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ISOLATION AND CULTURE OF HUMAN ADIPOSE DERIVED STEM CELLS FOR CLINICAL APPLICATION IN UROLOGY

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INTRODUCTION

Tissue engineered tissues provide a good alternative to conventionally used gastrointestinal tissues. Construction of neo-bladder or neo-conduit for patients with bladder cancer requires using of different than autologic bladder source of cells because of risk of cancer transmission. Adipose tissue appears to be an good source of cells for reconstructive urology because of the simple and minimally invasive method of harvesting and relatively large number of stem cells.

AIM: To select the best serum-free medium for human Adipose Derived Stem Cells (ADSC) culture.

MATERIALS AND METHODS

1.Study design

Lipoaspirate collection using ultrasound liposuction from the abdomen area of patients undergoing radical cystectomy (n=3)

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Isolation of ADSCs using enzymatic digestion with collagenase

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ADSC culture in the three serum-free media until fifth passage

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Analysis of morphology, number, proliferation rate and phenotype of ADSCs

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Selection of the best medium for human ADSCs culture for clinical application

2. Serum-free media

	MEDIUM I	MEDIUM II	MEDIUM III
Basal medium	DMEM:F-12	MEM alpha	StemPro® MSC SFM Basal Medium CTS™
Substitute of serum	10% human platelet lysate	10% human platelet lysate	StemPro® MSC SFM Supplement CTS™
Supplements	- penicillin (100 U/ml) - streptomycin (100 µg/ml) - amphotericin B (5 µg/ml)		- gentamycin (5 µg/ml) - L-glutamine (2 mM)

3.Analysis

Morphology of ADSCs

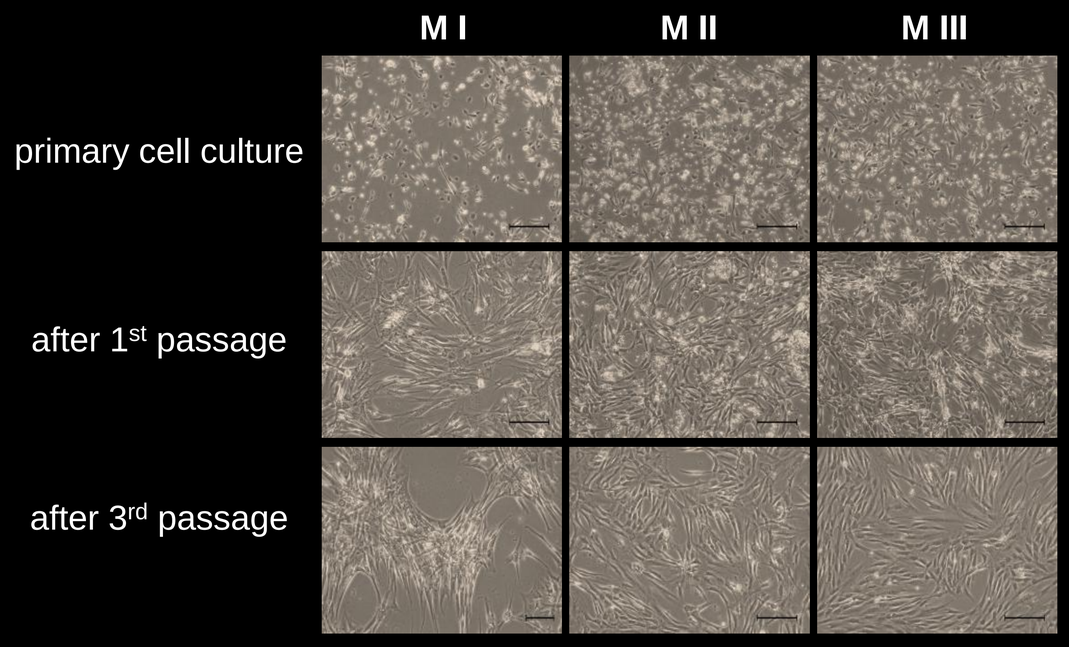
Number of ADSCs

Proliferation rate of ADSCs

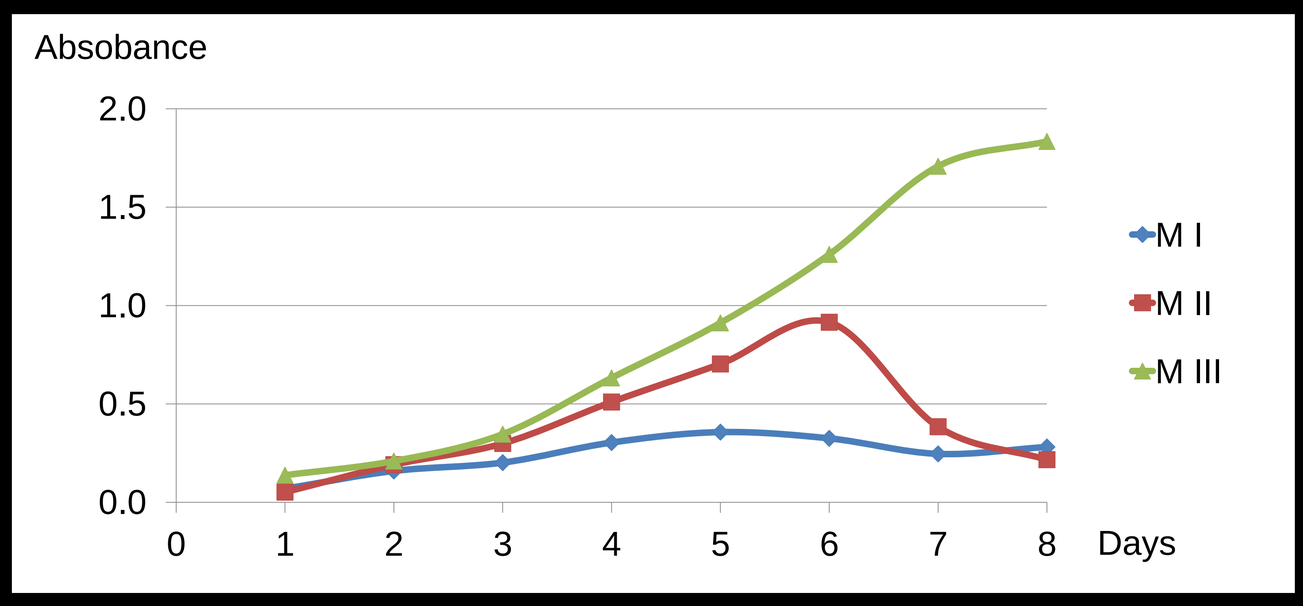
Phenotype of ADSCs

RESULTS

1. Analysis of ADSCs morphology.



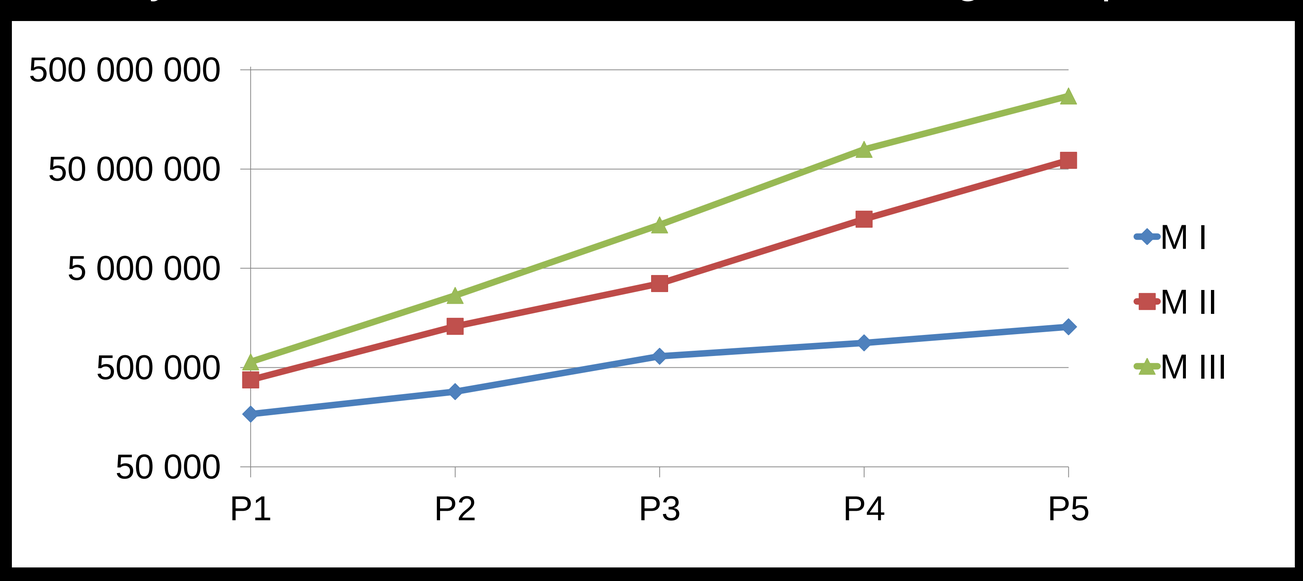
2. Analysis of ADSCs proliferation by MTT assay.



3. Analysis of ADSCs phenotype by flow cytometry.

	Medium I	Medium II	Medium III
Positive surface markers:			
CD44	99.7% ± 0.3	99.8% ± 0.1	99.4% ± 0.6
CD73	99.6% ± 0.4	99.8% ± 0.2	99.4% ± 0.6
CD90	99.6% ± 0.4	100% ± 0.0	99.8% ± 0.2
CD105	99.8% ± 1.0	97.8% ± 2.7	98.5% ± 1.3
Negative surface markers (CD45, CD34, CD11b, CD19, HLA-DR)	4.1% ± 4.8	0.9% ± 0.5	0.7% ± 0.1

4. Analysis of number of ADSCs isolated from 1 g of adipose tissue.



CONCLUSIONS

The composition of the culture medium significantly impacted on number of ADSCs obtained during the culture. Medium III is the best medium for ADSC culture for clinical application. Continuation of the study is necessary to confirm these results.

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