УДК: 546.41

The Formation of Liesegang Rings made of Calcium Phosphates in Agar Gel

Authors: Mervat Moussa Mohamed Eltantawy
Supervisors: Sviatlana A. Ulasevich, Ekaterina V. Skorb

ITMO University, International institute, Lomonosova str. 9, Saint Petersburg, 191002, Russian Federation

Nature is rich with examples of sophisticated materials displaying outstanding properties that emerge from their specific hierarchical structure. Materials such as bone, and dental enamel possess distinct structural organization at different length scales, which enhance their bulk material properties and functionality. Studying the processes of biomineralisation is very important for understanding the nature of bone formation and origin of life [1–3].

The aim of this study is to investigate the process of different patterns growth from calcium phosphates being not only the main component of bones' matrix but also crystalline components in human dental calculus and in other pathological calcifications. In our study calcium phosphate is precipitated for the first time in the agar gel matrix using single-diffusion gel growth technique. The method was chosen due to its simplicity to study the product of the reaction. It was also proven that the gel method of crystal growth is very suitable to provide direct access of observing the biomineralization process resembled to human physiological conditions, because of its viscous nature providing simulation of synovial fluid, cartilage and other biological fluids.

The precipitated Liesegang spherical bands in agar were obtained by employing Na_2HPO_4 and $CaCl_2$ as the inner and outer electrolyte, respectively. Effects of concentrations of the electrolytes as well as pH and temperature on the morphologies, periodicity, stability and number of Liesegang rings (LRs) were studied for 14 –21 days.

The 0.075 M Na_2HPO_4 solution was homogeneously distributed in agar. The 1 M $CaCl_2$ solution was added in a system after the gelatinisation of agar. This condition ensures that the pattern formation is governed by the diffusive flux of the outer (invading) electrolyte. We use the simplest case of that the outer electrolyte is directly poured on the top of a gel column containing inner electrolyte in a test tube. After a period of time, in different systems and different conditions, variety of the patterns have been observed including regular band patterns, concentric rings type Liesegang pattern, radial periodic pattern.

The pattern characteristic is that the distance between the bands/rings and their widths increases with the distance measured from the junction point of the two (outer and inner) electrolytes. Several models and empirical laws, such as the time law, spacing law, width law and Matalon–Packeter law, have been applied to explain the morphological characteristics. These different techniques will reveal the different phases formed under different conditions. These results will help to understand the promotion, modulation and inhibition of the Calcium phosphate self-assembled Liesegang rings in the agar gel.

Thus, here we report a self-assembling route to form calcium phosphate in agar gel for the first time to be utilized in the future in studying its effect on the differentiation, proliferation and migration of stem cells in the absence of osteogenic inducers providing important information that can be used in optimizing calcium phosphate -based scaffold

design to foster bone tissue growth and repair. Synthetic calcium phosphate exhibits good properties such as biocompatibility, bioactivity, osteoconductivity.

This study could be applied in the biomedical field and reconstructive surgery also suitable for synthetic bone substitution applications as it provides a permanent scaffold for new bone formation via its osteoconduction and the resorption properties which oversaturates the local environment with Ca^{2+} and PO_4^{3-} ions to accelerate this new bone formation.

Authors acknowledge RSF grant no. 19-79-10244 for the financial support of surface nanostructuring part. ITMO Fellowship and Professorship Program 08-08 is acknowledged for infrastructural support.

[1] Lotsari A. et al. Transformation of amorphous calcium phosphate to bone-like apatite //Nature communications. – 2018. – Vol. 9, №. 1. – P. 1-11.

[2] Elsharkawy S. et al. Protein disorder–order interplay to guide the growth of hierarchical mineralized structures //Nature communications. – 2018. – Vol. 9, №. 1. – P. 2145.

[3] Noorduin W. L. et al. Complete chiral symmetry breaking of an amino acid derivative directed by circularly polarized light //Nature chemistry. – 2009. – Vol. 1, №. 9. – P. 729.