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Review article

Design strategies and applications of nacre-based biomaterials

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ABSTRACT

The field of tissue engineering and regenerative medicine relies heavily on materials capable of implantation without significant foreign body reactions and with the ability to promote tissue differentiation and regeneration. The field of bone tissue engineering in particular requires materials capable of providing enhanced mechanical properties and promoting osteogenic cell lineage commitment. While bone repair has long relied almost exclusively on inorganic, calcium phosphate ceramics such as hydroxyapatite and their composites or on non-degradable metals, the organically derived shell and pearl nacre generated by mollusks has emerged as a promising alternative. Nacre is a naturally occurring composite material composed of inorganic, calcium carbonate plates connected by a framework of organic molecules. Similar to mammalian bone, the highly organized microstructure of nacre endows the composite with superior mechanical properties while the organic phase contributes to significant bioactivity. Studies, both *in vitro* and *in vivo*, have demonstrated nacre's biocompatibility, biodegradability, and osteogenic potential, which are superior to pure inorganic minerals such as hydroxyapatite or non-degradable metals. Nacre can be used directly as a bulk implant or as part of a composite material when combined with polymers or other ceramics. While nacre has demonstrated its effectiveness in multiple cell culture and animal models, it remains a relatively underexplored biomaterial. This review introduces the formation, structure, and characteristics of nacre, and discusses the present and future uses of this biologically-derived material as a novel biomaterial for orthopedic and other tissue engineering applications.

Statement of Significance

Mussel derived nacre, a biological composite composed of mineralized calcium carbonate platelets and interplatelet protein components, has recently gained interest as a potential alternative ceramic material in orthopedic biomaterials, combining the integration and mechanical capabilities of calcium phosphates with increased bioactivity derived from proteins and biomolecules; however, there is limited awareness of this material's potential. Herein, we present, to our knowledge, the first comprehensive review of nacre as a biomaterial. Nacre is a highly promising yet overlooked biomaterial for orthopedic tissue engineering with great potential in a wide variety of material systems. It is our hope that publication of this article will lead to increased community awareness of the potential of nacre as a versatile, bioactive ceramic capable of improving bone tissue regeneration and will elicit increased research effort and innovation utilizing nacre.

Contents

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1. Introduction

Nacre, commonly known as mother of pearl, is a naturally occurring composite material composed of aragonite (calcium carbonate) platelets cemented by a complex organic matrix. Nacre comprises the inner layer of pearl oyster and freshwater pearl mussel shells as well the pearls themselves, with pearl and shell nacre demonstrating similar chemical compositions as well as high strength, mechanical resilience, and iridescence.

Previous research has demonstrated nacre's biocompatibility, biodegradability, and osteogenic properties, marking nacre's potential as an alternative to other commonly utilized biomaterials in tissue engineering. Nacre has been shown to facilitate osteoblast proliferation and accelerate extracellular matrix (ECM) production and mineralization [1–4]. Additionally, nacre has demonstrated high mechanical strength, excellent biocompatibility, osteogenic capability, and biodegradability both *in vitro* and *in vivo*. The organic matrix contained within nacre has also been found to contain biological molecules capable of activating osteoblasts through chemical signaling; however, research into these organic molecules has been limited [5,6].

Nacre is a composite consisting of an inorganic mineral phase and an organic matrix, similar to the native structure of bone. This organized structure imparts nacre with its remarkable mechanical strength, while the presence of the organic matrix imparts improved osteoconductivity compared to synthetic materials such as titanium [7]. While orthopedic tissue engineering has previously relied on either autografts or allografts (limited by tissue availability and donor site morbidity) or permanent metal implants, biodegradable materials and composites are the focus of much research in order to provide grafting materials with high availability as well as the potential to be replaced over time by native tissue [8,9]. Nacre has recently emerged as a potential bone grafting material due to the above-mentioned favorable properties. Bulk nacre as well as composite materials containing nacre as the mineral phase and utilizing various polymers including poly(L-lactic acid) (PLLA), poly(D,L-Lactide) (PDLLA), poly(D,L-Lactide-co-glycolide) (PLGA), ultra-high molecular weight polyethylene (UHMWPE), Polyetheretherketone (PEEK), and poly(3-hydroxybu tyrate-co-3-hydroxyvalerate) (PHBV) have been tested as potential biomaterials.

Despite its demonstrated biocompatibility and bioactive properties, to date, no comprehensive review of nacre based materials has been reported and the potential role of nacre based materials in tissue engineering still remains relatively unknown. This paper summarizes current research on nacre, including its basic characteristics as well as its potential use as a biomaterial. Through an examination of relevant literature, the potential of nacre as an alternative to commonly used compositing materials such as hydroxyapatite (HA) and other calcium phosphate minerals for bone tissue engineering will be expounded. Elaboration of the previously conducted research on nacre will demonstrate its potential as a novel and relatively unexplored material for orthopedic biomaterials science and will stimulate continued research into nacre as an alternative to commonly used biomaterial ceramics such as hydroxyapatite.

2. Characteristics of nacre

2.1. Formation of nacre

Nacre is an organized organic-inorganic composite material, secreted by the epithelial cells of the mantle tissue of mollusks. Mantle epithelial cells secrete crystal precursors (Ca^{2+} and CO_3^{2-}) as well as amorphous calcium carbonate, along with organic matrix molecules consisting predominantly of proteins and polysaccharides [10]. In this manner, calcium carbonate forms between the outer layer of mantle epithelial cells and the periosteum membrane. The organic matrix is co-deposited along with the mineral phase, and serves as a critical mineralization regulator.

In nacre, sheets of beta-chitin are interspersed by a hydrophobic gel, containing acidic, aspartic acid rich proteins, forming a highly structured chitinous framework [11]. Amorphous calcium carbonate is deposited at crystal nucleation sites, where it acts as seed molecules for the growth of the aragonite plates that make up the mineral phase of nacre [12]. The process of nacre formation can be summarized as: (1) the formation of a layer of elongated calcite prisms, arranged tightly in a perpendicular orientation to the direction of shell growth, followed by (2) the crystallization of calcium carbonate within the platelets, converting amorphous calcite to a layer of flat, crystalline aragonite platelets [11]. This process is conserved among many species of mollusk (see Table 1).

Pearl formation, when occurring naturally as opposed to artificial seeding, is characterized as a host immune response to intrusion of a foreign body. Typically, a foreign particle or parasite enters the mollusk, prompting local irritation of the mantle tissue and subsequent formation of a pearl sac to cover the site. A similar process occurs during the pearl forming process when a seed is implanted in the mantle tissue of the mollusk to stimulate the above foreign body response and generate a pearl. Once a pearl sac is formed, nacre is secreted layer by layer to envelope and isolate the irritant, resulting in formation of a pearl after several years. Nacreous pearls are primarily produced by molluscan bivalves or clams. Of the many other molluscan species, few can produce pearls of interest as gemstones. Pearl producing species include the bailer shell *Melo*, the giant clam, *Tridacna*, various scallop spe-