

## RESEARCH ARTICLE

# In vitro evaluation of different organic matrices used to modulate silicon bioavailability

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## Abstract

Silicon (Si) has numerous health properties. It is an element of the extracellular matrix; it is involved in collagen synthesis, bone mineralization, and immune system modulation; and it reduces metal accumulation in Alzheimer's disease and the risk of atherosclerosis. Given its poor intestinal absorption, Si is ingested in the form of orthosilicic acid (OSA) to promote its bioavailability. The aim of this work was to compare different commercial dietary supplements containing stabilized OSA to ascertain their bioaccessibility, bioavailability, and safety in a model of human intestinal epithelium. Biocompatibility with the glycocalyx was also investigated. Supplements containing collagen, maltodextrins, and choline as OSA stabilizers were analyzed. Bioaccessibility was explored by means of an in vitro digestive process. Bioavailability was investigated using a Caco2 cell line alone, or co-culturing with a HT29-MTX cell line. The safety of the compounds tested (in terms of intestinal epithelium integrity) was judged on the grounds of MTS assay, transepithelial electrical resistance, and apparent permeability. The three formulations were also tested in a Caco2 cell model of intestinal glycocalyx Si retention. The choline-formulated OSA formulation outperformed the maltodextrin-stabilized supplement, with a Si bioavailability about 14 times higher ( $P < .05$ ). The choline-formulated OSA formulation increased cell permeability, with consequent intestinal epithelium disruption. The supplements' absorption and bioavailability (and harmfulness) differed considerably, depending on the OSA stabilizer involved. Of the three formulations tested, the collagen-formulated OSA represents the best Si dietary supplement.

## KEYWORDS

choline, collagen, maltodextrins, orthosilicic acid, silicon

## 1 | INTRODUCTION

Silicon (Si) is the second most abundant element in nature after oxygen, and the third most abundant trace element in

the human body.<sup>1</sup> Due to its unique features, it has been extensively used in many production sectors, including the cosmetics, food, and beverage industries. Humans may encounter Si through both environmental exposures and their

**Abbreviations:** HBSS, Hanks' balanced salt solution; ICP-MS, inductively coupled plasma mass spectrometry; LY, Lucifer yellow; OSA, orthosilicic acid; TEER, transepithelial electrical resistance.

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diet. Si deprivation has not been shown to interrupt the life cycle in mammals, or to have any specific biochemical effects, so Si is not generally accepted as an essential nutrient for higher animals and humans. Increasing evidence of the beneficial effects of Si on connective tissue and bone formation in higher animals and humans has nonetheless highlighted its importance to several physiological functions.<sup>2</sup> Si contributes to the structural integrity of the nails, hair, and skin, to overall collagen synthesis, to bone mineralization and to a healthy immune system, while reducing metal accumulation in Alzheimer's disease, and lowering the risk of atherosclerosis.<sup>3-5</sup> The market for a number of Si-based dietary supplements has consequently seen an exponential growth in recent years.

In the same way as for other nutrients, the beneficial effects of Si on the human organism depend on its bioavailability, or availability for use by the body.<sup>5</sup> Bioavailability is strongly influenced by the matrix involved, and a number of modulating factors (such as nutritional status) can also influence this factor in different individuals.<sup>6,7</sup> Si is typically present in food as orthosilicic acid (OSA), a form in which it is readily absorbed through the gastrointestinal tract in humans.<sup>8</sup> Although OSA is the main form of bioavailable inorganic Si, many other formulations have been developed to improve its bioavailability.<sup>9</sup>

In view of the important physiological role of Si in many biochemical pathways, the present study investigated the ability of three food supplements to modulate its interaction with the intestinal glycocalyx and bioavailability. Commercial collagen-, maltodextrin-, and choline-based OSA formulations were analyzed as they are probably the most readily available and commonly used Si-based dietary supplements. The three formulations were compared using an approach that combined an *in vitro* digestive process with two *in vitro* intestinal models.

## 2 | MATERIALS AND METHODS

### 2.1 | Properties of the formulations tested

The role of the matrix in modulating Si bioavailability and interaction with the intestinal glycocalyx was examined for the three commercial OSA-based formulations listed in Table 1.

### 2.2 | Si content in the form of OSA in the different formulations

Si content was measured using an in-house method and analyzed by ICP-MS (inductively coupled plasma mass spectrometry). Briefly, samples were placed in a vial, adding 0.5 mL of tetramethylammonium hydroxide (TMAH) and 0.5 mL of ultrapure water. Then the samples were heated at 70°C for 8 hours and, at the end of the digestion process, they were transferred to a vial and diluted to a final volume of 50 mL with ultrapure water. Three independent replicates per sample were prepared and analyzed by ICP-MS (NexION 300D, Perkin Elmer).

### 2.3 | Bioaccessibility of Si in the form of OSA in different powder formulations

In compliance with EFSA guidelines, Si bioaccessibility in the OSA-based formulations was measured using an *in vitro* model of the human digestive process. For each formulation, 10 mg of Si corresponding to the maximum recommended daily intake of the element in the commercial product G5 was submitted to the *in vitro* digestion procedure (designed to simulate the physiological process in the oral, gastric and intestinal compartments of the human digestive tract). The composition and volumes of simulant digestive fluids are described in Walczak et al.<sup>10</sup> The digestion started by adding saliva (pH = 6.8 ± 0.1) to the formulation and incubating the obtained bolus at 37°C for 5 minutes under constant head-over-heels agitation, to simulate the chewing phase. Subsequently, gastric juice (pH = 1.3 ± 0.1) was added to the bolus and pH checked and, if necessary, adjusted to 2.5 ± 0.5. Resulting chyme was further incubated at 37°C for 2 hours under constant head-over-heel agitation, to simulate gastric peristaltic movements. In the next phase, duodenal juice (pH = 8.1 ± 0.1), bile (pH = 8.2 ± 0.1) and sodium bicarbonate solution were added. The pH of obtained chyle was set at 6.5 ± 0.5 and it was rotated head-over-heels for another 2 hours at 37°C. Digestive fluids volumetric ratio was strictly respected, by adding saliva, gastric juice, duodenal juice, bile, and sodium bicarbonate in the following ratio: 1:2:2:1:0.3. This volumetric ratio, defined and described in Walczak et al,<sup>10</sup> was derived from the seminal works of Versantvoort et al,<sup>11</sup> and Oomen et al,<sup>12</sup> whose *in vitro* digestion models

Si Type	Formulation	Batch	Declared Si concentration (mg/kg)
Collagen-OSA	COLLASIL® OSA	0159.17	16700
Maltodextrin-OSA	SILICIU MAX®	OSP18184	15000
Choline-OSA	BIOSIL®	17C13	9101

**TABLE 1** List and composition of analyzed formulations

were based on the human digestion physiology as described by Guyton & Hall.<sup>13</sup>

At the end of the digestive process, Si concentrations in the complete digests were measured and compared with the amounts submitted for processing to estimate the overall recovery rate. After a centrifugation step at 2750× *g* for 5 minutes, Si was measured in the pellet and the supernatant (the latter corresponding to the bioaccessible fraction). Si concentrations were measured with the ICP-MS (NexIon 300D, Perkin Elmer).

## 2.4 | Intestinal bioavailability of Si

In the same way as for bioaccessibility, and again in compliance with EFSA guidelines, the absorption and bioavailability of the bioaccessible fractions of Si were measured using an in vitro model of the intestinal epithelium based on human adenocarcinoma-derived intestinal cells Caco-2 (ATCC, HTB-37), cultured as functional monolayers in Transwell inserts. Standard cell culture practices were performed as previously described.<sup>14</sup> Caco-2 cells (passage 28 to 40) were seeded in 12-well transwell culture insert (microporous membrane material: polyethylene terephthalate (PET), 1 μm pore size) at  $1.5 \times 10^5$  cells/well and cultured in Caco-2 cell culture complete medium (DMEM complemented with 4 mM glutamine, 100 μ/mL penicillin, 100 μ/mL streptomycin, 1% non-essential amino acids (NEEA), 10% heat inactivated foetal bovine serum (FBS)). To allow for Caco-2 differentiation in mature enterocytes, seeded Caco-2 were maintained for 21 days in complete medium, with medium refresh every other day. In these conditions, cells reach confluence in approximately 3 days and differentiate completely in 21 days.

Transwell inserts have two compartments, one apical (or luminal), and one basolateral (or serosal), separated by a microporous membrane. On the microporous membrane, Caco-2 monolayers are characterized by polarized cells with morphological and functional features typical of enterocytes, such as microvilli, tight junctions, and P-glycoprotein. Based on dose-response curve information and posology, the digested formulations were added to the apical side of the in vitro intestinal epithelium, while HBSS (Hanks' Balanced Salt Solution) buffer was placed in the basolateral compartment. After 1 and 3 hours of incubation, the apical and basolateral solutions were collected, and their Si content was established by ICP-MS. Si bioavailability, derived from three independent experiments, was expressed as: amount of Si absorbed (μg); percentage of absorption (%); and apparent permeability ( $P_{app}$ ) (cm/min). The proportion of Si recovered from the initial dose of digested Si was calculated for each formulation, considering: (i) the amount of Si digested; (ii) the percentage of Si present in the digestive fluids after

the in vitro digestion (bioaccessibility); and (iii) the fraction of bioaccessible Si absorbed at intestinal epithelium level (bioavailability).

## 2.5 | Impact of digested formulations on intestinal epithelium viability

To test their impact on the viability of the intestinal epithelium models based on Caco-2 and Caco-2/HT29-MTX, the digested formulations were serially diluted in digestive fluids and added to the apical side of the in vitro intestinal epithelia. HBSS buffer was placed in the basolateral compartment. Digestive fluids (without the formulations) were used as a negative control. After 3 hours of incubation, the viability of the intestinal epithelia was measured using MTS assay. This method is based on the reduction of MTS tetrazolium compound by the viable cells to generate a colored formazan product that can be quantified by measuring the absorbance at 490 nm. Cell viability is thus directly proportional to absorbance. For intestinal glycocalyx Si retention experiments, collagen-formulated OSA, choline-formulated OSA, and maltodextrine-formulated OSA formulations were also tested on a more complex intestinal epithelium in vitro model, comprising co-cultured Caco-2 cells and mucus-secreting, goblet-like HT29-MTX cells (ECACC, 12040401; passage 54 to 64). Bioavailability and intestinal glycocalyx Si retention experiments were performed using non-toxic concentrations as determined from dose-response curves.

## 2.6 | Impact of OSA-based powder formulations on intestinal epithelium integrity

After 1 and 3 hours of exposure to the digested formulations, cell viability and barrier integrity were assessed in the two intestinal epithelium models. Cell viability was examined using MTS assay, as described above. Barrier integrity was quantified by measuring transepithelial electrical resistance (TEER) and apparent permeability ( $P_{app}$ ). TEER is a rapid, noninvasive method for quantifying barrier tissue integrity by measuring the electrical resistance across the tissue. To obtain a comprehensive trend, the TEER of the intestinal epithelium was measured before treatment, 1 hour after treatment, and again after 24 hours (recovery). Apparent permeability ( $P_{app}$ ) was quantified by measuring the paracellular permeability (ie, passage through the tight junction) of a polar, fluorescent Lucifer Yellow (LY) probe. The apparent permeability coefficient ( $P_{app}$ , cm/s) was calculated as  $P_{app} = (\Delta C V)/(\Delta t A C_0)$ , where:  $\Delta C/\Delta t$  is the flow of the molecule being transported across the monolayer during the

incubation time (mM/s);  $V$  is the volume of the basolateral compartment ( $\text{cm}^3$ );  $A$  is the area of the membrane ( $\text{cm}^2$ ); and  $C_0$  is the initial concentration of the molecule in the apical compartment.

## 2.7 | Intestinal glycocalyx retention of Si released from the digested tested formulations

Based on dose-response curve information and posology, the digested formulations were added to the apical side of Caco-2/HT29-MTX intestinal epithelium model, while HBSS buffer was placed in the basolateral compartment. After 3 hours of incubation, intestinal glycocalyx was re-suspended as proposed by Jin and colleagues.<sup>15</sup> Briefly, the monolayers were washed twice with HBSS, then incubated with N-acetyl-L-cysteine (a substance known to loosen mucus) for 60 minutes at 37°C, under constant agitation. The re-suspended glycocalyx was collected and the Si content was determined by ICP-MS.

## 2.8 | Statistical analysis

All statistical analyses were run using Microsoft Excel and GraphPad version 5 (GraphPad Software, San Diego, CA, USA). Data were tested for normal distribution using the Kolmogorov-Smirnov test. The results were analyzed using Student's two-tailed  $t$  test for comparison between two groups or one-way ANOVA for comparison involving three or more groups. Experiments were run in triplicate, and the results are presented as mean  $\pm$  standard error of the mean (SEM). A  $P$  value of  $< .05$  was considered significant.

## 3 | RESULTS

### 3.1 | Comparison between declared and measured Si concentrations of the formulations tested

Si content was measured in the different OSA-based formulations (Figure 1), and compared with the nominal Si concentration (Table 1). The percentage of recovery was calculated as the ratio between the measured and nominal Si concentrations. As shown in Table 2, the declared and measured concentrations were compatible.

### 3.2 | SI release from the formulations tested during in vitro digestion

Bioaccessibility refers to the amount of active principle released from its matrix into the gastrointestinal tract and available for absorption. Table 3 shows the results of our experiment to measure the total amount of Si released from the matrix and available for absorption, or bioaccessible fraction.

### 3.3 | Effect of digested formulations on intestinal epithelium viability

The impact of the digested formulations on intestinal epithelium viability was assessed before measuring the Si bioavailability of the OSA-based formulations. The intestinal monolayers (both Caco-2- and Caco-2/HT29-MTX-based models) were exposed to increasing concentrations of the three formulations for 3 hours, dose-response curves were

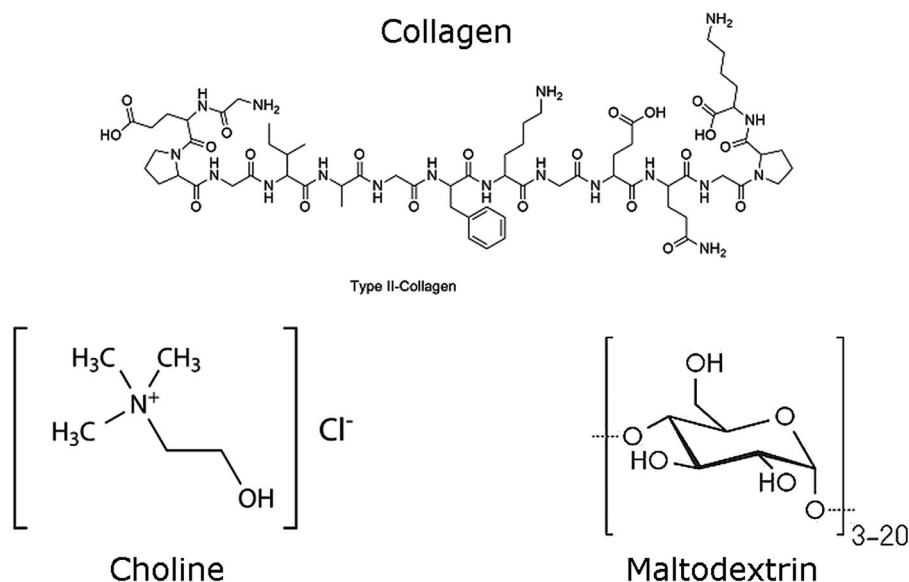


FIGURE 1 Chemical structure of three commercial OSA-based formulations analyzed

**TABLE 2** Si concentration in OSA-based formulations, expressed in mg/kg. Measured values were calculated as average  $\pm$  standard deviation of three replicates

	Declared concentration (mg/kg)	% Si on declared concentration	Measured concentration (mg/kg)	% Si on measured concentration	Recovery (%)
Collagen-OSA	16 700	1.67	16 468 $\pm$ 429	1.65 $\pm$ 0.43	99 $\pm$ 3
Maltodextrin-OSA	15 000	1.50	17 644 $\pm$ 270	1.70 $\pm$ 0.30	117 $\pm$ 2
Choline-OSA	9101	0.91	10 464 $\pm$ 62	1.05 $\pm$ 0.06	115 $\pm$ 1

**TABLE 3** Bioaccessible fraction of Si released from OSA-based formulations during in vitro digestion, expressed as percentage of measured Si at the end of the digestive process

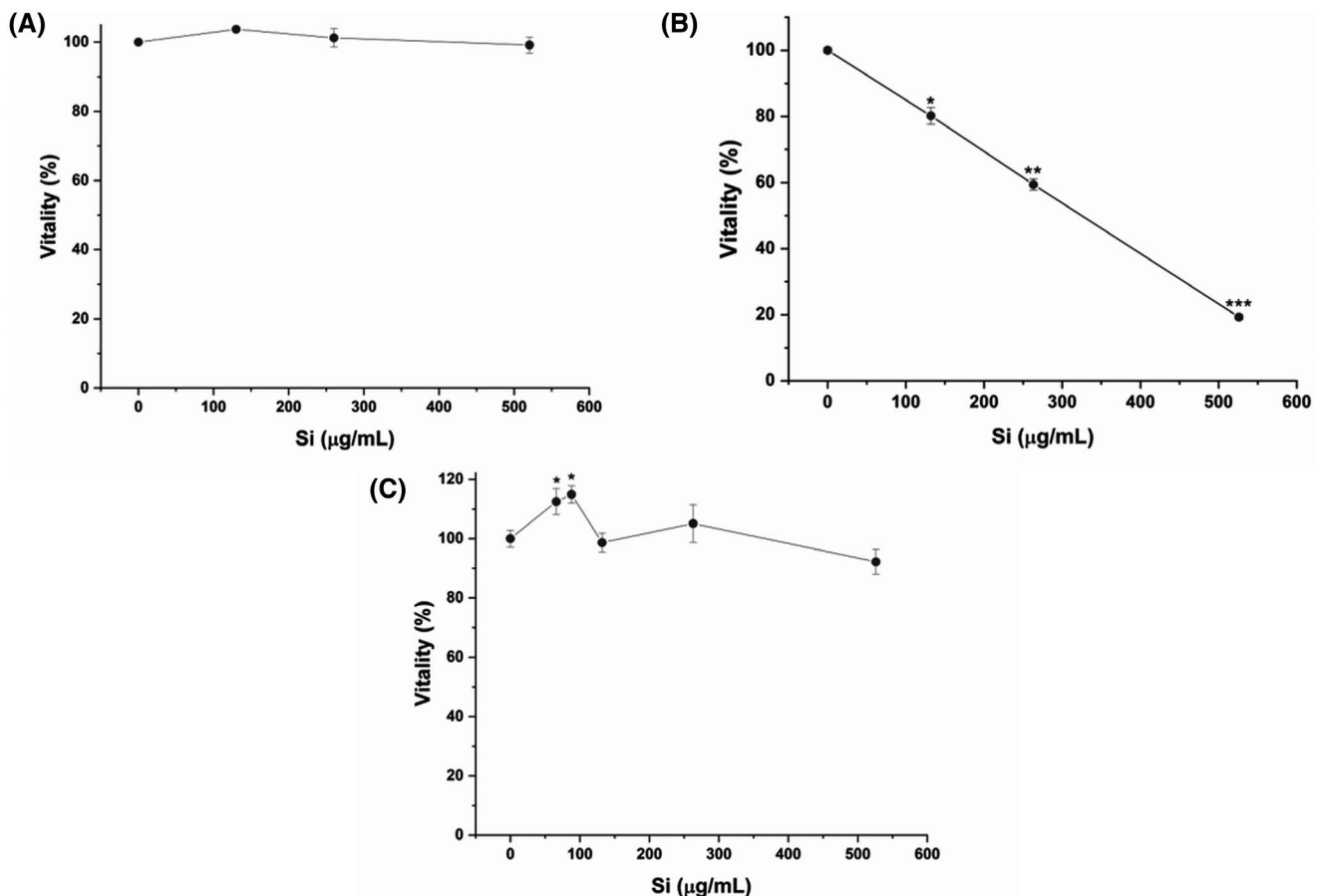
Formulation	Bioaccessibility (%)
Collagen-OSA	25.0
Maltodextrin-OSA	36.6
Choline-OSA	11.0

obtained (Figures 2 and 3). The digested collagen-formulated OSA had no effect on cell viability in either model (Figures 2A and 3A); the choline-formulated OSA reduced the cell

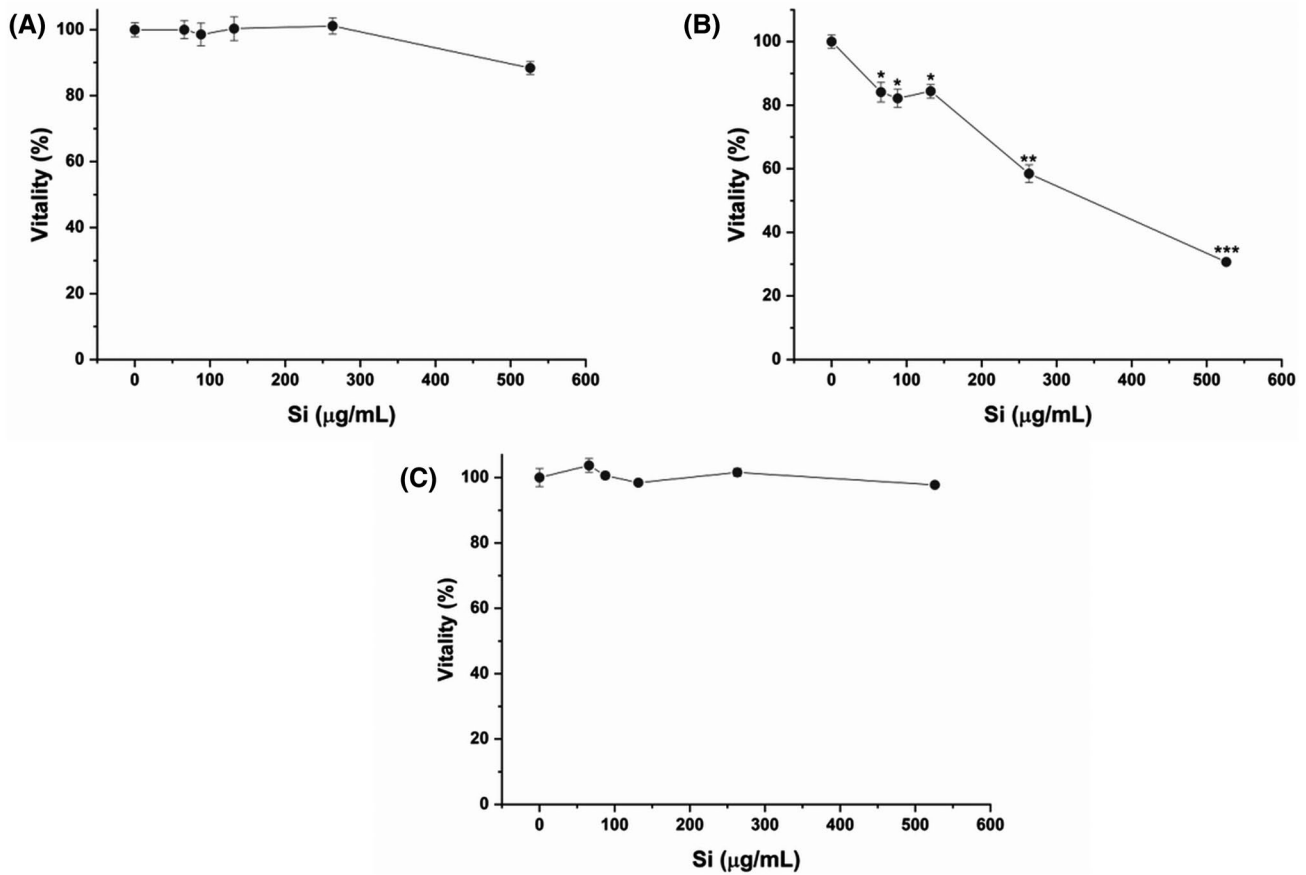
viability in both models at Si concentrations  $>132 \mu\text{g/mL}$  (Figures 2B and 3B); and the maltodextrin-formulated OSA had no effect on cell viability in Caco-2- and Caco-2/HT29-MTX-based cell models (Figures 2C and 3C).

### 3.4 | Intestinal absorption of Si following digestion of the formulations tested

Based on the impact on intestinal epithelium viability of the digested formulations, experiments were run to ascertain Si bioavailability. After exposure of the in vitro intestinal epithelia to the digested formulations for 1 and 3 hours, Si content was measured in both the apical (luminal) and the basolateral



**FIGURE 2** Impact of collagen-formulated OSA A, choline-formulated OSA B, and maltodextrin-formulated OSA C, on Caco-2-based intestinal mucosa viability evaluated by MTS assay. \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$



**FIGURE 3** Impact of collagen-formulated OSA A, choline-formulated OSA B, and maltodextrin-formulated OSA C, on Caco-2/HT29-MTX-based intestinal mucosa viability evaluated by MTS assay. \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$

**TABLE 4** OSA-based formulations Si bioavailability after 1 hours exposure expressed as amount of Si ( $\mu\text{g} \pm$  Standard Deviation (SD)), absorption (%), and apparent permeability ( $P_{\text{app}}$ ; cm/min)

Formulation	Si ( $\mu\text{g} \pm$ SD)	Absorption (%)	$P_{\text{app}}$ (cm/min)
Collagen-OSA	$79.7 \pm 0.1$	83	0.014
Maltodextrin-OSA	$11.9 \pm 3.7$	6	$1.6\text{E-}05$
Choline-OSA	$79.9 \pm 11.9$	98.4	0.025

(serosal) compartments. Bioavailability (the amount of Si in the basolateral/serosal compartment) was then calculated and expressed in terms of amount of Si, percentage of absorption, and apparent permeability. Tables 4 and 5 show the amount of Si absorbed, and the bioavailability of the OSA-based powder formulations after 1 and 3 hours of incubation, respectively. The results concerning the bioaccessibility and bioavailability of the formulations are given in Table 6. The percentage of Si recovery was calculated for each matrix with respect to the initial digested Si dose, taking both bioaccessibility and bioavailability into consideration (Table 6). The mean recovery was calculated as the product of the bioavailable and bioaccessible fractions vis-à-vis the initial dose.

**TABLE 5** OSA-based formulations Si bioavailability after 3 hours exposure expressed as amount of Si ( $\mu\text{g} \pm$  Standard Deviation (SD)), absorption (%), and apparent permeability ( $P_{\text{app}}$ ; cm/min)

Formulation	Si ( $\mu\text{g} \pm$ SD)	Absorption (%)	$P_{\text{app}}$ (cm/min)
Collagen-OSA	$70.5 \pm 0.3$	73.4	0.009
Maltodextrin-OSA	$8 \pm 0.8$	5.7	$1.1\text{E-}05$
Choline-OSA	$31.5 \pm 3.0$	73.4	0.107

### 3.5 | Effect of OSA-based powder formulations on intestinal epithelium integrity

When Caco-2 and Caco-2/HT29-MTX monolayer viability and barrier integrity were analyzed after exposure to the digested formulations, only the choline-formulated OSA formulation significantly lowered the viability in both cell models, and altered their barrier integrity. No significant changes were seen for the other two OSA-based formulations in either the Caco-2-based model (Figures 4A and 5A) or the Caco-2/HT29-MTX-based model (Figure 6A).

The increase in apparent permeability caused by exposure to choline-formulated OSA was paralleled by a reduction in

**TABLE 6** Summary table of Si bioaccessibility and bioavailability of OSA-based formulations

	Bioaccessibility (%)	Bioavailability (%)		Recovery (%)
		1 hours	3 hours	
Collagen-OSA	25.0	83.0	73.4	19.6
Maltodextrin-OSA	35.6	6.3	5.7	2.1
Choline-OSA	11.0	98.4*	73.4*	9.4

Considering bioaccessibility and bioavailability obtained percentages, an overall Si recovery was calculated.

\*These values are imputable to the strong adverse effect of choline-formulated OSA on intestinal epithelium measured by TEER and LY.

the TEER of the intestinal epithelia in both models (Figures 4B, 5B, 6B, 7). The OSA-based digested formulations and digestive fluids only reduced the TEER temporarily, however their values fully recovered within 24 hours, except in the case of choline-formulated OSA.

### 3.6 | Intestinal glycocalyx retention of Si released from the formulations tested after their digestion

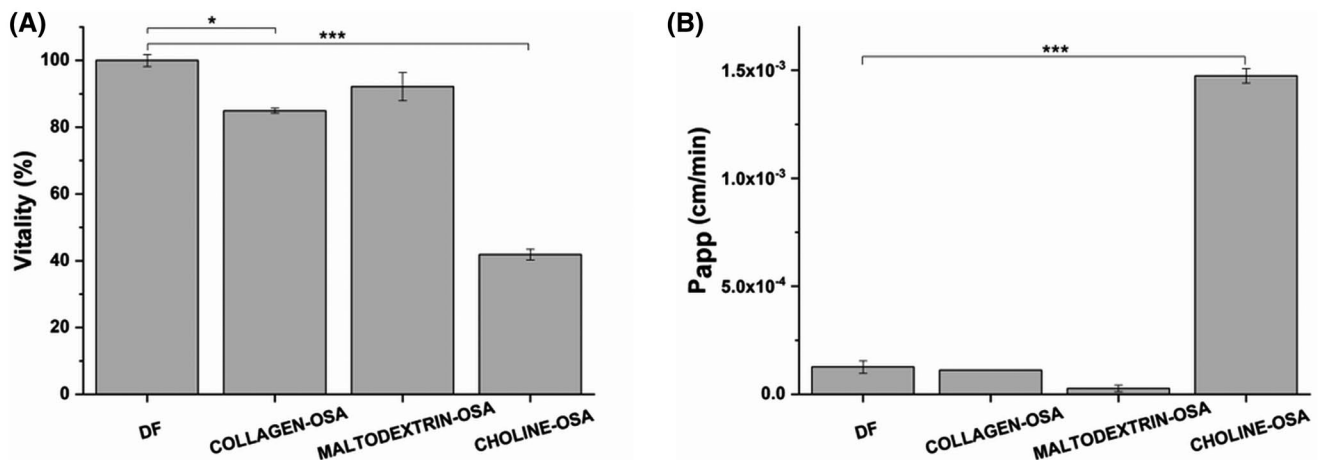
Based on the impact of the digested formulations on intestinal epithelium viability, experiments were run to measure Si retention at intestinal glycocalyx level after exposure of the in vitro intestinal epithelia to diluted digested formulations for 3 hours. As shown in Figures 8 and Table 7, the collagen-formulated OSA showed the highest Si retention by the intestinal glycocalyx or excreted mucus (32%, as opposed to approximately 6% for the choline-formulated OSA, and 2% for the maltodextrin-formulated OSA).

## 4 | DISCUSSION

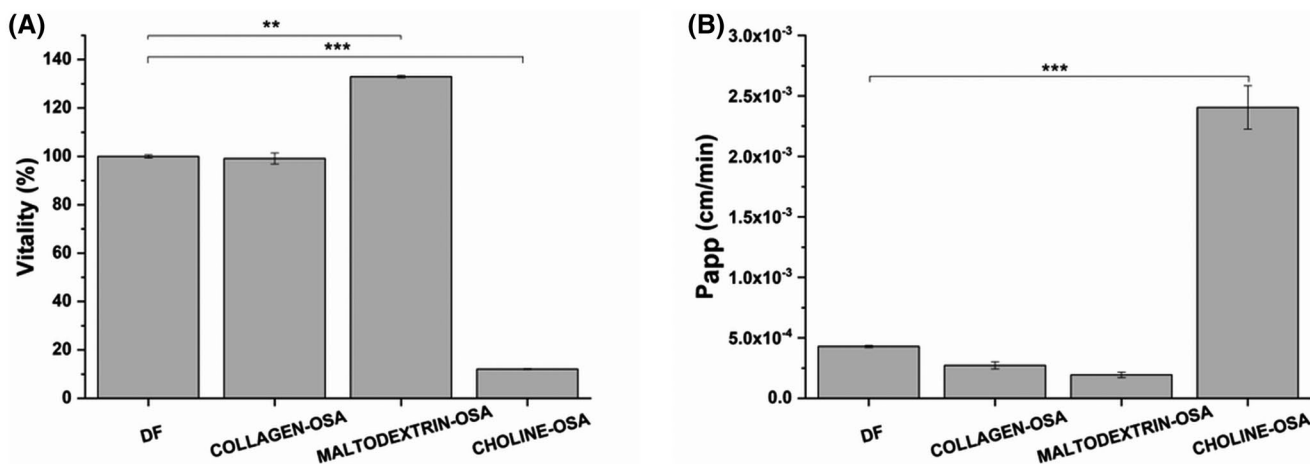
It has been suggested that Si has a beneficial impact on human health and many Si-based food supplements have

consequently been produced and marketed. Its effect is related to its absorption at intestinal level, however, and many forms of organic and inorganic, water-soluble, and potentially bioavailable Si have been proposed.<sup>3,16</sup> OSA is virtually the only form in which Si is carried in the natural environment and it is considered the main form of bioavailable inorganic Si. The present work investigated the role of the matrix used in different commercial OSA formulations in modulating Si bioaccessibility, bioavailability, and interaction with the intestinal glycocalyx, focusing on hydrolyzed collagen (gelatin), maltodextrin, and choline matrices. We used Caco-2 and Caco-2/HT29-MTX-based models to resemble as strictly as possible human intestinal epithelium. While Caco-2 is the most common and world-wide used reference cell line for studying intestinal barrier, Caco-2/HT29-MTX co-culture can mimic more faithfully the physiological conditions of the intestine due to extracellular matrix production by HT29-MTX cells.<sup>17</sup> Indeed, Caco-2/HT29-MTX model produces mucus which lines the top of the glycocalyx and this fact can impact on permeability and retention time.

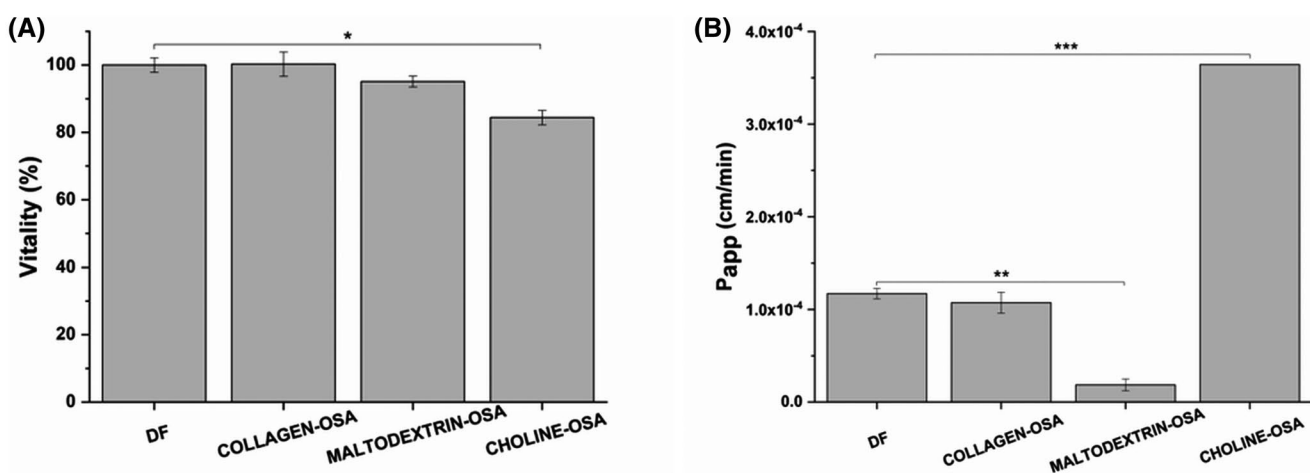
Our results indicate that maltodextrin-formulated OSA provides the most bioaccessible form of Si of the formulations tested: 36% of the Si was released from this matrix during the in vitro digestive process, as compared with 25% for the collagen matrix, and 11% for the choline matrix. Moreover, intestinal absorption was better for the



**FIGURE 4** Cell vitality A, and apparent permeability (Papp) B, of Caco-2-based in vitro intestinal epithelium exposed to digestive fluids (DF; control) and digested formulations for 1 hours. \* $P < .05$ , \*\*\* $P < .001$



**FIGURE 5** Cell vitality A, and apparent permeability (Papp) B, of Caco-2-based in vitro intestinal epithelium exposed to digestive fluids (DF; control) and digested formulations for 3 hours. \*\* $P < .01$ , \*\*\* $P < .001$



**FIGURE 6** Cell vitality A, and apparent permeability (Papp) B, of Caco-2/HT29-MTX-based in vitro intestinal epithelium exposed to digestive fluids (DF; control) and digested formulations for 3 hours. \* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$

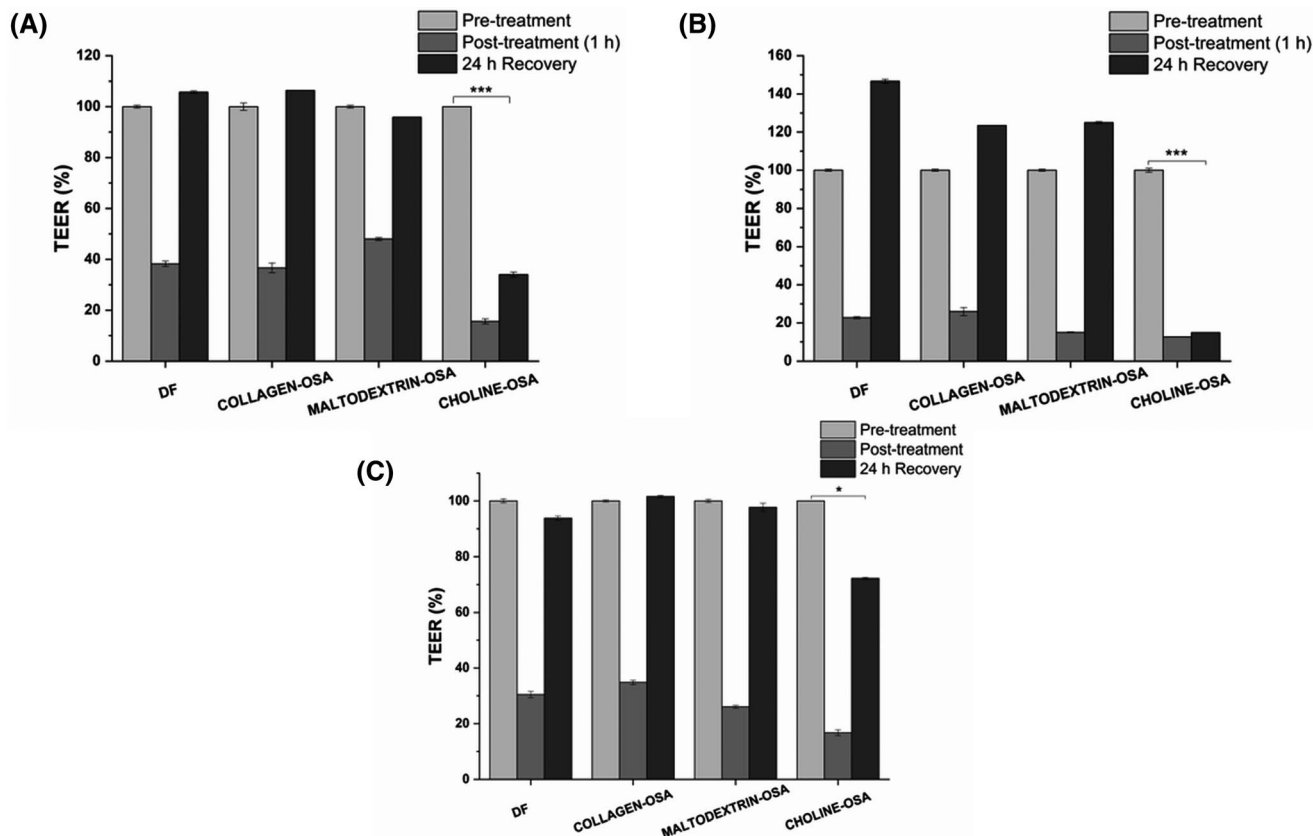
choline-formulated OSA than for the other two formulations. This could be due to the effect of this formulation on intestinal epithelium integrity. In fact, choline-formulated OSA induced an irreversible increase in cell permeability (as measured by TEER and LY), leading to a greater Si bioavailability. It should be noted, however, that the bioavailability of the collagen-formulated OSA was similar to that of the choline-formulated OSA without any side effects on the intestinal epithelium, and the Si bioavailability achieved with the collagen matrix was 14 times that of the maltodextrin-based SA formulation.

Our novel findings raise some concern regarding the chronic use of choline-formulated OSA supplements in humans. None of the published clinical trials on such choline-based products<sup>17-20</sup> examined their effects on Si bioavailability or recovery at intestinal level. They generally reported finding no related adverse events, but no specific biomarkers of intestinal integrity were analyzed. This point warrants careful consideration in future clinical trials.

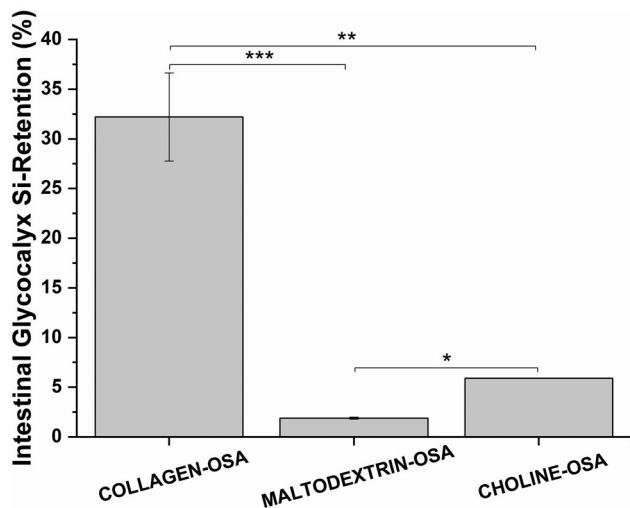
Taking the Si bioaccessibility and bioavailability data obtained in this in vitro study together, the percentage recovery of Si from the choline-based formulation is compatible with the findings of Sripanyakorn et al, confirming our model's reliability.<sup>7</sup> The good bioavailability of collagen-formulated OSA may be thanks to a high intestinal extracellular retention, which could effectively increase the amount of time the intestinal epithelium is exposed to Si. A collagen-based matrix thus improves Si bioavailability, supporting its retention by the intestinal glycocalyx and/or excreted mucus in Caco2/HT29-MTX model, prompting us to surmise that collagen is a good matrix for the purposes of Si delivery. Moreover, our Si bioavailability data for the choline-formulated OSA formulation may be misleading as a result of a disruption in the intestinal absorption model.

No information is available on Si recovery in animal models or higher organisms for the three OSA formulations considered here, so it is impossible to draw any comparisons with our in vitro data. The different behavior of the





**FIGURE 7** Trans-epithelial electrical resistance (TEER) trend of Caco-2-based and Caco-2/HT29-MTX in vitro intestinal epitheliums exposed to digestive fluids (DF; control) and digested formulations for 1 and 3 hours. \* $P < .05$ , \*\*\* $P < .0001$



**FIGURE 8** Intestinal glycocalyx Si-retention of OSA-based tested formulations. \*\* $P < .01$ , \*\*\* $P < .001$

OSA formulations in our in vitro experiments could have to do with the protein matrices involved: unlike the other two formulations, the OSA stabilized with collagen includes a hydrolyzed fish collagen peptide matrix, which probably facilitates Si bioaccessibility and bioavailability at intestinal level, favoring intestinal extracellular retention.

**TABLE 7** Intestinal glycocalyx Si-retention of OSA-based tested formulations expressed as percentage of Si

Formulation	Intestinal glycocalyx Si retention (% $\pm$ SD)
Collagen-OSA	32.2 $\pm$ 4.4
Maltodextrin-OSA	1.9 $\pm$ 0.1
Choline-OSA	5.9 $\pm$ 0.1

## 5 | CONCLUSIONS

Of the three formulations tested, collagen-formulated OSA revealed the highest bioavailability and intestinal extracellular retention, without affecting cell viability and intestinal barrier integrity. This is the first study to elucidate the mode of action of OSA-based formulations on the intestinal epithelium, suggesting that the best way to supplement Si in the diet is to supply it in a hydrolyzed collagen matrix.

## CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

## AUTHOR CONTRIBUTIONS

E. Tedesco, F. Benetti contributed to designing the study and completed the experimental procedures. E. Tedesco, F. Benetti, and R. Pezzani contributed to the study design, data interpretation, supervision, and drafting of the manuscript.

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