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HYPOTHERMIC PRESERVATION OF CELL SHEETS OF HUMAN ADIPOSE STEM CELLS

Presenter: Sara Ribeiro, BSc (Portugal)

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Authors: Ribeiro S, Costa M, Cerqueira MT, Marques AP, Pirraco RP, Reis RL

Introduction: Cell Sheet Engineering is based on the retrieval of cells from culture dishes as extracellular matrix (ECM)-rich sheets. These can then be used for the regeneration/engineering of several tissues and organs, as demonstrated in ongoing clinical trials. Cell sheet fabrication, while simple, requires cell culture facilities. Thus, ensuring adequate cell function from the fabrication site to the bedside is essential to its efficiency. We tested the ability of two commercially available compounds to preserve the viability of sheets of human adipose stem cells (hASC) at 4°C.

Methods: hASC were cultured in basal medium to hyperconfluence to produce cell sheets. Culture medium was then either replaced by a solution of Hypothermosol® (HTS) or supplemented with Rokepie® (RP) and cells were maintained at 4°C for 3 and 7 days. Controls with basal medium at 4°C and for all the conditions at 37°C were established. Non-confluent cultures mirroring all the conditions were also set up. At each time point, cells were imaged for morphology and viability was assessed using flow cytometry (7-AAD) and Alamar Blue.

Results: After 3 days, a sharp decrease in the viability at 4°C without preservation solutions was noticed when comparing with control at 37°C. This decrease was more evident in non-confluent cultures. The viability in the presence of HTS or RP was comparable but slightly lower than for the respective controls at 37°C. At day 7, few viable cells were detected in the 4°C conditions without supplementation, while none were found in the non-confluent counterparts. HTS- and RP-supplemented cultures presented 40% of the viability of the 37°C control. The relative viability in non-confluent conditions was much lower but RP accomplished better results than HTS conditions.

Conclusions: Both HTS and RP demonstrated an excellent capability of hypothermic preservation of cell sheets up to 3 days, but after 7 days the relative viability decreased to 40%. Furthermore, confluence apparently confers protection against hypothermic insult. Surface marker characterization, differentiation potential and caspase activity of cells after preservation are currently being assessed.

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MECHANICAL ISOLATION OF ADIPOSE STROMAL VASCULAR CELLS: A SAFE AND LESS TIME-CONSUMING ALTERNATIVE TO ENZYMATIC DIGESTION

Presenter: Tunc Tiryaki, MD (Turkey)

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Authors: Tiryaki T, Canikyan S, Conde-Green A

Introduction: Adipose tissue derived stromal vascular fraction (SVF) is a reliable source of stromal vascular cells with regenerative potential in plastic surgery and other specialties. The isolation of stromal vascular cells (SVCs) using enzymatic digestion relies on fat tissue dissociation by collagenase. Although the gold standard, this isolation method is expensive, requires expert personnel, raises legal and administrative concerns on the alteration of cell characteristics. We aimed to isolate SVCs by mincing, filtrating, incubating and centrifuging the lipoaspirate and compare them with the population obtained by enzymatic digestion.

Methods: Adipose tissue was harvested with the syringe from 25 healthy females aged 26-52 years undergoing liposuction. Twenty milliliters of fat was submitted to collagenase digestion, and 20 ml was minced with blades followed by different incubation protocols in crystalloid buffer solutions, and centrifuged. The SVCs obtained were quantified, assessed for viability and submitted to flow cytometry.

Results: The SVCs yields obtained by mechanical isolation were ($4.0 \pm 0, 8$ to $9,0 \pm 1,3 \times 10^5$ cells/ml lipoaspirate) representing 40 to 80% of the cell yield obtained by enzymatic isolation ($11 \pm 1,3 \times 10^5$ cells/ml lipoaspirate) (Table 1). An incubation time of 30 minutes at room temperature (Method 4) showed higher levels of CD34 expression. The mechanical and enzymatic isolation yields showed similar levels of CD44 expression. Overall, mechanically isolated SVCs showed higher CD90 and CD105 expression.

Conclusion: Our preliminary results suggest that our technique of mechanical SVCs isolation is safe, less time-consuming than enzymatic digestion. The fact that we can isolate 40 to 80% of the cell yield isolated enzymatically shows that it's a viable alternative. This technique does not require specialized personnel or environment, as it can be done in the operating room. As a minimally manipulated method, it may spare the surgeon from the regulatory issues encountered with enzymatic SVF isolation protocols. Further studies are warranted to determine whether these changes in cell yield or composition will affect our clinical outcomes.



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CLINICAL APPLICATION OF POLOXAMER 188 ENHANCED FAT GRAFTS

Presenter: Alfredo E. Hoyos, MD (Colombia)

Affiliation: Elysium

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TABLE 1. Comparison of cell yield and viability obtained with SVF enzymatic and mechanical isolation protocols

SVF isolation protocols	Cell Number/cc (x10 ⁵)	Cell Viability %
SVF Enzymatic digestion	11 ± 1,3	78,87%
Mechanical SVF isolation:		
1 hour incubation at 37 C (method 1)	6 ± 1.41	84,23%
1 hour incubation at room temperature (method 2)	4,0 ± 0,8	80,68%
30 min incubation at 37 C (method 3)	4,5 ± 0,5	85,2%
30 min incubation at room temperature (method 4)	9,0 ± 1,3	87,00%
Negative Control (no treatment)	0,5 ± 0,5	92,01%