Using machine learning for improving knowledge on antibacterial effect of bioactive glass

maria magdalena echezarreta, Maria Magdalena Echezarreta Lopez

International Journal of Pharmaceutics 453 (2013) 641–647

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Using machine learning for improving knowledge on antibacterial effect of bioactive glass

M.M. Echezarreta-López*, M. Landin

Departamento Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Campus Vida, Universidad de Santiago, Santiago de Compostela 15782, Spain

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 13 June 2013
Accepted 15 June 2013
Available online 24 June 2013

Keywords:
Bioactive glass
Antibacterial behaviour
Modelling
Artificial intelligence
Neurofuzzy logic
Machine learning

ABSTRACT

The aim of this work was to find relationships between critical bioactive glass characteristics and their antibacterial behaviour using an artificial intelligence tool. A large dataset including ingredients and process variables of the bioactive glasses production, bacterial characteristics and microbiological experimental conditions was generated from literature and analyzed by neurofuzzy logic technology. Our findings allow an explanation on the variability in antibacterial behaviour found by different authors and to obtain general conclusions about critical parameters of bioactive glasses to be considered in order to achieve activity against some of the most common skin and implant surgery pathogens.

1. Introduction

Since the introduction by Hench in the 1970s of Bioglass® (Hench et al., 1971; Hench and Paschall, 1973) this material has been extensively investigated. Bioactive glasses (BG) are special glass systems composed by SiO_2, CaO, P_2O, and NaO that can be produced by traditional melting or by sol–gel processes (Hench et al., 1971; Hench, 1998a). Their bioactive behaviour comes from their ability of bonding soft and hard tissues through complex reactions, which form strong and compliant interfaces between the glass and the tissues (Hench et al., 1971). BGs have excellent biocompatibility, osteoconductivity and osteostimulation (Sun et al., 2007). For these reasons BGs have been employed for clinical use for a variety of medical applications, including surgical orthopaedics and dentistry, mainly for repairing osseous, cystic, tumours and periodontal defects, or other lesions sites after resection in the appendicular skeleton (Hench, 1998b; Kellomäkiki et al., 2000; Vogel et al., 2001; Froum et al., 2002).

The use of implants in the body is associated with a risk of bacterial colonization of the material and the subsequent failure of the surgery (Gristina, 1987; Pye et al., 2009; Campoccia et al., 2006). Combinations of implant/drugs or implant materials with antibacterial properties would represent an excellent approach for prevention of potential postoperative infections. Different authors have pointed out the utility of the antibacterial activity of BGs in oral (Allan et al., 2001; Zehnder et al., 2006; Waltimo et al., 2009), orthopaedic implants (Xie et al., 2009; Gorriti et al., 2009; Misra et al., 2010) and wound dressings (Verrier et al., 2004; Day and Boccaccini, 2005).

A review of the literature allows the conclusion that antibacterial properties of BGs depend on their composition and morphological characteristics (Hu et al., 2009; Kalmodia and Molla, 2010), but the wide variety of conditions used to perform the studies (e.g. bacteria studied, antimicrobial conditions or glass pretreatment) hinders the specific factors influencing bactericidal capacity and the best combination of characteristics in achieving the maximum antibacterial effect.

The analysis of a database generated from historical data is difficult using traditional approaches. However, artificial intelligence tools allow for this, and the discovery of general or new patterns from this type of data in a process named “data mining” (Rowe and Colbourn, 2003). Among all methods, the neurofuzzy logic (NFL) approach has proven its utility in modelling complex non-linear relationships hidden in data, having a higher accuracy in prediction than classical statistics and helping the understanding of the complex relationships between variables (Shao et al., 2006; Landin et al., 2009; Gallego et al., 2011).

NFL combines the adaptive learning capabilities from artificial neural networks (ANN) with the generality of representation from fuzzy logic through simple rules. It has been demonstrated that ANN is effective in modelling complex processes. However, the

* Corresponding author. Tel.: +34 881815252; fax: +34 881815038.
E-mail addresses: mmagdalena.echezarreta@usc.es, ffmagda@yahoo.es (M.M. Echezarreta-López).

0378-5173/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.06.036

interpretation of an ANN is not always easy, especially if an important number of inputs are involved because just black box models are generated. For that reason other technologies such fuzzy logic are being coupled to ANN to help handling those complex models, which generates, after a proper fuzzification process of the variables, simple IF... THEN rules (García-Infante et al., 2010). Neurofuzzy logic can be applied to historical databases generating understandable and reusable knowledge in an explicit format. (Rowe and Landín, 2013)

We think that neurofuzzy logic can be useful in establishing the critical variables (composition, variables involved in the BGs production and/or variables used to demonstrate antibacterial properties) to detect antibacterial effect of BGs.

In this paper we have reviewed the literature on antimicrobial activity of bioactive glass, to extract and analyze together previous results. We have modelled results using neurofuzzy logic, intending to establish the critical aspects in the determination of the antimicrobial activity of the BGs.

2. Materials and methods

A large database (531 facts) on antimicrobial properties of several bioactive glasses was generated from 10 previously published articles from 2000 to 2010 (see table at the supplementary material) (Mortazavi et al., 2010; Zhang et al., 2010; Hu et al., 2009; Leppäranta et al., 2008; Munukka et al., 2008; Waltimo et al., 2007; Zehnder et al., 2006; Abou Neel et al., 2005; Bellantone et al., 2000, 2002).

In general, the antimicrobial activity studies intend to probe the antibacterial effect of BGs by culturing the pretreated or non-pretreated biomaterials (variable in composition, physical chemical properties and concentration) together with selected bacteria suspensions in specific culture media. After a preset time of cultivation, the pH is measured and a volume of the culture is transferred, cultivated on a new media and the growth index (GI) measured (Munukka et al., 2008; Mortazavi et al., 2010).

Two models were carried out. For model A, variables selected (21 inputs) which reduced the dataset to 348 facts can be classified in three groups: BGs characteristics, bacterial characteristics and microbiological experimental conditions.

Among the BGs characteristics, the production method, BGs composition regarding compound concentration (SiO_2, CaO, Na_2O, P_2O_5, MgO, K_2O, Al_2O_3, B_2O_3, Ag_2O, CuO) in the bioactive glass (w/w, %), particle size as mean size (μm), morphology as nanoparticles (nano), powder (po), particles (pa) and fibres (fib), and BG concentration (mg/mL) were registered.

For every experiment bacterial characteristics recorded were: microorganism specie including *Staphylococcus aureus* (Sa), *Staphylococcus epidermidis* (Se), *Enterococcus faecalis* (Ef), *Escherichia coli* (Ec), *Pseudomonas aeruginosa* (Pae) and *Salmonella typhi* (Sty), and morphology: bacillus (Ba) or coccus (Co). Bacteria were grown aerobically in different microbiological experimental conditions registered as bacterial concentration (CFU/mL), culture time (h) and culture media categorized in two groups named as buffered solutions (BS) (when used, simulated buffered fluid, phosphate buffered saline or solution saline) and nutrient solution (NS) (when used, Tryptone Soy broth, Lysogeny broth, Mueller Hinton broth or nutrient broth). Additionally, coculture time and coculture media (using the same categorization as previously noted) were registered.

For model B (Fig. 2), recorded final pH values were also included as an additional input. This approach reduced the number of facts to 129.

For both, model A and B, the output recorded was the growth index, measured as a function of the number of survival bacteria colonies. Absence of growth (GI = 0) indicates bactericidal effect. Very sparse, sparse and moderate values of GI (GI = 1 between 0 and 5 colonies, GI = 2 between 5 and 50, and GI = 3 between 50 and 300 colonies, respectively indicate moderate growth. The growth index of 4 (>300 colonies) indicates no effect.

Database with the inputs and the output from the sources are compiled in Table 1 of supplementary material.

Table 1
The training parameters setting with FormRules 3.31 for models A and B.

Minimization parameters
Ridge regression factor: 1 e^{-6}
Model selection criteria
Structural risk minimization (SRM)
Number of set densities: 2
Set densities: 2, 3
SRM parameters: C1 = 1 and C2 = 4.8
Adapt nodes: TRUE
Max. inputs per submodel: 4
Max. nodes per input: 15

2.1. The software tool: neurofuzzy logic

A commercial neurofuzzy logic software, FormRules v3.31 (Intelligensys Ltd., Stokesley, UK) was used.

The training process was conducted, in the same way, as reported previously by Shao and coworkers (2006). The accuracy of the neurofuzzy logic models was assessed using the ANOVA parameters and correlation coefficient (R^2) for the output "growth index".

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad (1)$$

where $\bar{y}$ is the mean of the dependent variable, and $\hat{y}$ is the predicted value from the model. Values of correlation coefficient between 70 and 99.9% together with ANOVA f values over its critical values for the corresponding degrees of freedom are an indication of reasonable model predictabilities (Colbourn and Rowe, 2009).

Within the statistical fitness criteria included in FormRules 3.31 software, structural risk minimization (SRM) was selected to give the model with the best correlation coefficient and, simultaneously, the simplest and more intelligible rule set. Selected parameters for training are presented in Table 1.

3. Results

The common factor in the published studies on antibacterial properties of BGs of the literature is the great variability in the conditions used with regard to the bacteria types, compositions and sizes of BGs as well as microbiological parameters used. In this situation to establish general conclusions on the optimal BGs properties to assure antibacterial activity is a complex task.

3.1. Model A characteristics

The 21 inputs selected for model A and the corresponding growth index reflect the main variables (independent and dependent) included in the articles from literature which made it possible to generate a database that can be modelled by neurofuzzy logic tool. A large database of 348 facts on antibacterial properties of BGs materials was successfully modelled (train set $r^2 = 76.31$) by neurofuzzy logic technology (see ANOVA model in Table 2) that allowed the selection of the critical factors or inputs which accurately explain the variability of the growth index, selected as output.

Fig. 1 presents the four submodels considered by neurofuzzy logic to explain variability in the GI and the inputs included by them. It is interesting to note the reduced number of inputs, five

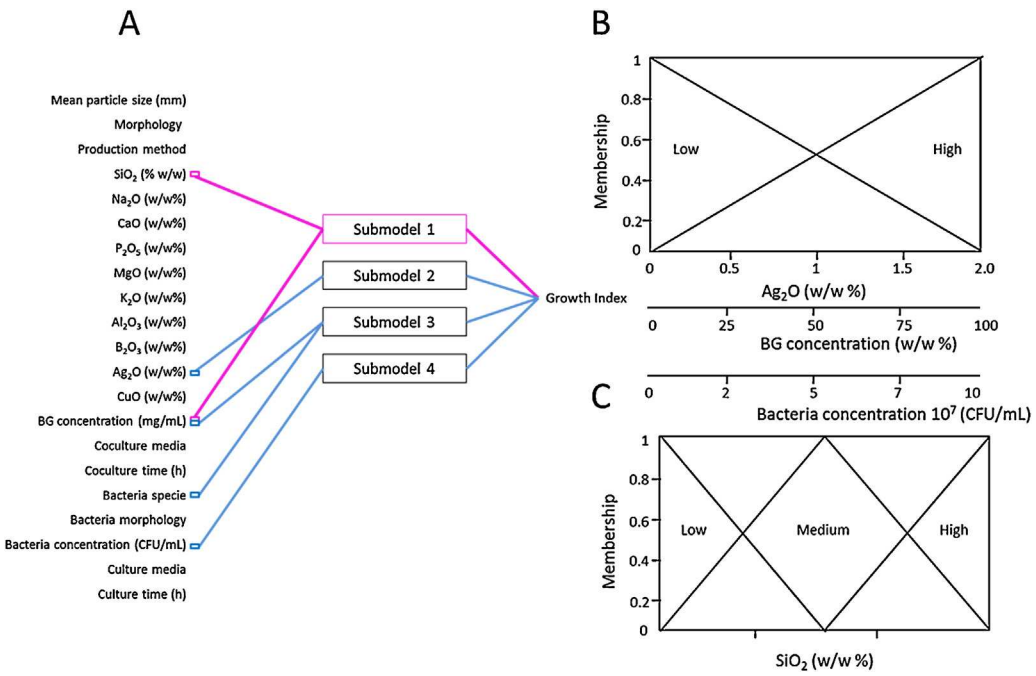

Fig. 1. Graphical example of model A with the significant submodels and their domains developed by neurofuzzy logic for the output GI. (A) Simplest submodels generated pointing out the significant input interaction of SiO_2 and BG concentration (in purple) as the main effect and the interaction of BG concentration and bacteria specie, the single effects of Ag_2O and bacteria concentrations affecting growth index; (B) domains established by neurofuzzy logic technology for the continuous variables BG concentration, Ag_2O and bacteria concentration; (C) domains established for the continuous input SiO_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(SiO_2 and Ag_2O concentration, BG concentration, bacteria specie and bacteria concentration) out of the twenty one that neurofuzzy logic has selected as the ones having important effect on the growth index or, in other words, enough to explain the GI variability.

Neurofuzzy logic technology allows a set of "IF ... THEN" rules per submodel with their corresponding membership degrees (Table 3) to be generated.

Those rules should be read as follows:

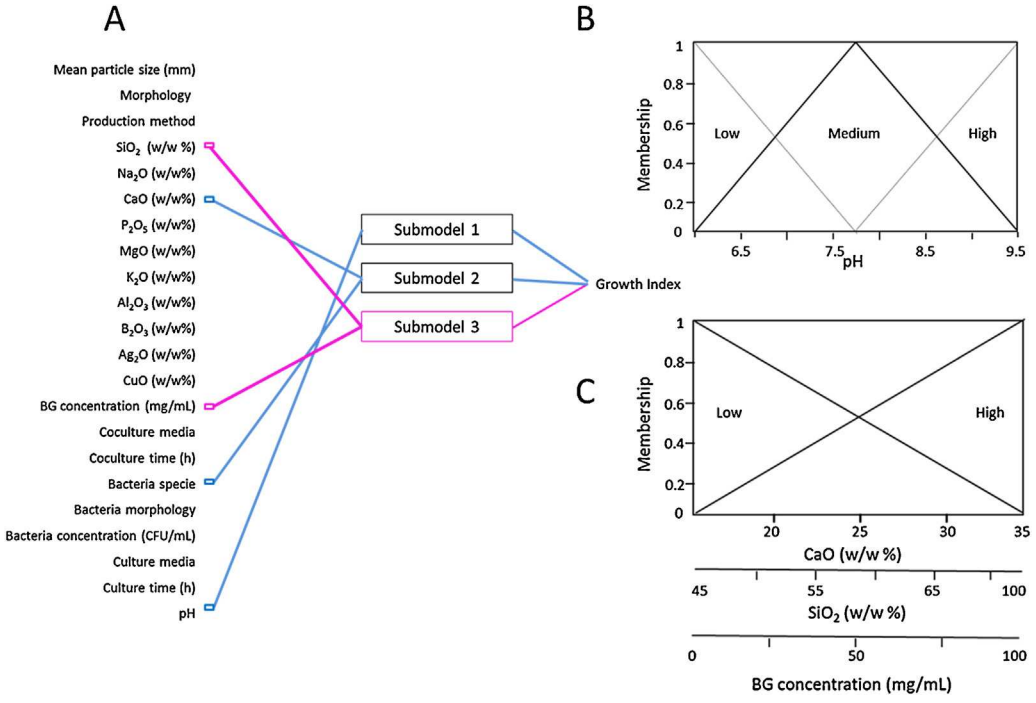

Fig. 2. Graphical example of model B with the significant submodels and their domains developed by neurofuzzy logic for the output GI. (A) Simplest submodels generated pointing out the significant input interaction of Si concentration and BG concentration as the main effect and the interaction of calcium concentration and bacteria specie, the single effect of pH; (B) domains established by neurofuzzy logic technology for the continuous input pH; (C) domains established for the continuous variables BG concentration, SiO_2 and calcium concentration.

Table 2
Analysis of variance for the model A developed. Computed *f* ratio is higher than 1.58 which is the critical *f* value (19 and 348 degrees of freedom and $\alpha < 0.01$) to assure statistical significance of the model A.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	Computed *f* ratio
Model	527.8	19	27.78	55.81
Error	163.7	329	0.49	
Total	691.6	348		

For example, for Rule 1 of Table 3: "IF the concentration of BG used is LOW AND the concentration of silicon oxide in the BG is LOW THEN the Growth Index founded is LOW with a confidence level or membership of 1.00.

The meaning of LOW, MID and HIGH for each parameter has been represented in Fig. 1B and C, which show the relationship between input values (*x*-axis) and the corresponding membership (*y*-axis) (Landín et al., 2009; Gallego et al., 2011; Colbourn and Rowe, 2009; Kavli and Weyer, 1994). Using those rules, neurofuzzy generates understandable and reusable knowledge on the antibacterial activity of BGs.

3.2. Model B characteristics

A second model was carried out including 22 inputs (21 from model A plus pH media value) and GI as the only output. The reduce number of facts, including a measured pH value, gives a reduced database (129 facts). Model predictability was higher than that obtained for model A with a training R^2 equal to 81.64 and statistically significant with a computed *f* ratio of 28.79 ($\alpha < 0.01$).

Despite the models pointing out different inputs the information reported by them is complementary, their main differences being interesting analyzed. Firstly, for model B (Fig. 2), pH (the new included variable) by itself is one of the parameters that explains the variability of GI. Additionally, the submodel 2 of model B includes the interaction between bacteria specie and calcium concentration instead of bacteria specie and BG concentration included in model A. This interaction is the most important, explaining the variability (purple lines) and helps in understanding the mechanism of antibacterial activity of these biomaterials. Finally, the Ag_2O concentration could not be included as a significant input in model B because its variability in the 129 facts is null, therefore this model, misses the differences regarding the BG inclusion of Ag ions pointed out by model A. Table 4 includes the rules derived from model B.

4. Discussion

Preventing and/or limiting bacteria colonization and/or contaminations when a biomaterial is introduced into the body, is the main concern of a number of studies over the recent years. The use of combined drug-materials, and especially biomaterials showing antimicrobial activity is bound up with a lower probability of surgery failure by infections and an increase of application areas. Among biomaterials, bioactive glasses of specific compositions have exhibited antibacterial activity whose identification and characterization have been demonstrated in a large range of experimental conditions that make general conclusions difficult to achieve.

The inclusion of data from different works and their modelling using artificial intelligence methods should allow those general conclusions to be established.

Artificial neural networks (ANNs) analysis has proved useful in finding the relationship between the BGs solubility behaviour and their chemical composition and if using a database large enough it may be possible to predict their solubility with sufficient accuracy (Brauer et al., 2007). ANNs have also been applied to characterize

Table 3
Rules for growth index generated by neurofuzzy logic for model A. Poor combinations have been highlighted.

Rule		[BG]	Si_2O	Ag_2O	Bacteria specie	Bacteria concentration		GI	Confidence level
Submodel 1									
1		LOW	LOW					LOW	1.00
2		LOW	MID					**HIGH**	**1.00**
3		LOW	HIGH					**HIGH**	**1.00**
4	IF	HIGH	LOW				THEN	LOW	1.00
5		HIGH	MID					LOW	1.00
6		HIGH	HIGH					**HIGH**	**1.00**
Submodel 2									
7				LOW				**HIGH**	**1.00**
8				HIGH				LOW	1.00
Submodel 3									
9		LOW			*E. faecalis*			**HIGH**	**1.00**
10		HIGH			*E. faecalis*			**HIGH**	**0.58**
11		LOW			*S. epidermidis*			LOW	1.00
12		HIGH			*S. epidermidis*			LOW	1.00
13		LOW			*S. typhi*			LOW	0.88
14	IF	HIGH			*S. typhi*		THEN	**HIGH**	**0.59**
15		LOW			*E. coli*			LOW	0.67
16		HIGH			*E. coli*			LOW	1.00
17		LOW			*S. aureus*			LOW	0.72
18		HIGH			*S. aureus*			LOW	1.00
19		LOW			*P. aeruginosa*			LOW	0.90
20		HIGH			*P. aeruginosa*			LOW	0.68
Submodel 4									
21	IF					LOW	THEN	**HIGH**	**0.97**
22						HIGH		LOW	0.72

Table 4
Rules for growth index generated by neurofuzzy logic for model B. Poor combinations have been highlighted.

Rule		pH	CaO	Bacteria specie	[BG]	Si_2O		GI	Confidence level
Submodel 1									
1		LOW						**HIGH**	**1.00**
2	IF	MEDIUM					THEN	**HIGH**	**1.00**
3		HIGH						LOW	1.00
Submodel 2									
4			LOW	*E. faecalis*				LOW	**0.55**
5			HIGH	*E. faecalis*				LOW	**0.70**
6			LOW	*S. epidermidis*				LOW	0.84
7			HIGH	*S. epidermidis*				LOW	0.89
8			LOW	*S. typhi*				LOW	1.00
9			HIGH	*S. typhi*				**HIGH**	**1.00**
10	IF		LOW	*E. coli*			THEN	LOW	1.00
11			HIGH	*E. coli*				LOW	1.00
12			LOW	*S. aureus*				LOW	1.00
13			HIGH	*S. aureus*				LOW	1.00
14			LOW	*P. aeruginosa*				LOW	1.00
15			HIGH	*P. aeruginosa*				**HIGH**	**1.00**
Submodel 3									
16					LOW	LOW		**HIGH**	**1.00**
17					LOW	HIGH		**HIGH**	**1.00**
18					HIGH	LOW		LOW	1.00
19					HIGH	HIGH		**HIGH**	**1.00**

the antimicrobial activity of a heterogeneous group of compounds and differentiate between active and inactive compounds (Garcia-Domenech and Ortiz, 1998) or to predict the antibacterial activity of a series of imidazole derivatives (Domingues et al., 2004).

In the present work we have used neurofuzzy logic technology which is an artificial intelligence tool that combines the adaptive learning capabilities from ANNs with the generality and the flexibility of representation from fuzzy logic allowing the growth index to be modelled (GI) as a function of the big set of parameters included as inputs. For the model A, using the whole dataset, neurofuzzy logic has selected 348 facts and GI was modelled using just 5 out the 21 parameters included.

Differences in BGs antimicrobial activity, regarding its production method have been extensively referenced in the literature, the sol–gel method being the best in producing high porosity particles that promote higher ion release to the medium (Mortazavi et al., 2010; Peltola et al., 1999).

Different authors have also stated that particle size is determinant when BG is showing antibacterial activity. Reduction in particle size, and as a result, the increase in the surface area of the material, has been pointed out as an approach of increasing the antibacterial activity of BGs (Gorriti et al., 2009; Misra et al., 2010; Waltimo et al., 2007; Balamurugan et al., 2008). Despite these arguments, neurofuzzy logic technology was unable to find even subtle effects of production procedure, particle size or morphology when the data are analyzed together, meaning that they cannot explain the variations in GI found from different authors in various experiments.

The main effect explaining the variability of growth index is the interaction between the silicon concentration and the concentration of BG (Table 3, model A, submodel 1) in the experiment, the BG having no effect when its concentration is low and the silicon concentration is medium or high (submodel 1, rules 2 and 3) or the BG concentration is high and the silicon concentration is also high (submodel 1, rule 6) (highlighted text). It has been shown that the antibacterial action of a BG can be affected by its chemical composition and its dissolution properties in the surrounding medium. In an aqueous environment, reactions occur on the BG particles surface, including release of soluble silica, sodium and calcium which result in an increase of the aqueous pH value (Hench, 1998a).

Our findings agree with previous authors which correlate BG antibacterial properties with the immediate release of alkaline species (Waltimo et al., 2007). The higher the silicon concentration the lower the alkaline species that can be release to the medium and the lower its bactericidal effect. The BGs concentration increases the total amount of ions available to be dissolved.

Some BGs, without specific ions, have shown antibacterial inhibition by attaching directly the bacteria by a biologically active carbohydroxyapatite (CHA) layer, a mechanism related to physical interactions more than chemical interactions (Stoor et al., 1999; Vaahtio et al., 2006; Stoor et al., 1998). However, the incorporation of particular ions into the silicate networks such silver, cooper or boron has been specifically investigated with the aim of developing antibacterial materials with promising results (Gerhardt and Boccaccini, 2010; Bellantone et al., 2002; Mulligan et al., 2003). Possible mechanisms for bacterial inhibition by those elements have been related to the disruption of bacteria functionality; the interference with electron transport, the binding to DNA or the interaction with the cell components (Balamurugan et al., 2008).

From our results from model A (Table 3), it can be deduce that silver is decisive for the BG antibacterial activity (submodel 2, rule 8) at the concentrations included in the study. Its effect is confirmed when the Ag_2O concentration is higher than 1.5%, which is in agreement with data from literature (Balamurugan et al., 2008; Bellantone et al., 2002). Verné et al. (2008) have found that antimicrobial effect of silver based BGs is due to the leaching of silver from the glass matrix, the change in pH and the ionic strength.

Different authors have related the BG antibacterial effect to the increase of aqueous pH value caused by the release of alkali ions from BG particles (Allan et al., 2001; Stoor et al., 1998). In general, all articles reviewed consider that the variation environmental pH is a critical parameter to the antimicrobial activity of bioactive glasses; Mortazavi et al., 2010; Bellantone et al., 2002; Zhang et al., 2010). Our results support those findings. When pH is included as input (model B) despite the number of facts being dramatically reduced (129) an acceptable model was produced. This simplest model missed the ability of detecting variations due to Ag ion concentration that remain constant in the smaller database but succeeded explaining the GI as a function of pH variability. The relationship silicon and final concentration is also clearly pointed out together with the significant effect of the interaction between calcium ion concentration and bacteria specie.

The database generated included six species of bacteria, three coccus and Gram-positive (*S. aureus*, *S. epidermidis* and *E. faecalis*)

and three bacillus and Gram-negative (*E. coli*, *P. aeruginosa* and *S. typhi*) which provided a structural and morphological variety, with different response against physical–chemical and environmental conditions (Allan et al., 2001; Munukka et al., 2008). Most of these are oral bacteria, including those associated with caries and periodontal diseases.

The susceptibility of species to antimicrobial agents varies regarding the presence or absence of a lipopolysaccharide layer (Gram-negative or Gram-positive) in their cellular wall and their kind of association and growth, or their biofilm formation capacity which is different for coccus and bacillus (Mortazavi et al., 2010; Zhang et al., 2010; Nikaido, 1993). In this study both characteristics were evaluated together as one input because all the coccus selected were Gram-positive and all the bacillus were Gram-negative, but neurofuzzy logic did not show this input as significant in explaining the variation of GI.

However, as it can be derived from model A (Table 3), submodel 3 (rules 9–20), which shows the interaction between the total BGs concentration and the bacterial specie, the susceptibility of the different species to the BGs treatment has great variability in confidence levels or membership values, *E. faecalis* and *S. typhi* being the most resistant species to treatment with BGs (highlighted text).

Those differences can be explained by variations in the structure or association of bacteria. Among the coccus studied the *E. faecalis* has been described to have associations by couples forming short chains. However, both *S. epidermidis* and *S. aureus* produce clusters. Those differences could justify variations in the susceptibility of these species (Nikaido, 1993).

S. typhi differs from the other two bacillus (*E. coli* and *P. aeruginosa*) in the structural organization of the lipopolysaccharide layer which gives bacteria resistance against BG.

As we have pointed out previously, model B (Table 4) helps to corroborate the differences in behaviour for the species studied and to understand the antibacterial mechanism of BG related to calcium ion variations. Submodel 2 (rules 4–15) specifies the significant interaction between those inputs. In general, the coccus (Gram-positive) species selected are more susceptible to treatment than bacillus (Gram-negative). For the coccus group, the higher the BG calcium concentration is the more important its antibacterial effect is. Comparing membership values it can be concluded that among coccus, *E. faecalis* can be pointed out as the most resistant specie to treatment (GI LOW but with memberships of 0.55 and 0.70), which is in agreement with conclusions from model A. Calcium ions are lethal for bacteria, but *E. faecalis* is able to survive in calcium ion rich environments (Nakajo et al., 2006). McHugh and coworkers evaluated the pH required to inhibit growth *in vitro* and showed that a pH higher than 11 is needed to eliminate this microorganism (McHugh et al., 2004). Nakajo group (Nakajo et al., 2006) suggested that the low effect of the pH increment may also be attributed to the resistance of the cytoplasmic membrane against acid or alkaline media along with the transport system ATP-linked proton.

The bacillus selected (all Gram-negative) have a thinner layer of peptidoglycan and an outer membrane. The outer membrane is a lipid bilayer and constitutes the major barrier permeability for this group of bacteria. The lipid bilayer seems similar to any biological membrane, but its structure is asymmetric and its composition differs from other living organisms. The semi-internal layer is comprised of phospholipids, and the external layer by a lipopolysaccharide (LPS). The stability of the LPS layer depends on the calcium concentration. Agents and/or processes that disturb the calcium ion equilibrium in the media can affect bacterial structure and as a consequence their integrity and bacteria mortality (Nikaido, 1993, 2003). In fact, controversy in the use of calcium hydroxide as an antimicrobial agent can be found in the dentistry literature (Siqueira and Uzeda, 1997; Estrela et al., 2001). Hu and coworkers have shown remarkable differences between Gram-negative (more sensitive to calcium ions) and Gram-positive (Hu et al., 2009).

Different functions have been described for calcium ions in Gram-negative bacteria depending on the amplitude and duration of the calcium transient and the physiological state of the organism; indispensable triggers like in chemostaxis, modulators of a specific function and structural elements of the membranes, nucleoids and spores (Norris et al., 1996).

Calcium ions act as a membrane stabilizer for *S. typhi* and *S. aeruginosa*. Calcium ions cannot be produced or destroyed by the bacteria, thus, bacteria should take calcium ions from the extracellular media which acts as the stabilizer, increasing bacteria survival and as a consequence reducing BGs bactericidal effect (Elliott, 2001).

Some Gram-negative species have membranes that can include organic and inorganic ion transporters with homeostatic functions able to capture or expelled specific ions (Jimenez and Cervantes, 2006). *E. coli* have specific transporters (related to nutrients active transport) to capture calcium ions which (Onoda et al., 1992). Osmotic stress produced by the increase of calcium in the media alters this function reducing the viability of *E. coli* (Roth et al., 1985). The presence of those specific channels justifies the differences in sensitivity of *E. coli* with regard to *P. aeruginosa* and *S. typhi*.

Neurofuzzy logic did not point out the influence of any of the microbiological parameters studied meaning that as far as the microbiological conditions (media and time of culture and coculture), are adequate for a particular specie, those variables have no effect on growth index obtained.

5. Conclusions

Artificial intelligence technology allowed a novel and integrated analysis of the results from literature on antibacterial activity of bioactive glasses. Neurofuzzy logic was able to model an extensive database on bioactive glasses, to determine the critical variables for BG antibacterial activity and to present conclusions in an explicit format.

In summary the antibacterial activity is mainly determined by the release of alkaline ions to the medium and an increase of pH.

Important variations in BG antibacterial activity on different species of bacteria are mainly linked to the composition of BG and, in particular, to their content of calcium ions. The differences found in the susceptibility of different bacterial species with respect to this chemical entity, should lead to addressing the study of the antibacterial activity of BGs on a wide selection of bacterial flora to the possible impact on the pathology or the process in which its use will be involved.

Microbiological conditions studied (culture and coculture media and time) do not have a significant impact on the results of the studies of BGs antimicrobial activity as soon as they are suitable for the culture of the species under investigation.

Conflicts of interest

Authors declare no conflicts of interest.

Acknowledgements

Authors thank Mrs. J. Menis for her help in the correction of the English version of the work. This work was supported by the POCTEP 0330IBEROMARE1P project, FEDER. ML (PR2010-0460) thanks the Spanish Ministry of Education for her financial support during their sabbatical year at Faculty of Science, University of Utrecht, The Netherlands.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2013.06.036.

References

Abou Neel, E., Ahmed, I., Pratten, J., Nazhat, S., Knowles, J., 2005. Characterisation of antibacterial copper releasing degradable phosphate glass fibres. Biomaterials 26, 2247–2254.

Allan, I., Newman, H., Wilson, M., 2001. Antibacterial activity of particulate Bioglass® against supra- and subgingival bacteria. Biomaterials 22, 1683–1687.

Balamurugan, A., Balossier, G., Laurent-Maquin, D., Pina, S., Rebelo, A., Faure, J., Ferreira, J., 2008. An in vitro biological and anti-bacterial study on a sol–gel derived silver-incorporated bioglass system. Dent. Mater. 24, 1343–1351.

Bellantone, M., Coleman, N.J., Hench, L.L., 2000. Bacteriostatic action of a novel four-component bioactive glass. J. Biomed. Mater. Res. 51, 484–490.

Bellantone, M., Williams, H.D., Hench, L.L., 2002. Broad-spectrum bactericidal activity of Ag_2O-doped bioactive glass. Antimicrob. Agents Chemother. 46, 1940.

Brauer, D.S., Rüssel, C., Kraft, J., 2007. Solubility of glasses in the system P_2O_5–CaO–MgO–Na_2O–TiO_2: experimental and modeling using artificial neural networks. J. Non-Cryst. Solids 353, 263–270.

Campoccia, D., Montanaro, L., Arciola, C.R., 2006. The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials 27, 2331–2339.

Colbourn, E.A., Rowe, R.C., 2009. Novel approaches to neural and evolutionary computing in pharmaceutical formulation: challenges and new possibilities. Future 1, 713–726.

Day, R.M., Boccaccini, A.R., 2005. Effect of particulate bioactive glasses on human macrophages and monocytes in vitro. J. Biomed. Mater. Res. A 73, 73–79.

Domingues, Z., Cortés, M., Gomes, T., Diniz, H., Freitas, C., Gomes, J., Faria, A., Sinisterra, R., 2004. Bioactive glass as a drug delivery system of tetracycline and tetracycline associated with [beta]-cyclodextrin. Biomaterials 25, 327–333.

Elliott, A., 2001. Recent developments in non-excitable cell calcium entry. Cell Calcium 30, 73–93.

Estrela, C., Bammann, L., Pimenta, F., Pécora, J., 2001. Control of microorganisms in vitro by calcium hydroxide pastes. Int. Endod. J. 34, 341–345.

Froum, S., Cho, S.C., Rosenberg, E., Rohrer, M., Tarnow, D., 2002. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: a pilot study. J. Periodontol. 73, 94–102.

Gallego, P.P., Gago, J., Landín, M., 2011. Artificial Neural Networks Technology to Model and Predict Plant Biology Process. Artificial Neural Networks—Methodological Advances and Biomedical Applications. Intech Open Access Publisher, Croatia, pp. 197–216.

García-Infante, J.C., Medel-Juárez, J.J., Sánchez-García, J.C., 2010. Evolutive neural net fuzzy filtering: basic description. J. Intell. Learn. Syst. Appl. 2, 12–18.

Garcia-Domenech, R., de Julian-Ortiz, J., 1998. Antimicrobial activity characterization in a heterogeneous group of compounds. J. Chem. Inf. Comput. Sci. 38, 445–449.

Gerhardt, L.C., Boccaccini, A.R., 2010. Bioactive glass and glass-ceramic scaffolds for bone tissue engineering. Materials 3, 3867–3910.

Gorriti, M.F., López, J.M.P., Boccaccini, A.R., Audisio, C., Gorustovich, A.A., 2009. In vitro study of the antibacterial activity of bioactive glass-ceramic scaffolds. Adv. Eng. Mater. 11, B67–B70.

Gristina, A.G., 1987. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 237, 1588.

Hench, L.L., 1998a. Bioactive materials: the potential for tissue regeneration. J. Biomed. Mater. Res. 41, 511–518.

Hench, L.L., 1998b. Biomaterials: a forecast for the future. Biomaterials 19, 1419–1423.

Hench, L.L., Paschall, H., 1973. Direct chemical bond of bioactive glass-ceramic materials to bone and muscle. J. Biomed. Mater. Res. 7, 25–42.

Hench, L.L., Splinter, R., Allen, W., Greenlee, T., 1971. Bonding mechanisms at the interface of ceramic prosthetic materials. J. Biomed. Mater. Res. 5, 117–141.

Hu, S., Chang, J., Liu, M., Ning, C., 2009. Study on antibacterial effect of 45S5 Bioglass®. J. Mater. Sci. Mater. Med. 20, 281–286.

Jiménez, R., Cervantes, C., 2006. Determinación de la topología de transportadores bacterianos. Revista de Educación Bioquímica, 3–11.

Kalmodia, S., Molla, A.R., 2010. In vitro cellular adhesion and antimicrobial property of SiO sub (2)-MgO–Al sub (2) O sub (3)-K sub (2) O 013; B sub (2) O sub (3)-F glass ceramic. J. Mater. Sci. Mater. Med. 21, 1297–1309.

Kavli, T., Weyer, E., 1994. IEE Colloquium on Advances in Neural Networks for Control and Systems , pp. 3/1–3/7.

Kellomäki, M., Niiranen, H., Puumanen, K., Ashammakhi, N., Waris, T., Törmälä, P., 2000. Bioabsorbable scaffolds for guided bone regeneration and generation. Biomaterials 21, 2495–2505.

Landín, M., Rowe, R., York, P., 2009. Advantages of neurofuzzy logic against conventional experimental design and statistical analysis in studying and developing direct compression formulations. Eur. J. Pharm. Sci. 38, 325–331.

Leppäranta, O., Vaahtio, M., Peltola, T., Zhang, D., Hupa, L., Hupa, M., Ylänen, H., Salonen, J.I., Viljanen, M.K., Eerola, E., 2008. Antibacterial effect of bioactive glasses on clinically important anaerobic bacteria in vitro. J. Mater. Sci. Mater. Med. 19, 547–551.

McHugh, C.P., Zhang, P., Michalek, S., Eleazer, P.D., 2004. pH required to kill *Enterococcus faecalis* in vitro. J. Endod. 30, 218–219.

Misra, S.K., Ansari, T.I., Valappil, S.P., Mohn, D., Philip, S.E., Stark, W.J., Roy, I., Knowles, J.C., Salih, V., Boccaccini, A.R., 2010. Poly(3-hydroxybutyrate) multifunctional composite scaffolds for tissue engineering applications. Biomaterials 31, 2806–2815.

Mortazavi, V., Nahrkhalaji, M.M., Fathi, M., Mousavi, S., Esfahani, B.N., 2010. Antibacterial effects of sol–gel-derived bioactive glass nanoparticle on aerobic bacteria. J. Biomed. Mater. Res. A 94, 160–168.

Mulligan, A., Wilson, M., Knowles, J., 2003. The effect of increasing copper content in phosphate-based glasses on biofilms of *Streptococcus sanguis*. Biomaterials 24, 1797–1807.

Munukka, E., Leppäranta, O., Korkeamäki, M., Vaahtio, M., Peltola, T., Zhang, D., Hupa, L., Ylänen, H., Salonen, J.I., Viljanen, M.K., 2008. Bactericidal effects of bioactive glasses on clinically important aerobic bacteria. J. Mater. Sci. Mater. Med. 19, 27–32.

Nakajo, K., Komori, R., Ishikawa, S., Ueno, T., Suzuki, Y., Iwami, Y., Takahashi, N., 2006. Resistance to acidic and alkaline environments in the endodontic pathogen *Enterococcus faecalis*. Oral Microbiol. Immunol. 21, 283–288.

Nikaido, H., 2003. Molecular basis of bacterial outer membrane permeability revisited. Microbiol. Mol. Biol. Rev. 67, 593–656.

Nikaido, H., 1993. Transport across the bacterial outer membrane. J. Bioenerg. Biomembr. 25, 581–589.

Norris, V., Grant, S., Freestone, P., Canvin, J., Sheikh, F., Toth, I., Trinei, M., Modha, K., Norman, R.I., 1996. Calcium signalling in bacteria. J. Bacteriol. 178, 3677.

Onoda, T., Oshima, A., Fukunaga, N., Nakatani, A., 1992. Effect of Ca2 and K on the intracellular pH of an *Escherichia coli* L-form. J. Gen. Microbiol. 138, 1265.

Peltola, T., Jokinen, M., Rahiala, H., Levänen, E., Rosenholm, J., Kangasniemi, I., Yli-Urpo, A., 1999. Calcium phosphate formation on porous sol–gel-derived SiO_2 and CaO–P_2O_5–SiO_2 substrates in vitro. J. Biomed. Mater. Res. 44, 12–21.

Pye, A., Lockhart, D., Dawson, M., Murray, C., Smith, A., 2009. A review of dental implants and infection. J. Hosp. Infect. 72, 104–110.

Roth, W.G., Leckie, M.P., Dietzler, D.N., 1985. Osmotic stress drastically inhibits active transport of carbohydrates by *Escherichia coli*. Biochem. Biophys. Res. Commun. 126, 434–441.

Rowe, R., Colbourn, E.A., 2003. Neural computing in product formulation. Chem. Educ. 8, 1–8.

Rowe, R., Landín, M., 2013. Artificial neural networks technology to model, understand and optimize drug formulations. In: Aguilar Diaz, J.E. (Ed.), Formulation Tools for Pharmaceutical Development. Biohealthcare Publishing Ltd., Baldock, pp. 10–40.

Shao, Q., Rowe, R.C., York, P., 2006. Comparison of neurofuzzy logic and neural networks in modelling experimental data of an immediate release tablet formulation. Eur. J. Pharm. Sci. 28, 394–404.

Siqueira Jr., J.F., de Uzeda, M., 1997. Intracanal medicaments: evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. J. Endod. 23, 167–169.

Stoor, P., Söderling, E., Grenman, R., 1999. Interactions between the bioactive glass S53P4 and the atrophic rhinitis-associated microorganism *Klebsiella ozaenae*. J. Biomed. Mater. Res. 48, 869–874.

Stoor, P., Söderling, E., Salonen, J.I., 1998. Antibacterial effects of a bioactive glass paste on oral microorganisms. Acta Odontol. 56, 161–165.

Sun, J.Y., Yang, Y.S., Zhong, J., Greenspan, D.C., 2007. The effect of the ionic products of Bioglass® dissolution on human osteoblasts growth cycle in vitro. J. Tissue Eng. Regen. Med. 1, 281–286.

Vaahtio, M., Munukka, E., Leppäranta, O., Zhang, D., Eerola, E., Ylänen, H.O., Peltola, T., 2006. Effect of ion release on antibacterial activity of melt-derived and sol–gel-derived reactive ceramics. Key Eng. Mater. 309, 349–354.

Verné, E., Ferraris, S., Miola, M., Fucale, G., Maina, G., Martinasso, G., Canuto, R., Di Nunzio, S., Vitale-Brovarone, C., 2008. Synthesis and characterisation of bioactive and antibacterial glass–ceramic. Part 1. Microstructure, properties and biological behaviour. Adv. Appl. Ceram. 107, 234–244.

Verrier, S., Blaker, J.J., Maquet, V., Hench, L.L., Boccaccini, A.R., 2004. PDLLA/Bioglass® composites for soft-tissue and hard-tissue engineering: an in vitro cell biology assessment. Biomaterials 25, 3013–3021.

Vogel, M., Voigt, C., Gross, U.M., Müller-Mai, C.M., 2001. In vivo comparison of bioactive glass particles in rabbits. Biomaterials 22, 357–362.

Waltimo, T., Brunner, T., Vollenweider, M., Stark, W., Zehnder, M., 2007. Antimicrobial effect of nanometric bioactive glass 45S5. J. Dent. Res. 86, 754.

Waltimo, T., Mohn, D., Paqué, F., Brunner, T., Stark, W., Imfeld, T., Schätzle, M., Zehnder, M., 2009. Fine-tuning of bioactive glass for root canal disinfection. J. Dent. Res. 88, 235.

Xie, Z.P., Zhang, C.Q., Yi, C.Q., Qiu, J.J., Wang, J.Q., Zhou, J., 2009. In vivo study effect of particulate Bioglass® in the prevention of infection in open fracture fixation. J. Biomed. Mater. Res. B: Appl. Biomater. 90, 195–201.

Zehnder, M., Waltimo, T., Sener, B., Soderling, E., 2006. Dentin enhances the effectiveness of bioactive glass S53P4 against a strain of *Enterococcus faecalis*. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 101, 530–535.

Zhang, D., Leppäranta, O., Munukka, E., Ylänen, H., Viljanen, M.K., Eerola, E., Hupa, M., Hupa, L., 2010. Antibacterial effects and dissolution behavior of six bioactive glasses. J. Biomed. Mater. Res. A 93, 475–483.