

GENOTOXICITY ASSESSMENT OF CONVENTIONAL AND CAD/CAM DENTURE BASE MATERIALS

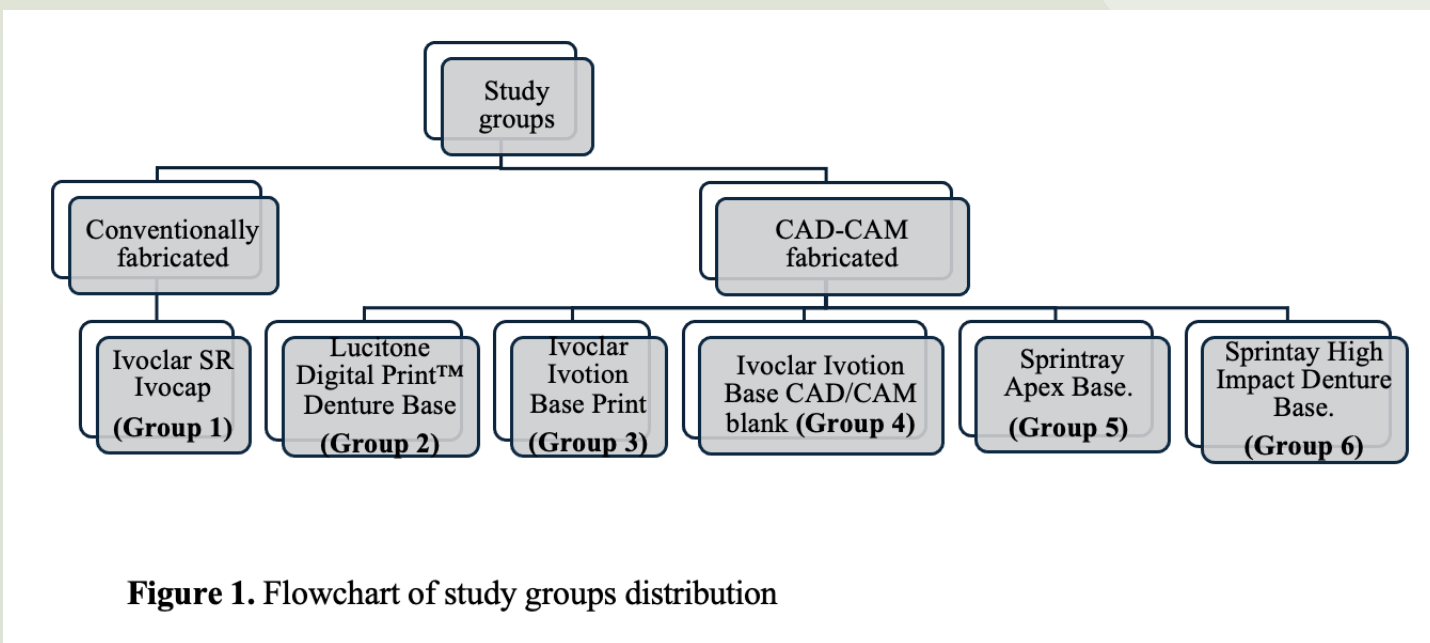
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INTRODUCTION

Advancements in techniques and materials have been witnessed in the field of prosthodontics. Among these materials is Polymethyl Methacrylate (PMMA), which was invented to replace vulcanized rubber. Nowadays, it serves as the foundation for removable dental prostheses. It is the most commonly used denture material, because of its superior aesthetics and mechanical properties. The chemical composition of PMMA consists of a polymer matrix composed of methyl methacrylate monomers (MMA) that undergo polymerization to form a solid material. Heat-cured PMMA resin is the most commonly used method of removable prostheses fabrication due to its superior physical and chemical properties, ease of processing, and low cost. Despite these advantages, there are a few limiting properties, the most important of which is the release of residual methyl methacrylate monomer. Polymethyl methacrylate (PMMA) biocompatibility and potential genotoxicity remain a concern due to the residual monomer release. Computer-aided design and manufacturing (CAD/CAM) denture base materials have emerged and offered some improved mechanical and physical properties compared to the standard conventional heat-cured denture base material. However, their long-term biocompatibility remains unstudied. Therefore, this study evaluates the genotoxicity effects of conventional and CAD/CAM denture base materials on human gingival fibroblasts (HGFs).

MATERIALS AND METHODS

Study groups: The study groups are structured into two main categories: Conventional fabrication, which will represent the control group, and CAD/CAM fabrication. The CAD/CAM category includes milled and 3D-printed denture base resins as shown in Figure 1.



Denture disk samples preparation: Sample preparations for all the groups have followed the International Organization for Standardization (ISO 10993-12:2012) guidelines. To fabricate the heat-cured Ivocap resin samples, the reference disk design (STL file) was used to 3D print ten wax patterns. These wax patterns were then processed conventionally with pink Ivocap (Ivoclar) denture base resin. The samples were left unpolished and unfinished to mimic the intaglio surface of a complete denture. The CAD/CAM samples were fabricated according to the recommended workflow for each material.

Preparation of denture resin-disk extracts: Resin disks were sterilized, incubated in DMEM medium under controlled conditions, and extracts were collected at 1 and 7 days for testing on human gingival fibroblasts with proper controls.

Cell culture: Human gingival fibroblasts obtained from biopsies were cultured in DMEM with supplements, expanded in flasks, detached using trypsin-EDTA, and seeded for experiments.

DNA Damage assay: γ -H2AX biomarker was used to detect DNA double-strand breaks in fibroblasts after exposure to resin extracts and controls.

Cell culture and treatment protocol: Fibroblasts seeded at 10,000 cells/well were exposed to DMEM, Etoposide (positive control), or resin extracts for 6 hours before processing with a DNA damage assay kit.

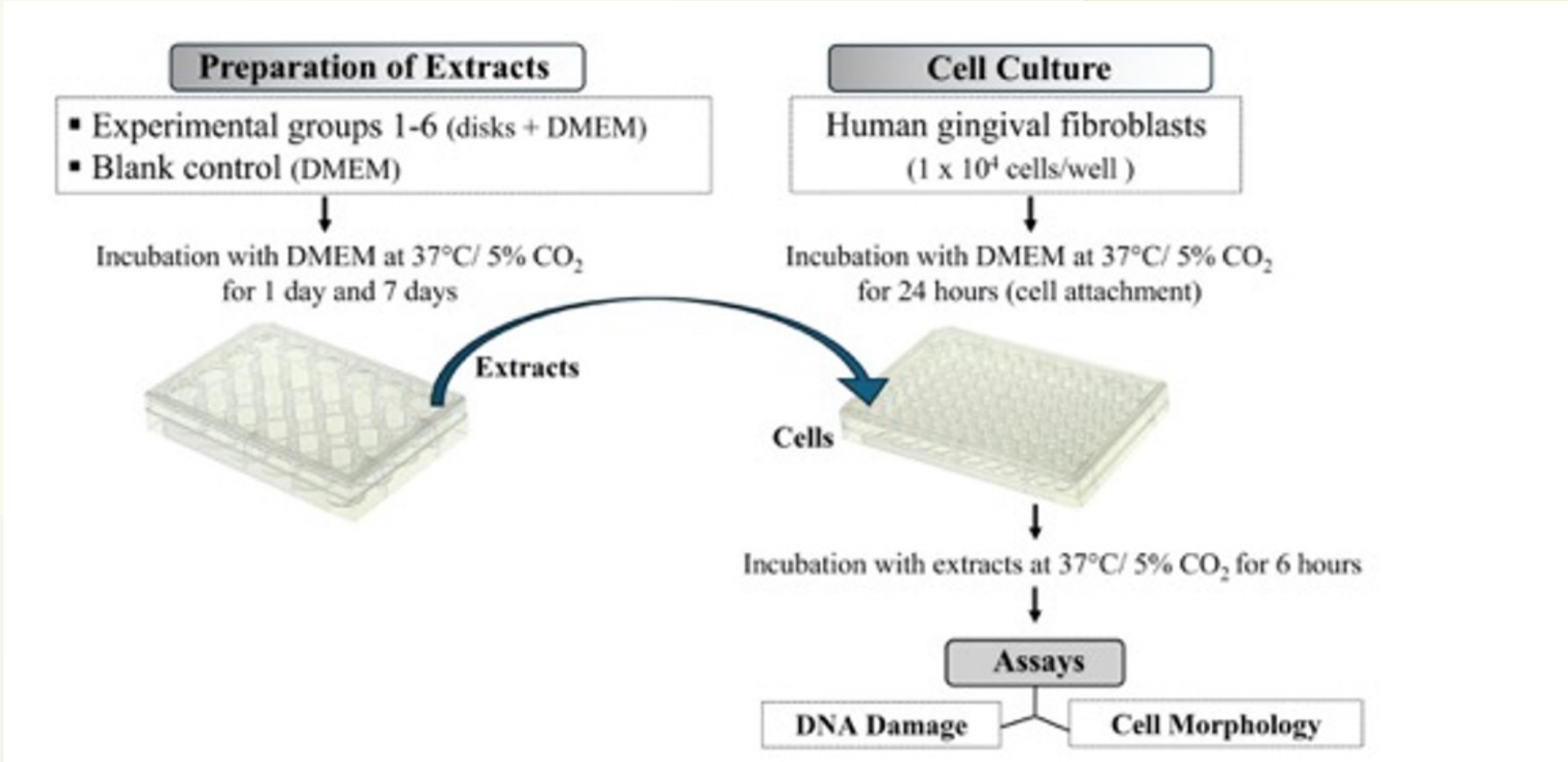


Figure 2. Preparation of extracts and cell culture diagram. DMEM: Dulbecco's Modified Eagle's Medium.

Cell fixation and membrane permeabilization: Cells were fixed with formaldehyde, washed, and permeabilized to prepare for antibody-based detection.

Blocking procedure: Endogenous peroxidase activity was quenched with hydrogen peroxide and blocked with buffer to prevent nonspecific binding.

Antibody incubation: Cells were incubated with primary anti- γ H2AX antibody, washed, and then treated with HRP-conjugated secondary antibody.

Colorimetric development and quantification: DNA damage was quantified by TMB substrate reaction, stopped with solution, and absorbance measured at 450 nm to calculate damage percentage.

Microscopic evaluation of cellular morphology: Fibroblast morphology after exposure to resin extracts was observed and documented using phase contrast microscopy.

Statistical analysis was performed using R (v4.3.2), where means and standard deviations of DNA damage were calculated, ANOVA tested group differences with day 1 and day 7 as factors, Tukey's post-hoc identified intergroup differences, paired t-tests compared within-group changes over time, and significance was set at $p < 0.05$.

RESULTS

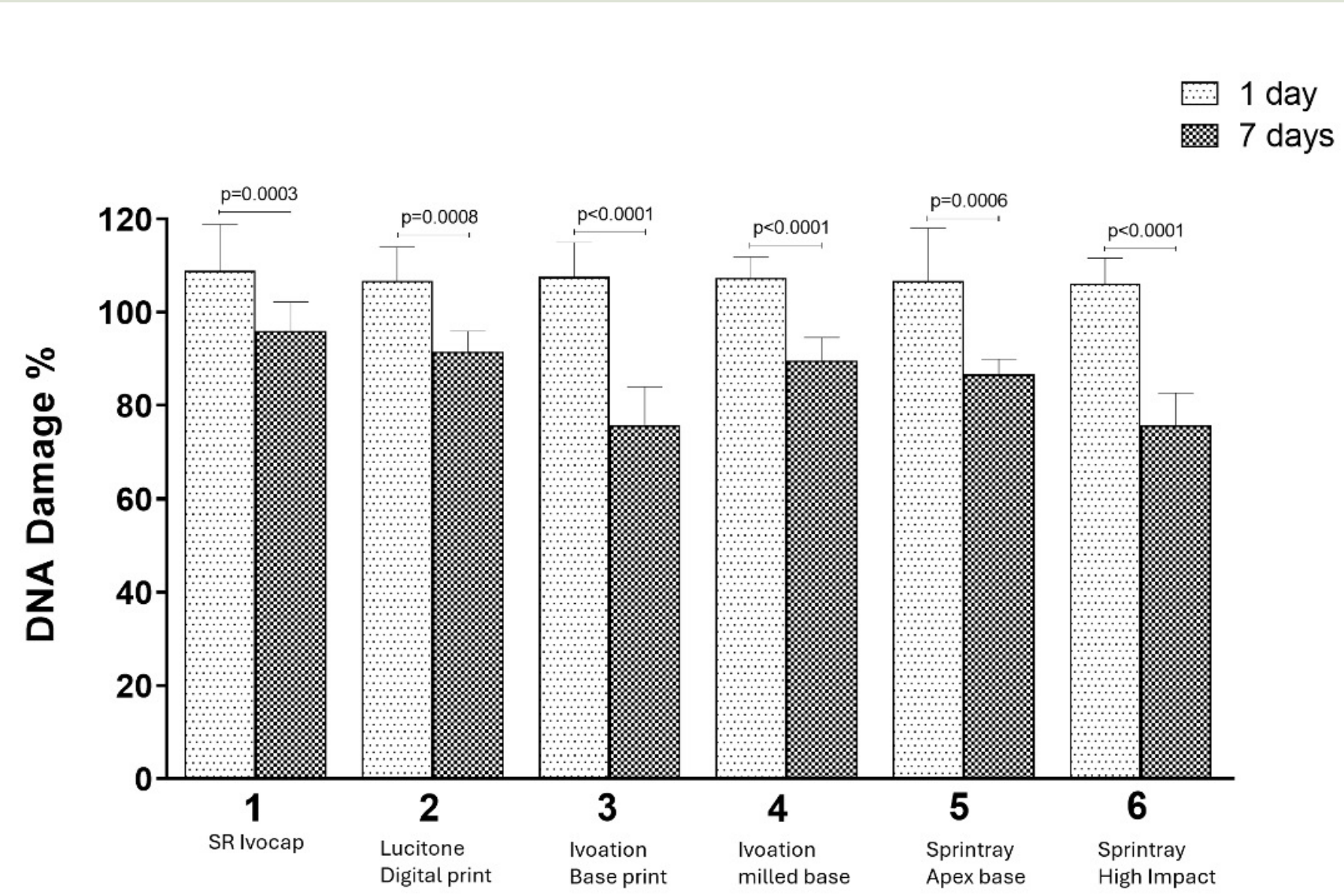


Figure 3. DNA damage after 6 hours of incubation with denture base resin disks extracts groups 1-6, from 1- and 7-days incubation periods. Results are presented as mean $\pm$ SD.

Groups	DNA damage Day-1		DNA damage Day-7	
	Mean	Std. Deviation	Mean	Std. Deviation
Ivoclar SR Ivocap Heat-Cure Polymer (Group 1)	108.8	9.9	96.0	6.1
Dentsply Sirona Lucitone Digital Print (Group 2)	106.7	7.2	91.4	4.4
Ivoclar Ivotion Base Print (Group 3)	107.5	7.3	75.7	8.2
Ivoclar Ivotion Base CAD/CAM blank (Group 4)	107.3	4.4	89.6	4.8
Sprinray Apex Base (Group 5)	106.6	11.3	86.6	3.2
Sprinray High Impact Denture Base (Group 6)	106.1	5.4	75.7	6.9

Nine samples from six groups were analyzed for DNA damage at day 1 and day 7. On day 1, Group 1 (Ivoclar SR Heat-cure) showed the highest DNA damage ($108.8 \pm 9.9\%$), while Group 6 (Sprinray High Impact) showed the lowest ($106.1 \pm 5.4\%$), with no statistically significant differences among groups ($p = 0.984$). By day 7, Group 1 maintained the highest DNA damage, whereas 3D-printed bases (Ivoclar Ivotion and Sprinray High Impact) showed the lowest levels with marked reduction from baseline. ANOVA confirmed highly significant group differences at day 7 ($p < 0.001$), and Tukey post-hoc tests revealed that Groups 1 and 2 were significantly different from most other groups, while Groups 3 and 6 showed no difference between them but differed significantly from all other materials.

Table 2: ANOVA between groups comparison for γ -H2AX at day1					
Source	SS (Sum of Squares)	DF (Degrees of Freedom)	MS (Mean Square)	F	p-value
Between Groups	328.1	5	65.62	1.87	0.123
Within Groups	843.7	24	35.15		

Table 3: ANOVA between groups comparison for γ -H2AX at day7					
Source	SS (Sum of Squares)	DF	MS	F-value	p-value
Between Groups	3432.0	5	686.5	19.61	7.62e-11*
Within Groups	1786.0	51	35.0		

Phase-contrast microscopy shows typical spindle-shaped morphology of Fibroblast cells at both time points, with the exception of Etoposide concentrations (20 μ M and 25 μ M) (Figure 4C, D), which displayed altered cellular morphology.

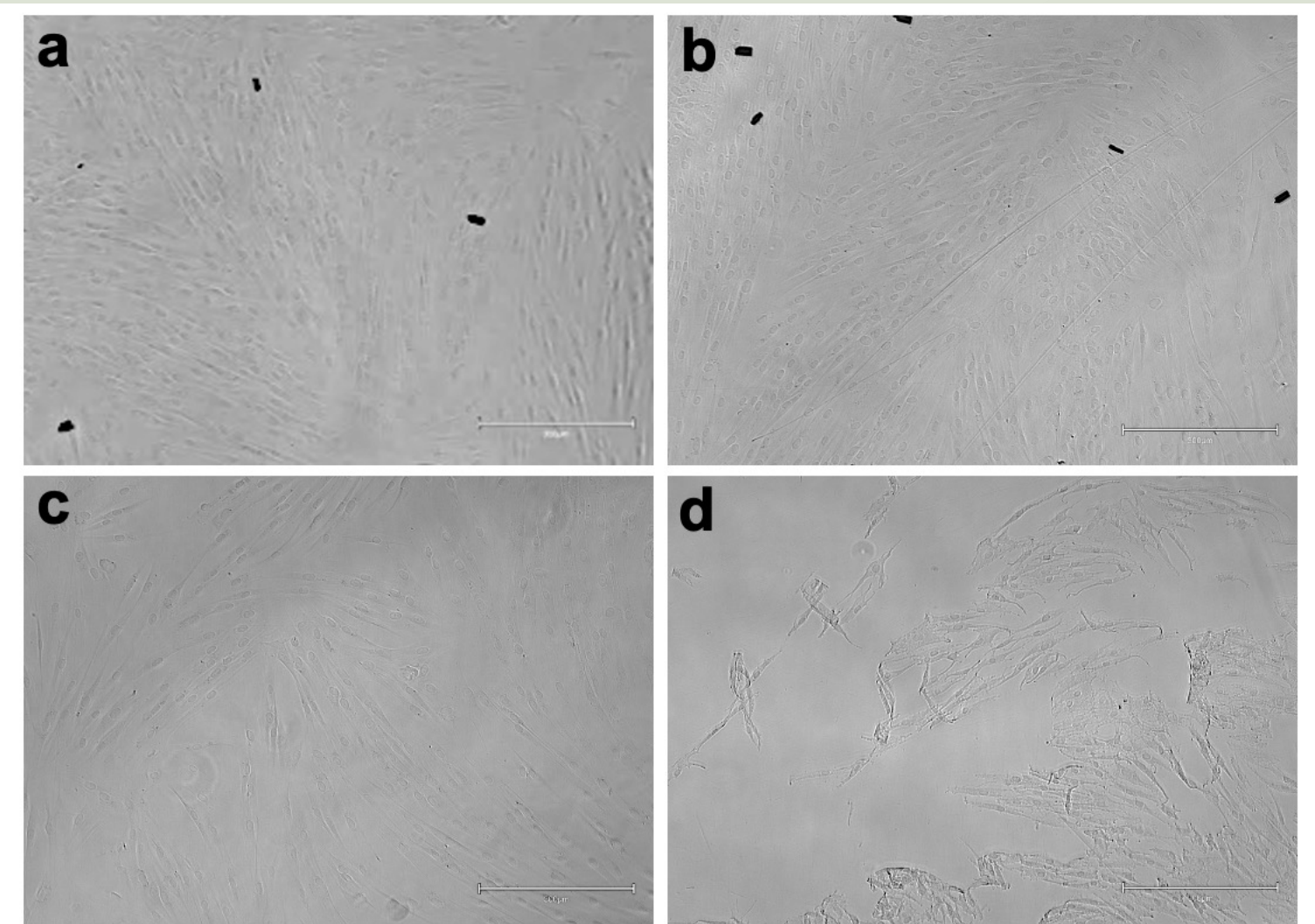


Figure 4. Representative photomicrographs of fibroblasts after 6h-incubation with Etoposide at 10 μ M (a), 15 μ M (b), 20 μ M (c) and 25 μ M (d). Scale bar: 300 μ m. Magnification 10x.

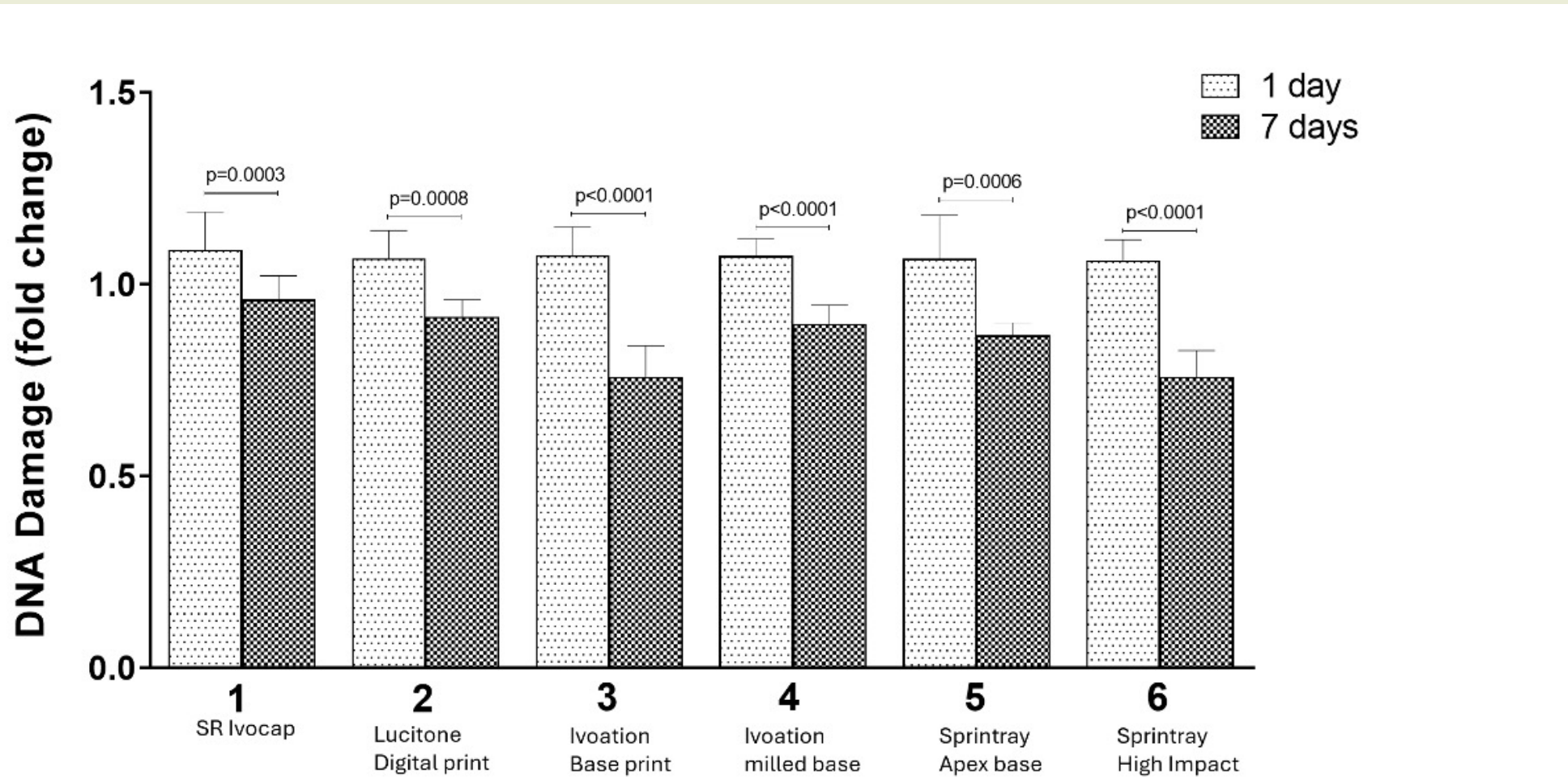


Figure 5. DNA damage fold changes after 6 hours of incubation with denture base resin disks extracts groups 1-6, from 1- and 7-days incubation periods. Results are presented as mean $\pm$ SD.

All materials showed initial DNA damage at baseline exposure (1 day). However, after 7 days, significant differences emerged. The 3D-printed resins (Ivoclar Ivotion Base Print and Sprinray High Impact Denture Base) showed the highest reduction in DNA damage, while heat-cured PMMA showed the least improvement. The other 3D-printed and milled PMMA resins displayed intermediate recovery.

DISCUSSION

This study evaluated the genotoxicity of conventional heat-cured PMMA versus CAD/CAM denture bases using the γ -H2AX assay on human gingival fibroblasts. All materials induced DNA strand breaks at day 1, with Ivoclar SR Ivocap showing the highest damage, but DNA damage significantly decreased by day 7. CAD/CAM resins, particularly Ivoclar Ivotion and Sprinray High Impact, showed the lowest DNA damage, reflecting enhanced biocompatibility and reduced monomer release. Findings suggest conventional PMMA poses higher genotoxic risk due to incomplete polymerization, while CAD/CAM resins demonstrate superior safety and performance, aligning with prior studies. Overall, all tested materials stayed below the 1.5-fold γ -H2AX threshold, indicating they are not genotoxic, though cytotoxicity considerations remain essential for clinical evaluation.

CONCLUSION

The results of our study provide a critical understanding of the genotoxicity of the conventional and CAD/CAM fabricated denture base materials and conclude that:

- Superior biocompatibility and minimal DNA changes were noticed with the 3D-Printed resins (Ivoclar Ivotion Base Print and Sprinray High Impact Denture Base), which is most likely attributed to the reduced residual monomer release and optimized polymerization.
- Heat-cured PMMA demonstrated the highest DNA changes compared to all the groups.
- Milled PMMA denture base material showed intermediate response to the DNA changes, which suggests that CAD/CAM fabricated denture bases generally have enhanced material biocompatibility compared to the conventionally fabricated denture base material.
- All the materials in both time points did not exceed the threshold of genotoxicity when assessed by γ -H2AX assay.
- Long-term and comprehensive evaluations of these material genotoxic potentials are recommended.