学 位 論 文 の 要 旨

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学 位 論 文 名　Applicability of Bacterial Cellulose as an Alternative to Paper Points in Endodontic Treatment

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論 文 内 容 の 要 旨

INTRODUCTION

Dental root canal treatment is required when dental caries progress to infection of the dental pulp. A major goal of this treatment is to provide complete decontamination of the dental root canal system. However, the morphology of dental root canal systems is complex, and many human dental roots have inaccessible areas. In addition, dental reinfection is fairly common. In conventional treatment, a cotton pellet and paper point made from plant cellulose is used to dry and sterilize the dental root canal. Such sterilization requires a treatment material with high absorbency to remove any residue, the ability to improve the efficacy of intracanal medication and high biocompatibility. Bacterial cellulose (BC), which is well known as the Asian dessert Nata de Coco, is produced by certain strains of bacteria. The properties and characteristics of BC are substantially different from those of conventional plant cellulose, and BC is distinguished by a high mechanical strength, and absorptive capacity. Furthermore, BC has recently been studied for a variety of biomedical materials. BC has different properties to plant cellulose, even though both exhibit similar properties when used as a traditional treatment material. We therefore hypothesized that BC will serve as a superior dental canal treatment material for intracanal asepsis. The aim of this study is to clarify the applicability of BC as a novel material for dental canal treatment with regard to solution absorption, expansion, tensile strength, drug release, and biocompatibility.

MATERIALS AND METHODS

Specimen preparation BC wet pellicles were produced by static culture of *Acetobacter hanseni*. BC wet pellicles were autoclaved in deionized water, and the process was repeated 3 times. BC sheets were then created with BC wet pellicles frozen and dried using a freeze dryer and pressed into sheets with a thickness of approximately 100 μm using a small press. BC sheets were rolled into a point form. Commercially available paper points (PP) were used as controls. The specific surface area of the sheets was estimated by the nitrogen absorption/desorption method.

Solution absorption and expansion Each specimen was immersed in three solutions overnight for estimation of solution absorption: saline, K^+ free electrolyte fluid, or electrolyte fluid. After overnight immersion, each specimen was weighed to obtain its final mass. The rate of increase of mass in each specimen was calculated as the difference between final and initial mass. The specimens were soaked in the three solutions to estimate expansion due to solution absorption. Specimen thickness was measured under an optical microscope at predetermined times up to 240 min, and specimen expansion was presented as the rate of change of thickness.

Tensile strength The specimen thickness was measured prior to testing with a digital caliper. Specimens were divided into 4 groups, one under a dry condition, and the other three under wet conditions. Wet-condition specimens were soaked in each of the three solutions. Specimens were then subjected to tensile stress on a micro mechanical testing machine. Tensile stress and Young's modulus were estimated.

Drug release One-percent trypan blue solution was prepared as an immersion fluid base. Ultraviolet absorbance (UV) was measured for each dilution using a UV spectrophotometer, and trypan blue concentration was determined by using the standard curve of concentration versus UV absorbance.

Biocompatibility Specimens were implanted in the subcutaneous layer on the back, into the latissimus dorsi muscle, and under the periosteum of the mandibular angle of rats to estimate biocompatibility. Rats were sacrificed by overdosing with anesthetic at 3, 7, 28, and 56 days after implantation. Specimens were explanted with the surrounding tissue, and samples were processed for routine paraffin embedding. Routine hematoxylin and eosin staining was then performed.

RESULTS AND DISCUSSION

Characterization of specimens BC had a unique laminated structure consisting of compact layers and porous interconnecting layers resembling a *mille-feuille* in scanning electron microscope image. BC had a larger specific surface area (49 m^2/g) than that of PP (1 m^2/g).

Solution absorption and expansion BC showed noticeably higher absorption than PP.

The absorption rate of BC was 85-fold its weight, while that of PP was about 8-fold its own weight. Therefore, BC is capable of effectively removing dental root canal fluid. BC showed significantly higher expansion than PP. BC expanded to about 3 times its initial thickness after 4 h, whereas PP showed no obvious expansion in thickness during the soaking period. Recently, calcium hydroxide paste and antibiotics have been widely used in intracanal medication, but their efficacy depends on concentration and duration of contact. A more detailed investigation is needed to elucidate the effect of pressing medicaments onto dental canal walls.

Tensile strength PP showed significantly higher tensile strength than that of BC under the dry conditions. However, PP was drastically weakened under the wet conditions, while the tensile strength of BC showed no significant change under either condition. Thus, the tensile strength of BC was higher than PP under wet conditions. This means that BC maintains tensile strength after absorption of root canal fluid or blood, and the inserted BC can be easily removed without fragmenting.

Drug release The cumulative release of trypan blue was significantly greater from BC than from PP. The average levels of trypan blue released from BC were around 20-fold larger than those from PP at 3 min. Therefore, BC can retain a large amount of liquid medicaments which might improve efficacy.

Biocompatibility In the animal experiments, histological examination showed that BC had not been resorbed under the periosteum of the mandibular angle. On day 3, BC was surrounded by inflammatory cells, and no cell invasion was seen, whereas PP seemed to fragment, and inflammatory cells had infiltrated the spaces between the PP fragments. Eight weeks later, a higher number of giant cells were observed for PP than for BC. BC maintained its physical integrity in all groups and did not fragment, and foreign body reaction was seen only in the area surrounding BC. BC in the subcutaneous layer and in the latissimus dorsi muscle showed similar results. The present findings indicate that BC is safer for use in biological tissues than more widely used filling materials. However, further clinical examinations that include application to the dental root are needed.

CONCLUSION

A novel material for dental canal treatment made from BC has favorable material and biological characteristics compared with conventional PP. The absorption rate of BC was 10-fold greater than that of PP, providing a significantly higher expansion of BC over PP. The tensile strength of BC was higher than that of PP under wet conditions. The animal experiments showed that BC maintains its physical integrity and does not fragment, and only a small foreign-body reaction was observed. This is the first study to demonstrate that BC has good potential for use as a material for dental root canal treatment.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

根管治療のポイントは、感染組織を機械的・化学的に除去することである。現行法では、植物由来のセルロースからなるペーパーポイントを治療材として、根管内感染組織の除去や薬剤の貼付を行っているが、根管内は複雑な形態を有するため、その治療材には、より高い吸収性と強度、さらなる消毒効果の増強や生体安全性が求められる。本研究は、酢酸菌が産生するバクテリアルセルロースを用いて新規治療材を開発し、その適用性を評価することを目的としたものである。

申請者はバクテリアルセルロースを乾燥・圧縮後成型して新規治療材（BC)を作製し、その性質を現行治療材ペーパーポイント（PP)と比較検討した。模擬体液として生理食塩水、1号ならびに3号輸液を用い、その吸水重量と、吸水時の厚み変化の測定から、BCは現行品の約10倍の吸水性と高い吸水膨張率を持つこと、乾燥状態と湿潤状態における引張り強度の測定から、PPの引張り強度が湿潤状態においては著しく減少するのに対し、BCは湿潤状態においても強度をそのまま保ち、吸水してもちぎれにくく根管内に残留しにくいことを示した。さらに、BCは薬剤放出性にも優れ、消毒薬の効果を高めること、組織埋入試験においてはPPより異物反応が少ないことも明らかにした。以上のようなBCの特性はそのミルフィーユ構造と細長い繊維の網目構造による比表面積の高さによるものであることを、走査型電子顕微鏡による表面や断面の観察、ガス吸着法による表面積の測定結果に基づいて考察している。本研究で得られたこれらの知見は学術的にも臨床的にも価値あるものであり、学位（医学）の授与に値すると判断した。

最終試験又は学力の確認の結果の要旨

申請者はバクテリアルセルロースの根管治療材としての適用評価を行い、現行品を凌ぐ優れた性質を見いだした。これは学術的にも臨床的にも高く評価できるものである。さらに最終審査では研究の背景、今後の展望と課題についても明解に述べ、博士の授与に値する高い学識と研究能力が認められた。　（主査　吉田正人）

申請者はバクテリアルセルロースによる根管治療材の開発を行い、現行素材との比較検討を行った。新素材は高い吸収性、生体低反応性などより安全性に優れ、今後、臨床治療成績をあげる可能性を示した。関連知識も豊富で学位授与に値すると判断した。　（副査　大平明弘）

申請者は新規根管治療材としてのバクテリアルセルロースと既存の根管治療材について諸所の観点から比較試験を行い、バクテリアルセルロースが優れた性質を有することを明らかにした。治療成績を上げる材料となる可能性が期待され、さらなる応用や今後の発展性についても明確であり、博士の学位に値すると判断した。　（副査　竹下治男）

（備考）要旨は、それぞれ４００字程度とする。