

Abstract

Bone tissue engineering attempts to mimic the natural behavior of hard connective tissues. Currently, there is an active research and development of new functional materials in this field. The main direction in the treatment of bone tissue defects is the use of biodegradable composite materials based on biopolymers enriched with an inorganic component, possibly in combination with a cellular component. In general, hydrogels play an important role in tissue engineering due to their properties, as they can respond relatively well to changes in environmental conditions and are used as a suitable medium for cells to grow and proliferate. Structure of hydrogels also allows nutrients and waste products to diffuse, and the last but not least, thanks to this they can also serve as drug carriers. A major advantage of hydrogels is that they can be injected with subsequent *in situ* gelation.

In this work, physically cross-linked hydrogels based on polyvinyl alcohol (PVA) and hyaluronic acid (HA) in different volume ratios enriched with hydroxyapatite (HAp), an inorganic component inherent to bone tissue, were compared. Incorporation of HAp into the organic matrix was performed in two different ways, by *in situ* synthesis directly in the PVA/HA matrix and by physical mixing of pre-prepared HAp. The composition of both types of HAp was analyzed using infrared spectroscopy and energy dispersive spectroscopy.

To determine the most suitable type of hydrogel for the treatment of a bone defect, the mechanical and biological properties and viability of MG-63 osteoblast-like cells was evaluated. Furthermore, the viability and differentiation of human mesenchymal stem cells BM-hMSC were verified on selected hydrogel types. The used differentiation medium was enriched with dexamethasone, ascorbate-2-phosphate, β -glycerol phosphate, vitamin K3 and vitamin D3. The degree of cell differentiation was assessed by expression of selected osteogenic genes using polymerase chain reaction, determination of alkaline phosphatase activity, and visual confirmation of calcium salt staining.

The achieved results showed that the preferred composition of PVA/HA/HAp hydrogels was in the volume ratios of 3:1:2 and 1:1:2, respectively. From the point of view of mechanical properties, the preparation of hydrogels, where HAp was synthesized *in situ*, appeared to be advantageous. As expected, the presence of HA also appeared to significantly enhance primary cell adhesion and the presence of HAp improved cell proliferation. The evaluation of differentiation showed that, with the exception of Runx2 gene expression, the hydrogel formulation with a 3:1:2 volume ratio of individual components was significantly preferable.

Key words: bone tissue engineering, hydrogel, PVA, hyaluronic acid, hydroxyapatite, MG- 63, mesenchymal stem cells, differentiation, osteoblasts