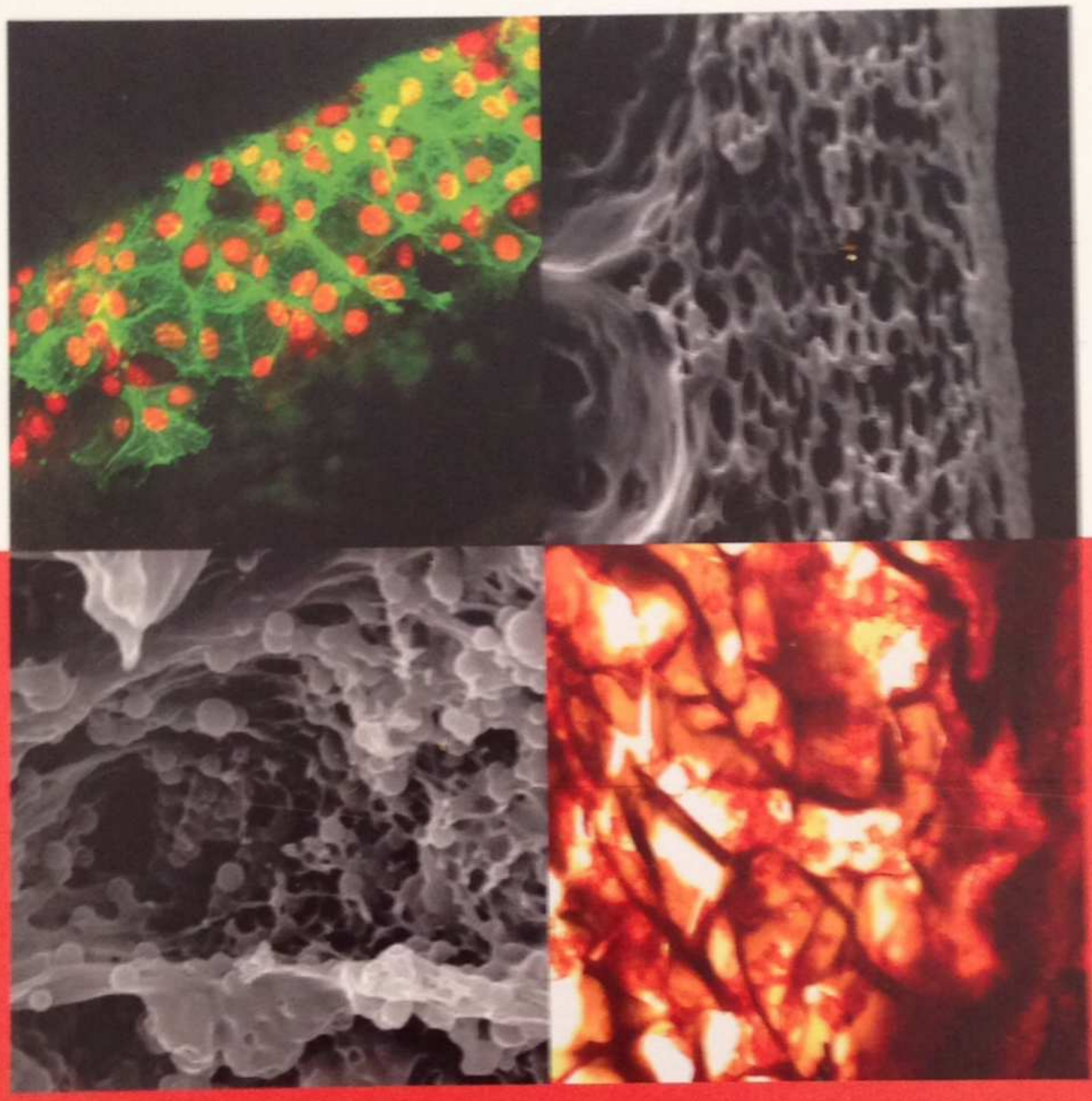
I MATERIALI BIOCOMPATIBILI PER LA MEDICINA

Convegno Nazionale della Società Italiana Biomateriali
Palermo, 2-4 luglio 2014



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WATER-BORNE, E-BEAM CROSSLINKED NANOGELS AS NANOMATERIALS PLATFORM FOR DRUG DELIVERY

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Introduction

The interest in nanotech applications for medicine is constantly growing as it holds the promise of radical improvements of current therapies. Several nanocarriers have been proposed in the literature, some of them have demonstrated highly successful in the application and are now being evaluated in clinical trials.[1] However, their road to the market is still long and difficult for several reasons, such as the huge gap between their *in vitro* properties and the *in vivo* behavior and the lack of "simple" and "clean" manufacturing processes for their production.[1] Newer promising nanocarriers have been developed in more recent times, which include nanogels.[2] In the present work, a very promising approach, based on e-beam radiation-induced radical crosslinking of a water soluble, biocompatible synthetic polymer has been developed for the generation of Poly-N-(Vinyl-Pyrrolidone) (PVP)-based nanogels. E-beam irradiation using industrial type accelerators demonstrated to be a viable manufacturing process for nanogels generation, since it grants high yields in terms of recovered product and high throughputs.[3-5] Moreover, through a proper selection of the experimental parameters, this approach allowed to obtain NGs with the desired properties, in terms of size, surface charge density, degree of crosslinking and functionality. It has been also demonstrated that all the NGs produced are biocompatible and able to be internalized by cells. Finally, the many functional groups grafted on the NGs have shown of being available for coupling reactions with bioactive molecules, such as targeting moieties, drugs and metal-ions chelating agents.

Materials and Methods

Chemicals: PVP k60 and acrylic acid (AA) were supplied by Aldrich and were used without further purification. (3-N-aminopropyl) methacrylamide hydrochloride (APMAM) was supplied by Polyscience.
PVP-based nanogels synthesis: PVP aqueous solutions at different concentrations (0.5, 0.25 and 0.1 wt%) have been prepared by overnight stirring, filtered with 0.22 μm pore size syringe filters and saturated with N_2O (N_2O ≥99.99%) prior to irradiation.[3-5] PVP aqueous solutions at 0.1 wt % with APMAM (1:100 and 1:50 APMAM/PVP's RU molar ratios) and PVP/AA aqueous solutions with two concentrations of PVP, 0.1 and 0.25 wt%, and a molar ratio between PVP's RU and AA equal to 50, have been prepared in the same conditions as described above. Amino-functionalized PVP nanogels have been coded as P*(0.1)APMAM(Y); carboxyl-functionalized nanogels have been coded as P*(X) AA(Y), where X is the polymer concentration in the feed and Y is the molar ratio between PVP's RU/monomer (APMAM or AA). Electron beam irradiation has been performed using two 10 MeV liner accelerators located at the ICHTJ of Warsaw (Poland), LAE 13/9 and Electronika 10/10. The irradiation set-ups are summarized in Table 1.

Accelerator	Elektronika 10/10	LAE
Frequency (Hz)	400	37.5
Pulse Length (μs)	5.5	10-12
Dose-rate (kGy/h)	13,000	100

Table 1. Summary of the irradiation conditions.

Characterizations: Hydrodynamic diameters (D_h) of NGs' dispersed in water were measured by dynamic light scattering (DLS).[3] DLS data were analyzed by the method of cumulants. Measurements were carried out on a minimum two samples from three independent runs.

Results and Discussion

The possibility of obtaining PVP-based NGs using high-energy radiation-induced radical crosslinking, as a reliable manufacturing approach, is explored. This synthetic route has been first applied for the generation of base PVP NGs and, then, further developed for the production of their functionalized variants. Since the main goal of these synthetic efforts has been the generation of a family of functional nanocarriers for drug delivery, the capability of the technology to grant particles dimensional control has been assessed. In Figure 1a-c the average hydrodynamic radii of the NGs obtained for three PVP concentrations (0.1, 0.25 and 0.5 wt%), at the variance of dose and dose-rate, is provided. Relative widths of nanoparticles size distributions are also reported as error bars. Two different dose-rates have been selected (100 kGy/h and 13,000 kGy/h) and each panel of Figure 1a-b refers to a different dose-rate. The total doses delivered to the materials have been 20, 40 and 80 kGy. The two lower doses are the upper and lower limits of the sterilization dose range. Samples have been irradiated also at twice the maximum sterilization dose, to investigate the effects of higher doses on the structure and properties of these polymer nanoparticles. Generally, e-beam crosslinked PVP NGs have shown particles size below 100 nm and not smaller than 20 nm. Furthermore, narrow particles size distributions have been obtained. Only for the 0.5 wt% systems, subjected to low lose-rate irradiation, particles size control has not been achieved. At all irradiation conditions, a strong influence of polymer concentration on particle size is evident.

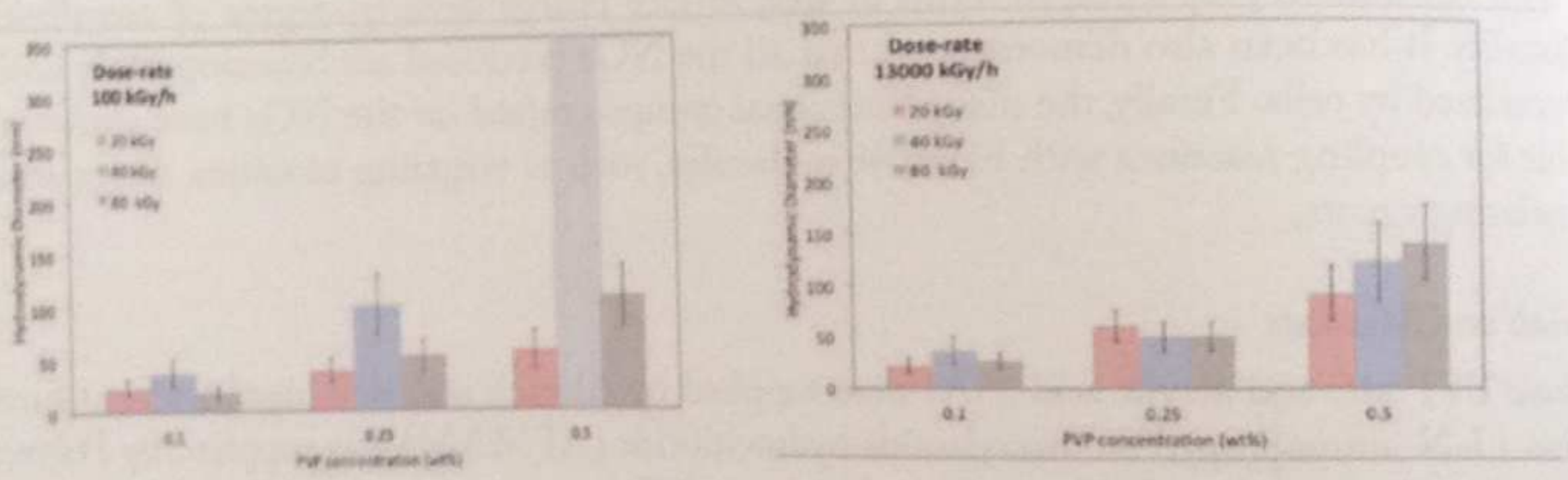

Fig. 1. Hydrodynamic radii of nanogels obtained for three PVP concentrations at the variance of dose, for dose- rates of a) 100 kGy/h; b) 13000 kGy/h.

In particular, at the increase of polymer concentration the hydrodynamic radius increases. Furthermore, both dose and dose-rate affect nanogels particles size, the more the higher is the polymer concentration. This study evidences one advantage of this synthetic route that is the possibility to fine-tune the size of the polymer nanoparticles by controlling - independently – the composition of the irradiated systems, the dose-rate and the total absorbed dose.

PVP has been also irradiated in the presence of monomer carrying functional groups. Table 2 reports the values of mean D_h for selected systems irradiated with Elektronika 10/10 at 40 kGy. In the chosen experimental conditions, APMAM has been successfully grafted on PVP, although when either the content of APMAM or the polymer concentration in water is too high the possibility of obtaining amino-functionalized PVP NGs with controlled particles size at the nanoscale is impaired by the attractive interactions occurring between the irradiated PVP (anionic) and APMAM (that is in the form of chlorine salt). These interactions promote nanoparticles aggregation and covalent bridging phenomena.[3] Conversely, when PVP is irradiated in the presence of AA, NGs dimensional control is always achieved and a variety of carboxyl-functionalized NGs have been generated by varying the concentration of polymer in solution.4 All PVP-based NGs have shown to be biocompatible and able t

be internalized by cells.[3] This collective evidence validates the generated nanostructures for the intent they have been designed for, i.e. as nanocarriers in the biomedical field. Functional groups grafted on PVP NGs have been then exploited for conjugation reactions with various (bio)molecules.[3,6,7] Furthermore, two different types of ligands have been efficiently conjugated on the same nanogel particle, proving that "multifunctional" nanodevices can be assembled. Finally, it has been proved that all the ligands conjugated to these NGs so far have preserved their biological activity upon conjugation. More in detail, folate-NG variants have been generated and shown the ability of being preferentially uptaken by tumor cells and that their uptake is faster than for bare NGs. Responsive NGs have been also generated by attaching a model drug through a linker with a redox-cleavable bond. In-vitro release studies have shown that these modified NGs quantitatively release the drug when the release is triggered by the presence of a reducing molecule. Moreover, in vitro intracellular release studies have demonstrated that the drug is not active when linked to the nanoparticle, while it exerts its action (cell-death) when it is released.[7] All considered, due to the great versatility showed, e-beam crosslinked PVP-based NGs can be regarded as a very promising "nanomaterials platform" for drug delivery. This research is further progressing with studies on relevant animal models.

System	Dh(nm)	Err (nm)
P*(0.1)AA(50)	14	5
P*(0.25)AA(50)	26	10
P*(0.1)APMAM(50)	1140	500
P*(0.1)APMAM(100)	33	12

Table 2. Mean hydrodynamic diameters (Dh) and their relative distribution widths for selected NGs.

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