ABSTRACT

Title of the thesis: Development and characterisation of different inorganic biocomposite materials suitable for craniofacial reconstruction: An in vitro and in vivo approach

Craniofacial bone reconstruction is preferred to correct large skull bone defects arising from the treatment of tumours, infections, trauma intracranial haemorrhage or necrosis. These defects cause both functional and aesthetic discomfort to patients. In worse case, this may cause swelling of brain tissue for which suitable solution is required. There is a long history of reconstructing large skull bone defects with autogenous bone, and it remains the gold standard of treatments. But disadvantages of this type of reconstruction are related to possible infection of the bone graft, donor site morbidity, and cumbersome handling of the bone graft.

To overcome these concerns, several alloplastic materials including metals, plastic, ceramics, and composites are used with limited success and most importantly these never achieved patient compliance. For reconstruction, patient specific implants can be manufactured directly or indirectly. Each method has a set of advantages and disadvantages and till now no perfect solution for craniofacial reconstruction is available.

Thus, in the present investigation biocompatible and osteoconductive composites were fabricated using HAp and bioinert E-glass as base or matrix. Nano-hydroxyapatite (n-HAp) was developed by sol-gel method (with Ca/P molar ratio close to 1.67) and suitably applied/ coated on E-glass substrates. Thorough physical, chemical, mechanical, and biological characterisation was carried out. XRD, FTIR, Raman spectroscopy were undertaken for phase analysis and evaluation, while FESEM-EDAX, TEM for detailed layer-wise microstructural characterisation; MTT assay, SEM and cell viability using MG63 and NIH3T3 cell line. Also *in vitro* studies, cell proliferation by Alamar blue assay, alkaline phosphatase assay, gene expression by real-time RT-PCR, and mineralization assay by ARS staining were done to predict the nature of the sample *in vivo.*

In our second study we tried to assess the beneficial effects of Sr^{2+} and Li^{+} doping on in vivo bone formation of an interconnected bioactive glass porous scaffold developed in the laboratory through rabbit bone defect model. Detailed phase, composition and microstructure analysis were performed prior using tools like X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR), differential thermal analysis-thermo-gravimetric analysis (DTA-TGA), quantitative EDAX analysis and scanning electron microscopy (SEM) respectively. The scaffolds were also assessed for its bioactivity in contact with simulated body fluid (SBF) and in vitro cyto-toxicity by MTT assay using NIH3T3. The *in vivo* bone regeneration was analysed using chronological radiography, fluorochrome labelling, SEM, histology, and micro-computed tomography (μ -CT).

On the other hand, plasma spray grade HAp and bioactive glass (S53P4) granules were used for coating on commercially available Mg-alloy implant varying the coating parameters including current, voltage, stand-off distance, powder flowability and primary/secondary gas. Mg-alloy is known for their bioresorbable properties with higher degree of corrosion in vitro. HAp/S53P4 coating on the substrate, thus, not only act as a source for bone bonding interface, but also protects from corrosion when used in physiological fluid. As before the coated substrates were studied for different physical, chemical, mechanical, and biological characterisation including XRD, FTIR, Raman spectroscopy for phase analysis and evaluation; FESEM-EDAX at the interface for detailed layer-wise microstructural characterisation; electrochemical corrosion test using SBF solution. *In vitro* biocompatibility assessments like cell viability and proliferation assay using MG63 cell line, alkaline phosphatase assay,

mineralization assay by ARS staining, gene expression by real time RT-PCR, cell morphology within the scaffold constructs by Laser confocal, were done prior to *in vivo* study.

Publications from the work:

In Journals:

1) **A. Mahato**, M. De, P. Bhattacharjee, V. Kumar, P. Mukherjee, G. Singh, B. Kundu, V.K. Balla, S.K. Nandi, J. Mater. Sci.: Mater. Med., 32 (2021) 55. **(Impact factor: 3.896)**
2) **A. Mahato**, P.K. Khan, B. Kundu, S.K. Nandi, P. Mukherjee, S. Datta, S. Sarkar, J. Mukherjee, S. Nath, V.K. Balla, Nature Scientific Reports 6 (2016) 32964. **(Impact factor: 4.380)**
3) **A. Mahato**, Z. Sandy, S. Bysakh, L. Hupa, I. Das, P. Bhattacharjee, B. Kundu, G. De, S.K. Nandi, P. Vallittu, Mater. Sci. Eng.: C 111 (2020) 110764. **(Impact factor: 7.328)**
4) **A. Mahato**, B. Kundu, P. Mukherjee, S.K. Nandi, Trans. Ind. Ceram. Soc. 76(3) (2017) 149-158. **(Impact factor: 1.729)**

In Book Chapters:

1) **A. Mahato**, B. Kundu, Bioactive glass based composites for cranioplasty implants, in: G. Kaur (Ed.) Clinical Applications of Biomaterials: State-of-the-Art Progress, Trends, and Novel Approaches, **Springer**, Gewerbestrasse, Switzerland, 2017, pp. 337-356.
2) S.K. Nandi, **A. Mahato**, B. Kundu, P. Mukherjee, Doped bioactive glass materials in bone regeneration, in: A.R. Zorzi, J.B. de Miranda (Eds.) Advanced Techniques in Bone Regeneration, **InTech Open**, Rijeka, Croatia, 2016, pp. 275.
3) S.K. Nandi, **A. Mahato**, B. Kundu, P. Mukherjee, Organic-inorganic micro/nanofiber composites for biomedical applications, in: V. Grumezescu, A.M. Grumezescu (Eds.) Materials for Biomedical Engineering: Biopolymer Fibers, **Elsevier**, Bucharest, Romania, 2019, pp. 21-55.
4) P. Barua, **A. Mahato**, P. Datta, S.K. Nandi, R. Sen, B. Kundu, Fiber-nano bio-compositions for cranioplasty and other orthopaedic applications, in: B. Han, S. Sharma, T.A. Nguyen, K. Subrahmanya Bhat, L. Longbiao (Eds.) Fiber-Reinforced Nanocomposites: Fundamentals and Applications, **Elsevier**, Amsterdam, Netherlands, 2020, pp. 525-558.

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