Grafting of N-vinyl pyrrolidone into silicone rubber for potential vascular prosthesis application

by

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INTRODUCTION

The development of a small diameter synthetic vascular graft suitable for coronary bypass or peripheral vascular replacement has shown limited success. With 320,000 coronary bypass surgeries alone being performed each year (Medtronic, Inc., 1987), a definite need exists for an off-the-shelf prosthesis. Dacron and expanded poly tetrafluoroethylene (EPTFE), commonly used for large and medium diameter vascular prostheses, have failed in their applications as small diameter vascular grafts. Promising nonthrombogenic polyurethanes have been compromised by biodegradation of the polymer. Therefore, new design methods are necessary to enhance the patency of the small diameter vascular grafts.

Hydrogels have long been suggested as good materials for blood and tissue contact purposes. A high water content and soft, rubbery consistency imparts a superficial resemblance of living tissue (Ratner and Hoffman, 1976). However, hydrogels are mechanically weak and must be applied to a support material to perform adequately in the human environment. Medical grade silicone rubber has been used routinely as a substrate for grafting hydrogels due to its good biocompatibility characteristics and its extensive use as catheter tubing.

Porosity of the graft material tends to be an important factor in determining the patency of the vascular graft. The openings act as ports for the anchoring of the developing neointima. This natural surface imparted to the implant decreases thrombogenesis and limits

cellular proliferation. Therefore, a microporous hydrogel grafted silicone rubber would seem to have potential as a new small diameter vascular graft.

This investigation was designed to determine how to graft a controlled, thin layer of N-vinyl pyrrolidone (NVP), a hydrogel, into silicone rubber tubing. This information could be useful to avoid interference with the favorable porous structure, when a graft is placed on a microporous material. Several formulations of NVP were luminally grafted using various irradiation doses into silicone rubber tubing substrates. Graft deposition was determined by gravimetric measurements, and the surface penetration of a graft was identified by staining techniques and analyzed using light microscopy and scanning electron microscopy.

REVIEW OF LITERATURE

Influences on Compatibility

According to Bruck (1974), materials selected for vascular prostheses should not cause the following:

- thrombosis,
- destruction of the cellular elements of the blood such as red blood cells, white blood cells and platelets,
- alteration of the plasma proteins,
- destruction of the enzymes,
- depletion of electrolytes,
- adverse immune responses,
- damage to adjacent tissue,
- cancer,
- toxic and allergic reactions, and
- deterioration in the biological environment or during sterilization with resultant changes in their physical, chemical, mechanical and surface characteristics.

The materials available today fulfill many of the above conditions, but there still is disagreement as to the importance of other key parameters such as porosity, surface texture, compliance and surface composition have on patency of the small diameter vascular prosthesis. This lack of agreement has been due to the diversity of graft materials, experimental conditions and techniques, and the animal models employed.

Porosity

Porosity of the synthetic vascular graft is one of the major factors determining blood compatibility and long term patency, according the the early work of Wesolowski et al. (1961). Using the

currently available synthetic materials, they recorded that deleterious changes in the patency of the graft were dependent on the porosity of the graft and independent of the material and its biological activity. The porosity contributed to the development of a nonthrombogenic neointima on the luminal surface. Successful 4 mm diameter grafts were developed when expanded polytetrafluoroethylene (EPTFE), with an average pore size of 22 μm or less, was introduced (Campbell et al., 1975).

Researchers have observed a correlation between the porosity of the material and the thickness of the neointima (also referred to as pseudointima) (Didisheim et al., 1984; Hess et al., 1984). Didisheim et al. (1984) suggested that nonporous materials do not permit the diffusion of platelet derived growth factor (PDGF) and thus the development of a thick neointima is stimulated. Hess et al. (1984) theorized that the blood contact surface must be ordered to allow the anchoring of cytoplasmic protrusions. If an anchoring capability is not provided, the neointima wall thickens, interfering with the patency or causing emboli to be constantly released.

Tizian et al. (1982) used the replamineform process to construct porous silicone microvascular prostheses of 1 mm internal diameter. In the rat abdominal aorta, a 88.6 per cent patency rate (sacrifice schedule of 3 days to 6 months) was realized. The replimineform process is very difficult. This was thought to preclude its application to clinical surgery (Tizian et al., 1981); however, this

problem has been eliminated by commercially availabile replimineform materials (White et al., 1987; Berman et al., 1986).

Compliance

A graft's compliance, the strain or elongational response to an applied stress, has also been implicated in thrombosis formation. Lyman et al. (1978) suggested that a compliance mismatch between the prosthesis and the natural vessel could disrupt the endothelium of the vessel and stimulate intimal hypertrophy at the anastomosis.

Distensibility, the radial expansion of the tubing under an applied pressure, is closely related to the material compliance. Walden et al. (1980) and Lelah et al. (1984) demonstrated that it would be necessary to match the distensibility of the synthetic material to that of the normal artery to enhance its compatibility. White and co-workers (1983; 1987) designed in vivo dog experiments where instantaneous, intraluminal distensibility parameters of implanted synthetic vessels could be measured using electromagnetic rheoangiometry. This unique capability permitted them to correlate compliance (distensibility) changes of the prosthesis to graft patency. Microporous replamineform silicone rubber prostheses remained "isocompliant" (matched the distensibility of the native vessel) for up to 8 months after implantation, whereas other materials tended to rapidly become "minimally compliant" (distensibility less than the native vessel). It was also found that excessively "over-compliant" materials (distensibility greater than native vessel) may be a factor influencing anastomotic hyperplasia and early graft occlusion.

Surface Texture

The significant influence that surface texture has on cellular adhesion and aggregation seems to be flow related (Didisheim et al., 1983). Microemboli can be trapped in surface grooves and disrupt the laminar flow of blood resulting in thrombus formation. Cumming (1980), using the Stagnation Point Flow Experiment, showed that both general surface contours and localized particulate inclusions induced thrombus formation to a greater degree than surface chemistry.

Criteria for roughness of a polymer surface, in terms of formed elements and plasma proteins, were defined by Merrill and Salzman (1976). A peak-to-peak distance or a peak-to-valley distance of 10^3 A is considered rough to proteins, whereas, 10^2 μm to 10^8 A is considered rough to cells. However, Baier (1978) states that surface irregularities of less than a micron in depth or breadth do not significantly influence the outcome of blood contact experiences. It is his contention that micro air bubbles entrapped in crevices are responsible for the thrombus formation and the poor patency results observed.

Surface Composition

When a foreign surface comes into contact with blood, plasma proteins quickly react to the material; they can be adsorbed onto its surface (Bruck, 1977). The composition and organization of that proteinaceous layer influences subsequent cellular events of thrombus formation, embolization, or passivation (Kim et al., 1974; Bruck, 1977;

Horbett, 1984). This initial reaction between the blood proteins and foreign material is believed by many to be the fundamentally most important step in determining the biocompatibility of the material (Horbett, 1984).

Hydrogels

Since their introduction for biological use by Wichterle and Lim (1960), hydrogels have been tried for a large number of products (e.g. contact lenses, catheters, arterial prostheses, sutures, etc.). Their high affinity for water and their soft, rubbery consistency make them attractive for simulating normal endothelial tissue (Ratner and Hoffman, 1976). Ratner and Hoffman (1976) list several advantages of hydrogels over other possible vascular materials:

- expanded nature of the hydrogel structure and its permeability allow effective extraction of additives which can interfere with the compatibility of the implant,
- physical characteristics minimize mechanical irritation to surrounding cells and tissue,
- low interfacial tension between a hydrogel surface and body fluid should reduce the tendency of adsorption and denaturation of proteins, and
- diffusion of small molecules through the hydrogel may enhance its in vivo performance.

The poor mechanical strength of hydrogels requires structural reinforcement through the use of a stronger support material. Several methods including radiation grafting, dip coating and heat polymerization have been used to provide the appropriate hydrogel/substrate combination. Radiation grafting is a very useful technique for preparing surfaces for analytical and medical applications (Hoffman, 1977). Radiation grafting avoids contamination from catalysts and oxidized residues that can adversely affect medical performance (Weathersby et al., 1975; Ratner and Hoffman, 1976). It also avoids biological "sideness" differences (i.e., air side vs mold side) produced during casting procedures (Lyman et al., 1978). With proper selection of substrate, monomer, and solvent, a high degree of product control can be exercised (Jansen, 1984; Chapiro et al., 1980; 1981; 1982; Chapiro, 1983).

Hydrogels have been extensively researched for possible use in the design of arterial prostheses. Andrade (1973) postulated that a hydrophilic surface with a low interfacial energy would promote a blood compatible environment. However, Ratner et al. (1979) suggested that a purely hydrophilic surface may strongly interact with the blood and cause a continuous shedding of microemboli. They further concluded that a balance of hydrophilic/hydrophobic sites would be important for blood compatibility.

N-vinyl pyrrolidone (NVP) is one hydrogel of interest for biomedical applications. It has been shown to be non-toxic and non-

thrombogenic when used intravenously as a plasma expander (Jenkins et al., 1956) and in hemodialysis membranes (Luttinger and Cooper, 1967). It has been radiation grafted into various substrates to provide a new type of blood contact surface (Hoffman and Harris, 1972; Chapiro et al., 1973; 1980).

N-Vinyl Pyrrolidone Grafting

The earliest work done on radiation grafting of N-vinyl pyrrolidone into silicone rubber was by Yasuda and Refojo (1964). A Van de Graaf accelerator was used to generate high energy electrons (3 million electron-volts) for the irradiation process. By varying the monomer water content and irradiation dose, surface grafting (ca. 15 μm depth) up through homogenous grafting (grafted throughout the 125 μm thick wall) was produced. Eosin stained samples were microtomed and examined using light microscopy to determine penetration depths of the grafted N-vinyl pyrrolidone.

A preswelling technique to regulate the monomer uptake and degree of graft penetration was used by Jansen and Ellinghorst (1979; 1981) for the irradiation grafting of various hydrogels into polyetherurethanes. They showed that grafting was dependent on preswelling time and irradiation dose. The results were demonstrated by measurements from gravimetric analysis and microscopic observations of stained microtomed samples. Further reports (Jansen, 1984) showed that the grafting yield of NVP preswelled tubes was also dependent upon

the concentration of the NVP in the preswelling monomer. It was noted that the mechanical properties of the polyetherurethane substrates were not significantly changed if grafting yields were less than 5 mg/cm^2.

Chapiro and coworkers (1973; 1980; 1981; 1982) have contributed significant information on NVP grafting. Their work on irradiation grafting of NVP into polytetrafluoroethylene (PTFE) identified several parameters, such as temperature, dose, dose rate, and concentration of monomer, which influenced the grafting process (Chapiro et al., 1973). It was also learned that the grafting process was complicated by the high viscosity of the reaction medium and the rate of diffusion of the monomer into the film.

Similar parameters that influenced grafting were found for the process of grafting NVP into silicone rubber (Chapiro et al., 1980; 1981). Homogeneous grafted (uniform silicone/poly-NVP composition throughout the thickness of the wall) and surface grafted (NVP incorporated only at the surface) samples of NVP into silicone rubber were produced by varying the solvent, monomer concentration, radiation dose, and temperature, and by using selective inhibitors(Chapiro et al., 1980; 1981; 1982; Chapiro, 1983). Early homogeneous bulk grafts of 30-40 percent graft ratio showed improved blood compatibility as demonstrated by in vivo carotid artery experiments in lambs. Examinations of the brain showed no, or very few, thrombi in cases where the prostheses remained clear. This indicated that the patency was not due to constant shedding of accumulated thrombi by a nonadhesive surface.

Tubes with a high graft ratio would swell in water and become very brittle in the dry state. In order to retain the good mechanical properties of the silicone rubber, the tubes were irradiation grafted in aqueous solutions of NVP so that only surface grafting would occur (Chapiro et al., 1981; 1982). Adjusted monomer concentrations and limited swelling of samples in the monomer solution yielded a series of samples with graft depths not exceeding 100 μm, and surface NVP contents greater than 30 per cent (Chapiro et al., 1981). These samples showed significant improvement in thromboresistance and patency (Chapiro, 1983). It is unfortunate that the details of irradiation dose rate, irradiation dose, swelling time, and aqueous formulation are not stated in these reports.

Vale and Greer (1982) used irradiation grafting and interpenetrating network (IPN) techniques to test the exclusive effect that wettability has on the biocompatibility of grafted hydrogels into and onto silicone rubber. The grafted films exhibited differences in water contact angle measurements (range 57° to 95°), yet ex vivo experiments in dogs using the grafted tubing, exhibited similar declines to each other in surface platelet populations after 60 minutes. The most promising formulation was one which produced a high percentage grafting of NVP. Follow-up work (Greer et al., 1985) using a similar series of hydroxyethyl methacrlyate/N-vinyl pyrrolidone (HEMA/NVP) mixtures in 15% methanol solvent, was performed for hydrogels grafted onto silicone rubber tubing having different silicone

rubber filler formulations. The 20% NVP/0% HEMA formulation grafted into a 0% filler content silicone rubber resulted in the best material-blood exposure characteristics, as determined by scanning electron microscopy (SEM).

Hoffman, Ratner and coworkers have studied the effects that various monomers (e.g., HEMA, NVP), solvent systems, temperatures, inhibitors and irradiation doses have on irradiation grafting using silicone rubber substrates (Hoffman and Harris, 1972; Ratner and Hoffman, 1974; 1975; Khaw et al., 1975). They found that the concentration of graft and graft water content could be varied over a wide range with small changes in grafting conditions. Grafted hydrogels were prepared which ranged continuously from 0.6 to 10 mg graft/cm^2, with water contents ranging from 10 to 65 %. When NVP is grafted into silicone rubber, it penetrates the surface and forms a covalently bonded homogeneous hydrogel-silicone rubber material, whereas, HEMA grafts just onto the surface layer of silicone rubber (Ratner and Hoffman, 1975).

Vena cava ring tests were performed to evaluate the blood's response to radiation grafted HEMA and NVP/Silastic® surfaces. The surfaces were relatively resistant to thrombus accumulation when compared with non-grafted silicone rubber surfaces (Ratner et al., 1978). However, results of canine renal embolus ring tests and baboon A-V shunt tests indicated that high water content gels tended to cause platelet destruction and shedding of emboli (Ratner et al., 1979).

They concluded that high water content materials may be detrimental to the blood and suggested that surfaces composed of a balance of hydrophilic (polar) and hydrophobic (apolar) sites would be important for optimum blood compatibility.

Polymer Extraction

The extraction of homopolymer and monomer from the silicone rubber substrate after grafting is an important step in the preparation of irradiation grafted materials. The extraction solvents should be nonsolvents of the substrate grafted polymers, yet should be able to remove leachable components and nongrafted polymer which can interfere with blood contact experimental results. Table 1 lists the diversity of extraction methods used to remove nongrafted NVP from silicone rubber.

Staining

Hydrogel grafted polymers can be examined by various methods (Ratner, 1980). Microscopic examination of stained samples allows for rapid, inexpensive analyses which can be performed in most laboratories. NVP grafted polymers can be stained by many dyes (Yasuda and Refojo, 1964; Chapiro et al., 1982). Yasuda and Refojo (1964) used a stain containing 2.5% eosin in 24% ethanol to study NVP grafted into silicone rubber; Chapiro et al. (1982) used a stain containing 0.1% fuchsin in methanol (concentration unlisted) to study NVP graft into silicone rubber; and Jansen and Ellinghorst (1979) used "Mallory's Azar II" (unreported concentration) to study NVP grafted into polyurethane.

TABLE 1. Silicone rubber extraction methods

Author/Date	Method
Yasuda and Refojo, 1964	Washed in water then extracted in a Soxhlet extractor for 3 days
Hoffman and Harris, 1972	24 hours acetone, 24 hours acetone/water, 48 hours water
Ratner and Hoffman, 1974; 1975	Scrubbed with acetone/water (50/50), agitated for 2 hours in acetone/water, stirred in water bath for 24 hours
Sasaki et al., 1976	30 minute and 2 hour acetone/methanol (50/50) wash, 24 hours in water
Chapiro et al., 1980	Boiling ethanol for 48 hours
Chapiro et al., 1982	Boiling methanol for 48 hours
Jansen, 1984	Ethanol/water mixture and pure water (polyurethane substrate)
Vale and Greer, 1982	Ethanol/water (50/50) rinses

PROCEDURES

The procedures were modified during the course of this work. The alphabetical listing of methods correlates to the sequence in which they were used.

The irradiation doses have been reported as supplied by the Nuclear Engineering Laboratory; however, our calibration analysis supports increasing the irradiation dose value as well as assigning a gradation of dose in relation to the axial position in the Cobalt-60 Unit. Details are given in the Appendix.

Grafting Methods

Method A

Fourteen cm sections of non-reinforced Silastic® tubing[1], 0.078" x 0.125", were boiled for three 1 hour periods in aqueous 2.0% sodium bicarbonate solution. They were then thoroughly rinsed in distilled carbon filtered water and subsequently dried in a desiccator (over Drierite®, $CaSO_4$) overnight. Selected ca. 200 μm thick cross sections were removed from the tubing and viewed under a stereomicroscope to take diameter measurements. The tubes were weighed on an analytical balance.

A series of 20, 40, 60, 80, and 100 v/o concentrations of N-vinyl pyrrolidone (NVP)[2] were prepared with the balance as 80, 60, 40, or 20%

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[1]Dow Corning Corp., Midland, MI., Lot HH063212.

[2]Polysciences, Inc., Warrington, PA., Lot 51721.

water, toluene, or methanol (100, 80, 60, 40, and 20 v/o methanol in water) to form a series of solutions for subsequent irradiations. NVP and solvents were individually bubbled with nitrogen for at least 30 minutes. An appropriate quantity of NVP and solvent to produce a 2 ml quantity of a specific formulation was pipetted into a 15 mm x 45 mm bottle. The solution was transferred to an inert nitrogen atmosphere in a glove bag and agitated to insure good mixing. A portion of the particular stock solution was drawn into a silicone rubber sample using a syringe, and the ends were sealed using two hemostasis clips placed at one cm from each end of the tubing. The tubing was placed into a 16 mm x 150 mm screw cap pyrex tube that had been flushed with nitrogen and the tube was sealed (teflon tape was previously wrapped on threads to insure air-tight fit). Samples remained in the glove bag until an irradiation series of sealed sample tubes was complete. These were stored in a nitrogen purged desiccator (over Drierite®) for one week. Samples were irradiated within a period of two weeks.

Each sample was individually given a 250 Krad dose from a ca. 360 Curies Cobalt-60 source (Nuclear Engineering Laboratory) at a dose rate of ca. 285,600 rads/hr. The center of the tubing was placed in the center of the irradiation field of the Cobalt-60 irradiation unit (104 cm depth). All irradiations were done at room temperature.

After irradiation, samples were removed from the irradiation tubes. The samples were flushed with acetone/methanol (50:50). This was followed by three 30 minute acetone/methanol (50:50) agitated

washes. Subsequently, the samples were rinsed in distilled water (three changes during a 24 hour rinse). Samples were placed in a desiccator (over Drierite®) overnight and were weighed the next day.

The series of NVP/water and NVP/100% methanol was repeated, except that an ethanol/water (50:50) post-irradiation wash was used rather than an acetone/methanol wash. Samples were flushed with ethanol/water, and then were agitated in a shaker bath for 2 hours in ethanol/water (3 changes). These samples were then rinsed in distilled water for 24 hours (three changes), dried, and weighed.

Method B

Ten cm lengths of Silastic® of the same lot as used for Method A were washed in a similar manner to those in Method A, and dry weights were recorded for those samples. The tubing samples were then extracted in acetone/methanol (50:50) for three 30 minute rinses under agitation. These samples were rinsed in 3 changes of distilled water during a 24 hour period. Samples were dried under vacuum (distinct from Method A) in a desiccator (over Drierite®) for 24 hours and weighed.

NVP[3] was bubbled with nitrogen for 30 minutes. This was performed in a nitrogen filled glove bag. Pure NVP monomer was drawn into the tubing using a syringe. The silicone rubber tubing was sealed with McKenzie hemostasis clips (4 mm) at 1 cm and 6 cm from the top (see Appendix). Three tubes were placed in each 16 mm x 150 mm test tube

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[3]Polysciences, Inc., Warrington, PA., Lot 71441.

for irradiation. Prior to sealing these irradiation tubes, they were flushed with nitrogen, and then sealed with teflon tape on the threads. Fifteen irradiation tubes were prepared in this manner. Another set of fifteen irradiation tubes were prepared in the same fashion, but the NVP solutions were drained from the tubing before they were irradiated. This allowed the measurement of the homogeneous grafting into the silicone rubber tubing due to equilibrium swelling. Subsequent comparisons of similarly prepared filled tubings could be used to calculate surface NVP graft concentrations. After these tubings were allowed to set for four to six hours with the NVP, they were drained. They were quickly dipped in acetone, and the inner and outer surfaces blotted dry with Whatman #1 filter paper. Hemostasis clips were applied at the tubing ends, and the tubes were placed in nitrogen flushed test tubes.

Each "filled" and "flushed" set was given one of the following irradiation doses: 50, 100, 150, 200, or 250 Krads. After irradiation, these samples were washed in ethanol/water (2 hours), rinsed in water (24 hours), dried, and then weighed.

Method C

The silicone rubber tubing samples were prepared in a similar fashion as in Method A, except they had an initial acetone/methanol extraction. Ten cm lengths of silicone rubber tubing which were washed in 2.0% aqueous sodium bicarbonate and extracted with acetone/methanol (done at same time as Method B) were dried under vacuum in a desiccator (over Drierite®) and weighed.

Five ml volumes of the following formulations were tested: 100% NVP[3], 80% NVP/20% methanol, and 60% NVP/40% methanol. Three samples of each formulation were prepared. These were bubbled with nitrogen at a controlled flow rate (ca. 1 bubble/second) for 30 minutes in a nitrogen purged glove bag. The specific formulation was drawn into the tubing using a syringe. The silicone rubber tubing was sealed with hemostasis clips at 1 and 6 cm from the top. The tubing was placed in individual test tubes and sealed. One sample from each received either 150, 200, or 250 Krad dose of irradiation. After irradiation, the tubing was washed in ethanol/water (2 hours), rinsed in water (24 hours), dried, and then weighed.

Method D

This procedure is similar to A in that the tubing was not pre-extracted with acetone/methanol, yet the filling process, nitrogen bubbling, sealing of the tubing ends, and final cleaning and drying follow Method C. Three samples of each formulation of either 100% NVP or 80% NVP/20% methanol were irradiated. Two samples of each formulation received a 50 Krad irradiation dose, while the other received 250 Krads.

Equilibrium Swelling

An equilibrium swelling method (adapted from ASTM D471-79, ASTM, 1986) was used to determine the percent of NVP monomer in Silastic® tubing. Also, the diffusion rate of the NVP monomer into the silicone

rubber was measured. Twenty-four 14 cm. lengths of Silastic® tubing, 0.125" x 0.078", were cleaned in an aqueous 2.0% sodium bicarbonate solution (designated as sets 1-4; 6 tubes per set). Sets 3 and 4 received an additional extraction in acetone/methanol (50:50), consisting of three 30 minute washes followed by a 24 hour rinse in distilled water. All samples were dried overnight in a desiccator (over Drierite®) and were weighed the next day. Each set consisted of three tubes which were totally immersed into 15 ml of NVP[4]. The other three tubes were filled with NVP and the ends of the tubes were closed using tubing clamp regulators. The tubes were then placed into a screw cap jar and the jar was sealed. Set 1 samples were weighed at 22, 46, 70, 166, 334, 502, and 607 hours. Samples in sets 2-4 were weighed at 1, 2, 4, 6, 12, and 24 hours. After the above designated NVP contact time, samples were separated from NVP and quickly dipped into acetone to remove remaining surface NVP. The outside of a tube was blotted with Whatman #1 filter paper, and then plugs of filter paper were gently pushed along the inside of the tubing using a metal rod. This removed all droplets (usually within three passes). The samples were then put into a pre-weighed jar, and this jar was sealed and weighed. After weighing, the samples were returned to the original solution or were refilled.

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[4]Polysciences, Inc., Warrington, PA., Lot 71411.

Graft Characterization Methods

General

Qualitative visual observations were made throughout the irradiation and cleaning process. Before samples were irradiated, any changes that had occurred in the appearance of the tubing or the interior of the test tube were noted. Before the clips were removed from the ends, the color of the tubing was noted as being unchanged, clear (homopolymerization of NVP occurred in the lumen leaving a clear color), or opaque (presence of grafted NVP into the tubing wall having an opaque color). When the tubing clips were removed, the solvent consistency was described as watery, viscous, or plugged (i.e., could not be removed from the tubing). The stiffness, color, and surface topography on dried tubing and tubing cross sections were evaluated subjectively.

Gravimetric analysis

Prior to irradiation, samples were cleaned in aqueous sodium bicarbonate and then rinsed in water. The samples were dried overnight under vacuum in a desiccator (over Drierite®). Each sample was weighed and then prepared for irradiation. Following post-irradiation cleaning in ethanol/water and water rinse, samples were dried as above, then were reweighed. The graft content was measured as follows: $(W_f - W_i)/\text{Area} = \text{mg/cm}^2$; where,

- W_f = post-irradiation weight of grafted section,
- W_i = initial weight of tubing - weight of unexposed ends,

- Area = final length of irradiated section * original circumference (0.628 cm).

Preparation of tubing cross sections

Several methods were tested to achieve tubing cross sections of uniform thickness. Best results were obtained by using size #0 cork as the supporting structure of the silicone rubber tubing. A hole was bored in the center of the cork using a 0.125" diameter metal tubing as a bit in a drill press. Short sections of samples (ca. 1 cm) were fitted in the cork and any overlapping cork was removed with a razor blade. The cork was fastened onto a chuck using super glue, and ca. 200 μm cross sections were taken using a Lancer Vibratome (Series 1000[5]). The sections were placed on a microscope slide and a coverslip was loosely placed on top.

Light and stereoscopic microscopy analysis

Graft penetration depths and wall thicknesses were measured at 100x and 400x magnification using a Balplan microscope[6]. Inner and outer diameter measurements of the tubing were made at 30x magnification using a stereomicroscope[7]. If a section was elliptical in shape, the major and minor axes were measured and its circumference determined. This circumference was used to obtain the equivalent diameter of a circle so comparisons could be made.

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[5]Division of Sherwood Medical, St. Louis, MO.

[6]Baush & Lomb, Inc., Rochester, NY.

[7]Nikon Corp., Tokyo, Japan.

Scanning electron microscopy

The depth of graft penetration into the Silastic® was evaluated on the 60% NVP/40% methanol formulation from Method A (ethanol/water rinse, e/w) using a JEOL-JSM 840[8] scanning electron microscope at 1.0 Kev. A 200 μm thick cross section and a 0.5 cm longitudinal section were mounted on carbon stubs with high purity silver paint[9]. The samples were then sputter coated with 300 A of gold prior to examination.

Staining Methods

General

0.1% basic fuchsin[10] in 100% methanol solvent was used on the initial samples prepared by Methods A and B. The dried irradiated tubing was immersed into the stain for 48 hours. After it was removed, it was rinsed with several flushes of water, and was dried in a desiccator (over Drierite®) before cross sections were cut.

0.1% acid fuchsin[11] in 10% methanol aqueous solvent was used for initial characterization of graft depth for Methods C and D. The grafted tubing was immersed in the stain for 48 hours, removed, quickly rinsed with water and methanol, and was dried under vacuum in a

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[8]JEOL USA Electron Optics, Peabody, MA.

[9]EMSL Supplies - Division of EMSL, Inc., Westmont, NJ., Lot H2002.

[10]Fisher Scientific Co., Fair Lawn, NJ., Lot 792591B, C.I. 42500.

[11]Fisher Scientific Co., Fair Lawn, NJ., Lot 794846, C.I. 42685.

desiccator (over Drierite®) before sections were cut.

Methanol concentration affects methods

Several tests were performed on grafted tubing samples to determine if the methanol concentration in the stain solvent affected the depth of graft or the penetration of the stain into the silicone rubber.

Silicone rubber tubing sections (0.5 cm lengths) of cleaned Silastic®, acetone/methanol extracted Silastic®, and grafted tubing of 100% NVP and 80%NVP/20% methanol formulations from Method A(e/w) were immersed in 0.1% acid fuchsin stains in 10, 50, and 100% methanol for 48 hours. After rinsing and drying, cross sections were cut. The penetration depth of the stain (i.e., graft depth) was measured.

Previously, 0.1% acid fuchsin in 10% methanol, stained samples of 100% NVP (Method A(e/w)), and 100% NVP, 80% NVP/20% methanol, 60% NVP/40% methanol (Method C), and all samples from Method D were immersed in 100% methanol for 48 Hours. These samples were then removed and placed into 0.1% acid fuchsin in 10% methanol for 48 hours. After a quick rinse in water, they were dried, cross sections were cut, and measurements were taken.

RESULTS

Grafting Methods

Method A(acetone/methanol post-rinse)

All samples, except the 100% NVP formulations, decreased in weight by an average of 1.7%. This was unexpected so three cleaned, nongrafted silicone rubber tubings were processed through the acetone/methanol post-irradiation wash. These tubings had an average weight loss of 1.8%. Later results on tubing prepared for Method C confirmed this 1.8% weight loss value. The acetone/methanol wash appears to remove leachable material from the silicone rubber. Since the wash was harsh to the silicone rubber, it may also have had an adverse effect on the graft, even though gravimetric analysis could not confirm this.

Using toluene as a solvent greatly distorted and weakened the silicone rubber. The first set of tubes was not usable since the tubing had expanded and had torn at the hemostasis clips. A second set, prepared and irradiated on the same day, also showed deterioration and some solvent leakage. Therefore, toluene was shown to be an unacceptable solvent for this method.

The water and methanol formulations tended to homopolymerize as the NVP concentration in the formulation increased (Table 2). This was evidenced by a clear tubing color and a plugged lumen. Also, the lower methanol concentration solvents tended to homopolymerize more readily than formulations of higher methanol concentrations.

TABLE 2. Observations on irradiated silicone tubing prepared by Method A(acetone/methanol post-rinse)

Formulation	Tubing color	Solvent consistency
20% NVP/80% water	unchanged	watery
40% NVP/60% water	clear	plugged
60% NVP/40% water	clear	plugged
80% NVP/20% water	clear	plugged
20% NVP/80% toluene	unchanged	watery
40% NVP/60% toluene	unchanged	watery
60% NVP/40% toluene	unchanged	watery
80% NVP/20% toluene	unchanged	watery
20% NVP/80% MeOH(20%)	unchanged	viscous
40% NVP/60% MeOH(20%)	clear	plugged
60% NVP/40% MeOH(20%)	clear	plugged
80% NVP/20% MeOH(20%)	clear	plugged
20% NVP/80% MeOH(40%)	clear	viscous
40% NVP/60% MeOH(40%)	clear	viscous
60% NVP/40% MeOH(40%)	clear	plugged
80% NVP/20% MeOH(40%)	clear	plugged
20% NVP/80% MeOH(60%)	unchanged	watery
40% NVP/60% MeOH(60%)	clear	viscous
60% NVP/40% MeOH(60%)	clear	plugged
80% NVP/20% MeOH(60%)	clear	plugged
20% NVP/80% MeOH(80%)	unchanged	watery
40% NVP/60% MeOH(80%)	unchanged	viscous
60% NVP/40% MeOH(80%)	unchanged	watery
80% NVP/20% MeOH(80%)	unchanged	watery
20% NVP/80% MeOH(100%)	unchanged	watery
40% NVP/60% MeOH(100%)	unchanged	watery
60% NVP/40% MeOH(100%)	unchanged	watery
80% NVP/20% MeOH(100%)[a]		
100% NVP	opaque	viscous

[a]This sample lost its contents before irradiation.

Subsequent staining (the top 7 cm of tubing was immersed in 0.1% acid fuchsin in 10% methanol) of the samples prepared by using formulations containing toluene, methanol, or water showed no signs of

grafting except for the 80% NVP/20% water sample. The graft was observed as a reddish-purple rim, averaging 24 μm in width, in the lumen wall. The rim diminished in size and uniformity 2 cm away from the 7 cm center and became negligible near the ends. Solvent formulations which did not produce a graft were evaluated on the basis of their lack of homopolymerization. Those samples which did not homopolymerize were considered as candidates for future graft experiments.

The 100% NVP formulation produced an opaque graft of 0.6 mg/cm^2; however, the staining revealed that the graft was unevenly distributed along the axial length of the tube. The rim width averaged 90 μm between 4 cm and 10 cm (measured from the bottom of the tube). The rim width was not measurable beyond those limits.

Method A(ethanol/water post-rinse)

The water solvent formulations produced a high degree of homopolymerization in the lumen without depositing any graft. This was shown by gravimetric analysis and lack of staining (Table 3). The homopolymerized plug hindered post-irradiation cleaning in ethanol/water. It could be removed after it dried, since the plug was not attached to the wall.

The tubing prepared using the 60% and 80% methanol formulations was more opaque, and the solvent viscosity was much greater than those prepared using Method A(acetone/methanol post-rinse). The 80% NVP/20% methanol formulation had twice the amount of graft per surface area as

TABLE 3. Observations on irradiated silicone tubing prepared by Method A(ethanol/water post-rinse)

Formulation	Tubing color	Solvent consistency	Graft mg/cm^2	Stainable
20% NVP/80% water	clear	viscous	none	no
40% NVP/60% water	clear	plugged	none	no
60% NVP/40% water	clear	plugged	none	no
80% NVP/20% water	clear/ opaque	plugged	negligible	negligible
20% NVP/80% MeOH(100%)	unchanged	watery	none	no
40% NVP/60% MeOH(100%)	unchanged	watery	none	no
60% NVP/40% MeOH(100%)	clear/ opaque	viscous	1.3	yes
80% NVP/20% MeOH(100%)	opaque	viscous plugged	2.5	yes
100% NVP	opaque	viscous plugged	10.6	yes

the 60% NVP/40% methanol formulation, yet there is little difference between their graft penetration depths (Table 4). This could signify a more concentrated graft.

TABLE 4. Average rim depths in grafted silicone rubber prepared by Method A(ethanol/water post-rinse)

Distance from bottom (cm)	Formulations (rim thickness in μm) 60% NVP/40% MeOH	80% NVP/20% MeOH	100% NVP
7	95	109	173
8	92	93	159
9	91	100	171
10	71	82	142
11	62	52	152
12	13	2	70

Several photographs and scanning electron micrographs were taken of the 60% NVP/40% methanol formulation. Figures 1 and 2 demonstrate the rim depth as shown by the dark color near the edge. A faint line delineates the graft from the silicone rubber in Figure 3 (sample coated with 300 A gold). Figure 3 also shows the surface topography of the grafted lumen. Figure 4 is a control section of silicone rubber. Figures 5, 6, and 7 show the material character differences between the grafted region and the nongrafted region.

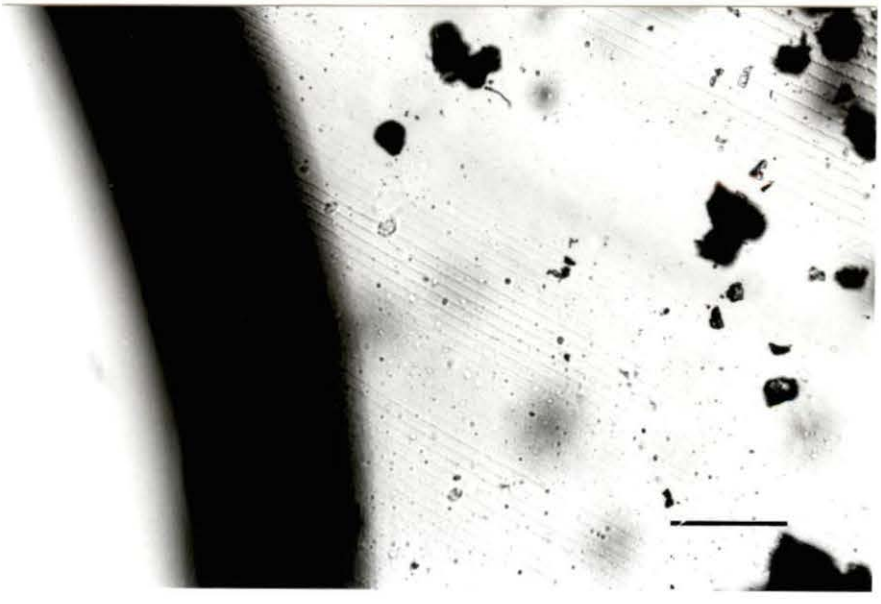

FIGURE 1. Optical microscope photograph of a cross section from the 60% NVP/40% methanol sample prepared by Method A(ethanol/water rinse) and stained in 10% basic fuchsin in 100% methanol (scale bar = 100 μm)

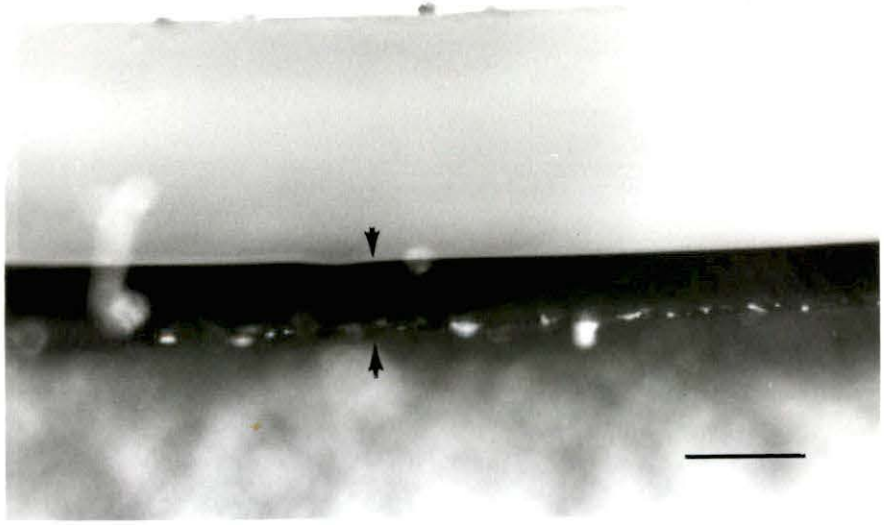

FIGURE 2. Optical microscope photograph of edge view of 60% NVP/40% methanol sample prepared by Method A(ethanol/water rinse) and stained in 10% basic fuchsin in 100% methanol (scale bar = 100 μm). Arrows denote boundaries of NVP graft

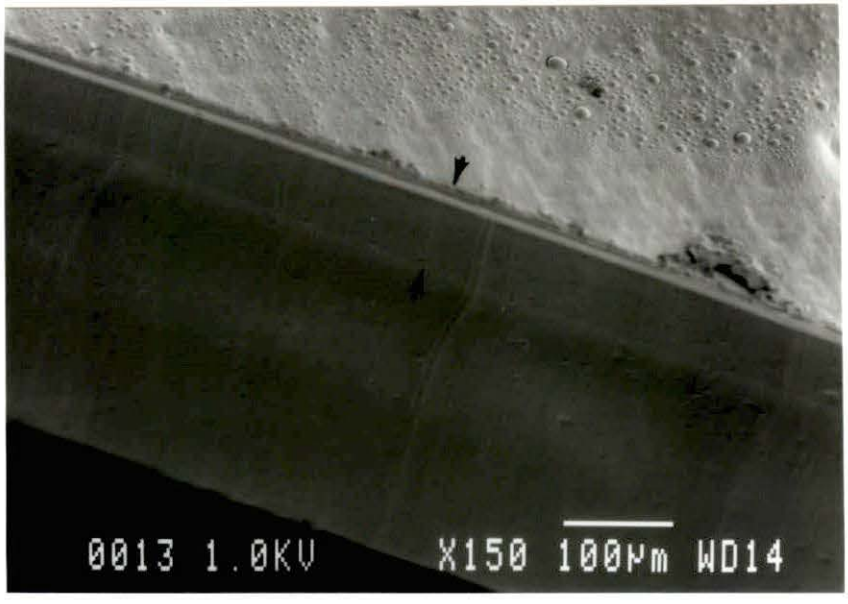

FIGURE 3. Scanning electron micrograph from edge of 60% NVP/40% methanol sample prepared by Method A(ethanol/water rinse) and stained in 10% basic fuchsin in 100% methanol. Arrows denote boundaries NVP graft

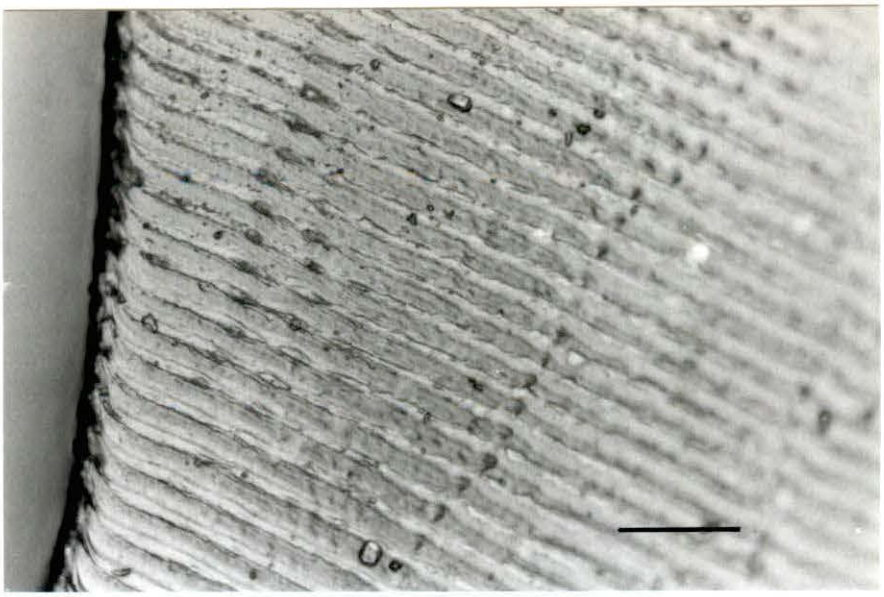

FIGURE 4. Cross section of cleaned, nongrafted silicone rubber (scale bar = 40 μm)

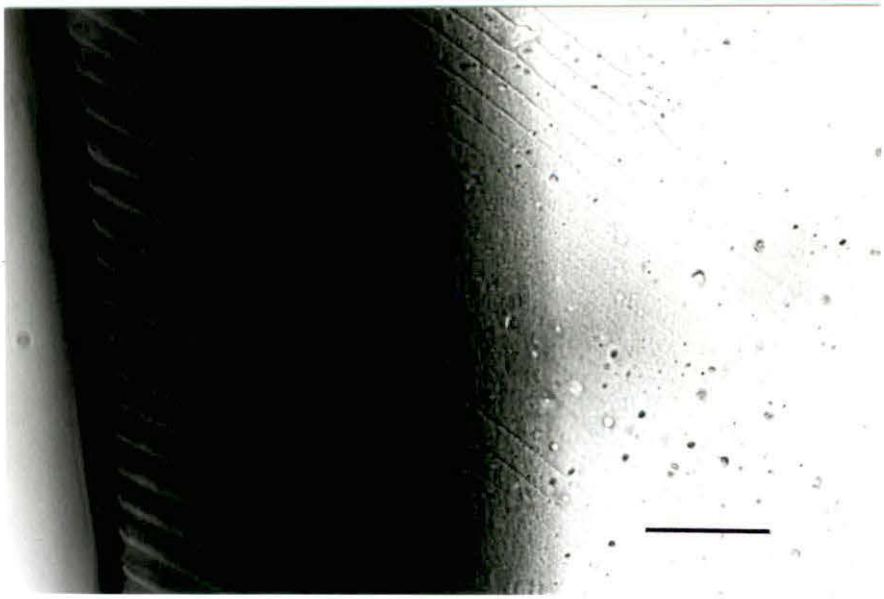

FIGURE 5. Optical microscope photograph of a cross section from the 60% NVP/40% methanol sample prepared by Method A(ethanol/water rinse) and stained in 10% basic fuchsin in 100% methanol (scale bar = 40 μm)

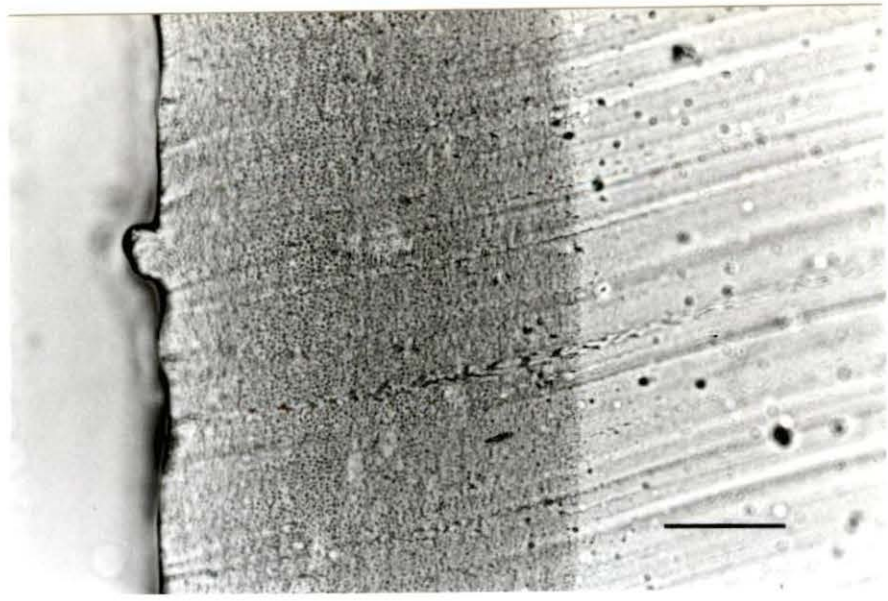

FIGURE 6. Optical microscope photograph of a 10 μm thick, nonstained cross section from the 60% NVP/40% methanol sample prepared by Method A(ethanol/water rinse) (scale bar = 40 μm). Note the appearance of the NVP rim

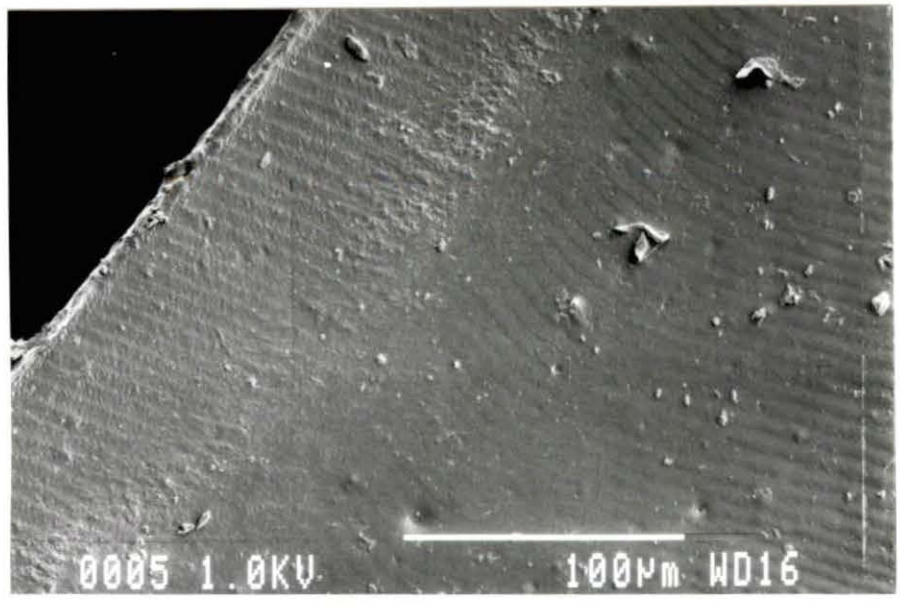

FIGURE 7. Scanning electron micrograph of a cross section from the 60% NVP/40% methanol sample prepared by Method A(ethanol/water rinse) and stained in 10% basic fuchsin in 100% methanol

Method B

Ten cm lengths of silicone rubber were used to allow positioning within a uniform irradiation dose region of the Cobalt-60 Irradiation Unit. This region of uniform irradiation dose was indicated by a calibration examination of the Cobalt-60 Unit (see Appendix).

There was no significant grafting in either the "filled' or "flushed" samples. Some homopolymerization occurred in filled samples receiving a dose of 150 Krads or greater, but no graft rims were evident. However, small areas of external surface graft were noticed where the tubings were in contact with each other.

A new lot of NVP was used for this experiment. This could have caused the difference; however, subsequent purity tests (Nuclear Magnetic Resonance, gas chromatography/mass spectrometry, and infra-red analysis) performed by Chemistry Instrument Services (Iowa State University, Ames, IA) indicated no significant differences between the lots. Routine irradiation grafting experiments using the two lots also did not reveal any differences. NVP polymerization decreases in the presence of oxygen. Since there are several steps in this procedure where oxygen contamination is difficult to avoid, further work to measure surface NVP concentration was discontinued.

Method C

All samples had a greater amount of graft and less homopolymerization (Table 5) than the samples prepared using the same formulations in previous experiments. However, bulk homoplymerized NVP

in the 100% NVP formulation (irradiated at 250 Krads) was not removable during the rinsing or drying processes. This inflated its graft value; therefore, no weight comparisons between this and the other irradiation doses or formulations could be made.

TABLE 5. Observations on irradiated silicone rubber tubing prepared using Method C

Formulation	Irradiation dose Krads	Tubing color	Solvent consistency	Graft mg/cm^2
100% NVP	250	opaque	plugged	38.6
100% NVP	200	opaque	viscous	13.5
100% NVP	150	opaque	viscous	10.2
80% NVP/20% MeOH(100%)	250	opaque	viscous	16.5
80% NVP/20% MeOH(100%)	200	opaque	viscous	15.6
80% NVP/20% MeOH(100%)	150	opaque	viscous	13.9
60% NVP/40% MeOH(100%)	250	opaque	viscous	12.0
60% NVP/40% MeOH(100%)	200	opaque	viscous	9.7
60% NVP/40% MeOH(100%)	150	opaque	watery	9.7

There are no correlations between graft concentration and irradiation dose within the methanol formulations. The 100% NVP formulation in the wet state was less flexible than the nongrafted tubing, whereas, the flexibility of the other formulations was slightly increased. All the tubings were stiff after drying.

The sample prepared using the 100% NVP formulation showed a thicker wall (0.025 to 0.027 in.) than the nongrafted Silastic® tubing (0.024 in.); whereas, the samples prepared using the methanol formulations showed no significant changes in width (Table 6). All

samples exhibited an increase in measurements of their outer diameters. The methanol formulation samples also had an associated increase in inner diameter. The 80% methanol formulation samples demonstrated larger increases in diameter than the 60% methanol formulation samples.

TABLE 6. Measurements on irradiated silicone tubing prepared using Method C (6 cm from bottom, stained using 0.1% acid fuchsin in 10% methanol)

Formulation	Irradiation dose Krads	Rim thickness (μm)	Wall thickness (in.)	Inner diameter (in.)	Outer diameter (in.)
100% NVP	250	311	0.025	0.080	0.131
100% NVP	200	262	0.026	0.074	0.126
100% NVP	150	268	0.027	0.075	0.128
80% NVP/20% MeOH(100%)	250	214	0.025	0.087	0.138
80% NVP/20% MeOH(100%)	200	226	0.025	0.088	0.137
80% NVP/20% MeOH(100%)	150	195	0.025	0.082	0.132
60% NVP/40% MeOH(100%)	250	268	0.025	0.081	0.130
60% NVP/40% MeOH(100%)	200	226	0.024	0.081	0.128
60% NVP/40% MeOH(100%)	150	220	0.024	0.081	0.129

Rim measurements were more difficult to obtain than previously on the 100% NVP formulation due to the rough surface texture of the lumen. There was not as significant a correlation between the graft rim depth and irradiation dose from samples prepared using the same formulations as there was in previous experiments.

The methanol formulations displayed a second internal ring of graft and a peculiar surface topography (Figures 8, 9, 10) which did not appear on previous samples. The second ring of grafted NVP was

0.018 to 0.029 in. in from the lumen. In some areas, where it protruded to the exterior of the wall, it was stained, otherwise it remained unstained (whitish-yellow color). In some samples these protrusions were more prominent and took on a clear, white, or purple speckled appearance (Figure 11). The clear regions were identified as nongrafted domains on the tubing. The white patches were grafted areas which were just under the the wall and had no access to stain. The purple was due to graft which erupted through the wall and accepted stain.

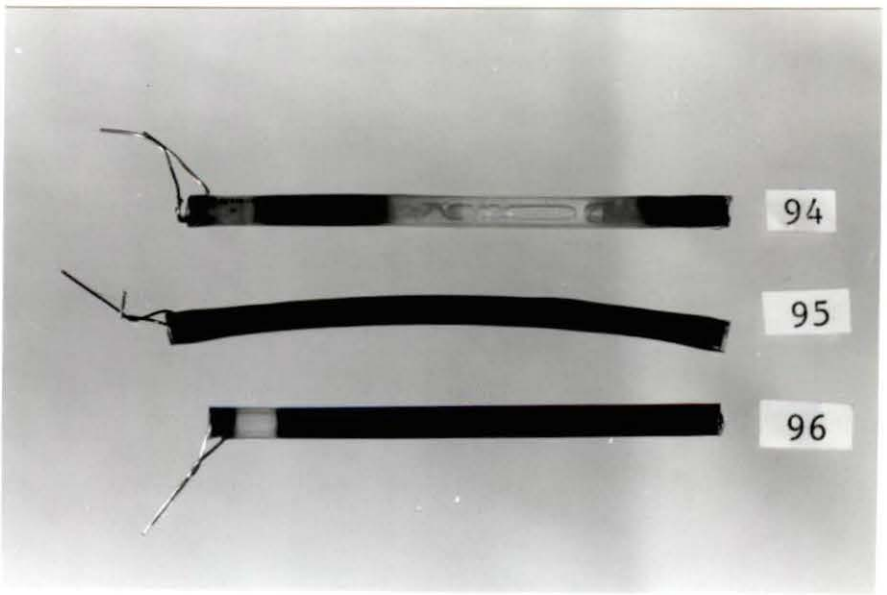

FIGURE 8. Stained irradiated tubing samples prepared using the 100% NVP formulation of Method C. 94 = 250 Krad dose, 95 = 200 Krad dose, 96 = 150 Krad dose

FIGURE 9. Stained irradiated tubing samples prepared using the 80% NVP/20% methanol formulation of Method C. 97 = 250 Krad dose, 98 = 200 Krad dose, 99 = 150 Krad dose

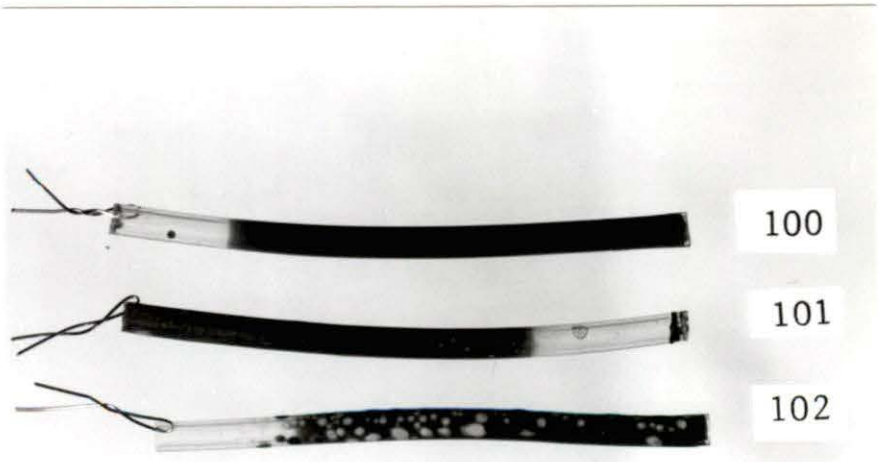

FIGURE 10. Stained irradiated tubing samples prepared using the 60% NVP/40% methanol formulation of Method C. 100 = 250 Krad dose, 101 = 200 Krad dose, 102 = 150 Krad dose

FIGURE 11. Magnified view of the sample prepared using the 80% NVP/20% methanol formulation (200 Krad dose) of Method C

Method D

The sample prepared using the 100% NVP formulation and irradiated at 250 Krads exhibited similar graft concentration values as in Method C. It also had homopolymerization in the lumen; this resisted removal using ethanol/water (Table 7). The two 100% NVP formulation samples irradiated at 50 Krads displayed similar graft concentrations, but they had higher graft concentrations than what was expected based upon previous trials. The graft concentration values for the methanol formulation samples were as expected. The methanol formulation samples had a similar solvent viscosity as those measured in the samples prepared by Method C, yet they lacked the peculiar surface topography. A second internal graft ring was present in all of the formulations,

but it was not as prominent nor did it have surface protrusions observed in the samples prepared by Method C.

TABLE 7. Observations on irradiated silicone rubber tubing prepared using Method D

Formulation	Irradiation dose Krads	Tubing color	Solvent consistency	Graft mg/cm^2
100% NVP	250	opaque	plugged	43.5
100% NVP	50	opaque	watery	19.3
100% NVP	50	opaque	watery	17.3
80% NVP/20% MeOH(100%)	250	opaque	viscous	17.2
80% NVP/20% MeOH(100%)	50	opaque	watery	5.2
80% NVP/20% MeOH(100%)	50	opaque	watery	5.5

The rim thicknesses for these formulations showed a different pattern than observed in earlier work. The graft penetrated deeper into the 50 Krad irradiated samples than in the 250 Krad irradiated sample (Table 8). Homopolymerization in the lumen of the 250 Krad irradiated sample may have caused the lack of NVP penetration into the wall. All the 100% NVP formulation samples and especially the 250 Krad irradiated 80% NVP/20% methanol formulation samples, showed an expansion in their diameters.

Whitish-yellow NVP grafts were apparent on the lumen of the 80% NVP/20% methanol formulation samples (50 Krads), yet the stain was not incorporated into that area as usual. There were areas of no stain, light stain, and heavy stain distributed within the rim. Subsequent

TABLE 8. Measurements on irradiated silicone tubing prepared using Method D (7 cm from bottom, stained using 0.1% acid fuchsin in 10% methanol)

Formulation	Irradiation dose Krads	Rim thickness (μm)	Inner diameter (in.)	Outer diameter (in.)
100% NVP	250	200	0.083	0.134
100% NVP	50	300	0.086	0.134
100% NVP	50	350	0.085	0.133
80% NVP/20% MeOH(100%)	250	232	0.091	0.139
80% NVP/20% MeOH(100%)	50	140	0.078	0.127
80% NVP/20% MeOH(100%)	50	122	0.079	0.127

staining of these samples using 0.1% acid fuchsin in 100% methanol produced a uniform rim of stain. It appears that the silicone rubber/NVP matrix was able to resist stain penetration in a low methanol concentration.

Equilibrium swelling

The type of contact, totally immersed versus lumen filled, did not significantly affect the equilibrium swelling of the silicone rubber tubing. The silicone rubber swelled to equilibrium in NVP (23° C) after one hour of contact for both methods. The Silastic® showed an NVP equilibrium swelling value of 4.1% for acetone/methanol extracted tubing and 3.6% for nonextracted tubing. The difference in percent is significant, yet there were no differences in grafting response that could be attributed to this extraction process.

Methanol concentration effects

Test 1 The grafted tubing lengths were removed from the lower previously unstained portion of the tubing. Serial sections were cut starting 7 cm from the bottom position (i.e., center of whole tubing).

The stain, when applied in 100% methanol, displayed finger-like projections which extended from the grafted rim into the wall. Only minor projections were seen after staining in 50% methanol, and none were present after staining in 10% methanol. The different methanol concentrations resulted in no absorbance of stain in the two nongrafted sections (Table 9). Samples stained in 10% methanol exhibited greater rim thicknesses than those stained in 50% or 100% methanol in both of the grafted samples. The rim thickness measurements obtained using the 50% and 100% methanol in this study agree with those in Method A(e/w) where 0.1% basic fuchsin in 100% methanol was used (see Table 3). These data tend to show that stains having methanol concentrations greater than or equal to 50% can affect rim thickness measurements.

Test 2 A decrease in rim thickness after the sample was rinsed in 100% methanol was evident in samples from Methods A and C (Table 10). The differences between rim thickness in Method D samples can not be considered significant due to the large range of measured graft values.

There is a reduction in the tubing diameters due to the 100% methanol exposure. This may indicate that some material, such as ungrafted NVP, homopolymerized NVP, or grafted NVP, was eroded from the

TABLE 9. Effects of methanol concentration on rim thickness measurements

Formulation	0.1% Acid fuchsin in: 10% MeOH Rim Thickness (μm)	50% MeOH Rim Thickness (μm)	100% MeOH Rim Thickness (μm)
untreated Silastic®	none	none	none
acetone/methanol extracted Silastic®	none	none	none
100% NVP Method A(e/w)	192	165	159
80% NVP/20% MeOH Method A(e/w)	140	104	104

silicone rubber/NVP matrix. This removal of material would allow the tubing to relax and contract to a smaller diameter.

TABLE 10. The effects of a 48 hour rinse in 100% methanol on samples stained with 0.1% acid fuchsin in methanol

Formulations	Rim Thickness (μm) Before	After	Diameters (in.) Before	After
Method A(e/w)				
100% NVP, 250 Krads	189	152	---	---
Method C samples				
100% NVP, 200 Krads	262	238	0.074 x 0.127	0.074 x 0.125
80% NVP/20% MeOH, 250 Krads	214	177	0.087 x 0.138	0.081 x 0.129
60% NVP/40% MeOH, 250 Krads	268	232	0.081 x 0.130	0.081 x 0.130
Method D samples (8 cm from bottom of tube)				
100% NVP, 250 Krads	200	201	0.085 x 0.135	0.082 x 0.129
100% NVP, 50 Krads	300	275	0.087 x 0.135	0.083 x 0.131
100% NVP, 50 Krads	350	300	0.083 x 0.133	0.081 x 0.129
80% NVP/20% MeOH, 250 Krads	214	171/ 238	0.086 x 0.134	0.081 x 0.128
80% NVP/20% MeOH, 50 Krads	140	134	0.080 x 0.127	0.078 x 0.126
80% NVP/20% MeOH, 50 Krads	122	98	0.079 x 0.127	0.078 x 0.126

DISCUSSION

The amount of rim deposited into silicone rubber tubing exposed to similar NVP/solvent formulations was highly variable among experiments. However, patterns did exist between grafts deposited in samples irradiated on the same day. This type of response was also observed by Ratner and Hoffman (1974). They saw as much as 8.7% difference in the degree of graft from experiments performed on different days. This difference was attributed to the nonregulated level of oxygen in the solution and to changes in manufacturing procedures and to possible composition differences between lots of Silastic®. In the current system however, the tubings were of the same lot, so it is assumed that oxygen contamination is the cause for the differences. Due to that problem, rim depths could not be predictably duplicated.

The results did not duplicate Ratner and Hoffman's (1975) results from experiments using 20% NVP concentrations in water and methanol. The samples of the 20% NVP concentration tended to homopolymerize; this is similar to their reported data, yet no detectable graft was deposited in the silicone rubber. The difference in $Cobalt^{60}$ source strength, 20,000 Curies compared to the Nuclear Engineering Laboratory $Cobalt^{60}$ source of 360 Curies, may be a factor infuencing these results. Ratner and Hoffman (1976) tried grafting only the lumens of tubes. The tubing was filled with monomer, ends clamped, and irradiated similar to the present methods, yet they also failed to observe any grafting. However, tubing processed in this manner has been shown to exhibit graft (Greer et al., 1985).

The results using high concentrations of NVP (60, 80, and 100%) compare very favorably with the grafting ratios of Chapiro et al. (1980, 1982). They were able to obtain consistent, reproducible results by immersing the tube into the monomer formulation. Our graft penetration depths were in the same range as theirs, yet our graft concentrations were approximately half of their values. This is reasonable since graft was deposited only on the inner surface of our samples, whereas graft was deposited on both the inner and outer surfaces of their samples.

Methanol in the monomer formulation reduced homopolymerization in the lumen and allowed for easier removal of the mixture from the tubing.

The lack of availability of monomer to the lumen surface was a problem for all the monomer formulations. As the tubing sits with the solvent before irradiation, some of the monomer mixture is absorbed into the tubing wall. Also, while it is being irradiated, more monomer diffuses into the tubing wall. This diminishes the availability of the monomer at the upper region and thus produces nonuniform grafting.

The stained or nonstained rim of grafted NVP into silicone rubber can be identified by optical microscopy and by scanning electron microscopy.

Acid fuchsin or basic fuchsin can be used to identify grafted NVP; however, the post-irradiation wash solution and the methanol concentration of the stain solvent can influence the measurement of the

depth of graft. Changes in the diameter and rim thickness of the samples after rinsing in 100% methanol indicated that the ethanol/water post-irradiation rinse did not remove all the noncovalently bound material (Ratner, 1980). This residue would inflate the graft concentration values and could result in undesirable reactions after implantation.

The equilibrium swelling values, 3.6 and 4.1%, of NVP into silicone rubber are slightly higher than those reported by Chapiro et al. (1980), 3%, and Yasuda and Refojo (1964), 1.7%. This difference is probably due to differences in the nature of the elastomer, degree of cure, filler content, and NVP product differences.

CONCLUSIONS

Several monomer/solvent formulations and irradiation doses were used in an attempt to impart a microthin layer of NVP into silicone rubber tubing. NVP rim thicknesses of 2 μm to 350 μm and graft concentrations ranging between 0.6 and 19.3 mg/cm^2 were grafted into silicone rubber tubing; however, these results were not consistently reproduced.

The greatest source of error in the filled tubing method of preparing samples is oxygen contamination. Equivalent monomer formulations irradiated with identical doses can yield substantially different graft results. Also, the whole tubing needs to have equal access to the monomer formulation in order to produce uniform grafts. The irradiation dose, likewise, must be uniform along the axial length of the tube to ensure symmetrical grafting.

The grafting methods and results will be improved if the tubing is immersed into the monomer formulation, rather than being filled with the formulation and clamped. This should help decrease the oxygen contamination. The amount of pre-exposure to the formulation should also be controlled. This would limit the amount of graft penetration into the wall. The solvents should not easily swell the silicone rubber. Ethanol/water should not be used as the post-irradiation rinse, since there is enough evidence to suggest that it may not adequately remove noncovalently bonded material from the graft.

Cleaned silicone rubber tubing samples immersed in 100% NVP solution, and then irradiated with less than 50 Krads, look promising for future experiments. These samples would not be allowed to pre-swell in the NVP before irradiation. A post irradiation wash using acetone/methanol is recommended.

Even though the results of this project did not identify a method which could impart a controlled thin layer of NVP into silicone rubber, useful technical considerations are documented which can provide a basis for further progress.

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APPENDIX: CALIBRATION RESULTS ON COBALT-60 UNIT

Introduction

The Cobalt-60 Irradiation Unit of the Nuclear Engineering Laboratory was used as the irradiation source to propagate the polymerization and grafting of N-vinyl pyrrolidone into silicone rubber tubing. During the course of the early experiments, it was noticed that there was a difference in the grafting concentration along the vertical axis of the grafted tubing. This difference could not be explained as solely due to availability of monomer. Hence, the irradiation uniformity along the vertical axis, and, consequently, the accuracy of the irradiation dose were in question. A calibration on the Cobalt-60 Irradiation Unit using Thermal Luminesence Dosimetry (TLD) was performed to define the intensity profile and exact dose of the unit.

Thermal Luminesence Dosimetry (TLD) is based on the principle that some thermoluminescent materials can store the effects of incident ionizing radiation. When stimulated by sufficient heat energy, the materials will emit a quantity of light proportional to the total energy of the received radiation (Eberline Instrument Corporation, 1975). TLD chips made from lithium fluoride provide the most stable and accurate results for large irradiation dose measurements.

Procedure

Chip irradiation characterization

Lithium fluoride chips, TLD-100[1], were annealed for one hour at 400° C in a Thermolyne®, Type 1400 Furnace[2] (courtesy of the Environmental Health and Safety Laboratory (EHSL)). The chips were then annealed for an additional 24 hours at 100° C in a Thelco® drying oven[3], The chips were carefully wrapped in two layers of aluminum foil and placed in a holder specifically made for the them. The holder was 8 cm x 8 cm of half inch plexiglass. Forty-nine 1/4" holes were radially situated from the center so that no hole extended beyond 3 cm from the center. The TLD chips were irradiated, using a Picker C-9 Cobalt Unit[4] (courtesy of Mary Greeley Medical Center), at a distance of 70 cm from the source in a 10 cm x 10 cm field size. The chips received 49,950 rads at an irradiation rate of 10,428 rads/hour.

Immediately following irradiation, the aluminum foil was removed from the chips and the ionizing radiation of the chips was measured using the TLD-Reader[5]. The TLD-Reader displayed irradiation counts in arbitrary units and needed to be calibrated to chips irradiated by a known dose source (i.e., the Picker C-9 Cobalt Unit). Each chip was

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[1]Harshaw Chemical Company, Solon, OH.

[2]Sybron Corporation, Dubuque, IA., Model No. F-B1310M.

[3]GCA/Precision Scientific, Chicago, IL., Model 28.

[4]Picker International, Cleveland, OH.

[5]Eberline Instrument Corporation, Santa Fe, NM., Model TLR-5.

given an identification number and placed in a separate vial. These results were designated as Standard A. A second irradiation was done using identical methods, except a high quality Lindberg muffler oven[6] was used for the one hour 400° C annealing. These count values were designated as Standard B.

Calibration design

The chips were annealed in the exact fashion as stated previously using the Lindberg muffler oven for the high temperature annealing. Three groups of twelve chips were chosen based upon their similarity of irradiation response in the Picker C-9 Unit. For each group the chips were individually wrapped in two layers of aluminum foil and placed into separate notches of a wooden support rack designed to fit into a 16mm x 150mm screw cap test tube. The notches started at one cm from the bottom of the rack and continued at one cm intervals from the notch base for twelve cm. The loaded rack was placed into the test tube and sealed.

The cobalt source rods in the Nuclear Engineering Laboratory Cobalt-60 Unit were placed in the closest position (multiplication factor of 17), emitting an irradiation dose rate of 279,300 rads/hour. The test tube was placed into sample carrier C and lowered into the cobalt unit to the same depth used in the grafting experiments (104 cm). Each sample was given an irradiation dose of 50 Krads.

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[6]Lindberg, Division of Sola Basic Industries, Watertown, WS., Type 59344.

The remaining 13 chips were used as controls and were again irradiated by the Picker C-9 Cobalt Unit following the chip characterization procedure.

After all samples were irradiated, the chips were stripped of aluminum foil and measured by the TLD-Reader. The count values of the 13 control chips were compared to their previous Picker C-9 count values as follows: (Trial count - Standard B count)/Standard B count = response difference (RD). If there was a difference in the control chip count compared to the Standard B count, then the TLD-Reader calibration had drifted from the previous reading, since individual chip response is repeatable to within 1% accuracy (personal communication in November, 1987 with Dr. Steven McKeever, Dept. of Physics, Oklahoma State University, Stillwater, OK). An adjustment, based upon an average of the RD's for that run, was calculated to compensate for the drift: Standard B count * (1 + average RD) = adjusted Standard B count. The irradiation dose received by the chips in the Nuclear Engineering Cobalt-60 Unit was calculated as follows: (Nuclear Engineering count * 49,950 rads)/adjusted Standard B count = irradiation dose. The irradiation dose was plotted against the chip position along the vertical axis of the Nuclear Engineering Cobalt-60 Unit. A second calibration was performed following the same procedure. The irradiation dose measurements of each position along the axis were averaged and confidence limits set.

Results

The TLD's initially characterized using the Picker C-9 Cobalt Unit demonstrated a very broad response, ranging between 21,862 to 45,929 counts (average = 35,732). This was unexpected since the TLD's were of the same lot and according to manufacturer's specifications should have been within 10% of the mean. These values were designated as Standard A. Subsequent comparisons of the controls from the first trial to their Standard A values showed responses ranging from an increase of 88.4% to a decrease of 14.0%. Such a diversity in the controls annulled any attempts to use this information for calibration purposes.

The same chips were reannealed and then recalibrated in the Picker C-9 Cobalt Unit, and the results designated as Standard B. The range of counts was much more uniform (26648 to 35530) with a mean of 30866. Using a 99.9% confidence limit, all samples were within 2.8% of the mean. A comparison of values for Standards A and B showed no pattern to the differences, as seen in Table 11.

The control count values of Trial 1 were compared to their Standard B values. Trial 1 controls had consistently and uniformly greater count values (mean = 23.8%, standard deviation = 5.5825, standard error = 1.548) than their Standard B counter parts. This indicated a calibration drift in the TLD-Reader, yet a good correlation existed between the values. Table 12 shows the count values and the calculated irradiation doses.

TABLE 11. Comparison of TLD-Reader counts between Standards A and B

Chip	A Value	B Value	% Dif	Chip	A Value	B Value	% Dif
1	30795	31366	1.8	26	44030	32678	-34.7
2	34851	30842	-13.0	27	43687	30888	-41.4
3	31304	31411	0.3	28	31854	30242	-5.3
4	35891	30387	-18.1	29	44692	31312	-42.7
5	34387	31330	-9.8	30	42569	31354	-35.8
6	35225	31051	-13.4	31	27197	29663	8.3
7	28449	31128	8.6	32	45212	33650	-34.4
8	36515	31369	-16.4	33	29170	29144	-0.1
9	33414	30908	-8.1	34	44784	32159	-39.3
10	35367	31278	-13.1	35	44566	30772	-44.8
11	29590	30740	3.7	36	45531	31504	-44.5
12	29963	30649	2.2	37	39687	29398	-35.0
13	28879	28883	0.0	38	44020	31146	-41.3
14	26538	31583	16.0	39	24620	30121	18.3
15	26797	30517	12.2	40	21862	30746	28.9
16	30267	27908	-8.5	41	39747	30295	-31.2
17	28388	30290	6.3	42	44227	28561	-54.9
18	29171	30160	3.3	43	28211	26697	-5.7
19	28482	28394	-0.3	44	26436	26648	0.8
20	33807	29517	-14.5	45	45602	32585	-39.9
21	32305	30764	-5.0	46	45795	35530	-28.9
22	29936	30171	0.8	47	44011	32045	-37.3
23	40081	31539	-27.1	48	45929	34072	-34.8
24	42077	33627	-25.1	49	42733	32805	-30.3
25	42214	32632	-29.4				

Standard B count values averaged 1.9% lower in value (standard deviation = 5.0883, standard error = 1.4112) than Trial 2 control chip values and showed a consistent deviation around the mean. Count values and calculated radiation doses are presented on Table 13.

Figure 12 shows the scatter between the samples of Trials 1 and 2.

Values for the average irradiation intensity based on position are shown in Table 14 and plotted in Figure 13. The irradiation exposure

TABLE 12. Comparison between Trial 1 and Standard B count values with adjusted irradiation doses

Chip	Position	Trl 1	Stnd B	% Dif	Calc Dose
40	control	41182	30746	33.9	54000
39	control	38684	30121	28.4	51800
44	control	32065	26648	20.3	48600
14	1	18868	31583	-40.3	24100
15	2	26841	30517	-12.0	35500
31	3	33888	29663	14.2	46100
43	4	36517	26697	36.8	55200
17	5	36867	30290	21.7	49100
7	6	39929	31128	28.3	51800
19	7	36689	28394	29.2	52100
13	8	38605	28883	33.7	53900
33	9	40437	29144	38.7	56000
18	10	39154	30160	29.8	52400
11	11	40332	30740	31.2	52900
22	12	36507	30171	21.0	48800
12	control	35802	30649	16.8	47100
16	control	32881	27908	17.8	47500
1	1	16459	31366	-47.5	21200
3	2	26867	31411	-14.5	34500
28	3	33393	30242	10.4	44600
21	4	37438	30764	21.7	49100
9	5	38430	30908	24.3	50200
20	6	38031	29517	28.8	52000
5	7	39988	31330	27.6	51500
2	8	40616	30842	31.7	53100
6	9	40920	31051	31.8	53200
10	10	40982	31278	31.0	52900
4	11	38982	30387	28.3	51800
8	12	37860	31369	20.7	48700
37	control	38065	29398	29.5	52200
41	control	37397	30295	23.4	49800
23	control	37781	31539	19.8	48300
24	1	20175	33627	-40.0	24200
25	2	28822	32632	-11.7	35600
30	3	36517	31354	16.5	47000
49	4	42825	32805	30.5	52700
27	5	42563	30888	37.8	55600
47	6	43422	32045	35.5	54700
38	7	46099	31146	48.0	59700
26	8	45551	32678	39.4	56200
42	9	43621	28561	52.7	61600
35	10	45906	30772	49.2	60200
29	11	43863	31312	40.1	56500

TABLE 12 (continued)

Chip	Position	Trl 1	Stnd B	% Dif	Calc Dose
34	12	43796	32159	36.2	55000
32	control	43192	33650	28.4	51800
36	control	41054	31504	30.3	52600
45	control	39233	32585	20.4	48600
46	control	42631	35530	20.0	48400
48	control	41002	34072	20.3	48600

time for Sample 4 of Trial 2 was not regulated properly, so that result was not included in determining mean values.

All samples demonstrated a definite difference in average dose along the vertical axis. The lower region, positions 1-4, showed a percent of expected dose of 46.1%, 69.8%, 90.1%, and 104.1% respectively. Such a pattern was expected since the NVP grafting experiments showed a decline of graft depth in that region. Between regions 4 and 12, the intensity field is fairly uniform. The average dose in this region is 53,200 ±2.7% rads (95% confidence level) and is shown in Figure 13. This value falls within the ±20% range for a 50 Krad dose as specified in the "Application for Cobalt-60 Irradiation" form provided by the Nuclear Engineering Laboratory. A correction factor of 1.064 can be multiplied to the reported Nuclear Engineering Laboratory dose to provide a more accurate irradiation dose.

TABLE 13. Comparison between Trial 2 and Standard B count values with adjusted irradiation doses

Chip	Position	Trl 2	Stnd B	% Dif	Calc Dose
44	control	27561	26648	3.4	52600
43	control	27894	26697	4.5	53200
16	control	26839	27908	-3.8	49000
13	1	15600	28883	-46.0	27500
33	2	25107	29144	-13.9	43900
37	3	31824	29398	8.3	55100
20	4	32501	29517	10.1	56000
31	5	36707	29663	23.7	63000
39	6	37386	30121	24.1	63200
18	7	37009	30160	22.7	62500
22	8	35179	30171	16.6	59300
28	9	38519	30242	27.4	64800
17	10	35836	30290	18.3	60200
41	11	36326	30295	19.9	61000
4	12	32904	30387	8.3	55100
19	control	27008	28394	-4.9	48400
42	control	30981	28561	8.5	55200
15	1	13656	30517	-55.3	22800
12	2	20403	30649	-33.4	33900
11	3	27048	30740	-12.0	44800
40	4	31485	30746	2.4	52100
21	5	29403	30764	-4.4	48600
35	6	34875	30772	13.3	57700
2	7	32140	30842	4.2	53000
27	8	35482	30888	14.9	58500
9	9	32268	30908	4.4	53100
6	10	32837	31051	5.8	53800
7	11	32142	31128	3.3	52600
38	12	31426	31146	0.9	51400
10	control	29259	31278	-6.5	47600
29	control	31559	31312	0.8	51300
5	control	28978	31330	-7.5	47100
30	control	30976	31354	-1.2	50300
1	1	14143	31366	-54.9	23000
8	2	21626	31369	-31.1	35100
3	3	26423	31411	-15.9	42800
36	4	31629	31504	0.4	51100
23	5	30989	31539	-1.7	50000
14	6	32867	31583	4.1	53000
47	7	31621	32045	-1.3	50200
34	8	35565	32159	10.6	56300
45	9	34230	32585	5.0	53500
25	10	33460	32632	2.5	52200

TABLE 13 (continued)

Chip	Position	Trl 2	Stnd B	% Dif	Calc Dose
26	11	34455	32678	5.4	53700
49	12	31042	32805	-5.4	48200
24	control	32805	33627	-2.4	49700
32	control	33442	33650	-0.6	50600
48	control	31717	34072	-6.9	47400
46	control	32790	35530	-7.7	47000

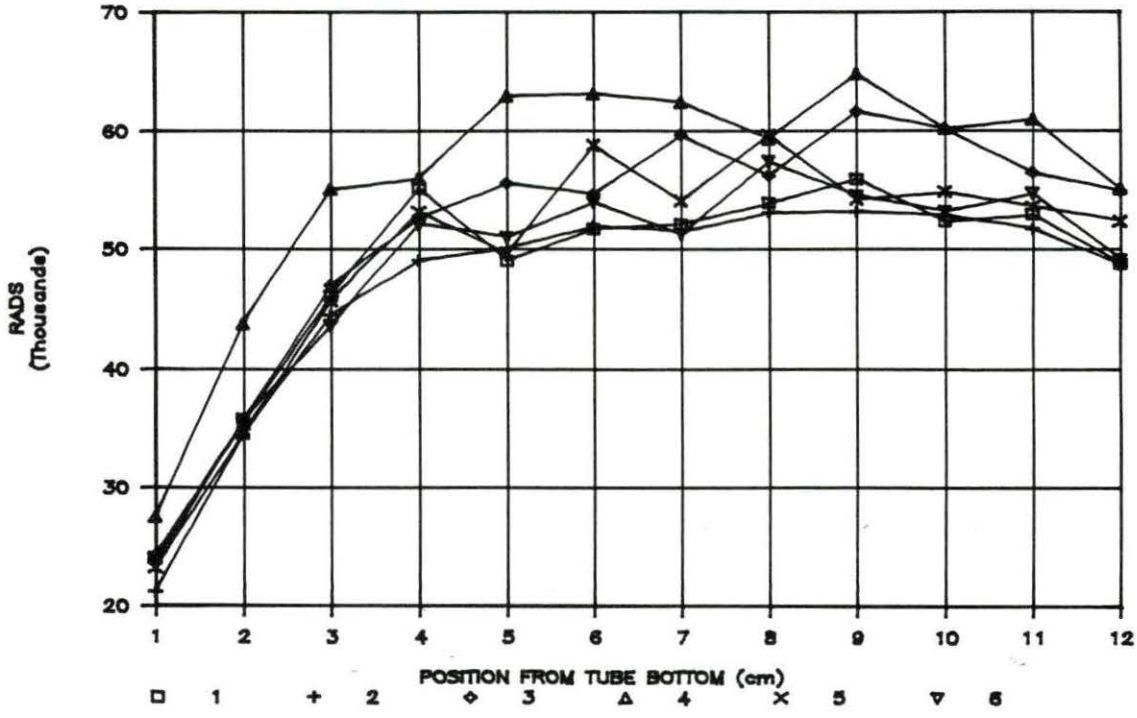

FIGURE 12. Graph of intensity profiles for Trial 1 (samples 1-3) and Trial 2 (samples 4-6) showing similarities of irradiation dose vs position. Position 7 is center alignment for 104 cm chamber depth

TABLE 14. Average dose and percent of expected dose

Position	Smpl 1	Smpl 2	Smpl 3	Smpl 5	Smpl 6	Average Dose	Percent Expected Dose
1	24100	21200	24200	22800	23000	23000	46.1
2	35500	34500	35600	33900	35100	34900	69.8
3	46100	44600	47000	44800	42800	45000	90.1
4	55200	49100	52700	52100	51100	52000	104.1
5	49100	50200	55600	48600	50000	50700	101.4
6	51800	52000	54700	57700	53000	53800	107.6
7	52100	51500	59700	53000	50200	53300	106.7
8	53900	53100	56200	58500	56300	55600	111.2
9	56000	53200	61600	53100	53500	55500	111.0
10	52400	52900	60200	53800	52200	54300	108.6
11	52900	51800	56500	52600	53700	53500	107.0
12	48800	48700	55000	51400	48200	50400	100.8

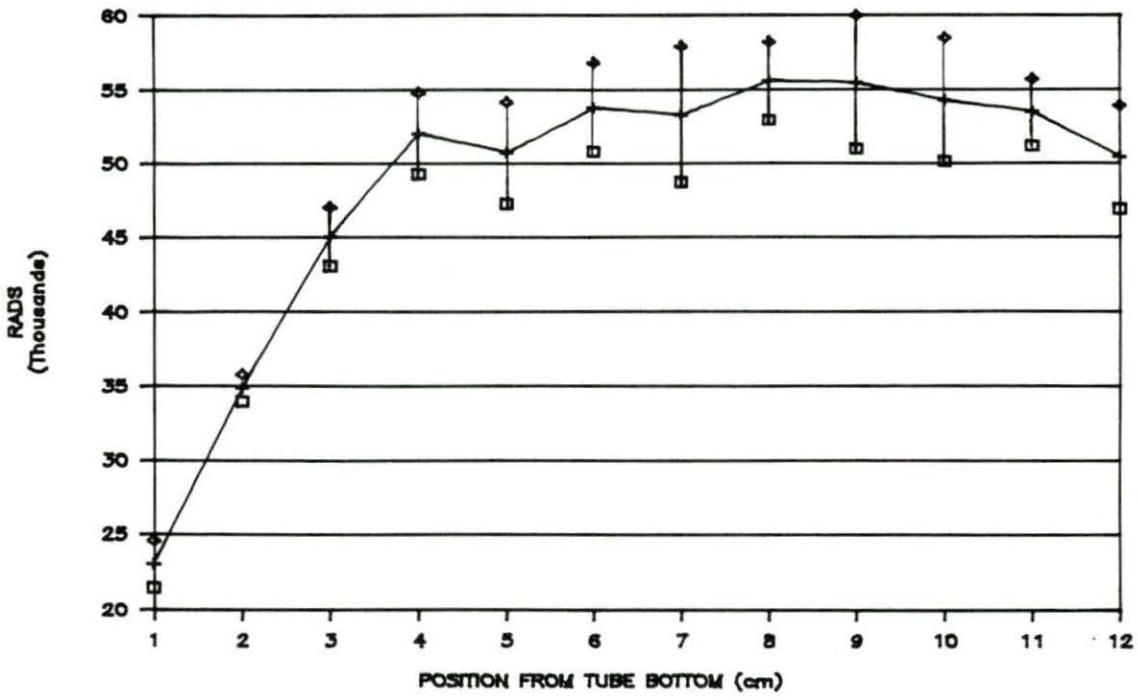

FIGURE 13. Graph of average intensity profile of irradiation dose vs position in the Nuclear Engineering Cobalt-60 Unit for samples 1, 2, 3, 5 and 6 of Trials 1 and 2 (error bars at 95% confidence)

Discussion

A large difference was found between the initial calibration of the chips on the Picker C-9 Cobalt Unit (A value of Table 11) and the second calibration (B value of Table 11). This was most probably due to the difference in the annealing procedure. The chips were initially annealed in the Environmental Health and Safety Laboratory oven. The thermostat on that oven is not reliable, and the initial heat up extends beyond the target annealing temperature of 400° C. After

annealing, the chips were placed near a cool window and then transported about 1.5 miles for the second annealing. For the latter three chip preparations, the first annealing was done in a well controlled Lindberg muffler oven. The chips were quickly removed, allowed to slightly cool, and then transported only a few feet to the second oven.

After these experiments were performed, information was found which stated that the annealing procedure is very critical for lithium fluoride (TLD-100), and that if the procedures are not strictly the same, significantly different results can be obtained from repeated irradiations to the same exposure (Busuoli, 1981). This would explain the large diversity between Standards A and B.

The following are other factors that affect the sensitivity and reproducibility of the TLD chips:

- should have an oven devoted to preparing TLD chips, to avoid contamination which produces distorted signals,
- should have rapid, consistent cool down of the chips after the 400° C annealing to assure high sensitivity,
- repeated large irradiation doses can produce permanent radiation damage to the crystal and thus decrease response,
- readout instruments must be stable, including heating rate, cooling rate, maximum readout temperature, and time held at maximum readout temperature,
- different geometries for calibration fields and unknown fields affect sensitivity,
- handling of TLD chips can cause damage over time (Regulla, 1981),
- the same irradiation source should be used for calibration as for experimental irradiation, otherwise the possible

> difference in energy response of the phospher to the calibration and the experimental irradiation energies must be corrected for (Cameron et al., 1968).

The quality of the TLD-Reader caused difficulty in getting reliable results. The drift in its calibration forced the use of standards every time to insure accuracy. This made the exercise more expensive, time consuming, and error prone; however, significant results were obtained.

These data appear to show that the center of the intensity field may be miscalculated by 1 cm (8 cm center rather than 7 cm); however, if the Nuclear Engineering Laboratory calibration tables are used, the degree of freedom of the mechanical system of the Cobalt-60 Unit could compensate for misalignment.

Conclusions

The accuracy of the Nuclear Engineering Laboratory Cobalt-60 Unit can be reported at an increased level of confidence for the (17 * current dose rate) position. Instead of the present ±20% accuracy, these data would support narrowing it to ±2.7% (95% confidence level) for the 4-12 region. It has also been shown that the intensity sharply decreases from 4 cm to the bottom. This information is important to researchers desiring knowledge of exact dosages given to specimens as a function of position in the Nuclear Engineering Laboratory Cobalt-60 Unit.

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