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 REVIEW

Review on the Biocompatibility and Bioactivity of Forsterite: *In Vitro* and *In Vivo* Studies

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Abstract. *There is an increasing demand for new materials in orthopedics, biomaterials that can stimulate osseointegration and vascularization, either repairing damaged tissue or producing new one. Currently, the forsterite (FS, Mg_2SiO_4) is actively researched in regards to bone tissue engineering due to its biocompatibility and high bioactivity. The present review focuses on summarizing the research regarding the in vitro (from apatite formation in simulated body fluid, SBF, to cells) and in vivo studies on forsterite.*

Keywords: forsterite, orthopedic implants, in vitro effect, in vivo action

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1. Introduction. The ideal orthopedic implant

Bone defects can follow a variety of conditions such as infections, physical trauma, neoplastic or congenital processes. All issues regarding bone health have led to an increasing need to find the ideal orthopedic implant, namely a biocompatible one with a high bioactivity and good mechanical properties. It should be able to offer proper support regardless of load or mechanical stressors, in the meantime being able to help bone completely regenerate whilst also preventing any infections related to orthopedic surgery as well as any other potential problems. Not unexpectedly, research concerning this perfect material is still ongoing.

However, a rapid progression can be seen within this field of research. Figure 1 presents a schematic classification of biomaterials. The first generation of biomaterials started as being inert due to the fact that the human body is a highly corrosive medium. Also, they had to be able to withstand all stressors, fatigue and high wear level that come with day to day use. However they were not developed specifically for medical use, and instead were already available industrial materials [1]. Hence, they did not interact with the host tissues and this poor integration led to possible implant rejection. Also, no matter how inert the material, it was still a foreign body being able to be recognized as such by the host, thus triggering an inflammatory response [2].

The need for a better integration of the implant gave rise to bioactive, biodegradable materials. These bioactive materials interact with bone tissue leading to a better osseointegration and thus preventing implant failure.

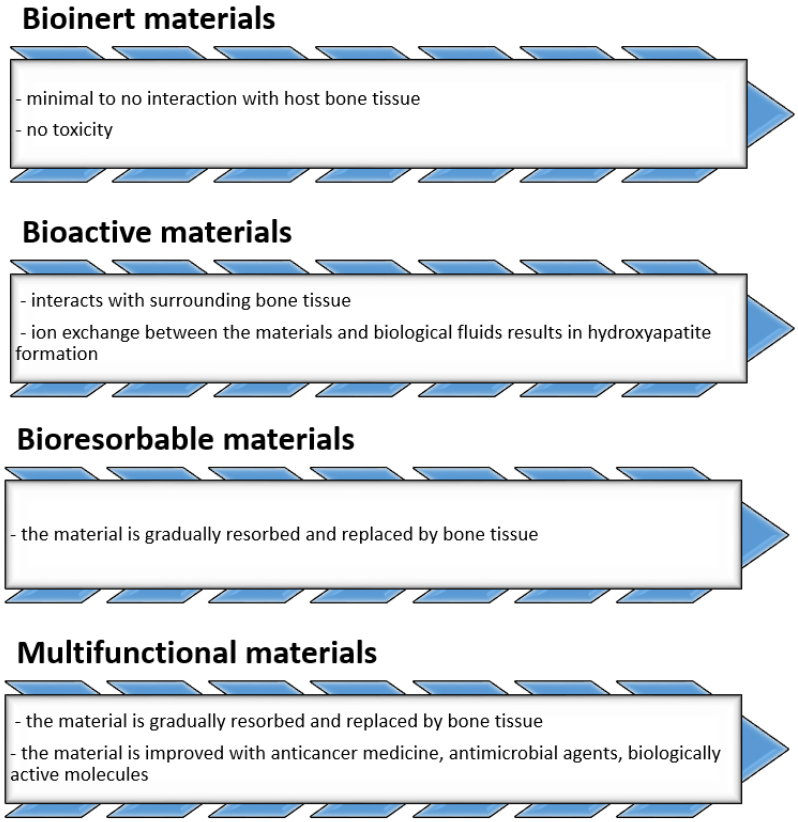

Figure 1. Classification of biomaterials

Of course research went further with materials that are bioresorbable during the new bone formation, the ions in their composition already being involved in multiple biological processes. These new generation materials would improve the self-healing properties of bone, being able to adapt to the host environment and lead to a complete bone replacement. These properties would seem ideal regarding healing of fractures and other mechanical damages. In spite of that, these materials do not help with other conditions in orthopedics, such as bone cancers or post-surgery infections. This led to further research into the functionalization of biodegradable scaffold with different anticancer drugs, antimicrobials and a variety of biologically active biomolecules.

After a biomaterial is implanted, a temporary matrix is formed around it due to a dynamic interaction that occurs between its surface and plasma proteins, a reaction named the Vroman effect [3]. This is of course dependent upon the properties of the biomaterial's surface, such as surface charge, topography, or wettability [4, 5]. An ionic composition close to that of human bone also plays a key part in the acceptance of the implant and promotion of bone regeneration.

It has to be noted that bone is a complex tissue, composed both of organic and inorganic material. The inorganic mineral phase (about two thirds) includes hydroxyapatite (HAP, $C_{10}(PO_4)_6(OH)_2$), dicalcium phosphate ($Ca_2P_2O_7$), dibasic calcium

phosphate ($CaHPO_4$), tricalcium phosphate ($Ca_3(PO_4)_2$) alongside a small amount of amorphous calcium phosphate. Considering that hydroxyapatite is a major component in natural bone and that it is chemically stable at the environmental conditions of the human body (pH and temperature) [6], synthetic hydroxyapatite has been the biomaterial of choice for a large variety of studies both in its stoichiometric form as well as substituted with different biologically important ions [7-40].

However, it is worth noticing that hydroxyapatite in bone is not necessarily 'pure' as in the stoichiometric one. Minor differences within different bone compositions are due to the presence of certain ions (carbonate – CO_3^{2-}; citrate - $C_6H_5O_7^{4-}$; fluoride – F^-; hydroxyl – OH^-; hydrogen phosphate - HPO_4^{2-}; sodium – Na^+; strontium – Sr^{2+}) [41, 42]. As a consequence, some research teams focused on biocompatible materials that can actively generate hydroxyapatite in situ, within the host.

One such promising candidate is forsterite (FS, Mg_2SiO_4), mainly due to the magnesium and silicate ions in its structure, that are essential in the mineralization of bone tissue [43-47]. At a concentration of 0.625 mM, Si has been reported to significantly amplify the mineralization and proliferation processes in bone, alongside gene expression and bone matrix proteins [48]. Mg also plays a key role, low concentrations of it causing hydroxyapatite crystals to become larger and better organized, thus leading to brittle bones [49, 50]. Hence, forsterite having Mg in its composition would potentially help with this unwanted issue by leaching Mg ions and increasing its concentration.

2. Forsterite synthesis

Choosing the right reagents and synthesis method is quite important for biomaterials as they can influence the particle size, surface characteristics and overall morphology of the final powder. Up to present day, a large variety of methods have been developed for the synthesis of forsterite. Some of these methods include solid-state proceses [51-53], mechanical activation [54-57], sol-gel [58-64] and sol-gel variations [65-70], microwave synthesis [71, 72], precipitation [73, 74] and hydrothermal [75].

In terms of obtaining a material with high bioactivity, choosing the right synthesis technique is critical as it will influence this parameter. Smaller, nanometer-sized particles have a higher bioactivity and a better influence on cell proliferation than micrometer-sized particles. This will be discussed later on. The choice of synthesis precursors is also crucial as it can limit the range of methods to be used. Also, some synthesis routes, such as solid-state, require a higher temperature during the thermal treatment which is detrimental to the size of nanoparticles as it increases it. Wet methods (i.e. sol-gel) are highly preferred when it comes to obtaining homogenous powders with really small nanoparticles.

2.1. Synthesis of substituted and doped forsterite

In biomaterials-related literature 'doping' and 'substituting' are used interchangeably. This is somewhat understandable if one considers the end application. For example, an inclusion of strontium is going to add a boost in cell proliferation, no matter how strontium was added. So, both 'doping' and 'substituting' improve upon the properties of a biomaterial. However, it is worth mentioning the fact that they do mean different things. Substitution is the replacement (either partially or totally) of an anion or

cation in any given structure. Doping on the other hand is adding a small amount of a substance to an already existent material. Of course, depending on the nature of the dopant and other factors like further processing (i.e. thermal treatment), it can replace some atoms but it does not have to.

Gheitanchi et al. [76] investigated how strontium substitution influenced the forsterite structure as well as its chemical and biological properties. The samples were synthesized through a sol-gel method. Different substitution ratios were experimented upon ($Mg_{2-X}Sr_XSiO_4$, where x= 0.05; 0.1; 0.2 and 0.4). XRD and Rietveld refinement performed on both pure and substituted forsterite (thermally treated at 1000 ^oC) revealed changes in the phase composition. Due to the bigger lattice parameters of strontium secondary phases might appear. For x=0.05 and 0.1 a small amount of $MgSiO_3$ (enstatite) was formed (7.35 and 4.08 wt% respectively). For 0.1, 0.2 and 0.4, a progressive increase in $Sr_2MgSi_2O_7$ can be observed. MgO also appears for 0.1 and 0.2 substitutions. The size of the nanoparticles falls within the 40-62 nm range depending on the Sr content.

Devi et al. [77] also worked with strontium, namely a doping of 1, 2 and 3% SrO. Samples were prepared through solid-state. XRD analysis coupled with Rietveld refinement performed on ceramics sintered at 1200 ^oC confirmed the formation of secondary phases (MgO, $MgSiO_3$, $MgSr_2Si_2O_7$) with the addition of SrO.

2.2. Further processing of forsterite for biomedical applications

Before discussing the biocompatibility and bioactivity of forsterite, the obtaining of scaffolds has to be mentioned. While the biocompatibility and bioactivity of forsterite can be tested in vitro by using nanopowder, further processing is needed for it to be tested in vivo. Considering the potential application in orthopedics, two processing techniques are studied, namely the obtaining of porous forsterite ceramics and the application of FS powder as a coating on already used inert metallic substrates.

2.2.1. Forsterite ceramics

Bioceramics are a good option for orthopedic implants due to their higher biocompatibility when compared to inert metals. The choice of synthesis route and parameters (i.e. temperature and duration of thermal treatment) are crucial as a homogenous particle shape and size distribution would lead to better ceramics. Conversely, a powder with differently-shaped nanoparticles that fall within a wide size range would introduce defects in the microarchitecture of the ceramic, shortening its lifespan.

Compactness is an important parameter that will influence mechanical properties such as resistance or deformation under loads. However, an appropriate porosity is also needed since the distribution and size of pores is known to help the degradation rate of the ceramic, the acceptance of the ceramic implant and thus minimize any possible rejection [78, 79]. Here an adequate sintering process is pivotal as higher temperatures will lead to stronger, more compact ceramics and lower ones, to more porous ceramics. The plateau (time at which a sample stays at a certain temperature) is also a parameter of interest. So, thermal treatment conditions should be chosen based on the final use of the ceramic (i.e. higher temperatures for load-bearing applications).

One parameter that is closely associated with porosity in ceramic processing is linear shrinkage. With the increase in temperature during the sintering process a densification process takes place that results in a volume reduction.

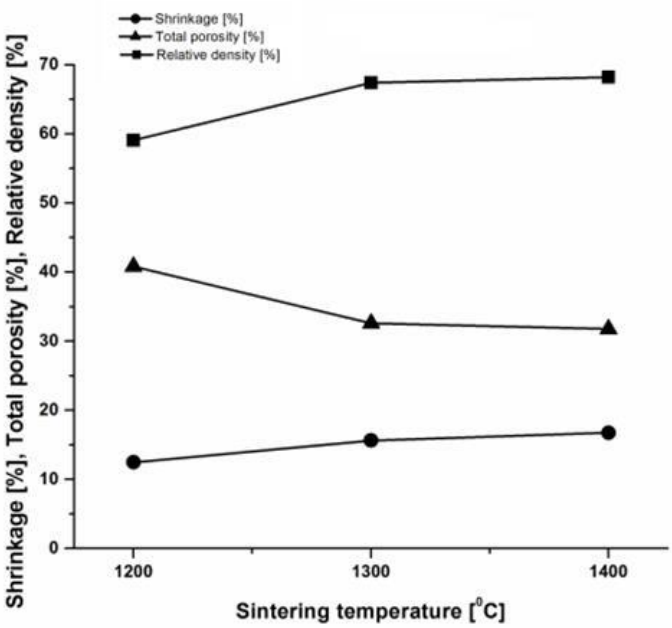

Figure 2. Linear shrinkage, total porosity and relative density for forsterite ceramics versus sintering temperature [80]

Figure 2 offers a more visual aid to the variation of compactness parameters of some forsterite ceramics with an increase in temperature (1200 ^{o}C, 1300 ^{o}C, and 1400 ^{o}C). Unlike phosphates that are not stable at very high temperatures, forsterite, being a silicate, tends to maintain its structure intact, within a wide thermal range, therefore being a lot easier to work with.

2.2.2. Forsterite as coatings on metallic implants

Metal implants are widely used in orthopedics due to their low cost and high mechanical properties. However, they are only suitable for patients that can heal rapidly and have normal bone microarchitecture [81]. These types of implants might not have a proper osseointegration due to the poor adhesion of the metallic substrate to the host tissue. This is also crucial in terms of resistance to infections, without it, mechanical irritation leading to the formation of vesicles that helps bacterial propagation [82]; such problems can be solved by either chemically modifying the substrate to induce cellular adhesion or to cover the substrate with a ceramic layer [1]. While both methods are widely studied in literature, the covering of metallic implants with a ceramic layer shows more promise as the layer can either have a similar composition to that of bone or help induce hydroxyapatite in situ, thus leading to a better osseointegration. This method also

includes a chemical activation of the metal substrate, followed by the application of a binder layer so that the bioactive powder is uniformly distributed on the substrate.

Of course, due to its similarity to the inorganic phase of bone tissue and its ability to facilitate osseointegration, hydroxyapatite has been widely preferred and used as a coating for metallic orthopedic implants. In situ, once in contact with bodily fluid and due to the ionic exchange during which Ca and phosphate ions are released while proteins from the fluids are deposited on the implant, HAP would induce a layer of carbonated hydroxyapatite on the surface [83].

In relation to forsterite, a literature review shows that studies on the coating of metal-based implants with nanoforsterite are still limited. Several research groups have reported experiments involving the use of forsterite powders as coatings on the surface of metal alloys. Electrophoretic deposition on steel [84] and Ti [85], dip coating on stainless steel substrate [86], as well as Ti6Al4V alloy plasma spraying [87] have been employed.

In the following, both in vitro (SBF and cell cultures) and in vivo studies will be presented to the best of the authors' knowledge to obtain a better understanding of the biocompatibility and bioactivity of forsterite.

3. Biocompatibility and bioactivity of forsterite

Due to the biological importance of the ions in its structure, many studies have been focused on the potential use of forsterite as a candidate for tissue regeneration. Its prospective use as a bone implant material has to be confirmed by in vitro testing. For this purpose, two methods are commonly used in regards to bioceramics [88]:

- The hydroxyapatite formation ability by Simulated Body Fluid (SBF) immersion for different periods of time.
- Evaluation of the effect of the material on the osteogenic differentiation in cell cultures

3.1. In vitro SBF studies

The clinical application viability for biodegradable materials is correlated with a degradation rate comparable to new bone formation. This is studied by evaluating hydroxyapatite formation with the immersion in Simulated Body Fluid (SBF) a fluid that is similar in ionic concentration and pH to human plasma (Na^+:142.0 mM; K^+: 5.0 mM; Mg^{2+}: 1.5 mM; Ca^{2+}: 2.5 mM; Cl^-: 103.0 mM; HCO_3^-: 27.0 mM; HPO_4^{2-}: 1.0 mM; SO_4^{2-}: 0.5 mM [89]. It was introduced by Kokubo as a better alternative to the tris-buffer solution in order to reproduce the surface structural changes in vivo [90]. Due to the fact that the original SBF lacked SO_4^{2-}, the formulation was improved upon in later papers by Kokubo [91, 92]. The SBF immersion study is performed by either maintaining the same SBF for said amount of time (static method) or by periodically changing it to better mimic in vivo environments (dynamic method). Of course, over time, several slightly altered formulations of SBF have appeared. A comparison among some formulations, ordered by time progression, found by the authors, is presented in table 1.

Table 1. Ionic concentrations of human plasma and SBF formulations (mM)

Medium	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}	Ref.
Plasma	*142.0*	*5.0*	*2.5*	*1.5*	*103.0*	*27.0*	*1.0*	*0.5*	93
Simulated body fluids	142.0	5.0	2.5	1.5	148.8	4.2	1.0	0	90
	142.0	5.0	2.5	1.5	147.8	27.0	1.0	0.5	92
	142.0	5.0	2.5	1.5	125.0	27.0	1.0	0.5	94
	142.0	5.0	2.5	1.5	103.0	27.0	1.0	0.5	95
	142.0	5.0	2.5	1.5	103.0	10.0	1.0	0.5	96
	142.0	5.0	2.5	1.5	147.8	4.3 (pH 7.4) 4.7 (pH 6.5)	1.0	0.5	97
	165.0	5.0	2.5	1.5	147.8	27.3 (pH 7.4) 28.2 (pH 6.5)	1.0	0.5	
	142.0	5.0	2.5	1.5	103.0	4.2	1.0	0.5	93
	141.5	5.0	2.5	1.5	124.5	27.0	1.0	0.5	89
	142.0	5.0	2.5	1.5	147.8	4.2	1.0	0.5	98

3.1.1. In vitro SBF studies on forsterite

The chemical nature of the material has a role in its ability to form hydroxyapatite while immersed in SBF. Most literature that proposes forsterite as a bone substitute is done by analyzing the ability of forsterite to lead to hydroxyapatite formation on its surface. One study [51] revealed the behavior of FS powder (synthesized through a solid state route) immersed in SBF (1.5 mg/ml) from 7 to 28 days. FTIR (Fourier-transform infrared spectroscopy) spectra revealed the presence of hydroxyapatite specific bands (OH bands - 1660 cm^{-1}, 3450 cm^{-1}, 3690 cm^{-1}; PO_4 group bands - 496 cm^{-1}, 1074 cm^{-1}). The authors observed that nanometer-sized FS (obtained at 1100 ^oC) powders have a higher ability to support HAP formation than micrometer-sized ones (obtained at 1200 ^oC). Another study from the same group [99] examines the performance in SBF of FS nanopowder obtained through a different method, namely sol-gel, synthesized at 900 ^oC. The conditions were kept the same as in the previous work and HAP formation was evaluated by X-ray diffraction (XRD), FTIR and Scanning Electron Microscopy (SEM) coupled with Energy-dispersive X-ray spectroscopy (EDX). It was revealed that with an increase in soaking time, the confirmed HAP aggregates tended to increase in size, leading to a more compact sample surface.

On the other hand, Gorea et al. [80] examined hydroxyapatite formation on forsterite ceramics (sintered at 1200 and 1400^{o}C) kept in SBF for 1, 2 and 3 months respectively. The XRD coupled with FTIR revealed a progression with time for HAP formation. Here, SEM images on FS ceramics obtained at 1400 ^oC show an evident difference in morphology between the ceramic surface and the newly formed phase. This can be better observed in Figure 3. The EDX performed on the newly formed phase confirmed the presence of phosphorous and calcium ions specific to hydroxyapatite.

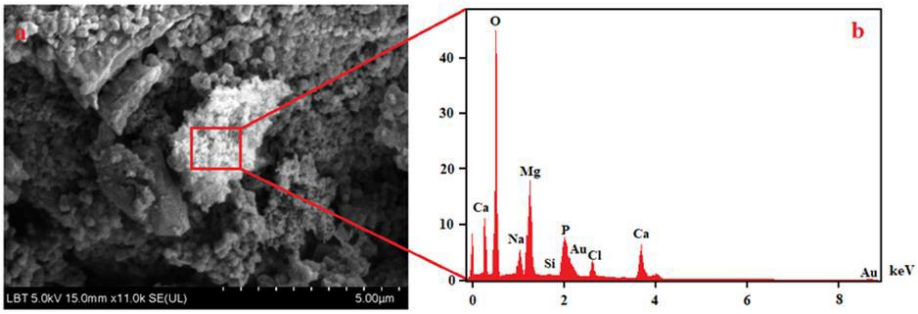

Figure 3. SEM image (a) and EDS spectrum (b) for forsterite ceramic, FC-1400, fired at 1400 ºC, after 3 months of immersion in SBF [80]

Tavangarian and Emadi [100] also experimented on FS ceramics (from powder obtained through mechanical activation) kept in SBF for 1, 2, 4, 7, 14, 21, and 28 days. Hydroxyapatite formation was confirmed through FTIR, SEM-EDX. The HAP particles increased with prolonged soaking time, for example from 7 µm at 14 days to 10 µm at 28 days. Unlike the previous studies, here the authors also performed an Atomic absorption spectroscopy (AAS) analysis on the SBF after the removal of ceramics, revealing a release in Mg ions from the ceramics into the SBF. The AAS experiment is better explained in another study from the same authors [101]. In this case two samples were compared, namely F1 (annealing at 1000 ºC for 1h) and F2 (annealing at 1000 ºC for 2 min). With the immersion of FS samples in SBF, an increase in Mg ions and pH was observed, primarily during the first day. It was reported that ceramic F2, with a lower crystallinity, was able to release more ions and thus lead to more HAP formation.

To conclude, regardless of the synthesis method by which forsterite powder was obtained or the further thermal processing to manufacture ceramics forsterite is able to produce hydroxyapatite in vitro when immersed in simulated body fluid.

3.1.2. In vitro SBF studies on substituted and doped forsterite

Gheitanchi et al. [76] investigated how strontium substitution influenced the behavior of forsterite samples ($Mg_{2-X}Sr_XSiO_4$, where x= 0.05; 0.1; 0.2 and 0.4. All samples were immersed in SBF at 37 ºC for 30 days. After 28 days of soaking the authors report cauliflower-like hydroxyapatite particles completely covering the samples, confirmed by EDX spectra. The Ca/P ratios for the samples with strontium substitutions were determined to be 1.6, 1.04, 1.53 and 1.33, a little different when compared to that of 1.67 for stoichiometric hydroxyapatite.

On the other hand, Devi et al. [77] also reported on SBF studies on SrO doped forsterite ceramics (1, 2 and 3% SrO). The addition of SrO led to an increase in pH and weight of samples after 8 weeks of SBF soaking. With time, there was an increase in the release of magnesium ions, alongside strontium and silicon ions. The sample with 3% SrO presented quite a rapid initial weight loss reported as being due to its smaller grain size of about 126 nm.

3.2. In vitro cell studies

3.2.1. In vitro cell studies on forsterite

The monitoring of material-cell interaction is of crucial importance in the field of biomaterial research for tissue engineering. However, while studies on in vitro cell lines can offer a multitude of insights in regards to the response of cells to said biomaterial, they can also have certain drawbacks. These drawbacks come in the form of differences between isolated cell cultures and living organisms and have to be taken into account considering that the investigated biomaterials will be used in living organisms. Isolated cell cultures in vitro are easier to work with due to multiple factors, such as the fact that there is no immune system present, no disease and there is an excess of nutrients/ factors [102]. Due to the lower cost, lack of ethical issues and higher reliability, in vitro cell cultures are preferred as part of the initial testing for any type of new material intended as a future implant.

While most studies, dealing with forsterite as a biomaterial, focus only on its apatite formation ability, there are some that have chosen testing on cell cultures to get a better grasp of its interactions. Table 2 presents a summary of simple forsterite, either in nanopowder or in ceramic form, and its effect on different cell lines.

Table 2. Brief information regarding cell lines and the effect of forsterite

Type of material	Sintering temperature of ceramic	Cell line used	Effect	Ref.
Forsterite ceramics	1350 – 1550 ºC Not mentioned which temperature was used for the sintering of the ceramic used for the in vitro testing	Osteoblast cells isolated from calvaria of neonatal (less than 2 days old) Sprague–Dawley rats	An obvious attachment and spreading was observed after 24 h (SEM imaging). The MTT test revealed that osteoblasts exhibited a high degree of proliferation that increased with increasing culture time. No cytotoxicity was observed.	103
Forsterite ceramics	1. 600 ºC for 60 min 2. 900 ºC for 6 min 3. 750 or 850 ºC for 2-15 h	osteoblast-like G292 cells	After 3 days, the culture cells adhered and spread onto the ceramic surface. After 7 days, cells formed a layer on all samples.	104
Forsterite nanopowder	-	osteoblast-like G292 cells	Cell numbers increased slowly at concentrations of 50-200 mg/ml culture medium. Cells proliferated rapidly at concentrations of 6.25-50 mg/ml culture medium.	

Forsterite block	Not mentioned	L929 mouse fibroblast cell line (ATCC)	No signs of toxicity after 48 hours. The cells formed a monolayer	105
Forsterite nanopowder	-	osteoblasts	Cell proliferation increases with the culture time. Better proliferation is observed at lower concentration (6.25 mg/ml medium) as opposed to higher ones (12.5 and 25 mg/ml medium).	99
Forsterite ceramics	1000 ºC for 6 min 750 ºC for 7 h	Osteoblast-like MG63, a human osteosarcoma cell line	Cell proliferation was enhanced from 1 to 5 days. An increase in ALP activity was revealed up to 14 days when compared to the control group.	72
Forsterite ceramics	1200 °C for 3 h	human lung fibroblasts HFL	Rapid increase in cell proliferation up to 7 days. No. significant cytotoxic effect.	106
Forsterite ceramics	800 ºC for 2 h	hBMSCs isolated from human bone marrow obtained from subjects (50-70 years old)	Forsterite supports cell attachment and proliferation. Osteogenic commitment of the cells was induced.	107
Forsterite ceramics	1200 °C for 2 h	human lung fibroblasts (ATCC number: CCL-153, normal lung fibroblasts)	Rapid increase in cell proliferation up to 7 days. After 14 days, the cell proliferation increased only slightly.	80

While the studies in Table 2 have experimented on different types of cell cultures, they all suggest that there is no toxicity and that forsterite actually leads to cell proliferation. Figure 4 can provide a better visual support. The rapid increase in number of living cells (fluorescent green) can be clearly observed from day 1 to day 3 and day 7. After 7 days of culture it can be observed that almost all the surface of the forsterite ceramic scaffold was covered in living cells.

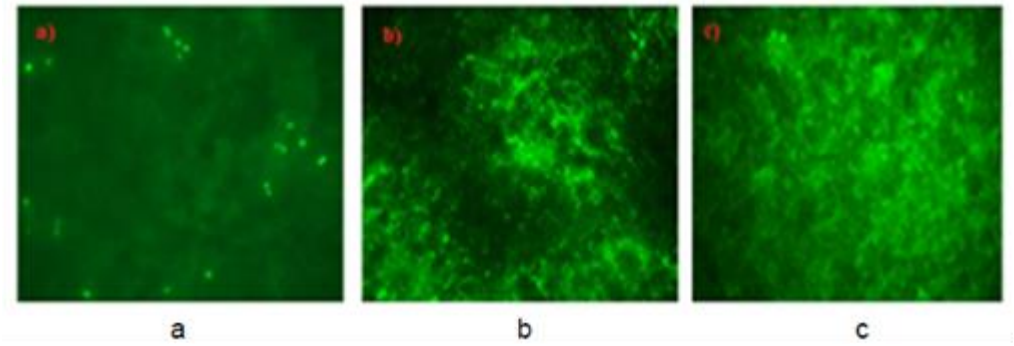

Figure 4. Optical microscopy images of FDA stained (in green) HFL after different incubation time, a) 1 day (1d), b) 3d, and c) 7d, on the surface of forsterite ceramic scaffolds, fired at 1200 ºC [106]

The fact that forsterite is generally reported to aid cell proliferation might be explained by the ions in its composition with both silicon (Si) and magnesium (Mg) playing crucial roles in a wide range of metabolic processes, some of which involve skeletal development [44, 48-50, 108]. A study reporting on how different Mg concentrations can affect osteoblast cells showed that it can increase their viability and differentiation [46]. On the other hand, Si is involved in osteogenesis and angiogenesis, an inadequate Si concentration leading to potential growth defects [47].

Surface area also plays a part in eliciting the desired biological effects. In the case of forsterite powders smaller nanoparticles lead to a more rapid effect due to their larger surface area that allows a higher ionic leaching in the culture media. On the other hand, in case of forsterite ceramics, a higher porosity leads to a larger surface area. In this case, a good control of nanoparticles size and shape as well as the temperature and duration of the thermal treatment are crucial factors for obtaining bioceramics with a desired porosity. Notably, non-toxic organic pore forming agents can also be used as they tend to disappear during the thermal treatment and thus not influence the biological properties of the final ceramic.

3.2.2. In vitro cell studies on substituted and doped forsterite

In regards to this, there is some literature, albeit little, that discusses research on either doped or substituted forsterite on certain cell cultures. For example, one study [76] explores the effect of strontium substituted forsterite ($Mg_{2-X}Sr_XSiO_4$ where 'x'=0.05, 0.1, 0.2, 0.4) on MG63 osteoblast-like cell line. The authors observed an increased proliferation from 1 to 7 days, with the Sr-substituted samples leading to enhanced proliferation.

One later study [77] compares simple forsterite ceramics (thermally treated at 1200 ^{o}C for 2 h) with those doped with SrO (1, 2, 3 wt%) on a Murine osteoblast cell line MC3T3-E1. While a significant increase in live cells after 3 days was observed for ceramics doped with 1 and 2 wt% SrO, a moderate increase was revealed for pure forsterite and that doped with 3 wt% SrO.

The effect obtained with the addition of strontium comes through the fact that this ion is known to promote osteogenesis through the Wnt/β -catenin pathway activation, thus increasing Glycogen synthase kinase 3 beta (GSK3β) phosphorylation [5]

4. In vivo studies

In vivo studies are necessary due to the presence of an immune system and multiple types of cells that are not isolated as with in vitro experiments. Everything leads to a specific response and getting a better understanding of these responses would help in developing better materials with a greater degree of biocompatibility. However, choosing the right animal model is crucial in regards to the clinical potential of a biomaterial and there are multiple factors that have to be taken into careful consideration. Such factors include the size and age of the chosen animal (size and age of bone), the size and depth of the defect as well as its position.

On account of ethical issues, high cost and profundity of animal experiments, there is little literature reporting on in vivo forsterite studies. Devi et al. [109] evaluated the in vivo osteogenesis of forsterite ceramics, both pure and doped with zinc oxide (0.25 and 0.5 wt % ZnO) on white New Zeeland rabbits. Cylindrical shapes of pure and doped FS were implanted in the femoral condyle. After 30 days post implant, bone starting forming on both pure and doped samples, revealing the osteoconductive behavior of forsterite. However, the sample doped with 0.5 %wt ZnO presented large portions of trabecular bone at 30 days, followed by a significant increase in implant degradation up to 60 and 90 days. In comparison, a lower degradation coupled with less bone formation was evidenced for simple forsterite and that doped with 0.25 % ZnO.

Another work from the same group [77] reported on the effect of strontium oxide (1, 2, and 3 wt % SrO) doped forsterite on the osteogenesis in white New Zeeland rabbits. Cylindrical implants were inserted in the distal metaphysis femur of the rabbit. It was reported that after 90 days from implantation, the pure forsterite implant did not present any changes, while the one doped with 1 wt % SrO presented irregularities, indicating resorption. In addition, forsterite doped with 2 wt % SrO vas revealed to have a considerable reduction in volume and density after 30 days, suggesting to the host acceptance of the implant. The authors also confirm a high amount of bony tissue on the implant surface, tissue that increased both in amount and thickness after 90 days post implant. Forsterite doped with higher amount of SrO were reported to have a better coverage of new bone formation.

The fact that the studies were conducted on New Zeeland white rabbit models is understandable considering the fact that they have an accelerated skeletal maturity and much more rapid bone turnover than that found in primates [110]. However, this turnover could also introduce a long-term study bias as it is hard to interpret the results in relation to human biology also considering the fact that rabbit bones are the most different from that of humans [111].

Of course, more studies are needed to get a better grasp on how forsterite ceramic scaffolds would behave over prolonged periods of time within a host. However, the results regarding osseointegration and bone formation do look quite promising.

5. Way forward

All discussed papers showed forsterite to be biocompatible with no cytotoxic effect and to possess a good bioactivity. Some research groups went even further, by studying forsterite-based composite materials [70, 112-118]. These composites combine the properties of forsterite with those of other biocompatible materials both inorganic such as hydroxyapatite, wollastonite, diopside, as well as polymers like polycaprolactone, in an attempt to tailor new possibilities for medical implant design. While the results are promising, some materials are more complex so more data is needed in terms of how they would perform long term compared to forsterite ceramics. All things considered, the research of biocomposites containing FS is still emerging, there being very few in vitro studies, and the further study of this field would lead to implants that can possess a wide variety of biological and mechanical properties. Also, a functionalization of forsterite (either as a standalone material, simple or substituted, or in composites) with medicine, different biologically active molecules or metallic nanoparticles could help develop more advanced implant materials.

Furthermore, as the surface of the material plays a crucial role in its interaction with living cells, modifying these surfaces at a molecular level by incorporating specific biomolecules could potentially diminish the problems associated with both implantation pathologies as well as other conditions. For example carotenoids [119-133] are valuable molecules of interest in pharmaceutics due to their known antioxidant and potential anti-tumor activity. Going even further, some groups have studied the prospective antimicrobial activity of some carotenoids on different strains [134-138]. In particular, one study by Keceli et al. has to be mentioned as it reports an antibacterial effect of some carotenoids extracted from *Rhodotorula glutinis* on *Staphylococcus aureus* (*S. aureus*) [139]. In addition, Kusminta et al. [140] revealed an inhibition of methicillin resistant *S. aureus* (MRSA) and Multi-Drug Resistant *Escherichia coli* (MDR *E. coli*) with the use of bacterial carotenoids. This is quite relevant when it comes to the further synthesis of better orthopedic implants as *S. aureus* (gram positive) is the most prevalent pathogen related to orthopedic surgeries while around 20% of such infections are caused by E. coli (gram negative) [141].

As the increase in bacterial resistance is quite a serious problem another research alternative would be the incorporation of metal nanoparticles with proven antimicrobial resistance. Of course, silver nanoparticles (AgNPs) [23, 25, 142-145] have been widely studies as antimicrobial agents. However, gold nanoparticles (AuNPs, GNPs) are another alternative due to their biocompatibility, photothermal effects and the fact that they can be easily modified and loaded with various therapeutic agents [146-162].

Of course with more complex implant systems there is a need for a better understanding of molecule self-assemblies into monolayers [163-173], bilayers [174-176] or Langmuir-Blodgett layers [177-181]. A good cognizance of how these systems would interact with different model membranes would facilitate a better interpretation of in vivo studies.

Conclusions

This review focuses on the behavior of nanostructured forsterite related to biological environments. Its biocompatibility and bioactivity were discussed both in vitro (Simulated Body Fluid testing as well as cells) and in vivo. All things considered, the good biocompatibility and bioactivity of FS, either in powder or ceramic form, was demonstrated by a variety of analysis by multiple studies. Due to the enhanced SBF degradability of FS the ions in its composition are released in the media. However as the ions are already involved in biological processes they should not pose a problem. This was confirmed by the histological analysis of organs in in vivo studies. This promotes forsterite as a suitable candidate for biomedical applications, especially for bone tissue engineering. Of course, obtaining a better understanding of the different signaling pathways in bone formation and the influence of different ions or molecules might lead to a more precise tailoring of future biomaterials.

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