Dental glass ionomer cements (GIC) are unique restorative materials: they are self-adhesive to enamel and dentin, they are able to release fluoride and they are moisture tolerant1,2. They can be used for routine restorations (in non-stress bearing areas such as cervical caries, or in primary teeth), provisional restorations (pending final restoration, for caries control, or for endodontic access preparations), as well as base material3).

In order to improve their properties, it was proposed to incorporate resin components, usually 2-hydroxyethyl methacrylate (HEMA). These resin-modified glass ionomer cements (RMGIC) show greater mechanical strength, higher bond strength to enamel and dentin, decreased early moisture sensitivity and extended working time4-6). Their setting involves two reactions: an acid-base reaction between polyalkenoic acid and fluoro-alumino-silicate glass particles and a polymerization of HEMA monomers. Previous studies showed that these reactions may rely on and/or compete one another7,8): the addition of HEMA9) and/or photopolymerization10) significantly reduces the ionic reaction rate. Thus, time of light-activation after mixing determines the ratio between both reactions.

It was also reported that delaying light-activation increases the release of free HEMA11), suggesting a lower conversion rate, a lower crosslinking and a poorer biocompatibility. Thus, it seems pertinent to minimize this delay. However, a significant increase of conversion rate was reported with the extension of this delay, reaching its maximum value at 41.9 % for 10 min delay, before decreasing for more extended delays11). The inflection point would correspond to the best compromise between acid-base and polymerization reactions.

But what about the microstructure and mechanical properties of RMGIC with delayed light-activation? Analysis of differential scanning calorimetry data suggested that varying light-cure initiation time could result in different structured materials8). Few studies focused on the delayed light-activation of RMGIC and its effects on diametral tensile strength4), water uptake13), wear rates14), fluoride release14), and bracket enamel bond strength15). No studies have studied both structural and mechanical impact of delayed light-activation. Scanning electron microscopy (SEM) examination and mechanical tests could characterize the resulting materials as a function of light-cure initiation time.

The aim of this study was first to examine, by SEM, the microstructure of an RMGIC after various delays of light-activation; secondly to test flexural strength and microhardness of this RMGIC after various delays in short (24 h) and long (12 months) term.

**MATERIALS AND METHODS**

**Sample preparation and experimental conditions**

The RMGIC selected for this study was the Fuji II™ LC (GC, Tokyo, Japan) supplied in capsules. The manufacturer product specifications including batch
number and composition, sample set designation and polymerization conditions are summarized in Table 1. The experimental design consisted in 5 sample sets spanning various light-activation delays i.e., light-curing 1 min after mixing (P1), 5 min after mixing (P5), 10 min after mixing (P10), 15 min after mixing (P15) and no light-curing (i.e., infinite delay) (NP).

A sample set was defined as 24 replicate bars, which were prepared in (25×2×2) mm silicone molds. Each capsule was mixed 10 s in a mechanical mixer and injected into the mold. The mold was covered with a Mylar strip; a glass plate was placed atop the Mylar strip to help apply even pressure on the sample and express excess material, resulting in a homogeneous flat surface. The bars were then light-cured with a series of three 20 s irradiations using a LED unit (Radii, SDI, Victoria, Australia) set to a light output power of 1,200 mW/cm² as measured with a commercial radiometer (Dentsply Caulk, Milford, DE, USA). Irradiation was applied in the middle and at each extremity of each given sample. All the samples were kept in the dark before the light-activation and then prepared under dimmed conditions to reduce background reaction due to ambient light. The samples were removed from their molds 15 min after mixing, then immersed in deionized water.

Microstructural characterization
Among the 22 replicates bars of each group (n=110), 20 (n=100, 20×5) were polished with abrasive discs of decreasing grit size (400, 800, 1200, 2400, 4000 SiC) on a water-irrigated grinding wheel. Ten of them (n=50, 10×5) were stored during 24 h at 37°C; the 10 others (n=50, 10×5) were stored during 12 months.

1. Flexural strength
All the samples (n=100) were measured with a digital calliper (Mitutoyo, Kawasaki, Japan) before being tested. Flexural strength was tested on 10 samples of each group (n=50, 10×5) after 24 h, and the 10 others of each group (n=50, 10×5) after 12 months. Flexural strength was determined by loading the samples in a three point bending device on a universal testing machine, computer controlled (Lloyd LRX, Lloyd, Fareham, UK), with a 20 mm span between the supports, at a crosshead speed of 1 mm.min⁻¹. It was calculated using the load at fracture, on the software NexyGen® (Lloyd).

2. Microhardness
The samples tested in flexural strength were then used to determine the surface microhardness, i.e. 10 samples for each group (n=50, 10×5) at 24 h, and 10 others for each group (n=50, 10×5) after 12 months. Their surfaces were coated with a thin gold layer (10 nm), in a sputter-coater (SC500, Bio-Rad), in order to improve reading. Surface microhardness was measured using a Vickers indenter (MH3, Metkon, Bursa, Turkey), under a 200-N load and a 20-s dwell time. Thirty indents were performed on the 10 samples of each group (3 indents per bar). The microhardness of each group was taken from the average microhardness of these 30 indents.

Statistical analysis
For flexural strength and microhardness, the means and standard deviations of the results were calculated. Then, data were analyzed by two-way ANOVA for the factors “light-activation delay” and “ageing period”, followed by Tukey post-hoc test for comparisons between the groups. Statistical significance for all tests was set at p<0.05. Statistical calculations were performed using XLSTAT software.
RESULTS

Microstructural characterization: SEM examination
Figure 1 shows the microstructure of Fuji II LC after various delays of light-activation when etched with HF acid at ×5,000 magnification. The acid dissolves the glass phase derived from acid-base reaction, which reveals crystalline particles and exposes the acid-resistant polymeric network derived from polymerization reaction. The polymeric network appears more dense and continuous for P5 (Fig. 1b). In fact, it is less dense for P1 (Fig. 1a) and when the delay increases for P10 (Fig. 1c), then P15 (Fig. 1d), the polymeric network becomes less and less dense as well as discontinuous: it is aggregated in some area. Finally for NP (Fig. 1e), it seems to be almost totally absent and the particles appear smaller.

Mechanical characterization: flexural strength and microhardness tests
Table 2 shows the flexural strength of Fuji II LC light-cured at various time delays, respectively after 24 h and 12 months of storage. Two-way ANOVA showed that flexural strength was significantly influenced by the delay of light-activation and time of storage. At 24 h, flexural strength increased significantly when the delay increased, until a maximum value for P5 (64.4 MPa), then decreased for more extended delay, with a minimum value for NP (14.8 MPa). At 12 months, it was significantly higher for P1 (28.2 MPa) than for all other groups with longer delays from P5 (23.9 MPa) to NP (10.5 MPa). Moreover, values after 12 months storage were lower than those after 24 h, whatever the delay.

Table 3 shows the microhardness of Fuji II LC surface, light-cured at various time delays, respectively after 24 h and 12 months of storage. The two-way ANOVA showed that microhardness was significantly influenced by the delay of light-activation and time of storage. At 24 h, microhardness increased significantly when the delay increased, until a maximum value for P10 (72.1 HV), then decreased for more extended delays, with a minimum value for NP (42.7 HV). At 12 months, it was not significantly different for P1, P5 and P10 (between
Table 3  Means and standard deviations of the microhardness of Fuji II LC light-cured after various time delays, at 24 h and 12 months

| Time delay before light-curing | Vickers microhardness at 24 h (HV) | Vickers microhardness at 12 months (HV) |
|-------------------------------|------------------------------------|---------------------------------------|
| P1                            | 58.4±7.5a                          | 60.5±8.3A                             |
| P5                            | 65.1±9.0b                          | 62.4±9.8A                             |
| P10                           | 72.1±8.2c                          | 60.3±9.7A                             |
| P15                           | 59.3±8.2a                          | 48.5±7.5b                             |
| NP                            | 42.7±13.9d                         | 25.1±10.8c                            |

Values with the same superscript letter are not significantly different at \( p<0.05 \)

60.3 and 62.4 HV), but was significantly reduced for P15 (48.5 HV) and NP (25.1 HV). Besides, values for P1 and P5 were not significantly different at 24 h and 12 months storage, but they were reduced for delays longer than P10.

**DISCUSSION**

This study provided information about the setting of RMGIC and the repercussions on its microstructure, and its global and surface properties, i.e. flexural strength and microhardness.

*Microstructural properties according to the delay of light-activation*

SEM examination of Fuji II LC after various delays of light-activation clearly showed different images of the material according to the delay. The polymer network revealed by HF etching seemed to be the most homogeneous and continuous for P5. It was looser for P1, increasingly sparse for P10 and P15 and almost entirely absent for NP. In fact, for increased delays (from P10) and in an extreme manner for NP, acid-base reaction is favored and gradually forms the polysalt matrix; and when the light-curing is later initiated, the formed polysalt matrix impedes the mobility of HEMA monomers and their subsequent polymerization. Thus, HEMA monomers polymerize in some areas, resulting in polymer clusters. These results corroborate those of Kakaboura *et al.* who reported that light-curing 20 min after mixing decreases the degree of conversion (DC) of C=C double bonds from 60 to 30 % for Fuji II LC and from 45 to 35 % for Photac Fil, but on the contrary, does not affect that of Vitremer, which contains also a chemical initiator system. This is also consistent with the results of Dursun *et al.* showing a decrease of DC after 10 min delay. In fact, they determined by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrophotometry the DC of the same RMGIC after delays of light-activation of 2, 5, 10, 15 min and without light-activation. They reported DCs of 18.5, 33.7, 41.9, 33.1 and 17.1%, respectively. They explained the initial increase by a phase separation, with HEMA groupings. These groupings could promote the polymerization at the initiation of light-curing. However, when the delay is too extended (after a 10 min delay), the formation of the acid-base polysalt is obtained, that would constrain mobility of the HEMA monomers and hinder the formation of polyHEMA, causing a decrease of DC. (The low but no null DC of their NP group was explained by background polymerization due to the light beam of the spectrometer, during the spectrum acquisition.) The competition between molecules involved in the acid-base reaction and free HEMA mobility for polymerization can thus be shown structurally.

*Short- and long-term mechanical properties according to the delay of light-activation*

Flexural strength and microhardness were significantly improved when light-cured initiation time was respectively delayed from 1 to 5 min (39.5 to 64.4 MPa) and from 1 to 10 min (58.4 to 72.1 HV). However, when the delay was more extended (above respectively 5 and 10 min), flexural strength and microhardness decreased. These results corroborate those obtained by SEM examination. In fact, the formation of acid-base polysalt impedes the mobility of HEMA monomers, which hinders the formation of polyHEMA, decreasing mechanical properties.

Furthermore, when light-cured initiation time is delayed, the RMGIC is more produced by the acid-base reaction and should exhibit physical properties closer to those of GICs, which also explains the decline of mechanical properties. On the contrary, when light-cured initiation time occurs early, the acid-base reaction is reduced and the resulting material should exhibit physical properties closer to those of composite resins, with higher mechanical properties. However, the highest mechanical properties were not reached for the shortest delay. The maximum mechanical properties were reached: at P5 for flexural strength and P10 for microhardness. These inflection points would correspond to the best compromise of acid-base/polymerization reaction ratio. A little delay would allow the acid-base reaction to be favored and to compensate for the fact that it is a slower reaction than polymerization. These results corroborate those of other mechanical tests, reported by two other studies. Yelamanchili and Darvell
also reported a critical value of optimal light-curing in terms of duration and initiation time, with decreased compressive strength with prolonged exposure and increased delay. Li et al.\textsuperscript{20} found a significant decrease of diametral tensile strength from 10 min delay for the Photoc Fil, but no reduction for Vitremer.

Besides, the maximum value reached with a shorter delay for flexural strength (P5) compared than microhardness (P10) would suggest that acid-base reaction (i.e. the formation of the polysalt matrix) would more contribute to increase the microhardness than polymerization reaction; and conversely, polymerization reaction (i.e. the formation of polyHEMA) would more contribute to increase the flexural strength. Thus, the competition between calcium and aluminium ions involved in the acid-base reaction and free HEMA for polymerization was structurally highlighted.

It is well known that RMGIC strengthens with time, because of its maturation\textsuperscript{17}, however no studies have performed mechanical tests after long-term water storage. Yet it is precisely another interesting point. After 12 months of storage, the shortest delay always showed the best mechanical properties. Flexural strength was reduced: for P1, the decrease may be explained by the separation of polymer chains by water molecules and the softening of the organic matrix due to hydrolysis, which have been described for resin-based materials\textsuperscript{18}).

For extended delays (P5, P10 and P15), flexural strength was even more decreased probably because of the poorer formation of the polyHEMA, favoring the hydrolysis phenomenon. For NP, the numerous unpolymerized monomers could also cause water absorption or be released, implying a loss of cohesion of the material. Microhardness was less affected by water storage. There were no differences at 24 h and 12 months of storage for P1, P5 and P10. However, microhardness decreased for higher delays from P15. This can be explained by the fact that microhardness reflects the surface properties, while flexural strength reflects the global strength of the material\textsuperscript{19}). Surface properties were affected by water storage only when the polymerization reaction was very limited, from P15.

Clinical implications
The delay of light-activation should be adapted to the clinical situation. It would be pertinent to wait a few minutes before light-activation for transitional restoration. In fact, for a short-term indication, mechanical properties would be improved. On the contrary, an early light-activation could be preferred for routine restoration, ensuring the best mechanical properties in long term. Moreover, it could also limit the HEMA release and could be more biocompatible\textsuperscript{21}).

Besides, when RMGIC is placed in block for deep caries (depth>2–3 mm), the light source does not reach the bottom of the restoration\textsuperscript{20,21}). This behavior can be modelled by the NP sample set. The bottom of the restoration would therefore develop inferior mechanical properties to those expected. This corroborates the results of Robert et al.\textsuperscript{20} reporting reduced Knoop hardness of RMGIC placed in bulk, as well as those of Kim et al.\textsuperscript{23} showing by SR-FTIR spectroscopy in conjunction with the Kramers-Kronig transformation that the depth of cure increased over time by the continuous acid-base reaction rather than photopolymerization. Thus, an incremental placement could be recommended and ideally a first layer with a GIC to provide improved biocompatibility close to the pulp, then a second with an RMGIC to improve mechanical strength. Decreasing the thickness of the latter would have the added benefit of minimizing free HEMA release.

CONCLUSION
Microstructure SEM examination and mechanical tests of an RMGIC after various delays of light-activation highlighted the competition between the acid-base and polymerization reactions, resulting in the formation of a structurally and mechanically different material for each delay, according to the extent of each of those reactions. It can be concluded that a 5-to-10 min delay of light-activation give the best mechanical properties in short-term. However, a delay of light-activation do not improved these properties in long term.

Within the limitations of this in vitro study, it can be suggested that the delay of light-activation should be adapted to the clinical situation. As well, an incremental placement could be recommended in case of deep restoration.

REFERENCES
1) Hotz P, McLean W, Seed I, Wilson AD. The bonding of glass-ionomer cements to metal and tooth substrates. Br Dent J 1977; 142: 41-47.
2) Forsten L. Fluoride release from a glass ionomer cement. Scand J Dent Res 1977; 85: 503-504.
3) Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. J Funct Biomater 2016; 7: E16.
4) de Gee AJ, Leloup G, Werner A, Vreven J, Davidson CL. Structural integrity of resin-modified glass ionomers as affected by the delay or omission of light activation. J Dent Res 1998; 77: 1658-1663.
5) Rusz JE, Antonucci JM, Eichmiller F, Anderson MH. Adhesive properties of modified glass-ionomer cements. Dent Mater 1992; 8: 31-36.
6) Mathis RS, Ferracane JL. Properties of a glass-ionomer/resin-composite hybrid material. Dent Mater 1989; 5: 355-358.
7) Yelamanchili A, Darvell BW. Network competition in a resin-modified glass-ionomer cement. Dent Mater 2008; 24: 1065-1069.
8) Berzinis DW, Abey S, Costache MC, Wilkie CA, Roberts HW. Resin-modified glass-ionomer setting reaction competition. J Dent Res 2010; 89: 82-86.
9) Eliades G, Palaghias G. In vitro characterization of visible light-cured glass ionomer cement. Dent Mater 1993; 9: 198-203.
10) Kakaboura A, Eliades G, Palaghias G. An FTIR study on the setting mechanism of resin-modified glass ionomer restoratives. Dent Mater 1996; 12: 173-178.
11) Dursun E, Nguyen JP, Tang ML, Attal JP, Sadoun M. HEMA release and degree of conversion from a resin-modified glass ionomer cement after various delays of light activation. Dent Mater 2016; 32: 640-645.
12) Li J, von Beetzen M, Sundström F. Strength and setting behavior of resin-modified glass ionomer cements. Acta Odontol Scand 1995; 53: 311-317.
13) Jevnikar P, Jarh O, Sepe A, Pintar MM, Funduk N. Micro magnetic resonance imaging of water uptake by glass ionomer cements. Dent Mater 1997; 13: 20-23.
14) Yoda A, Nikaïdo T, Ikeda M, Sonoda H, Foxton RM, Tagami J. Effect of curing method and storage condition on fluoride ion release from a fluoride-releasing resin cement. Dent Mater J 2006; 25: 261-266.
15) Ando F, Komori A, Kojima I. Tensile bond strength of light-cured resin-reinforced glass ionomer cement with delayed light exposure. Odontology 2001; 89: 45-48.
16) Hamid A, Okamoto A, Iwaku M, Hume W. Component release from light-activated glass ionomer and compomer cements. J Oral Rehabil 1998; 25: 94-99.
17) Zoergiebel J, Ilie N. An in vitro study on the maturation of conventional glass ionomer cements and their interface to dentin. Acta Biomater 2013; 9: 9529-9537.
18) Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater 2006; 22: 211-222.
19) Heintze SD, Zimmerli B. Relevance of in vitro tests of adhesive and composite dental materials, a review in 3 parts. Part 1: approval requirements and standardized testing of composite materials according to ISO specifications. Schweiz Monatsschr Zahnhed 2011; 121: 804-816.
20) Burke FM, Hamlin PD, Lynch EJ. Depth of cure of light-cured glass-ionomer cements. Quintessence Int 1996; 21: 977-981.
21) Young AM. FTIR investigation of polymerisation and polyacid neutralisation kinetics in resin-modified glass-ionomer dental cements. Biomaterials 2002; 23: 3289-3295.
22) Roberts HW, Berzins DW, Charlton DG. Hardness of three resin-modified glass-ionomer restorative materials as a function of depth and time. J Esthet Restor Dent 2009; 21: 262-272.
23) Kim YK, Kim KH, Kwon TY. Setting reaction of dental resin-modified glass ionomer restoratives as a function of curing depth and postirradiation time. J Spectrosc 2015; 462687.