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# SIZE- AND SURFACE-DEPENDENT UPTAKE OF COLLOID PARTICLES INTO THE LYMPHATIC SYSTEM

F. Ikomi, G.K. Hanna, G.W. Schmid-Schönbein

Department of Bioengineering and Biomedical Engineering, University of California San Diego (FK,GWSS) and Alliance Pharmaceuticals (GKH), San Diego, California, USA

*ABSTRACT*

*PURPOSE: To examine the effect of particle size and surface characteristics on colloidal particle uptake from subcutaneous tissue into the lymphatic system.*

*MATERIALS AND METHODS: Perfluorocarbon emulsion ZY13163 (median particle diameter, 0.08 μm; surfactant, egg yolk phospholipid), ZY13164 (median particle diameter, 0.36 μm; surfactant, egg yolk phospholipid), ZY13199 (0.18 μm, surfactants Tetronic904) and ZY14001 (0.11 μm, surfactant Pluronic L121) were individually injected into the dorsal skin of the hind foot of rabbits. Lymph flow rates and particle concentrations were determined in prenodal lymph fluid after injection, with and without massage of the skin over the injection site.*

*RESULTS: In the first 24 hours after injection, the decreasing order of colloid flux without massage was as follows: ZY13199 > ZY14001 > ZY13163 > ZY13164. Lymph flow rates, lymph leukocyte concentrations and colloid concentrations increased substantially with mechanical skin massage.*

*CONCLUSIONS: Transport of colloids into lymphatic vessels depends on particle size and surface characteristics. Without massage, colloids with egg yolk phospholipid exhibit size dependent decrease in uptake into lymphatics, while with massage only a weak correlation with particle size is present.*

*INTRODUCTION*

Lymphography serves to observe intranodal architecture for the detection of tumor metastasis in lymph nodes (12,26). Reliable tumor staging is the basis for therapy planning and prognosis in patients with malignant tumors. To determine tumor staging it is necessary to distinguish intact lymph nodes from metastatic ones. While lymphangiography has the particular advantage of providing information about the internal structure of lymph nodes, it is invasive, the use of oily contrast media may cause a number of serious side effects (4), and only a limited number of lymph nodes can be imaged (26).

In addition to lymphangiography, more recently, a less invasive technique of percutaneous lymphography has been proposed (33). In percutaneous lymphography, radiopaque colloids are administered into the interstitium. The colloids are taken up from the interstitium into the initial lymphatics and accumulate in regional lymph nodes (5,14,29). Wolf et al. (33) have shown that percutaneous lymphography performed with computer tomography effectively depicts the intranodal distribution of macrophages in normal lymph nodes. There is evidence that smaller colloids are taken up more easily into lymphatic system compared to larger colloids (7,19,30). No systemic study, however, has been carried out regarding the relationship

between the characteristics of colloids and their lymphatic uptake from the interstitium.

Accumulation of colloids in lymph nodes requires (a) uptake of particles from interstitial tissue into the lymphatic vessels, (b) transport along the lymphatic vessels into the lymph nodes, and (c) binding to cells in the node. The individual steps are, however, not well defined. In this report, we will focus on the first step, that is, the uptake of interstitially administered colloids into the *initial* lymphatic vessels (lymphatic channels with endothelium but no smooth muscle and without spontaneous contraction). We hypothesize that particle size and surface characteristics of colloids, which may depend on the choice of the surfactant, are the main determinants for their movement from the interstitium into the lymph nodes. To examine this hypothesis, we measured the amount of colloid transport in prenodal lymph fluid after subcutaneous injection of several colloid suspensions with different particle diameters and with different surfactants.

## *MATERIALS AND METHODS*

### *Animal Preparation.*

The experiments were performed on 32 male New Zealand White rabbits (2.0-3.0 kg, Simunec, Vista, CA). The animals were housed in a heated and light controlled facility. The studies were previously reviewed and approved by the Animal Subjects Committee of the University of California San Diego.

Rabbits were anesthetized with ketamine chloride (20 mg/kg iv.) and pentobarbital sodium (20 mg/kg iv.). Intermittent boluses of pentobarbital sodium were administered during the experiment as dictated by a toe-pinch test. The trachea was cannulated to ensure patent airways. A catheter was inserted into the right external jugular vein for administration of drugs and into the carotid artery for the measurement of systemic blood pressure. Esophageal temperature was monitored and maintained at 38 to 39°C with a heating pad.

Lymph was collected by cannulating one of the afferent lymphatics of the popliteal node in the lower left hind leg as described previously (7,9). PE-10 tubing was used as lymphatic cannulae. Cannulations were made under a stereomicroscope. To prevent escape of lymph into other lymphatics pathways and to increase lymph flow rates, the free tip of the lymph drainage cannula was placed 5 cm below the cannulation site. Lymph fluid was collected in 1 ml syringes.

The volume of the collected lymph was measured with a 50 µl microsyringe. The lymph flow rate was computed from the ratio of volume and lymph collection time. The lymph leukocyte counts were determined with a hemocytometer.

### *Perfluorocarbon Colloids.*

PFC colloids with different particle diameter and different surfactants were used. Egg yolk phospholipid (EYP) was used as surfactant in the suspensions ZY13163 and ZY13164 (Alliance Pharmaceutical Corp., San Diego, CA). The surfactants Tetronic 904 and Pluronic L121 were used in ZY13199 and ZY14001, respectively. The composition of PFC and surfactant of each suspension are summarized in *Table 1.* The median diameter of the particles were determined by photosedimentation (Capa 700; Horiba, Kyoto, Japan) for ZY13164 and ZY13199 as well as sedimentation field-flow fractionation (model 5101; FFFractionation, Salt Lake City, Utah) for ZY13163 and ZY14001. The median diameter values for LA11063 and ZY12149 were previously measured (7).

### *Measurements of Colloid Concentration.*

PFC colloid concentrations of all lymph samples were determined by gas chromatography (GC).

To determine the concentration of ZY13164 particles, the following labeling

**TABLE 1**
**Particle Diameter and Composition of Each Colloid, and Assay for Concentration of Lymph Perfluorocarbon**

| colloid | particle diameter median ± SD (μm) | surfactant (% wt/vol) | PFC (% wt/vol) | assay for lymph PFC concentration |
|---|---|---|---|---|
| LA11063 | 0.34 ± 0.24 | EYP (4) | PFOB (60) | fluorescence[a] |
| ZY12149 | 0.06 ± 0.03 | EYP (6.7) | PFOB (40) + PFDB (20) | fluorescence[a] |
| ZY13163 | 0.08 ± 0.05 | EYP (4) | PFOB (40) + PFDB (20) | GC |
| ZY13164 | 0.36 ± 0.20 | EYP (4) | PFDB (60) | fluorescence and GC |
| ZY13199 | 0.18 ± 0.10 | Tetronic904 (4) | PFOB (55) + PFDB (5) | GC |
| ZY14001 | 0.11 ± 0.06 | Pluronic L121 (4) | PFOB (55) + PFDB (5) | GC |

(a, data from ref. (7). PFC, perfluorocarbon. EYP, egg yolk phospholipid. PFOB, perfluorooctyl bromide (perflubron). PFDB, perfluorodecyl bromide. GC, gas chromatography.

technique was used. A $10^{-3}$ M stock solution of the fluorescent membrane phospholipids dye PKH-26 (Zynaxis Cell Science, Inc., Malvern, PA) was added to the emulsion (final concentration of $10^{-5}$ M) (7, 24). The mixtures were gently stirred in the dark for 5 min at room temperature. Subsequently, the mixture of PKH-26 and ZY13164 emulsion was added in equal volume to rabbit plasma and gently mixed for 1 min to stop the staining reaction. The final concentration of fluorescently stained ZY13164 was 30% w/v PFDB. ZY13164 uses egg yolk phospholipid as surfactant and each colloidal particle is presumed to be covered by a phospholipid layer on its surface.

Colloidal particles of fluorescently stained LA11063 and ZY12149 were observed under a fluorescence microscope using a 50x oil immersion objective (N.A. 1.0, Leitz, Wezlar, Germany). Each sample was placed in a fluid layer (thickness 100 μm) between a glass slide and a glass coverslip. To elicit fluorescent images, the samples were illuminated with a 200 W mercury lamp. The light was passed through a quartz collector, heat filter (Model KG-2, Carl Zeiss Inc., Thornwood, NY), and an excitation filter (515-560 nm, E. Leitz Inc., Rockleigh, NJ) to epi-illuminate the sample. Fluorescence emission from the sample was passed through a band-pass filter (580 nm, E. Leitz Inc.) and recorded by a silicone intensified target television camera (Model 66, Dage-MIT. Inc., Michigan City, IN). The images were stored on video tape and analyzed on a monitor. The number of particles were counted on the monitor 5 times for each sample. ZY13164, per se, or just saline (Baxter Healthcare Corporation, Deerfield, IL) showed no fluorescence with this staining protocol. In order to calibrate the particle concentration

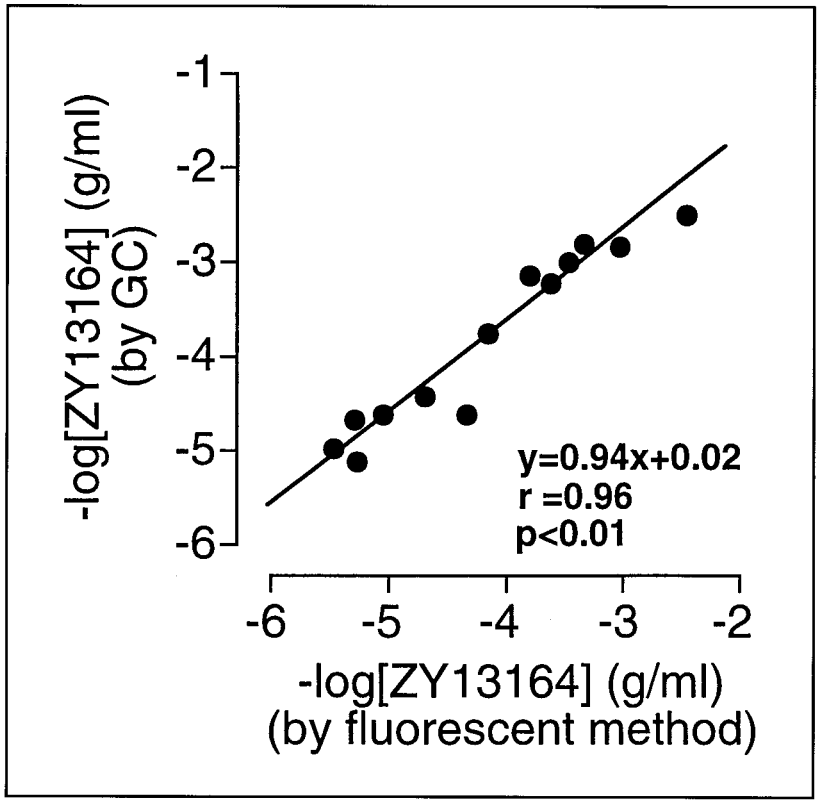


*Fig. 1. Correlation between Fluorescent method and GC for particle concentration measurements. Each sample was divided into two groups and measured by use of each technique. The gradient of the regression equation was not significantly different from 1 ($p > 0.4$) and the intercept was not significantly different from 0 ($p > 0.5$).*

measurements, a sample of the fluorescently stained ZY13164 stock solution with known particle count was diluted in saline with 0.5 % bovine serum albumin (BSA; Sigma Chemical Company, St. Louis, MO) ($10^{-4}$, $10^{-5}$, $10^{-6}$ dilutions) and at each dilution the particle concentrations were determined. ZY13164 concentrations in each lymph sample were determined by means of this calibration curve.

PKH-26 does not stain pure PFC particles without phospholipids, but stains phospholipid membranes without PFC. To verify therefore the perfluorocarbon particle concentration measurements with the fluorescent technique, randomly selected samples were compared with measurements obtained by GC. Such a comparison yielded a close linear correlation (n=13 samples) *(Fig. 1).*

*Lymph Collection.*

All lymph collections reported in this study were carried out in the presence of a slow leg rotation driven by a crank attached to an electric motor, since otherwise insufficient lymph fluid volume could be collected for measurements (6,9). The leg was rotated in a sagittal plane with frequency of 0.3 Hz and a diameter of about 8 cm at the level of the toes. To study enhancement of lymph transport by local mechanical deformation of the dermis, gentle hand massage was performed on the rabbit's skin over the site of the colloid injection. For this purpose, the fore finger, middle finger and third finger were positioned on the skin with slight pressure and rotated parallel to the skin surface at a frequency of 2 to 3 Hz with

diameter of about 3 cm. Massage was applied for 15 min at the end of each experimental protocol during the slow leg rotation. The massage was carried out by a single operator for all studies and gave reproducible lymph flow rates.

*Protocol.*

After successful lymphatic cannulation, a stabilizing period of about 30 min with passive whole leg rotation was maintained. Lymph collection was performed 2 hrs (n = 4 rabbits) and 24 hrs (n = 4 rabbits) after injection of the colloids (0.1 ml of 30% wt/vol) into the dorsal skin of the rabbit's foot. To compare the present data with previous results, colloids were added in equal volume to rabbit plasma. The final concentration of subcutaneously injected colloids were 30% wt/vol PFC. Colloids were injected separately in different animals but in all rabbits close to the same anatomical site. A rabbit was used only for one time point. Lymph fluid was collected in each rabbit for 2 hrs without massage followed by 15 min of collection with massage. The samples were assayed for lymph flow rate, leukocyte count, and colloid concentrations. After this procedure, an albumin solution conjugated with Evans blue dye was injected at the same location as the stained perflubron emulsion. The blue color serves to delineate the drainage area of the cannulated lymphatic duct.

*Statistical Analysis.*

All results except colloid diameter are expressed as mean ± standard error (SE). Colloid particle diameters are expressed as mean ± standard deviation (SD). Two tailed Student's t test for paired or unpaired data, or a one-way analysis of variance followed by Newman-Keuls' test was used to test for significance between groups. Significance of simple linear regression was tested by analysis of variance. Other tests for simple linear regression were carried out according to Zar (34). Differences between groups were considered significant at $p < 0.05$.

*RESULTS*

*GC and Fluorescent Method*

A close correlation was found between ZY13164 concentration of lymph samples measured by GC and by the direct counting technique. The regression equation derived from paired values of several lymph samples was: $y = 0.94 x + 0.02$ ($n = 13$ samples, $r = 0.96$, $p < 0.01$) *(Fig. 1)*. The slope of the equation was not significantly different from 1 ($p > 0.4$) and the intercept was not significantly different from 0 ($p > 0.5$). Thus the current measurements can be compared with our previous results obtained by the fluorescent labeling and counting technique (7).

*Lymph Flow Rate and Lymph Leukocyte Count after Injection of ZY13163 and ZY13164.*

During lymph collection, the rabbit foot was rotated passively in a vertical circular direction at a frequency of 0.3 Hz with diameter of 8 cm. This passive leg movement served to increase the lymph flow rate (0.188 ± 0.024 ml/h) from a much lower value without such motion (0.007 ± 0.002 ml/h) (6). Injection of LA11063, a PFOB emulsion with EYP, did not yield significant difference in lymph flow rate and lymph leukocyte concentration compared with saline injection (8).

No statistically significant changes in lymph flow rate were observed over 24 h, either with or without massage *(Fig. 2a)*. In all rabbits and at all times foot massage served to increase the lymph flow rate significantly compared to measurements without massage and no statistically significant differences in lymph leukocyte count were observed over 24 h *(Fig. 2b)*. All rabbits had significantly increased lymph leukocyte counts after massage compared to measurements without massage.

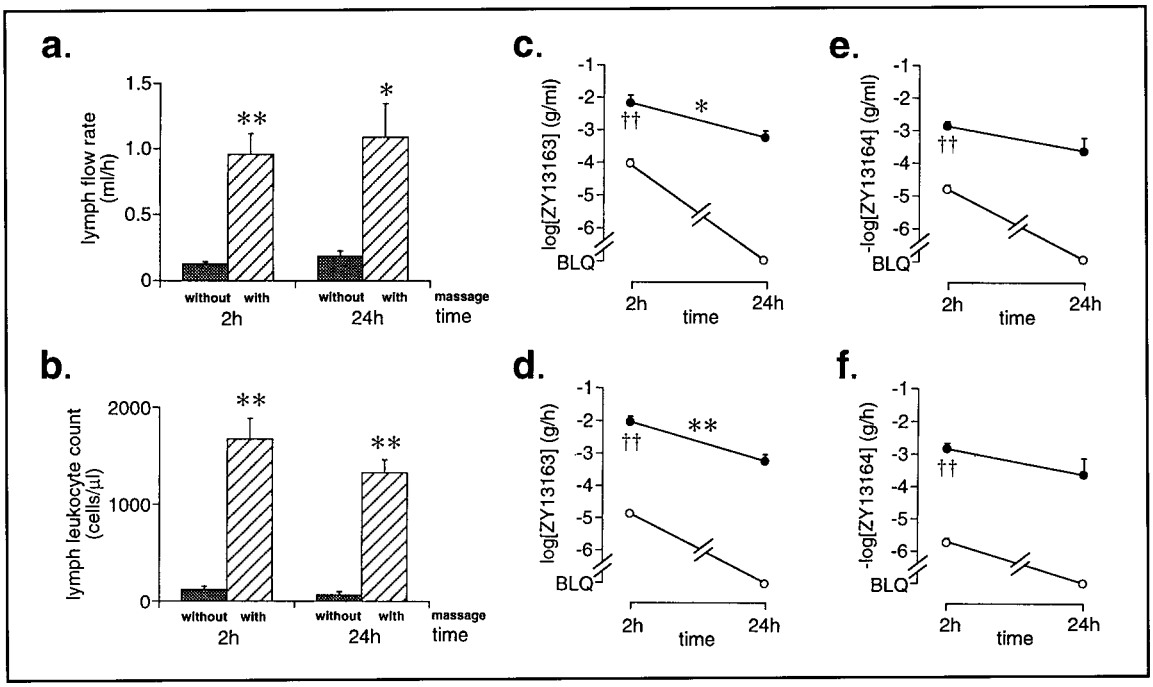


*Fig. 2. (a) Lymph flow rate at 2 hrs and 24 hrs after ZY13164 injection in rabbit foot dorsal skin with (striped column) and without (gray column) massage. No significant differences were observed between 2 hrs and 24 hrs. *p<0.05, **p<0.01 with vs. without massage. (b) Lymph leukocyte count at 2 hrs and 24 hrs after ZY13164 injection in rabbit foot dorsal skin with (striped column) and without (gray column) massage. No significant differences were observed between 2 hrs and 24 hrs. **p<0.01 with vs. without massage. (c) and (d) ZY13163 concentration in lymph samples (c) and flux through an afferent lymphatic (d) at 2hrs and 24hrs after subcutaneous injection with (closed circle) and without (open circle) massage. The ordinate shows the concentration (g/ml) and flux (g/h) of ZY13163 on a logarithmic scale. The abscissa denotes the time after ZY13163 injection in rabbit's foot dorsal skin. (e) and (f) ZY13164 concentration in lymph samples (e) and flux through an afferent lymphatic (f) at 2hrs and 24hrs after subcutaneous injection with (closed circle) and without (open circle) massage. The ordinate shows the concentration (g/ml) and flux (g/h) of ZY13164 on a logarithmic scale. The abscissa denotes the time after ZY13164 injection in rabbit's foot dorsal skin. Each value is presented as a mean ±SE (vertical bar). Animal number n = 4 in each group. BLQ = below limit of quantitation. *p<0.05, **p<0.01. ††p<0.01 with vs. without massage.*

Almost identical trends in lymph flow rates and lymph leukocyte concentrations were observed with the ZY13164 emulsion.

*Lymph Concentration after Injection of ZY13163 and ZY13164.*

All lymph samples contained ZY13163 emulsion particles. But the concentration was about 3 orders of magnitude lower than the injected concentration (30% wt/vol PFC). Both with and without massage, the ZY13163 concentration decreased in a time dependent manner. LA11063 concentration with massage was significantly greater than the concentration without massage. After 24 h all rabbits showed undetectable levels of ZY13163 concentration without massage, although with massage the levels of ZY13163 could be detected again *(Fig. 2c, Table 2)*.

Since both lymph flow rates and ZY13163 concentrations were all increased after massage, the values for the colloid flux with versus without massage resulted in a large difference in magnitude *(Table 3)*. The time course of the ZY13163 flux exhibited a similar pattern as the ZY13163 concentration since the lymph flow rates remained

**TABLE 2**
**Summary of Colloid Concentration in Lymph Fluid**

| Time After Massage Injection | | Flux* ZY13163 | ZY13164 | ZY13199 | ZY14001 |
|---|---|---|---|---|---|
| 2 hr | No | 104.1±25.7 | 14.5±3.7 | 353.5±202.4 | 264.5±218.7 |
| | Yes | 12854±5457 | 1506±510 | 17360±2538 | 17736±5989 |
| 24 hr | No | BLQ | BLQ | 115.6±40.0 | 24.3±7.1 |
| | Yes | 821±401 | 537±307 | 1084±384 | 4369±1252 |

* Values are mean _ SE. The data are given in micrograms per millilitter. Sample size n = 4 rabbits in each group. BLQ = below limit of quantitation.

**TABLE 3**
**Summary of Colloid Flux through Lymphatics**

| Time After Massage Injection | | Flux* ZY13163 | ZY13164 | ZY13199 | ZY14001 |
|---|---|---|---|---|---|
| 2 hr | No | 13.2±1.3 | 2.0±0.6 | 49.6±29.3 | 50.0±44.6 |
| | Yes | 10893±4087 | 1738±712 | 17629±5505 | 15688±5658 |
| 24 hr | No | BLQ | BLQ | 16.7±6.1 | 1.7±0.9 |
| | Yes | 741±261 | 797±537 | 1106±411 | 4118±1610 |

* Values are mean ± SE, The data are given in micrograms per hour. Sample size n = 4 rabbits in each group. BLQ = below limit of quantitation. Colloid flux = lymph perfluorocarbon concentration x lymph flow rate.

relatively constant with respect to time *(Fig. 2d, Table 3).*

Lymph concentration of ZY13164 after 2 hrs of collection was about 4 orders of magnitude lower than the injected concentration (30% w/v PFC). Without massage, the lymph ZY13164 concentrations decreased with time. ZY13164 concentrations with massage were significantly greater than without massage *(Fig. 2e, Table 2).*

Similar to the case of ZY13163, since lymph flow rate and ZY13164 concentration were increased after massage, the colloid flux values during massage increased also by several orders of magnitude compared to the situation without massage *(Fig. 2f, Table 3).*

*Lymph Flow Rate, Leukocyte Count and Lymph Concentration after Injection of ZY13199 and ZY14001.*

The time course of the lymph flow rate and the lymph leukocyte count after ZY13199 and ZY14001 injection were similar

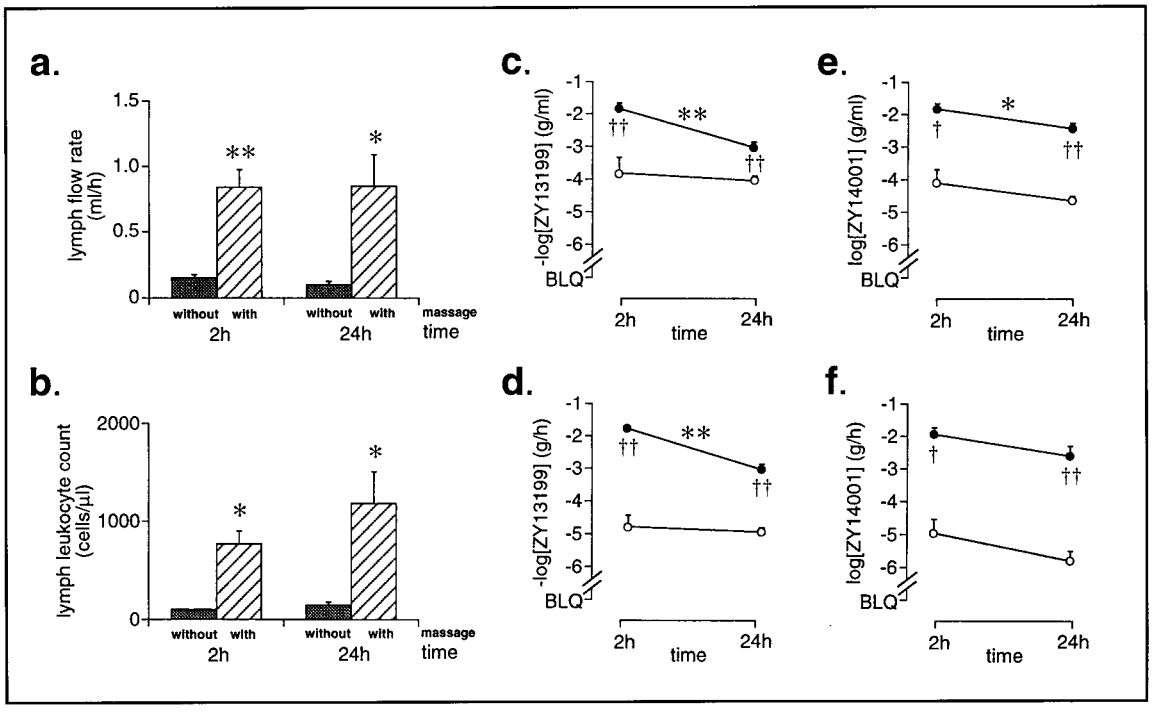


*Fig. 3. (a) Lymph flow rate at 2 hrs and 24 hrs after ZY14001 injection in rabbit foot dorsal skin with (striped column) and without (gray column) massage. No significant differences were observed between 2 hrs and 24 hrs. *p<0.05, **p<0.01 with vs. without massage. (b) Lymph leukocyte count at 2 hrs and 24 hrs after ZY14001 injection in rabbit foot dorsal skin with (striped column) and without (gray column) massage. No significant differences were observed between 2 hrs and 24 hrs. *p<0.05 with vs. without massage. (c) and (d) ZY13199 concentration in lymph samples (c) and flux through an afferent lymphatic (d) at 2hrs and 24hrs after subcutaneous injection with (closed circle) and without (open circle) massage. The ordinate shows the concentration (g/ml) and flux (g/h) of ZY13199 on a logarithmic scale. The abscissa denotes the time after ZY13199 injection in rabbit's foot dorsal skin. (e) and (f) ZY14001 concentration in lymph samples (e) and flux through an afferent lymphatic (f) at 2hrs and 24hrs after subcutaneous injection with (closed circle) and without (open circle) massage. The ordinate shows the concentration (g/ml) and flux (g/h) of ZY14001 on a logarithmic scale. The abscissa denotes the time after ZY14001 injection in rabbit's foot dorsal skin. Each value is presented as a mean with SE (vertical bar). Animal number n = 4 in each group. BLQ = below limit of quantitation. *p<0.05, **p<0.01. †p<0.05, ††p<0.01 with vs. without massage.*

to the patterns found following ZY13163 or ZY13164 injection. No significant changes in lymph flow rate were observed over 24 hrs, with and without massage *(Fig. 3a)*. With all rabbits and at all times, foot massage increased the lymph flow rate significantly compared to values without massage. No significant changes in lymph leukocyte counts were observed over 24 h *(Fig. 3b)*. Massage also increased lymph leukocyte counts in all rabbits *(Fig. 3b)*.

Lymph concentration of ZY13199 or ZY14001 after 2 hrs of collection were about 3 to 4 orders of magnitude lower than the injected concentration (30% wt/vol PFC). With massage, the particle concentrations decreased in time. ZY13199 and ZY14001 concentrations with massage were significantly greater than the concentration without massage *(Fig. 3c,e, Table 2)*. Similar to the case of ZY13163, since lymph flow rate and ZY13199 and ZY14001 concentrations increased after massage, the colloid flux values also showed a large rise with massage *(Fig. 3d,f, Table 3)*.

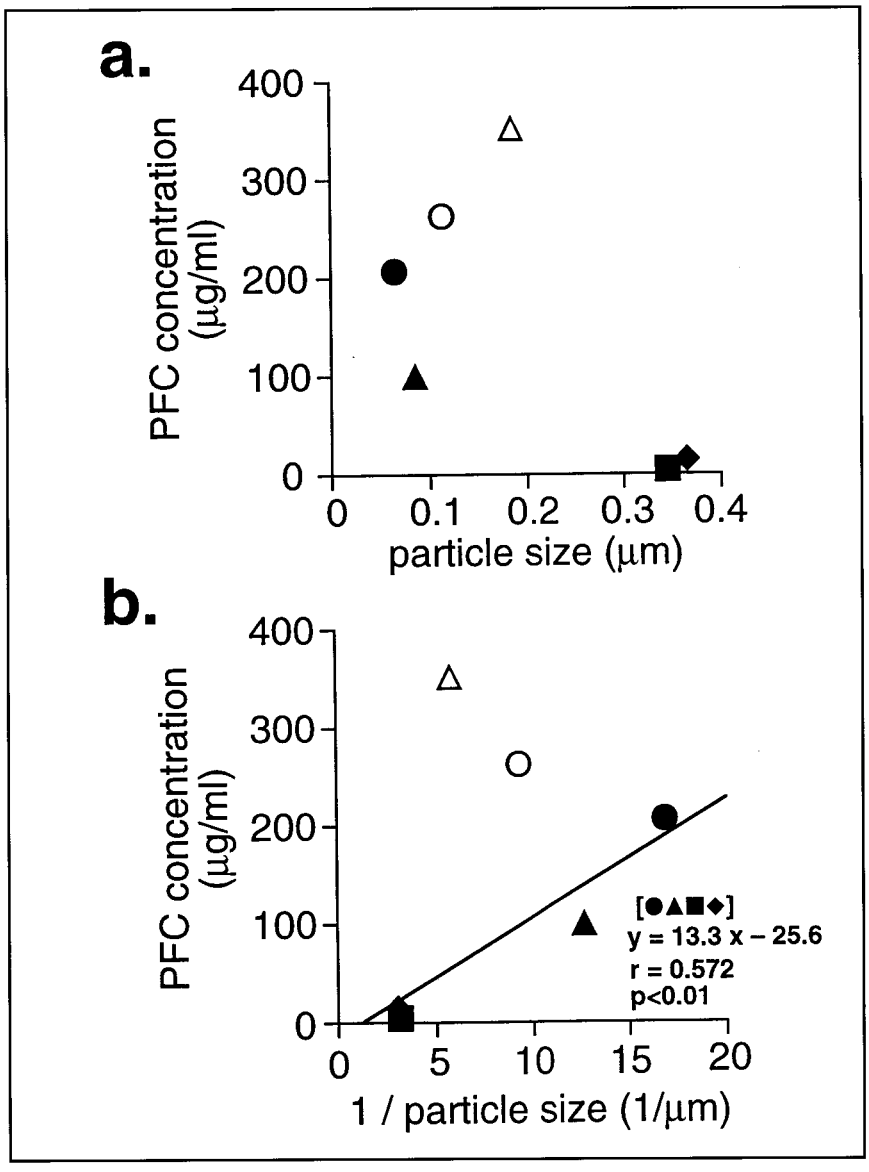


*Fig. 4. Relationship between median particle diameters (particle size) and PFC concentration in the lymph sample without massage at the point of 2 hrs. Data of LA11063 (■) and ZY12149 (●) are from reference no. (7). Closed symbols indicate colloids with surfactant egg yolk phospholipid (LA11063, ■, n = 6; ZY12149, J, ● = 7; ZY13163, ▲, n = 4 and ZY13164, ◆, n = 4), open triangle are with Tetronic904 (C, n = 4) and open circle are with Pluronic L121 (E, n = 4). The ordinate shows the concentration of PFC. The abscissa denotes particle size (a). When the abscissa was converted to a reciprocal number of particle size (b), a significant linear regression was observed in the particles with egg yolk phospholipid (■, ●, ▲, ◆; n = 21 data points, r = 0.57, p < 0.01).*

*Comparison of Transport between the Colloids.*

The median particle diameter of the colloid emulsions were inversely related to the PFC concentration in the lymph sample *without* massage at 2 hrs after injection *(Fig. 4)*. The reciprocal value of the average particle size was linearly correlated with PFC particles concentration in the emulsion with

EYP (LA11063, n = 6; ZY12149,n = 7; ZY13163, n = 4 and ZY13164, n = 4). The concentrations of LA11063 and ZY12149 were determined in our previous study under identical conditions (7). Colloids with Tetronic 904 (n = 4) and Pluronic L121 (n = 4) as surfactant had markedly higher particle counts than emulsions with EYP. In contrast, *with* massage a weaker correlation was detected between the particle size or its inverse and the lymph particle concentration *(Fig. 5).*

*DISCUSSION*

Interstitially injected colloid particles accumulate in regional lymph nodes. By using this characteristics of colloids, Taupitz et al (31) detected lymph node metastasis of cancer by magnetic resonance (MR) lymphography with interstitial injection of supermagnetic iron oxide (SPIO) particles (0.05 μm in mean diameter) as contrast medium. For treatment of cancer metastasis in lymph node, Sherman et al (29) have injected radioactive colloidal gold into the interstitium where it was transported to regional nodes, trapped, and concentrated. For more precise diagnosis and more effective treatment of cancer metastasis in lymph node, it should be necessary to find colloid suspensions which are specifically taken up into lymphatics.

In the present study, we demonstrated that transport of colloids into lymphatic vessels with the same surface characteristics depends on particle size. Perfluorocarbon (PFC) emulsion made with egg yolk phospholipid (EYP), where each particle is coated by a monolayer of EYP molecules (7, 23), exhibits significant correlation between the inverse of the mean particle size and lymph PFC concentration. In the absence of tissue massage, the regression equation, $y = 13.3 x - 25.6$, where x = reciprocal number of mean particle size (in 1/μm) and y = lymph PFC concentration (in μg/ml), indicates that the amount of colloid uptake into lymphatic system decreases in a size dependent manner. The relationship also suggests that without massage over the injection site, subcutaneously injected particles with an average diameter of more than 0.52 μm are less likely to appear in lymph fluid. In the presence of tissue massage, lymph particle concentrations are significantly elevated and the correlation with particle size becomes weaker.

All rabbits showed a large increase in lymph flow rate and leukocyte count with massage. Skin massage increases lymph flow rate (3,9), lymph cell count (7,8), and lymphatic pressures (1,11). Massage may affect lymph formation by periodic compression and expansion of the initial lymphatic vessels that are tethered to the surrounding tissues (10,27). The enhancement of lymph flow rates during massage suggests an increase in convective fluid flow in the tissue, a process that increases convective transport of particles. Lymphatic valves permit only one way direction of fluid, cell, and particle movement (21,27). Skin massage also serves to increase clearance of albumin, colloidal gold and rhenium sulfide colloids from pig skin (18).

Local edema also affects colloid transport from subcutaneous tissue with enhanced colloid flux compared with normal non-edematous tissue (8). Inter-endothelial gaps in the initial lymphatics may be opened in edematous tissue (2,13) thereby facilitating the uptake of particles into the lymphatics. The uptake of particles during edema is however not as effective as the enhancement of particle uptake achieved with massage even without edema (8).

At least two transport mechanisms for colloids from subcutaneous tissue into lymphatic vessels have been described. One of the mechanisms is extracellular transport (dispersed particle) (20) and the other is intracellular transport (particles phagocytosed by macrophages) (5). Our previous study has served to demonstrate that both extracellular and intracellular pathways are utilized for transport of colloids (average

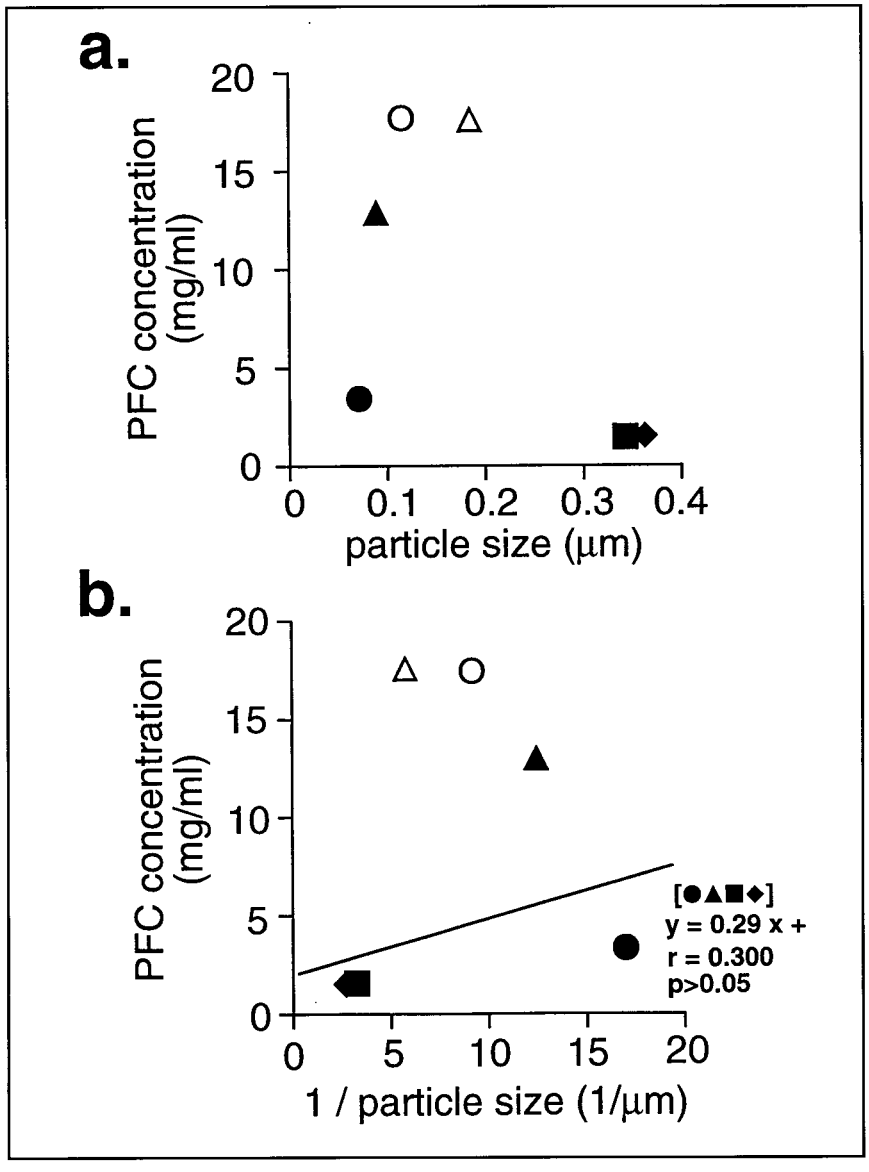


*Fig. 5. Relationship between median particle diameters (particle size) and PFC concentration in the lymph sample with massage at the point of 2 hrs. Data of LA11063 (■) and ZY12149 (●) are from reference no. (7). Closed symbols indicate colloids with surfactant egg yolk phospholipid (LA11063, ■, n = 6; ZY12149, ●, n = 7; ZY13163, ▲, n = 4 and ZY13164, ◆, n = 4), open triangle are with Tetronic904 (C, n = 4) and open circle are with Pluronic L121 (E, n = 4). The ordinate shows the concentration of PFC. The abscissa denotes particle size (a). When the abscissa was converted to a reciprocal number of particle size (b), no significant linear regression was observed in the particles with egg yolk phospholipid ■, ●, ▲, ◆; n = 21 data points, r = 0.30, p > 0.05).*

diameter, 0.06 μm and 0.08 μm) (7). Up to 24 h, however, the extracellular colloid transport pathway tends to dominate; thereafter the intracellular transport following phagocytosis of the colloids in the tissue by macrophages tends to assume increasing importance. Most colloids were transported in the form of freely suspended particles via an extracellular

transport mechanism and the intracellular transport even during elevated leukocyte counts remained negligible.

In the present study, EYP, Tetronic 904 and Pluronic L121 were used as surfactants. The main components of EYP are phosphatidylcholine and phosphatidylethanolamine. Each surfactant covers the PFC particles (7,23). The surface characteristics of the particles might affect their motility in the tissue and the adhesion to the extracellular matrix. The present study showed that PFC emulsions made with Tetronic 904 and Pluronic L121 can more easily enter the lymphatic system than emulsions made using EYP. Surface modification of particles may also affect phagocytic uptake by interstitial leukocytes (23). It is also possible that surface modifications alter permeation of particles through the initial lymphatic wall.

Brominated fluorochemicals, including PFOB and PFDB, are known to have the ability to carry oxygen (32) and to be radiopaque (15). By use of these physical characteristics, PFOB emulsion has been used as X-ray contrast medium (15,16), as an adjuvant to tumor radio therapy (32), and for other use in diagnosis and therapy (17, 22). After phagocytosis of these emulsions by macrophages or related cells, liver or spleen tumors (16) and inflammatory tissues (25) can be distinguished with computer tomography. PFOB emulsions are also reported to be useful for percutaneous lymphography (33).

In summary, particle size- and surface-dependent colloid uptake from subcutaneous tissue to lymphatics are clearly demonstrated in this study. Massage of the skin over the colloid injection site leads to a large increase in colloid transport. These results are consistent with those from earlier studies on lymph fluid formation (28) (2) and lymphatic colloid uptake (7,8). From a clinical point of view, it is necessary to develop materials that are selectively taken up into the peripheral lymphatic system for staging cancer.

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*REFERENCES*

1. Calnan, JS, JJ Pflug, ND Reis, et al: Lymphatic pressures and the flow of lymph. Br. J. Plast. Surg. 23 (1970), 305-317.
2. Castenholz, A: Morphological characteristics of initial lymphatics in the tongue as shown by scanning electron microscopy. Scanning Electron Microscopy 1984 (1984), 1343-1352.
3. Drinker, C K, ME Field: *Lymphatics, Lymph and Tissue Fluid.* Baltimore, Williams & Wilkins, 1933.
4. Fuchs, W A: Complications in lymphography with oily contrast media. Acta Radiol. 57 (1962), 427-441.
5. Harmsen, AG, BA Muggenburg, MB Snipes, et al: The role of macrophages in particle translocation from lungs to lymph nodes. Science. 230 (1985), 1277-1280.
6. Ikomi, F, G Hanna, GW Schmid-Schönbein: Intracellular and extracellular transport of perfluorocarbon emulsion from subcutaneous tissue to regional lymphatics. Art. Cells Blood Subs. Immob. Biotech. 22 (1994), 1441-1447.
7. Ikomi, F, G Hanna, GW Schmid-Schönbein: Mechanism of colloidal particle uptake into the lymphatic system: Basic study of percutaneous lymphography. Radiology 196 (1995), 107-113.
8. Ikomi, F, J Hunt, G Hanna, et al: Interstitial fluid, plasma protein, colloid and leukocyte uptake into initial lymphatics. J. Appl. Physiol. 81 (1996), 2060-2067.
9. Ikomi, F, GW Schmid-Schönbein: Lymph pump mechanics in the rabbit hind leg. Am. J. Physiol. 271 (1996), H173-H183.
10. Ikomi, F, GW Schmid-Schönbein: Lymph transport in the skin. Clin. Dermatol. 13 (1995), 419-417.
11. Ikomi, F, BW Zweifach, GW Schmid-Schönbein: Fluid pressures in the rabbit popliteal afferent lymphatics during passive tissue motion. Lymphology 30 (1997), 13-23.
12. Kinmonth, J.B: Lymphography in man: A

method of outlining lymphatic trunks at operation. Clin. Sci. 11 (1952), 13-21.
13. Leak, LV, JF Burke: Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am. J. Anat. 118 (1966), 785-810.
14. Lock-Andersen, J, N Rossing, KT Drzewiecki: Preoperative cutaneous lymphoscintigraphy in malignant melanoma. Cancer 63 (1989), 77-82.
15. Long, D M, FK Multer, AG Greenburg, et al: Tumor imaging with X-rays using macrophage uptake of radiopaque fluorocarbon emulsions. Surgery 84 (1978), 104-112.
16. Mattrey, RF, DM Long, F Multer, et al: Perfluoroctylbromide: A reticuloendothelial-specific and tumor-imaging agent for computed tomography. Radiology 145 (1982), 755-758.
17. Mattrey, RF, FW Scheible, BB Gosink, et al: Perfluoroctylbromide: A liver/spleen-specific and tumor-imaging ultrasound contrast material. Radiology 145 (1982), 759-762.
18. Mortimer, PS, R Simmonds, M Rezvani, et al: The measurement of skin lymph flow by isotope clearance - reliability, reproducibility, injection dynamics, and the effect of massage. J. Invest. Dermatol. 95 (1990), 677-682.
19. Nagai, K, Y Ito, N Otsuka, et al: Experimental studies on uptake of $^{99m}$Tc-antimony sulfide colloids in RES - A comparison with various $^{99m}$Tc-colloids. Int. J. Nucl. Med. Biol. 8 (1981), 85-89.
20. Nopajaroonsry, C, GT Simon: Phagocytosis of colloidal carbon in a lymph node. Am. J. Pathol. 65 (1971), 25-42.
21. Ohhashi, T, T Azuma, M Sakaguchi: Active and passive mechanical characteristics of bovine mesenteric lymphatics. Am. J. Physiol. 239 (1980), H88-H95.
22. Riess, J G, JL Dalfors, GK Hanna, et al: Development of highly fluid, concentrated and stable fluorocarbon emulsions for diagnosis and therapy. Biomat. Artif. Cells Immob. Biotech. 20 (1992) 839-842.
23. Rudt, S, H Wesemeyer, RH Müller: In vivo phagocytosis assay of nano- and microparticles by chemiluminescence. IV. Effect of surface modification by coating of particles with poloxamine and Antarox CO on the phagocytic uptake. J. Control. Release 25 (1993), 123-132.
24. Samlowski, WE, BA Robertson, BK Draper, et al: Effects of supravital fluorochromes used to analyze the in vivo homing of murine lymphocytes on cellular function. J. Immunol. Methods 144 (1991), 101-115.
25. Sartoris, DJ, J Guerra, Jr, RF Mattrey, et al: Perfluoroctylbromide as a contrast agent for computed tomographic imaging of septic and aseptic arthritis. Invest. Radiol. 21 (1986), 49-55.
26. Schaffer, B, PR Koehler, CR Daniel, et al: A critical evaluation of lymphangiography. Radiology 80 (1963), 917-930.
27. Schmid-Schönbein, GW: Microlymphatics and lymph flow. Physiol. Rev. 70 (1990), 987-1028.
28. Schmid-Schönbein, GW, BW Zweifach: Fluid pump mechanisms in initial lymphatics. News Physiol. Sci. 9 (1994), 67-71.
29. Sherman, AI, M Bonebrake, WM Allen: The application of radioactive colloidal gold in the treatment of pelvic cancer. Am. J. Roentgenol. Rad. Therapy. 66 (1951), 624-638.
30. Strand, SE, BRR Persson: Quantitative lymphoscintigraphy I: Basic concepts for optimal uptake of radiocolloids in the parasternal lymph nodes of rabbits. J. Nucl. Med. 20 (1979), 1038-1046.
31. Taupitz, M, S Wagner, B Hamm, et al: Interstitial MR lymphography with iron oxide particles: Results in tumor-free and VX2 tumor-bearing rabbits. Am. J. Radiol. 161 (1993), 193-200.
32. Thomas, C, J Riess, M Guichard: Influence of the 100% w/v perfluorooctyl bromide (PFOB) emulsion dose on tumour radiosensitivity. Int. J. Radiat. Biol. 59 (1991), 433-445.
33. Wolf, GL, J Rogowska, GK Hanna, et al: Percutaneous CT lymphography with perflubron: Imaging efficacy in rabbits and monkeys. Radiology 191 (1994), 501-505.
34. Zar, JH: *Biostatistical Analysis*. Second Edition. Englewood Cliffs, New Jersey, Prentice Hall, 1984.

**Geert W. Schmid-Schönbein, Ph.D.**
**Department of Bioengineering and**
**Institute for Biomedical Engineering,**
**University of California, San Diego,**
**Rm. 5601 Engineering Building Unit 1,**
**9500 Gilman Drive**
**La Jolla, CA 92093-0412 USA**
**Tel: 619-534-4272**
**Fax: 619-534-5722**