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Brief communication

Osteoprogenitor viability in cell populations isolated from rat femora is not affected by 24 h storage at 4 °C ☆

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Abstract

This study was initiated to determine whether partially dissected bones of rats could be refrigerated for 24 h in saline without losing viability of progenitor cells, specifically osteoprogenitors. This is directly applicable to studies involving bone tissue requiring overnight shipment, for example, studies involving space flown animals, grafting experiments, or transplantation. We evaluated cell populations isolated from the proximal femur of 6-week-old male Fisher 344 rats. Explants from the left femur were prepared and placed into culture immediately following dissection, while the right femur was cleaned, fragmented, and stored in saline at 4 °C for 24 h, after which explant cultures were initiated. After 11 days of explant culture, cells were collected from outgrowths, counted, and plated to initiate experiments. Plated cells were grown for either 15 or 21 days. To determine if storage affected the total number of colony forming progenitors, alkaline phosphatase positive colonies, or the number of osteoprogenitors, were counted. There was no significant difference in any of the types of colony forming units examined between cell populations derived from freshly prepared samples or those stored for 24 h, indicating that storage at 4 °C of bone tissue for 24 h in saline does not affect the osteogenic potential or the number of osteoprogenitors of the cell populations isolated.

Keywords: CFU-O; Storage; Bone; Bone nodule; Alkaline phosphatase; Explant culture

It has been determined, in experiments involving space flight, bed rest, and/or hind limb unloading, that exposure to microgravity in both humans and animals results in extensive physiological changes including bone loss. This manifests as a decrease in bone mineral density and bone formation rate [1–3,17,18,20]. A major cause of the decrease in bone formation rate is likely due to a decrease in the number of osteoblasts and osteoprogenitors in the periosteum and metaphysis [4,18,19]. The mechanisms by which these changes are produced are still relatively unknown and requires further clarification.

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Extensive research has been done on the role of unloading on the skeleton using the NASA designed rodent tail-suspension model [13] and studies using rats that have been flown in space [17,18,20]. One major obstacle in space experiments is the lack of laboratory facilities at the landing site, particularly with the Biocosmos experiments. Thus, the question as to whether bone tissue can be refrigerated and transported to the laboratory for examination without losing the biological integrity of the sample arises. This study was performed to determine whether bone fragments taken from rats could be refrigerated for 24 h in saline without losing viability of progenitor cells, specifically the osteoprogenitors. These results directly apply to studies involving space flown rats, but are also relevant for experiments involving a delay in tissue processing like those involving grafting and/or transplantation.

Evidence to date suggests that short term refrigeration of osteoblast-like cells has fewer detrimental effects on cell growth than storage at −80 °C [9,15,16]. Moreover, transport of refrigerated samples is often more feasible than transport of frozen samples, and there are fewer complicating factors with refrigeration (i.e., the use of cryoprotective agents, antioxidants, and rapid versus slow thawing of samples). In a previous study that examined the effect of long-term refrigeration on osteoblastic cells harvested from murine calvaria using sequential enzymatic digestion, it was shown that cultures that were refrigerated for more than 48 h no longer grew, as assessed by alkaline phosphatase activity in vitro [8], suggesting a 2 day cut-off period for cell viability once cells are plated. In a shorter term study, it was shown that osteoblast-like cell populations isolated from explants of iliac cancellous bone of 4-month-old pigs had similar proliferative and differentiative potential (as assessed by [^{3}H]thymidine incorporation and alkaline phosphatase activity, respectively) compared to those isolated and stored in saline at room temperature for 2 h [12].

In our study, we evaluated whether cells could be grown from explants of bone refrigerated for 24 h and whether the cell populations isolated from these explants were different from those isolated from freshly prepared explants with respect to the proliferative capacity of osteoprogenitor cells. To do this, we prepared explant cultures from the proximal femur of 6-week-old male Fisher 344 rats from the left side of the skeleton immediately following dissection. The proximal femurs from the right side of the skeleton were dissected, cleaned, and fragmented. These fragments were stored in phosphate-buffered saline (PBS) and antibiotics (100 μg/ml gentamycin sulfate, 200 μg/ml penicillin G, and 0.6 μg/ml fungizone; Sigma–Aldrich Canada, Oakville, ON) at 4 °C for 24 h, after which explant cultures were prepared. Bones were fragmented prior to storage to remove bone marrow, to allow for more rapid cooling of the trabecular bone, and to aid in the explant preparation on the following day. PBS and antibiotics were used to store bones since this is the same solution that is used to wash bones upon collection at putative landing (collection) sites and is the solution used during explant culture preparation. Unsupplemented medium was not used as a storage solution to limit the number of solutions one would need to bring or prepare to a putative landing (collection) site and to decrease the amount of time and handling of samples prior to refrigeration.

The explant culture procedure used was as described previously [10]. Briefly, bone tissue was aseptically dissected and minced into ~1 mm^3 chips termed 'explants.' These were placed in plasma clots composed of 10 μl of 15% citrated bovine plasma (Sigma–Aldrich Canada, Oakville, ON) and 85% α-minimal essential medium (α-MEM) supplemented with 20% FBS (Invitrogen Canada, Burlington, ON) and antibiotics (50 μg/ml gentamycin sulfate, 100 μg/ml penicillin G, and 0.3 μg/ml fungizone) with 18 explants per 60 mm tissue culture dish (Falcon, BD Biosciences, San Diego, CA). Plasma clots were used to anchor explants to the surface of the dish, to provide additional growth factors and to act as support for the outgrowth. After clotting for 2.5 h, α-MEM containing 10% FBS and antibiotics (50 μg/ml gentamycin sulfate, 100 μg/ml penicillin G, and 0.3 μg/ml fungizone) was added and explants were cultured for 11 days. Explant cultures were grown for 11 days to allow sufficient cellular outgrowth required for subsequent experiments but not long enough to observe differentiation of the outgrowth population [10]. Cell outgrowth size at day 11, visualized