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The Effect of Nanozirconia Mixed with Glass-ionomer on Proliferation of Epithelial Cells and Adhesive Molecules

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Abstract

Background and aims. The aim of the present study was to evaluate the effect of nanoparticles of zirconia mixed with glass-ionomer on the proliferation of epithelial cells and adhesive molecules (ICAM-1).

Materials and methods. Zirconia nanoparticles were mixed with glass-ionomer powder in weight percentages of 0%, 5%, 50%, 70%, and 100%. The powders were then mixed with glass-ionomer liquid in 2:1 weight ratios. The paste was then inserted into a steel ring mold (5 mm in diameter and 0.5 mm in thickness) sandwiched between two glass slides. Glass-ionomer was then cured using a light-curing unit. Seven samples (discs) were prepared for each mixing percentage. Cell cultivation (epithelial) and MTT tests were performed to assess the cytotoxicity of specimens containing different nanozirconia contents. Finally, human ICAM-1 platinum ELISA test was performed for quantitative diagnosis of human ICAM-1 epithelial cells.

Results. Statistically significant differences ($p<0.001$) were observed in the cytotoxicity of specimens with different nanozirconia contents after 1 and 24 hours and one week. There were no significant differences between the specimens in relation to the ICAM-1 molecules released from epithelial cells.

Conclusion. The results revealed that incorporation of zirconia nanoparticles (except for the pure zirconia particles) stimulated the adhesion of epithelial cells to the specimens, making the zirconia-containing glass-ionomers promising biomaterials for dental applications. The highest biocompatibility was obtained for 70 wt% of zirconia after 24 hours.

Key words: **Epithelium, glass-ionomer, ICAM-1, zirconia.**

Introduction

There is gingival recession of 1 mm or more in more than 50% of people in one or more locations.[1] Gingival recession, for one reason or another, worries patients and clinicians and its proper treatment, when sufficient keratinized gingiva exists apical to the recession, consists of a pedicled autograft.[2]

However, in some cases on exposed root surfaces in the recession area, there are carious lesions or restorations that make treatment difficult, and that is why the graft does not remain on the restoration for a long time and recedes again.[3] We assumed a combination of glass-ionomer and zirconia nanoparticles is a suitable filling material, based on literature review. Selection of glass-ionomer was based on the chemical and micromechanical bond with tooth root and ability of fluoride release.[4,5] Zirconia nanoparticles were selected because of better biologic compatibility of zirconia due to minimum release of ions,[6,7,8] promotion of connection and proliferation of fibroblasts and osteoblasts on titanium–zirconia discs rather than by titanium only,[9] better coronal repositioning of gingival margin and reconstruction of gingiva automatically around zirconic collar area when titanium implants are used with zirconia collar,[10] better and easier connection of human gingival fibroblasts on zirconium nitride surfaces rather than titanium and titanium nitride surfaces[11] and less inflammatory reaction of soft tissue around zirconia healing cap rather than titanium oxide alone.[12] Schreiber et al[13] evaluated surface roughness of TiN and ZrN cover and examined formation of focal adhesion contacts (FACs) by gingival fibroblasts in an in vitro study in 2006. Samples were examined on the third day after seeding. The greatest numbers of FACs were observed on surfaces with the least surface roughness (ZrN, TiN or Ti). They observed extracellular fibrinectins, vitronectins and intracellular actins and vancolites in FAC surfaces by immune gold-labeling assay; again, the greatest numbers of the gold particles were related to surfaces with less roughness. These surfaces, especially zirconium nitride, facilitate attachment of human gingival fibroblasts.[14] Bianchi et al carried out an in vitro study to compare connection and proliferation of fibroblasts and osteoblasts on implants made from titanium, titanium with zirconia collar (TBRZ) and thermanox polymeric substrate on tetragons fixed with yttrium oxide (Y-TZP) and titanium discs with a diameter of 0.4 cm and sterilized for 2 hours at 160˚C in standard conditions. In this study after 6 hours of cellular incubation, connection of cells in two experimental and one control group (thermanox polymeric substrate) was compared and it was concluded that connection of cells on titanium disks with zirconia cover was more than that in the control group; in addition, cell connections were more numerous in the control group than titanium disks. Furthermore, after 4 days of cellular incubation, they concluded that cell proliferation on titanium disks with zirconia cover was more noticeable than the control group and in the control group was more than titanium disks.[10] In 2006 Degidi et al compared inflammatory reaction of soft tissue around titanium healing cap with that of soft tissue around zirconium oxide (Y-TZP) healing cap. They evaluated biopsies of patients' soft tissue and reported that inflammatory infiltration in peri-implant soft tissue (mainly in submucosal areas) around zirconium oxide cap is less than that around titanium oxide caps. Capillary density was less than titanium oxide caps and NOS-1 and NOS-2, indicators of NO synthase, were less noticeable in tissues around zirconium oxide. They finally concluded that the tissue around zirconium caps is subject to processes of less inflammatory grading.[15] Bianchi et al published the results of a human clinical trial showing the advantages of a transmucosal titanium implants with a bioactive zirconia (Y-TZP) collar (i.e. hybrid system) on soft and hard tissues in a nonsubmerged approach (one-time surgery) for a two-year period. By analyzing various parameters, such as plaque index, bleeding on probing, and measures of mucosal sulcus depth around implants via clinical and radioscopic analyses, the authors reported that zirconia collar type implant offers better tissue stabilization than titanium. Their observation was also corroborated by in vitro adhesion, spreading and proliferation of fibroblasts and osteoblasts, showing that zirconia-coated titanium improves all the three cell parameters for both cellular types. However, in humans, with this one-time surgical intervention, the collar composed of zirconia is not directly in contact with bone tissue compared with titanium but is closer to soft tissue. Therefore, the authors demonstrated an improvement in the biocompatibility of zirconia. According to this very recent research, it can be concluded that surface roughness and thus the "finishing" (polishing) of zirconia is of utmost importance for osseo-integration of this biomaterial. However, for humans, available hybrid systems for dental implantology, composed of titanium and zirconia collar, would improve the periointegration by preserving both mucosal and bone levels.[16] Considering recent cases and limited number of research studies in this regard on epithelial cells, one comes to the conclusion that it is possible to fill exposed root surfaces with a combination of zirconia nanoparticles and light-cured glass-ionomer to not only save gingiva from recession after root coverage but also to make perio-integration possible.[17,18] Therefore, the aim of this study was to determine the effect of zirconia nanoparticles mixed with glass-ionomer on the number and proliferation of epithelial cells and adhesive molecules (ICAM-1). The results

of this study might help select the best curing policy, especially in cases of exposed roots.

Materials and Methods

Preparation of Glass-ionomer Containing Zirconia Nanoparticles

At first the weight percentages of 0%, 5%, 50%, 70%, and100% of zirconia nanoparticles (ZrO_2) with dimensions of 40-60 nm (Nanoshel, HARYANA, India) were measured to 4 decimal figures by a digital weighing machine and then each weight percent was separately added to glass-ionomer powder (Fuji II LC, GC,Tokyo Japan). It means that zirconia nano-particles were not added to 0% group, which was considered the control group. Then the 5% group received 0.0313% gr of nanoparticles; 50% group received 0.3125% gr of nanoparticles; 70% group received 0.4375% gr of nanoparticles; and 100% group received 0.625% gr of nanoparticles. For suitable combination and distribution of particles a mortar and a pestle were used for 20 minutes. The nanopaticles were completely mixed with the powder of each weight group separately to achieve an equal and uniform distribution of particles. Glass-ionomer powder with different percentages of Zirconia nanoparticles with specified ratio of liquid-to-powder (0.625 gr powder and 0.375 gr liquid) was mixed. The mold was completely filled with the material after mixing for 25 seconds on a glassy clock screen (Figure 1a). The metal mold used in this research was ring form and after filling with the material and curing, the discs produced were 5 mm in diameter and 0.5 mm in thickness. The samples were light-cured with an LED light-curing unit (Figure 1b). The specimens were immediately stored in distilled water in an incubator (100% relative humidity at 37˚C) after curing. The samples of epithelial cell cultivation included the following:

- 7 samples consisting of 0% zirconia nano-filler
- 7 samples consisting of 5% zirconia nano-filler
- 7 samples consisting of 50% zirconia nano-filler
- 7 samples consisting of 70% zirconia nano-filler
- 7 samples consisting of 100% zirconia nano-filler

Cell Cultivation and MTT Tests

In this study, the cells were A431 epithelial cells (Figure 1c). At first, usable cells were reproduced. The number of cells was supposed to be 500,000 cells in mililiter. Counting was performed by Neobier

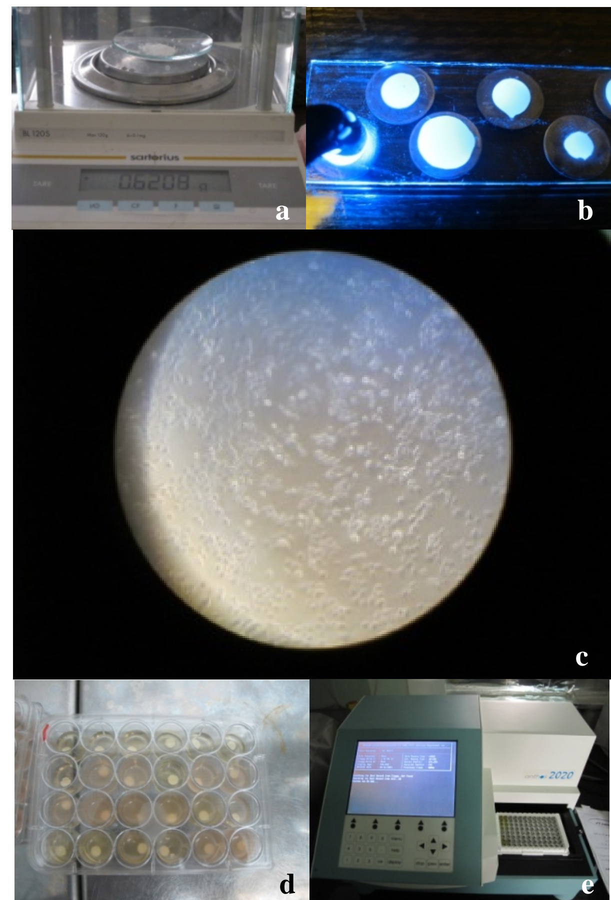

Figure 1. Glassy clock screen including zirconia mixed with glass-ionomer powder on a digital weighing machine (a). Curing samples sandwiched between two glass slides with the light-curing unit (LED) (b). A431 epithelial cells under a light microscope (c). Contiguity of cells with samples (discs) in plate wells (d). ELISA Densitometer (ELISA Reader) (e).

LAM. These ready cells were added to 24 cultivation plate wells. The number of cultivation plate wells was selected in proportion to the number of samples added to the plate. Contiguity of the cells with the samples (discs) was checked at 1-hour, 24-hour and 1-week intervals (Figure 1d). Two hundred mL of the mixture in the wells was retrieved and poured in microtubes and kept in a refrigerator at 70˚C to follow ICAM-1 by ELISA test. In order to carry out this test, at first the samples and culture media were retrieved from the wells. The cells underwent an MTT test, which demonstrates cell vitality and activity. Two hundred µL of 4% acid–alcohol (4% acid choloridric + isopropyl alcohol) was added to all the wells. This acid–alcohol tears the bottom cells of the wells and can revive MTT and change it to formasan to pour out of the cells. Then 100 µL from each well was taken and added to 96 plate wells specially used for ELISA. Then by using an ELISA densitometer

(ELISA Reader) a wavelength of 490 nm was recorded (Figure 1e). In this case the system gives us an optical density (OD) and as OD increases and shows that the sample has had a simulation effect on the cells; when OD decreases, it means that the sample has exerted an inhibitory effect on the cells or has destroyed them.

Human ICAM-1 Platinum ELISA Test

ELISA is an enzyme-linked immunosorbent method for quantitative diagnosis of human ICAM, which is only used for research not for diagnostic or therapeutic purposes. The plate was washed twice with 400 µL of buffer. From the standards prepared, 100 µL was added to the standard plates. 100 µL from the diluting solution was added to blank wells. 60 µL from the diluting solution was added to other wells and then 40 µL from the samples was added to all the wells. Plate cover was placed and heated for one hour on a shaker with a rate of 100/min at room temperature. After one hour the plates were washed 3 times with 400 µL of buffer. 50 µL from HRP solution was added to all the wells. It was placed on a shaker for 1 hour at room temperature. After 1 hour, it was washed 3 times again. 100 µL from TMB substrate was added to all the wells. It was placed in dark for 10 minutes. 100 µL from the stop buffer solution was added to all the wells. Photon absorption of ELISA reader was recorded at 450 nm with a reference of 620 nm.

Statistical Analysis

In this research in addition to descriptive statistical indicators such as averages, standard deviations etc analytical statistics methods were used for assumptions test. To evaluate normal distribution of data Kolmogorov-Smirnov, one-sample test and to test variance equality Levene test were used. One-way ANOVA was used to compare cytotoxicity of different materials and Tukey HSD test was used for two-by-two comparisons. In order to compare cytotoxicity of every group at different time intervals repeated measures ANOVA was used. Statistical significance was defined at A $p<0.05$ was considered statistically significant.

Results

The differences in the means of cytotoxicity of zirconia different percentages (0%, 5%, 50%, 70%, and 100% mixed with glass-ionomer) on epithelial cells after one hour, 24 hours and one week were statistically significant ($p <0.001$; Figures 2, 3 and 4). Cytotoxic effects of the group with 100% zirconia on epithelial cells at 1-hour (1), 24-hour (2), and 1-week (3) intervals were as follows: The differences between 1 and 3 were significant but the differences between 2 and 3 were not. Cytotoxic effects of the group with 70% zirconia on epithelial cells at 1-hour (1), 24-hour (2), and 1-week (3) intervals were as follows: The differences between 1 and 3 were not significant; in

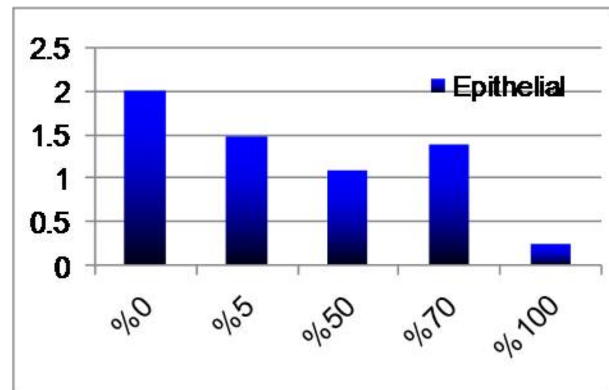

Figure 2. Cytotoxicity of different zirconia concentrations after1 hour.

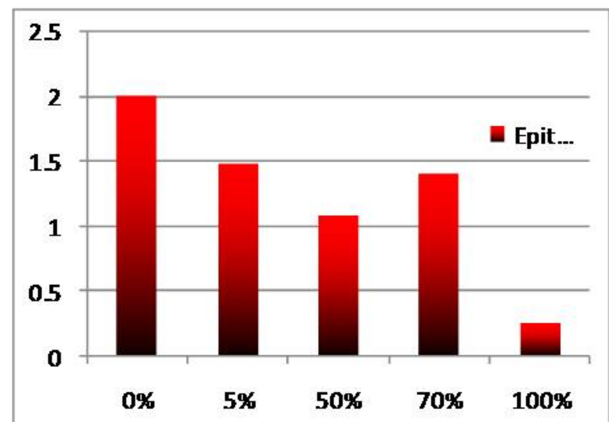

Figure 3. Cytotoxicity of different zirconia concentrations after 24 hours.

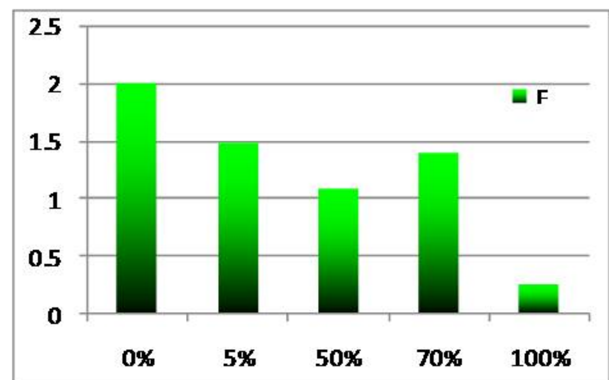

Figure 4. Cytotoxicity of different zirconia concentrations after 1 week.

addition, the differences between 2 and 3 were not significant. Cytotoxic effects of the group with 50% zirconia on epithelial cells at 1-hour (1), 24-hour (2), and 1-week (3) intervals were as follows: The differences between 1 and 3 were not significant but the differences between 2 and 3 were significant. Cytotoxic effects of the group with 5% zirconia on epithelial cells at 1-hour (1), 24-hour (2), and 1-week (3) intervals were as follows: The differences between 1 and 3 and also between 2 and 3 were significant. Cytotoxic effects of the group with 0% zirconia on epithelial cells at 1-hour (1), 24-hour (2), and 1-week intervals (3) were as follows: The differences between 1 and 3 were not significant but the differences between 2 and 3 were significant. There were no significant differences between the specimens in relation to the ICAM-1 molecules released from epithelial cells (Table 1).

Discussion

The results of this study showed that 1 hour after cultivation of the cells, different ratios of zirconia exhibit cytotoxic effects on epithelial cells, with statistically significant differences between the zirconia concentrations used as follows: 100% > 70% > 50% > 5% > 0%.

Two-by-two comparison of the groups showed statistically significant differences between the 100%, 70%, and 50% groups on one hand and the 5% and 0% groups on the other. However, no significant differences were observed between 100% and 70%, 100% and 50%, 70% and 50%, and 5% and 0% groups.

At 24-hour evaluation after cultivation, 70% concentration exhibited the lowest cytotoxic effects on epithelial cells. Therefore, the cytotoxic effects of different concentrations of zirconia can be summarized as follows: 100% > 50% > 5% > 0% > 70%.

With the exception of 0% concentration, there were significant differences between the other concentrations and 70% concentration regarding cytotoxic effects on epithelial cells. It is noteworthy that the differences between 100% ratio and all the other groups were significant. In addition to what was mentioned above there were significant differences between 50% and 5%, and 50% and 0%; however, no significant differences were observed between 0% and 5% concentrations.

Table 1. Comparison of five specimens in relation to ICAM-1 molecules released from the epithelial cells

Item	*N*	*Mean*
100%	7	0.067219
70%	7	0.063859
50%	7	0.063859
5%	7	0.066859
0%	7	0.063859

Evaluation of the epithelial cells one week after cultivation showed that 0% concentration had the lowest cytotoxic effect on epithelial cells. Therefore, the cytotoxic effect of different concentrations of zirconia can be summarized as follows: 100% > 50% > 70% > 5% > 0%.

Two-by-two comparison of the groups showed significant differences between 100% concentration and the other groups and between 0% and 50%, and 50% and 70% groups.

Nothdurft & Pospiech[19] did not report any inflammatory reactions around prefabricated zirconium for posterior single teeth at 12-month interval, and the tissue around them was healthy. The differences between the results might be attributed to differences in the methods of the two studies (in vivo vs. in vitro) and the study period.

Gomes et al[20] reported that adding zirconium oxide as a radiopaque factor to Portland cement does not induce cytotoxic effects on mouse PDL cells after 24 hours. In the present study, 70% zirconia exhibited a minimal cytotoxic effect after 24 hours. This discrepancy between the results might be attributed to differences between the densities and the zirconium type. In the research study cited above zirconium was added to Portland cement rather than directly to the culture medium at 100% concentration, while in the present study after 24 hours this concentration of zirconia (100%) exhibited significant differences from other ratios as to cytotoxic effects.

Bianchi et al[21] reported that connection and proliferation of fibroblasts and osteoblasts to titanium–zirconia discs is definitely higher than titanium disks (one proposal). As is seen, there is much similarity between the results of the research above and those of the present study, i.e. in the present study, too, the highest ICAM-1 ratio was observed in disks having 5% and 50% zirconia concentrations.

Conclusion

The results revealed that incorporation of pure zirconia nanoparticles should be avoided and zirconia nanoparicles would probably be more efficient in concentrations less than 5%.

References

1. Kassab MM, Cohen RE. The etiology and prevalence of gingival recession. *J Am Dent Assoc* 2003;134:220-5.

2. Newman MG, Takei HH, Klokkevold PR, Carranza FA. *Clinical Periodontology*, 10th ed. St. Louis: Elsevier Saunders; 2006:1016-21.
3. Alkan A, Keskiner I, Yuzbasioglu E. Connective tissue grafting on resin ionomer in localized gingival recession. *J Periodontol* 2006;77:1446-51.
4. Momoi Y, Hirosaki K, Kohno A, McCabe JF. Flexural properties of resin-modified "hybrid" glass-ionomers in comparison with conventional acid-base glass-ionomers. *Dent Mater J* 1995;14:109-19.
5. Mohammadi Basir M, Haj Ali Mohammadi M. Flextural strength evaluation of six type of restorative glass-ionomer cements in three different times. *Journal of Islamic Dental Association of Iran* 2008;2:118-150.
6. Müller K, Valentine-Thon E. Hypersensitivity to titanium: clinical and laboratory evidence. *Neuro Endocrinol Lett* 2006;27:31-5.
7. Stejskal J, Stejskal VD. The role of metals in autoimmunity and the link to neuroendocrinology. *Neuro Endocrinol Lett* 1999;20:351-364.
8. Depprich R, Zipprich H, Ommerborn M, Mahn E, Lammers L, Handschel J, Naujoks C, Wiesmann HP, Kübler NR, Meyer U. Osseointegration of zirconia implants: an SEM observation of the bone-implant interface. *Head Face Med* 2008;4:25.
9. Bianchi AE, Bosetti M, Dolci G Jr, Sberna MT, Sanfilippo S, Cannas M. *In vitro* and *in vivo* follow-up of titanium transmucosal implants with a zirconia collar. *J Appl Biomater Biomech* 2004;2:143-50.
10. TBR Research Center. Aesthetic benefits of the Zirconia-titanium technology: an *in vivo* study. Available from: http://www.tbr-group.com.
11. Degidi M, Artese L, Scarano A, Perrotti V, Gehrke P, Piattellr A., Inflammatory infiltrate, microvessel density, nitric oxide synthase expression, Vascular endothelial growth factor expression, and proliferative activity in peri-implant soft tissues around titanium and Ziroconium oxide healing caps. J Periodontal 2006;77:73-80.
12. Williams JA, Billington RW. Changes in compressive strength of glass ionomer restorative materials with respect to time periods of 24 h to 4 months. *J Oral Rehabil* 1991;18:163-8.
13. Hisbergues M, Vendeville S, Vendeville P. Zirconia: Established facts and perspectives for a biomaterial in dental implantology. *J Biomed Mater Res B Appl Biomater* 2009;88:519-29.
14. Trowbridge SHO, Emling RC. *Inflammation: A Review of the Process*, 5th ed. Chicago: Quintessence Publishing; 1997.
15. Degidi M, Artese L, Scarano A, Perrotti V, Gehrke P, Piattelli A. Inflammatory infiltrate, microvessel density, nitric oxide synthase expression, vascular endothelial growth factor expression, and proliferative activity in peri-implant soft tissues around titanium and zirconium oxide healing caps. *J Periodontol* 2006;77:73-80.
16. Rimondini L, Cerroni L, Carrassi A, Torricelli P. Bacterial colonization of zirconia ceramic surfaces: an *in vitro* and *in vivo* study. *Int J Oral Maxillofac Implants* 2002;17:793-8.
17. Mallmann A, Ataíde JC, Amoedo R, Rocha PV, Jacques LB. Compressive strength of glass ionomer cements using different specimen dimensions. *Braz Oral Res* 2007;21:204-8.
18. Momoi Y, Hirosaki K, Kohno A, McCabe JF. Flexural properties of resin-modified "hybrid" glass-ionomers in comparison with conventional acid-base glass-ionomers. *Dent Mater J* 1995;14:109-19.
19. Nothdurft F, Pospiech P. Prefabricated zirconium dioxide implant abutments for single-tooth replacement in the posterior region: evaluation of peri-implant tissues and superstructures after 12 months of function. *Clin Oral Implants Res* 2010;21:857-65.
20. Gomes SC, Miranda LA, Soares I, Oppermann RV. Clinical and histologic evaluation of the periodontal response to restorative procedures in the dog. *Int J Periodontics Restorative Dent* 2005;25:39-47.
21. Bianchi AE, Bosetti M, Dolci G Jr, Sberna MT, Sanfilippo S, Cannas M. *In vitro* and *in vivo* follow-up of titanium transmucosal implants with a zirconia collar. *J Appl Biomater Biomech* 2004;2:143-50.