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Use of Autologous Fibrin as a Substrate for *in vitro* Endothelialization of Small-Diameter Vascular Grafts

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It is well known that the replacement of arteries with purely synthetic vascular grafts often leads to the failure of such reconstruction when small-diameter (≤ 6 mm) or low-flow configurations are concerned, due to the thrombogenicity of the artificial blood contacting surface. One of the most promising approaches to improve long-term patency of these grafts is to cover the luminal surface with endothelial cells (ECs) that provide thromboresistence to circulating blood.

Because the synthetic vascular grafts placed in humans do not spontaneously form an ECs monolayer, their *in vitro* endothelialization is the only chance to obtain a thromboresistant surface.

Several studies showed that ECs do not adhere to the most commonly used materials, therefore the coating with commercial substances known to facilitate the cellular adhesion, such as fibronectin and fibrin, is necessary.

The aim of this work was to find optimal conditions for the *in vitro* endothelialization of synthetic small-diameter vascular grafts. In particular, a new type of coating to improve ECs attachment and growth onto the inner surface of microporous grafts was investigated. Unlike the other known coating procedures, the innovative idea was to employ patient own plasma to realise a fibrin coating of vascular grafts. The plasma was put in contact with the synthetic surface; as result of this contact the coagulation process begins and at the end of this process fibrinogen is converted in fibrin by thrombin, factor XIII and calcium. In this way, a resistant and homogeneous fibrin layer deposited on the surface.

The experiments were carried on polyurethane/silicone (PU/PDMS) vascular grafts of 5 mm internal diameter and 5 cm length, obtained by an instrument named *spray-machine*. The grafts manufacturing process is based on a physical principle applicable to polymer solutions known as *phase inversion*. Briefly, the pre-phase inversion polymer solution and distilled water (as non-solvent) are sprayed to intersect on a rotating mandrel through two spray-guns, which are mounted on a carriage. Where polymeric solution meats the non-solvent a sudden phase inversion of the material happen and the result is the deposition of microporous layers onto the mandrel.

The sterilized PU/PDMS grafts were fitted in a new developed pulsatile flow system, known as bioreactor. The bioreactor is a closed circuit constituted by four different units: a unit for rotation of the vascular graft, a unit for control of fluid dynamic parameters, a peristaltic pump for dynamic perfusion and a vascular-graft chamber. The chamber is a Plexiglas modular tank equipped with two adapters for the connection of the vascular graft with the perfusion system. The whole system is an isolated cell culture setting, which provides a high level of sterility, gas supply and fits into a standard humidified incubator.

The bioreactor allowed to realise the coating of graft, the seeding and the growth of ECs.

Before the ECs seeding, the re-calcified plasma was slowly circulated in the perfusion system at r.t., by the peristaltic pump, to obtain the fibrin coating of the microporous graft. After 1 hour of treatment, the entire surface of the graft showed, by light microscopy, an homogeneous fibrin layer that completely covered the underlying PU/PDMS material.

As control, a fibronectin coating was realised incubating the graft with human fibronectin solution (20 μg/ml) for 1 hour at 37°C in static condition.

The ECs suspension at density of $1x10^5$ cells per cm^2 was injected into the lumen of the grafts coated with plasma or fibronectin. Then, the graft had been slowly rotated for 4 hours to guarantee an even distribution of ECs on the luminal surface. Finally, the seeded graft was cultured for twenty-four hours in the bioreactor under flow of culture medium to supply nourishments to the cells and to remove the waste products.

At the end of incubation period, the adherent cells were stained with 0.1% Giemsa in methanol. Light microscopy observation showed that on the luminal surface of plasma coated vascular grafts the ECs appeared to be completely spread, showing their characteristic morphology and were at confluence after only 24 hours of incubation.

On fibronectin coated grafts cell adhesion and growth were lower than plasma coated grafts and the cells were at confluence only in some areas. The Alamar Blue assay confirmed the microscopical observations.

In conclusion, this study showed that the plasma coating improves the adhesion and growth of ECs into PU/PDMS vascular grafts, because the fibrin appears to fill micropores of the material providing sufficient cell attachment area.

Moreover this treatment has several advantages: 1) the synthetic surface is covered with a biological matrix of human origin, therefore it does not induce toxic effects on cells and tissues unlike the synthetic products; 2) it is economic, in fact a few millilitres of peripheral blood are enough to obtain the plasma amount to coat the PU/PDMS graft; 3) it is fast, only few steps are required to realise a complete coating; 4) it is safe, all matrix constituents have autologous origin, avoiding risk of viral transmission and antigenic reaction.

For the above mentioned reasons, this new coating procedure might constitute an interesting alternative to the adhesive commercial molecules and might be used clinically to allow the complete endothelialization of vascular grafts in order to obtain tissue-engineered medical devices.