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Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth

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ABSTRACT

Objective: The aim of this paper was to compare the chemical composition of human teeth with other mammal species that are likely candidates for replacing them in studies that test dental material.

Design: Dentine and enamel fragments extracted from 400 sound human, bovine, porcine and ovine – 100 teeth per species – incisors and molars were mechanically ground up to a final particle size of less than 100 μm. C/N analysis, thermogravimetric analysis coupled to mass spectrometry (TG–MS), and wavelength dispersive X-ray fluorescence (WDXRF) were used to analyse the samples' composition.

Results: Elemental analysis showed more organic carbon and nitrogen in dentine than in enamel. Human enamel was the most highly mineralised, with C and N values close to hydroxyapatite. Bovine dentine and enamel were the most similar to human. TG–MS: in all species, enamel contained less carbon and organic matter than dentine. Thermal decomposition of human enamel showed great similarity to synthetic hydroxyapatite, and large differences from bovine, ovine and porcine enamel. Thermal decomposition showed the greatest similarity between human and bovine dentine. WDXRF: Dentine contained larger quantities of Mg, S, Sr and Zn than enamel. Enamel contained larger quantities of P, Ca, Cl, Cu, K and Ca/P ratio than dentine. Human enamel and dentine contained a higher Ca/P ratio, larger quantities of Cl and Cu and lower quantities of Mg, S, Zn than the animal species.

Conclusions: Elemental analysis, TG–MS and WDXRF have shown that human and bovine enamel and dentine show the greatest similarity among the species analysed.

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1. Introduction

The majority of *in vitro* testing of dental materials is performed on extracted human teeth, which would appear to be the perfect candidates for these studies. However, the use of human teeth suffers several limitations: they are often difficult to obtain in sufficient quantity and with adequate quality, since many are extracted due to extensive caries lesions and other defects; it is hard to establish sample homogeneity because it is difficult to control the source and age of the teeth; finally, increasing awareness of the infection hazard and other ethical issues have led to increased restrictions on their use. As a result, alternative substrates have been proposed and have entered into use in dental research. Several types of non-human teeth, such as primate, bovine, swine, ovine and equine teeth, have been used as substrates for *in vitro* dental experiments. The main criteria for the choice of animal teeth are that the physico-chemical, structural, and biological characteristics should be similar to human teeth.[1–3]

Bovine teeth have been the most widely used substitute for human teeth in dental studies. However, their chemistry and structure are not identical. Indeed, it has been shown that acid etching of bovine enamel causes the formation of a rougher surface and the hydroxyapatite crystals are oval shaped and narrow, in contrast to the round shape observed with human enamel.[4]

The mineral phase of vertebrate teeth contains one or more types of phosphate minerals, predominantly calcium phosphates. The most abundant mineral in human teeth is a basic calcium phosphate idealised as calcium hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$. Other calcium phosphates and magnesium phosphates have been identified with or without association with apatite: brushite ($CaHPO_4{\cdot}2H_2O$), octacalcium phosphate ($Ca_8H_2[PO_4]_6{\cdot}5H_2O$), tricalcium phosphate or whitlockite (β-TCP, β-$Ca_3[PO_4]_2$), calcium pyrophosphate dehydrate ($Ca_2P_2O_7$), and amorphous calcium phosphates, struvite ($MgNH_4PO_4{\cdot}6H_2O$), newberyite ($MgHPO_4{\cdot}3H_2O$), and amorphous calcium magnesium pyrophosphates.[5]

Enamel and dentine are predominately composed of hydroxyapatite crystals. Enamel consists of an inorganic matrix (96%, w/w) and organic constituents (*i.e.* proteins and lipids) and water (4%, w/w), which occupy the gaps among the apatite crystals in the enamel. Hydroxyapatite crystals in enamel are hexagonal and bundled to form approximately 4 μm diameter rods. Mature dentine is about 70% mineral, 20% organic matrix, and 10% water by weight. The hydroxyapatite crystals in dentine are in the form of flattened plates with approximate dimensions of 60–70 nm length, 20–30 nm width, and 3–4 nm thickness. The calcium and phosphorus (as phosphate) content of the teeth range 34–39% and 16–18% by weight, respectively. Various cations and anions are incorporated into cationic (Ca^{2+}) and anionic centres (OH^-, PO_4^{3-}) of the hydroxyapatite matrix. Sodium (Na^+), potassium (K^+), and magnesium (Mg^{2+}) can substitute in the calcium position, fluoride (F^-) and chloride (Cl^-) in the hydroxyl position and carbonate (CO_3^{2-}) in the hydroxyl and phosphate positions. Close to 40 elements have been reported to be present, ranging from ≥1000 ppm (*i.e.*, Zn, Sr, Fe, Al, B, Ba, Pb, etc.) to ≤100 ppb (*i.e.*, Ni, Li, Ag, As, Se, Nb, Hg, etc.).[6,7]

The presence of these trace elements will determine different physico-chemical and biological behaviour; in this way, Scholfield et al.[8] observed a high correlation between tooth hardness and zinc content.

Various analytical methods have been applied for identifying trace elements in teeth. They include atomic absorption spectrophotometry,[9] proton induced X-ray emission,[10] electrothermal vaporisation inductively coupled plasma-mass spectrometry,[11] laser ablation ICP-MS,[12] and energy-dispersive X-ray elemental analysis.[13]

In order to determine which will be the best substrate for replacing human teeth, the elemental composition of enamel and dentine in each species must be known. Although several studies have compared animal with human teeth, none have conclusively warranted the use of animal teeth in laboratory tests.

The aim of this study was to determine the chemical composition of the enamel and dentine in human, bovine, porcine and ovine teeth, using C/N analysis, thermogravimetric analysis linked to mass spectrometry (TG–MS) and wavelength dispersive X-ray fluorescence spectrometry (WDXRF).

2. Materials and methods

2.1. *Experimental groups. Enamel and dentine samples*

The dental substrates studied were bovine enamel, bovine dentine, ovine enamel, ovine dentine, porcine enamel, porcine dentine, human enamel and human dentine; hydroxyapatite powder (Reference #04238, Sigma–Aldrich, St. Louis, MO, USA) was also analysed as a control substrate.

The study used 400 incisors and molars freshly extracted and free from enamel cracks, caries, and fillings: 100 human, 100 bovine, 100 porcine, and 100 ovine. The teeth were washed in water to remove any traces of blood and then stored in distilled water, which was changed daily to avoid deterioration. In no case was a tooth stored for more than a month after extraction.

The teeth were sectioned, removing enamel and dentine, with a water-cooled diamond saw (Horico, Berlin, Germany). To be sure that the selection of fragments had been performed correctly, they were observed with a stereoscopic microscope (NIKON SMZ-U, Yokohama, Japan). Samples were hand-milled with an agate mortar and any pieces larger than 100 μm were ground with a mill (Disc mil HSM 100H, Herzog, Maschinenfabrik, Osnabrick, Germany). Every disc-milling process was refrigerated with 2 mL of hexane (*n*-hexane 95%, Panreac, Barcelona, Spain) in order to avoid structural changes in the specimens. The resulting dust was sieved in order to ensure a controlled comminution.

Samples were dried at 60 °C for 24 h in a furnace (UFP 500, Memmert, Nuremberg, Germany), in order to release most of the free water, and then ground by hand-milling with an agate mortar (NAHITA, Navarra, Spain) and with a disc mill for 1 min, to give a final particle size of less than 100 μm. Lastly, 5 g of powder samples were pelletised using a semiautomatic