ABSTRACT

Bone diseases represent a major global health challenge, affecting individuals across all age groups and genders. Among people over the age of 50, these conditions account for nearly half of all chronic illnesses. The most prevalent bone disorders–osteoarthritis and osteoporosis–affect approximately 7.6% and 2.5% of the global population, respectively.

Bone tissue undergoes continuous remodelling driven by the coordinated activity of bone cells. However, in cases of pathological fractures or extensive bone defects, the intrinsic regenerative capacity of bone may be insufficient. This regenerative potential is further compromised by systemic diseases, infections, or poor vascularization. Although autologous bone grafts remain the clinical "gold standard" for bone repair, their use is limited, and alternative methods often fail to ensure predictable and effective regeneration.

In response to these challenges, my doctoral research focused on the design and development of composite materials intended for use in implants for bone defect repair. These composites were engineered to function as temporary cellular scaffolds and to promote the regeneration of damaged bone tissue.

The primary aim of the study was to develop innovative composite materials with pro–regenerative properties for applications in bone tissue engineering. To achieve this, I designed, synthesized, and characterized three types of composites composed of chitosan, a 2–Maleimido acetic acid, and selected peptides derived from growth factor sequences. The composites differed in the mode of peptide integration into the chitosan matrix and in their peptide release mechanisms.

The first composite type consisted of chitosan covalently conjugated with the biologically active peptide ug4 via a maleimide linker. The second was a physical mixture of chitosan and free peptide ug51, which underwent slow autohydrolysis, gradually releasing active sequences into the bone tissue environment. The third composite incorporated chitosan and peptide fibrils of ug52, which exhibited high stability and enabled controlled, sustained peptide release, making them a promising platform for long–term therapeutic application.

The designed peptides included sequences recognized by matrix metalloproteinase–7 (MMP–7), enabling enzymatically controlled release under physiological conditions directly at the site of implantation. Peptide stability in aqueous and culture media, as well as enzymatic cleavage sites, were investigated using liquid chromatography and mass spectrometry.

Biological evaluation–including cytotoxicity assays and osteoblast proliferation studies in the presence of the developed materials–was carried out in collaboration with the Institute of Biotechnology and Molecular Medicine in Gdańsk. Based on the biological activity and cost–effectiveness analysis, the most promising peptide was selected, and a pilot–scale synthesis procedure was developed and optimized.

I synthesized an active ester of maleimidoglycine and established a method for chitosan functionalization via conjugation with a maleimidoamino acid. The degree of substitution was quantified using high–performance liquid chromatography (HPLC), following optimization of chromatographic conditions and selection of an external calibration standard. These results enabled the design and optimization of the peptide–chitosan conjugate via covalent coupling. The efficiency of peptide conjugation was assessed by HPLC under validated analytical conditions.

The project also included the fabrication and detailed physicochemical characterization of pro–regenerative peptide fibrils. This characterization involved aggregation assays, circular dichroism (CD) spectroscopy, thioflavin T staining, and transmission electron microscopy (TEM).

In summary, the work optimized synthetic procedures for bioactive peptides, established a method for chitosan functionalization, and comprehensively evaluated the physicochemical and biological properties of the resulting peptide–chitosan conjugates and fibrillar structures. The developed composites were designed to provide a bioactive environment conducive to bone tissue regeneration and to facilitate the controlled release of therapeutic agents.

Composite discs were prepared in collaboration with the Institute of Ceramics and Building Materials in Warsaw, a member of the Łukasiewicz Research Network. The materials were analysed for their physicochemical and biological properties. For the composite exhibiting the highest regenerative potential, implantation studies were conducted at the Centre for Preclinical Research of the Wroclaw Medical University, assessing acute toxicity and local tissue response following bone implantation.

This dissertation presents an interdisciplinary research approach and contributes novel findings to the global body of knowledge in the field of bioactive bone–filling composite materials.