

DEVELOPMENT AND CHARACTERIZATION OF A NOVEL HIGHLY EXTENDABLE MEMBRANE FOR CELL CULTURE

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INTRODUCTION: Adherent cells tend to change their phenotype in standard culture conditions; this may be partly due to repeated passaging involving enzymes which destroy cell surface proteins. In order to avoid unnecessary passaging, it has been proposed to culture adherent cells on surfaces which can be controllably augmented [1]. By these means it is possible to maintain constant cell density while total cell number increases. However, current commercially available and biocompatible silicone elastomer membranes have limited extendibility of ~50%. In the present study we have developed a highly extendable membrane with a maximum extendibility of ~1000%. We further demonstrate that this novel surface is biocompatible, promotes cell attachment and proliferation and can be provided with a microtopology.

METHODS: The silicone elastomer A-221 (Factor II, Inc., USA) was selected as cell culture substrate for its transparency, nontoxicity, and capacity for elongation near 1000%. A-221 is supplied as a kit with two parts which were mixed in a 1:1 ratio by weight. After mixing and degassing under vacuum for 5 minutes, the mixture was poured onto flat open-faced molds, then cured at 100°C for 48 hours to produce elastomer membranes 0.5 mm thick. The mold with microstructure was fabricated on silicon and photoresist, we employed Bosch process² technique (Fig1).

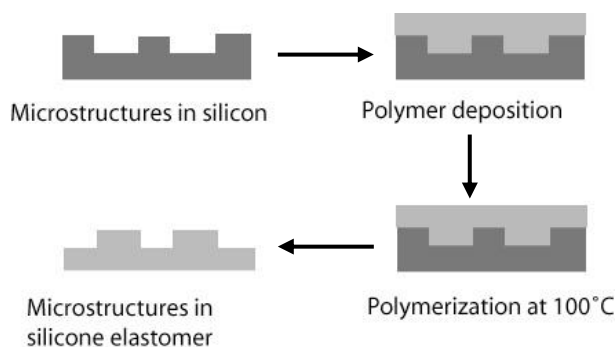


Fig. 1: Principle of membrane microstructuring

Elastomer culture surfaces were then exposed to an oxygen plasma (Tegal) for 45 s at 50 W under 300mTorr. Pilot experiments indicated that the resulting surface chemical activation improved cell attachment if membranes were stored in deionized

water after plasma exposure and prior to use in experiments.

Rat lung fibroblasts were seeded onto standard cell culture petri dishes and onto non-structured elastomer membranes at an initial density of 5,200 cells/cm². Cell growth was assessed after 1-10 days without passaging and without membrane extension.

RESULTS: Fibroblast morphology on highly extendable transparent elastomer membranes appeared identical to that on standard petri dishes throughout the culture period. Cell attachment and proliferation on non-surface treated elastomer membranes was satisfactory (although apparently somewhat reduced) in comparison to standard cell culture plastic (Fig 2a). With photolithography and etching technology we can develop different structure and design on silicone elastomer surface (Fig 2b).

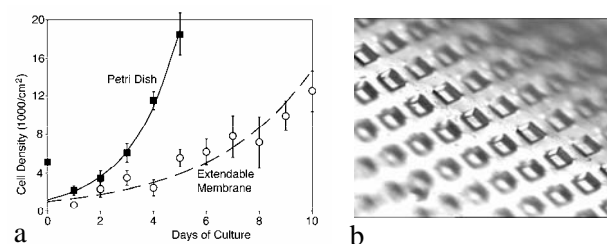


Fig. 2: a) Evolution of cell density on petri dishes versus non-structured extendable membranes (mean \pm sem, n=3)

b) Membrane with micro squares of 100µm x 100µm and 50µm depth

DISCUSSION & CONCLUSIONS: With no surface treatment, cell attachment and proliferation on highly extendable elastomer membranes was somewhat lower than on standard cell culture plastic, but nevertheless adequate for future experiments designed to explore effects of substrate stretch. In future we will exploit the combined use of microstructured surfaces topology with elongation to influence cell density and proliferation. Methods for improvement of cell attachment on silicone elastomer surfaces are currently under development.

REFERENCES: ¹Frei+, WO03020871, 2003, ²Hilbert+; *Deep Anisotropic Etching Using Low Pressure High Density Plasma*; <http://cmi.epfl.ch/>