Title- "Osteoblast Microtissues as Profunctional Modules for Bone Tissue Engineering Applications"

Abstract:

Although a significant advancement has been made in the field of bone tissue engineering, engineered tissue constructs often fail to transmit the desired therapeutic response. Incidentally, cellular microtissues (also known as microaggregates/ microspheres / micromass) possessing higher order of structural and functional coordination, were hardly explored in bone tissue engineering. Concurrently, bottom up approach of tissue engineering relies on the exploitation of social characteristic of cells via creating microscale profunctional cellular constructs, which in turn could act as modules for the reparative or regenerative process. Keeping this perspective in mind, an effort is being made to explore the potentials of osteoblast microtissues in bone tissue engineering applications. It is hypothesized that use of osteoblast microtissues along with suitable 3D matrix may lead to superior bone tissue repair *in vivo*. Experimental validation of the hypothesis was preceded by preparation of ostoeblast microtissues from primary murine neonate calvarial osteoblast cells, and characterization in terms of gap-junctional communication, bone specific marker gene expression and mechanosensitivity. Secondly, a novel injectable gel composed of Tyrosinase enzyme – carboxymethyl chitosan – gelatin –nanohydroxyapatite was developed and characterized in view of using it as a non-invasive delivery vehicle of microtissues to ectopic site for exploring their osteogenic property *in vivo*. Lastly, a macroporous scaffold composed of Gelatin-nanohydroxyapatite was fabricated via a novel combination of ultrasonic foaming-freeze drying method and was characterized thereof. Osteoblast microtissues, seeded onto the macroporous scaffold, were tested for their critical skull defect healing potentials in murine model. The *in vivo* studies revealed that osteoblast microtissue carrying implants showed superior expression of bone specific markers, mineralization, and vascularization than isolated non-aggregated cells seeded implants as revealed from RT-PCR, X-ray radiography, and histochemical experiments. Higher vascularization *in vivo* may be one of the reasons for faster bone formation in osteoblast microtissue seeded implants, which may be attributed to high *in vitro* VEGF production by osteoblast microtissues. The results indicated that osteoblast microtissues were functionally closer towards bone tissues and can induce faster bone formation ectopically as well as at skull defects. In conclusion, it could be said that use of osteoblast microtissues in cell bone based tissue engineering may ascertain faster healing.

Keywords: Primary murine osteoblast, Osteoblast microtissue, nano-hydroxyapatite, Injectable gel, Ectopic bone, Macroporous scaffold, Critical skull defect, Bone tissue engineering.