Sprayed Microgels onto 2D and 3D scaffolds as drug eluting coatings

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ABSTRACT

Big advances are being achieved in the design of new implantable devices. Coated scaffolds capable of releasing bioactive agents for inhibiting totally or partially the inflammatory response of the surrounding tissues, are now being regarded as potential useful systems.

Polymeric nanoparticles are known to be able to provide a programmable and sustained local drug delivery [1-3]. In addition, biodegradable and biocompatible scaffolds having a highly open porous structure and good mechanical strength are needed to provide an optimal microenvironment for cell proliferation, migration, and differentiation, and guidance for cellular in-growth from host tissue [4].

This study describes preliminary results on a novel coating method using spraying techniques for coating micro-nanometric crosslinked hydrogels on 3D biodegradable scaffolds.

Keywords: spraying, microgel, scaffolds, drug eluting devices

1 INTRODUCTION

The first objective of this work was to coat different substrates with nanometric crosslinked hydrogels and study their spatial distribution along these supports. Furthermore, it has also been studied the unique properties of these particles to arrange themselves spontaneously in two dimensions toward regular patterns in a size-specific way.

1.1 Spray coating technique

There are several coating techniques used in the field of material science for modifying the surface to improve its tribological characteristics: (i) physical and chemical vapour deposition, (ii) ion implantation, (iii) surface welding, (iv) thermal spraying, (v) laser glazing and alloying, (vi) friction surfacing, (vii) explosive cladding, (viii) electroplating [5].

The spray coating technique is a fast and easy coating technique. Spray coating technique was developed to overcome challenges that other coating techniques could not solve, such as spin coating and electrodeposition. Spray coating works properly on surfaces with different topographies. It consumes less material than other techniques and it can be used to coat irregularly shaped substrates. It can also coat many substrates simultaneously, deposit a protective coating on top of fragile structures and perform underfill steps [6]. Moreover, it can be use as well, for conductive or insulating materials [7]. Spray coating technique is used in different applications and to coat different materials: 3D microstructures [8], wood [9], and metallic surfaces [10].

In the spray coating technique there is a substrate that wheels and a solution or dispersion is sprayed on its surface. It is important the choice of the solvent in order to obtain a good dispersion. The spray coat technique requires a solvent: (i) which does not dissolve the substrate where it is going to be sprayed, (ii) with low density to allow it to be sprayed, (iii) with low boiling point to facilitate a fast evaporation.

The purpose of the present study is the analysis of the spatial distribution of sprayed sensitive microgels on different surfaces: glass, 2D and 3D biodegradable polymeric scaffolds.

In future works is aimed to study the use of 3D biodegradable scaffolds combined with nanometric hydrogels as transporters and releasing agents of several types of biomolecules.

1.2 Microgels

Microgel or nanogel particles (depending on the source) are defined as intramolecularly crosslinked macromolecular networks ranging in size from 10 to 1000 nm. They form colloidally stable dispersions instead of solutions because of their porous structure, crosslinked nature and small size [11].

A previous work investigated their two dimensional packing structures [12]. This work showed that microgels show minor tendency towards aggregation in solution, the ordered structure formation essentially takes place during evaporation of the solvent and the resulting high microgel concentration. They arrange in an unique self-organization process, in which the rate at which the solvent evaporates, the differences in the particle sizes and polydispersity are the main driving forces. It was found that the size of the aggregates is dependent on the polydispersity of the samples

Another characteristic of microgels is their high surface-volume ratio, which is responsible for their fast swelling-deswelling properties, making them more sensitive to changes in environmental conditions than macroscopic

hydrogels, solving the problem of slow response to external changes [13].

Due to their fast swelling-deswelling response, three-dimensional structure and small size, nanometric gels can be used as effective controlled release systems.

The design of new synthetic devices with enhanced stimuli-responsive sensitivity, specific ligands and functional groups is a promising field for the development of specific delivery systems. One of the pathways to achieve this aim is the use of functionalized nanodevices such as microgels. For example, T-sensitive (NIPAM) or pH-sensitivite functional groups (AA) can increase the selectivity of the targeting devices for specific applications. The new devices would lead to a reduction in the minimum effective dose of the drug required for each target [14].

1.3 Polymeric scaffolds

Biodegradable polymeric scaffolds for tissue engineering have received much attention since they provide a temporal and spatial environment for cellular growth and tissue in-growth. An ideal polymeric scaffold requires several structural and chemical features: (i) a three-dimensional architecture with a desired volume, shape, and mechanical strength, (ii) a highly porous and well interconnected open pore structure to allow high cell seeding density and tissue in-growth, (iii) chemical composition such that its surface and degradation products are biocompatible causing minimal immune or inflammatory responses and (iv) their degradation rate finely tuned in a pattern that it provides sufficient support until the full re-growth of impaired tissues[4].

Polymeric scaffolds can be designed to function more actively in tissue remodelling and regeneration by incorporating bioactive factors such as growth factors. Local and sustained delivery of cell proliferation, survival, migration and/or differentiation, may greatly enhance tissue remodelling. These factors can be incorporated into the scaffold matrix by bulk encapsulation, specific or non-specific surface adsorption, or by adding microparticles encapsulating them [4].

In this study the scaffolds have been coated with microgels by spraying technique. This system will be able to release or carry several types of biomolecules such as drugs, vitamins, growth factors, genes or enzymes.

2 MATERIALS AND METHODS

2.1 Materials

Microgels preparation

Microgels were prepared by precipitation polymerization in water using NMBA (N,N´-methylene-bis-acrylamide) as crosslinker and KPS (potassium persulfate) as initiator. The reaction conditions and microgel characterization are described elsewhere [15].

They were composed of N-Isopropyl acrylamide (NIPAM) and acrylic acid (AA).

The copolymer composition was checked by ^{1}H-NMR. Particle size distributions were measured by Quasy-Elastic Light Scattering (QELS): particle sizes of 450 ± 12 nm were obtained from Microgel dispersions swollen in water. SEM confirmed that Microgel morphology was spherical .

Scaffolds preparation

2D scaffolds preparation

2D scaffolds were prepared by solvent casting. The poly(L-lactic) acid (PLLA) polymer was dissolved in trichloromethane under vigorous stirring. The solvent was evaporated off and the remaining solid was heated until 200º C for 1 h. In order to obtain a flat and non porous substrate pressure is made on the solid.

3D scaffolds preparation

Scaffolds were fabricated by the TIPS process. The poly(L-lactic) acid (PLLA) polymer (8,10 or 15wt%) were dissolved in an 87/13 (v/v) mixture of 1,4-dioxane/ water by warming at 60ºC. The clear polymer solution was quenched at -16ºC to create a two-phase solid [16, 17].

The solidified solvent was removed by sublimation leaving a porous polymer scaffold.

2.2 Methods

Dispersion

The solvents used to prepare the microgel dispersions were used as received. The selection of the solvent is very important. It should provide a good dispersion of the microgels. In this case a good dispersion would be a transparent one, this status is sign of good affinity between the solvent and the microgel, where the gel is swollen in the dispersion. On the other hand, if the dispersion is cloudy it means that the microgel is in a deswollen status due to its low affinity with the solvent.

Spraying process

The spray coater machine used was an EVG 101. The spray coating technique is similar to the spin-coating method. There is a substrate that wheels where a solution or dispersion is sprayed. Through this technique is easy to reach a valley and a hidden zone of porous and rough substrate.

3 EXPERIMENTAL PART

Dispersion

The study was made in a dispersion of < 0.8 mg µgel / ml of solvent. The dispersion was left 2 minutes sonicating in an ultrasound bath and the status of the dispersion was checked. The change of the pH was performed by adding NaOH and/or HCl. The swelling/deswelling status of the dispersion was characterised by visual technique.

Spraying process

Dispersions at different concentrations were prepared. These dispersions were sprayed onto different substrates: glass, 2D scaffolds and 3D scaffolds.
The spraying parameters used were: speed 60rpm; acceleration 500 rpm/s; height 3100000; route edge to edge; sprayed times.

4 RESULTS

Dispersions

The best solvent selected for NIPAM/AA was ethanol. The resulting dispersions were transparent and stable. Moreover, ethanol is a good solvent to be used for the spraying technique because it has low density and high evaporation rate.

Spraying process

The spray coating technique gives the chance to spray microgels dispersion onto glass, 2D PLLA scaffolds and 3D PlLLA scaffolds. Figure 1 shows the characteristic spherical morphology of the microgels, it can be observed in Figure 2 that this is maintained after the spraying process. Figure 2a shows the coating formed by sprayed microgels onto the surface of glass. The microgels organize themselves forming small clusters in coexistence with isolated microgels. In Figure 2b the microgels have been sprayed three times, and more layers are observed. Finally, Figure 2c shows the microgels when they are sprayed at high concentration.

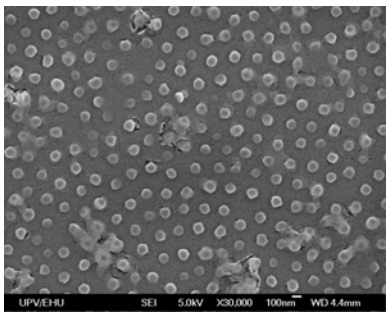
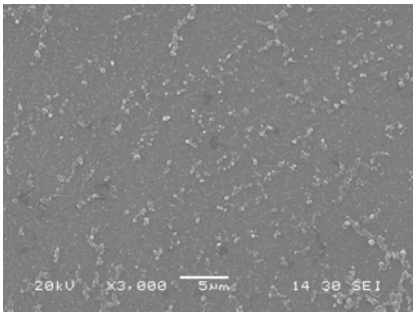
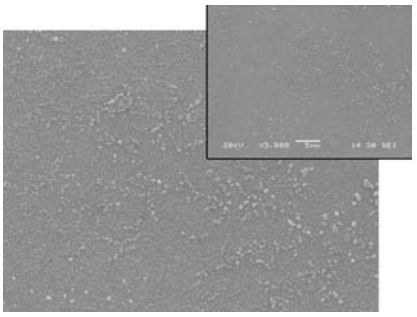


Figure 1. SEM picture of microgels before spraying

(a)



(b)



(c)

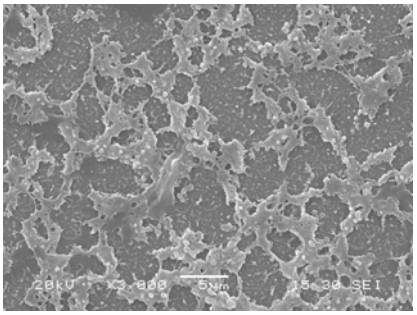


Figure 2. SEM pictures of microgels sprayed onto glass substrate : (a) sprayed one time, (b) sprayed three times. (c) sprayed at high concentration.

5 DISCUSSION

Spraying process

The microgels organize themselves forming clusters. The coupled size of the clusters depends on the polydispersity: the higher the polydispersity the smaller the clusters [12]. Figure 2a shows samples composed of larger ordered clusters in coexistence with some isolated microgels which obviously did not find their way to condense into the cluster.

In Figure 2b several layers of microgels one on top of the other, can be observed. This fact is not visible in Figure 2a. This is a consequence of how many spraying-stages have been used. The first layer arranges in an ordered structure, while the following deposit over it. It is important to ensure a proper drying of the solvent between the spraying stages, to obtain a correct deposition of the layers.

Figures 2a and 2c proves that higher concentrations of microgels in the evaporating solvent lead to larger structures [12]. When highly concentrated dispersions are sprayed formation of larger aggregates is observed and they trend to arrange in an ordered structure over the substrate. Due to the high concentration of the microgels in the dispersion, the evaporation of the solvent is difficulted, for this reason, the drying process is important.

For 2D scaffolds a good loading of the sprayed dispersion is reached. The microgels coating is not so good as in glass substrate because the PLLA has some roughness that causes inhomogeneities in the microgels layer.

In the case of 3D scaffolds it is also possible to spray microgels dispersions onto them. However, because of the porous structure of the scaffold the microgels distribution is not homogeneous, they are more visible in the surface of the scaffold than inside it. The interconnected structure of the foam difficulties the gel infiltration into the inner side of the scaffold.

6 CONCLUSIONS

The choice of an adequate solvent for a specific type of microgels lead to stable microgel dispersions that can be applied in the spray coating technique. This process is a good challenge to load 3D scaffolds with nanometric crosslinked hydrogels. The new system will play an important role as a bioactive molecule carrier and as a device for controlled release, that can be very useful for tissue engineering and regenerative medicine applications.

In future works the technique of the spray coating of microgels onto several polymeric scaffolds will be improved.

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