Novel Characterization Techniques for Prediction of Tissue In-Growth in Reticulated Porous Materials

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Abstract

Pore volumes accessible to flow and liquid permeability of un-reticulated and reticulated foams were measured. The foams were implanted in rabbits, pigs, and dogs. The harvested implants were examined for tissue in-growth. Un-reticulated foam with low pore volume and liquid permeability showed no in-growth. Reticulated foam had high pore volume and liquid permeability and showed considerable tissue in-growth. Characterization techniques for pore volume and liquid permeability were very effective in predicting tissue in-growth characteristics of foams.

Introduction

Biocompatible substrates can serve as scaffolds onto which cells may attach, grow and proliferate and this tissue engineering approach can be used to regenerate new tissues and to replace and repair defective ones. Open cell biocompatible elastomeric and resilient foams have significant potential for use as scaffolds for repair and regeneration of defective tissue in orthopedic, wound healing, vascular emobilization, and aneurysm control. A high void content together with an interconnected and intercommunicating network of pores that provide fluid permeability throughout the implantable device can permit cellular in-growth and proliferation leading to eventual bio-integration of the elastomeric and resilient foam substrate or implant.

Suitable characterization techniques for the interconnected and intercommunicating network of pores will, thus, be of great value for prediction of tissue in-growth in open cell porous materials. We have developed two novel techniques for quantifying parameters that can be used as predictors for tissue in-growth. The parameters are liquid permeability and accessible pore volume. These material characteristics were further correlated to the tissue response by examining the behavior of these materials as implants in animals.

Materials

- Porous matrix made from biodurable cross-linked elastomeric and resilient polyurethane was obtained by foaming using water as a foaming agent.
- The thin windows formed between the pores during the foaming process were removed by reticulation.
- Reticulation created a continuous passage throughout the entire porous matrix characterized by an inter-connected and inter communicating pore structure, improved the mechanical response by reinforcing the struts with deposition of the melted polymer from the cell windows, and enhanced the material's potential for fluid transport and tissue in-growth and proliferation.

Experimental Techniques

Liquid Extrusion Porosimetry

Principle

In this technique, the sample is placed on a membrane whose largest pore diameter is smaller than the smallest pore of interest in the sample that is being measured. The pores of the sample and the membrane are filled with a wetting liquid. The liquid from pores of the sample is displaced by application of differential pressure on a non-reacting gas on the sample. The gas pressure is progressively increased until all the fluid is removed from pores. Pressure needed to displace the wetting liquid from a pore is related to pore diameter by the following relation [1].

$$p = 4\,\gamma \cos\theta / D \tag{1}$$

where:
p = differential pressure on the liquid in the pore; γ = surface tension of wetting liquid
θ = contact angle of the wetting liquid on the sample; D = pore diameter

Pressure required to displace liquid from the pores of the sample is insufficient to remove liquid from pores of the supporting membrane. Therefore, gas does not flow through the membrane, but the liquid displaced from pores of the sample flows out through the membrane, and is collected and measured. Measurement of pressure yields pore diameter and corresponding measurement of displaced liquid yields pore volume and pore distribution.

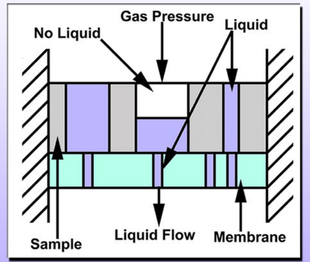

Figure 1. Principle of liquid extrusion porosimetry

Instrument

The fully automated instrument performs the test, regulates the pressures, acquires data, and calculates results in a controlled and reproducible fashion.

Capability

- Uses very low pressures. Thus, the distortion of pore structure is minimal.
- Does not use any toxic material.
- Customer specified liquid can be used in the test.

Liquid Permeametry

Principle

Liquid permeability is computed from the liquid flow rates measured through the sample using the liquid permeameter. In this instrument, the application liquid is pushed from the top or bottom of the sample and the liquid is made to flow under desired differential pressure applied using an inert gas. The pressure difference across the sample and the flow rate of the liquid through the sample are accurately measured [2]. Liquid permeability is computed from flow rate using Darcy's law [3]:

$$v = -(k/\mu)(dp/dx) \tag{2}$$

where
v = linear fluid flow rate; k = permeability; μ = viscosity of fluid
p = pressure; x = linear displacement

Assuming permeability to be a constant over the thickness of the sample and converting linear velocity, v, to volume flow rate, F, integration of Equation 2 yields:

$$F = -(k A / \mu l)(p_i - p_o) \tag{3}$$

where
l = thickness of the sample; p_i = inlet pressure; p_o = outlet pressure
A = area of sample; Permeability, k, is computed in Darcies

Figure 2. Liquid Extrusion Porosimeter

Figure 3. Liquid Permeameter used in this study

Instrument

The fully automated instrument performs the test, regulates the pressures, acquires data, and calculates results in a controlled and reproducible fashion.

Capability

- Minimal pressure on sample and minimal structural distortion of the pores
- Customer specified liquid usable in the test
- Wide range of measurement capability from very high to very low permeability
- Permeability measurable on materials subjected to different compression

Animal Test

Study 1 - Implant placed in the carotid artery of rabbits

- Implant: Oversized occlusive implant measuring 3 mm diameter and 10 mm length
- Implants placement: By open surgical procedure
- Animals sacrifice: At 24 hours, 2 weeks and 4 weeks
- Harvested implant: Examined for tissue in-growth, biocompatibility and biointegration

Study 2 - Implant placed in the right iliac artery of pigs

- Implant: Oversized occlusive implant measuring 4 - 8 mm diameter and 15 mm length
- Implants delivery: Percutaneously via catheter for peripheral embolization
- Animals sacrifice: At 1 week, 1 month and 3 months
- Harvested implant: Examined for tissue in-growth, biocompatibility and biointegration

Study 3 - Implant placed in the spinal annulotomy of mini pigs.

- Implant: Implant measuring 12 mm diameter and 18 mm length
- Implants delivery: By open surgical procedure
- Animals sacrifice: At 3 week and 6 week
- Harvested implant: Examined for tissue in-growth, biocompatibility and biointegration

Study 4 - Implant placed adjunctive to stent-graft in aneurysm sac in dogs

- Implant: Implants measuring 10 mm diameter and 20 mm length
- Implants delivery: Percutaneously by femorally accessed catheters to prevent and treat Type II endoleaks
- Animals sacrifice: At 1month and 3 months
- Monitoring of pressure: Intra aneurysmal pressure (defined as the pressure on the aneurysmal vessel wall from any blood flow within the perigraft space in the sac) and systemic pressures
- Harvested implant: Examined for tissue in-growth, biocompatibility and biointegration

Results and Discussion

SEM Foam Structure

Figure 4. Reticulated foam structure showing an inter-connected and inter-communicating pore structure. Reticulation created a continuous passage throughout the entire foam.

Material Properties

- Material has been tested extensively to demonstrate that it is non-cytotoxic, non-mutagenic, non-toxic, non-pyrogenic, and non-clastogenic

Density	2.70 – 3.20 pcf
Tensile Strength	15 – 35 psi
Elongation at Break	175 – 225 %
Compressive Strength @ 50% strain	0.75 – 1.25 psi
Compression Set	5 – 10 %
Average pore size by SEM	250 – 300 microns
Dynamic Recovery (90%)	< 5 s after 10 minutes @ 75 % strain

Characterization of Pore Structure

A – Accessible Pore Volume and Volume Distribution

- Pore volumes of reticulated (Figure 5) and un-reticulated foams were measured
- Inert wetting liquid Galwick® was used for the test
- Less than 0.5 psi pressure was needed for the test

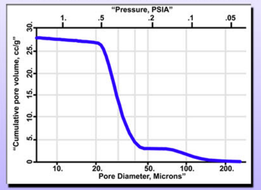

Figure 5. Measured cumulative pore volume of the reticulated foam

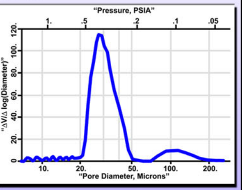

Figure 6. Measured pore volume distribution of the reticulated foam

- Distribution function (Figure 6) is such that area under the curve in any pore diameter range is the volume of pores in that particular range.
- In this technique as the liquid is displaced in the pore structure consisting of cells connected to each other through small openings or windows, the volume of the liquid in the pore downstream from the window is measured as the volume of the pore having the diameter equal to the diameter of the window. Thus, the diameter of the windows, which normally would have gone undetected is detected and measured by this technique.
- The technique measures cell diameters in the range of about 80 -300 microns, which is in the same range provided by microscopic observations. However, the technique also provides additional information about average small openings or window diameters of around 30 microns not measurable by microscopy.
- Measurement of window diameter is important because it provides the primary resistance to flow as well as in-growth and proliferation of tissue through adjacent pores in the overall pore structure.

B - Liquid Permeability

- Liquid (water) permeability of reticulated and un-reticulated foams were measured (Figure 7).

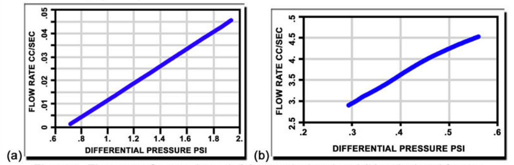

Figure 7. Flow rate of water through (a) un-reticulated and (b) reticulated foams

- Liquid permeability was computed from flow rate using Darcy's law
- The flow rate is very low for un-reticulated and high for reticulated matrix.
- Effects of compressive strain or compressive stress on the foam can change the permeability of the implant considerable. These effects can be quantitatively evaluated.

C - Effect of Reticulation on Accessible Pore Volume and Liquid Permeability

Foam Characteristics	Intrusion volume, cc/gm	Permeability, Darcy [Flow rate of water = (Darcy)×4.08×10-3 l/min-(psi/cm)-cm2]
Un-reticulated	4	0.54
Reticulated	28	205

- Reticulation significantly improved the fluid accessibility by the presence of the continuous passage throughout the matrix.
- It is measured by the increase in intrusion volume or the volume of inter-connected and intercommunicating pores which permit fluid flow, which in turn is determined by the dimensions of the opening of the window between cells.
- Tissue in-growth can occur in fluid accessible continuous passage and the extent and amount of proliferation can be potentially predicted depending on intrusion volume.
- Liquid Extrusion Technique was able to differentiate between un-reticulated foam with very small volume of accessible pores and reticulated foam with much larger volume of accessible pores.
- Un-reticulated foam permitted very little flow of fluid while reticulated foam, owing to inter-connected and intercommunicating pores, permitted flow that is several orders of magnitude higher.
- Presence of higher flow again indicates potential for tissue in-growth and proliferation.
- Liquid Permeability was sensitive in characterizing the transformation in the matrix morphology as it relates to flow of fluid through the matrix.

D - Effect of compression on Darcy's Constant

	Percentage Drop in Darcy's Constant as a function of Compression Strain			
	0% compression	25% compression	50% compression	100% compression
Reticulated Matrix	198.90	130	56	89

- The variability of flow arising out of different compressive strains on the matrix was measurable.
- Changes in Liquid Permeability was measured as a function of compressive strain.

Animal Studies

Study 1 - Implant placed in the carotid artery of rabbits

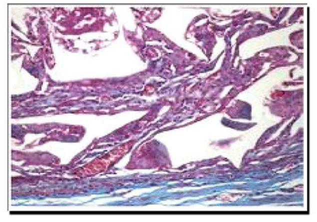

Figure 8. Histology of harvested carotid artery implanted with reticulated foams at 4 weeks

Study 2 - Implant placed in the right iliac artery of pigs

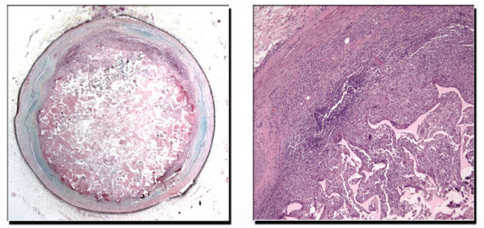

Figure 9. Harvested pig iliac artery containing reticulated foams and the associated histology at 3 months

Study 3 - Implant placed in the spinal annulotomy of mini pigs.

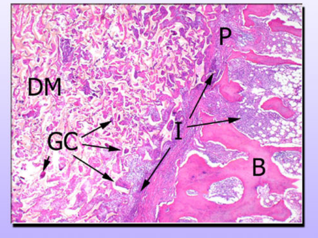

Figure 10. Histology of harvested mini pig spine annulus with reticulated foams at 6 weeks

Study 4 - Implant placed in the aneurysm sac adjunctive to stent-graft in dogs

Pressure Measurements of Treatment and Control Animals in an Canine Model of AAA Endoleaks

Test Systems	Systolic Pressure*	Mean Pressure*	Endoleak Patency
Patent Type II Endoleak	0.702	0.784	Patent
Polyurethane Treated Type II Endoleak	0.183	0.142	Thrombosed
Control (No Endoleak/ No Branches)	0.172	0.137	Thrombosed
Systemic Pressure	1.0	1.0	NA
P-Value (Patent vs. Polyurethane Treated)	<0.001	<0.001	<0.001

Note *All pressures listed were measured after antegrade AAA exclusion and are indexed as a percentage the systemic pressure

- All arterial vessels into which the implant were placed showed total occlusion following placement of the implant and remained as occlusive barrier through the end of the study.
- Treatment of endoleaks with reticulated implants leads to complete elimination of intra aneurysmal pressure in the sac making it indistinguishable from controls with no aneurysm.
- As the healing progressed, extensive tissue in-growth and proliferation occurred into the reticulated matrix accompanied by organizing fibrin/platelet rich thrombus.
- No evidence of fibrous encapsulation was observed for any of reticulated implants – a phenomenon expected and observed with un-reticulated matrices.
- The extent of tissue infiltration scaled fairly well with the accessible pore volume of the implants.
- Expected cellular response to porous materials with no necrosis, mild to moderate inflammation that subsides with time and none to decreasing granular tissue formation across multiple animal studies
- Implants were atraumatic to vessel walls.
- Overall, the reticulated implants were successfully bio-integrated [4, 5].

Summary and Conclusions

- A reticulated, biodurable, cross-linked, elastomeric, and resilient polyurethane matrix was used for this study.
- Implants made from this matrix were surgically implanted in a variety of tissues using different animal models through various time points from 1 week through 3 months.
- Material supported extensive tissue in-growth and proliferation including soft tissue (rat RCR and rabbit subcutaneous – not shown here), fibrocollagenous (mini-pig & rabbit annulus), and vascular (carotid artery, abdominal & carotid bifurcation aneurysms, external iliac).
- No adverse reaction was noticed and the material was shown to be very biocompatible with complete bio-integration across various tissue types.
- Accessible Pore Volume and Liquid Permeability of un-reticulated and reticulated matrices were measured using novel techniques developed by Porous Materials, Inc, Ithaca, NY.
- Measurement of pore volume accessible to flow and, thus, to tissue is an excellent qualitative and quantitative indicator for predicting the degree or extent of tissue in-growth and proliferation. The extent of tissue infiltration scaled fairly well with the accessible pore volume of the implants
- Permeability of matrix under relaxed and compressed conditions is indicative of the degree of interconnectivity of the matrix. These results can be used as indicators for tissue in-growth and proliferation especially when the implant configuration and geometry are expected to change from post implantation to delivery.

References

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