**Monoclonal antibody controlled and targeted delivery: the atherosclerosis case study**

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**1.Introduction**

In the last years drug delivery systems have been deeply studied for the ability of preserving the payload activity and to control the release in order to maintain the drug concentration in the therapeutical window for prolonged time. The encapsulation of monoclonal antibody with a therapeutic activity represents an interesting new strategy that has been explored in the oncology fields, with encouraging results [1]. In this field, the external surface decoration of the drug delivery device can allow a targeted therapy with the main objective of decreasing the side effects since the drug is released directly in the pathological site.

For the controlled and targeted delivery of therapeutic monoclonal antibodies different materials have been tested for the micro and nanoparticles production such as synthetic polymers (i.e. poly (lactic-co-glycolic acid) (PLGA), poly (ε-capro-lactone) and poly (lactic acid)) [2], natural polymers (i.e. alginate [3] which is extracted from brown algae) and phosphatidylcholine (PC) for the production of liposomes [4].

In this project it will be described a comparative study of the preparation of different type of carrier which encapsulate a therapeutic monoclonal antibody Bevacizumab (BEV). It is a monoclonal antibody commercialized as Avastin® used to decrease the angiogenic correlated diseases [5]. In particular, this angiogenesis inhibitor has demonstrated to have positive effect on atherosclerotic plaques of various stages for which it demonstrated advanced inhibition of neovascularization. Therefore, a targeted delivery of this therapeutic protein directly on the target site is of great interest for cardiovascular field. The production of BEV loaded liposomes and PLGA micro and nanoparticles were optimized in order to obtain a good control over particles size distribution, suspension stability, high drug encapsulation efficiency and stability of entrapped therapeutic protein. Drug release kinetics were studied under simulated physiological conditions at 37 °C. Furthermore, the targeting of particles towards specific tissues was obtained via surface modification with the attachment of specific marker for angiogenesis.

**2. Methods**

To product polymeric particles, *water/oil/water* technique was used. Briefly, the PLGA was dissolved in ethylacetate until complete dissolution, then the aqueous inner phase was added (bevacizumab solution, at 25 mg/mL,or PBS solution for loaded or empty microparticles, respectively). The first w/o solution was obtained homogenising for 2 minutes (30 sec on-off) using a Vibra-Cellᵀᴹ ultrasonic probe with 60% of amplitude. After that, the first emulsion was added to 80 mL of ethyl acetate saturated water previously prepared. PVA was used as surfactant at 2% w/w in the aqueous solution. The secondary emulsion was obtained using a rotor-stator emulsifier (Silverson L5T) at 7000 rpm for 6 minutes. Finally, the obtained emulsion was left under magnetic stirring at 300 rpm in a fume hood to obtain the complete evaporation of the solvent. The obtained particles suspension was washed with Milli-Q water three times by centrifugation, 10.000 × g for 15 minutes to remove the PVA excess.

Liposomes were produced using the thin-film hydration method. Briefly, 200 mg of PC was dissolved in chloroform. Then, the organic solvent was removed using a rotary evaporator (model Laborota 4000, Heidolph, Schwabach, Germany). The aqueous solution (with Bevacizumab) was used to hydrate the obtained thin-film layer. The solution was left under magnetic stirring for 3 h at room temperature. Then, it was homogenized for 2 min using the same Vibra-Cell™ ultrasonic liquid processor reported above at the same conditions. The obtained liposome solution was then centrifuged at 12.984× g for 30 min at 4 °C three times and the pellet was washed with deionized water to remove the non-entrapped Bevacizumab. Liposome suspensions were stored at 4 °C after preparation. Empty nanoliposmes were also produced following the same procedure and used as control during the entire experimentation.

The functionalization with specific markers for angiogenesis was performed using the coupling reaction in the presence of carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS).

**3. Results and discussion**

Empty and loaded PLGA microparticles and nanoparticles and PC liposomes were produced and optimized.

Polymeric microparticles were produced used the double emulsions evaporation process, whereas liposomes were produced using the thin layer hydration method. In general polymeric particles allowed to achieve higher entrapment efficiency, 77.15%. Indeed, the method is based on the formation of a stable emulsion in which the drug is contained in the inner water phase of the emulsion. In the case of liposomes, instead, the drug is dissolved in the water medium used for the hydration of the lipid layer. As a consequence, only a part of the water used to hydrate the layer effectively goES inside the vesicles, resulting in a lower entrapment efficiency of 51.13%.

Regarding the size of the particles and liposome, it is possible to precisely tune the desired dimensions from the nanometric to the micrometric one. In the case of PLGA particles the conditions of emulsification are the most responsible of emulsion droplet dimensions and then particles diameters. In the case of liposomes, since the thin layer hydration method is a spontaneous process, micrometric liposomes are generally obtained. In order to reduce liposome dimensions to nanometric level a post processing step of sonication is required. Changing the intensity and the duration of the sonication treatment it is possible to obtain the desired particle size distribution.

Regarding particles morphology an example of FESEM image of produced PLGA microparticles is reported in Figure 1.

Immagine che contiene mucchio, frutta, diverso, gruppo

Descrizione generata automaticamente

Figure 1. FESEM image of PLGA loaded BEV microparticles

Very different drug release kinetic can be obtained from the drug delivery devices produced, as shown in Figure 2. Indeed, polymeric particles allow the obtainment of a sustained release of BEV over very long time of observation, instead, liposomes, under simulated drug delivery conditions, rapidly release the cargo. The principle of drug release at the basis of the carrier systems selected is very different. This allow a modulation of the drug release according to the therapeutic need.

Figure 2. Drug release kinetics at 37°C

The possibility to drive the encapsulated BEV towards selected tissues was achieved by immobilizing the markers for angiogenesis on the produced carriers. The effective immobilization was demonstrated by FTIR and by circular dichroism analysis. Furthermore, after all the production steps, it was verified that BEV was structurally in its active form, thus maintaining its biological properties. Cell viability and hemocompatibility with human endothelial cells and human red blood cells confirmed the compatibility of this innovative drug delivery system as next generation therapy for angiogenesis based diseases, as atherosclerosis.

**4. Conclusions**

In this work a complete study about the encapsulation and targeting of Bevacizumab using different drug carriers was presented. At the optimized conditions of production, the best carrier for the application was selected in the PLGA nanoparticles. This carrier allowed the higher entrapment efficiency, the best particles size distribution and particle stability, the possibility of efficient and oriented attachment on the surface of functionalizing molecules, able to make specific targeting to selected tissues.

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