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Physiological Polyphosphate: a New Molecular Paradigm in Biomedical and Biocomputational Applications for Human Therapy

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Physiological polyphosphate: a new molecular paradigm in biomedical applications for human therapy.

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Abstract. Inorganic polyphosphates (polyP) consist of linear chains of orthophosphate units, linked together by high-energy phosphoanhydride bonds. The family of polyP molecules are evolutionarily old biopolymers and are found from bacteria to man. PolyP is exceptional, no other molecule concentrates as much (bio)chemically usable energy as polyP in animals, including humans. Before this discovery, we found that the long-neglected polymer provides ortho-phosphate units, required for bone (hydroxyapatite) synthesis. Hence, polyP is a cornerstone for bone synthesis and repair, especially in higher animals. Besides of its importance for regenerative medicine, especially for the reconstitution of osteo-articular impairments/defects, a further imperative property could be attributed the polyP. This polymer is the only extracellular generator of metabolic energy in the form of ATP. While the mitochondria synthesize ATP in large amounts intracellularly, it is the polyP, which functions as the storage for extracellular ATP. After enzymatic hydrolysis of polyP through the alkaline phosphatase (ALP) the released free energy is partially stored in ADP (during the transition from AMP), which in the second step is up-phosphorylated to ATP by the adenylate kinase (ADK). In turn, the two enzymes ALP and ADK are the bio-catalytic proteins that conserve the released free energy and store it in ATP, especially in the extracellular space. In a proof-of-concept we could demonstrate that polyP is an essential component for human regeneration processes, especially in those regions which are poorly vascularised, like in bone, cartilage and wounds (including chronic wounds).

Keywords

Regenerative medicine • polyphosphate • biomaterial • metabolic energy • morphogenetic activity • tissue regeneration • bone, cartilage • chronic wounds

1 Introduction

During the last years, a group of polymers has attracted increasing attention due to their unique ability to provide metabolic energy, which is essentially needed for tissue regeneration and repair. It is the physiological, inorganic polyphosphate (polyP). The polyP polymers are polyelectrolytes, composed of multiple phosphate (P_i) residues linked together by high-energy phosphoanhydride bonds. Structurally, the polyP chains are composed of tetrahedrally coordinated P_i units that are interconnected to one another via shared oxygen atoms [1, 2].

PolyP has been identified in yeast already during 1880 and its importance was disclosed later in bacteria by Lohmann, Langen, Holzer/Lynen, Kulaev/Belozerskij, and Kornberg. Our group focused on polyP already in 1998, when we found that *in vitro* polyP modulates the bio-mineralization process. Subsequently, we described that bio-minerals are synthesized by enzymes, a finding which opened up a new window in therapeutic approaches also for human repair processes. The first enzyme in this field was silicatein, which is synthesizing the skeleton of the most basal animals, of the sponges. After that, focusing on human bone, we pinpointed that for bone formation both the alkaline phosphatase (ALP) and the carbonic anhydrase are needed. After these observations it became possible to sketch the bone anabolic processes with the following sequences: During endochondral ossification the hyaline cartilage acts as a template mold for the initial mineralization, most likely of calcium carbonate. In parallel an ingrowth of blood vessels occurs, followed by the formation of the primary ossification centers in the diaphysis. Later, spongy bone is formed in the epiphyses at the secondary ossification centers. In these two regions of the hyaline cartilage remaining on the surface of the epiphysis (articular cartilage) and the epiphyseal plate (growth region) between the epiphysis and the diaphysis final hydroxyapatite bone formation takes place. Appositional growth of the bone proceeds in the absence of a cartilage template occurs [2, 3].

2 Methods, Results and Discussion

The, mineral deposition involves two enzymes; first, carbonic anhydrase and second, alkaline phosphatase (ALP). This process can be subdivided into four stages. Phase I: bio-seed deposition catalyzed by carbonic anhydrase; it is postulated that the product is amorphous calcium carbonate. Phase II: hydrolytic cleavage of polyP by ALP allowing, phase III: the released phosphate units to be transferred non-enzymatically to the calcium carbonate under conversion to amorphous calcium phosphate. Phase IV: maturation of the calcium phosphate to crystalline hydroxyapatite [3].

A further important property of polyP was determined in *in vitro* experiments – the generation of extracellular metabolic activity. Using the appropriate cells, the human umbilical vein endothelial cells (HUVEC) it was disclosed that these cells require a considerable amount of metabolic energy, of ATP. Incubation of HUVEC with polyP the extracellular space, this polymer was hydrolyzed *via* the ALP and together with the adenylate kinase, the final ATP was formed which became the energy for the migration of the cells for the initial vascularization. These processes were abolished by apyrase (eliminates extracellular ATP). ATP was also identified as a signal for the chemotactic migration of the cells during the vascularization. These data demonstrated that polyP is the storage for extracellular ATP and also a signaling molecule for an autocrine chemotactic pathway of ATP during with polyP endothelial cell migration [4].

After the disclosure of the bio-mineralization processes for bone it was demonstrated that amorphous Ca-phosphate (ACP) particles, stabilized by inorganic polyP, is a suitable matrix for cell growth and attachment and showed pronounced osteoblastic and vasculogenic activity *in vitro* and also *in vitro* angiogenesis events in the tube forming assay. A possible involvement of an ATP gradient, generated by polyP during tube formation of human umbilical vein endothelial cells, was confirmed by ATP-depletion experiments. In order to assess the morphogenetic activity of the hybrid particles *in vivo*, experiments in rabbits using the calvarial bone defect model were performed. The particles were encapsulated in poly(D,L-lactide-*co*-

glycolide) microspheres. In contrast, to crystalline Ca-phosphate amorphous ACP caused pronounced osteoinductive activity even already after a six-week healing period. The synthesis of new bone tissue was accompanied by an intense vascularization and an increased expression of mineralization/vascularization marker genes. The data show that amorphous polyP-stabilized ACP, which combines osteoinductive activity with the ability to act as a precursor of hydroxyapatite formation both *in vitro* and *in vivo*. PolyP is a promising material for bone regeneration [1, 4].

As a strong proof-of-concept it is shown that polyP is a powerful curative molecule for wound healing [5, 6]. Wound healing, especially of chronic wounds, are a very relevant burdens for patients and also for the health system. In the United States, around 15% of all Medicare beneficiaries suffer from at least one type of wound or wound infection. Among them diabetic wounds have the second highest prevalence; and the annual cost expenditures for wound care estimates range between $30 and $100 billion. We succeeded to provide a protocol for a complete healing of chronic wounds. In the “bench to bedside” process, our protocol had been introduced into the clinical application. The polyP was engineered into collagen-based mats and applied onto human wounds. Those mats impressively accelerated the re-epithelialization rate, with a reduction of the wound area to 65% after 3 weeks and to 36.6% and 22.5% after 6 and 9 weeks, respectively. Complete healing was achieved and no further treatment was necessary (Fig. 1). Biopsy samples from the regenerating wound area showed predominantly myofibroblasts.

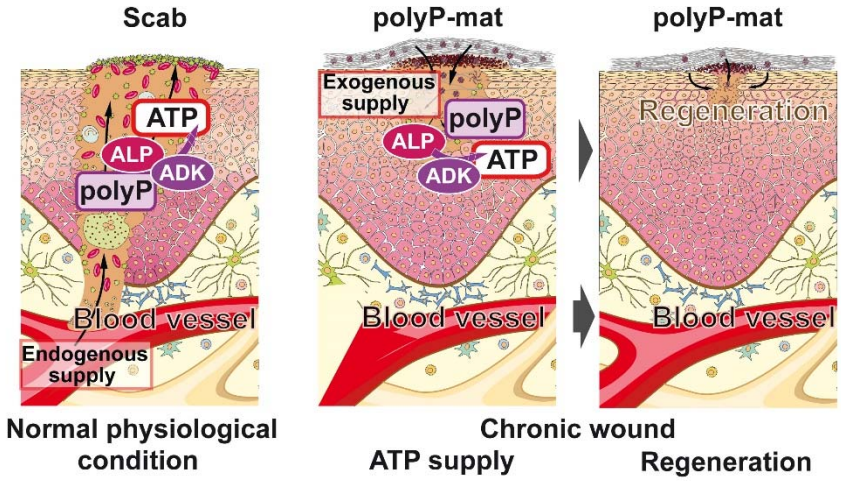

Fig. 1. Polyphosphate, the first biomaterial, which heals chronic wounds. Under normal physiological conditions polyP is delivered *via* the blood stream to the wound and supplies enough polyP, which is required for wound healing. However, chronic wounds are totally dependent on exogenous polyP in order to allow regeneration. This polymer is supplied via mats to the injury.

3 Conclusion

We applied new concepts for biology-based processes in human biomedicine from nature, preferably from metazoans. Both bioinspired and biomimetic approaches are possible. It is demonstrated that polyP, a physiological inorganic polymer, is a new powerful materials in

biotechnology solving important needs in human biomedicine, like for bone repair and wound healing, as worked out by us.

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References

1. Müller, W.E.G., Schröder, H.C., Wang, X.H.: Inorganic polyphosphates as storage for and generator of metabolic energy in the extracellular matrix. Chem. Rev. **119**, 12337-12374 (2019)
2. Wang, X.H., Schröder, H.C., Müller, W.E.G.: Amorphous polyphosphate, a smart bioinspired nano-/bio-material for bone and cartilage regeneration: Towards a new paradigm in tissue engineering. J. Mat. Chem. B **6**, 2385-2412 (2018)
3. Müller, W.E.G., Ackermann, M., Al-Nawas, B., Righesso, L.A.R., Muñoz-Espí, R., Tolba, E., Neufurth M., Schröder H.C., Wang X.H.: Amplified morphogenetic and bone forming activity of amorphous versus crystalline calcium phosphate/polyphosphate. Acta Biomaterialia **118**, 233–247 (2020)
4. Müller, W.E.G., Neufurth, M., Lieberwirth, I., Wang, S., Schröder, H.C., Wang, X.H.: Functional importance of coacervation to convert calcium polyphosphate nanoparticles into the physiologically active state. Materials Today Bio **16**, 100404 (2022)
5. Schepler, H., Neufurth, M., Wang, S., She, Z., Schröder, H.C., Wang, X.H., Müller, W.E.G.: Acceleration of chronic wound healing by bio-inorganic polyphosphate: In vitro studies and first clinical applications. Theranostics **12**, 18-34 (2022)
6. Müller, W.E.G., Schepler, H., Neufurth, M., Wang, S., Ferruccic, V., Zollo, M., Tan, R., Schröder HC, Wang X.H.; The physiological polyphosphate as a healing biomaterial for chronic wounds: Crucial roles of its antibacterial and unique metabolic energy supplying properties. J. Mat. Scie & Technol. **135**, 170-185 (2023)