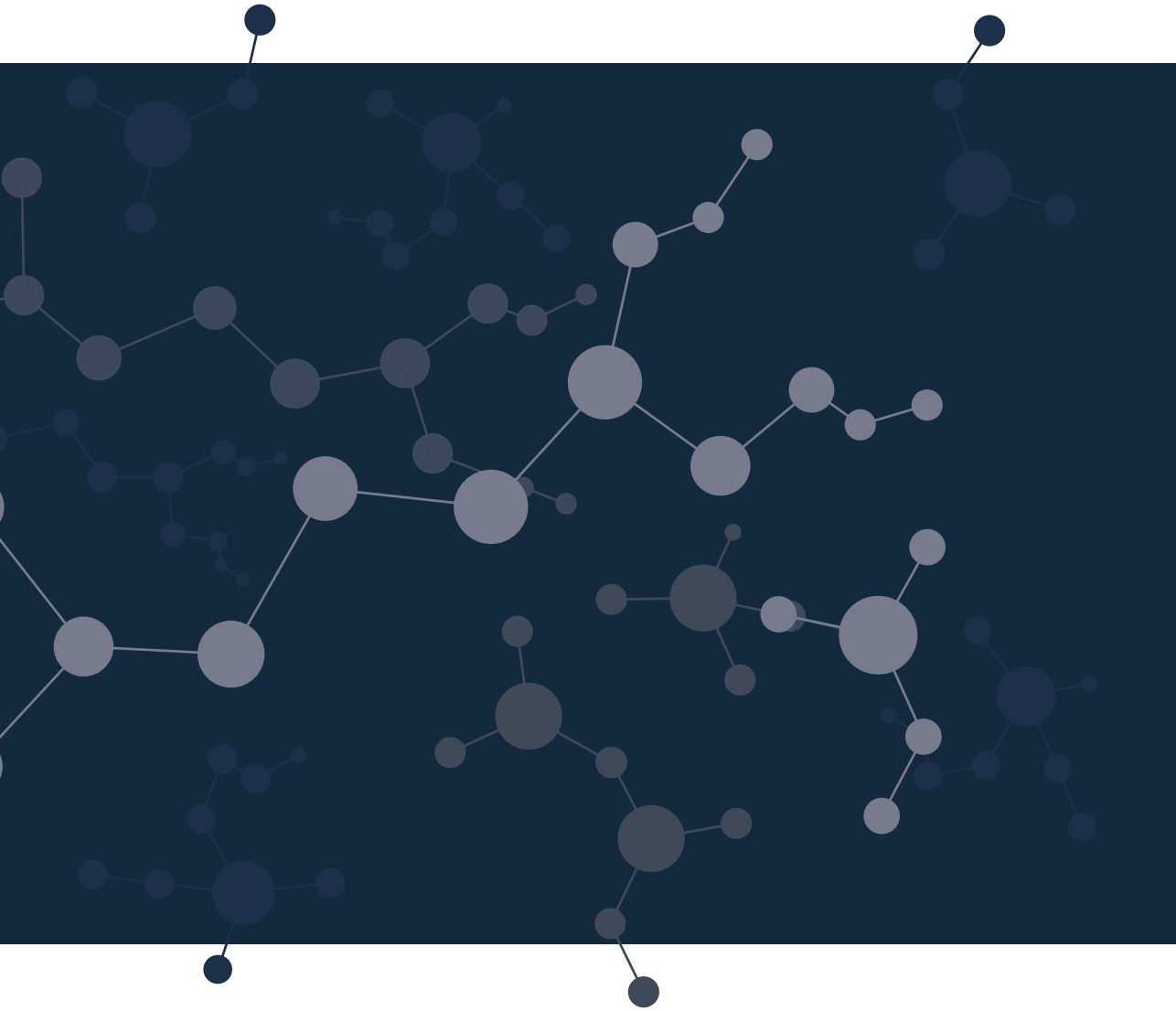
Polymer- and hybrid-based biomaterials for delivering biotherapeutic molecules in bone and cartilage tissue

García Couce, J.



CHAPTER 1

General Introduction

Jomarien García-Couce

INTRODUCTION

Diseases and injuries related to bone and cartilage severely affect the function of the musculoskeletal system, causing loss of mobility and patient quality of life. The most common conditions in these tissues are osteoarthritis, osteoporosis, cancer, severe mechanical trauma, infectious diseases such as osteomyelitis, and birth defects. [1,2]. Due to global population aging and also to the high incidence of vehicular accidents, many of these conditions have been increasing and currently constitute an important health problem that is accompanied by a significant financial burden for health systems and patients themselves who suffer from them. Osteoarthritis is one of the most prevalent disabling joint disorders worldwide, described in 2016 as a serious disease by the Osteoarthritis Research Society International (OARSI) [3]. In 2017, it reached a number of 303 million people affected worldwide and in 2019, the costs of its treatment exceeded 300 billion dollars in the United States [3,4]. On the other hand, a study carried out in six European countries showed that fragility fractures may increase by 23.3% between 2017 and 2030, with a growth in annual costs of 27% [4]. Considering reports from 2018 in the European Union, 3.5 million osteoporotic fractures were treated per year at an approximate cost of 37 billion euros [5] and the therapy of a patient with a bone defect of critical size can cost between € 10,000 and € 100,000 [6].

Bones and cartilage are tissues with very important functions and very peculiar structural characteristics. Bone, the main component of the musculoskeletal system, is a highly complex tissue that forms the structural framework of the body, providing rigidity to the skeleton, and is involved in locomotion, mineral homeostasis, and protection of internal organs [7,8]. Bone tissue is composed of an inorganic matrix of hydroxyapatite nanocrystals and an organic matrix made up mainly of type I collagen and water [8]. The hierarchical structure ranging from the nanoscale to the macroscale ensures the high mechanical strength and structural complexity required to withstand the force applied to these tissues [1]. Articular cartilage, unlike bone, is an avascular and aneural tissue, which covers the bone surface in synovial joints and provides a low-friction interface and mechanical support necessary to maintain normal joint functionality [1,9-11]. Articular cartilage is composed of a single cell type, chondrocytes, distributed within a dense extracellular matrix made up mostly of type II collagen and proteoglycans [1,12]. Because it is a tissue lacking blood circulation, it has a limited capacity for self-repair and the arrival of systemically administered drugs to this area is poor [8,9,12,13].

The treatments applied to all the ailments associated with these tissues of the musculoskeletal system are diverse and range from non-pharmacological treatments to surgical procedures. In the case of fractures and bone defects,

for example, the most common in clinical practice are autologous bone transplants or allogeneic bone grafts, but these have limitations. In the first case, it is limited by the insufficient supply of the donor site, for which patients have to undergo more than one intervention, increasing the risk of infections, hematomas and the cost of the procedure. Allogeneic grafts, on the other hand, have a high risk of rejection due to the patient's immune response to the implanted tissue, as well as transmission of infections after transplantation [2,14]. The implantation of different biomaterials and the treatment with pharmaceutical agents and soluble growth factors have also been investigated. Metallic implants, for example, provide immediate mechanical support but generally have poor integration with the tissue, cause infection and suffer from fatigue fracture [8]. Pharmacological treatments, on the other hand, generally do not achieve an optimal effect due to the low concentration that it is reached at the damaged site and high doses often cause side effects that lead to the suspension of treatment by the patient [2]. In the case of osteoarthritis, which is the most prevalent cartilage disease, physical and pharmaceutical therapy are the most common forms of treatment. Frequently used medications are non-steroidal anti-inflammatory drugs and corticosteroids that serve to reduce pain and inflammation but their long-term use causes cardiovascular, digestive and other side effects, in addition to reaching the joint cavity long after administration obtaining a low concentration in the synovial cavities [15,16].

As it has been seen so far, the peculiarities of bone and cartilage structures mean that the therapeutic and surgical treatments developed to date, have a series of disadvantages and limitations in order to achieve adequate effectiveness against the diseases and damage suffered by these tissues. Specifically, in pharmacological treatments, it happens that the drug concentrations required to obtain the desired biological response in the affected area are sometimes insufficient, causing low efficacy. Additionally, it is mentioned that systemically administered drugs are absorbed into the bloodstream, distributed throughout the body through the circulatory system and require frequent administration, which can cause systemic toxicity [17]. Consequently, the development of new strategies for administering drugs that favor localized delivery is very important to achieve increasingly better treatments.

Drug delivery systems for bone and cartilage tissue

Drug delivery systems (DDS) have been developed as an alternative to conventional systems to effectively deliver the required amount of drug to the appropriate target sites and to maintain desired drug levels [18]. Among the main advantages of these systems is (i) increased drug bioavailability, (ii) they can be used in treatments for chronic diseases, reducing the side effects

produced by conventional treatments, (iii) they help to improve treatment compliance by the patient due to the reduction in the number and frequency of doses required and (iv) reduce costs by developing new carriers for existing molecules [18]. In the specific case of delivery systems for bone and cartilage, local administration in these tissues achieves greater efficacy by avoiding metabolic catabolism and elimination compared to systemic administration [19]. In addition, it prevents damage to other tissues due to the action of drugs considering the administration directed specifically to the damaged site [18].

Research to achieve systems with adequate properties for the localized application of drugs in bone and cartilage has been extensively studied, especially given that the types of materials, processing conditions and morphology of the carriers, mainly due to the particular structural characteristics of these tissues. The morphology of the vehicle or matrix is a factor that influences the release kinetics and the stability of the incorporated drug [10]. In the studies carried out to date in this field, a variety of drug delivery matrices with different morphologies have been explored, such as nano/microparticles, liposomes, dendrimers, hydrogels and porous scaffolds (**Fig. 1**) [1,10,20]. These matrices will not only have the function of transporting the drug but will often serve as a temporary substrate for cell infiltration, growth and proliferation [10].

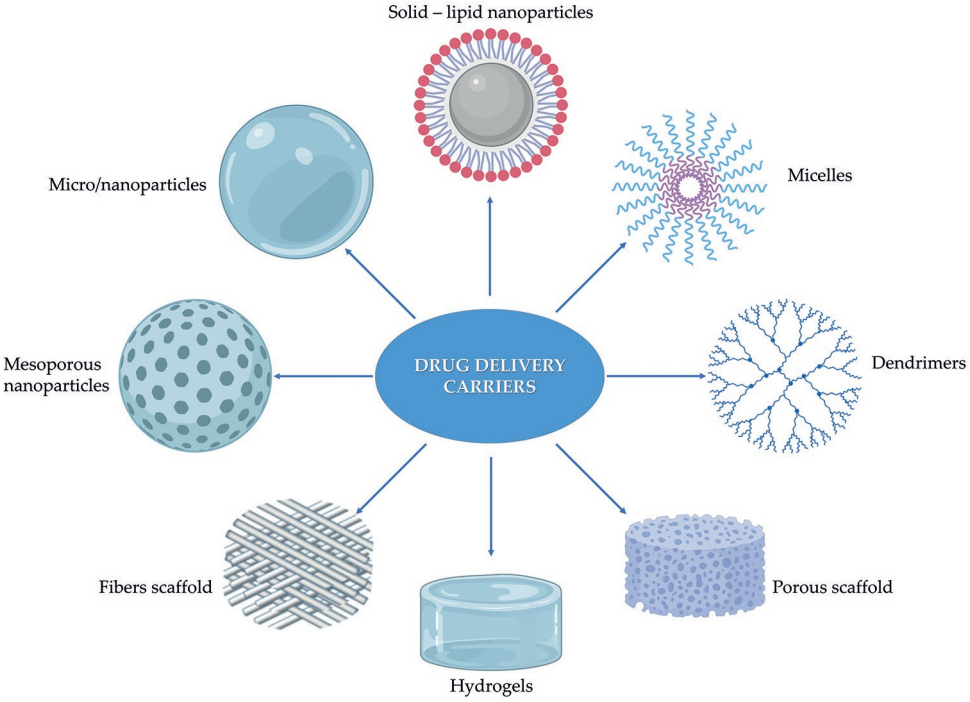


Figure 1. Schematic illustration of the different types of carriers in bone and cartilage drug delivery systems

Hydrogels

Hydrogels are important hydrophilic polymeric matrices with a three-dimensional network structure that can absorb large amounts of water or fluids without disintegrating and absorb up to several times their dry weight [8,21]. Compared to other types of biomaterials, hydrogels have good biocompatibility, biodegradability, drug transport capacity, and controllable drug release capacity [22]. The high content of water that they retain causes them to have a large number of pores in their structure and to be highly permeable, which favors a rapid diffusion of oxygen and nutrients within the matrix, this allows the cells to adhere and proliferate satisfactorily. Therefore, hydrogels can simulate a natural tissue environment and provide structural support for the implantation site [21].

Hydrogels can be classified in different ways based on source, charge, crosslinking, response, or physical properties. Depending on the source of the polymer(s) that compose it, they can be natural or synthetic and according to the crosslinking, they can be physical or chemical hydrogels if a chemical crosslinking agent is used or not [23,24]. Physical crosslinking is preferred for the preparation of hydrogels since it does not involve the use of chemicals that can be toxic. It is mainly achieved by inter and intramolecular interactions, hydrophobic associations, hydrogen bonds or polyelectrolytic interaction [23,25]. The hydrogels that are classified based on the response are the so-called "smart hydrogels", which undergo changes in response to stimuli such as light, temperature, pH, among others [23,24]. From this group, temperature-sensitive hydrogels (or thermosensitive) are the most studied since temperature is an easy variable to control. They have been widely developed because, unlike traditional hydrogels that must be surgically implanted, these can be applied by injection in liquid form and gel in situ within the treated area due to the change because the action of body temperature. They also have advantages as vehicles for delivery systems since the encapsulation of the drug in a fluid state allows uniform dispersion of the therapeutic agent and cells administration [7,24].

Hybrid materials (ceramic/polymer)

Although we have previously seen the benefits of polymeric hydrogels as vehicles for the localized administration of drugs, they have some limitations for their application in bone tissue. In a way, the low mechanical resistance that the majority of conventional hydrogels could be cause their rupture in an environment such as bone, the inability to unite with natural bone without the formation of a fibrous capsule, and the lack of osseointegration

[7,14]. Although the mechanical properties of hydrogels can be modified by increasing chemical crosslinking or matrix density, this alternative can limit cell migration, proliferation, and morphogenesis [26]. For this reason, the incorporation of an inorganic phase within the hydrogel reinforces the structure and is one of the ways to solve mechanical resistance problems. A variety of nanometric ceramics has been incorporated into different matrices, both of natural and synthetic origin, to enhance the osseointegration and mechanical properties of the developed matrices [7,27-30]. Ceramic nanoparticles have gained more space than conventional ceramics due to the decrease in particle size improves cell anchorage, which promotes the proliferation, differentiation and mineralization of osteoblasts [7,31]

Materials used in preparation of the matrices

The main criteria taken into account for the selection of materials to be used in the design and production of carriers such as hydrogels and hybrid matrices are: to be biocompatible, non-cytotoxic, non-immunogenic and to be degraded by enzymes and/or surrounding biological fluids. They must also promote cell adhesion and proliferation of surrounding cells in the area of application [4,5,10]. There are also some specificities, as in the case of materials for use in bone tissue, they must also have osteoinductive and osteoconductive properties for a better proliferation of bone cells [6]. The types of materials most be used to develop carrier platforms are natural and synthetic polymers, as well as inorganic compounds such as mostly of the calcium phosphates or combination between them.

Natural polymers have a series of properties that make them very attractive for their use in the design of carriers for bone and cartilage, they are highly biocompatible, biodegradable, favor cell growth and have structural similarities to the natural components that make up these tissues [4,7,17,20]. Within this group of materials, proteins such as collagen, gelatin, silk fibroin and polysaccharides such as chitosan, cellulose, hyaluronic acid and sodium alginate have been used [10,13,17,20]. Synthetic polymers have also gained great interest, among other reasons, because the carriers designed with them have better mechanical properties and high reproducibility [10,32]. Polymers of α-hydroxy esters such as lactic acid (PLA), glycolic acid (PGA) and copolymers of these two monomers (PLGA) are commonly used, also other polymers such as polyethylene glycol (PEG), poly(vinyl alcohol) (PVA) and polyacrylamide (PAM) [10,32]. Due to its biocompatibility and structure similar to that of natural bone, ceramics are a favorable group of biomaterials. Among them, hydroxyapatite, β-tricalcium phosphate and others, are the most used materials in the design of vehicles for bone applications [33].

Mathematical modeling

So far, we have briefly discussed the main types of matrices and materials developed to date as carriers for the localized release of molecules in tissues such as bone and cartilage. Another important aspect of research in this field is the study and analysis of mathematical models developed to predict the behavior of the release from these carriers. Mathematical modeling today has a key role in the drug development and delivery systems. Mathematical models allow to know the main release mechanisms that are occurring in the designed device. Due to this fact, it is possible to predict if the molecule release is occurring by a diffusion process, or degradation of the matrix, the swelling of the structure, etc. [34,35]. Knowledge of these elements makes it possible to predict the output behavior of the molecule once the system has been applied and also to optimize the design of the devices according to the applications for which they have been designed. The first mathematical models contribution in the field was the equation proposed by Professor Takeru Higuchi in 1961 [36], known as Higuchi's equation and used to these days [35]. Although there are a few mathematical models already described for different applications, a study carried out in 2019 by Cacavo showed that the most applied adjustment models in release studies from hydrogels are the zero-order equation; the first order; the Higuchi; Peppas and the Hixon-Crowell because they are simple equations that provide important information on the release behavior [37]. Drug delivery modeling is a continually growing field, thanks to the continuous development of new methods, software optimization, and increased computational power [35].

Aims of this thesis

Although much progress has been achieved in the development of controlled release systems, it is still a challenge to obtain formulations or devices that enables a therapeutic substance to selectively reach its site of action, which should also be compatible with the surrounding tissue and jointly regulate the drug release. That is why the main objective of this thesis was to obtain and investigate matrices for drug delivery systems in bone and cartilage tissue, in order to achieve formulations with potential applications for the treatment of conditions in these tissues. The specific objectives to cover this global objective are described in the chapters of the thesis:

Chapter 1: Introduction

The controlled drug delivery systems are described, in greater detail those intended for the treatment of conditions in the bone and articular cartilage. The most common materials used in the investigations of these systems are

presented. The main characteristics and damages that occurs in the bones and articular cartilage are also briefly described, as well as some aspects associated to the mathematical models used to study the release processes in these systems.

Chapter 2: Targeting Polymeric Nanobiomaterials as a Platform for Cartilage Tissue Engineering

The structure and composition of articular cartilage is explained in detail. Important aspects about osteoarthritis and the most frequently condition in this type of tissue are exposed; pointing out the incidence, risk factors, conventional treatments and the development of novel controlled release platforms for the disease pharmacological treatment.

Chapter 3: A novel information criterion to elucidate a drug delivery mechanism from poly (acrylamide-co-2-hydroxyethyl methacrylate) reinforced with hydroxyapatite composite. (Objective 1)

An hybrid polymer/ceramic platform composed of poly(acrylamide-co-hydroxyethyl methacrylate) with hydroxyapatite, which is the main component of bone, was designed and prepared. The influence of the formulation's composition on the hydrolytic and mechanical properties of the final composites was investigated. The capacity of the composites as drug-controlled delivery systems of sodium cephazolin, using a novel information criterion to make decisions, was also studied.

Chapter 4: Synthesis and Evaluation of AlgNa-g-Poly(QCL-co-HEMA) Hydrogels as Platform for Chondrocyte Proliferation and Controlled Release of Betamethasone. (Objective 2)

The hydrogels prepared in this work combine a natural polymer and a synthetic copolymer in their formulations, and the effect of the composition on morphology, swelling and betamethasone release was investigated. In addition, the cytocompatibility of each sample against chondrocytes and fibroblasts was evaluated to determine if some formulation was cytotoxic because of the composition

Chapter 5: Chitosan/Pluronic F127 Thermosensitive Hydrogel as an Injectable Dexamethasone Delivery Carrier (Objective 3)

Injectable thermosensitive hydrogels from the physical mixture between the chitosan and the thermosensitive polymer (Pluronic) were prepared. The initial formulation was modified by incorporating a cross-linking agent

to evaluate the influence in time-control of the dexamethasone release incorporated into the matrix and whether or not modified the release kinetics, which was analyzed using several mathematical models. The viability of chondrocyte cells in contact with hydrogels samples and the residence time of hydrogels in the joint of the mice employed in the study were also studied.

Chapter 6: Thermo-sensitive injectable hydrogel for intraarticular delivery of Etanercept (Objective 4)

As a continuation of the previous chapter, a similar hydrogel was evaluated, in this case using another crosslinking agent. Etanercept, a tumor necrosis factor (TNF-a) inhibitor, was incorporated into this new system to evaluate the release process from the designed hydrogel, quantify its blood concentration in treated mice, and assess whether its incorporation into the hydrogel can reduce articular cartilage damage in mice with induced osteoarthritis.

Chapter 7: General discussion

The results of researches are discussed and future experiments will be considered, based on the results presented in this thesis

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