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HIGH FLOW RATE MICROSIEVE
FOR BIO MEDICAL APPLICATIONS

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Abstract

A new composite filtration membrane having a thin filtration or sieving layer has been developed. This filtration membrane with a high pore density and a narrow pore size distribution on a macro porous support shows good separation behaviour and a high flow rate.

Because of the construction method, the openings are no longer restricted to circular shapes, but can be chosen freely. In this case a microsieve with slits will be presented. The main advantage of a slit over a circular opening is the higher flow rate.

In this paper a process description for manufacturing is presented and the flow rate behaviour of the membrane with slits will be compared with a membrane with circular perforations.

Also results of experiments of leukocyte removal from erythrocyte concentrates with a silicon microsieve will be discussed.

1 Introduction

Inorganic membranes and in particular ceramic membranes (Chan and Brownstein, 1991) have a number of advantages above polymeric membranes like high temperature stability, relatively inert to chemicals, applicable at high pressures, easy to sterilize and recyclable.

However, because of their high costs and relatively poor control in pore size distribution, they have not been used extensively. Also the thickness of the effective membrane layer is very high in comparison to the mean pore size (typical 50-1000 times), which results in a reduced flow rate (Porter et al., 1990).

In the filtration membrane presented here lithographic techniques are used for the construction of a micro filtration membrane made of inorganic materials as siliconnitride and silicon to overcome the disadvantages of the ceramic membranes (van Rijn and Elwenspoek, 1995).

This filtration membrane has a high pore density and a narrow pore size distribution (see figure 1) and shows a good separation behaviour and a high flow rate. A macro porous support contributes to the mechanical strength of the total composite membrane.

The openings in the support should be made as large and numerous as possible in order to maintain the flow rate of the membrane layer and to reduce the interaction of the support with the fluid.

An established use of inorganic membranes with very thin membrane layers, in particular microsieves with high flow rates, will result in an energy- and cost-saving separation technology for present and future innovative applications, like micro liquid handling, modular fluidic systems or micro total analysis systems (van den Berg and Bergveld, 1995)

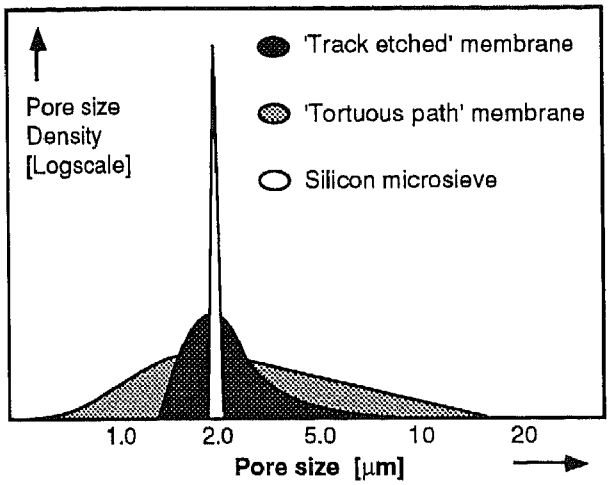

Figure 1, pore size distribution of different membrane filters.

2 Construction

Figures 2 to 5 show in cross-section subsequent stages of a process for production of the microsieve consisting of a support and a membrane layer.

On the surface of the support (see figure 2), a single crystalline 3" <100>-wafer with thickness of 380µm, a thin layer of siliconnitride (Si_3N_4) with a thickness of 1µm is deposited by means of 'Chemical Vapour Deposition'.

This layer is formed by the reaction of dichloresilane (SiH_2Cl_2) and ammonia (NH_3) at elevated temperature 850°C and low pressure (LPCVD).

On the siliconnitride layer a photosensitive lacquer layer with a thickness of 1.8µm is formed by spincoating lacquer (Shipley Europe Resist S1818) at 4.000r.p.m. (see figure 2).

The lacquer layer (see figure 3) is then exposed to a mask pattern with the use of a suitable UV-source, here with a Karl Süss projection system using hard contact projection.

The mask pattern is made of a circular field with radius of 22mm. This field is filled with membrane areas of 1000µm by 1000µm. These membrane areas are separated at spacings of 100µm.

In case of circular openings, each membrane area has 100 x 100 circular perforations with diameter 4µm. The mutual distance between the centre of the perforations is 10µm.

In case of slits, each membrane area has approx. 1750 slits of 2µm x 10µm.

After exposure the lacquer layer is developed for 45 seconds in a diluted NaOH solution giving a mask pattern in the lacquer layer on the siliconnitride layer (see figure 3).

In the siliconnitride layer the mask pattern is etched by means of CHF_3/O_2 reactive ion-etching at 10mTorr and 75Watt for 15 minutes forming the perforations in the membrane layer (see figure 4).

Next openings of 1000µm x 1000µm are etched in the silicon support anisotropically along the <111>-planes using the backside siliconnitride layer as an etch mask.

For this a 10 % KOH solution is used at 70°C until the membrane layer is reached (see figure 5).

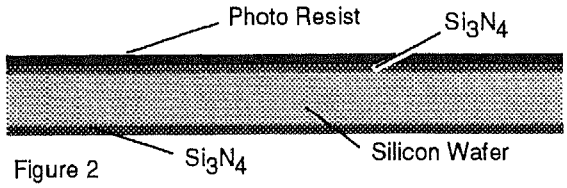

Figure 2

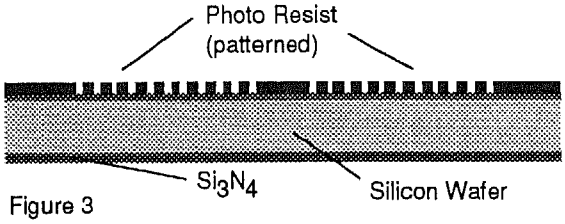

Figure 3

Figure 4

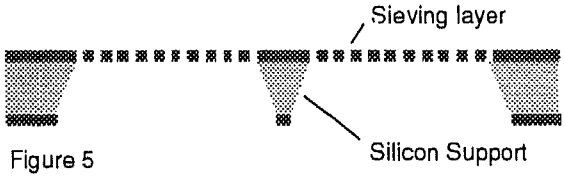

Figure 5

Figures 2 to 5, process steps to construct a microsieve

3 Surface smoothness

Membrane layers with an overall flatness smaller than the pore size have a proven advantage in 'cross-flow' applications. In the photograph in figure 6 the overall flatness of silicon micro machined sieve can be seen clearly.

Also the uniform diameter of the perforations by the anisotropic etch can be seen in figure 6.

By changing the etch recipe also a diverging diameter can be obtained for applications where fragile particles (cells) must be sieved (see figure 7).

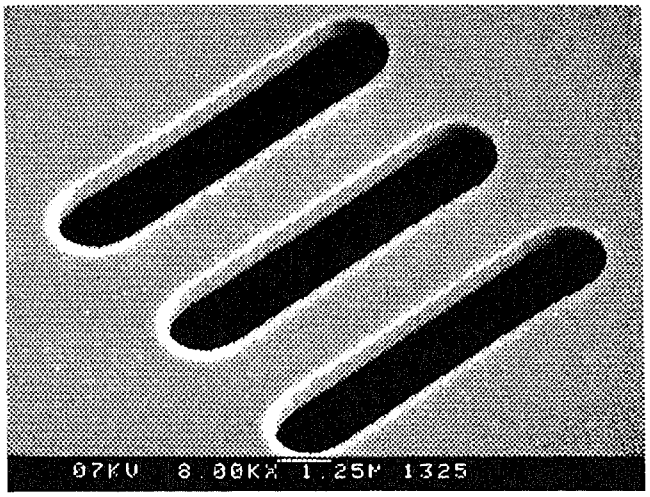

Figure 6, detailed SEM photograph of a microsieve with slits. The uniform diameter and the overall flatness can be seen.

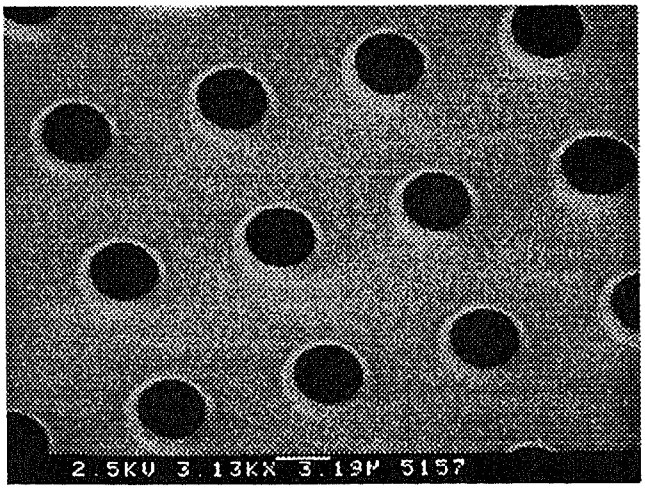

Figure 7, detailed SEM photograph of a microsieve with circular perforations. Here the diverging diameter can be seen.

4.1 Flow rate through perforations in a thin plate

In Happel and Brenner (1983) the volumetric flow rate Q_c through a circular perforation with radius R is given by:

$$Q_c=\Delta p\frac{R^3}{3\eta} \quad (1)$$

with Δp the pressure difference across the plate and η the viscosity of the fluid.

In Tio and Sadhal (1994) the volumetric flow rate Q_s through a slit with length l and width $2R$ is given by:

$$Q_s=\Delta p\frac{\pi lR^2}{8\eta} \quad (2)$$

In order to compare the volumetric flow rate of both type of perforations the perforated area of a number of circular perforations must be equal to the perforated area of a single slit

$$x=\frac{2lR}{\pi R^2}=\frac{2l}{\pi R} \quad (3)$$

with x the number of perforations.

Comparing these two perforations, the ratio of Q_s and Q_c is

$$\frac{Q_s}{Q_c}=\frac{3\pi l}{x8R} \quad (4)$$

When the perforated area of a number of circular perforations is equal to the perforated area of a single slit it follows that the ratio $\frac{Q_s}{Q_c}=1.85$.

4.2 Flow rate through decreased perforations in a thin plate

The process (photolithography) to define the circular holes and slits in the siliconnitride membrane layer have many restrictions in the dimensions. With our equipment it is possible to make holes with a radius R up to 1μm, whilst normally the spacing between these holes is larger than 2μm.

In order to make very small and smooth perforations, a thin siliconnitride layer is deposited on the microfiltration membrane and in the perforations. This is done by LPCVD after the final step in manufacturing. Although there is more space for perforations on the membrane, the number of perforations stays the same.

Now the advantage of a slit over a circular perforation can be seen because the volumetric flow rate of the sieve with slits scales with R^2 and the volumetric flow rate of the sieve with circular perforations scales with R^3. So the ratio

$$\frac{Q_s}{Q_c}\sim\frac{1}{R} \quad (6)$$

will increase when the perforations are made smaller, without changing the number of perforations.

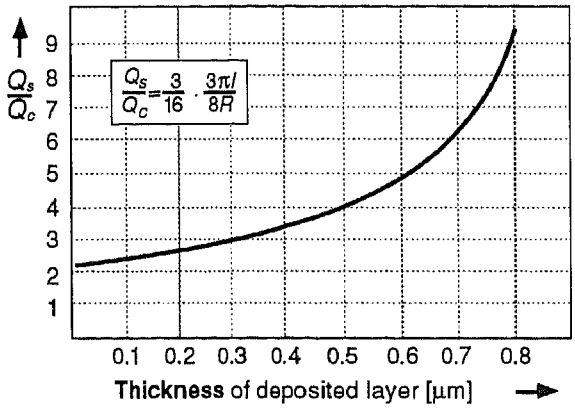

Figure 8, the ratio $\frac{Q_s}{Q_c}$ when the perforations are made smaller.

In figure 8 the ratio is shown as a function of the thickness of the deposited layer with 3 slits (2µm x 10µm) in an area versus 16 circular holes (R=1µm) in an area of equal size.

Formulas 1 and 2 are only valid if

$$d \ll R \qquad (7)$$

with d the thickness of the membrane and the thickness increases when a layer is deposited. However, the thickness of the membrane in the opening is smaller than the radius R due to the non-uniform step coverage in the opening (see figure 9).

Figure 9, profile of an opening after depostition of an extra layer.

5 Bio Medical Applications

A microsieve with perforations essentially of one well defined size will have potentially many interesting applications, like absolute sterile filtration, critical cell-cell separation and cell deformability tests.

Depending on the specific application (Philip et al., 1993) bio or blood compatible materials should be used for the construction of the membrane. With use of thin film technology biocompatible materials may be provided on the used membrane surfaces like titanium, titaniumoxide, titanium nitride, siliconnitride etc. Some of these materials are presently used in dental and chirurgical applications, e.g. as a non-corrosive dental coating (Brauner, 1993) or as a biocompatible film on pacemakers.

The biocompatibility of these materials are strongly related to the low surface roughness (Williams, 1981) and their intrinsic hydrophilic nature, however for every different application the biocompatibility should still be thoroughly tested.

Interesting applications are possible whenever the membrane layer has a thickness that is smaller than the pore diameter, typically between 0.5µm and 5µm. This is particularly usefull if particles or cells should be filtered with a high flux against bigger particles or cells.

	Average size	Number of blood cells per ml	Perc. WBC= Leukocytes
Granulocytes:	8 µm		
Neutrophils,		5,4 x 10^6	60 %
Eosinophils,		2,75 x 10^5	3 %
Basophils		35 x 10^4	0,4 %
Monocytes	20 µm	5,4 x 10^5	6 %
Lymphocytes	6 µm	2,75 x 10^6	31 %
Erythrocytes = Red Blood Cells	non spherical: diam. = 8µm height = 2µm	5 x 10^9	
Blood Platelets	2-4 µm	3 x 10^8	

Table I gives normal number values for different blood cells. Blood consists for 45% of blood cells, together called the hematocrit. The red blood cells (RBC or erythrocytes) transport oxygen, while the white blood cells (WBC or leukocytes) defence the body against infections. The blood platelets (thrombocytes) repair holes in vessels.

5.1 Leukocyte depletion

Nowadays whole blood is separated in red blood cell and blood platelet concentrates with use of advanced centrifugal techniques. Both concentrates however are still being contaminated with relatively large amounts of leukocytes (white blood cells). The remaining white blood cells in the concentrates are further depleted mainly with use of non woven filters. The mechanism of depletion (Steneker, 1994) is adsorption to the fibers (depth filtration) through enhancing the activation of the white blood cells. By this not only the white cells but also the blood platelets are activated, resulting in a loss blood platelets.

A depletion mechanism solely based on sieving with use of thin perforated membrane plates would be a good alternative, however most existing membranes are not suitable for cell-cell separation because of the low flow rate and the adsorption of cells to the membrane surface often with a high surface roughness comparable with the size of the cells.

6 Experiments

Aquamarijn Microsieves® (SiN1.5, SiN1.4 and SiN1.3) with a siliconnitride membrane layer with a thickness of 1μm and pore sizes of 5μm, 4μm and 3μm were being used for performing leukocyte depletion experiments. Before trial they were cleaned in fuming and subsequently boiling nitric acid. The membranes were examined before and after filtration on a light microscope. Units of whole blood were centrifuged and processed within 3 hours of donation. One buffy coat depleted red blood cell unit was subsequently filtered using an Asahi R2000 Sepacell filter in order to decrease the leukocyte concentration.

Filter Pore size [μm]	Experiment	Volume [ml]	Vol. loss after exp.	Hema-tocryt	RBC $*10^9$/ml	Incr. of Hemolysis	WBC before experiment $* 10^3$/ml	WBC depletion after experiment	Filtration time [sec.]	Membrane Pressure [cm H_2O]
4	Dead-end	200	1.5 %	55 %	6.5	0.01 %	11	70 %	100	50
5	Dead-end	200	2 %	64 %	7.5	0.15 %	8500	95 %	150	75
3	Cross-flow	25	5 %	45 %	5.1	0.01 %	500	>99 %	200	5
5	Cross-flow	25	8 %	48 %	5.2	none	1500	>99 %	120	3

Table II, leukocyte depletion of red blood cell concentrates using a microsieve with various pore sizes.

When filtrating the cell concentrates the pressure across the membrane slightly rises in the beginning with a few cm H_2O. Cautions have been taken to reduce this effect at very low transmembrane pressures during experiments. The addition of a small amount of plasma is therefore been used in wetting the membrane surface with pore size 3μm, for larger pore sizes wetting is not necessary. The pore density for all microsieves is 1 x 10^6 per cm^2. All experiments were performed with a surface area of $12cm^2$. Dead-end and cross-flow configuration (see figure 10) of the filtration system have been used. In cross-flow configuration the height of the cross-flow channel above the membrane surface is less than 50μm. A pre-filter with a pore size of 20μm is used to avoid 'microcluts' (Hitzler, 1993) when using a cross-flow channel with a very small height.

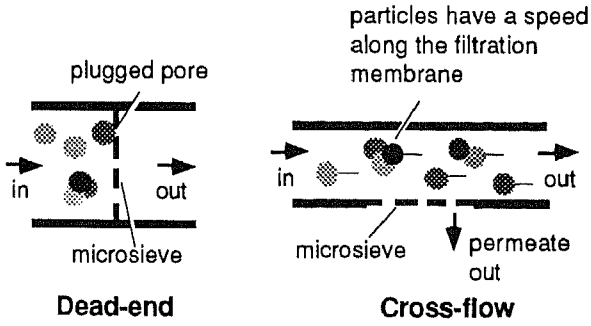

Figure 10, dead-end and cross-flow filtration setup.

7 Discussion

In a 'dead-end' configuration the leukocyte depletion of the concentrated cell suspensions is complicated due to the build up of leukocytes at the sieving surface. A second layer is then formed by the retained leukocytes and at a high concentration this layer may even act as a second filter for smaller cells or even proteins that could have passed through the pores of the sieve. This phenomenon is known as 'concentration polarization' and is being circumvented by using a 'cross-flow' configuration through continuously transporting all cells from the surface larger than the pore size to the cross-flow exit. Moreover the interaction time of the (polymorphonuclear) leukocytes with each individual pore is then severely reduced, avoiding them from slipping through. Pulsatile flow may be used to inhibit the slipping effect. The membrane surface may also be cleared by pulsatile back-flushing during depletion. In a next study we will report on this and present also some results for separating bloodplasma from whole blood using a microsieve with a pore size below 1μm.

8 Conclusions

Microsieves have been made with silicon micro machining. These microsieves are biocompatible due to a very low surface roughness and may therefore be used for medical applications.

Experiments on leukocyte filtration of red blood cell concentrates show promising results.

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