Preparation of hydroxyapatite/poly(lactic acid) hybrid microparticles for local drug delivery.

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Abstract. Calcium phosphate (CaP) bioceramic is well known as bioactive and biocompatible material in bone tissue regeneration applications. Apatitic CaP, especially nano sized hydroxyapatite (NHAp), is more similar to the natural apatite presented in the bone tissue than CaP bioceramics. In the current research NHAp was modified using biodegradable polymer - poly(lactic acid) (PLA) to develop composites providing bone regeneration and local drug delivery. NHAp/PLA microcapsules were prepared using solid-in-water-in-oil-in-water (s/w₁/o/w₂) encapsulation technology. The impact of primary and secondary emulsion stability on the emulsion droplet and microparticle properties was evaluated. The stability of final emulsion can be increased by varying the process parameters. Stable s/w₁/o/w₂ emulsion using 3ml of NHAp suspension, not less than 100ml of 4% PVA water solution and 10ml of 10% PLA solution in dichloromethane can be obtained. S/w₁/o/w₂ microencapsulation method can be effectively used for the preparation of multi-domain microcapsules achieving high NHAp encapsulation efficacy (93%).

1. Introduction
Musculoskeletal diseases or disorders such as osteonecrosis, osteoporosis, bone fracture, bone tumor etc. affect hundreds of millions of people all over the world [1]. All these diseases may lead to the weak and fragile bones, increasing the need for bone repair and reconstruction materials as well as for new drug delivery devices.

Bone is a living tissue, which continuously rebuilds its structure and is capable of regeneration. Natural bone is a complex composite, mainly constituted of biologic apatite, responsible for the bone mechanical properties and natural polymer collagen, responsible for the bone elasticity and toughness [2-3]. To promote the bone regeneration and healing, bioactive composite materials have been used for
the filling and supporting defects, voids or damaged bone parts [4]. Synthetic calcium phosphates, especially hydroxyapatite (HAp) and β-tricalcium phosphate, are the most prospective materials for bone tissue replacement and regeneration due to their unique properties – biocompatibility, bioactivity, osteoconductivity and similarity to the natural bone mineral phase [5]. Last two decades such biodegradable polymers as poly(lactic acid) (PLA), poly(glycolic acid) and poly(ε-caprolactone) have been widely used as biomaterials [6-7]. Biodegradability, biocompatibility and non-toxicity are the main advantages of these polymers. Combination of PLA and HAp will result in materials with properties necessary for the bone fillers or drug delivery systems in the field of bone regeneration.

In the current research nano sized HAp/PLA microcapsules were prepared using solid-in-water-in-oil-in-water (s/w/o/w) encapsulation technology. The impact of primary and secondary emulsion stability on the emulsion droplet and microparticle properties was evaluated. Prepared composite materials could be the potential candidates for the scaffold preparation using additive technology as well as effective drug delivery systems in orthopedic trauma surgery.

2. Materials and methods

Poly(L-lactide) (Biomer L9000) with molecular weight of 200-300 kDa and polyvinyl alcohol (PVA) with molecular weight of 25 kDa (98 mol % hydrolyzed) were purchased from Polysciences (Warrington, USA). All solvents were distilled before use.

Nanosized hydroxyapatite (NHAp) was synthesized using precipitation reaction between calcium hydroxide suspension and orthophosphoric acid solution (wet precipitation method) described elsewhere before [8-9]. The solid loading in NHAp suspension was 10% wt.

Microcapsules were prepared using modified water-in-oil-in-water emulsification method described before [10-11]. An aqueous suspension of NHAp (0.5 ml, 1 ml, 1.5 ml, 2ml and 2.5 ml) was added to the PLA solution in dichloromethane (1%, 3%, 5%, 10%). S/w/o primary emulsion was properly mixed and added to 100 ml of 4% aqueous PVA solution. S/w/o/w secondary emulsion was emulsified for 60s, using a mechanical stirrer. After emulsification, the organic solvent was extracted in 2l of water for 25 min. Formed microcapsules were separated by filtration and dried at ambient temperature for 24h.

S/w/o/w emulsion stability was evaluated using light microscopy equipped with Image Pro+ software. Light microscopy was also used for characterization of the microparticle form, particle size distribution, as well as for the detection of agglomerates. Scanning electron microscopy was used to evaluate the microparticle surface morphology and inner structure.

3. Results and discussion

The main precondition for the successful microencapsulation process is to provide stable s/w/o/w emulsion. Emulsion stability was expressed as a time after interruption of homogenization process, when water phase and organic phase retain homogenous and do not decompose into two separate phases. NHAp suspension was used as primary water phase and the impact of its amount (0.5 ml, 1 ml, 1.5 ml, 2ml and 2.5 ml) as well as concentration of the organic phase (1%, 3%, 5%, 10%) on the s/w/o emulsion stability was evaluated. Results obtained showed that increasing the primary water phase content and polymer concentration in the organic phase, stability of s/w/o emulsion increased (see figure 1). At the same time the average droplet size of s/w/o emulsion increased from 80µm up to 500µm due to the changes in the organic phase viscosity.
In order to provide stable s/w/o/w secondary emulsion, polyvinyl alcohol as surface active substance was added to the secondary water phase. Variations of different amounts of secondary water phase (30ml, 60ml, 100ml and 150ml) containing 4% of emulsifier on the s/w/o/w emulsion droplet size distribution and tendency to agglomerate was estimated. Results showed that the increase of secondary water phase decreased the emulsion droplet agglomeration. The average size of emulsion droplets increased from 200µm-300µm, if 30ml of 4% PVA solution was used up to 600µm-800µm, if 150ml of 4% PVA solution was used (see figure 2).

**Figure 1.** Stability of s/w/o/ emulsion.

**Figure 2.** Light microscopy images of s/w/o/w emulsion droplets. The time after homogenization interruption if 30ml of 4% PVA are used: a) 1min; b) 2min; b) 5min. The time after homogenization interruption if 150 ml of 4% PVA are used: a) 1min; b) 2min; b) 5min.
Impact of the secondary water phase variations on the obtained NHAp/PLA microparticles was determined (see figure 3).

Figure 3. SEM microphotographs of: a)surface morphology of NHAp/PLA microcapsules; b)cross-section of blank NHAp/PLA microcapsules; c)NHAp.

Results showed that s/w<sub>1</sub>/o/w<sub>2</sub> microencapsulation method can be effectively used for the preparation of NHAp loaded PLA microparticles with multi-domain microcapsule structure. Encapsulation efficiency of NHAp strongly depends on the microencapsulation process parameters used (water content in w<sub>1</sub> phase and amount of w<sub>2</sub> phase) and it varies from 77% in the case of 30ml of 4% PVA up to 93% in the case of 150ml PVA. During the investigation it was found that increasing the volume of w<sub>2</sub> phase the number and size of NHap loaded PLA domains increased.

4. Conclusions

The stability of final emulsion could be increased, by varying the process parameters and stable s/w<sub>1</sub>/o/w<sub>2</sub> emulsion using 3ml of NHAp suspension, not less than 100ml of 4% PVA water solution and 10ml of 10% PLA solution in dichloromethane can be obtained. S/w<sub>1</sub>/o/w<sub>2</sub> microencapsulation method can be effectively used for the preparation of multi-domain microcapsules, where NHAp encapsulation efficacy reaches 93%.

5. References

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