Ex-vivo large scale expansion of human RBCs from hematopoietic progenitors on a novel nanofiber scaffold mimicking bone marrow milieu

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Abstract

Introduction:
Various transmittable diseases from donor blood led to explore in-vitro manufacturing, cost effective, clinically applicable RBCs in large scale with several defined protocols worldwide. Commercially available alternative blood substitutes and medicines to increase the blood constituents are unstable with less oxygen carrying capacity and have not been successful in replacing donor derived RBCs. Large scale production of RBCs in-vivo depends on potential cell sources, bio-physiological parameters, erythroid inducers and sterile, non reactive, non-tumorigenic expansion media, to produce stable and functionally efficient RBCs. We formulated an efficient bone marrow milieu considering the physiological factors, favouring the differentiation of haematopoietic progenitors to clinically applicable and pathogen free mature RBCs.

Method:
The mononuclear cells were isolated from cord blood and bone marrow of living human donor with a pre-informed consent. The cells were nourished with media derived from human plasma of same blood group, bone marrow milieu and erythropoietic differentiation inducers (following laboratory proprietary protocol). The Temperature, Oxygen concentration, pH, Osmolarity of the cultures were maintained optimally. Finally, the cultured RBCs were subjected to various assays for cell proliferation, differentiation, haemoglobin content, and structural and functional properties of cultured RBCs such as oxygen affinity and dissociation ability etc.

Results:
The day 4 onward burst colony forming units were observed proving the erythroid lineage, which was monitored and documented on daily basis till the transformation happened from nucleated to enucleated adult RBC formation on 17th day. The scalability of the functional RBCs appeared to be 15 times of the originally seeded mononuclear cell number (2x10⁶/ml of culture media) in the culture flask and it was reproducible. The cells were in majority mature RBCs (95% by FACS) with adult haemoglobin, with trivial presence of other lineages. The adult haemoglobin had been proved by haemoglobin electrophoresis. Cultured RBCs were morphologically and functionally efficient. The scalability of production was assured in bioreactor culture expansion with indigenous novel biocompatible nano-fiber scaffold. Oxygen affinity and dissociation were efficient with five sets of experiments.

Conclusion:
This method of producing RBCs in large scale with a simple in-vitro reproducible protocol for making scalable and functional RBCs which is devoid of WBCs would be advantageous for frequently transfused patients, who are suffering from blood dyscrasias and exsanguination due to severe injury.

Further in-vivo stability evaluation with various animal experiments and clinical trials would make the possibility of clinically applicable, off the shelf packed RBCs which do not require screening for viral diseases as the cell source and media are pre-tested.