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Review

# Laminin coatings on implant surfaces promote osseointegration: Fact or fiction?

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ABSTRACT

To our knowledge from indexed literature, the role of laminins in the expression of osteogenic biomarkers and osseointegration enhancement has not been systematically reviewed. The aim of the present systematic review was to assess the role of laminin coatings on implant surfaces in promoting osseointegration. To address the focused question, "Do laminin coatings on implant surfaces influence osseointegration?", indexed databases were searched from 1965 up to and including November 2015 using various combination of the following keywords: "Bone to implant contact"; "implant"; "laminins"; and "osseointegration". Letters to the Editor, case-reports/case-series, historic reviews, and commentaries were excluded. The pattern of the present systematic review was customized to primarily summarize the pertinent data. Nine studies were included. Six studies were prospective and were performed in animals and 5 studies were *in vitro*. Results from 8 studies showed that laminin coatings enhanced new bone formation around implants and/or bone-to-implant contact. One study showed that laminin coated implants surfaces did not improve osseointegration. On experimental grounds, laminin coatings seem to enhance osteogenic biomarkers expression and/or osseointegration; however, from a clinical perspective, further randomized control trials are needed to assess the role of laminin coatings in promoting osseointegration around dental implants.



**Contents**



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## 1. Introduction

Osseointegration plays an essential role in the long-term success and survival of implants. A variety of therapeutic protocols have been proposed in an attempt to enhance bone formation around implant surfaces. These include the use of growth factors (such as the platelet derived growth factor, basic fibroblast growth factor, insulin-like growth factor-I and bone morphogenetic protein 2) and placement of osteogenic coatings on implant surfaces (Alghamdi et al., 2013; Chang et al., 2012; de Jonge et al., 2010; Javed, Vohra, Zafar, & Almas, 2014; Javed et al., 2015, 2016; Lan, Wang, Wang, Wang, & Cheng, 2006; Nagayasu-Tanaka et al., 2016; Yoo et al., 2014). It has also been reported that modifications in topography and the surface chemistry enhances cell attachment, proliferation and expression of osteogenic genes and angiogenic factors, compared to turned pure titanium surfaces (Wang et al., 2015; Xuereb, Camilleri, & Attard, 2015; Yeo, 2014). To date, only a limited number of studies (Bougas, Stenport, Currie, & Wennerberg, 2011; Bougas, Jimbo et al., 2012; Bougas, Stenport, et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Min et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) have investigated the role of laminins coatings on implant surfaces on osseointegration and new bone formation (NBF) around implants.

Laminins are glycoproteins and major structural components in the basal lamina of most cells and organs tissues, including brain, skeletal muscle, and peripheral nerves. (Rohde, Wick, & Timpl, 1979; Timpl et al., 1979) Laminins present a heterotrimeric structure with 3 chains ($\alpha$, $\beta$ and $\gamma$), forming a cross-like structure. Laminin $\alpha$2 chains present a large globular (LG) domain-like module capable to bind cell transmembrane molecules, including integrins, syndecans and dystroglycans. (Timpl et al., 2000) This binding property confers to laminins biological activities, including cell adhesion, differentiation and migration, angiogenesis and tumor metastasis (Colognato and Yurchenco, 2000; Suzuki, Yokoyama, & Nomizu, 2005). Twelve different heterotrimers have been identified and numbered in the order discovered. (laminin 1 to laminin 12). (Aumailley et al., 2005; Burgeson et al., 1994)

The effect of different laminin heterotrimers and isoforms on osseointegration has been reported (Kang et al., 2013; Yeo et al., 2015). Results from *in vitro* studies have shown that laminin-1 stimulates osteoblastic alkaline phosphatase (ALP) production (Vukicevic, Luyten, Kleinman, & Reddi, 1990) and osteoprogenitor cells proliferation through an integrin $\beta$1-dependent cell attachment effect (Roche, Goldberg, Delmas, & Malaval, 1999). *In vitro* studies (Bougas et al., 2011; Bougas, Stenport et al., 2012) have shown that laminin-1 increases the precipitation of calcium phosphate (CaP). Likewise, results from other *in vivo* studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014) have also reported that laminin-1 coatings improve osseointegration around implants. Laminin-2 derived peptides have been studied as novel therapeutic agents due to their smaller molecular weight and lower antigenicity. Laminin-2-P3 and Laminin-2-LG3 have been reported to enhance bone cell function *in vitro* (Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) and to induce faster osseointegration around titanium implants *in vivo*. (Kang et al., 2013; Yeo et al., 2015) Moreover, *in vitro* studies have shown that Laminin-5 enhances epithelial cell attachment and spreading, and hemidesmosome assembly around titanium discs (El-Ghannam, Starr, & Jones, 1998; Tamura et al., 1997; Werner et al., 2009). It is therefore hypothesized that laminin coatings play a role in enhancing osseointegration. However, controversial results have been also reported regarding laminins effect on implant osseointegration. Schwartz-Filho et al. (2012) reported significantly higher levels of osteoblastic and osteoclastic markers, but no significant difference in bone apposition around implants coated with laminin-1 compared to control.

From the currently available evidence, there seems to be a relationship between laminin coatings and osseointegration of implants. However, to our knowledge from indexed literature, the role of laminins in the expression of osteogenic biomarkers and osseointegration enhancement has not been systematically reviewed. Therefore, the aim of the present systematic review was to assess the role of laminin coatings on implant surfaces in promoting osseointegration.

## 2. Methods

### 2.1. Focused question

The addressed focused question was "Do laminin coatings on implant surfaces influence osseointegration?"

### 2.2. Eligibility criteria

The eligibility criteria were as follows: (a) clinical studies, (b) experimental studies (*in-vivo* and *in-vitro*), (c) inclusion of a control group (osteogenic biomarkers expression and/or osseointegration around non-coated implants); and (d) intervention: effect of laminin coating on osseointegration around implants. Letters to the Editor, historic reviews, commentaries, case-series and case-reports were excluded.

### 2.3. Literature search protocol

PubMed/Medline (National Library of Medicine, Washington, DC), EMBASE, Scopus, Web of knowledge and Google-Scholar databases were searched from 1965 up to and including February 2016 using various combination of the following keywords: (a) laminins + osseointegration; (b) laminins + implants; (c) laminins + implants + osseointegration; (d) bone to implant contact + laminins; (e) bone to implant contact + laminins + osseointegration. Search titles and abstracts were initially screened by one author (SVK) to exclude articles that were clearly outside the scope of the review. The remaining titles and abstracts of studies identified using the above-described protocol were screened by two authors (FJ and SVK) and checked for agreement. Full-texts of studies judged by title and abstract to be relevant were read and independently evaluated for the stated eligibility criteria. Reference lists of potentially relevant original and review articles were hand-searched to identify any studies that could have remained unidentified in the previous step. Once again, the articles were checked for disagreement via discussion among the authors. The initial search yielded 176 studies. One hundred and sixty seven studies that did not abide by the eligibility criteria were excluded. In total, 9 articles (Bougas et al., 2011; Bougas, Jimbo et al., 2012; Bougas, Stenport et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Min et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) were included and processed for data extraction (Fig. 1).

### 2.4. Quality assessment

Quality Assessment of studies was performed using the Critical Appraisal Skills Program (CASP) Cohort Study Checklist (Zeng et al., 2015). This was done to grade the methodological quality of each study included in the present systematic review. This tool is based on 12 criteria that are as follows: 1) Study issue is clearly focused; 2) Cohort is recruited in an acceptable way; 3) Exposure (laminin administration) is accurately measured; 4) Outcome (osseointegration and/or new bone formation [NBF] around implants, expression of osteogenic biomarkers) is accurately measured. 5) Confounding factors are addressed; 6) Follow-up is long and complete; 7) Results are clear; 8) Results are precise; 9) Results are credible; 10) Results can be applied to the local population; 11) Results fit with available evidence; and 12) There are important clinical implications. According to the CASP scale, each criterion is given a response of either "Yes", "No", or "cannot tell" and the maximum score a study could have was 12.

## 3. Results

### 3.1. General characteristics of the studies included

Amongst the 9 studies (Bougas et al., 2011; Bougas, Jimbo et al., 2012; Bougas, Stenport et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Min et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) included, 6 studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) were prospective and were performed in animals and 5 studies (Bougas et al., 2011; Bougas, Stenport et al., 2012; Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) were *in vitro*.

- *In vitro* studies

In two studies (Bougas et al., 2011; Bougas, Stenport et al., 2012) the effect of laminin-1 coating on CaP precipitation was assessed in 3 different bioactive titanium surfaces in simulated body fluid. Min et al. (2013) and Kang et al. (2013) evaluated laminin-2-P3 (DLTIDDSYWYRI motif) effectiveness, with concentrations ranged between 21 and 23 $\mu g/cm^2$, in cell attachment around titanium discs using human osteosarcoma (HOS) osteoblast-like cells and osteoblast-like MG63 cells respectively. Yeo et al. (2015) performed cell adhesion, spreading and migration assays around titanium discs, using laminin-2-LG3 (PPFEGCIWN motif, 14.3 $\mu g/cm^2$) and human placental laminin (1.4 $\mu g/cm^2$) in HOS osteoblast-like cells and osteoblast-like MG63 cells. In all the *in vitro* studies (Bougas et al., 2011; Bougas, Stenport et al., 2012; Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) the follow up period ranged between 1 h and 14 days.

- *In vivo* studies

Four studies (Bougas et al., 2013; Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) were performed in male rabbits, and one study (Bougas et al., 2014) used male and female rabbits. Bougas, Jimbo et al. (2012) used male rats. Four studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Schwartz-Filho et al., 2012) evaluated the effectiveness of laminin-1 coating (250 µl solution. 26 Å of protein thickness) to improve osseointegration around implants. Kang et al. (2013) and Yeo et al. (2015) assessed NBF around implants coated with Laminin-2-P3 (1 $mg/cm^2$) and Laminin-2-LG3 (1 $mg/cm^2$) respectively. In all *in vivo* studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) the follow up period ranged between 3 days and 28 days (Table 1).

### 3.2. Implant-related characteristics of the studies included

- *In vitro* studies

In all studies (Bougas et al., 2011; Bougas, Stenport et al., 2012; Kang et al., 2013; Min et al., 2013; Yeo et al., 2015), titanium discs were used. Three studies (Bougas et al., 2011; Bougas, Stenport et al., 2012; Min et al., 2013), reported the total numbers of the

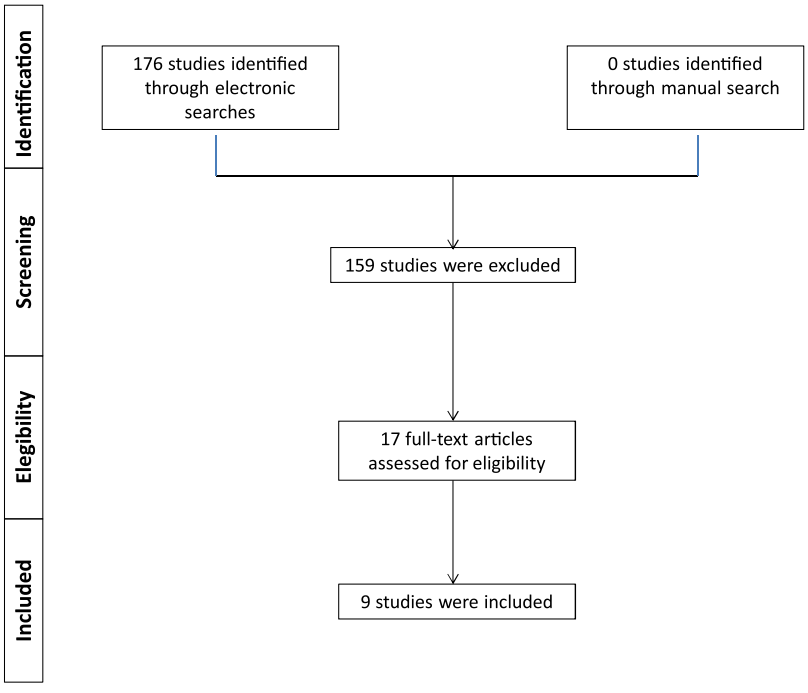


**Fig. 1.** Article selection flow chart for the systematic review according to PRISMA guidelines.

**Table 1**
Characteristics of the studies included.

| Authors et al. | Study design | Study Subjects | Study groups | Dose | Follow-up | Analysis Methods |
|---|---|---|---|---|---|---|
| Bougas et al. (2011) | *In vitro* | NA | Group 1: B + Lam-1<br>Group 2: Uncoated Ti + B<br>Group 3: B + AH + Lam-1<br>Group 4: B + AO + Lam-1<br>Group 5: B + HA + Lam-1 | 300 ng/ml | 1 h, 3 days and 7 days | Iodine labeling<br>SEM/EDX |
| Bougas et al. (2012t) | *In vitro* | NA | Group 1: B + Lam-1<br>Group 2: Uncoated Ti + B<br>Group 3: B + AH + Lam-1<br>Group 4: B + AO + Lam-1<br>Group 5: B + HA + Lam-1 | Lam-1<br>250 μl solution<br>Protein thickness: 2.6 nm | 14 days | SEM/EDX |
| Min et al. (2013) | *In vitro* | Human osteosarcoma osteoblast-like cells | Group 1: AO + Scrambled peptide<br>Group 2: AO + Lam-2-P3<br>Group 3: AO<br>Group 4: Ti + Scrambled peptide<br>Group 5: Ti + Lam-2-P3<br>Group 6: Uncoated Ti | Lam-2-P3 (DLTIDDSYWYRI motif)<br>Groups 2 and 5: 23 μg/cm$^2$ | 1 h, 1 day and 7 days | SEM<br>ALP assay<br>Real time RT-PCR |
| Kang et al. (2013) | *In vitro* and in vivo prospective | ***In vitro:*** Osteoblast-like MG63 cells<br>***In vivo:***<br>9 Male rabbits | ***In vitro***<br>Group 1: Uncoated Ti<br>Group 2: Ti + Scrambled peptide<br>Group 3: Ti + Lam-2-P3<br>Group 4: AO<br>Group 5: AO + Scrambled peptide<br>Group 6: AO + Lam-2-P3<br>***In vivo:***<br>Group 1: Uncoated Ti<br>Group 2: Scrambled peptide<br>Group 3: Lam-2-P3 | ***In vitro:***<br>Lam-2-P3 (DLTIDDSYWYRI motif)<br>Groups 3 and 6: 21 μg/cm$^2$<br>***In vivo:***<br>Lam-2-P3 (DLTIDDSYWYRI motif)<br>Group 3: 1 mg/cm$^2$ | ***In vitro:***<br>1 h, 1 day and 7 days<br>***In vivo:***<br>7, 14 and 28 days | ***In vitro:***<br>SEM<br>Real time RT-PCR<br>ALP assay<br>***In vivo:***<br>HIST<br>ALP<br>Histochemistry |
| Yeo et al. (2015) | *In vitro* and *In vivo* prospective | ***In vitro:***<br>Human osteosarcoma osteoblast-like cells and Osteoblast-like MG63 cells<br>***In vivo:***<br>48 Male rabbits | ***In vitro:***<br>Group 1: Uncoated Ti<br>Group 2: Scrambled peptide<br>Group 3: Lam-2-LG3-P2<br>Group 4: Lam-2-LG3-P2-DN3<br>Group 5: Placental Lam<br>***In vivo:***<br>Group 1: Uncoated Ti<br>Group 2: Scrambled peptide<br>Group 3: Lam-2-LG3-P2<br>Group 4: Lam-2-LG3-P2-DN3 | ***In vitro:***<br>Lam-2 PPFEGCIWN motif<br>Groups 3 and 4: 14.3 μg/cm$^2$<br>Placental Lam<br>Group 5: 1.4 μg/cm2<br>***In vivo:***<br>Lam-2 PPFEGCIWN motif<br>Groups 3 and 4: 1 mg/cm$^2$ | ***In vitro:***<br>1 h, 3 h and 3 days<br>***In vivo:***<br>7, 14 and 28 days | ***In vitro:***<br>SEM<br>ALP assay<br>Real time RT-PCR<br>***In vivo:***<br>Histology<br>ALP<br>Histochemistry |
| Bougas, Jimbo et al. (2012) | In vivo prospective | 45 Male rats | Control: Uncoated Ti<br>Test: Lam-1 coated implants<br>Group 1:<br>15 Test + control, | Lam-1<br>250 μl solution<br>Protein thickness: 26 Å | Group 1: 3 days<br>Group 2: 7 days | Real time RT-PCR<br>HIST |

**Table 1** (*Continued*)

| Authors et al. | Study design | Study Subjects | Study groups | Dose | Follow-up | Analysis Methods |
|---|---|---|---|---|---|---|
| | | | 3 days<br>Group 2:<br>15 Test + control,<br>7 days<br>Group 3:<br>15 Test + control,<br>21 days | | Group 3:<br>21 days | |
| Bougas et al. (2013) | In vivo prospective | 14 Male rabbits | Control: HA implants<br>Test: Lam-1 coated HA implants<br>Group 1:<br>7 Test + control,<br>14 days<br>Group 2:<br>7 Test + control,<br>28 days | Lam-1<br>250 μl solution<br>Protein thickness: 26 Å | Group 1:<br>14 days<br>Group 2:<br>28 days | MicroCT<br>Histology |
| Bougas et al. (2014) | In vivo prospective | 22 Female and male rabbits | Group 1: Control (T)<br>Group 2: Control (AH)<br>Group 3: T + Lam-1<br>Group 4: AH + Lam-1 | Lam-1<br>250 μl solution<br>Protein thickness: 26 Å | 14 and 28 days | Removal Torque<br>HIST<br>Nanoindentation |
| Schwartz-Filho et al. (2012) | In vivo prospective | 9 Male rabbits | Group 1: Uncoated Ti<br>Group 2: Lam-1 coated | Lam-1<br>250 μl solution<br>Protein thickness: 26 Å | 14 days | Real time RT-PCR<br>SEM |

LAM: Laminin; RT-PCR: Reverse-Transcription Polymerase Chain Reaction; HIST: Histomorphometry; SEM: Scanning electron microscopy; EDX: Energy dispersive Xray; RTQ: Removal torque; AH: alkali and heat treatment.
AO: Anodic oxidation; HA: Hydroxyapatite; B: Blasted; T: Turned.

titanium discs, which ranged between 24 and 90 discs. In 2 studies (Kang et al., 2013; Yeo et al., 2015), the total number of discs studied was not reported.

In all studies (Bougas et al., 2011; Bougas, Jimbo et al., 2012; Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) dimensions (diameter × length in millimeters) of discs used ranged between 8 × 1 and 50 × 0.5 millimeters. In 2 studies (Bougas et al., 2011; Bougas, Stenport et al., 2012), roughed surfaced discs were used and in 1 study (Yeo et al., 2015), the implants had smooth surfaces. In the studies by Kang et al. (2013) and Min et al. (2013) rough and smooth discs surfaced were used.

- *In vivo* studies

In five studies (Bougas, Jimbo et al., 2012; Bougas et al., 2014; Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015), and in one study (Bougas et al., 2013) titanium and Hydroxyapatite implants respectively, were placed in the tibiae. The total number of implants placed ranged between 18 and 90 implants. In all studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) dimensions (diameter × length in millimeters) of implants used ranged between 1.5 × 2 and 4.2 × 9 millimeters. Cylindrical and screw-

**Table 2**
Characteristics of the implants included in all the studies.

| Authors et al. | Number of implants | Implant dimensions (DxL in mm) | Location of implant placement | Implant Shape | Implant Surface Characteristics (Median roughness) |
|---|---|---|---|---|---|
| Bougas et al. (2011) | 75 Ti discs | 8 × 1 | NA | Discs | Rough |
| Bougas, Jimbo et al. (2012) | 90 Ti discs | 8 × 1 | NA | Discs | Rough |
| Min et al. (2013) | 24 Ti discs | 20 × 0.5 | NA | Discs | Smooth and Rough |
| Kang et al. (2013) | ***In vitro:*** | ***In vitro:*** | ***In vitro:*** | ***In vitro:*** | ***In vitro:*** |
| | Ti discs (NA) | 20 × 0.5 | NA | Discs | Smooth and rough |
| | ***In vivo:*** | 50 × 0.5 | ***In vivo:*** | ***In vivo:*** | ***In vivo:*** |
| | 27 Ti implants | ***In vivo:*** 3.5 × 8 | Tibia | Screw | Smooth |
| Yeo et al. (2015) | *In vitro*: | ***In vitro:*** | ***In vitro:*** | ***In vitro:*** | ***In vitro:*** |
| | Ti discs | 20 × 0.5 | NA | Discs | Smooth |
| | In vivo: | 50 × 0.5 | ***In vivo:*** | ***In vivo:*** | ***In vivo:*** |
| | 48 Ti implants | ***In vivo:*** 3.5 × 8 | Tibia | Screw | Smooth |
| Bougas, Jimbo et al. (2012) | 90 Ti implants | 1.5 × 2.5 | Tibia | NA | Smooth (0.28 μm) |
| Bougas et al. (2013) | 28Hydroxyapatite implants | 4.2 × 9 | Tibia | Cylindrical | Smooth (0.08 μm) |
| Bougas et al. (2014) | 88 Ti implants | 3.5 × 7 | Tibia | NA | Smooth |
| Schwartz-Filho et al. (2012) | 18 Ti implants | 1.5 × 2 | Tibia | NA | Rough |

type implants were placed in 1 study (Bougas et al., 2013) and 2 studies (Kang et al., 2013; Yeo et al., 2015), respectively. In 3 studies (Bougas, Jimbo et al., 2012; Bougas et al., 2014; Schwartz-Filho et al., 2012) the shape of the implants used was not reported. In 5 studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Yeo et al., 2015), smooth surfaced implants were used and in 1 study (Schwartz-Filho et al., 2012), rough surfaced implants were used (Table 2).

### 3.3. Assessment of osteoblast differentiation and osseointegration

- *In vitro* studies

In 3 studies (Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) scanning electronic microscopy (SEM) was used to examine the spreading of human osteogenic cells cultured in titanium surfaces. In two studies (Bougas et al., 2011; Bougas, Stenport et al., 2012) SEM/energy dispersive X-ray analisis (EDX), was used to assess the total amount of CaP of titanium discs. In three studies (Kang et al.,

**Table 3**
Results of the studies included.

| Authors et al. | Implant Surface characterization | Gene expression | Histomorphometry |
|---|---|---|---|
| Bougas et al. (2011) | Group 3 presented a significantly higher Sds compared to the rest of the groups. Group 2 presented the lower Sds. | NA | Group 3 presented higher Ca and P contents, compared to the rest of the groups after 72 h. Group 2 showed lower levels of Ca and P compared to the rest of the groups. |
| Bougas et al. (2012t) | Group 3 presented a significantly higher Sds compared to the rest of the groups. | NA | Groups 1, 3 and 4 presented higher Ca and P contents, compared to groups 2 and 5. |
| Min et al. (2013) | NA | Groups 2 and 5 presented higher ALP activity (day 1) and higher expression of ALP and bone sialoprotein (day 7) compared to the rest of the groups. | Group 2 presented higher cell attachment compared to the rest of the groups. Group 5 presented significantly higher cell attachment compared to group 6. |
| Kang et al. (2013) | ***In vitro:*** NA ***In vivo:*** NA | ***In vitro:*** Group 3 after 7 days presented increased expression of osteogenic biomarkers mRNAs (OST, T1C, bone sialoprotein and Cbfa-1) compared to group 2. Group 3 showed increased ALP activity compared with groups 1 and 2. ***In vivo:*** Group 3 showed increased ALP expression compared with groups 1 and 2. | ***In vitro:*** Group 3 presented significantly higher cell attachment, compared to groups 1 and 2. No significant difference cell attachment between groups 3 and 6. ***In vivo:*** Group 3 presented higher NBF levels compared to groups 1 and 2 after 1 week. Group 3 presented higher BIC compared to group 2 after 4 weeks. |
| Yeo et al. (2015) | NA | ***In vitro:*** Group 3 and 4 after 3 days presented increased expression of osteogenic biomarkers: OST, osteonectin, T1C, and RUNX-2. ***In vivo:*** Groups 3 and 4 presented significantly ALP at week 1 compared to groups 1 and 2. | ***In vitro:*** Groups 3 and 4 presented significantly higher spreading of osteogenic cells compared to groups 1 and 2. ***In vivo:*** Groups 3 and 4 presented significantly higher BIC compared to groups 1 and 2. |
| Bougas, Jimbo et al. (2012) | NA | NA | Group 1: NBF, BIC and BA were higher in test group compared to control, but not statistical significant. Group 2: No significant NBF, BIC and BA difference between test and control. |
| Bougas et al. (2013) | NA | NA | Group 1and 2: NBF, BIC and BA were higher in test group compared to control. |
| Bougas et al. (2014) | No significant difference in implant topography between groups 1 and 3. Group 4 presented higher Sa and Sdr values compared with group 2. No significant difference in Sds between groups 2 and 4. | NA | Groups 2 and 3 presented higher RTQ compared to groups 1 and 4 after 2 weeks. No significant difference among the 4 groups after 4 weeks. Group 3 presented higher BA and BIC compared to groups 1, 2 and 4, after 4 weeks. No significant difference among the 4 groups in elastic modulus and hardness. |
| Schwartz-Filho et al. (2012) | NA | Group 2 showed higher levels of osteoblast markers: RUNX-2, OST, ALP, T1C; and inflammatory markers IL-10, TNF-α, compared to group 1. Group 1 showed higher levels of all osteoclast markers: TRAP, calcitonin receptor and ATPase, compared to group 2. | No significant difference in NBF between groups 1 and 2 |

OST: Osteocalcin; ALP: alkaline phosphatase; T1C: Type 1 collagen; Runx-2: runt-related transcription factor 2; TRAP: tartrate-resistant acid phosphatase; Sds: density of summits; Ca: Calcium; P: Phosphorous; Sa: height deviation from the mean plane; Sdr: Total surface of the implant; IL: Interleukin; TNF: Tumor necrosis factor; Cbfa-1: Core-binding factor alpha-1 BIC: Bone-to-implant contact BA: Bone Area NBF: New bone formation.

2013; Min et al., 2013; Yeo et al., 2015) ALP activity assay and quantitative real-time polymerase chain reaction (RT-PCR) were performed to assess ALP and mRNA levels of specific marker genes respectively. Bougas et al. (2011) used iodine labeling to quantify the laminin adsorption in titanium surfaced discs.

- *In vivo* studies

In three studies (Bougas, Jimbo et al., 2012; Bougas et al., 2014) and two studies (Bougas et al., 2013; Yeo et al., 2015), osseointegration and NBF was assessed using histomorphometric analysis and histology respectively. In one study (Bougas et al., 2014) biomechanical testing (removal torque and nanoindentation) was performed to assess the strength of NBF around implants. Bougas et al. (2013) and Schwartz-Filho et al. (2012) assessed NBF using three-dimensional microcomputed tomography (MicroCT) and SEM respectively. RT-PCR was used in two studies (Bougas, Jimbo et al., 2012; Schwartz-Filho et al., 2012) to assess the gene expression of osteoprogenitor and osteoclastic markers, and proinflammatory cytokines. In 2 studies (Kang et al., 2013; Yeo et al., 2015) ALP histochemistry was used to detect osteoblast differentiation and osteogenic effects in laminin coated implants (Table 1).

### 3.4. Main outcomes

- *In vitro* studies

Results from 3 studies (Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) showed that Lam-2-P3 and Lam-2-LG3 enhanced cell adhesion and spreading of osteogenic cells around titanium surfaced discs. In 3 studies (Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) Laminin-2-P3 and Laminin-2-LG3 increased the ALP and bone sialoprotein activity, and the expression of osteogenic biomarkers mRNAs including osteocalcin, type I collagen (TIC), Cbfa-1 (Core-binding factor alpha-1), osteonectin and runt-related transcription factor 2 (Runx-2). Bougas et al. (2011) and Bougas, Stenport et al. (2012) showed that laminin-1 raised CaP deposition and the density of summits (Sds) suggesting a potential use as an osseoinductive coating agent around titanium implants.

- *In vivo* studies

In 5 studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Yeo et al., 2015) laminin coating (Laminin-1, Laminin-2-P3 or Laminin-2-LG3) enhanced NBF, bone to implant contact (BIC) and/or bone area (BA) around implants. Schwartz-Filho et al. (2012) reported that Laminin-1 coating did not improve BIC or BA around titanium implants. Bougas et al. (2014) showed that after 2 weeks of healing Laminin-1 coated implants demonstrated higher implants removal torque values compared to non-coated implants (Table 3).

In 3 studies (Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) laminin coated implants raised ALP levels. One study (Schwartz-Filho et al., 2012) showed that Laminin-1 after 2 weeks of healing increased the gene expression of osteoblast markers Runx-2, osteocalcin, and TIC; osteoclast markers: tartrate-resistant acid phosphatase (TRAP), calcitonin receptor and ATPase; and inflammatory markers: interleukin-10 and tumor necrosis factor-α as compared to non-coated implants.

### 3.5. Quality assessment of included studies

Quality assessment showed that 5 studies (Bougas et al., 2011; Bougas, Stenport et al., 2012; Kang et al., 2013; Min et al., 2013; Yeo et al., 2015) were conducted *in vitro*, and 6 studies (Bougas, Jimbo et al., 2012; Bougas et al., 2013, 2014; Kang et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) were conducted on experimental animals and the total quality score ranged from 8 to 9. The most common shortcoming among all studies was the short term and incomplete follow up of the experimental groups. Furthermore, as all studies were performed *in vitro* or in animals, the application of these results to human population is still limited. Thus, on average, the quality of included studies on the impact of laminins coatings on the osseointegration of implants was good, limitations of short term follow up and lack of clinical studies limit the clinical application of these study outcomes. Quality assessment of the individual papers is summarized in Table 4.

## 4. Discussion

To our knowledge from indexed literature, the present study is the first one to systematically review the efficacy of laminins coatings enhancing osseointegration and NBF around implants. Results from ~91% *in vitro* and *in vivo* studies showed that laminins are effective in enhancing the gene expression of osteogenic biomarkers and/or the osseointegration and NBF around implants. These results seem convincing enough to conclude that laminin coatings on implants surfaces enhances osseointegration. However, it seems difficult to replicate these experimental results in a clinical setting due to a number of reasons. Firstly, it seems challenging to choose a precise laminin heterotrimer and/or isoform that would significantly increase NBF and BIC. For example, in studies by Bougas, Jimbo et al. (2012), Bougas et al. (2014) and Schwartz-Filho et al. (2012) laminin-1 coating was applied in titanium implants; whereas in the study by Kang et al. (2013) and Yeo et al. (2015) laminin-2 peptides derivatives were used. (Laminin-2-P3, DLTIDDSYWYRI motif and Lamini-2-LG3-P2-DN3, PPFEGCIWN motif respectively)

Secondly, it is notable that the experimental studies were performed for a maximum follow-up period of 28 days. Studies

**Table 4**
CASP quality assessment of the reviewed papers.

| Authors | Item 1 | Item 2 | Item 3 | Item 4 | Item 5 | Item 6 | Item 7 | Item 8 | Item 9 | Item 10 | Item 11 | Item 12 | Total quality score (0–12) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bougas et al. (2011) | Yes | No | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 8 |
| Bougas, Jimbo et al. (2012) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |
| Bougas, Stenport et al. (2012) | Yes | No | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 8 |
| Min et al. (2013) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |
| Kang et al. (2013) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |
| Yeo et al. (2015) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |
| Bougas et al. (2013) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |
| Bougas et al. (2014) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |
| Schwartz-Filho et al. (2012) | Yes | Yes | Yes | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | 9 |

(Bougas, Jimbo et al., 2012; Bougas et al., 2013) suggest that laminins enhanced bone formation during early stages of osseointegration and decreases in time. However, it remains unclear if laminin coated implants would increase BIC and contribute to long-term success and survival of dental implants. Further long-term clinical studies are needed in this regard. The authors however, emphasize that a longer follow-up of studies included in the present systematic review would provide stronger evidence regarding the efficacy of laminin coatings on the osseointegration of implants.

It is pertinent to note that there was a lack of standardization towards the selection of implants in the control groups of the studies included. For example, in the study by Bougas et al. (2011) rough surfaced titanium discs were used as controls; whereas, in another study (Schwartz-Filho et al., 2012) titanium implants with rough and smooth surfaces were used as controls. From a clinical perspective it may be argued that the implant surface roughness itself is a critical factor that affects osseointegration. Therefore, its contribution towards NBF around implants (regardless of whether laminins are used or not) cannot be disregarded. The authors of the present review propose that additional studies based on the following groups are warranted: (a) rough surface implants + laminin coating (test group); (b) rough surfaced implants without laminin coating (control group 1); (c) smooth surface implant + laminin coating (control group 2); (d) smooth surface implant without laminin coating (control group 3). From the literature reviewed only two experimental studies (Bougas et al., 2014; Kang et al., 2013) followed a protocol similar to our proposal. However, for the long clinical trials based on the proposed study groups are needed to assess the role of laminin coatings on implant surfaces in promoting osseointegration.

Interestingly, Schwartz-Filho et al. (2012) showed conflicting results, reporting that laminin did not improve the osseointegration and NBF around implants; however, the results of real-time RT-PCR presented higher expression of osteogenic and osteoclastic markers. An explanation in this regard is that their results were based on SEM observation. Although SEM is a valuable imaging technology, histological analysis is the "gold standard" for assessing NBF around implants (Nevins, Camelo, Koo, Lazzara, & Kim, 2014). Moreover, it is speculated that the early time point for evaluation (2 weeks) the mineralization could be still in process and similar bone formation present in control and test groups. Furthermore, the possibility of risk of infection after the implant placement in rabbits' tibiae; *peri*-implant infections that may jeopardize osseointegration cannot be disregarded. (Figueiredo, Camps-Font, Valmaseda-Castellon, & Gay-Escoda, 2015) It is worth mentioning that Schwartz-Filho et al. (2012) did not perform a microbiological analysis of the osseous tissues, which could have revealed valuable information with reference to impaired osteogenesis.

Is well established that confounding parameters, such as poorly controlled diabetes mellitus, osteoporosis, psychological stress, immunodeficiency, increasing age, female gender, deficient oral hygiene, and tobacco habits that may also impair healing and are significant risk factors of alveolar bone loss (Javed et al., 2007; Javed, Altamash, Klinge, & Engstrom, 2008; Javed, Al-Rasheed, Almas, Romanos, & Al-Hezaimi, 2012; Javed, Al-Askar et al., 2013; Javed, Tenenbaum et al., 2013; Renvert and Persson, 2004). Since all studies (Bougas, Jimbo et al., 2012; Bougas, Stenport et al., 2012; Bougas et al., 2011, 2013, 2014; Kang et al., 2013; Min et al., 2013; Schwartz-Filho et al., 2012; Yeo et al., 2015) included in this systematic review were performed *in vitro* or in animals, it remains to be determined whether or not laminin coated implants in a clinical scenario would facilitate NBF in patients with a poor plaque control, elderly individuals, systemically compromised and habitual tobacco product users. Hence, additional studies are warranted in this regard.

## 5. Conclusions

On experimental grounds, laminin coatings seems to enhance osteogenic biomarkers expression and/or osseointegration; however, from a clinical perspective, further randomized control trials are needed to assess the role of laminin coatings in promoting osseointegration around dental implants.

## Conflict of interest

The authors declare that they have no conflicts of interest related to the present study.

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