Influence of zinc oxide enriched biogas on proregenerative properties of chitosan based bio composites for bone tissue regeneration

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Cytotoxic and pro-regenerative effects of biogas: 12 – well plates with 1.5 ml of medium per

Well and inserts with or without biogas and 0.5 ml were preincubated in 34°C, 90% of humidity and 5% CO2 to the time of cells' seeding. Next 2 x 105 hFOB 1.19 cells were seeded into each well. Plates were incubated for 48 h in previous descripted conditions then Proliferation and cytotoxicity test were conducted. Cytotoxic and proregenrative effects of composites.

Direct method: Prior the experiment the composites were inserted into 24 well plates and Immersed in 1 ml of culture medium for 1 hour. Sterilized Ranching rings were placed on the top of the composites followed by hFOB 1.19 cell seeding. For each well 5x104/cm2 hFOB were Seeded inside the Ranching ring. Plates were incubated for 1 hour in 34°C and 5% CO2 to allow cells attachment to the composites. After 1 hour rings were removed, and cells were incubated for 48 h. After that time the cell viability was estimated by mitochondrial activity measurement (WST-1 test) and Cytotoxicity was measured by the leakage of lactate dehydrogenase (LDH cytotoxicity assay). Indirect method: Different composites were inserted into 24 – well plates and immersed in 1,1ml culture medium for 24 hours in 34°C and 5% CO2. After that time the extract was collected. Day prior the experiment hFOB 1.19 cell were seeded at density 5x104/cm2 on 24 – well plates. On the day of experiment medium from wells were removed and collected extracts were added On the cells and then incubated for 48 h 34°C and 5% CO2. After incubation time the cell Viability was estimated by mitochondrial activity measurement (WST-1 test) and cytotoxicity.