A Study of MXP Cell Process and BioArchive Cryopreservation with Human Bone Marrow

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thermogenesis
From stem cells to solutions.

Background

The MarrowXpress Platform (MXP) was developed for automated processing of human bone marrow for various tissue regeneration and cell therapy applications. The system reduces bone marrow volume by removing excess plasma and red cells. The buffy coat rich product is harvested in a functionally closed disposable in less than one hour. The freezing bag of the MXP system can be stored in the BioArchive which is an automated and freezing rate controlled system for long term storage of stem cells. The MXP has received regulatory authorization from the FDA as a class I laboratory device indicated for the preparation of cell concentrates from bone marrow either in a point of care or in a laboratory setting. The goals of this study were to verify the MXP process for volume reduction with high stem cell recovery and to determine if the cell product is compatible with BioArchive system for cell cryopreservation.

Methods

- The study was designed and performed to characterize the efficiency of MNC, TNC, CD34+ cell, ALDH^{br} cell and colony forming unit (CFU) recovery after MXP processing and BioArchive cryopreservation.
- Bone marrow unit was collected from volunteer donors using heparin as the anticoagulant. The bone marrow was processed in the MXP system within 1-2 days of collection, and cryopreserved with BioArchive system after MXP process using DMSO as the cryoprotective agent.
- The MXP process was performed per operator manual. Samples were collected before and after processing to determine stem cell, mononuclear cell and cell viability of the blood.
- The BioArchive cryopreservation is a completely automated and freezing rate-controlled process. The process took approximately 25 min for freezing two samples spontaneously and 10 min for retrieving the frozen sample. Samples were stored for at least 1 week prior to thawing and analysis. Samples were thawed at 37ºC until the ice melted and then DMSO removed by cell washing using dextran-albumin thawing solution, based on New York Blood Center thawing protocol. (PNAS 92:10119, 1995)
- Cell counts were measured using Sysmex 2100 automatic cell counter.
- CD34+ cells and cell viability were detected and quantified using Beckman Coulter's Stemkit and Coulter's flow cytometer.
- ALDH^{br} cell concentration was determined using ALDEFLUOR assay kit purchased from StemCell Technologies Inc.
- CFU assay was performed using StemCell Technologies' MethoCult kit.

Results

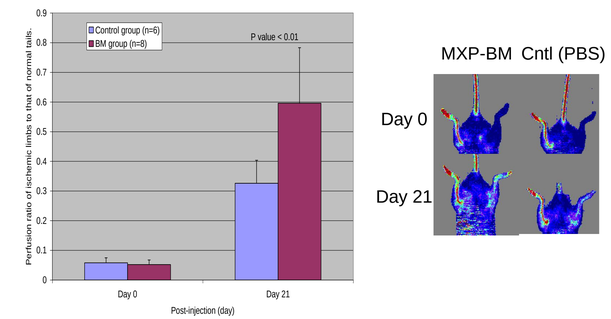


Fig 5. Angiogenesis effect of MXP-processed bone marrow product in critical limb ischemia mouse model. The blood flow of the lower limbs was measured using a laser Doppler perfusion color image (LDPI) analyzer, followed by calculation of the perfusion ratio of the surgically induced ischemic limbs (right) to normal limbs (left). In right panel, representative LDPI at indicated time points. The color changes from blue to red represent the increase in perfusion. In left panel, quantitative measurement of perfusion ratio of ischemic limbs to that normal limbs (n=8) is provided.

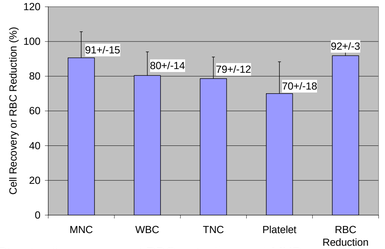


Fig 1. Blood cell recovery and RBC reduction post-MXP process. Human bone marrow sample of pre-MXP and post-MXP process were aseptically taken and measured for MNC, WBC, TNC, RBC and platelet using Sysmex 2100 cell counter. The value of cell count was used to calculate the cell recovery dividing total cell count in post-MXP sample by total cell count in pre-MXP sample. The value is expressed as a percentage (mean+/-S.D.).

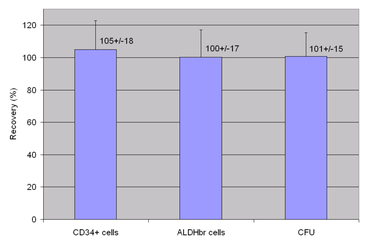


Fig 2. Hematopoietic stem cell recovery post-MXP process. Human bone marrow sample of pre-MXP and post-MXP process were aseptically taken. The samples were measured for CD34 cells using Coulter's StemKit flow cytometry, for ALDHbr cells using StemCell technologies' ADLEFLUOR assay and CFU using StemCell technologies' MethoCult. The value of the cell number or the colony number was used to calculate the cell recovery dividing total cell number in post-MXP sample by total cell number in pre-MXP sample. The value is expressed as a percentage (mean+/-S.D.).

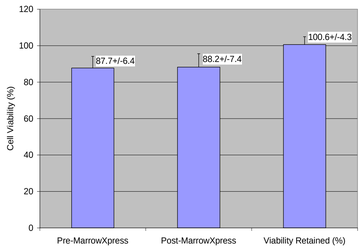
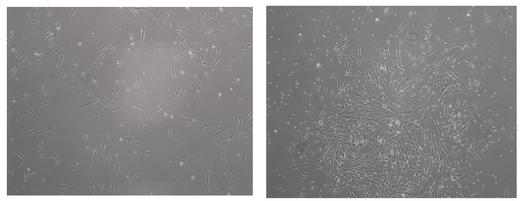


Fig 3. The MarrowXpress process did not affect cell viability. There was no significant difference of cell viability between pre-MarrowXpress and post-MarrowXpress samples (p value = 0.586). Cell viability was determined by flow cytometry with Stem-Kit using 7-ADD.



Day 2 of post-culture Day 7 of post-culture

Fig 4. MSC culture of MXP-processed bone marrow product. Human post-MXP bone marrow buffy coat was aseptically collected from the product bag. After RBC lysis, the nucleated cells were cultured in StemCell's MesenCult MSC basal medium containing MSC stimulatory supplements. An example of MSC colony was microscopically observed at day 2 (left) and day 7 day (right).

Table 1 Stem cell recovery after cryopreservation with BioArchive.

	THAWED CELL RECOVERY (%)			
Bone Marrow #	MNC	CFU	ALDH^{br} cells	CD34 cells
Bone Marrow 1	51	104	121	145
Bone Marrow 2	86	137	136	129
Bone Marrow 3	89	108	120	102
Mean	**75**	**116**	**126**	**125**
SD	**21**	**18**	**9**	**22**

Note: Thawed cell recovery was calculated dividing the total cell number in the post-thawed sample by the total cell number in the pre-frozen sample.

Conclusions

- **The MarrowXpress cell processing technology can be successfully used to reduce excess volume as well as concentrate stem cells from human bone marrow.**
- **Excellent hematopoietic stem cell recovery is coupled with reproducible volume reduction and RBC reduction without loss of cell viability.**
- **The cell product from MXP process shows angiogenesis activity and contains mesenchymal stem cells.**
- **The cell product from MXP process is compatible for cryopreservation in the BioArchive system.**