

An update on delivery systems of Icarin for bone regeneration in Periodontics: a Review

Dr Aditi Yadav¹, Dr K.T.Chandrashekar², Dr Rohit Mishra³,
Dr Priyanka Mahawar⁴, Dr Shailendra Dass⁵

¹(Post Graduate Student, Department Of Periodontics And Implantology, Hitkarini Dental College And Hospital, India)

²(Professor and Dean, Department Of Periodontics and Implantology, Hitkarini Dental College And Hospital, India)

³(Professor , Department Of Periodontics and Implantology, Hitkarini Dental College And Hospital, India)

⁴(Post Graduate Student, Department Of Oral Medicine and Radiology, Hitkarini Dental College And Hospital, India)

⁵(Post Graduate Student, Department Of Periodontics And Implantology, Hitkarini Dental College And Hospital, India)

Abstract: Epimedium contain active compounds exhibiting wide pharmacological activities especially in bone healing. Primary bone tissue cells are osteoblasts, osteocytes and osteoclasts. Bone is a highly dynamic tissue which is continuously formed by osteoblasts and resorbed by osteoclasts. This is a highly complex process maintaining equilibrium in healthy tissue. Any imbalance between the activity of these cells result in pathological conditions like osteoporosis, periodontitis, etc. Such conditions require bioactive molecule with effective delivery vehicle to promote bone healing and maintain tissue homeostasis. Icarin extracted as the main active ingredient of Epimedium has osteoinductive effects which when used with effective delivery system is of huge therapeutic importance as it is proven to possess wide range of osteoprotective effects. This review article highlights various methods available to effectively deliver icarini to targeted tissue and achieve bone repair.

Keyword: Epimedium, Icarin, bone, Alkaline Phosphatase, Sustained .

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I. Introduction

Traditional Chinese medicines (TCM) have been successfully employed in bone regeneration and repair for thousand of years. [1] Herbal medicines are healer and promoter and are widely accepted due to their low cost, easy availability, safety and efficacy. Herb Epimedium contain active molecules which account for its pharmacological activity. One such active flavanoid is Icarin which has been used in TCM for over 2000 years.[2] Icarin is the most abundant bioactive component of this herb constituting about 77% and is chosen as the marker for quality control in Chinese Pharmacopeia.[3,4] It has osteogenic potential, however, due to its poor water solubility, extensive first pass metabolism, low availability and lack of tissue targeting ability, there arises a desperate need to design effective delivery system for icarini so that it can be locally delivered by biomaterials to the targeted tissue.[5]

II. Rationale behind use of icarini in periodontitis

Icarin has an osteoinductive potential for bone tissue engineering. The study done by Zhang et al proved regenerative, anti-inflammatory and immunomodulatory properties of icarini in periodontitis model.[6]

III. Structure of icarini

Icarin (C₃₃H₄₀ O₁₅) is a small monomer of molecular weight 676.67. It is a prenyl flavonoid glycoside. It has a glucosyl group on C-3, rhamnosyl group on C-7, methoxyl group on C-4 and a prenyl group on C-8.[Fig.1].

The prenyl group on C-8 is the active part of the molecule which is responsible for its potency and participate in osteoblastic differentiation. [1, 6]

IV. Delivery systems for icariin

Delivery of herbal drugs require standardization and modification to ensure safety, quality, strength and purity. An effective delivery system should allow sustained release so that drug level is maintained for prolonged period of time especially for those drugs which are metabolized and eliminated too fast by our body. Targeted drug delivery is more effective as it increases the drug concentration in a particular site which improve therapeutic index and reduce side effects.[7]

The approaches for sustained drug delivery system includes:[8]

- Attaching drug with a molecule which can increase its circulation time.
- Membrane based approach which encapsulate the drug in a reservoir membrane.
- Entrapment of drug in a matrix. (drug + polymer system)
- Osmotic system
- Pumping system

4.1 Composites containing icariin:

Porous composites containing hydroxyapatite (HA) , herb Epimedium (EP) and chitosan (CS) are fabricated using freeze drying technique. The porous structure provide space for blood vessels and new bone and maintain stability. The rapid movement of body fluid through porous structure supply oxygen and nutrients to osteoblasts which allow them to gather, differentiate and secrete osteoid. These composites show homogenous bone formation after 12 weeks of in vivo implantation. The rationale behind this composition include:

- Biocompatibility and osteoconductivity of HA.
- High bond strength, hydrophilicity, biodegradability and adhesive property of CS.
- Bone regenerative property of EP.

Such composites utilize the ability of EP in promoting cellular proliferation and osteogenesis and yield a large quantity of new bone tissue.[9]

A three dimensional Composite construct containing icariin, allogenic bone marrow derived mesenchymal stem cells and siliceous mesostructured cellular foam can be used for repairing critical size and large volume bone defects in clinic.[10]

4.2 Biomineral Binding Liposomes:

This system provides a platform for effective delivery of antimicrobials to the skeletal tissue. They are fabricated using combined technique of thin film dispersion and mechanical extrusion.

Structure of liposome:

It is a nano sized spherical vesicle with cholesterol as the major component.

- One or more lipid bilayer incorporate hydrophobic substances.
- Central aqueous core incorporate hydrophilic substances.

These liposomes quickly bind onto the bone, HA, HA coated implant surface and help in concentrating the drug locally and prevent bacterial colonization thereby improving infection. This delivery system overcome problem of lack of targeting ability of icariin. This enable accumulation of icariin at the local diseased site. [5,11]

Sun et al designed a novel pyrophosphate-tri (ethyleneglycol)-cholesterol conjugate taking pyrophosphate as the targeting part because of its affinity for bone. These liposomes anchor on HA surface and slowly release icariin. [5]

The advantages of BBL include:

- Stimulation of bone formation and suppression of bone resorption.
- Restore bone microarchitecture.
- Improve mechanical properties of bone.
- Activate bone remodeling.

4.3 Icariin incorporated BioCaP granules :

Biomimetic calcium phosphate (BioCaP) based materials have the capacity to carry and deliver bioactive agents without compromising their efficacy and specificity. Their fabrication involves precipitation, incubation, filtration and compression to form tablet (0.4 mm thick). The sustained release of icariin and BMP-2 has dual advantage because icariin could not maintain an appropriate concentration locally and BMP adsorption due to BMP burst has low osteoinductive efficiency. Icariin show synergistic action when used with BioCaP and BMP

-2 as it enhance osteogenic effect of BMP-2, increase blood vessels, osteocalcin, alkaline phosphatase and matrix mineralization.[12][Figure 2,3]

4.4 Local drug delivery systems :

Calcium phosphate powder when mixed with an aqueous solution produce a paste of calcium phosphate cement (CPC) which harden within 15-30 min. This produces an injectable scaffold which is used for local release of antibiotics. A dual drug delivery system containing 2 mg icariin, 20 mg vancomycin and calcium phosphate was fabricated by Huang et al. The two drugs are adsorbed by CPC using freeze drying technique. This local antibiotic release system can be used to treated infectious bone diseases and can release antibiotic for more than 30 days. This system can be used for targeted delivery of antibiotic to control infection and cover selected gram positive and gram negative bacteria locally there by eliminating risk of systemic toxicity. [3]

Local injection of 0.1 micro gram mL⁻¹ of icariin promoted periodontal tissue regeneration and exerted anti-inflammatory and immunomodulatory function in minipig model of periodontitis. (Zhang et al 2017).[6]

Icariin loaded on rod shaped micro/nano hydroxyapatite granules is useful in repairing bone defects by enhancing osteogenesis and angiogenesis.[13]

4.5 Treatment of titanium surface with icariin :

Different methods have been employed to create bioactive and functional implant surface.[14]. Figure 4 summarizes various surface modification methods.

Icariin when loaded on titanium surface via layer by layer (LBL system) improve surface osteogenesis. The multilayer within LBL system contain chitosan and hydroxyapatite. Chitosan loaded icariin layer is of prime importance because icariin adherence to chitosan provide sustained drug release for weeks.

Icariin functional coating composed of poly-lactic-glycolic acid (PGLA) on TiO₂ nanotubes surface promote cell adhesion, proliferation and differentiation in vitro and osseointegration in vivo. PGLA exert antibacterial action, enhance osseointegration and allow controlled release of icariin on TiO₂ surface.[Figure 5]

4.5 Micelle system:

This is a biocompatible nanoparticle delivery system. Amphiphilic polymer micelles contain hydrophobic and hydrophilic segments. Hydrophilic segment is larger than hydrophobic segment. The micelle contain the core and the shell[15].[Figure 6]

V. Discussion

Plants contain bioactive molecules which possess bone protective effects due to their significant action on osteoblasts, osteoclasts and have role in bone remodeling. Herb Epimedium contain icariin as the most active flavanoid which has multiple effects on bone cells. [16] The therapeutic effect of icariin on bone is possible only when it is delivered via suitable delivery vehicle to the targeted site. Figure 7 depicts the ideal requirements of a delivery vehicle. The aim of this review article is to highlight various modes of icariin delivery. [figure 8]

Systemic antibiotic therapy is effective but is associated with disadvantages and limitations like low bioavailability, limited penetration to the site of infection, systemic toxicity, drug resistance, etc.[17]. Therefore there is a need of new drug delivery system which allow targeted and sustained release of antibiotic thereby improving its therapeutic efficacy and eliminating drug resistance and side effects.

Bone Morphogenetic protein (BMP-2) is approved by United States Food and Drug Administration(US FDA) and has been widely used in bone regenerative procedures. However it is associated with unwanted calcification at high dose, ectopic bone formation, abnormal bone resorption and stimulation of cancerous growth. [12] Icariin may be used as a substitute for BMP or as a promoter to enhance properties of BMP. [Zhang et al, 2013][1]

Icariin has been widely used to cure bone related diseases in China for centuries. [12] It is safe, non-toxic, inexpensive and osteoinductive.[Wu et al, 2009][1]. However the only drawback with icariin is its inability to maintain an appropriate concentration in bone defects for required period of time. Therefore there is an urgent need to develop a delivery vehicle/system for local and sustained release of icariin.

BioCaP granules, liposomes, surface modification, micro/nano hydroxyapatite granules, dual delivery injectable systems, phase transited lysozyme priming and layer-by-layer self assembly system are an attractive tool for treatment of infectious diseases. Controlled drug delivery depend on the device and not the environment. Polymer matrices can be used to deliver large molecules over days and weeks.[Robert langer and Judah Folkman][8]

Liposomes are earliest targeted system discovered in 1990s. Doxil , the liposomal encapsulation formulation of doxorubicin, an anti-cancer agent was the first Food and Drug Administration (FDA) approved liposomal drug delivery system. Liposomes have numerous advantages. They protect drug from metabolic

digestion, carrier for both lipid and water soluble drugs, target specific, sustainable, increase half life of drugs, reduce toxicity, increase efficacy and therapeutic index, protect sensitive tissues from toxic drugs, rapid clearance by monocyte macrophage system. The limitations of using liposomes include high cost, complicated sterilization, short shelf life and stability, toxic at higher doses, etc[8]

Icariin incorporated with chitosan(CS) and hydroxyl apatite(HA) is the best system for controlled release of icariin. Icariin is not only released in controlled form but also in a bioactive form from this system. The physical and biological properties of icariin is totally preserved in this system without any chemical change during the production. The introduction of icariin in the system does not change morphology, porosity and mechanical strength of the system and the icariin release kinetics last for more than 90 days. [18]

BioCaP granules loaded with icariin is a novel drug delivery system. These granules carry icariin without compromising its bioactivity. Biomimetic materials are capable of eliciting specific cellular response and direct new tissue formation. [12]

Calcium phosphate cement is a type of injectable scaffold. It is able to release icariin for more than 30 days. It has excellent osteoconductivity and bone replacement capability.[3] icariin could also be loaded on beta tricalcium phosphate using soaking method.[14]

Layer by layer self assembly system is based on principle of electrostatic attraction. The system uses positively charged Phase transition lysozyme nanofilm and hitosan and negatively charged polyelectrolytes and hyaluronic acid. Icariin adhere well to chitosan and encapsulated by it so icariin is loaded on chitosan layers.[14][Figure 5]

Polyethylene glycol (PEG) molecule increases the circulation time of a drug in the bloodstream by creating a highly hydrated volume around the drug. The most common form of targeting system involves nanoparticles or liposomes covered by PEG to reduce their aggregation and promote transport.[8]

Polyethylene glycol monomethyl ether is a derivative of polyethylene glycol has good hydrophilic properties and stable chemical structure. PEG is a hydrophilic polymer used to modify the surface of nanoparticles (NP). This modification reduce the adsorption and prevent removal of NPs by macrophages in circulation. PEGylated drugs dissolved macromolecules diffuse in the interstitial spaces and help the drug to enter ischaemic sites. This monomer is non-toxic and non-irritant and modify insoluble properties of icariin. This system has been used by Yongqiang Zheng et al for treating myocardial ischaemia.[19]

VI. Figures

Figure 1: Chemical Structure Of Icariin (Ma et al,2011)

Figure 2 : Synergism of Icariin with BMP-2 and BioCaP

Figure 3: Increase in bone formation when BioCap, BMP-2 and Icariin are used together. BioCap molecule has a centre depot in which protein molecule is incorporated.

Figure 4: Titanium surface modification methods with their disadvantages

Figure 5: A modified titanium surface using icariin as an osteoinductive material. Amyloid – like microfibre net PTL film has funtional groups(carboxyl , amide , thiol, etc).This positively charged layer is linked with negatively charged hyaluronic acid layer via electrostatic interactions . HA layer is linked with positively charged chitosan layer. In this way, layer by layer system is formed in which icariin is loaded on CS layer as it adheres well to this layer. Icariin is either incapasulated by CS or present in micro interspaces of this layer.

Figure 6: Structure of a micelle

Figure 7 : Requirements of a delivery vehicle

Figure 8: Various modes of icariin delivery

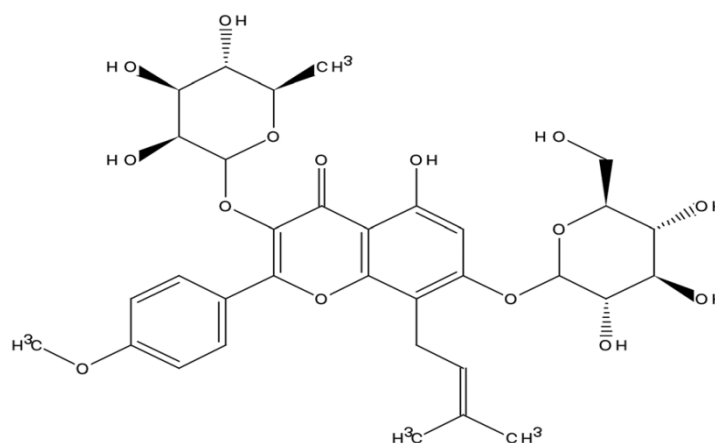


Figure 1

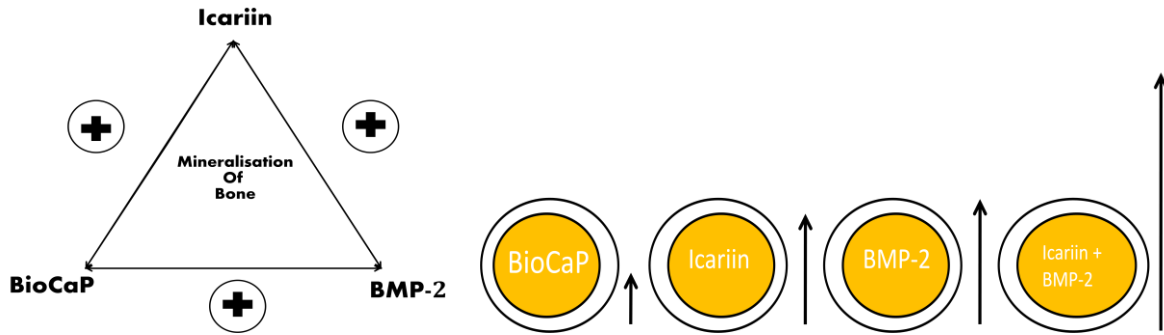
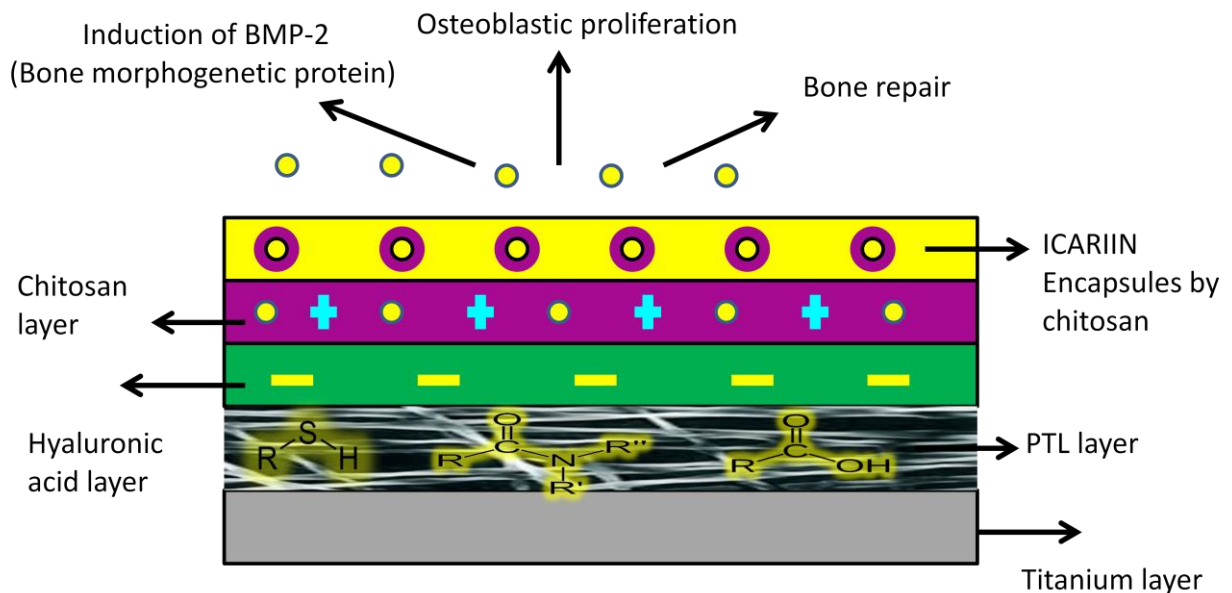
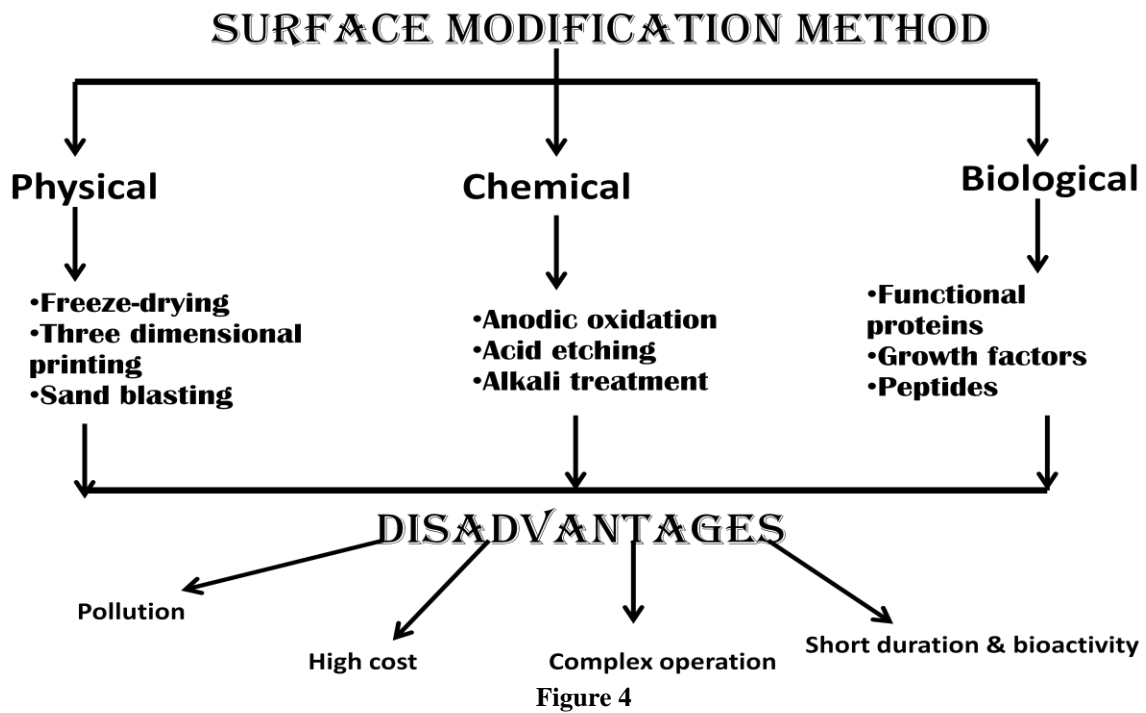
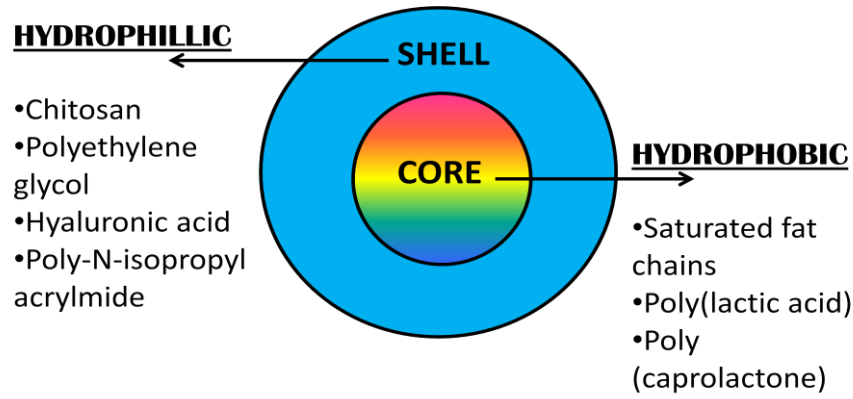


Figure 2

Figure 3





STRUCTURE OF A MICELLE

Figure 6

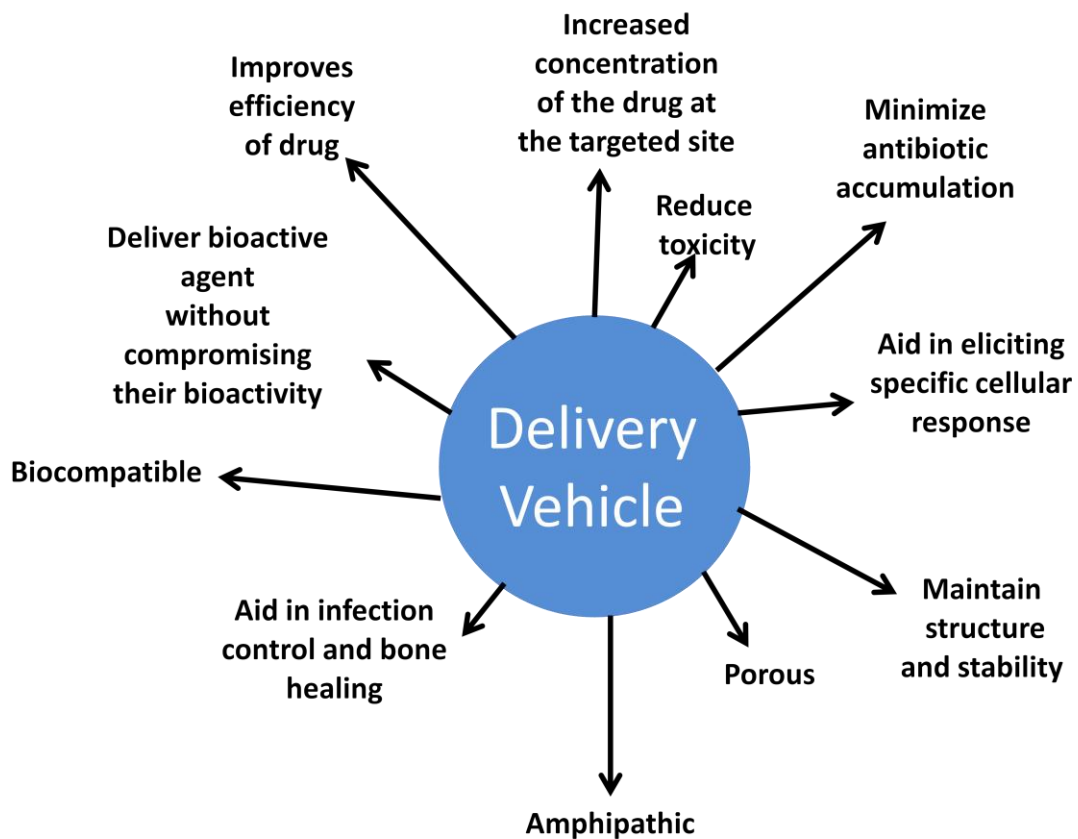


Figure 7

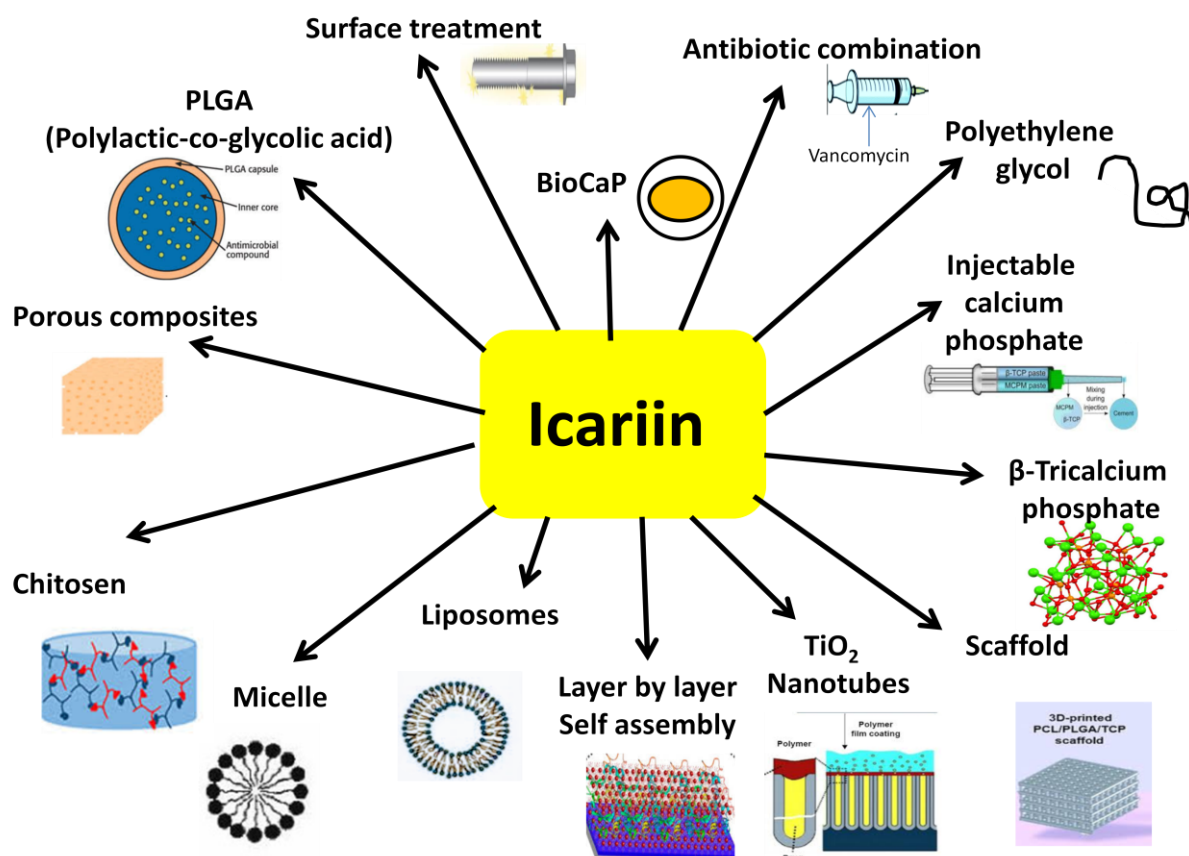


Figure 8

VII. Conclusion

Herbal medicines contain natural active phytochemicals which are highly effective substitute to antibiotics. One such molecule is icariin. The only means to extract therapeutic benefit of icariin is through an efficient delivery vehicle. This article provide an update of various drug carrier system for icariin. However more intensive research should be performed on various carrier system to validate their use in periodontitis so that they can complement current available vehicles in the battle against periodontitis. More studies should be conducted on herbal plants so that more active ingredients are researched for their regenerative properties.

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