RADIATION-ENGINEERED FUNCTIONALIZED NANOGELS AS PLATFORM FOR BIOMEDICAL NANOCARRIERS AND BIO-HYBRID, HIERARCHICALLY ASSEMBLED NANOSTRUCTURES

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Summary

Radiation technologies can be considered as choice methodologies for the creation of new functional materials at the nanoscale, the challenge being now the integration of these and other novel nanomaterials into new materials and products. The possibility of generating nanoscalar PVP-based hydrogels particles, with reactive functional groups for subsequent bioconjugation, using industrial type accelerators has been demonstrated. These functional nanoparticles are under evaluation as nanocarriers for targeted release of drugs, but can also be considered as useful building blocks for the assembly of nanostructured materials with controlled architecture. In particular, molecular recognition strategies can be developed to tailor the structural and functional properties of the composite by attaching complementary sequences of molecules from biological source (peptides or oligonucleotides) that will tie nanoparticles together.

Under the present CRP, biodegradable nanoparticles will be developed using xyloglucan, a relatively inexpensive polysaccharide as base material, in alternative to PVP. Chemical modification of xyloglucan will be attempted with the purpose of generating radiation cleavable crosslinked micro/nanoparticles. These micro/nanoparticles will incorporate stabilizers (antioxidants, such as quercetin) or pro-degrading agents (enzymes) and will be either dispersed into a biodegradable film forming polymer or self-assembled to form a supramolecular networked film or scaffold. For the purpose, suitable surface modification will be pursued either to promote compatibilisation with the matrix polymer or to efficiently drive the self-assembly process. UV or quantum beam irradiation will be investigated as trigger for the release of the entrapped actives from micro/nanoparticles.

1. Introduction

Hydrogels have been often proposed as matrices for incorporation and controlled release of drugs, owing to their benign toxicological profile, soft and rubbery consistency, tailored chemical and physical properties. Opposite features can be conferred by design: inertness or stimuli responsiveness; absence of tackiness or enhanced skin or muco-adhesive properties; or combined, such as high permeability to water-loving solutes and the presence of hydrophobic pockets to host lipophilic drugs. Further opportunities are offered by the control of size and shape of hydrogels down to the nanoscale. Nanogels, or nanoscalar water swollen networks, offer unique advantages over other micro and nanoscalar delivery systems stemming from the their water swollen networked structure, including a large and flexible surface for bio-conjugation with targeting moieties; the possibility to entrap large, even macromolecular drugs or to embed active metal or mineral cores for imaging or phototherapeutic purposes.[1,2] Moreover, conformability and flexibility make these nanoparticles able to penetrate through small pores and channels through shape modification. Major synthetic strategies for the preparation of nanogels belong either to micro-fabrication methodologies (photolithography, microfluidic, micromoulding) or to self-assembly approaches that exploit ionic, hydrophobic or covalent interactions.[3] The availability of inexpensive and robust preparation methodologies is at the basis of the development of effective nanogel-based theragnostic devices.

It has been already established that nanogels can be produced under high-dose pulse irradiation of dilute aqueous solutions of water soluble polymers.[4] In these conditions polymer macroradicals form and recombine mainly within the same polymer coil, i.e. intra-molecularly. As a result of this process, internally crosslinked single macromolecules are formed.

First aim of our research is the assessment of structure-functionality preservation of designed nanogels with novel or the pre-defined properties and behavior, when translated into scalable industrial systems related to their applications.

2. Results and discussion

We have assessed the possibility of generating biocompatible nanogels with controlled dimensions in a range of tens up to hundred of nanometers and relatively narrow particle size distributions, using existing industrial-type electron accelerators and set-ups. Poly(N-vinyl-pyrrolidone) has been chosen as base material for its known propensity to crosslink under irradiation in water and because it is favorably accepted in pharmaceutical and cosmetic formulations. A functional monomer, specifically amino propyl methacrylamide (APMAM) was added to the polymer aqueous solution before irradiation, at different concentrations (see Fig. 1).

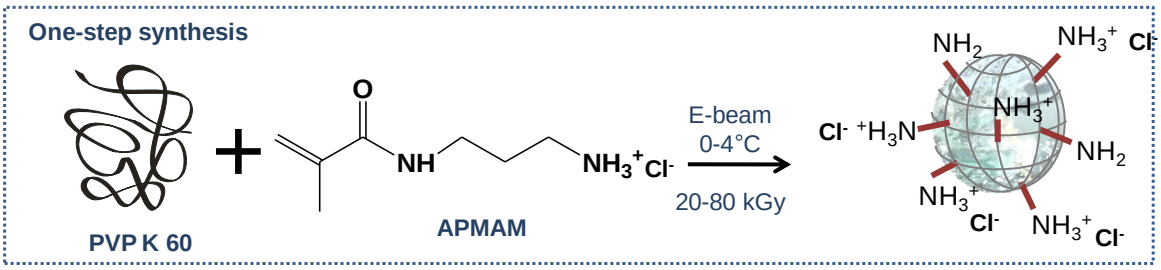

Fig. 1. One-step synthesis of amino functionalized PVP nanogel via e-beam irradiation. Both PVP and APMAM are present in the irradiated solution.

Radiation crosslinking of crosslinkable polymer solutions fixes a specific polymer conformation, depending on the chosen irradiation conditions, thus conferring to the formed particles shape and functionality in virtue of the specific polymeric chemical structure and degree and density of T or X bonds in the network. Varying the irradiation conditions (polymer concentration in water, pH, irradiation dose and dose-rate) at controlled temperature (0-4°C), it is possible to control the size and size distribution of the micro-/nanoparticles formed.

In table 1, the median hydrodynamic diameters (D_h) is reported for base PVP systems at the variance of polymer concentration in water (0.25-0.1-0.05 % wt.), dose rate (lower for L40-100 and higher for E40) and integrated doses (lower for E40 and higher for E80). D_h values were calculated from dynamic light scattering experiments (DLS) at controlled temperature, pH, ionic strength, dilution and upon a standardized filtration procedure (0.22 μm pore size filters) to eliminate dust and other possible contaminants.

TABLE 3. MEDIAN HYDRODYNAMIC DIAMETERS (DH) AND STANDARD DEVIATION OF BASE PVP AQUEOUS DISPERSIONS FROM DLS AT 25°C

System (PVP % in water)	Irradiation conditions	D_h±Std. dev. (nm)
0.25	L40kGy-100KGy/h	99.8±28
0.1	L40kGy-100KGy/h	25.4±8.8
0.05	L40kGy-100KGy/h	18.3±8.7
0.25	E40	42.2±13.6
0.25	E80	43.3±12.9

It can be observed that both decreasing the concentration of polymer in water and increasing the dose rate the particle size of the produced nanogels significantly decrease. Conversely no effects in the particle size distribution are observed by an increase of irradiation dose.

For comparative purposes we have also synthesized structurally "equivalent" nanogels through thermally activated inverse microemulsion free-radical polymerization and crosslinking, starting from vinyl pyrrolidone monomer (VP) and methylene bisacrylamide (MBA) as crosslinking agent. This process suffers for lack of robustness and need of laborious purification procedures for surfactant removal. Moreover, in order to improve dimensional control and yields both the reaction initiator system and the purification protocols need to be adjusted at the variance of the monomer ratio in the reactor feed (e.g. in the presence of ionic monomers).

In tables 2 and 3, the hydrodynamic diameters for two sets of "equivalent" nanogels, one generated starting from the monomers in w/o inverse microemulsion at 37°C and the other with an electron accelerator are presented. For the chemically synthesized nanogels, when reaction conditions and purification procedures are optimized for one formulation, e.g. for VP:APMAM with an intermediate value of the ratio between the two monomers (medium), by both increasing or decreasing this ratio, the control of particle size distribution becomes worse. Conversely, PVP-graft-APMAM systems produced via e-beam irradiation show good control of particle size distribution varying the ratio between repetitive units of PVP and APMAN, until the concentration of the APMAM becomes too high and this is likely due to association, driven by electrostatic interaction and leading to covalent intermolecular crosslinking, between cationic APMAM and slightly anionic PVP nanogels.

TABLE 4. MEDIAN HYDRODYNAMIC DIAMETERS (DH) AND STANDARD DEVIATION OF VP/MBA/APMAM NANOGELS PRODUCED VIA INVERSE MICROEMULSION FREE-RADICAL POLYMERISATION AT 37°C.

System Mol(VP):mol (APMAM)	D_h±Std. dev. (nm)
high	284±200
medium	250±24
low	200±58

TABLE 5. MEDIAN HYDRODYNAMIC DIAMETERS (DH) AND STANDARD DEVIATION OF PVP-GRAFT-APMAM NANOGELS PRODUCED VIA E-BEAM IRRADIATION (E80).

System Mol(PVP RU):mol (APMAM)	D_h±Std. dev. (nm)
high	53±31
high 0,22 μm filtered	32±8
medium	52±27
medium 0,22 μm filtered	36±8
low	1270±1224
low 0,22 μm filtered	No signal

Efforts have been also paid to the characterization of nanogel internal structure. In particular, the study of the profile of the scattered light as a function of scattering angle I(q) from static light scattering measurements is particularly useful to obtain information on the characteristic fractal dimension (d_f) of the network, when particle size is sufficiently big (approx. D>100 nm). In particular, for mass fractals, d_f, is indicative of the "openness" of the fractal structure and it can vary from 1, for a rod-like structure, to 3 for a compact spherical particle. Nanogels presented values of fractal dimension varying in the range 1.8-2.2, thus suggesting an interconnected, but yet open structure, that is an important characteristic for drug delivery and tissue engineering applications.

Assessment of availability of nanogel functional groups to chemical attachment with targeting ligands has been performed using a fluorescent probe. Specifically, fluorescein isothiocyanate (FTIC) has been covalently reacted with the amino groups of the amino-functionalised nanogel variants in mild conditions and the same protocol has been applied to react the amino groups with bovine serum albumin (BSA) as model protein.

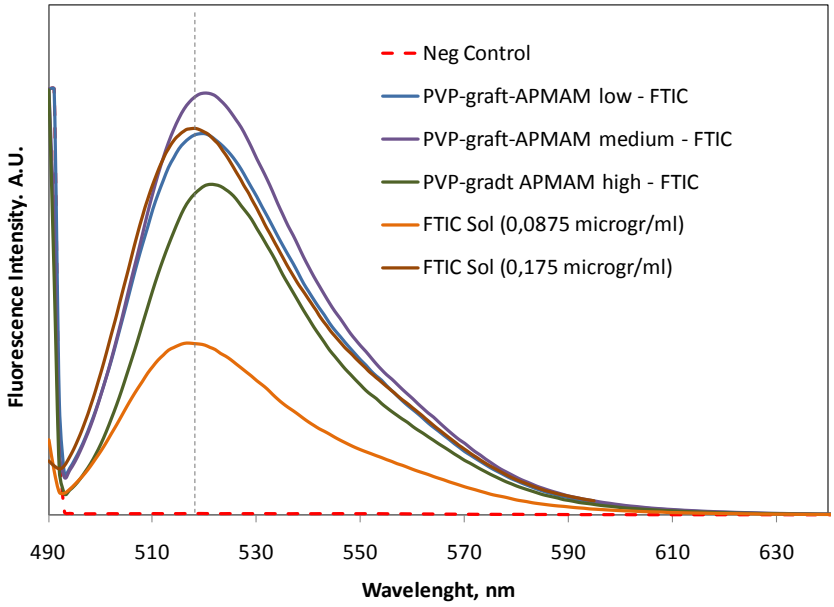

Fig. 2. Fluorescence emission from fluorescein isothiocynate (FTIC) solutions and fluorescein-conjugated PVP-graft-APMAM nanogels. Negative control is the PVP-graft-APMAM nanogel not conjugated. Temperature 25°C and □exc= 490 nm.

Figure 2 shows the fluorescence emission from fluorescein-conjugated PVP-graft-APMAM nanogels. Emission peak shift of the conjugated FTIC with respect to the free probe in aqueous solutions (FTIC sol) suggests strong interaction with the substrate.

Different in vitro intra-cellular localization, geno and cyto-toxicity studies have been performed on both the base and amino functionalized PVP nanogels. The results so far collected are very promising and support the development of these nanogels as nanocarriers for target release of therapeutic biomolecules or for specific cellular targeting in diagnostics. Additionally, these nanoparticles can be considered useful and relatively inexpensive nanoscalar building blocks for the bottom-up assembly of bio-hybrid, hierarchically assembled structures to be exploited in advanced drug delivery and regenerative medicine.

In alternative to PVP, bioresorbable polymers are considered and xyloglucan, in particular. Xyloglucans are a major class of structural polysaccharides found in the primary cell walls of higher plants. Xyloglucan skeleton is formed by β-(1,4) D-glucan, partially substituted by α-(1->6)-linked xylose units. Some of the xylose residues are β-D-galactosylated at the O-2 and some galactose unit can be fucosilated. [5]

The collapse of the transient water soluble network formed in aqueous solution by addition of an alcohol is a well known mechanism of gelation of certain biopolymers, such as gelatin by addition of a large amount of sugar alcohol. [6]

Xyloglucan from tamarind seed, that is a not fucosilated variant of xyloglucan, is approved for use as a food additive and it should not possess any significant toxicity in humans, therefore it has been evaluated as *in-situ* gelling polymer for generating drug delivery depots in vivo. Moreover, xyloglucan can be functionalized to form carboxylated, sufonated or aminated xyloglucans [7, 8] or reacted with RGD cell adhesion peptides, to enhance adhesion and proliferation of endothelial cells, when proposed for applications in which it gets in

contact with blood: such as catheters, blood bags, blood vessel replacements, membranes for dialysis, cardiac valves, etc. [9]

Xyloglucans are water soluble, but macromolecules tend not to be individually hydrated and dissolved in water. The balance between the hydrophilic and hydrophobic character and the chain stiffness of the cellulose-like backbone facilitate intermolecular interactions, among different chains and with solutes. Therefore, aggregation characteristics of diluted xyloglucan aqueous solutions are under investigation, in order to assess if micro/nanoparticles can form, incorporate and protect different kind of solutes.

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Work plan under the present CRP

Under the present CRP, biodegradable nanoparticles will be developed using xyloglucan, a relatively inexpensive polysaccharide as base material, in alternative to PVP.

During the first eighteen months of the project, the optimal conditions for the formation of xyloglucan micro/nanoaggregates from solutions will be investigated, varying the degree of degalactosylation, molecular weight, temperature, concentration and presence of co-solutes .

Chemical modification of xyloglucan with suitable groups will be attempted with the purpose of generating radiation–cleavable crosslinked micro/nanoparticles through the recourse to specific linkers and/or by irradiation.

These micro/nanoparticles will be used to incorporate stabilizers (natural antioxidants, such as quercetin) or pro-degrading agents (such as enzymes or metal oxide nanoparticles) and will be either dispersed into a biodegradable forming polymer, such as variants of xyloglucan or poly lactic acid, or self-assembled to form a supramolecular nanocomposite film or a polymeric scaffold.

UV or quantum beam irradiation will be considered as trigger for the preferential cleavage of crosslinks of micro-nanoparticles leading to degradation of the encapsulant material and the release of the entrapped actives in and through the biopolymer matrix.

Further development of this research will investigate degradation and/or bioresorption properties of these nanocomposite films or scaffolds.

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