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Osteoblastic maturation on graphene film onto titanium via dry transfer technique

Titanium is widely used for implants due to its ability to integrate to native bone. The time for dental implants to integrate usually takes three months in the mandible and up to six in the maxilla. Notably, both the long-healing time is one area that can be improved in implant-based therapies. A possible solution could be graphene produced by chemical vapour deposition (CVD) that can induce the osteogenic differentiation of stem cells by increasing the expression of osteogenic-related genes and proteins (e.g., RUNX2, OCN and OPN) and mineralized nodule deposition (Xie et al., 2015). Regardless of the osteogenic potential, the deposition of graphene films onto implants is hindered by the transfer methods. The classical wet transfer technique often traps water between the target substrate and graphene film that can create large cracked areas upon evaporation (Li et al., 2009). Alternatively, the direct dry transfer technique relies on the application of pressure to promote the intimate contact between a graphene/polymer film and the target substrate. Thereafter, the polymer is peeled off leaving graphene behind. Despite of its versatility, the osteogenic potential of graphene deposited via dry techniques remain unknown. The objective of our study was to evaluate the osteogenic potential of graphene coating on commercially pure titanium (CpTi) via wet (WGp) and direct dry transfer technique (DGp). For this, graphene used was grown by chemical vapor deposition (CVD) and transferred to titanium (CpTi) via the traditional wet technique (WGp) or dry technique (DGp) where a graphene/PDMS film as placed in contact with CpTi and hot pressed (150 N, 4 min at 150°C) followed by the peeling of the PDMS. Thereafter, pre-osteblastic cells (MG-63) were seeded onto the samples and cultured with basal growth medium. The cytocompatibility of the graphene-based coatings were assessed by the MTS assay. Both WGp and DGp presented significantly higher proliferation compared to CpTi after 120 h ($p<0.05$). The percentage of LDH released by cells on all substrates was statistically similar denoting no cellular membrane damage provoked by the graphene coatings. The osteogenic potential was assessed in both gene and protein level by qPCR and Western blot, respectively. Except for RUNX2 at 24 h, both WGp and DGp increased the expression of all osteogenic-related genes and time points tested comparing to CpTi. Both WGp and DGp also increased the OCN gene and protein expression. The calcium content per ng of DNA on graphene-coated substrates were significantly higher than the amount obtained with CpTi for all time points evaluated. In summary, the dry-transfer technique yielded to coat more than 90% of the commercially pure titanium sample with graphene without changing the morphological characteristics of the substrate. The graphene coating accelerated the osteogenic differentiation and increased the amount of calcium deposition of pre-osteoblast cells.

References

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