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Plasminogen purification with affinity membranes for ophthalmology applications

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Some eye conditions like diabetic rethinopaty, macular pukers, retinal detachment may benefit from vitreoctomy. Enzymatic vitrectomy with plasmin is envisaged to augment or even replace conventional vitrectomy by proposed means of less surgical risks, less surgeon time, lower costs. In addition, the use of autologous plasmin is beneficial since avoid rejection problems and the search of compatible donors [1]. Plasmin has properties to hydrolize a variety of glycoproteins, including laminin and fibronectin, by degrading the links between these components of the vitreoretinal interface and the inner limiting membrane, therapeutic posterior vitreous detachment has become possible.

Plasmin can be obtained by conversion of plasminogen by a variety of enzymes, including tissue plasminogen activator and (tPA), urokinase plasminogen activator (uPA). The purification of plasminogen from blood is normally performed with bead-based affinity chromatography [2].

Due to the limitations of bead-based chromatography [3], affinity membranes are ideally suited for the development of a device to be used by the surgeon in the operating theatre. Membranes can be easily packed in a disposable device in a process which is fast and economic.

In this work we prepared affinity membranes for plasminogen purification using L-lysine as affinity ligand. To this aim regenerated cellulose membranes, Sartobind epoxy™, were used as a support for ligand immobilization. L-lysine was coupled by soaking the membranes in an aqueous solution of $Na_2B_4O_7$/NaOH and 1.4 dioxane at pH 10 and 80°C at different reaction times. Ligand density was measured using a colorimetric assay with Orange 7 as indicator. As it can be observed from Fig. 1, ligand density increases with time, but after about 54 hours of reaction the membranes started to loose their mechanical resistance and the reaction was interrupted.

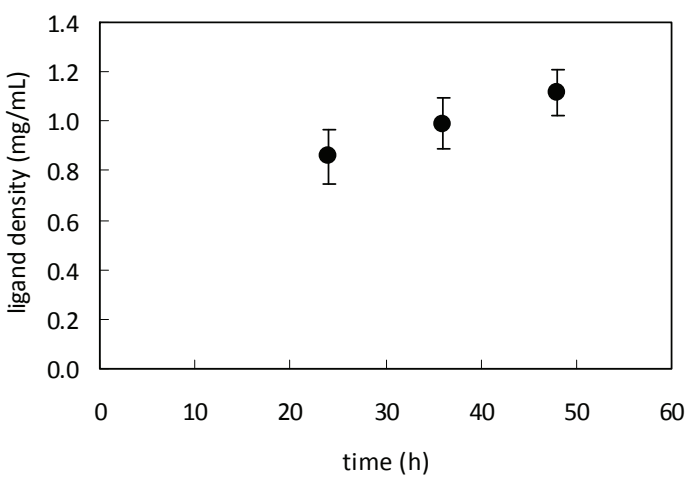

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Figure 1. Ligand density expressed as mg of immobilized lysine per mL of membrane support as a function of reaction time.

The membranes have been characterized in batch and in dynamic experiments using both pure plasminogen and human serum. Pure plasminogen was obtained, in house, by purification from human serum with a commercial affinity chromatography resin, Pall Lysine HyperD, packed in 1 mL chromatography column and used to characterise the affinity membranes.

During experiments with human serum, fractions have been collected in all chromatography steps, and analysed with both HPLC and SDS-PAGE electrophoresis. In particular from the HPLC SEC analysis of the eluate it can be shown a well defined plasminogen peak indicating that the lysine affinity membranes are suitable for the purification of plasminogen.

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