Nativus, a Novel Tissue Processing Method, Promotes Human Stem Cell Survival, Attachment and Proliferation

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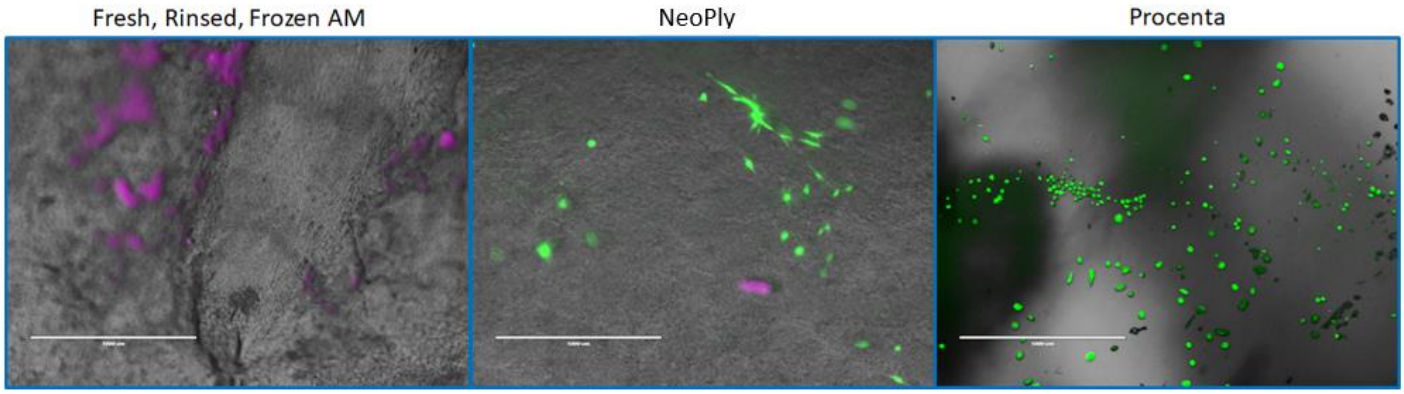
Introduction:

Historically, human tissue allographs are highly processed and/or require specific storage requirements to avid degradation of the product while "on the shelf". Recently, Lucina BioSciences has engineered a method where human tissues are processed to preserve innate soluble factors associated with the tissue matrix, remain in a hydrated form (as they are in normal physiology) and are room temperature stable for 3 years without loss of bioactivity. This unique processing method avoids harsher treatments which denature (or collapse) structural proteins and render soluble factors useless. Such processing steps traditionally used in tissue processing facilities including chemical/alcohol/peroxide-based washes, freezing and lyophilization[1-4]. In this investigation, we challenge freshly collected, saline rinsed and frozen tissue and Nativus-processed tissues (NeoPly and Procenta) to establish human mesenchymal stem cell cultures without supplemental growth media. It is well known in the art that key soluble factors are required for cells to 1). Survive, 2). Adhere and 3). Proliferate[5-7]. Where these factors are lacking, cell culture will ultimately fail over a short time course[8].

Method:

Nativus-processed amniotic membrane (NeoPly) and fresh, rinsed, previously frozen amniotic membrane (AM) from a matched donor were combined with human bone marrow-derived MSCs in non-coated 12-well plates. Amniotic membranes and were then placed in non-supplemented DMEM (no growth media (e.g. FBS) and seeded with 10,000 MSCs. Procenta, a soft-tissue integumental tissue processed via Nativus was also seeded with cells and stained as described. Cells were incubated for 72 hours before labeling for viability. Calcein AM was added at 2.5µM and Sytox Red at 10nM final concentration and incubated at 37˚C for 60 minutes. Wells containing MSC-seeded tissues were image using an AMG EVO FL microscope and images were taken using LED light cubes to detect the respective fluorescent dyes (GFP cube Ex. 470/22 Em. 510/42 and Cy5 light cube Ex. 628/40 Em. 692/42). Live cells are green, dead are purple. Scale bar = 1 millimeter.

Figure 1:



Results:

Nativus processed tissues clearly established hMSC cultures whereas the fresh, rinsed, previously frozen AM counterpart failed to show viable cell populations. Further, we observe a clear difference in the morphology and distribution of hMSCs between the amniotic membrane NeoPly and the flexible soft tissue matrix, Procenta. Principally, the hMSCs show a fibroblast-like morphology on the epithelial side of NeoPly whereas they retain a "chondrocyte-like", spherical morphology in the Procenta tissue. This is due to the difference in the structural components of the two respective tissues, where ridged collagen networks are known to exist in amniotic membrane compared to the looser collagen network and hydrophilic, glycosaminoglycan-rich nature of Procenta.

Discussion:

The rationale for the study was to determine the innate ability of the tissue samples to allow cell attachment, survival and proliferation. For each of these processes, soluble factors are required. As no growth supplement (e.g. fetal bovine serum, platelet lysate, human serum) was added to the basal media, the ability of the cells to establish on the available scaffold is based on the inherit characteristics of the respective tissues.

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