

EXTRACELLULAR MATRIX, COLLAGEN TYPE I EXPRESSION LEVELS, CELL DENSITY AND DNA CONTENT IN DECELLULARIZED HUMAN OVARIAN TISSUES: EFFECTS OF IONIC AND NON-IONIC DETERGENTS COMBINED WITH ENZYMATIC TREATMENT

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Abstract Body

This study aimed to investigate efficacy of ionic and non-ionic detergents combined with enzymatic digestion to preserve extracellular matrix (ECM), eliminate cell and DNA content in ovarian scaffold using tissue decellularization technique. Use of ovarian tissues (donated from six premenopausal patients; 42-47 years old) were approved by Institutional Review Board. Cortex and medulla were randomly allocated into five groups; (1) fresh control, native tissue decellularization using ionic detergent (2) 0.5 % SDS, (3) 1.0 % SDS, non-ionic detergent (4) 1.0 % Triton-X, (5) 5.0 % Triton-X. After 24-h, tissues were supplemented with DNase I enzyme and followed by detergent residual removal. Glycosaminoglycans (GAGs) and Collagen Type I immunohistochemistry staining level (%), total number of ovarian cell per 100 μm^2 , DNA content (spectrophotometer) and detergent residual concentration (colorimetric assay) from each tissue were evaluated. Major findings indicated that GAGs and Collagen Type I levels in decellularized tissues were comparable between ionic and non-ionic detergents (i.e. GAGs in cortex; 43.3 ± 4.7 (Group 2) vs 40.3 ± 2.7 (Group 3) or Collagen Type I in cortex; 51.6 ± 5.7 (Group 2) vs 49.9 ± 5.9 (Group 3); mean \pm SD). Additionally, designed decellularization protocols did not effect ECM level compared to fresh tissues ($P > 0.05$). SDS effectively eliminated cell and DNA content in cortex (1.9 and 1.1 times higher than Triton-X; $P < 0.05$) while Triton-X was effective in medulla part. Detergent residuals were present in all treated tissues which were increased by detergent concentration (26.5 ± 4.6 ng/mL vs 32.6 ± 5.4 ng/mL of 1.0 % and 5.0 % Triton-X residuals in cortical tissues). In conclusion, bioengineered ovary with well-preserved native niche could be constructed either by ionic or non-ionic detergents which was depended on ovarian compartment. In vitro follicle growth under these created milieus should be further interrogated.