femtosecond laser ablation in liquid ambient (deionized water, acetone). The inclusion of nanoparticles in the electrospinning process could affect the final thickness of the nanofibers. The nanofibers functionalized by nanoparticles demonstrated stability of mechanical parameters and the improvement of thermal characteristics. The incorporation of Au and TiN NPs did not lead to any significant toxicity effects, as it was concluded from cellular studies. The fabrication of polymer nanofibers functionalized with bare laser-synthesized NPs opens up novel avenues toward the implementation of functional scaffolds for tissue engineering enabling advanced biomedical modalities. The employment of laser-synthesized NPs as functional modules for tissue engineering is still in a very early stage. Intensive research is still required to assess all potential benefits from such nano-engineered systems.

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Conflict of Interests
The authors declare no conflict of interest.

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