Effect of Laser Beam Welded AISI 2205 Duplex Stainless Steel on the Viability of Fibroblast Cells

Ceyhun Köse[1], Ramazan Kaçar[2], Aslı Pınar Zorba[3], Melahat Bağırova[3], Emrah Şefik Abamor[3], Adil M. Allahverdiyev[3]

[1]Gaziosmanpaşa University, [2]Karabük University, [3]Yıldız Technical University - Türkiye

Abstract

Duplex stainless steel is widely used in the oil, chemical industry and to a lesser extent in the medical industry, because of the high mechanical properties, corrosion resistance and good weldability. In recent years, because of these properties, it can enable the use in the biomedical applications. Accordingly, duplex stainless steel material can be highly important to examine the effect on the cells. In this study, the effect of the AISI 2205 duplex stainless steels which are joined by CO_2 laser beam welding on the fibroblast cells has been studied *in vitro* for the first time. The effect of the laser beam welded samples and samples of the base metal of AISI 2205 duplex stainless steel with L929 fibroblast cells as an element of connective tissue under in vitro conditions has been studied. To study the effect of the base metal and the laser welded test specimens on the viability of the fibroblast cells were kept in DMEMF-12 medium for 7 days. The viability study was experimentally studied using the MTT method for 7 days. The cell viability of the laser welded sample has been detected to be higher than that of the base metal and the control based on 7^{th} day data. According to the obtained results reveals that laser beam welded and base material AISI 2205 duplex stainless steel has been found suiatable to study for biomedical applications.

1. Introduction

Duplex stainless steels (DSSs) consist of a two phase microstructure involving δ-ferrite and γ-austenite. DSS as suitable alternatives to conventional austenitic stainless steels. Duplex stainless steel shows such excellent mechanical properties and corrosion resistance due to the fact that its internal structure consists of ferrite and austenite phases at equal rates [1,2]. The duplex microstructure enables the steel to become especially highly resistant to stress corrosion cracking as well as intergranular and pitting corrosion. AISI 2205 stainless steel, with 22% chromium, 56% nickel, and 3% molybdenum, is a nitrogen-alloyed duplex stainless steel with excellent corrosion resistance and mechanical properties. Due to its superior corrosion resistance, this type of steel is used in steam boilers, chemical tanks and heat exchanger pipes as well as in a wide range of areas in the chemical and petroleum chemical industry and less in the medical industry [3-5]. Weldability of duplex stainless steel is very good and it can be joined by many fusion welding techniques, such as submerged arc welding (SAW), shielded metal arc welding (SMAW) and tungsten inert gas welding (TIG) and laser beam welding [6].

AISI 316L austenitic stainless steel is known as the most commonly used orthopaedic and orthodontic bracket material because of its favourable mechanical properties, and relatively good corrosion resistance in various aqueous environments [7]. However, it is regularly challenged by the aggressive environment in the human body, as it is highly susceptible to localized corrosion in environments containing chloride [7]. Therefore, austenitic stainless steels are currently often replaced with duplex stainless steels, such as AISI 2205 duplex stainless steel.

There are many studies on the laser beam welding process of AISI 2205 duplex stainless steel [8-10]. However, there is no study regarding the clinical examination of *in vitro* and *in vivo* relation between laser beam welded AISI 2205 duplex stainless steel and fibroblast cells in the

literature. Welded implants' and prosthesis' biological properties such as biocompatibility and cell toxicity should be examined under *in vitro* conditions prior to its clinical application. The effects of the AISI 2205 duplex stainless steels which are joined by CO_2 laser beam welding on the fibroblast cells has been studied *in vitro* for the first time with this study.

2. Experimental Procedure

2.1. Material, welding process and microstructure analyses applied to the welded joint

In this study, AISI 2205 duplex stainless steel is widely used in various industries, such as chemical and petroleum chemical industry. The chemical composition of AISI 2205 stainless steel is given (weight %) in Table 1.

Table 1. The chemical composition of AISI 2205 stainless steel (weight %)

C % 0,028	Si 0,333	Mn 1,814	P 0,011	S 0,0072	Cr 22,88	Mo 3,105	Ni 5,450	Al 0,019	Co 0,119
Cu 0,224	Nb 0,036	Ti 0,0089	V 0,116	W 0,050	Pb 0,0038	Sn 0,012	Zn 0,034	N 0,058	Fe 65,68

AISI 2205 stainless steel plates were fixed in a horizontal position onto the fixture and the welding was performed with TRUMPF LASERCELL 1005 model, 4 kW power rated CO_2 laser beam welding machine without any filler metal with the parameters given in Table 2.

Table 2. The welding parameters.

Laser power (W)	Travel speed (cm/min)	Shielding gas	Gas flow rate (lt/min)	Focal length (mm)	Heat input (kJ/mm)
4000	270	50% Ar + 50% He	10	200	0.088

The process of electrolytic etching was applied using a solution of 10g oxalic acid + 100 ml pure H_2O. Microstructure examinations were carried out by using optical microscope (OLYMPUS) at magnifications between 5x-100x.

2.2. The preparation of the test specimen and the examination of their effects on the cell culture

AISI 2205 duplex stainless steel is commercially obtained and a pair of AISI 2205 stainless steel joined by CO_2 laser beam welding method. Samples have been polished by 200-1200 grit emery paper after being cut in sizes of 3 x 5 x 20 mm and perpendicular to the welding direction so that the welding seam is left in the middle.

The experiments to study the effect of the laser beam welded AISI 2205 Duplex Stainless Steel material and the laser beam welded joint on L929 fibroblast cell culture. The test materials have been wiped with alcohol and bathed in distilled water and then sterilized in 170 ^oC temperature for 90 minutes. They have been incubated in an incubator containing 5 % CO_2 at 37 ^oC temperature by placing 3 ml of DMEMF12 medium and the materials in a sterile falcon. The supernatants of the main material and the specimen joined by welding which have been in a DMEMF12 medium along 7 and their viability effect on the L929 cells have been examined via the MTT (Thiazolyl Blue tetrazolium bromide) method. The same order of procedures have been carried out for all three time periods set in this study. L929 fibroblast cell plantation has been carried out in a way that 10.000 cells are in each well on a plate of 96.

Sample wells in threes have been taken for the main material and the sample joined by laser welding and the testing apparatus has been set on the control cells by only adding from the medium so that there samples are in threes. As a result of the cells fully covering the wells, the excess medium has been removed and 100µ of the DMEMF12 medium in which the samples were kept has been placed instead and they have been incubated in an incubator with 5% CO_2 at 37 ^oC for one day.

Following the incubation, the MTT experiment protocol has been applied to determine the cell viability. It has been dispersed and vortexed with MTT solution of 10mg/ml (Phosphate Buffered Saline). The MTT solution prepared has been put in each well in 10 µl's after being filtered with 0,45 µl filters and incubated for 4 hours at 37^oC and then the crystallization has been observed under the microscope. 100 µl's of DMSO have been added to each well by pipetting onto the MTT for the dissolution of the formazan crystals formed on the cells and kept in the dark for 30 minutes. After the dissolution of the crystals with the DMSO, they have been read in 570 nm by ELISA and the results have been obtained.

2.3. Statistical analysis

In multiple comparisons, the Tukey test has been applied as a post-hoc test for the ANOVA one-way analysis of variance on the GraphPad statistics software. In the assessment of the results, the significance levels of $p<0.001$, $p<0.01$ and $p<0.05$ have been used as a basis.

3. Results and Discussion

3.1. Microstructural examination of the laser welded sample

The microstructural examination of the base metal, the heat affected zone (HAZ) and the weld metal of the AISI 2205 duplex stainless steel joined by CO_2 laser beam welding used in the study and the result are shown in Fig.1.

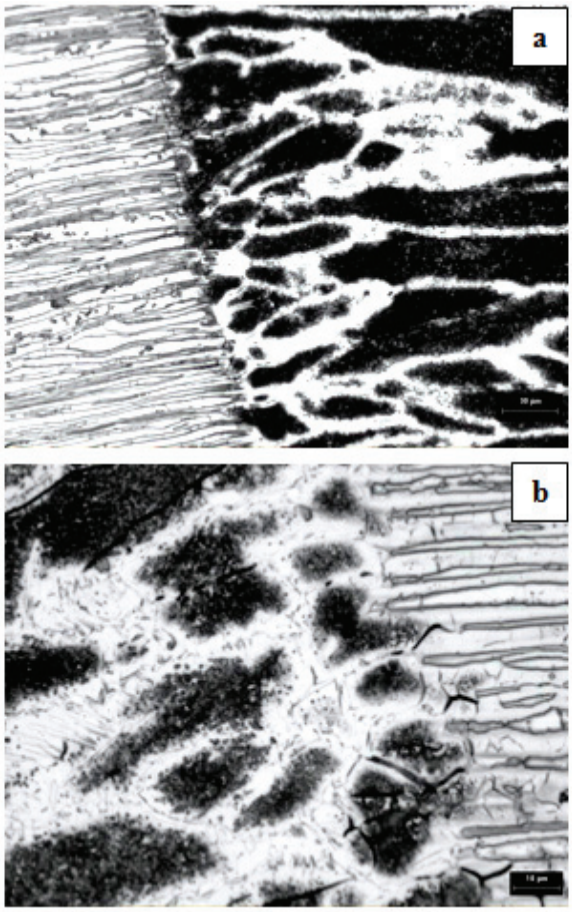

Figure.1. Microstructure of laser welded joint, a) HAZ (200X), b) HAZ (500X)

As can be seen in Figure 3, the weld metal structure of AISI 2205 stainless steel as it was provided consists of ferrite phase (70 %) and austenite phase (30 %) and it has almost fully ferritic structure with some precipitation of austenite decorate to the grain boundaries. The light colored grains in the structure indicate the austenite phase while the dark colored ones indicate the ferrite phase. As it is known, the high tensile strength and stress corrosion resistance of duplex stainless steel results from the delta ferrite phase in its structure while its toughness and general corrosion resistance results from the austenite phase in its structure. The micrographic examination shows that the extension of the heat affected zone is negligible (Fig.1a and 1b). Formation of a very narrow heat affected zone is an expected result since low heat input, which is a characteristic of laser beam welding, causes fast cooling.

3.2. Cell Studies

The effect of CO_2 laser beam welding method applied to AISI 2205 duplex stainless steel on the viability of the fibroblast cells was studied by placing the base metal and laser welded samples directly in the cell culture medium for particular periods. MTT result is given in Fig.2. and Fig.3. The basemetal and the laser welded sample show better cell viability effect when compared to the control group (Fig.) As a result of the direct interaction of the L929 cell culture and the control, main material and laser welded sample, while an increase in the cell proliferation of main material and the laser welded sample has been observed in comparison with the control group ($p<0.001$)

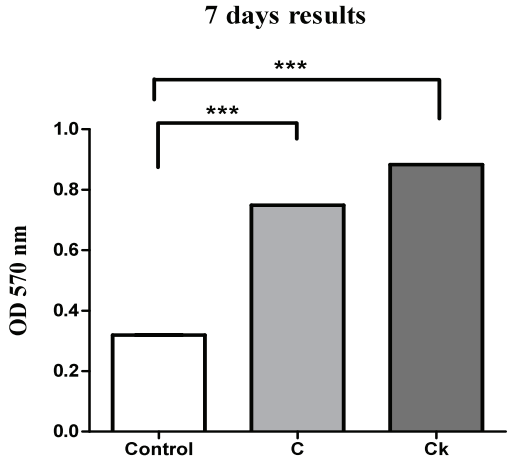

Figure 2. The results of the MTT which is applied as a result of the direct interaction of the L929 cell culture and the samples, C: Control, C: Base metal, Ck: Laser welded sample

When the results regarding the 2205 duplex stainless steel main material and laser welded sample are studied, after the first week, the effect of the supernatant samples obtained from the mediums in which the samples were kept on the cell viability was higher for laser welded sample when compared with the main material. In laser welding, weld metal has finer grains and therefore, larger grain boundary due to high solidification rate. The grain boundaries have higher energy than inside the grains [11]. Therefore, this excessive energy in grain boundary combined with high ferrite phase content (70 %) and austenite phase (30 %) and it has almost fully ferritic structure with some precipitation of austenite decorate to

the grain boundaries. Weld metal is considered for the reason of the denser cells at the beginning of the process. However, the grain boundaries are more chemically reactive than the grains themselves as a consequence of this grain boundary energy [11] and the rapid ion exchange along the grain boundaries can be responsible for less dense cells in welded sample compare with base metal end of the process.

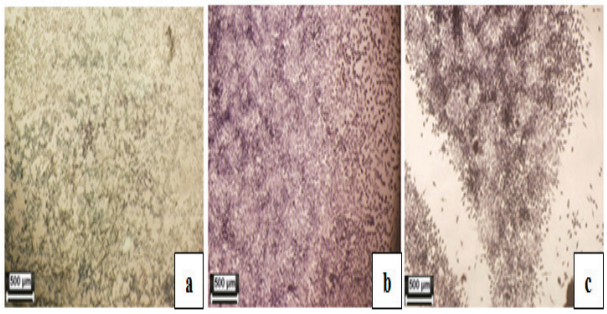

Figure 3. Formazan crystal formation after 7 days MTT (10x) **a)** L929 control has been incubated only with the DMEMF12 culture medium. The control cells and the density of the formazan crystals which indicate the viability are scarcer than the cells shown in figures **b)** and **c)** The post MTT image of the L929 cell culture which has been incubated with the supernatant taken from the medium in which the main material was kept for 7 days. Density of the cells and formazan crystals has been observed to be higher than the control. **c.** The post MTT image of the L929 cell culture which has been incubated with the supernatant taken from the medium in which the laser welded sample was kept for 7 days. It has been observed that the L929 cells were denser in one direction and that they contained darker formazan crystals when compared with that of the control and the main material.

4. Conclusions

According to the results acquired, the interaction of the CO_2 laser beam welded AISI 2205 duplex stainless steel sample and base metal with the L929 fibroblast cells which were connective tissue elements were studied under in vitro conditions.

The findings obtained can be summarized as follows:

1. It was found that the low heat input and high cooling rate of laser beam welding method had an effect on the microstructure and morphology of the weld metal. It was discovered that the ferrite-austenite balance of the weld metal and the HAZ differed from that of the base metal as to be in favor of ferrite phase, also, the low heat input caused a very narrow HAZ.

2. In the data obtained after the first week (7 days), the effect of the laser welded sample on the cell viability was greater than that of the base metal. It is believed that higher grain boundary energy, the presence of high delta ferrite phase content (70%) in weld metal and higher surface energy of welded region due to weld thermal cycle can be responsible for the denser cells at the beginning of the process.

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