# Biological Evaluation of a New Organically Modified Ceramic-Based Dental Restorative Resin

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**ABSTRACT:** Material tissue interactions of a newly developed organically modified ceramic (ormocer)-based dental restorative composite based on a resin containing mixture of alkoxides of silicone and calcium with polymerizable methacrylate end groups are discussed in this study. Admira, a commercially available ormocer was used as control. A sol–gel process has been used to synthesize organically modified ceramic resins from liquid precursors, which allow one to produce inorganic–organic hybrid polymer materials, which can be functionalized to optimize their physical and chemical properties. Besides, the resulting material properties have been significantly modified by further technological processing such as photochemical curing of the materials by incorporating dimethacrylate groups as organically polymerizable units. Intracutaneous (intradermal) irritation test is found not to elicit any gross signs of tissue reaction. The results of maximization test for delayed hypersensitivity also did not show any adverse skin reaction during the induction or challenge period. Histological analysis after 1, 4, and 12 weeks of subcutaneous implantation of restorative composite resin on the dorsal surface on one side of the spinal column of Wister rats is also found not to reveal any inflammatory response after the implantation. Granuloma and material debris is found to be absent in all three periods of implantation in both test and control. No necrosis was observed around the implanted materials, which were found to be encapsulated by fibrous connective tissue consisting of predominantly fibroblasts and inflammatory cells. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 620–629, 2012

**Key words:** visible light cure; dental composite; organically modified ceramic; calcium containing resin; biocompatibility

## INTRODUCTION

With rapid changes happening in dental materials research, material choices for treatment today are enormous. Currently, the most popular direct restorative materials are polymeric composites, though they still have problems such as polymerization shrinkage and wear resistance. The postgel polymerization shrinkage causes significant stresses in the surrounding tooth structure and composite tooth bonding leading to premature restoration failure. Other problems such as uncured organic monomers leaching from the dental composites into the surrounding gum tissue have been reported to cause cytotoxic effects.[1,2]

Organically modified ceramic hybrid materials are used in wide range of applications such as wear resistant coating, in microelectronics, micro-optics, electro-optics, photonics as matrices for dental composites,[3] and scaffolds for tissue engineering.[4] Calcium hydroxide, $Ca(OH)_2$, was incorporated during resin synthesis, because it is known that $Ca(OH)_2$ is used as dressing in paste form in dentistry during dental root canal procedure. $Ca(OH)_2$ is known to have a strong antimicrobial effect and is a bone-regeneration stimulant. $Ca(OH)_2$ has long been used to stimulate dentine bridge formation for dental regeneration, although its mechanism of action has remained largely elusive.[5] New organically modified ceramic-based dental restorative composite consisting of inorganic–organic hybrid resins, containing mixture of alkoxides of silicone and calcium with polymeric methacrylate groups were used in this study.

Detailed description of synthesis, characterization, and structural elucidation of the novel resin used in this article has been published.[6] The calcium containing material under study was earlier subjected to extensive physico-mechanical[6] and cytotoxic[7] evaluation on mouse L929 fibroblast cell line refractive index, Fourier transform infrared spectroscopy (FTIR), FTRaman, thermogravimetry, high performance liquid chromatography (HPLC) data of the resin

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along with diametral tensile strength, flexural strength, flexural modulus, Vickers hardness number, depth of cure, linear polymerization shrinkage, water sorption, solubility, and monomer conversion data of cured composite were published.[6–11] They showed excellent hardness, good shrinkage[7] characteristics, excellent thermal stability,[8] and noncytotoxic[7] character to L929 fibroblast cell line. The data on comparison of the new composite with existing composite published in Refs. 6 and 7 showed that the present material has better physical properties than existing composites.

The results of intracutaneous (intradermal) irritation studies, maximization test for delayed hypersensitivity, and subcutaneous implantation are discussed in this article. Intracutaneous (intradermal) reactivity test provide an understanding of irritation level. Delayed hypersensitivity studies usually give indication of any adverse skin reaction during the induction or challenge period. Though the restorative material is intended to replace enamel and dentine during clinical applications, chances of soft tissues coming into contact with gum tissues and dental pulp are possible. Therefore, subcutaneous implantation studies were performed. Subcutaneous implantation is frequently used[12] to evaluate biocompatibility of biomaterials. We implanted cured composite discs[13–15] subcutaneously in rats and analyzed the inflammatory tissue response from 1 to 12 weeks after implantation using light microscopy. The tissue responses are discussed. Ideally, a dental material that has to be used in the oral cavity should be harmless to all oral tissues. Furthermore, it should not contain any toxic, leachable, or diffusible substance that can be absorbed into the circulatory system, causing systemic toxic responses, including teratogenic or carcinogenic effects. The material should also be free of agents that could elicit sensitization or an allergic response in a sensitized patient. Dental materials standards require biological testing along with physico-chemical and mechanical properties. Proper sterilization of the prepared sample is required to avoid infections.[16] When a new composite material is being developed for medical applications, it is important to assess the biocompatibility of the whole system, even if the individual materials are already proved to be biocompatible.[17] A disadvantage of *in vivo* biocompatibility testing is that it is often influenced by multiple factors. The surgical procedure itself indicates an inflammatory response. Additionally, the presence of implant also provides a continuous inflammatory stimulus. The problems of correlating *in vitro*, animal and usage biocompatibility tests have long been identified and extensively reviewed. Today, it is recognized that both *in vitro* and animal tests play an important role in the biological evaluation of dental materials. However, if meaningful data are to be obtained from these tests, then the experimental procedures must be closely scrutinized to ensure that the testing conditions are as relevant as possible.[18,19] Biocompatibility studies of resin composites subcutaneously implanted in rats with experimentally induced arthritis showed[20] that the health status of the animals and the materials used did not influence tissue response.

The inflammatory response is a sequential, localized tissue reaction to the foreign material and involves an initial infiltration of neutrophiles followed by macrophages.[21] In the initial stages of inflammation, the predominant cell type present is the neutrophil. It has been postulated that in short-term implantation, a high fibrocyte density and presence of few macrophages might be an indication of biocompatibility.[22] The presence of fibrous tissue isolating the PEEK/β tricalcium phosphate biocompatible composite implant from the surrounding bone was visible during the histopathological evaluation.[23]

Studies on cell cultures have demonstrated that methacrylate and dimethacrylate monomers commonly used in restorative polymer technology may affect recruitment of leukocytes to inflammation sites by decreasing the intercellular adhesion molecule-1 expression in cells simulated with the cytokine Tumor necrosis factor (TNF)-[24] and by inducing the enzymatic activity and cytokine/growth factor expression in a cell specific manner.[25] Moreover, resin monomers have been found to suppress the mitochondrial activity of macrophages and alter the normal macrophage-induced inflammatory responses.[26,27]

Biocompatibility studies such as intracutaneous (intradermal) reactivity test provided an understanding of irritation level. A lot of efforts to improve the performance of composites for dental fillings are focused on reducing the polymerization shrinkage and improving the marginal adaptation, abrasion resistance, and biocompatibility.[28,29] For this purpose, ormocers based on urethane or carboxy-functionalized methacrylate alkoxysilanes[30,31] were introduced into the $SiO_2$ glass network via the sol–gel approach. Since Bowen[32,33] introduced in the 1960s bisphenol A/dimethacrylate (BisGMA), few structural variations in the organic matrix of dental composites have been proposed[34] until recently with the advent of organically modified ceramics (ormocers). An ormocer is a hybrid ceramic composite in which the main chain is formed by polysiloxane linkages containing pendant unsaturated carbon chain molecules. Such structures can be considered prepolymers and are therefore less susceptible to polymerization contraction because of a lower number of polymerization sites. Additionally, an ormocer is expected to present higher biocompatibility than standard BisGMA-based composites as well as lower sensitivity.[34] Although ormocers are very promising,

few investigations have confirmed the potential of ormocers as biomaterials or low-contraction materials applied to teeth restoration. The two composites (Definite® (Degussa AG, Hanau, Germany) or Admira® (Voco GmbH, Cuxhaven, Germany) available in the market based on ormocer technology still contains conventional dimethacrylates like BisGMA and triethylene glycol dimethacrylate (TEGDMA). For example, according to the suppler,[34] Admira composite was 78% inorganic particles (barium and aluminum silicate) with an average size of 0.7 μ, and the organic fraction was composed of BisGMA, TEGDMA, and urethane dimethacrylate, which are the conventional organic dimethacrylates. The composition of the organic portion was 65.5% BisGMA and 34.5% TEGDMA.[34] Our new composite contains ormocer resin diluted with TEGDMA as the resin part with complete elimination of BisGMA is a new concept. These materials with superior shrinkage characteristics[7] are expected to avoid marginal leakage and secondary caries usually associated with the existing composites. As the material contains calcium, it is expected to enhance bone remodeling as the rich Ca resources aid bone regeneration locally,[35] which may extend its application in orthopedics.

The aim of this study was to characterize the biological tissue response to a new organically modified ceramic-based dental restorative resin and compare the tissue responses of the new material with a commercially available control material.

## EXPERIMENTAL

### Materials

The materials used in this study are 3-(methacryloxy propyl trimethoxy silane), TEGDMA sodium lauryl sulfate, cottonseed oil ( Aldrich Chem. Co. Milwaukee), Laboratory Rasayan (LR) grade calcium hydroxide, sodium hydroxide (LR), Analytical Rasayan grade hydrochloric acid, and specially dried LR grade diethyl ether (S.D. Fine Chemicals, Mumbai, India).

All the animals used in this study were in-house bred animals in the Laboratory of Animal Sciences of our Institute.

### Synthesis and characterization of organically modified ceramic hybrid materials (Ormoresin R17)

Synthesis details of Ormoresin R17 were previously reported.[6–11] One mole of 3-trimethoxy silyl propyl methacrylate was hydrolyzed with 3 moles of distilled water in presence of 6*N* HCl under stirring for 6 h. The hydrolyzed silane was kept at room temperature overnight for postcondensation. This was then extracted with ether, washed with distilled $H_2O$, till acid free, and dried after evaporating ether. The hydrolyzed silane was neutralized with 1*N* NaOH solution and dissolved in diethyl ether to get 40% solution of the polycondensed silane in diethyl ether. The ether solution was mixed with 1% calcium hydroxide and 0.5% silica and stirred well at room temperature and dried at 37°C.

Molecular weight of the resin was determined by gel permeation chromatography (GPC) (Shimadzu Prominence Series UFLC, Shimadzu, Tokyo, Japan) using 0.5% solution in chloroform with refractive index (RI) detector and phenogel column at a flow rate of 1 mL/min.

Organically modified ceramic resin (50 parts) diluted with TEGDMA (50 parts) was used as the resin,[10] and purified, silanated quartz[13] was used as the filler for the preparation of restorative paste. (−)Camphorquinone (CQ) (Aldrich Chem. Co., Milwaukee) was used as the photoinitiator. Other chemicals used are 4-(dimethyl amino) phenethyl alcohol, 4-methoxy phenol, phenyl salicylate, 2-hydroxy-4 methoxy benzophenone, and 2,6 di-*tert*-butyl-4-methyl phenol (all from Aldrich Chem. Co., Milwaukee). Admira (VOCO, Cuxhafen, Germany) was used as the control restorative resin.

### Dental composite paste preparation

The dental composite paste (Ormo 48) was prepared using the reported method.[6,10] TEGDMA was used as the diluent monomer for the organically modified ceramic resin. It also acts as the crosslinking agent. Fifty parts of organically modified ceramic resin was mixed with 50 parts of TEGDMA. To this resin mixture, photoinitiator (CQ), catalyst, inhibitor, and ultraviolet stabilizer (100 ppm to 0.25% w/w of resin mixture) were mixed to prepare the pastes. The prepared resin mixture was mixed with 300% of silanated[6] quartz and 12% pyrogenic silica in an agate mortar to get a uniform paste.

### Evaluation of dental composites

We performed the biocompatibility studies as per the international standard ISO 7405[14] [ISO 10993-10 2002 (E) and ISO 10993-6-1994 (E)]. Samples were prepared as per [ISO 10993-12-2002 (E) and ISO 10993-5-2002 (E)]. As per the standard,[15] sterility of the test material has to be considered. Sterilization by autoclaving at 120°C and 15 psi for 20 min is a standard procedure before biocompatibility studies including hydrogels, which are water soluble. Moreira et al.[16] used autoclaving for dextrin based hydrogels. During autoclaving, sample is exposed to steam and vacuum simultaneously. Chances of leaching are less. During extraction, all the possible leachants are expected to be collected in the extract. This extract was used for biocompatibility evaluation

(intracutaneous (intradermal) reactivity test and maximization test for delayed hypersensitivity). If the material contains toxic ingredients, it will remain cytotoxic even after washing and autoclave sterilization.[7] In our previous study,[7] we compared the cytotoxic behavior of three different composites, which sterilized with the same conditions. One out of the three was found to be mildly cytotoxic, while the two were noncytotoxic.

### Preparation of samples for *in vivo* biological evaluation

Stainless steel moulds with 10 mm diameter and 1 mm thickness were used for the preparation of specimens for *in vivo* toxicity evaluation. The mould was kept on a transparent sheet on a metallic plate. The paste was packed onto the mould, and a second transparent sheet was put on the top followed by a second metallic plate. The mould and strip of film between the metallic plates were pressed to displace excess material. The plates were removed, and the paste was exposed to visible light for duration of 40 s on both sides through the transparent sheet. The cured samples were removed from the mold and stored at 37°C for 24 h. The samples were cleaned using an ultrasonic cleaner. Samples were treated with 1% soap solution for 5 min at 25°C followed by washing in running water till free from soap and with distilled water for 5 min at 25°C twice in ultrasonic cleaner, dried at 60°C for 12 h and packed for sterilization.

The discs were used for implantation in subcutaneous test. For intradermal reactivity test and maximization test for delayed hyper sensitivity studies, powdered composite samples were used. The cured composite was powdered in a centrifugal ball mill (Retsch, Germany). All the samples were sterilized using an autoclave at 120°C for 20 min.

### Animal studies

For maximization test for delayed hypersensitivity, 15 Guinea pigs (adults of weight range 300–500 g of either sex) were used. For intracutaneous (intradermal) reactivity test, three Albino rabbits (adults of weight not less than 2 Kg of either sex) were used. For local effects after implantation tests, 20 Wistar rats (adults of weight not 200 to 300 g of 10 each from both sex) were used. For all experimental purposes, animals bred in the Division for Laboratory Animal Sciences (DLAS) of our Institute were used.

All animal studies were conducted according to the ethical principles for animal experimentation of The Committee for the Purpose of Control and Supervision of Experiments on Animals, India, guidelines and approved protocol and ISO 7405[12] [ISO 10993-2 : 2006 (E)] specifications with the prior approval of institutional animal ethics committee where ever required. High standard of care and accommodation were given to all experimental animals in the well-established animal house of DLAS, SCTIMST, India. All animals were supplied with food and water *ad libitum* in proper environmental conditions. All surgical procedures were performed on anesthetized animals, with extra care to avoid intraoperative sepsis. Animals for maximization test for delayed hypersensitivity tests and intracutaneous (intradermal) reactivity tests were housed 20–30 days. Animals for local effects after implantation were housed 7–84 days (six animals each for 1, 4, and 12 weeks, respectively).

### Intracutaneous (intradermal) reactivity test

Intracutaneous reactivity test was performed as per ISO 7405 [ISO 10993-10 2002 (E)][12] to evaluate the local responses to the extract of the composite material under test following intracutaneous injection into rabbit. The physiological saline and cotton seed oil extracts of powdered composite was prepared in 10 mL of the medium at 70 ± 2°C with constant stirring at 50 rpm for 24 ± 2 h. Two adult albino rabbit not less than 2 kg was used for the experiment. The physiological saline (NS) and cotton seed oil (CSO) extracts of the test material was aseptically injected into five sites (0.2 mL/site) on the upper left-hand side and right-hand side of the rabbits. Both physiological saline and cotton seed oil alone was taken and separately injected into five sites on the lower left-hand side and lower right-hand side of the same rabbit as control material. The grading of erythema and edema of test and control sites of all animals were recorded at 24, 48, and 72 h. The grading was given using arbitrary numbers from 0 to 4 indicating 0 = No erythema/edema, 1 = Very slight erythema/edema, 2 = Well-defined erythema/edema, 3 = Moderate erythema/edema, and 4 = Severe erythema/edema.

### Maximization test for delayed hypersensitivity

Maximization test for delayed hypersensitivity was performed as per [ISO 10993-10 2002 (E)] to determine the potential of the material to produce skin sensitization in guinea pigs. The physiological saline extract of (2 g) of powdered composite was prepared in 10 mL of the medium at 70 ± 2°C with constant stirring at 50 rpm for 24 ± 2 h. Fifteen Albino Guinea pigs 300–500 g was used for the experiment per material extract. Sample for intradermal injection was prepared as follows:

1. A 50 : 50 (v/v) mixture of Freund's complete adjuvant mixed with solvent.
2. The test material extract/control alone was prepared.

**TABLE I**
**Statistical Evaluation of Histological Studies of Ormo48 and Admira**

| | | 1 week | | 4 weeks | | 12 weeks | |
|---|---|---|---|---|---|---|---|
| | | Mean ± SD | *P* value | | *P* value | Absent | *P* value |
| Granuloma | Ormo 48 | Absent | | Absent | | Absent | |
| | Admira | Absent | | Absent | | Absent | |
| Material debris | Ormo 48 | Absent | | Absent | | Absent | |
| | Admira | Absent | | Absent | | Absent | |
| Tissue in-growth | Ormo 48 | Not applicable | | Not applicable | | Not applicable | |
| | Admira | Not applicable | | Not applicable | | Not applicable | |
| Polymorphonuclear cells | Ormo 48 | 1.53 ± 0.94 | 0.36345 | 0.38 ± 0.66 | 0.412 | 0.017 ± 0.06 | |
| | Admira | 1.81 ± 0.48 | | 0.2 ± 0.37 | | 0 ± 0 | |
| Lymphocytes | Ormo 48 | 0.75 ± 0.35 | 0.74531 | 0.64 ± 0.43 | 0.9545 | 0.47 ± 0.18 | 1.21594 E −11 |
| | Admira | 0.8 ± 0.39 | | 0.65 ± 0.26 | | 0.52 ± 0.13 | |
| Plasma cells | Ormo 48 | 0.25 ± 0.19 | 0.85254 | 0 ± 0 | 0.328 | 0 ± 0 | |
| | Admira | 0.23 ± 0.24 | | 0.02 ± 0.06 | | 0 ± 0 | |
| Macrophages | Ormo 48 | 0.7 ± 0.57 | 0.80859 | 0.37 ± 0.32 | 0.59 | 0.22 ± 0.25 | 0.512151 |
| | Admira | 0.75 ± 0.41 | | 0.3 ± 0.29 | | 0.17 ± 0.08 | |
| Giant cells | Ormo 48 | 0 ± 0 | | 0 ± 0 | | 0 ± 0 | |
| | Admira | 0 ± 0 | | 0 ± 0 | | 0 ± 0 | |
| Necrosis | Ormo 48 | 0 ± 0 | | 0 ± 0 | | 0 ± 0 | |
| | Admire | 0 ± 0 | | 0 ± 0 | | 0 ± 0 | |
| Neovascularization | Ormo 48 | 1.08 ± 0.51 | 0.23703 | 0.92 ± 0.29 | 1 | 1 ± 0 | |
| | Admira | 1.3 ± 0.49 | | 0.92 ± 0.29 | | 1 ± 0 | |
| Fibrosis | Ormo 48 | 0 ± 0 | | 1.25 ± 0.62 | 0.379352 | 1.67 ± 0.49 | 0.237032 |
| | Admira | 0 ± 0 | | 1 ± 0.74 | | 1.42 ± 0.51 | |
| Fatty infiltrate | Ormo 48 | 0.5 ± 0.52 | 0.29765 | 0.17 ± 0.39 | 0.454804 | 0.08 ± 0.29 | 0.293562 |
| | Admira | 0.75 ± .62 | | 0.33 ± 0.65 | | 0.25 ± 0.45 | |

$P < 0.05$ was considered as statistically significant. Significant difference between control and test material was observed for lymphocytes at 12 weeks implantation.

3. The test material extract/control emulsified in 50 : 50 (v/v) Freund's adjuvant mixture.

A pair of 0.1 mL intradermal injections of each of the above solutions (1,2,3) were made at three respective sites one on either side in the clipped intrascapular region. Seven days after intradermal injections, the test and control extracts (in physiological saline) were topically applied to intrascapular region of each Guinea pigs. After 48 h, the dressings and patches were removed. Twenty-four hours before topical application, the intradermal sites were pretreated with 10% sodium lauryl sulfate. Fourteen days after topical application, the test and control animals were challenged with test material extract. The patches and dressings were removed after 24 h. The appearance of the challenge skin sites of test and control animals were observed at 24, 48, and 72 h after removal of dressings and patches.

The skin reactions for erythema and edema were scored and recorded the numerical grading as per ISO 10993-10 : 2002/Amd 1 : 2006 (E). The grading was given using arbitrary numbers from 0 to 3 indicating 0 = No visible change, 1 = Discrete or patchy erythema, 2 = Moderate and confluent erythema, and 3 = Intense erythema and swelling.

**Local effects after implantation**

To assess the biological response of subcutaneous tissue to the implanted material, 10 mm diameter and 1 mm thick cured discs [ISO 10993-6 1994 (E)] were used for implantation studies. Cured discs of commercially available Admira were used as control material. Before implantation, the material was steam sterilized. Eighteen healthy Wister rats weighing not less than 180 g and of either sex were used. Before test, the fur on either side of the vertebral column was clipped. The implantation procedure was performed under clean and aseptic condition. Rats were anesthetized using Ketamine (100 mg/Kg) + Xylaxin (5 mg/Kg). The skin of the anesthetized rats were lightly swabbed using 70% alcohol and air dried. Implantation was done by making a subcutaneous tunnel pocket on the dorsal surface on one side of the spinal column by blunt dissection such that the base of the pocket is 10 mm away from the line of incision. The test or control is then pushed into the tunnel. Two test materials were implanted subcutaneously on the right side of the animal. Similarly, two control materials (Admira) were implanted on the left side of the animal. The incisions were then closed using sterile sutures. This procedure was conducted on 18 rats. Observation period was 1, 4, and 12 weeks. Sacrificed the animals (6 rats/period) at the end of 1, 4, and 12 weeks and

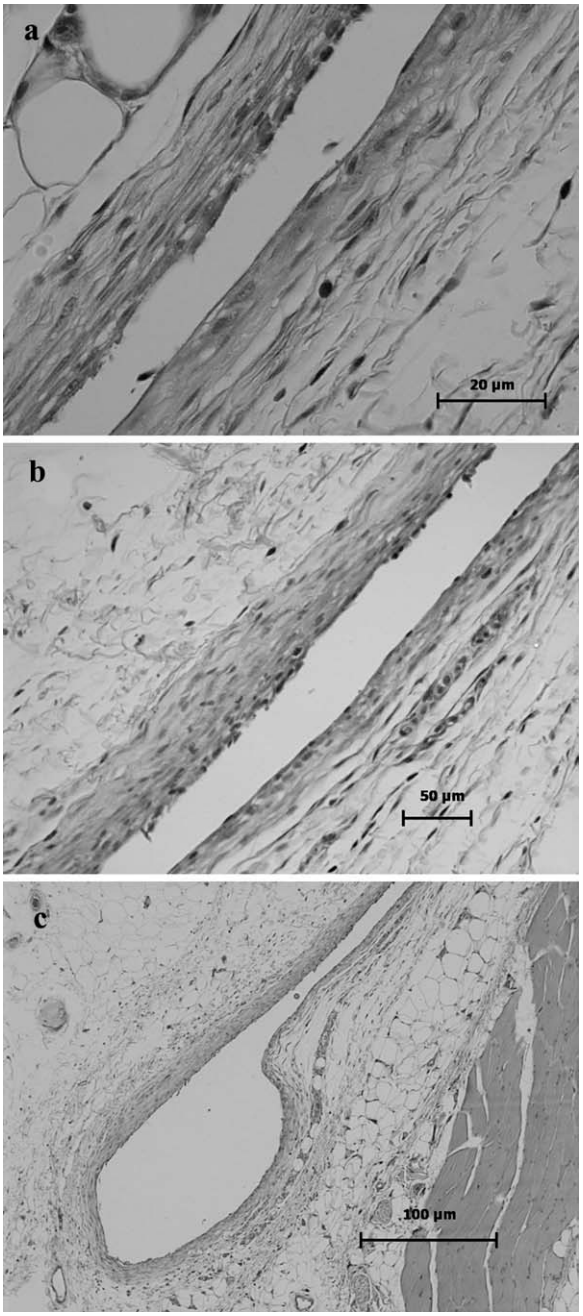


**Figure 1** Light microscopic images of Ormo 48 at 1 week postimplantation: (a) Fibrous capsule around implant site, 40× H&E, (b) cellular infiltration around implant site, 20× H&E, and (c) neovascularization and fatty infiltration at the implant site, 4× H&E.

collected the material along with surrounding tissue for histopathological evaluation.

### Gross and histopathology

The skin along with subcutaneous implant was removed completely and was fixed immediately in 10% neutral buffered formalin. Sections were cut perpendicular to skin surface and central section was processed for histology in an automatic tissue processor Leica ASP300 (Leica Micro-systems GmbH, Wetzlar, Germany) and embedded in paraffin. Longitudinal sections of 5 µm thickness were prepared (Leica RM2255, Wetzlar, Germany) and stained (Leica Autostainer XL, Wetzlar, Germany) with hematoxylin and eosin (H&E, Merck, Germany). The types and distribution of cells around the implants were evaluated under a trinocular light microscope (Nikon

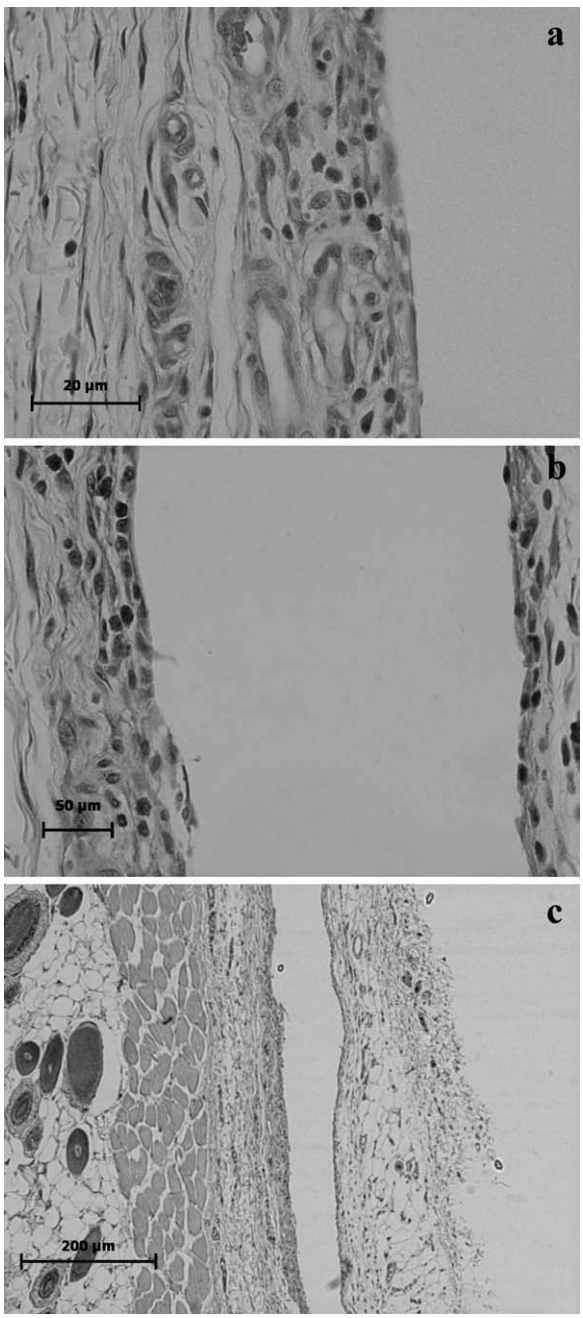


**Figure 2** Light microscopic images of Admira at 1 week postimplantation: (a) Fibrous capsule around implant site, 40× H&E, (b) cellular infiltration around implant site, 20× H&E, and (c) neovascularization and fatty infiltration at the implant site, 4× H&E.

OPTIPHOT, Japan) and photographed using a digital camera (Evolution MP color, MediaCybernetics) attached to the microscope. Five randomly chosen sites for the following parameters were evaluated for degeneration, necrosis, neovascularization, inflammatory cells, fatty infiltration, and extent of fibrous capsule formation. Twelve specimens per each period of implantation were used for evaluation. The cellular response was described by assessing the cellular densities of granulocytes, lymphocytes, macrophages, and foreign body type giant cells in each specimen. Necrosis was determined by cell debris and inflammation. Neovascularization was determined by the number of newly formed capillaries at the implant site. Fatty infiltrate was determined by amount of fat tissues present around the implant site. Group total (sum total at one time period), mean group average (group total divided by number of samples/sections evaluated at one time period), and score (derived by deducting the mean group average of control from test) were assessed. Comparison between cell numbers in different samples were made for each cell type and expressed as arbitrary units (au) ranging from 0 to 4. In the case of polymorphonuclear cells, lymphocytes, plasma cells, and macrophages, grading is based on the number and distribution of cells at higher magnification (0 = 0 cells, 1 = 1–5 cells, 2 = 6–10 cells, 3 = heavy infiltration, and 4 = packed cells). For giant cells, 0 = 0 cells, 1 = 1–2 cells, 2 = 3–5 cells, 3 = heavy infiltration, and 4 = packed cells. In the case of neovascularization, 0 = no capillaries, 1 = 1–3 capillaries, 2 = 4–7 capillaries, 3 = broad blood vessels, and 4 = extensive vascularization. Fibrosis measured by fibrous capsule thickness was graded 0 = absent, 1 $\leq$ 5μm, 2 = 6–15 μm, 3 = 16–30 μm, and 4 $\geq$ 30 μm. Necrosis and fatty infiltrate were graded as 0 = not present, 1 = minimally present, 2 = mild degree, 3 = moderate degree, 4 = severe degree. All above evaluations were done at higher magnification (400×).

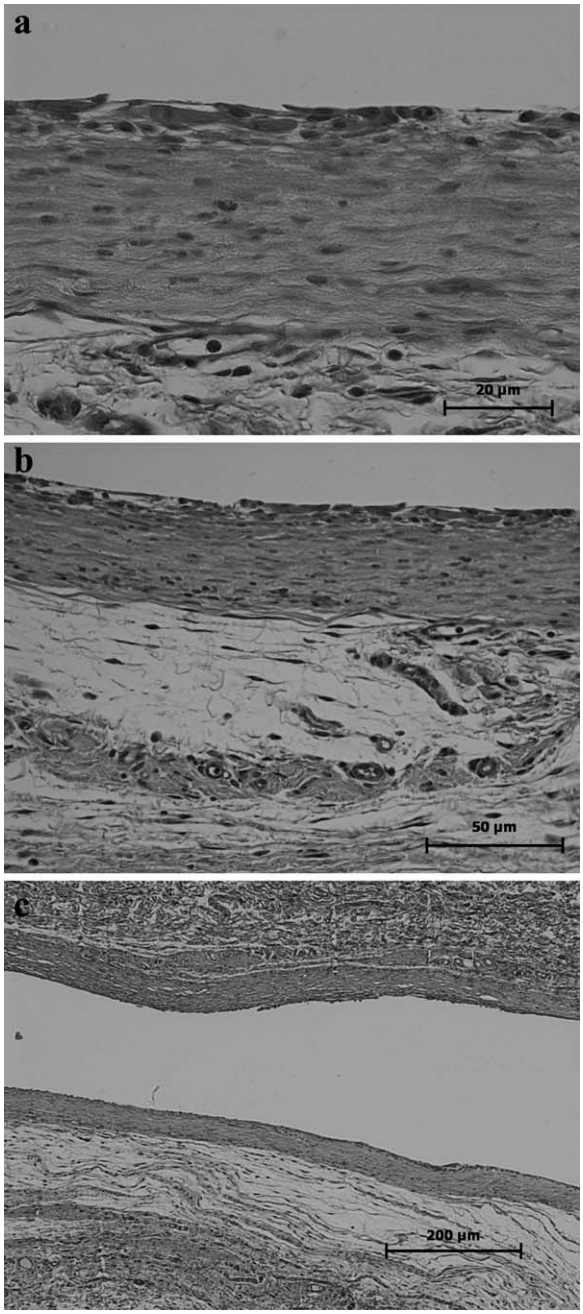


**Figure 3** Light microscopic images of Ormo 48 at 4 weeks postimplantation: (a) Fibrous capsule around implant site, 40× H&E, (b) cellular infiltration around implant site, 20× H&E, and (c) neovascularization and fatty infiltration at the implant site, 4× H&E.

## RESULTS AND DISCUSSION

Molecular weight determination of the resin by GPC showed that the resin has number average molecular weight ($M_n$) 23,109, weight average molecular weight ($M_w$) 29,507, sedimentation average molecular weight ($M_z$) 46,053, and a poly dispersity index 1.27. The polydispersity index value showed that the resin has a narrow molecular weight distribution.

The results of intracutaneous (intradermal) reactivity test showed that the test material produced an average irritation score of 0.40 in cotton seed oil extract and 0.0 in physiological saline extract following intradermal injection. As per international standard, the requirements of the test are met if the difference between the test sample mean score and control mean score is 1.0 or less. Intracutaneous reactivity studies performed on rabbit showed that the extract of the material did not evoke any edema and erythema.

Maximization test for delayed hypersensitivity results showed that none of the animals in the test and control groups showed any adverse skin reaction such as erythema and edema during the induction or challenge period. The physiological saline extract of

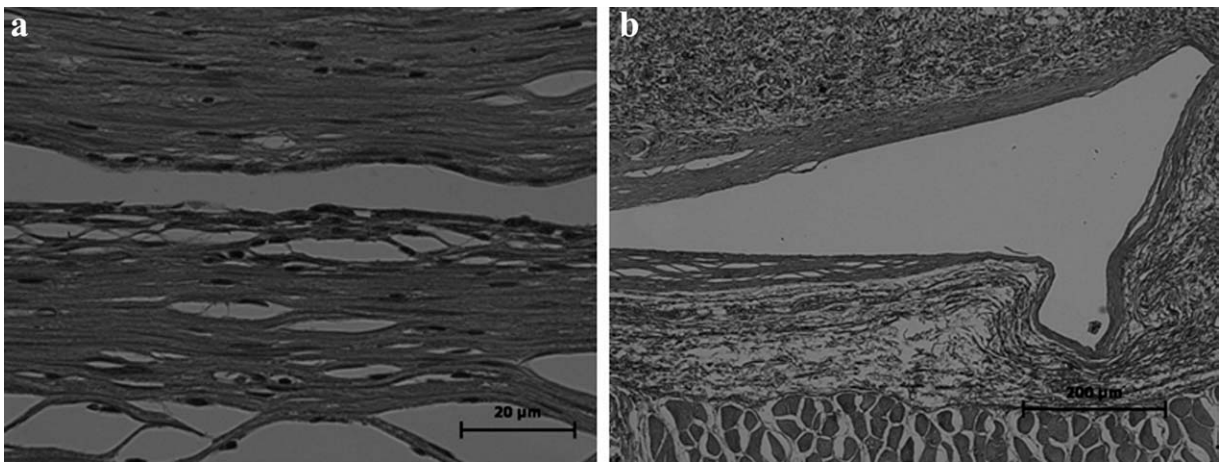


**Figure 4** Light microscopic images of Admira at 4 weeks postimplantation: (a) Fibrous capsule around implant site, 40× H&E and (b) neovascularization and fatty infiltration at the implant site, 4× H&E.

test material induced a numerical grading of "0" for erythema and edema, which showed that the material did not produce any hypersensitivity reaction and exhibits encouraging signs of healing response. The grading of maximization test for delayed hypersensitivity indicated that grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen on control animals.

Subcutaneous implantation of restorative composite resin in Wistar rats showed that all the experimental animals tolerated the surgery well. The increase in body weight and feed intake was normal during the experimental period of all animals. None of the animals showed any abnormality or behavioral change during these periods. There were no cases of infection, paralysis, and mortality during the observation period. The site of implantation healed without any complication for both test and control materials. All the implant materials were retrieved at the end of 1, 4, and 12 weeks. At the time of implant retrieval, there were no grossly noticeable signs of infection in any of the surviving animals.

Table I showed the results of histopathological evaluation of test and control material for 1, 4, and 12 weeks. From the table, it can be seen that for both test and control materials, granuloma, and material debris were absent in all three periods of implantation. No necrosis was observed around the implanted material. The implants were encapsulated by fibrous connective tissue consisting of predominantly fibroblasts and inflammatory cells. At 1 week, presence of polymorphonuclear cells, lymphocytes, plasma cells, and macrophages were noticed around the implant site for both test [Fig. 1(a,b)] and control [Fig. 2(a,b)] materials. Necrosis and fibrosis were absent. Neovascularization and fatty infiltration were noticed at the implant site [Figs. 1(c) and 2(c)]. At 4 weeks, presence of polymorphonuclear cells and plasma cells were subsided, lymphocytes and macrophages were noticed around the implant site of both test [Fig. 3(a,b)] and control [Fig. 4(a)]. Necrosis was found absent. Fibrosis, neovascularization, and fatty infiltration were noticed at the implant site [Figs. 3(c) and 4(b)]. At 12 weeks, presence of lymphocytes and macrophages were noticed around the implant site of both test [Fig. 5(a,b)] and control [Fig. 6(a,b)]. Necrosis was absent. Fibrosis, neovascularization, and fatty infiltration were seen around implant of test [Fig. 5(c)] and control [Fig. 6(c)]. No significant change (Table I) was observed between test and control material except for lymphocytes at 12 weeks. For test and control materials, polymorphonuclear cells, lymphocytes, plasma cells, macrophages, neovascularization, and fatty infiltrate were subsided with duration of implantation. For test and control material, fibrosis was comparable, and no statistically significant variation at 12-week period, which showed that the healing responses of both materials were comparable. Foreign body giant cells were absent for both test and control material during the whole implantation period indicated the absence of chronic inflammation. Necrosis was absent for both test and control materials, which indicated no cell death occurred due to material during the whole implantation period. The irritation index average score for 1 week is 0, for 4 weeks is 0.58, and for 12 weeks is again 0, which indicated that the *in vivo* toxicity studies, which were assessed on Wister rats by the implantation of modified composite specimens, revealed nonirritant response at 1, 4, and 12 weeks after the implantation of restorative composite resin. The polymorphonuclear cells present (Table I) at the initial stages of implantation would have resulted from the surgical procedures

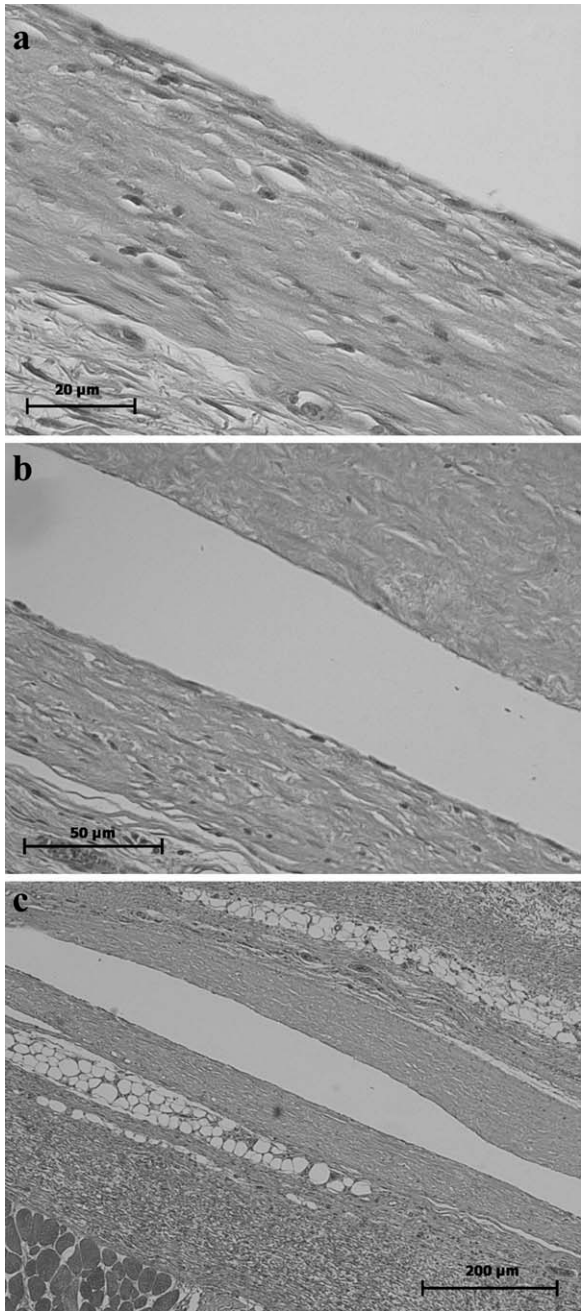


**Figure 5** Light microscopic images of Ormo 48 at 12 weeks postimplantation: (a) Fibrous capsule around implant site, 40× H&E, (b) cellular infiltration around implant site, 20× H&E, and (c) neovascularization and fatty infiltration at the implant site, 4× H&E.

used for implantation, which disappeared during 12-week implantation period.

The results of intracutaneous (intradermal) irritation studies did not show any gross signs of tissue reaction. Histological observations at 12 weeks postimplantation revealed that the initial presence of lymphocytes, plasma cells, and macrophages subsided with time which indicated that inflammation was subsided and had good healing response. Both materials induced a moderate cellular response consisting predominantly of fibroblasts, granulocytes, lymphocytes, and occasional giant cells, resulting in a fibrous capsule. Formation of effective bonding between the organic and inorganic parts of the resin during synthesis[6] and better crosslinking in presence of TEGDMA with the dimethacrylate group of these new resins are expected to be the reason for the better biocompatibility performance of the cured composite.

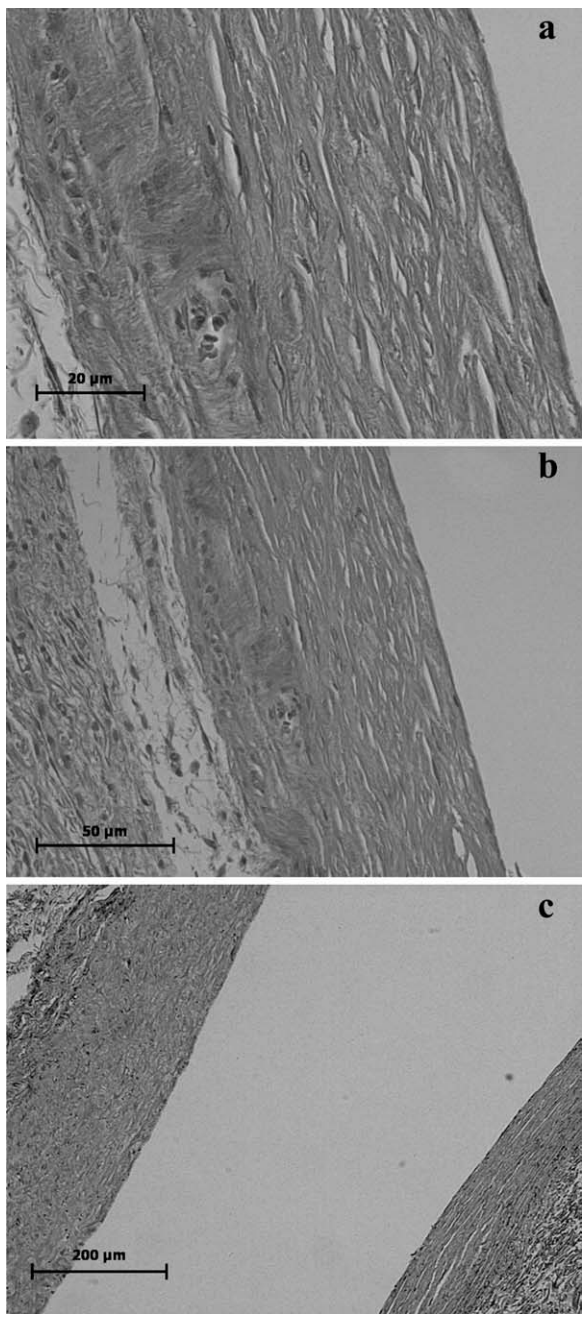


**Figure 6** Light microscopic images of Admira at 12 weeks postimplantation: (a) Fibrous capsule around implant site, 40× H&E, (b) cellular infiltration around implant site, 20× H&E, and (c) Neovascularization and fatty infiltration at the implant site, 4× H&E.

## CONCLUSIONS

The biological response of living tissue to a newly developed dental restorative material was investigated by *in vivo* methods. Tissue response to intracutaneous injections from the extracts of the material did not elicit edema and erythema. The extract of test material induced a numerical grading of "0" for erythema and edema in maximization test for delayed hypersensitivity. Subcutaneous implantation studies showed no inflammation response at 1, 4, and12 weeks after the implantation of restorative composite resin. Histopathological studies revealed that the inflammatory and healing responses of test material were as good as control material at all time periods, indicating the novel test material's biocompatibility. All the results indicated that the novel organically modified ceramic-based dental restorative composite material, which has excellent proved physicomechanical propertied is biocompatible and can be recommended for restorative application after the successful completion of the pulp and dentine test as per standards.

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