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LIFORLAB® - SOLUTION: A NEW OXYGEN-ENRICHED MEDIUM FOR TUMOR TISSUE TRANSPORT

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ABSTRACT

Introduction: Up to day transfer of human biopsies without damaging the tissue is time limited. Transport of human material for genomic and proteomic analysis at room temperature (RT) is nearly impossible without affecting the experimental outcome. To offer tumor based treatments to patients, sample storage is necessary without formalin fixing or snap frozen conservation. In this study, we examined a new physiological, oxygen enriched solution for transport and storage of human tumor samples.
Methods: The effect of Liforlab® solution (LS) on DNA and RNA stability and on cell viability was tested. Therefore, tumor samples of venous tumor types were stored in LS at RT and 4°C, respectively for up to 6 days. In addition, tumor biopsies were frozen in LS at -170°/-196°C. The content and quality of RNA and DNA was determined, using standard procedures. In addition, standard viability assays were utilized.
Results: Using LS, tumor biopsies of venous tumor types could be transferred at RT for up to 96h and showed more than 80% viability. Moreover, after transportation in LS, these samples could be frozen in the same solution for long time storage without addition of cryoprotective agents like DMSO and HES. Tumor samples stored in LS for up to 6 days at RT and 4°C did not show any degradation. Furthermore, stem cell storage at 4°C for up to 7 days lead to 95% viability. Granulocytes could be stored in LS for up to 24h without reduced activity.
Conclusions: Liforlab® solution makes transport of tumor-tissue/biopsies at RT feasible for up to 72h without any impact on viability. Furthermore, cryoconservation of tumor material and single cell suspensions can be earned out in LS without addition of cryoprotective agents.

METHODS AND RESULTS

Composition of Liforlab®-solution (LS): Physiological, oxygen enriched solution, containing inorganic salts, amino acids, vitamins, adenosine, cholesterol, glucose, dextrane 70 the growth factors EGF, VEGF, HGF and low protein. LS is free of serum.

Transport and cell viability in LS: Tumor samples: **A.** various tumor cell lines (e.g. lung, gastric, colorectal, cervical, epidermal, pancreatic, prostate, ovarian and bladder) and **B.** fresh tumor tissue (prostate, lung and thyroid) were stored in LS or in regular medium, respectively at RT for up to 96h. After that tumor samples were recultured or established as a primary culture in regular medium, respectively. Tumor cells did not lose viability and could be re-cultured **(Figure 1)**. Tumor tissue stored in LS could be cultivated in regular medium whereas tissue stored in regular medium showed a significant reduced rate of proliferation **(Figures 2 and 3)**. Furthermore, tumor samples were cryoconserved in LS without DMSO and HES for up to 3 months. Cells did not lose viability and could be cultured in regular medium **(Figure 4)**.

Vitality of hematopoetic cells in LS
1. Phagocytosis activity of granulocytes: Granulocytes were isolated with polymorphPrep (Axis-Shield, Norway) and stored in LS for up to 48h. Phagocytosius activity was determined by **A.** Nitroblue-tetrazolium-assay (NBT-assay, established by R. Baehner and D. Nathan) and **B.** Flow Cytometry analysis, respectively. Positive gating on leucocytes was done by CD45. Panmyeloic marker CD13 and phagocytosis correlated marker, CD18 and CD11a were measured. Cell vitality was analyzed with the vitality dye 7-AAD Granulocytes activity (phagocytosis) was stable in LS for at most 24h **(Figure 5)**
2. Viability of peripheral stem cells: Isolated stem cells (10^6cells/ml) were stored in serum ± **A.** 10% LS and **B.** 50% LS at 4°C. The percentage of vital and dead cells was determined up to 168h, respectively. Per experiment 5 samples were estimated; (n=3). Standard derivation was determined by T-test. Stem cells could be stored in LS (10%, 50%) at 4°C over at least 168h without DMSO with at least ≤95% vital cells **(Table 1 and Figure 8)**.

DNA/RNA stability of tumor samples in LS: DNA/RNA was isolated using DNeasy Tissue kit and RNeasy kit (Qiagen, Germany). **DNA/RNA content and quality** was determined using standard procedures. Tumor samples were stored in LS **A.** at RT for up to 72h, **B.** at 4°C for up to 5 days and **C.** at -196°C for up to 3 months. Isolated RNA did not show any degradation **(Figure 7)**. Furthermore tumor samples (prostate, stomach, esophagus and skin tumors) were stored in LS and RNALater (Quiagen, Germany); (storage interval 2-8 days at 4°C and RT). Samples were prepared as single cell suspensions by mechanical homogenization and cells were cultured in regular medium or in LS respectively. It was feasible to establish a stable growing colon carcinoma cell line (in regular medium) from tumor samples stored in LS, whereas it was not possible from cells stored in RNALater (data not shown). The quantity of DNA and RNA isolated from tissue samples stored in either LS or RNALater was not significantly different as measured as described. Storage of tissue samples at RT and at 4°C in LS for more than 5 days led to an increased uptake of medium.

PSA values of prostate cancer samples were determined prior resection of tumor tissue and once a week from **A.** enzymatically and **B.** mechanically homogenized established single cell suspensions in LS. PSA of Patients prior surgery was in the range of 0.37 - 30.92 ng/ml (median =6,7 ng/ml, n= 26 biopsies). PSA values measured in enzymatic prepared cell suspensions were compared to the non-enzymatic prepared samples significantly lower **(Figure 6)**.

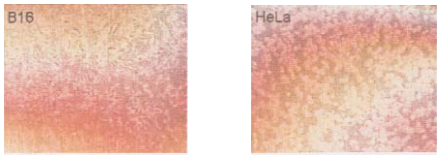

Figure 1: Tumor cells recultured in regular medium (4 days) after storage in LS up to 4 days. (B16: melanoma (mouse), HeLa: human cervical cancer).

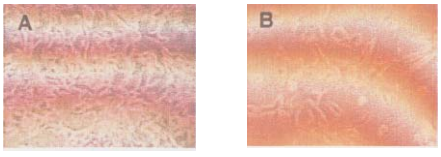

Figure2: A. Thyroid cancer tissue and B. Lung cancer tissue stored in LS for up to 72 h at RT and cultured in regular medium for 4 days and 7 weeks respectively.

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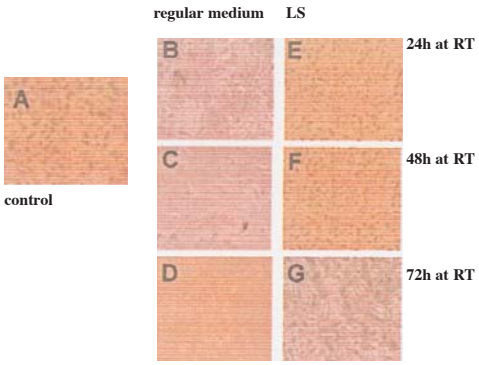

Figure3: Prostate cancer tissue was stored in regular medium and LS for up to 72h. Tumor samples were treated with collagenase/dispase and cultured in regular medium for 23 days. **A:** Fresh tissue cultured in regular medium (control). **B,C,D:** Tumor tissue stored in regular medium (RT for up to 72h) and cultured in the same medium. Cells showed a significant loss of their rate of proliferation. **E,F,G:** Tumor tissue stored in LS for up to 72h and cultured in regular medium with no difference to control.

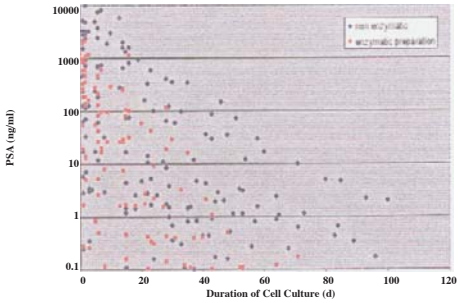

Figure6: PSA values measured in prostate cancer single cell suspensions in LS (up to 100 days). Two out of 49 cell suspensions of the non enzymatic preparations reached the LLQ of 0.1 ng/ml. PSA was significantly lower in the enzymatic than in the non enzymatic prepared samples.

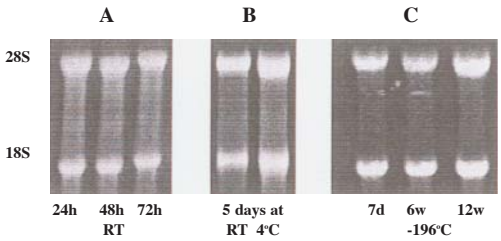

Figure 7: RNA of tumor samples stored in LS: **A.** PC-3 cells for up to 72h at RT, **B.** Lung Cancer tissue 5 days at RT and 4°c, respectively and **C.** PC-3 cells for up to 3 months at -196°c. RNA did not show any degradation.

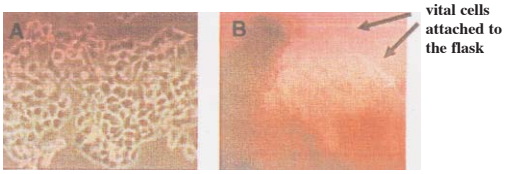

Figure 4: Tumor samples stored in LS at -196°c without DMSO and HES **A.** HCT-8 Ceils frozen in LS for 3 months and cultured in regular medium (4 days) and **B.** Tumor tissue shipped (RT), frozen in LS, thawed at 37°C and cultured in regular medium (2 weeks).

LiforCell for Stem Cell Preservation

	[%] Solution	[%] death cells	p
24h/4°C	control	15.2 ± 2.9	
	10% LS	7.4 ± 2.7	0,002
	50% LS	4.2 ± 1.6	0.0001
72h/4°C	control	18.0 ± 4.9	
	10% LS	7.5 ± 3.3	0.004
	50% LS	4.2 ± 1.6	0.0001
168h/4°C	control	70.6 ± 4.5	
	10% LS	11.6 ± 3.5	0.0001
	50% LS	5.8 ± 1.3	0.0001

N=3

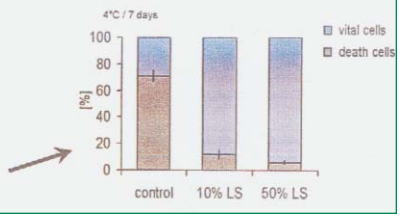

Table 1: The death rate of peripheral stem cells stored in LS (10% / 50%) at 4°C for up to 7 days is significantly lower than in control.

Figure 8: Vitality of stem cells stored in LS at 4°C for 7 days was ≤ 95% whereas only 30% in control was measured.

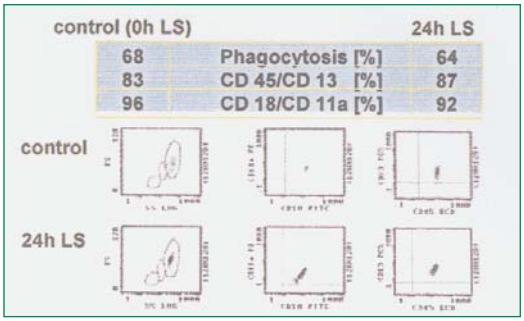

Figure 5: Stable phagocytosis activity of granulocytes stored in LS for 24h at RT. There was no percentage difference in the expression of panmyeloic (CD45/CD13) and phagocytosis correlated marker (CD18/CD11a) to control.

Conclusions

Liforlab® - solution (LS) is a high oxygen enriched solution. It allows storage and transport of tumor tissue samples at room temperature for at least 96 hours. It is possible to conserve tissue and cell samples in LS at -196°C without cryoprotective agents up to 3 months. Tumor cells could be stored in LS for more than 4 days and recultured in, regular medium. Tumor tissue could be stored in LS for up to 96h at room temperature and cultured in regular medium. In addition, hematopoetic cells could be stored in LS **A.** granulocytes for up to 24h without losing activity and **B.** peripheral stem cells (in LS 10%; 50%) for up to 7 days with more than 90% viability. LS did not effect DNA - and RNA - stability of all tumor samples tested. Current investigations are directed to establish permanent cell cultures in LS. Experiments to proof genomic and proteomic stability of these cultures are in progress.