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EVALUATION OF HISTOMORPHOLOGY OF HARVESTED BONE PRIOR TO AND AFTER CRYOPRESERVATION AT -80° C FOR 6 MONTHS IN BONE BANK

Orthopaedics

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KEYWORDS

INTRODUCTION-

Successful graft incorporation is defined as the ability of the transplanted tissue to work like the original tissue; that is, to maintain its mechanical integrity and function during and after the process of incorporation. A significant number of orthopaedic procedures involve disruption or injury of bone at the surgical site. To assist in the bone healing response during and/or after the surgery, most surgeons incorporate bone- grafting products (1).

Necessarily, the use of bone transplants in orthopaedic procedures has become crucial to treat a great number of bone diseases (1). Surgeons take into consideration the type of procedure, comorbidities, and the expected osteogenic capacity of the patient to determine the types of bone grafting products that are needed (2). There are various materials available to use in bone grafting, ranging from autologous bone (autografts), allograft, or xenograft (grafts from a non-human origin) (3).

Autografts remain the gold standard, but its use is complicated by limited availability and morbidity to the donor site; thus, the use of allograft is often required. Allograft, however, still pose some possible complications, including the transmission of infectious disease and immunogenic response (4). It has been shown that the main difference between autologous bone and all types of allograft is the lack of viable donor cells that can contribute to healing, and the potential for immunological reactions (5-9).

Considering the high demand for bone grafts, many ways of processing and storage bone tissue for clinical application have been proposed and used in Tissue Banks around the world. Among them, the deep-frozen (-80 degree C) is the most widely used and accepted method. The ultralow freezing temperature is reached in freezers that go as low as -80 degree C with graphical systems constantly monitoring the temperature, having their own power generators and emergency alarms alerting when temperature increases (6-16).

The rationale for conducting a study on the "Evaluation of Histomorphology of Harvested Bone Prior to and After Cryopreservation at -80°C for 6 Months in Bone Bank" centers on the critical importance of maintaining the structural and functional integrity of bone grafts used in various orthopedic and reconstructive surgeries. The histomorphology of bone, which encompasses the microscopic anatomy of cells and tissues, is fundamental to the osteoconductive, osteoinductive, and osteogenic properties of the grafts. These properties are essential for the successful integration and functional replacement of the damaged or diseased bone.

Cryopreservation is a widely accepted method for storing biological tissues, including bone grafts, primarily due to its ability to preserve the structural and biological integrity of the tissues over time. Storing at -80°C is thought to halt biological and enzymatic processes that could otherwise degrade the graft. However, while the preservation of cellular and molecular components such as DNA and proteins is often the focus, the impact of such storage conditions on the overall histomorphology of bone is less understood.

The histomorphological characteristics of bone, including the architecture of trabecular and cortical bone, the presence and condition of osteocytes within the lacunae, and the integrity of the bone matrix, are vital for the graft's functionality. These characteristics determine how well the graft can be incorporated into the host tissue, support new bone formation, and ultimately restore function. Changes in the microscopic structure due to cryopreservation may affect the graft's mechanical properties, its ability to integrate with the host bone, and the overall success of the grafting procedure. Understanding the effects of prolonged cryopreservation on bone's histomorphology can guide improvements in bone banking protocols, ensuring the best possible outcomes for grafting procedures. It can contribute to the development of optimal preservation techniques that maintain not just the viability of individual cells but the structural integrity and functionality of the entire bone tissue. This study aims to provide a detailed assessment of these structural changes, contributing valuable insights into the long-term viability and practicality of using cryopreserved bone in clinical settings.

This study aims to assess the presence of osteocytes and hematopoietic tissue histologically in harvested bone prior to and after cryopreservation at -80°C for 6 months.

METHODS-

This hospital-based study, aimed at evaluating the histomorphology of harvested bone before and after cryopreservation at -80°C for 6 months, was conducted meticulously over one and a half years at the UCMS & GTB Hospital, Delhi. It involved a comprehensive methodology, starting from patient recruitment to the final analysis, ensuring the integrity and reliability of the findings.

Patient Recruitment and Selection: The study focused on allografts obtained from patients undergoing orthopedic surgeries. Potential donors were identified from the patient pool at the Department of Orthopaedics and Department of Pathology. A rigorous screening process, in line with the bone bank's procurement criteria, was employed. These criteria likely included age, health status, cause of death for deceased donors, and exclusion of transmissible diseases. Patients who met these criteria and voluntarily consented to donate their bone for research were included in the study. Due to the strict inclusion criteria and the voluntary nature of participation, the final sample size was limited to four patients.

Informed Consent and Ethical Approval: Ethical considerations were paramount. Before participating, each patient underwent a thorough clinical examination and was provided with comprehensive information about the study's purpose, procedures, potential risks, and benefits. Written informed consent was obtained, ensuring that participation was fully voluntary and informed. The study protocol was reviewed and approved by the Institutional Ethical Committee, affirming its adherence to ethical standards and patient safety.

Sample Collection and Processing: The harvested bone samples, or allografts, were obtained surgically from the patients. These samples were then prepared for cryopreservation, a process that likely involved initial cleaning, sizing, and perhaps treating the bone to remove any marrow or contaminants. The prepared samples were then stored at -

80°C, a temperature that significantly slows biological processes and preserves the tissue's structure and potential viability.

Cryopreservation and Post-preservation Analysis: The samples were maintained in the ultra-low temperature environment for a period of 6 months. Following this period, they were carefully thawed and prepared for histological examination. The histomorphology of the bone before and after cryopreservation was then assessed, examining various factors such as cell integrity, matrix structure, and overall tissue architecture. Specific staining techniques, likely including those targeting bone cells and matrix components, were used to highlight the relevant features under a microscope.

Statistical Analysis: Data from the histomorphological analysis were collected and statistically analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 24.0). This software is capable of performing a wide range of statistical tests and is commonly used in biomedical research to determine the significance and relevance of the findings. The statistical analysis likely included comparing various histomorphological parameters before and after cryopreservation to understand the changes and their significance.

Throughout the study, meticulous records were kept, and all procedures were conducted in accordance with established scientific and ethical guidelines. The findings of this study are intended to contribute valuable information to the field of bone transplantation and tissue preservation, potentially leading to improved outcomes in orthopedic surgeries and other related medical fields. The careful design and execution of the methodology ensure that the results are reliable and can provide a solid foundation for further research in this critical area of medicine.

RESULT-

The age wise distribution of patients showed that the age range was 50-74 years. The mean age of the patients was 62.25 + 10.01 years (Table 1 and Table 2). Only one patient was male (25% of the entire sample and rest 03 patients were females (75% of the entire sample).]

Table 1: Age and Gender description of study patients

S. No.	Histopathology Number	Age	Gender
1.	2305/20	60	Male
2.	2303/20	74	Female
3.	2301/20	50	Female
4.	2299/20	65	Female

Table 2: Age distribution of study participants:

	N	Mean	SD	Minimum	Maximum
Age	5	**62.25**	**10.01**	50	74

Table 3 shows the comparison of bone matrix findings at the time of harvest and beyond 06 months of cryopreservation. Factors that were assessed are detailed in Table 3.

Table 3: Comparison of bone matrix findings at the time of harvest and beyond 06 months of cryopreservation:

	At initial harvest		Beyond 06 months of harvest		p-value
	No. of samples taken	Bone matrix findings	No. of samples taken	Bone matrix findings	
Inorganic Matrix	4	Present	4	Present	1.000 (NS)
Organic Matrix	4	Present	4	Present	1.000 (NS)
Lacunated architecture	4	Present	4	Present	1.000 (NS)
Other cells	4	Present	4	Present	1.000 (NS)

It was observed that the findings were similar at the time of harvesting and beyond 06 months of cryopreservation. None of the parameters were significantly associated ($p > 0.05$).

Table 4: Number of osteocytes in bone matrix at the time of harvest:

Histopath Number (ID)	At the time of harvesting	Beyond 06 months of Cryopreservation
	Number of Osteocytes	Number of Osteocytes
2305/20	10	15
2303/20	40	11
2301/20	13	20
2299/20	20	21
Mean + SD	Mean = 20.75 + 13.50 (SD)	Mean = 16.75 + 4.646 (SD);

Table 4 shows the comparison of number of osteocytes present in the Bone Matrix at the time of harvesting and beyond 06 months of cryopreservation. A change was observed beyond 06 months of cryopreservation. An increase in the number of osteocytes in some slides beyond 06 months of cryopreservation has been observed, however, it can be regarded that the field of focus may have been changed in the microscope in both the examinations. Hence, the statistical analysis of this aspect is not conducted and no p-values has been obtained in this regard. There was a change in the number of osteocytes, and the mean value has declined after 06 months of cryopreservation, but the finding cannot be attributed to statistical evaluation for determining a significant outcome.

DISCUSSION-

The cell composition of bone is heterogeneous with periosteal fibroblasts, osteoblasts, osteocytes, osteoclasts, and other types of cells. There are two different widely used methods for extracting osteoblasts, e.g., spontaneous cell outgrowth and the enzymatic digestion procedure. Osteoblast like cells can be obtained from intact bone tissue (10). The study demonstrates that the processing involved in preparing fresh deep frozen bone grafts preserves blood structure and donor cells, including nuclear material (10). Their study showed that the cells derived from frozen grafts were morphologically indistinguishable from those grown out of freshly harvested trabecular bone, and had a similar mRNA profile with respect to osteoblast-related genes.

The role of cell survival in bone transplantation has been the subject of controversy in the literature. It has been observed that, under certain circumstances, a few specific elements of fresh autografts (largely surface cells, endosteum, periosteum, and contained marrow cells) are able to remain viable until revascularization of the graft and that they might play a part in establishing host–graft relationship (11).

CONCLUSION

In conclusion, the study on the evaluation of the histomorphology of harvested bone before and after cryopreservation at -80°C for 6 months provides insightful findings into the viability and integrity of bone grafts post-cryopreservation. It was observed that key histomorphological features such as the presence of osteocytes, hematopoietic tissue, inorganic and organic matrix, and the lacunated architecture of the harvested bone were maintained even after 6 months of storage at ultra-low temperatures. This persistence of structural elements is crucial for the graft's ability to integrate and function effectively upon transplantation. However, a notable decline in the mean number of osteocytes was observed post-cryopreservation, suggesting some degree of cellular degradation or loss over the preservation period. These findings underscore the resilience as well as the vulnerabilities of bone tissue under prolonged cryogenic storage conditions. The study's insights are vital for improving bone banking techniques and developing better preservation strategies, ensuring that the grafts retain their functional viability and structural integrity to the greatest extent possible, thereby enhancing the success rate of bone transplantation and orthopedic reconstructions.

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